



**HAL**  
open science

# Importance of AJ and cell polarity in the regulation of Fas signaling

Hala Awina

► **To cite this version:**

Hala Awina. Importance of AJ and cell polarity in the regulation of Fas signaling. Cellular Biology. COMUE Université Côte d'Azur (2015 - 2019), 2019. English. NNT : 2019AZUR6025 . tel-02948926

**HAL Id: tel-02948926**

**<https://theses.hal.science/tel-02948926>**

Submitted on 25 Sep 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# THÈSE DE DOCTORAT

## Importance des jonctions adhérentes et la polarité cellulaire dans la régulation des signaux engendrés par le récepteur Fas/CD95

**Hala AWINA**

Équipe Hueber- Institut de Biologie Valrose

**Présentée en vue de l'obtention  
du grade de docteur en interactions  
cellulaires et moléculaires  
d'Université Côte d'Azur**

**Dirigée par :** Anne-Odile Hueber

**Co-encadrée par :** Laurent Gagnoux-  
Palacios

**Soutenue le :** 19 Décembre 2019

**Devant le jury, composé de :**

Anne-Odile Hueber, Directrice de recherche  
INSERM, Institut de Biologie Valrose

Laurent Gagnoux-Palacios, Charge de  
recherche INSERM, Institut de Biologie  
Valrose

Jean Paul Borg, Professeur des universités,  
Institut Paoli-Calmettes

Daniel BOUVARD, Charge de recherches,  
Institut pour l'Avancée des Biosciences

Philippe Naquet, Professeur des  
universités, Centre d'Immunologie de  
Marseille Luminy

# **Importance des jonctions adhérentes et la polarité cellulaire dans la régulation des signaux engendrés par le récepteur Fas/CD95**

## **Jury :**

Président du jury :

Jean Paul Borg, Professeur des universités, Institut Paoli-Calmettes

Rapporteur :

Daniel BOUVARD, Charge de recherches, Institut pour l'Avancée des Biosciences Philippe

Naquet, Professeur des universités, Centre d'Immunologie de Marseille Luminy

Co-Directeur de thèses :

Laurent Gagnoux-Palacios, Charge de recherche INSERM, Institut de Biologie Valrose

Directrice de thèses :

Anne-Odile Hueber, Directrice de recherche INSERM, Institut de Biologie Valrose

## Titre :

Importance des jonctions adhérentes et la polarité cellulaire dans la régulation des signaux engendrés par le récepteur Fas/CD95

## Résumé:

Fas (CD195/TNFRSF6) est une protéine transmembranaire appartenant à la superfamille des tumor necrosis factor receptor (TNFR). Même si la signalisation induite par ce récepteur suite à la liaison avec son ligand FasL (CD178/TNFSF6), a surtout été étudiée dans un contexte de mort cellulaire, on sait à présent qu'elle conduit aussi, notamment dans un contexte cancéreux, à la survie de la cellule en induisant sa prolifération ou sa migration. L'équipe du Dr. AO Hueber s'efforce depuis plusieurs années de disséquer les mécanismes moléculaires sous-tendant la versatilité de ce récepteur. En effet, comprendre le contrôle des signaux émis par Fas dans le contexte de l'équilibre de la décision mort/vie de la cellule, est crucial pour le développement de stratégies thérapeutiques anti cancéreuses et notamment pour le cancer colorectal, qui est la troisième cause la plus courante de cancer dans le monde et au 2ème rang des décès par cancer.

S'il était connu que les cellules intestinales, en perpétuel renouvellement, mourraient suite à la perte des contacts cellule-cellule ou cellule-matrice extracellulaire, le rôle possible des jonctions intercellulaires et de la polarité cellulaire dans le contrôle de la signalisation du récepteur de mort Fas avait été jusqu'à présent largement inexploré. L'objectif principal de mon sujet de doctorat a été d'étudier le rôle des jonctions adhérentes et de la polarité cellulaire dans la modulation de la mort cellulaire induite par FasL dans des cellules épithéliales de côlon.

Nous avons pu démontrer que l'établissement de la polarité cellulaire et la formation de jonctions adhérentes contrôlaient la signalisation pro-apoptotique du récepteur Fas en montrant que : (i) le récepteur Fas se concentre avec l'E-cadhérine aux jonctions intercellulaires ; (ii) l'association Fas-cadhérine protège les cellules de la mort cellulaire induite par Fas.

Une approche protéomique nous a permis d'identifier plusieurs nouveaux partenaires interagissant avec un site d'interaction à domaine PDZ localisé dans la partie C-terminale du récepteur. Parmi ceux-ci se trouve la molécule de polarité/échafaudage Dlg1. Nous avons démontré que Dlg1 interagit directement avec Fas et réduit la formation du complexe de mort suite à l'engagement avec le ligand, ce qui constitue un mécanisme supplémentaire de protection contre la mort cellulaire.

L'ensemble de ces résultats nous ont conduit à proposer que le complexe Fas-cadherine-Dlg1 en contrôlant l'inhibition de la mort cellulaire induite par Fas non seulement participe à l'homéostasie épithéliale en protégeant l'épithélium de l'apoptose mais également participe à l'élimination des cellules endommagées non-polarisées freinant ainsi le développement de cellules cancéreuses.

Dans la deuxième partie de mon doctorat, j'ai étudié le rôle de plusieurs partenaires de Fas identifiés dans notre analyse protéomique, et notamment de l'E3 ubiquitine ligase LNX2. J'ai pu montrer que (i) LNX2 se lie directement à Fas (ii) induisait la dégradation du récepteur

activé par son ligand au niveau des lysosomes, conduisant à une inhibition des signaux de mort. L'ensemble de ces résultats suggèrent que LNX2 protège les cellules épithéliales coliques de la mort cellulaire induite par FasL. La protéine LNX2 étant surexprimée dans le cancer du côlon, le mécanisme d'action de LNX2 identifié sur la signalisation Fas pourrait expliquer la résistance des cellules cancéreuses du côlon à la mort par Fas.

Mots-clés : Mort cellulaire, le récepteur Fas, jonction adhérente, polarité cellulaire, épithélium.

## Title:

Importance of AJ and cell polarity in the regulation of Fas signaling

## Abstract:

Fas is a transmembrane cell death receptor mostly known for its function in inducing cell death through apoptosis, but it is also recognized to be implicated in a wide range of non-death functions in various cell types and contexts. The disequilibrium between cell death and survival signals due to defective apoptosis is a key factor in tumorigenesis. Fas is found ubiquitously expressed in human tissues including many epithelia such as in the intestine. Thus, the activation of Fas signaling in this tissue should be rigorously regulated in order to maintain the balance between cell death and non-death signaling.

At a cellular level, the choice of life and death, is regulated by different environmental cues including cell-cell junctions and cell polarity through the involvement of various sets of specialized macromolecules. The possible role of cell-cell contacts and cell polarity in the control of Fas signaling has been largely unexplored. Therefore, the main aim of my PhD was to investigate the role of adherens junction and cell polarity in the modulation of the FasL-induced cell death signaling in colon epithelial cells.

We were able to demonstrate that both cell polarity establishment and adherens junction formation control the pro-apoptotic signaling of the death receptor Fas. We found that the Fas receptors concentrate at cell-cell junctions together with E-cadherin and that Fas-cadherin association protects cells from FasL-induced cell death. Using a proteomic approach, we identified several novel partners of Fas that interact with the C-terminal PDZ-binding site of Fas including the polarity/scaffold molecule Dlg1. We demonstrated that Dlg1 interacts directly with Fas and decrease the death-inducing complex formation upon Fas activation, therefore, providing an additional mechanism to protect against cell death. Altogether, our data show that inhibition of FasL-induced cell death by Fas-cadherin-Dlg1 complex helps to maintain epithelial homeostasis by protecting normal epithelia from apoptosis and promotes elimination of compromised non-polarized cells to avoid development of pathological conditions such as cancer development.

In the second part of my PhD, I investigated the role of several partners of Fas identified in our proteomic analysis, including the E3 ubiquitin ligase LNX2. I demonstrate that LNX2 binds directly Fas, and targets activated Fas to lysosomal degradation, therefore preventing FasL-induced cell death. My results suggest that LNX2 protects colon epithelial cells against FasL-induced cell death. Interestingly, LNX2 is found overexpressed in colon cancer, which may explain how colon cancer cells evades Fas-mediated apoptosis by promoting Fas degradation following its activation.

**Keywords:** Apoptosis, Fas receptor, adherens junction, cell polarity, epithelium

## Acknowledgements:

I would like to start by thanking my PhD director **Dr. Anne-Odile HUEBER** for accepting me to be part of her team for the last few years, and for giving me the opportunity to carry out my thesis in her lab. Moreover, want to thank her for her support, encouragement and for proofreading my manuscript.

I would like also to thank my PhD co-director **Dr. Laurent GAGNOUX** for accepting to conduct my studies under his supervision. I am also very grateful for his teaching, support and patience. Through a lot of discussions, he helped me have new insights to science. I want to thank him also for proof reading my thesis.

I want to thank all the members of my team including Sebastian Huault for taking care of our lab needs and Dr.Aurelie Rossin for her help and guidance through several experiments.

To my colleague, whom I would like to think of as a friend, Georgia Miloro for her support and praising, I will always remember our mind-blowing chats and discussions about science, food and most importantly music.

I will not forget the immense help and teaching I received from the engineering platform. From understanding a certain technique till the usage of the machine

At last I want to thank my family for their support even from afar, especially my mother who was always there for me when I needed her and for being my inspiration and back-bone. To my father and my two sisters, Noor and Riham, who never stopped encouraging me and supporting me throughout my journey.

## Table of Contents

<b>Résumé:</b> .....	<b>3</b>
<b>Abstract:</b> .....	<b>5</b>
<b>Acknowledgements:</b> .....	<b>6</b>
<b>List of Abbreviations</b> .....	<b>10</b>
<b>I. Literature review</b> .....	<b>14</b>
<b>I.A The TNF and TNF receptor superfamilies</b> .....	<b>15</b>
I.A.a History and evolution.....	15
I.A.b Tumor necrosis factor superfamily (TNFSF).....	17
I.A.c Tumor necrosis factor receptor superfamily (TNFRSF) .....	18
I.A.c.1 The death domain containing receptors .....	19
I.A.c.2 The TRAF-interacting motif containing receptors.....	19
I.A.c.3 The decoy receptors .....	19
<b>I.B The Fas/FasL system</b> .....	<b>21</b>
I.B.a FasL.....	21
I.B.a.1 Structural domains.....	22
I.B.a.2 FasL Expression.....	23
I.B.a.3 Proteolytic processing of FasL generates different forms with different functions.....	23
I.B.a.4 FasL function: .....	24
I.B.b Fas.....	25
I.B.b.1 Structure .....	25
I.B.b.2 Expression of Fas .....	27
I.B.b.3 The multi-signaling machinery of Fas.....	27
I.B.b.3.1 Cell death signaling pathway of Fas .....	27
I.B.b.3.2 Non-death signaling pathway of Fas .....	31
I.B.b.4 Fas/FasL signaling is regulated and fine-tuned at many levels.....	33
I.B.b.4.1 Fas signaling regulated at the receptor and ligand level .....	33
I.B.b.4.1.1 Role of endocytosis.....	33
I.B.b.4.1.2 Role of post-translational modifications.....	35
I.B.b.4.2 Fas signaling regulated by signaling modulators.....	36
I.B.c Functions of the Fas / FasL system .....	37
I.B.c.1 Fas /FasL function in physiology .....	37
I.B.c.2 Fas/FasL function in disease.....	39
I.B.c.2.1 Fas/FasL in auto-immune disorders .....	39
I.B.c.2.1.1 Type I autoimmune lymphoproliferative syndrome .....	39
I.B.c.2.1.2 Inflammatory bowel disease.....	39
I.B.c.2.1.3 Multiple sclerosis .....	40
I.B.c.2.2 Fas/FasL function in cancer.....	40
I.B.c.2.2.1 Fas/FasL system as a tumor suppressor .....	40
I.B.c.2.2.2 Fas/FasL system as a tumor promoter .....	40
I.B.c.2.2.3 Role of Fas/FasL system in colon cancer .....	42
I.B.d Potential Fas/FasL targeted therapies .....	45
<b>I.C Epithelial tissue architecture and organization</b> .....	<b>48</b>
I.C.a Organization and functions of epithelial tissues.....	48
I.C.b Polarity and cell-cell adhesion function in epithelia .....	51
I.C.b.1 Polarity in epithelia.....	51
I.C.b.2 Intracellular junction types in epithelia .....	52
I.C.b.2.1 Tight junction.....	53



I.C.b.2.2	Adherens junction.....	54
I.C.b.2.3	Desmosomes.....	54
I.C.b.2.4	Gap junction.....	55
<b>I.D</b>	<b>Adherens junction .....</b>	<b>55</b>
I.D.a	Organization of Adherens junctions.....	55
I.D.a.1	Cadherin-Catenin complex.....	56
I.D.a.1.1	Classical cadherins and the cadherin superfamily.....	56
I.D.a.1.1.1	Structure and function of cadherins.....	56
I.D.a.1.1.2	Expression in adult and during development of classical cadherins.....	60
I.D.a.1.2	Catenins.....	62
I.D.a.1.2.1	$\beta$ -catenin.....	62
I.D.a.1.2.2	$\gamma$ -catenin.....	63
I.D.a.1.2.3	$\alpha$ -catenin.....	63
I.D.a.1.2.4	p120-catenin .....	64
I.D.a.2	Nectin-Afadin complex.....	64
I.D.a.2.1	Nectin.....	64
I.D.a.2.2	Afadin .....	65
I.D.a.3	Mechanisms of AJ assembly and disassembly.....	65
I.D.a.3.1	Cadherin clustering and AJ formation .....	66
I.D.a.3.1.1	Clustering driven by cadherin ectodomains.....	66
I.D.a.3.1.2	Cadherin-catenin clusters and actin .....	67
I.D.a.3.2	Regulation of cadherin stability at the plasma membrane.....	70
I.D.b	AJ functions in physiology and pathology .....	73
I.D.b.1	AJ in physiology .....	73
I.D.b.1.1	Cell-cell adhesion: maintenance of tissue integrity in adult and during embryogenesis.....	73
I.D.b.1.2	Cell-cell recognition and cell sorting .....	77
I.D.b.1.3	Inflammation .....	78
I.D.b.1.4	Apico-basal polarity.....	78
I.D.b.1.5	Cell migration.....	79
I.D.b.1.6	Cell proliferation .....	81
I.D.b.1.7	Cell survival.....	82
I.D.b.2	Adherens junction role in human disease.....	85
I.D.b.2.1	Adherens junction in non-cancer diseases .....	85
I.D.b.2.2	Adherens junction in cancers.....	86
I.D.c	Regulation of intracellular signaling pathways by AJ .....	89
I.D.c.1.1	Role of AJ in “transcriptional regulation” .....	90
I.D.c.1.1.1	Catenins.....	90
I.D.c.1.1.2	AJ control the YAP/TAZ in Hippo signaling pathway .....	93
I.D.c.1.1.3	NF- $\kappa$ B activity.....	94
I.D.c.1.2	Regulation of cell surface receptors.....	95
I.D.c.1.2.1	EGFR.....	95
I.D.c.1.2.2	FGFR.....	97
I.D.c.1.2.3	VEGFR.....	97
I.D.c.1.2.4	TGF- $\beta$ receptor .....	98
I.D.c.1.2.5	Overview on receptor regulation by AJ.....	98
<b>I.E</b>	<b>Apico-basal Polarity .....</b>	<b>103</b>
I.E.a	Definition of polarity.....	103
I.E.b	Molecular complexes involved in establishment of Apico-basal polarity .....	104
I.E.b.1	The Par complex .....	107
I.E.b.2	Crb complex.....	109
I.E.b.3	Scrib complex .....	111
I.E.b.3.1	Structure.....	112
I.E.b.3.1.1	SCRIB.....	112
I.E.b.3.1.2	LGL .....	113
I.E.b.3.1.3	DLG.....	114
I.E.c	Polarity complexes in establishment and maintenance of epithelial polarity.....	116

I.E.d	Other molecules involved in apico-basal polarity.....	119
I.E.e	The Scrib complex.....	120
I.E.e.1	The Scrib module function in physiology and in physiopathology.....	120
I.E.e.1.1	Scrib module function in physiology.....	120
I.E.e.1.1.1	In polarity.....	120
I.E.e.1.1.2	Cell-cell junction formation.....	125
I.E.e.1.1.3	Cell proliferation.....	127
I.E.e.1.1.4	Cell survival.....	129
I.E.e.1.2	Scrib module role in disease.....	130
I.E.e.1.2.1	In non-cancer.....	130
I.E.e.1.2.2	In cancer.....	131
I.E.e.2	Role of the Scrib complex in modulation of Signaling Cascades.....	133
I.E.e.2.1	Hippo signaling.....	133
I.E.e.2.2	Role in regulating cell surface receptors.....	135
I.E.e.2.2.1	Receptor tyrosine kinase.....	135
I.E.e.2.2.2	Thyroid stimulating hormone receptor.....	136
I.E.e.2.2.3	Glutamate receptor.....	136
<b>II.</b>	<b><i>Rational and objectives of the thesis</i></b> .....	<b>139</b>
II.A	<b>My thesis objective 1: Characterization of the role of AJ and polarity in the regulation of Fas cell death signaling.....</b>	<b>141</b>
II.B	<b>My thesis objective 2: Investigation of the role of novel interactants of Fas in the modulation of receptor signaling and trafficking.....</b>	<b>142</b>
<b>III.</b>	<b><i>Results</i></b> .....	<b>144</b>
III.A	<b>Project 1: Characterization of the role of AJ in the regulation of Fas cell death signaling</b>	<b>145</b>
III.B	<b>Project 2: Investigating Fas novel interactants and their role in the receptor signaling</b>	<b>148</b>
<b>IV.</b>	<b><i>Discussion</i></b> .....	<b>160</b>
<b>V.</b>	<b><i>References</i></b> .....	<b>167</b>

## List of Abbreviations

ABCP: apico-basal cell polarity  
AJ: Adherens junctions  
CARD: caspase recruitment domain  
c-FLIP: cellular FLICE-like inhibitory protein  
CRD: cysteine-rich domain  
DD: death domain  
DED: death effector domain  
DISC: death-inducing-signaling complex  
ERK: extracellular-signal regulated kinase  
FADD: Fas-associated death domain  
MHC: major histocompatibility complex  
NK: natural killer  
PCP: planar cell polarity  
PDZ domain : PSD-95/Dlg-A/ZO-1 domain  
PI3K: phosphoinositide 3-kinase  
PLAD: pre-ligand assembly domain  
PRD: proline-rich domain  
SCN: sphingolipid- and cholesterol-rich nanodomains  
SFK: Src-Family-Kinase  
THD: TNF homology domain  
TJ: Tight junction  
TNF tumor necrosis factor  
TNFRSF: TNF receptor superfamily  
TNFSF: TNF receptor superfamily  
TRADD: TNFR-associated death domain  
TRAF: TNF receptor associated factors  
ZA: zonula adherens

Figure 1: Select members of the TNF and TNFR superfamily [23].....	15
Figure 2: Human Fas ligand protein structure [3].....	22
Figure 3: Human Fas receptor structure .....	25
Figure 4 A) The Fas DD-FADD in tetrameric model [21]. Color: Fas(green), FADD (blue). and B) The Fas DD-FADD model and the locations of the three types of contacts in 5:5 model [22], Color Fas (purple), FADD (blue). .....	26
Figure 5: Scheme of procaspase-8 and procaspase-10 processing [1].....	28
Figure 6: Apoptosis signaling pathway: the extrinsic and intrinsic pathway [2] .....	29
Figure 7: Type I and Type II apoptotic pathways of Fas (Yurchenko et al., 2012) .....	31
Figure 8: Fas-induced cell migration pathway [7].....	33
Figure 9: Multiple levels of regulation that affect the strength of apoptotic signaling (Flusberg e Sorger, 2015).....	33
Figure 10: Fas/Fas-L induced apoptosis in physiological immune response [10] .....	37
Figure 11: Fas and FasL expression in small intestine and colon (Hoogwater, Steller et al. 2012).....	44
Figure 12: Cells of Epithelial Tissue. Simple epithelial tissue is organized as a single layer of cells and stratified epithelial tissue is formed by several layers of cells.[185] .....	49
Figure 13: Tissue organization and cell junction composition in two different epithelia (intestine and epidermis).[4] .....	52
Figure 14: Domain structure of the cadherin-catenin complex components. Schematic overview of the domain structure of a classical cadherin and its three associated catenins: $\alpha$ - catenin, $\beta$ -catenin, and p120. (Niessen et al., 2011) .....	59
Figure 15: Organization and cytoskeletal relationships of cadherin–catenin and nectin–afadin complexes. [8].....	62
Figure 16: Molecular structures of nectin and afadin. [12] .....	64
Figure 17: Models for the intercellular adhesion activities of cadherin [12].....	66
Figure 18: Schematic representation of cadherin-mediated adhesion stages in epithelial cells [16] .....	69
Figure 19: Schema presenting mechanisms regulating cadherin stability at cell plasma [19] .....	72
Figure 20: Role of cadherin in cell-cell recognition and cell sorting. [11].....	77
Figure 21: Polarity complexes localization at cell-cell junction in simple epithelia .....	78
Figure 22: Scheme displaying the regulation of major intracellular signaling pathways by Cadherin-catenin complexes. [6] .....	90
Figure 23: Hippo signaling pathway regulated by various mechanisms . [18].....	93
Figure 24: Simple epithelia vs basal keratinocyte molecular organization [9].....	105
Figure 25: Structure and interactions of mammalian polarity complex proteins. [14] .....	106
Figure 26: Schematic diagram of the domain organization of SAP97 isoforms. [17] .....	115
Figure 27: Interchange between polarity proteins of the different complexes is possible in multiple ways, (I) involving competition of binding of Lgl1/2 and Par3 to Par6/aPKC, (II) binding of proteins from different complexes, (III) phosphorylation of Par3, Lgl1/2 and Crb3 by aPKC and (IV) mutual exclusion of the Scrib complex and the apically located Par and Crumbs complex.[20] .....	117
Figure 28: The crosstalk between different polarity complex in differentiated epithelium cell. [13] .....	118
Figure 29: Planar cell polarity .....	122
Figure 30: Regulation of the cytoskeleton by Scrib complex during cell polarity [15] .....	124

Figure 31: N-cadherin expression in invasive cancer cells associates with Fas and Dlg1 and restrains Fas cell death signaling .....	147
Figure 32: Screening the effect of Fas partners on Fas signaling .....	151
Figure 33: RING ub ligase LNX2 binds to Fas receptor through its c-ter PDZ binding domain (SLV).....	153
Figure 34: LNX2 silencing increase cell death induced by Fas-L.....	154
Figure 35: LNX2 regulates Fas total expression.....	157
Figure 36: Fas degradation requires LNX2.....	158
Figure 37: Localization of LNX2 in colon cell line.....	159
Figure 38: Schematic model proposing the inhibitory role of LNX2 on Fas cell-death signaling. ....	159

Table 1: Diseases caused by mutations in the TNF superfamily, receptors, and receptor-associated proteins [25] .....	16
Table 2: TNF receptors superfamily, their ligands and their intracellular binding partners [46] .....	20
Table 3: Table of Fas targeting drugs.[178] .....	46
Table 4: Summery of epithelial tissues function and localization [185] .....	50
Table 5: Members of the cadherin superfamily [5] .....	57
Table 6: Nectin and Nectin-like family members [252] .....	65
Table 7: Cadherin regulating growth factor receptor signaling .....	99
Table 8: Components of the polarity complexes in different species, adapted from [14] ....	111

## I. Literature review

## I.A The TNF and TNF receptor superfamilies

### I.A.a History and evolution

In the late 19<sup>th</sup> century, the observation made by Coley William on the regression of sarcomas from patients who previously contracted bacterial infection, led to the treatment of some human cancers by bacterial extracts. Later, it was discovered that it was the bacterial lipopolysaccharide (LPS) isolated from these extracts that was inducing tumor regression. It was not until 1975 that Carswell *et al.* biochemically and genetically characterized the factor present in the serum responsible of the tumor cells necrosis [24]. This factor was called “tumor necrotizing factor” before being renamed “tumor necrosis factor” (TNF). The discovery of TNF laid the foundation for the isolation and identification of the larger family of cytokines and their target receptors, known respectively as the TNF superfamily (TNFSF) and TNF receptor superfamily (TNFRSF).

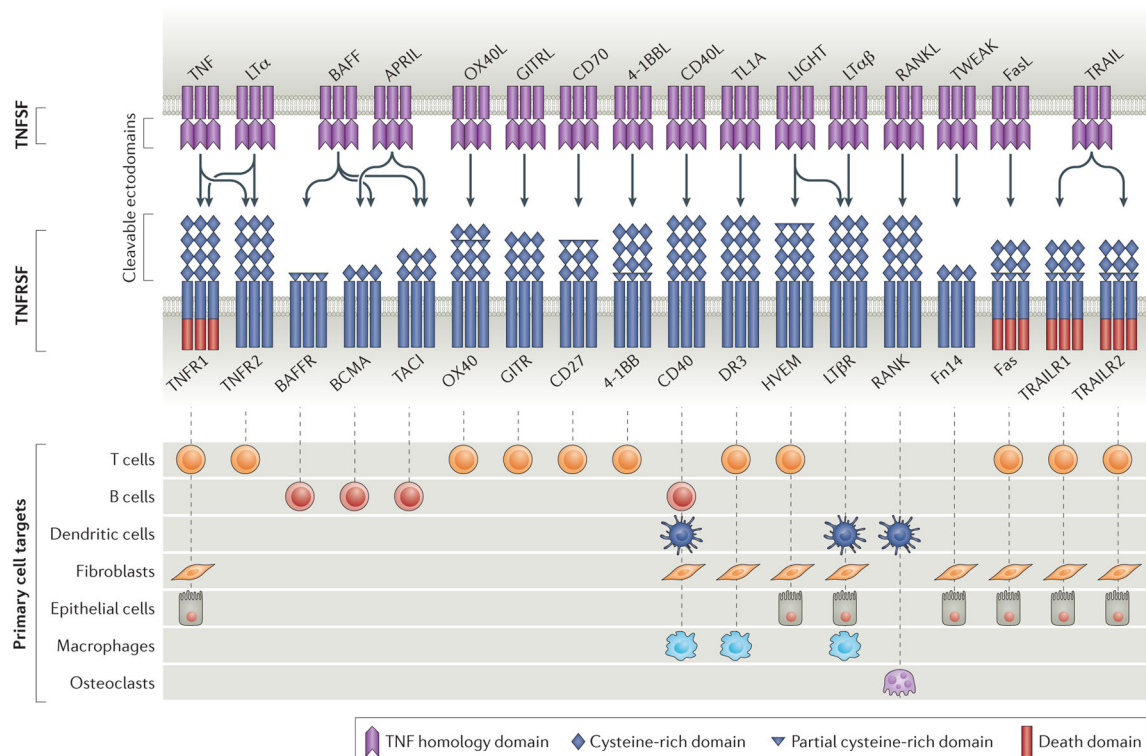


Figure 1: Select members of the TNF and TNFR superfamily [23]



TNFSF and TNFRSF, one of the major ligand-receptor families currently composed of 19 ligands and 29 related receptors, play a pivotal role in controlling countless aspects of the immune system development and function. The ligand-receptor interaction primarily leads to cell death and inflammation but can also induce several signaling pathways inducing cell proliferation, survival and differentiation. As a consequence of this versatile and dual signaling, most of the TNFSF/TNFRSF members could have both beneficial and harmful effects and it is therefore not surprising that they have been linked with diverse human diseases including cancer, cardiovascular, neurologic, pulmonary, autoimmune, and metabolic disorders [25] [26]. Several examples of diseases caused by mutations of TNFSF/TNFRSF members are presented in table 1. They, therefore, constituted potential targets in therapeutic strategies against human diseases including autoimmune disorder and cancer.

Table 1: Diseases caused by mutations in the TNF superfamily, receptors, and receptor-associated proteins [25]

Gene	Study	Disease
<b>Cytokine</b>		
<i>TNF-β (LT-α)</i>	Human	Cerebral infarction
<i>TNF-α</i>	Human	Cerebral infarction
<i>CD40L</i>	Human	X-linked hyper-IgM syndrome
<i>FasL</i>	Mouse	Generalized lymphoproliferative disease
<i>EDA-A1</i>	Human	Ectodermal dysplasia
<i>EDA-A2</i>	Dog	X-linked hypohidrotic ectodermal dysplasia
<b>Receptor</b>		
<i>TNFR1</i>	Human	TNFR1-associated periodic syndrome
	Human	TRAPS associated with SLE
	Human	Crohn disease
<i>TNFR2</i>	Human	Crohn disease
<i>Fas</i>	Mouse	Autoimmune lymphoproliferative syndrome
	Human	Generalized lymphoproliferative disease
<i>RANK</i>	Human	Familial expansile osteolysis
<i>OPG</i>	Human	Idiopathic hyperphosphatasia
	Human	Juvenile Paget disease
<i>TACI</i>	Human	Common variable immunodeficiency
<i>BAFFR</i>	Mouse	Lupus-like syndrome (B cell–mediated autoimmunity)
<i>EDAR</i>	Human	Hypohidrotic ectodermal dysplasia
<b>Adaptors</b>		

<i>TRAF3</i>	Human	Herpes simplex encephalitis
<i>TRAF6</i>	Mouse	Hypohidrotic ectodermal dysplasia
<i>EDARADD</i>	Mouse	Hypohidrotic ectodermal dysplasia
<i>Act1</i>	Mouse	B cell—mediated autoimmune phenotypes

TNFSF/TNFRSF are evolutionary conserved ligands and receptors, and can be followed back to single copy genes of Arthropods [27]. *Drosophila melanogaster* possess a very simple TNF ligand-receptor system, with one TNF homologue, Eiger, and one TNFR homologue, Wengen [28]. The Eiger-Wengen pathway plays vital roles in *Drosophila* development, cell death, and immune function. More recently, another TNFRSF member, Grindelwald, has been defined to have a role in mediating the pro-apoptotic effects of Eiger, resulting in JNK activation as well as the promotion of tumor invasiveness and JNK-dependent neoplastic growth [29].

In higher vertebrates, the various members of the TNFSF/ TNFRSF system originated from multiple whole genome duplication events. Interestingly phylogenetic tree construction and sequence alignment have disclosed that in human, the emergence of the adaptive immune system and the replication of the MHC (major histocompatibility complex) must have happened at the same time than extensive duplications in TNFSF genes [30].

#### I.A.b Tumor necrosis factor superfamily (TNFSF)

The TNF ligands are type II transmembrane proteins characterized by a common structural motif at the C-terminal extracellular called the TNF homology domain (THD). This domain is a 150 amino acid long sequence containing a conserved framework of aromatic and hydrophobic residues. THDs share a virtually identical tertiary fold and associate to form trimeric proteins. In addition, this domain has the ability to bind to the cysteine-rich domain (CRD) located at the extracellular N-terminus of its cognate receptor of the TNFSFR.

One TNFSF member can bind to one or more of the TNFRSF member (Figure 1). The expression of almost all TNFSF members is found in cells of the immune system, including B and T cells, natural killer (NK) cells, monocytes and dendritic cells [25].

The TNFSF proteins exist under several forms. The TNF is a good illustration of this multiple representation. The membrane-bound TNF (mTNF) is expressed at the cell surface, forms non-covalently-bound homotrimers, and can be shed by proteolysis releasing a soluble form (sTNF)

[31]. The sTNF allows a distant ligand-receptor interaction, away from the TNF bearing cells which can, therefore, spread the apoptosis induction effect [32].

#### I.A.c Tumor necrosis factor receptor superfamily (TNFRSF)

The TNFR member is a type I protein, characterized by the presence of one or several CRD in its extracellular part, that bind to the THD of its cognate TNFSF ligand [33].

Two models of receptor aggregation have been proposed so far in the structural organization of TNF/TNFR complexes: (a) the ligand induced trimerization model and (b) the pre-ligand assembly model.

a) The nuclear magnetic resonance (NMR) and crystallography studies lead to the ligand-induced trimerization model. It was proposed that the trimer TNF ligand recruits three individual chains of the receptor inducing its trimerization [34, 35]. Evidence supporting this model comes also from the crystal structure of TRAIL (Apo2L) in complex with one of its receptor, the protein DR5 (TNFRSF10B) revealing an architecture in which the three monomeric ligand interlocks with the three receptor chains forming a hexameric complex [36].

b) The pre-ligand assembly model proposes on the other hand that a preassembled trimeric complexes could be formed at the cell surface independently of the ligand binding, thanks to a domain localized in the membrane distal CRD, and therefore called later pre-ligand assembly domain (PLAD) [37]. Nowadays, it is widely recognized that many TNFRSF members predominantly exist as a pre-assembled complexes [38]. Depending on the form and composition of the receptor complexes found at the plasma membrane, the pre-ligand binding model offers different representations. For instance, the pre-assembled receptor complexes of the TNFR1 is trimetric, whereas, DR5 was found rapidly as a dimer, followed by the recruitment of a third DR5 monomer [39].

The receptors of the TNFRSF can be classified into three groups according to the nature of their cytoplasmic part, responsible of the type of interactions with intracellular adaptors and consecutively of the signaling outcome.

### *1.A.c.1 The death domain containing receptors*

The members of this group harbor a conserved cytoplasmic protein-protein interaction domain known as the death domain (DD) (see more detailed description page 26). There are eight human DD containing receptors originally called death receptors (DRs) : Tumor necrosis factor receptor 1 (TNFR1/TNFRSF1A), Fas (CD95/TNFRSF6), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1/DR4/TNFRSF10A), TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2/DR5/TNFRSF10B), death receptor 3 (DR3/APO-3/TNFRSF25), DR6(TNFRSF21), p75-nerve growth factor receptor (p75-NGFR/ TNFRSF16), ectodermal dysplasia receptor (EDAR) [40]. The DD of Fas, DR4 and DR5 binds to the adaptor protein Fas-associated death domain (FADD), whilst TNFR1 and DR3 binds to the TNFR-associated death domain (TRADD). The DD of each proteins interacts with one of several DD-containing adaptor molecules, leading to the formation of scaffolding complex that initiates the recruitment and activation of caspases and eventually induction of apoptosis (see section cell death signaling pathway of Fas) [41]. In spite of their name, death receptors induce also other signals notably, but not only, through the activation of transcription factors and consequent gene activation. For example, the most prominent role of TNF in inflammatory cascade is due to the induction of cytokines and chemokines [42]. Non-death activities of Fas are also important and will be detailed in page 31.

### *1.A.c.2 The TRAF-interacting motif containing receptors*

Some of the TNFRSF members lack a DD but harbor a TRAF-interacting motif (TIM) that allow the binding to the TNF receptor associated factors (TRAFs) and the initiation of non-death signaling pathways. Currently, seven TRAF proteins have been characterized in mammals: TRAF1 to TRAF7. According to the nature of the TRAF recruited, different signal transduction pathways are activated such as NF- $\kappa$ B, JNK, ERK and phosphoinositide 3-kinase (PI3K) [43]. Receptors with TIM domain are presented in table 2.

### *1.A.c.3 The decoy receptors*

Some TNFRSF serve as decoy receptors as these do not contain any functional intracellular signaling domains or motifs. So far four receptors belong to this group: DcR1 (TNFRSF10C), DcR2(TNFRSF10D), DcR3 (TNFRSF6B) and Opg (TNFRSF11B). Despite the fact that these receptors cannot transmit intracellular signals by themselves, they possess an important role as they can compete with other TNFRSF members for their corresponding ligands and/or by

forming signaling-incompetent heteromeric receptor complexes [44]. For instance, DcR3 can bind FasL and therefore neutralize the Fas/FasL signaling. Therefore, the function of these DcRs is to hinder the activation of signal transduction pathways through other TNFRSF receptors [45].

Table 2: TNF receptors superfamily, their ligands and their intracellular binding partners [46]

<b>TNFRSF receptor (other names)</b>	<b>TNFSF ligand (other names)</b>	<b>Intracellular binding partner</b>
<b>Death receptors</b>		
Fas (TNFRSF6, CD95)	FasL (TNFSF6, CD178)	FADD
TNFR1 (TNFRSF1a, CD120a)	TNF (TNFSF2, TNF- $\alpha$ ), LT $\alpha$ (TNFSF1, TNF- $\beta$ ), LT $\beta$ (TNFSF3)	TRADD, FADD, RIP
TRAILR1 (TNFRSF10A, DR4, CD261)	TRAIL/Apo2L (TNFSF10, CD253)	FADD, TRADD, RIP
TRAILR2 (TNFRSF10B, DR5, CD262)	TRAIL/Apo2L (TNFSF10, CD253)	FADD, TRADD, RIP
NGFR (TNFRSF16, p75NTR, CD271)	NGF (not a TNFSF member)	NADE
DR3 (TNFRSF25 or 12, TRAMP)	TL1A (TNFSF15, VEGI), TWEAK (TNFSF12)	TRADD, FADD
DR6 (TNFRSF21, CD358)	N-APP (not a TNFSF member)	TRADD, RIP
EDAR	EDA-A1	EDARADD
<b>Receptors with TRAF interacting motif</b>		
TNFR2 (TNFRSF1b, CD120b)	TNF (TNFSF2, TNF- $\alpha$ ), LT $\alpha$ (TNFSF1, TNF- $\beta$ ), LT $\beta$ (TNFSF3)	TRAF1–3
LT $\beta$ R (TNFRSF3)	LT $\beta$ (TNFSF3), LT $\alpha$ $\beta$ 2	TRAF2, TRAF3, TRAF5
OX40 (TNFRSF4, CD134)	CD40L (TNFSF5, CD154)	TRAF1–3, TRAF5, TRAF6
CD27 (TNFRSF7)	CD27L (TNFSF7, CD70)	TRAF2, TRAF3, TRAF5
CD30 (TNFRSF8)	CD30L (TNFSF8, CD153)	TRAF1–3, TRAF5
4-1BB (TNFRSF9, CD137)	4-1BBL (TNFSF9, CD137L)	TRAF1–3
RANK (TNFRSF11A, CD265)	RANKL (TNFSF11, TRANCE, CD254)	TRAF1–3, TRAF5, TRAF6
Fn14 (TNFRSF12A, TWEAKR; CD266)	TWEAK (TNFSF12)	TRAF2, TRAF6
TACI (TNFRSF13B, CD267)	APRIL (TNFSF13, CD256)	TRAF2–3, TRAF5, TRAF6
BAFFR (TNFRSF13C, BR3, CD268)	BAFF (TNFSF13B/20, BLys, THANK, CD257)	TRAF2, TRAF3, TRAF6

HVEM (TNFRSF14, CD270)	LIGHT (TNFSF14, CD258), LT $\alpha$ (TNFSF1, TNF- $\beta$ )	TRAF1–3, TRAF5
BCMA (TNFRSF17, CD269)	APRIL (TNFSF13, CD256), BAFF (TNFSF13B/20, BLys, THANK, CD257)	TRAF1–3, TRAF5, TRAF6
GITR (TNFRSF18, AITR, CD357)	GITRL (TNFSF18, AITRL, TL6)	TRAF1–5
TROY (TNFRSF19, TAJ)	?	TRAF2, TRAF5, TRAF6
RELT (TNFRSF19L)	?	TRAF1
XEDAR (TNFRSF27)	EDA-A2	TRAF3, TRAF6
<b>Decoy receptors</b>		
TRAILR3 (TNFRSF10C, DcR1, CD263)	TRAIL/Apo2L (TNFSF10, CD253)	
TRAILR4 (TNFRSF10D, DcR2, CD264)	TRAIL/Apo2L (TNFSF10, CD253)	
OPG (TNFRSF11B)	TRAIL/Apo2L (TNFSF10, CD253), RANKL (TNFSF11, TRANCE, CD254)	
DcR3 (TNFRSF6B)	FasL (TNFSF6), TL1A (TNFSF15, VEGI), LIGHT (14, CD258)	

## I.B The Fas/FasL system

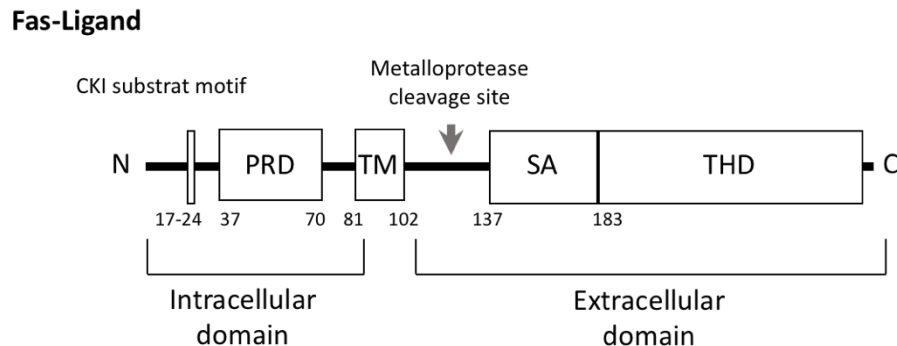
The cell surface bound receptor Fas (CD95/TNFRSF6) was first discovered in 1989 by two independent groups as a target of two monoclonal antibodies that induced the death of the cells by apoptosis (a cell death process displaying specific morphological and biochemical features that I will described in details in page 28 of the manuscript) [47] [48]. The receptors responsible of cell death were cloned by the Nagata' and Krammer's teams, in 1991 and 1992, respectively [49, 50]. Later, in 1993, the cognate ligand of Fas was identified as FasL (TNFSF6/CD95L) [51].

### I.B.a FasL

FasL (TNFSF6/CD95L/CD178), is a cytokine belonging to the TNFSF. FasL, a type II transmembrane protein of 40-kDa, is mainly expressed in activated T lymphocytes [51] and natural killer (NK) cells [52], and is constitutively expressed in tissues of immune-privilege sites such as the testis and eye [53, 54].

### *I.B.a.1 Structural domains*

Human FasL is encoded by a gene located on chromosome 1q23, which consists of 4 exons [55]. FasL is constituted of an extracellular domain, a transmembrane domain and an intracellular domain.



*Figure 2: Human Fas ligand protein structure [3]*

- The **extracellular domain** of FasL contains 179 amino acids. In addition to the THD (see page 17), it contains, the self-assembly (SA) domain, crossing amino acids 137 to 183, which is responsible for FasL biological activity as a homotrimeric complex [56, 57] (Figure 2).
- The intracellular domain (ICD) of FasL consists of 80 amino acids. It contains a binary casein kinase I (CKI) binding sites, which can be found in other TNF family members. This motif is involved in reverse signaling by FasL (see below)[58, 59]. Moreover, FasL comprises an extended proline-rich domain (PRD) (45–65 amino acids), Contrary to all other members of the protein family. It acts as a docking site for proteins containing proline-interacting Src-homology 3 (SH3) or WW domains (module of around 40 aa defined by the presence of two conserved tryptophan residues). This domain is responsible for FasL distribution to lipid rafts [60] that is essential for a maximum Fas receptor-Fas ligand contact and an optimal cell death signaling [61].

### *1.B.a.2 FasL Expression*

In contrast to Fas, FasL expression is limited and highly regulated. It is expressed by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells to selectively kill virus infected or tumorigenic cells [62]. FasL is also found expressed on the immune privileged sites, where development of an inflammatory response has to be limited to avoid inflammation-related destruction of the integrity and function of the tissues. Such privileged sites are found in the eye, testis, placenta or pregnant uterus as well as in neurons and astrocytes of the central nervous system [63]. Other organs express FasL only during periods of extreme inflammatory responses and during cancer development, such as the liver and small intestine [64, 65].

### *1.B.a.3 Proteolytic processing of FasL generates different forms with different functions*

Proteolytic processing by ectodomain shedding is a common feature of various membrane proteins. This is the case for FasL, therefore it can be found as soluble forms. Indeed different matrix metalloproteinases (MMPs) have been involved in FasL shedding, such as MMP3 [66], MMP7 [67], MMP9 [68]. However, the soluble FasL (sFasL) generated by MMP7 and MMP9 had a pro-apoptotic activity in contrast to previous described sFasL fragments. This suggest that the pro- or anti-apoptotic function and the exact nature of sFasL produced by ectodomain shedding seems to depend both on the cellular microenvironment and on its interactions with specific components of the extracellular matrix. Furthermore, our lab in collaboration with the team of Dr. Zoernig identified a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) as a major FasL sheddase in T-cells [69].

Only the membrane FasL (mFasL) leads to strong activation of apoptosis upon Fas engagement [70, 71]. As for sFasL, it inhibit the apoptotic signal triggered by the membrane ligand [72]. Biochemical studies led to propose that aggregation of multiple pre-assembled Fas trimers on the surface of responding cells is needed to induce an optimal cell death signaling [73]. Indeed, sFasL is not able to efficiently induce Fas death signaling, while in the presence of a cross-linking antibody, the efficiency of apoptosis-induction by sFasL was greatly increased, suggesting that the lesser pro-apoptotic potency of sFasL reflects an inability to induce trimerization of the Fas receptor [74]. These results are coherent with the results by P. Legembre's group illustrating that only the naturally cleaved trimeric FasL, in contrast to the oligomerized forms of FasL, induces cell migration signaling upon Fas binding (**see Fas**



**signaling** section)[75]. Suggesting that the mFasL triggers a better Fas aggregation than soluble FasL trimers.

In addition to these membrane and soluble forms, FasL can also exist in a membrane form with cytotoxic activity, on the surface of small vesicles called exosomes. Indeed, several studies have demonstrated that FasL containing exosomes are released by many types of FasL-producing cells, including NK cells, cytotoxic T cells, retinal pigment epithelial cells, and placental trophoblasts [76]. Moreover, a study by Abusamra *et al.* revealed that tumor cells expressing FasL, can also produce FasL positive exosomes that target tumor-specific CD8<sup>+</sup> T cells and promote tumor immune escape [77].

#### *1.B.a.4 FasL function:*

The membrane bound FasL has double roles, it can diffuse signals both as a ligand and as a receptor in a cell-to-cell contact-mediated manner. This means that mFasL not only mediates the forward signals to Fas in the target cells through cell-to-cell contact (described in section below) but also transmits reverse signals back into the mFasL-bearing cells in a process called reverse signaling [58].

Our lab reported that upon FasL shedding by ADAM10, the remaining membrane anchored 17-kDa N-terminal fragment, is further cleaved by a member of the signal peptide peptidase-like 2a family of intramembrane-cleaving proteases (SPPL2a). This cleavage liberates a smaller and highly unstable fragment that has been shown to be able to translocate to the nucleus and capable of inhibiting gene transcription in T cells [69]. This FasL reverse signaling has been found important in negative fine-tuning of certain immune responses [78].

Others molecular explanations for the FasL reverse signaling have been reported. It has been shown that FasL proline-rich sequence initiates reverse signaling which is involved in co-stimulation of CD8<sup>+</sup> T cells, optimal thymocyte maturation, and antigen-driven proliferation of mature T cells [58]. Upon Fas activation, FasL is phosphorylated as well as other signaling molecules such as AKT, ERK, and c-Jun N-terminal kinase (JNK) that will activate transcription factors and enhance IFN- $\gamma$  production [79]. Another study showed that the serine/threonine residues in CKI binding motif is needed for the FasL-mediated activation of nuclear factor of activated T cells (NFAT) and co-stimulation of T cells [59]. These results suggest that FasL reverse signaling can induce different outcomes depending on the context.

## I.B.b Fas

Fas (TNFRSF6/CD95) is a type I transmembrane receptor belonging to the TNFRSF. Fas is best-known for inducing cell death through apoptosis, but it is now well recognized that it could also be involved in a wide range of non-death functions in many cell types and contexts involving cell survival, proliferation, inflammation, migration, and invasion of cells [80, 81].

### Fas

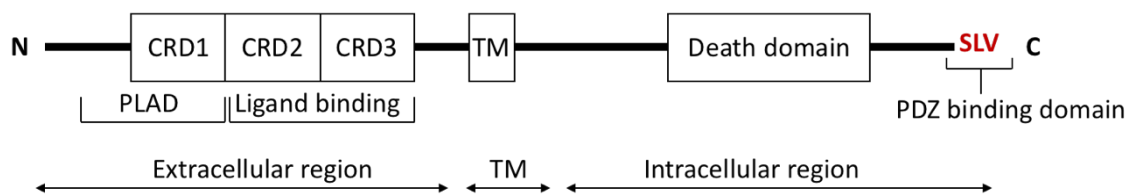


Figure 3: Human Fas receptor structure

### I.B.b.1 Structure

The human Fas encoding gene located on the long arm of chromosome 10 (10q24.1), contains nine exons and eight introns encoding a protein of a 335 amino acids [82].

Fas is divided into 3 domains: an extracellular domain, a transmembrane domain, and a cytoplasmic domain. Exons 1 through 5 encode the extracellular region, Exon 6 encodes the transmembrane region while Exons 7-9 encode the intracellular region [83, 84].

- **The extracellular domain** contains three extracellular CRDs. CRD2 and CRD3, the two CRDs of Fas closest to the plasma membrane, are required for Fas ligand binding [56, 85]. Fas pre-exists at the plasma surface as a homotrimer, in the absence of ligand [37]. The extracellular region of pre-ligand assembly domain (PLAD) makes it possible to homotrimerize, by homophilic bonds. This PLAD domain is localized at CRD1 [73] and is essential for FasL binding as well as for apoptotic signal transduction (Figure 3). The extracellular domain harbor also a conserved extracellular glycosphingolipid-binding motif (GBM) identified as one of the regulatory elements in the decision of the nature of the receptor internalization route used [86].
- The **transmembrane region (TM)** has 19 amino acids that forms a stable homo-trimer of Fas in the lipid bilayer. The intramembrane trimerization of Fas is necessary for

efficient activity of the receptor [87]. Moreover, the absence of TM by alternative signaling of Fas mRNA, produce a soluble form of Fas that act as decoy and prevent cell death [88].

- The **cytoplasmic domain** is constituted of 145 amino acids, containing the death domain (DD) and a lysin-rich region (LRR) at the proximal-membrane region of Fas. The DD is a region of approximately 80 amino-acid residues containing a series of six  $\alpha$ -helices. Fas induces cell death signaling through its DD, that is capable of establishing homotypic interactions with a DD-containing molecules such as Fas-associated DD (FADD) [89]. Mutations in the DD halts ligand-induced apoptosis and causes autoimmune lymphoproliferative syndrome (ALPS). [22]. There are two models of Fas DD /FADD recruitment. Scott and co-workers describe that the Fas-FADD complex is formed by dimer units (where two Fas-DD binds to two FADD-DD), that can additionally dimer into a tetramer structure (4 Fas plus 4 FADD) [21]. Meanwhile, Wang's team reported that the Fas DD/FADD complex is rather composed of 5 FADD units layered on a 5-member Fas DD [22] (Figure 4).

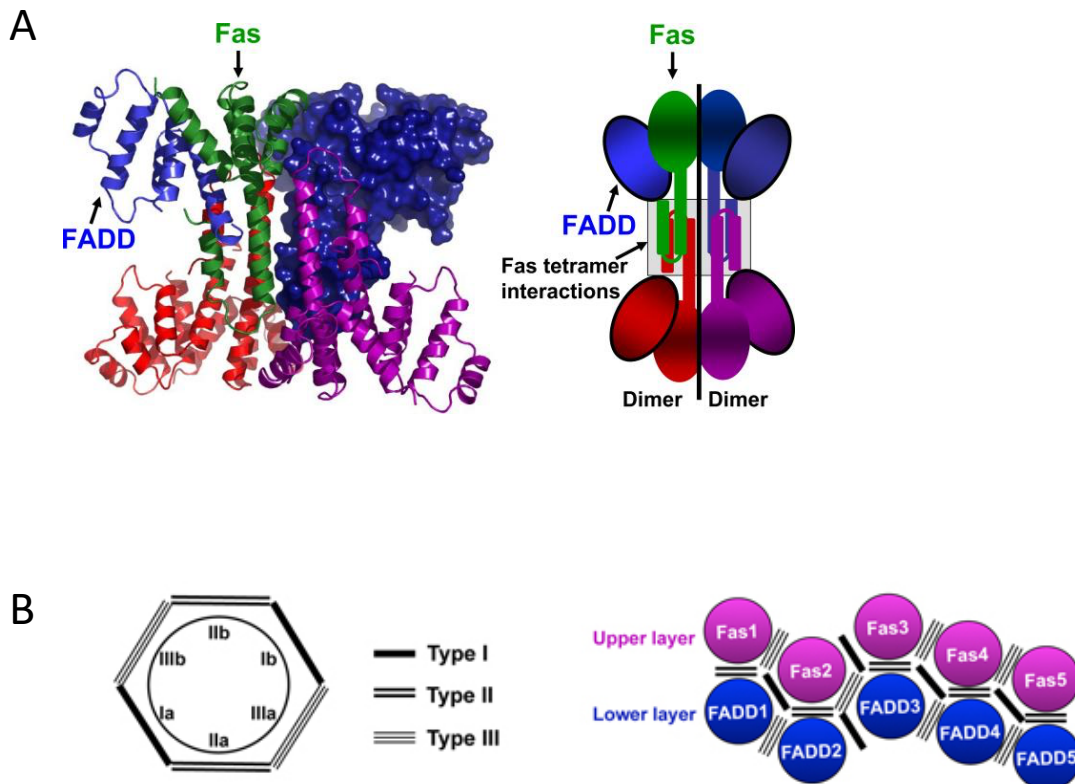


Figure 4 A) The Fas DD-FADD in tetrameric model [21]. Color: Fas(green), FADD (blue). and B) The Fas DD-FADD model and the locations of the three types of contacts in 5:5 model [22], Color Fas (purple), FADD (blue).

The finding of the interaction of Fas with the Fas-associated phosphatase-1 (FAP1), a protein containing a PSD-95/Dlg-A/ZO-1 (PDZ)-domain, lead to the identification of a PDZ-binding domain at the C-terminal part of Fas (three amino acids 332-335 SLV (serine-leucine-valine amino acids)) [90]. The PDZ-binding domain allow the interaction with a PDZ domain containing proteins known to play a key role in the formation and function of signal transduction complexes. Interestingly, the murine Fas lacks the three amino acids SLV at its C-terminal part. This suggests that the PDZ-binding domain of Fas emerged in humans and is not conserved through species [91]. In my thesis, we used the PDZ-binding domain of human Fas as a bait in a proteomic screen in order to identify molecular partners of Fas. This led us to the discovery of 19 new interactants of Fas including FAP-1 (see Results section).

### *1.B.b.2 Expression of Fas*

In contrast to FasL, the expression of Fas is ubiquitous. For example, Fas expression is constitutively expressed in several epithelial tissues including the biliary tract, the reproductive system of both genders and the intestine [92]. Fas is also abundant in thymus, liver, kidney and heart [93]. This expression can be nevertheless modulated.

The increase of Fas expression is observed in several situations including: (i) during the T lymphocytes activation or infection by viruses, such as human leukemia virus T (HTLV-1), the virus human immunodeficiency (HIV-1) or Epstein-Barr virus (EBV) [94-96]; (ii) through the action of Interferon- $\gamma$  or cytokines involved in inflammation [92, 97]. (iii) during hematopoietic cell differentiation, immature CD34+ hematopoietic progenitors gradually increase Fas expression at their surface during their differentiation [98].

On the other hand, a downregulation or a loss of Fas expression is frequently found in the progression of a number of human malignancies, including colon, breast, lung, and liver carcinoma [99].

### *1.B.b.3 The multi-signaling machinery of Fas*

#### *1.B.b.3.1 Cell death signaling pathway of Fas*

Apoptosis is a form of cell death that depends on the activation of a cascade of cysteine aspartyl-specific proteases (caspases), which are known to induce cleavage of critical cell's components leading to cell death. Of note these class of enzymes are also involved in other

signaling process including inflammation [100]. Caspases are produced in cells as catalytically inactive procaspases and must undergo proteolytic activation during apoptosis. Depending on their position in the death signaling cascade, two groups of caspases could be distinguished: the initiator caspases (including caspase-2, 8, -9 -10) and the downstream effector caspases (including caspase-3, -6 and -7 -14) that are thought to be more directly responsible of the cell execution. The initiator caspases are activated through dimerization which is promoted by an adapter protein through a protein-protein interaction domain in the prodomain of procaspase (Figure 5). The initiator inflammatory caspases-2 and -9 contain a caspase recruitment domain (CARD), while the initiator caspases-8 and 10 involved in apoptosis contain as death effector domain (DED). The particular protein-protein interaction domain in different caspases allow the interaction with distinct adaptors.

Apoptosis is characterized by blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay.

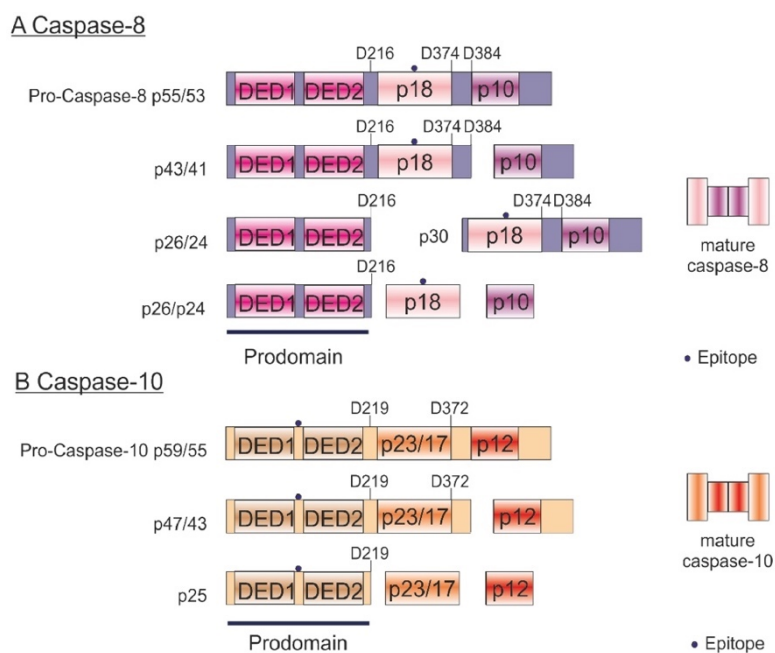


Figure 5: Scheme of procaspase-8 and procaspase-10 processing [1]

Apoptotic signaling pathway is activated through two mechanisms (Figure 6) [101]:

- i. The intrinsic pathway, also known as the mitochondrial pathway, that is activated upon detection of cytotoxic internal stimuli, such as DNA damage or growth factor deprivation.
- ii. The extrinsic pathway also known as the death receptor pathway, that is initiated by the binding of death ligands to death receptors (excepted for the dependence receptors that are able to induce apoptosis in absence of ligand binding).

Generally, both of these apoptotic pathways converge to activate caspases, which cleave key cellular proteins and destruct the cell [102].

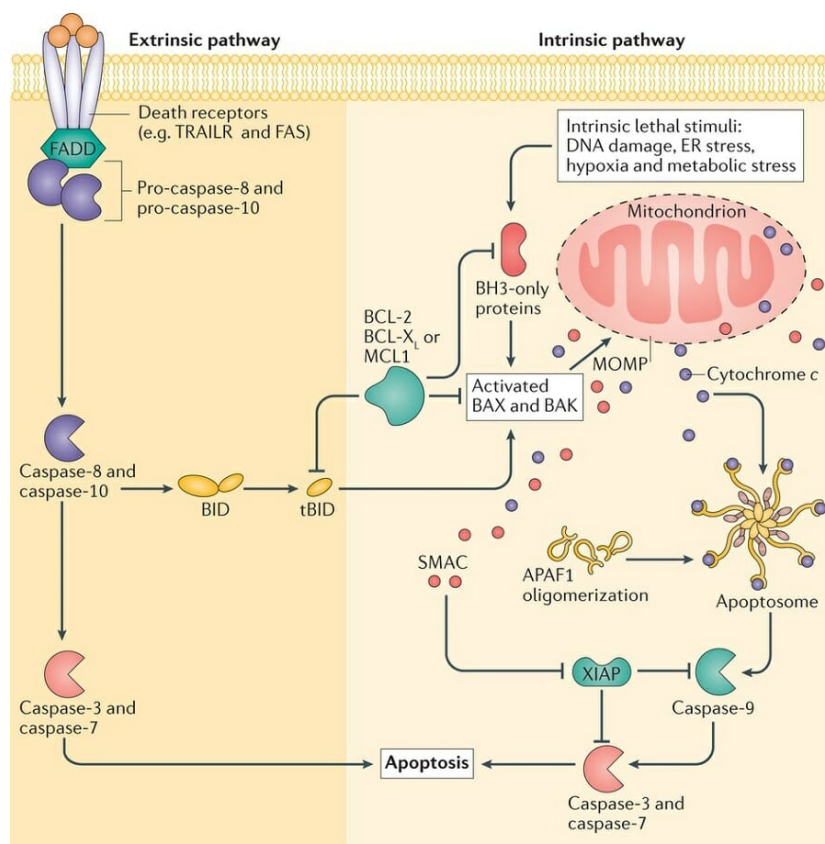


Figure 6: Apoptosis signaling pathway: the extrinsic and intrinsic pathway [2]

FasL binding triggers the recruitment of several cytosolic adaptor proteins to Fas cytoplasmic part leading to the formation of a death-inducing-signaling complex (DISC), which ultimately induces rapid apoptosis in a range of sensitive cell types [103]. The DISC is composed of the oligomerized receptors, the DD-containing adaptor molecule FADD, the initiator caspases, procaspase-8 and 10, and the regulator of procaspase-8 c-FLIP (Figure 7). It has been shown

that Fas stimulation upon FasL binding leads to conformational changes in Fas's intracellular DD, promoting the aggregation of additional Fas molecules into large clusters. This expanded complex is then capable of recruiting FADD through homotypic ligation and initiating DISC formation [21]. FADD also carries a N-terminal protein-protein interaction domain, the death effector domain (DED), and by homologous interaction recruits the DED-containing procaspase-8 (FLICE) and form the DISC [104]. Through close proximity, DISC-bound procaspase-8 dimers undergo autocatalytic cleavage to stabilize caspase-8 in its catalytically active conformation [105]. Active and mature caspase-8 is then released from the DISC into the cytosol in a hetero-tetrameric form, consisting of two p10 and two p18, where it activates downstream effector caspases, including caspase-3 and caspase-7, to drive apoptosis [100]. In human, another initiator caspase known to be recruited to the Fas DISC is the caspase-10, which is highly homologous to caspase-8 [106]. However, they exhibit a selective substrates cleavage, which may suggest that their biological function may differ [107].

Two different Fas-induced apoptosis pathways were described depending on the cell type, type I and type II, presents in (Figure 7). In contrast to type I cells, the DISC formation and the activated caspase-8 is not sufficient to induce FasL-mediated apoptosis in the type II cells, and therefore the mitochondrial pathway is required. Caspase-8 cleaves the pro-apoptotic BH3 domain only containing Bcl-2 family member, Bid, that is responsible for the activation of mitochondrial pathway. Truncated-Bid (t-bid) translocate to the mitochondria where it induces the activation of Bax and Bak. Active Bax and Bak cause mitochondrial outer membrane permeabilisation (MOMP), enabling the release of apoptotic factors including cytochrome c and Smac/Diablo into the cytoplasm. Cytochrome c forms a complex with dATP, apoptotic protease activating factor 1 (Apaf-1) and the initiator procaspase 9 forming a multimeric complex known as the 'apoptosome'. The apoptosome activates caspase 9, that can then cooperate with caspase-8 to cleave and activates effector caspases in the cytosol. Meanwhile, Smac/Diablo promote caspase activation through neutralizing the inhibitory effects to inhibitor of apoptosis proteins (IAPs) , which finally enhance the apoptotic signal [108].

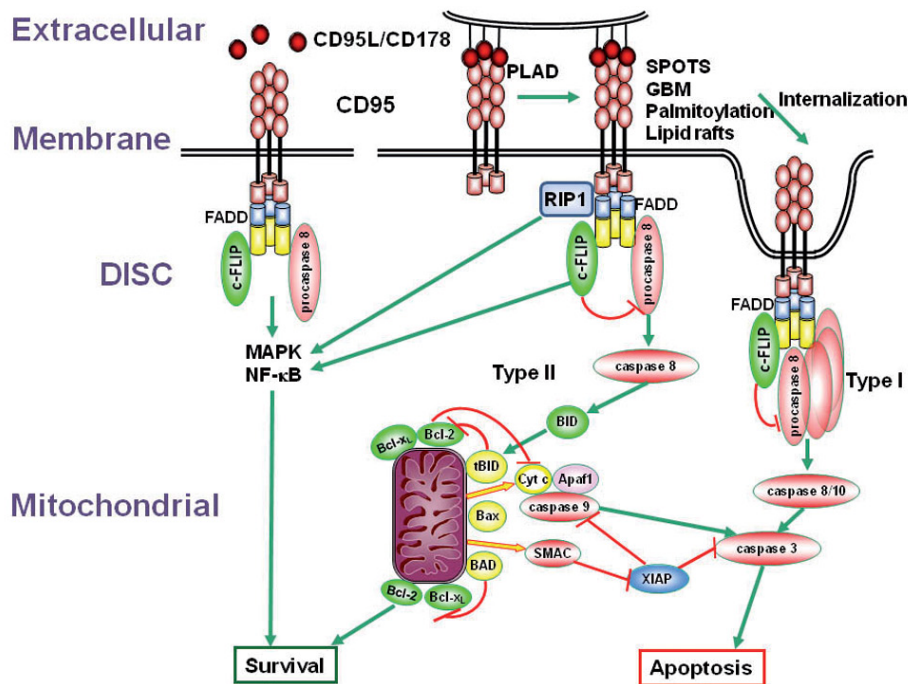


Figure 7: Type I and Type II apoptotic pathways of Fas (Yurchenko et al., 2012)

#### I.B.b.3.2 Non-death signaling pathway of Fas

Fas is an important activator of major signaling pathways other than cell death depending on the context and cell type [80, 81]. The non-death signals of Fas have been reported early on in the Fas research field. Alderson and coworkers, established that FasL engagement promotes proliferation of TCR stimulated T-cells and thymocytes [109]. Likewise, another study by Aggarwal *et al* reported the proliferation effect of Fas stimulation on the human diploid fibroblasts in a dose-dependent manner [110]. Moreover, Freiberg and co-workers demonstrated that Fas aggregation in dermal fibroblasts may initiate dual signaling programs either apoptosis or proliferation depending on the magnitude of Fas aggregation where high aggregation of Fas induces apoptosis and low aggregation leads to non-apoptotic signaling [111]. As mentioned above, to date there is a plethora of evidence of the non-death function of Fas involving cell survival, proliferation, inflammation, migration, and invasion of cells [81]. I will give here few examples of such functions.

- In tumor cell resistant to apoptosis, FasL engagement enhanced cell motility and invasiveness in a manner dependent on the activation of NF- $\kappa$ B, ERK, and caspase-8 [112].
- Fas is described as an activator of the PI3K pathway and as a critical trigger of the basal



invasion of glioblastoma through activation of MMPs that facilitates tumor migration [113].

- Fas stimulation leads to proinflammatory signaling in macrophages, DCs, fibroblasts, hepatocytes, and keratinocytes have been shown in several studies [114]. Fas cytokine stimulation includes IL-6, IL-8, IL-1 $\beta$ , TNF $\alpha$  and monocyte chemoattractant protein (MCP-1). While cytokines such as IL-6 and TNF $\alpha$  transduce powerful inflammatory signals in a broad fashion, chemokines like IL-8 and MCP-1 attract neutrophils and monocytes/macrophages to inflammatory sites, which act to further enhance immune signals.
- Fas is expressed on primary sensory neurons and induces neurite growth through sustained activation of the extracellular-signal regulated kinase (ERK) pathway and the consequent upregulation of p35, a mediator of neurite outgrowth [115].

Although the molecular mechanisms underlying these non-death pathways are less described than the cell death ones, accumulation of knowledge could be noticed in the last few years: Fas stimulation leads to increase of Src-Family-Kinase (SFK) members activity (including Yes, Fyn and Lck) that will transduce signals by tyrosine phosphorylation of different cellular targets. Fas activation leads to the formation of a PI3K-Activation-Complex (PAC) including the SFK member Yes and the PI3K regulatory subunit, p85, in human glioblastoma cells. PAC formation and activation of PI3K led to increased migration of glioblastoma cells [116].

The activation of Fas by naturally cleaved FasL in activated T lymphocytes induces the motility-inducing signaling complex (MISC) [75](Figure 8). Upon Fas stimulation, phospholipase C $\gamma$ 1 (PLC $\gamma$ 1) is recruited to the membrane-proximal intracellular domain known as calcium-inducing domain (CID). PLC $\gamma$ 1 produces inositol triphosphate (IP3) that activates IP3 receptors and promote the release of endoplasmic reticulum (ER) calcium stores. The intracellular increase in calcium (Ca<sup>2+</sup>) activates protein kinase C  $\beta$ 2 (PKC $\beta$ 2), limiting the recruitment of FADD and caspase-8 by activating [117]. In the MISC, the recruitment and activation of NADPH oxidase 3 lead to reactive oxygen species production, causing the activation of the Src kinase c-yes and the following induction of cell migration [7]. This pathway has been also described in glioblastoma cells (Kleber, Sancho-Martinez et al. 2008) and Triple-negative breast cancer (TNBC) cells [80, 118].

Nevertheless, the molecular mechanisms controlling the activation of such pathways are not fully understood and their contribution to the final phenotype is still unclear.

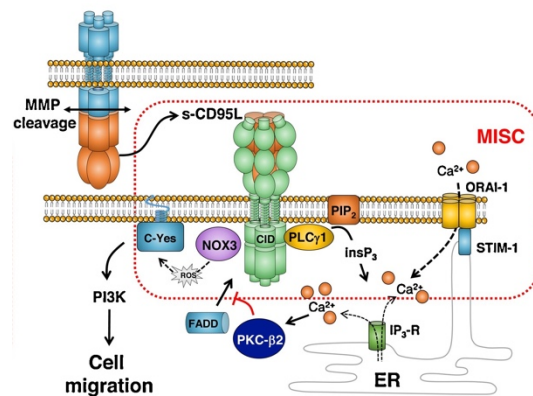


Figure 8: Fas-induced cell migration pathway [7]

#### I.B.b.4 Fas/FasL signaling is regulated and fine-tuned at many levels

The mechanisms underlying the switch from death to non-death signaling upon FasL engagement is still poorly understood. Several studies showed that Fas induced death and non-death signaling pathways determination might occur in the early steps of Fas signaling, through the regulation of signaling modulators and regulation of endocytosis, localization and post-translational modification of Fas at the plasma membrane level (Figure 9).

Receptors	DISC	IAPs	Mito
Lipid Rafts	FLIP:C8 Ratio	XIAP:C3 Ratio	<u>Pro-apoptotic</u>
Ligand Presentation	FLIP isoforms	cIAP	Bax
Receptor Clustering	Death DISC vs. Survival DISC	Survivin	Bak
O-Glycosylation	Kinases		<u>Anti-apoptotic</u>
Palmitoylation	NF-kB		Bcl-2
Nitrosylation	C8 Ubiquitination		Bcl-XL
Decoy receptors	C8 Partial vs. Full Processing		<u>BH3-only</u>
	C8 DED Chains		<u>Activator</u>
			Bid
			Bim
			<u>Sensitizer</u>
			Puma
			Noxa
			Bad

Figure 9: Multiple levels of regulation that affect the strength of apoptotic signaling (Flusberg e Sorger, 2015)

#### I.B.b.4.1 Fas signaling regulated at the receptor and ligand level

##### I.B.b.4.1.1 Role of endocytosis

Fas-induced apoptotic and non-apoptotic signaling pathways depends also on Fas receptor

internalization. Lee and coworkers demonstrated the requirement of Fas internalization in type I cells upon FasL engagement in order to induce DISC amplification, caspase activation and apoptosis. It was demonstrated that activated Fas moves into an endosomal compartment where the recruitment of the DISC components principally occurs. The blockage of Fas internalization impaired DISC formation and apoptosis. Cells unable to internalize Fas results in activation of proliferative ERK and NF- $\kappa$ B signaling pathways upon FasL stimulation. Moreover, Fas internalization was observed in co-culture experiments with cells expressing a non-cleavable form of membrane-bound FasL. The degree of receptor internalization was comparable to experiments using oligomerized soluble forms of FasL [119]. Therefore, the subcellular localization and internalization pathways of Fas play important roles in controlling activation of distinct signaling cascades to determine divergent cellular fates [120]. In contrary to Type I cells, Fas internalization was not observed in type II cells which suggest the difference in signaling in both types [120].

Moreover, a study from our lab identified an extracellular glycosphingolipid-binding (GBM) motif of Fas as a regulatory element in the selection of the internalization route and accordingly the signals transmitted upon ligand binding. We established that the GBM motif is needed for clathrin-mediated internalization of Fas, enabling the transduction of the apoptotic signal. The loss of function of this motif targets the activated receptor to an alternative raft/clathrin-independent but ezrin-dependent internalization route, thus suppressing its cell death signal while promoting its non-death functions [86]. Moreover, a study by Rossin *et al* reported a membrane-proximal region of Fas, a lysine-rich region (LRR) in the cytoplasmic domain, as a key determinant in modulating Fas localization to the sphingolipid- and cholesterol-rich nanodomains (SCN), also called rafts, and modulates Fas interaction with the cytoskeleton [121]. Mutation in this region excluded Fas from lipid rafts and decreased significantly cell death. However, it did not impair the non-death signaling of Fas. Indeed, one of the first studies demonstrating the essential role of membrane rafts in the initiation of Fas-mediated cell death signaling was by Hueber *et al* [122]. This study report that in mouse thymocytes a significant fraction of Fas is constitutively segregated into SCNs, and that this distribution is crucial in recruiting the DISC and inducing apoptosis upon Fas ligation. Other studies confirmed the importance of Fas distribution in lipid rafts on Fas-mediating apoptosis in several other cell types [123].

A more recent study by Sharma et al identified endosome associated trafficking regulator 1 (ENTR1), as a negative modulator of Fas-induced apoptosis through the regulation of Fas cell surface levels [124]. ENTR1 localizes to early and recycling endosomes where it binds to FAP-1, which interacts with Fas (see page 27). The FAP-1/ENTR1 complex regulates Fas endocytic trafficking and promotes Fas degradation, through the sorting of Fas into intraluminal vesicles of multivesicular bodies and consequently stopping Fas induced signaling transduction [124].

#### I.B.b.4.1.2 Role of post-translational modifications

Our lab pointed out two PTMs essential in the versatility of the Fas signaling:

(i) After a demonstration that Fas localization in SCN was necessary for Fas-induced cell death, we reported that Fas is internalized through a clathrin-dependent mechanism in these domains following FasL engagement and that this internalization is necessary for transducing an optimal death signal. In several cells, we demonstrated that some specific **lipid interactions** are regulating these very early steps. Indeed, we identified Fas palmitoylation, a reversible addition of a palmitic acid on a specific cysteine residue located in the cytoplasmic membrane-proximal domain of Fas, represents a key signal targeting Fas to the SCNs. More importantly, it allows the association of Fas with the actin cytoskeleton through the recruitment of the protein Ezrin, which is essential for internalization to occur. We carried out a screen that permitted the identification of DHHC7; one of the 23 members of the palmitoyl acyltransferase DHHC family, as the Fas palmitoylating enzyme. We define the modification of Fas by palmitoylation as a novel mechanism for Fas expression regulation via its capacity to avoid its degradation by lysosomal proteolysis [125].

(ii) the outcome of Fas signaling is clearly defined by the phosphorylation status of two tyrosines (Y232 and Y291) in its death domain which is regulated by the Src-family kinase/SHP-1 system. Dephosphorylation of both tyrosines in the death domain of Fas triggers apoptotic signal whereas the tyrosine phosphorylation suppresses the pro-apoptotic signal and triggers the prosurvival signal. Additionally, Fas tyrosine phosphorylation profile was correlated with different contexts of human cancer. Observing higher levels of pY232 and/or pY291 in colon, breast, and ovarian malignant tissues than their corresponding normal tissues. These results can permit the use of Fas tyrosine phosphorylation screening to help Fas-related therapeutic design and maximize the chance of therapeutic success [126, 127].

We also show that the status of Fas tyrosine phosphorylation strongly influences the signaling of the epidermal growth factor receptor (EGFR) pathway in colorectal cells: Fas in its prosurvival state, phosphorylated at Y291 (pY291-Fas), interacts with EGFR. This interaction notably increases EGFR signaling in anti-EGFR-resistant colorectal cancer cells through the Yes-1/STAT3-mediated pathway. Upon EGF treatment, the pY291-Fas accumulates in the nucleus and induce the nuclear localization of pEGFR and pSTAT3, the expression of cyclin D1, the activation of STAT3-mediated Akt and MAPK pathways, cell proliferation and migration [128].

#### I.B.b.4.2 Fas signaling regulated by signaling modulators

A complex network of pro- and anti-apoptotic regulators modulates the outcome of Fas engagement. The DISC is a complex structure comprising multiple regulators that together determine whether apoptosis becomes activated in response to diverse stimuli in different cell types. Among these regulators is the cellular FLICE-like inhibitory protein (c-FLIP), which is recruited to the DISC and interfere with caspase-8 activation. In addition to inhibiting caspase-8 processing, c-FLIP<sub>L</sub> also promotes Fas-stimulated activation of NF- $\kappa$ B and ERK in TCR-triggered cells [129, 130]. Furthermore, Lavrik *et al* show that the initial concentration of c-FLIP at the DISC is one of the factors contributing to the cellular decisions for Fas-induced death and non-death signaling [131]. FLIP therefore acts as a determinant of Fas signaling output rather than as a simple apoptosis-suppressor. Likewise, a recent study by Horn *et al.* discovered that the activity of caspase-10 within the DISC can trigger a switch from FasL and caspase-8-dependent cell death to DISC-mediated activation of NF- $\kappa$ B signaling and cell survival, since the depletion of caspase-10 inhibited FasL-mediated I $\kappa$ B $\alpha$  degradation/phosphorylation [132]. These studies demonstrate how Fas signaling modulators can be involved in generating either death or non-death signaling.

## I.B.c Functions of the Fas / FasL system

### I.B.c.1 Fas /FasL function in physiology

Fas/FasL system plays a major role in immune homeostasis through anti- and pro-apoptotic regulation. Here I will briefly introduce Fas/FasL role in immune system.

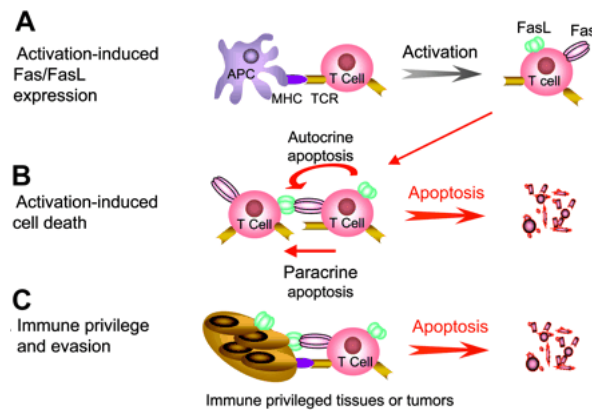


Figure 10: Fas/Fas-L induced apoptosis in physiological immune response [10]

In the thymus, T cells mature and are positively or negatively selected, depending on the affinity of their T-cell antigen receptors (TCRs) for self-major histocompatibility (MHC) antigens. T cells with a high affinity for self MHC molecules and peptide are eliminated to ensure tolerance to normal tissues and to prevent autoimmunity. Fas/FasL system is involved in this negative selection in the thymus when T cells encounter high antigen concentrations, thus, revealing the importance of Fas/FasL in regulating auto-immunity and central tolerance [133]. Moreover, FasL expression is induced in cytotoxic T cells (CTL) upon activation and induce cell death of targeted cells expressing Fas. CTL also kill target cells including infected cells and cancer cells or damaged cells, through another mechanism by the release of cytolytic perforin and granzyme B (GrB) that finds its way into the target cell and can kill it by directly cleaving and activating caspase-8 [134]. Following activation, T cells express Fas and FasL and upon recurrent antigenic stimulation become sensitive to Fas/FasL- mediated autocrine and paracrine apoptosis to be able to maintain the pool size of antigen activated T-cell clones and regulate immune response. Fas engagement leads to activation-induced cell death (AICD) in T-cell, (Figure 10). This mechanism is well-recognized to removing activated effector T cells after an immune response to a threat, preventing the tissue damage that might otherwise be

caused by an over-long immune response [104]. B cells activation as T-cells leads to the expression of FasL and killing of Fas-expressing target cells by B cells [135, 136]. Further, B cell specific Fas-deficient mice develop lymphoproliferation like *lpr* mice [137].

As mentioned earlier the absence or very limited FasL expression in certain tissues confers an immunologic privileged status to these sites and protect them from destructive effects through apoptosis mediated by immune cells (Figure 10).

Fas is also known for its function outside the immune system, that I will describe here.

Fas also play an essential role in liver homeostasis by contributing in the elimination of senescent cells [138]. Furthermore, activation of Fas is correlated with hepatocyte apoptosis in a broad variety of liver diseases including liver failure, fibrosis, and carcinogenesis [64].

Fas/FasL-mediated non-death signaling are also essential in inducing dendritic cells (DCs) maturation and proliferation of T cell and neurites:

- Fas triggers functional maturation of DCs as well as the secretion of proinflammatory cytokines that attract neutrophils and T cells. Fas is incapable to induce DC death due to a constitutive c-FLIP expression [139, 140].
- As mentioned above Fas mediates T-cell proliferation through Fas-mediated co-stimulation of TCR-driven T-cell which appears to involve caspase activation without subsequent apoptosis induction [130]. Moreover, Fas engagement can controls TCR/CD3-driven signal initiation in a dose-dependent manner. High doses of immobilized FasL almost completely silence T cells by blocking early TCR-induced signaling events, however, lower amounts of the same agonists dramatically augment TCR/CD3-driven activation and proliferation [141].
- Meanwhile, Fas can mediate proliferation and regeneration of neurons [115]. Furthermore, Fas appears to regulate neuronal branching in hippocampal neurons [142] and Fas activation increases neural progenitors cell survival [143].

### I.B.c.2 *Fas/FasL function in disease*

#### I.B.c.2.1 *Fas/FasL in auto-immune disorders*

##### I.B.c.2.1.1 *Type I autoimmune lymphoproliferative syndrome*

The critical role of the Fas/FasL role in immune system control was definitively brought with the spontaneous development of autoimmune disease symptoms in mutant mouse strains lacking Fas or FasL function [144]. In *lpr* (lymphoproliferative disease) mice, a mutation in Fas gene leads to dramatic reduction in Fas mRNA expression and therefore to the protein expression [145]. In *gld* (generalized lymphoproliferative disease) mice, a mutation in FasL gene leads to a nonfunctional FasL protein [146]. In *lprcg* mice, which have an *lpr*-like phenotype, there is a point mutation in the Fas DD that abrogates the recruitment of FADD and caspase-8, consequently abolishing apoptotic signaling [147]. All these mutant mice are characterized by a considerable accumulation of lymphocytes as well as high levels of auto-reactive antibodies and the development of several related pathologies [80].

Similar anomalies can be observed in human patients who suffer from autoimmune lymphoproliferative syndrome (ALPS) and develop chronic lymphadenopathy and splenomegaly and frequently develop autoimmunity [148]. In these patients, an expanded population of abnormal CD4<sup>-</sup> / CD8<sup>-</sup> double-negative T cells can be found. Patients with the type Ia form of the disease display dominant-negative *FAS* mutations, while type Ib patients carry FasL mutations. These defects parallel those in mice with *lprcg* and *gld* mutations, respectively. ALPS type Ia cells bear a heterozygous mutation inside the Fas DD that blocks the Fas-induced apoptotic cascade. whereas mutations in the *CASP10* gene lead to ALPS II, and cases without defined mutations are termed ALPS III. Other authors refer to the latter form as ALPS-like disease (ALD) [149].

##### I.B.c.2.1.2 *Inflammatory bowel disease*

Inflammatory bowel disease (IBD) is characterized by inflammatory conditions of the gastrointestinal (GI) tract, giving rise to progressive damage to the inflamed gut tissue and disruption of the intestinal epithelial barrier. The two major forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD). CD is characterized by inflammation of the distal ileum and colon, with discontinuous, patchy inflammatory lesions, whereas UC presents with continuous inflammation from the rectum to proximal parts of the colon. Several factors are known to



influence their pathogenesis, including genetics, environmental risks, abnormal microbiota, and dysregulated immune responses [150]. The extent of endothelial FasL expression was elevated in the submucosa and associated with an elevated number of lymphoid follicles in samples from patients with either CD or UC. In IBD, ulcers and areas with an elevated density of mononuclear cells expressing FasL also displayed a high density of blood vessels with endothelial FasL expression. These detailed expression pattern suggests that endothelial FasL expression has a role in the regulation of lymphocyte access to the peripheral lymphoid tissues, comprising the intestinal mucosa [151].

#### I.B.c.2.1.3 Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease characterized by the accumulation T cells in the central nervous system (CNS) compartment. T cells expand clonally, and they are responsible for oligodendrocyte loss, demyelination, and neuronal damage by targeting specific antigens. The regulation of the unlimited accumulation of these cells is achieved also by Fas/FasL-mediated apoptosis, and its therapeutic improvement could be useful to reduce pathogenic T cells in MS. [152]

#### I.B.c.2.2 Fas/FasL function in cancer

##### I.B.c.2.2.1 Fas/FasL system as a tumor suppressor

The pro-apoptotic function is the most well-established activity of Fas/FasL system, as discussed above. Indeed, Fas activity in mediating the death of either virus-infected cells or cancer cells when engaged by CTL is well documented. In addition to the perforin/granzyme pathway, Fas/FasL is a direct major system that both CTLs as well as cytolytic effector T cells use to eliminate neo-plastically transformed cells [153, 154]. Furthermore, FasL is a chemo-attractor, inducing the migration of neutrophils and phagocytic cells to the inflammatory site, although the mechanism of how FasL exerts its chemotactic effect is unknown [74].

##### I.B.c.2.2.2 Fas/FasL system as a tumor promoter

Likewise, various teams have revealed through extensive studies, that Fas could also be tumor promoter.

Actually, many carcinomas were found to express Fas at high levels such as hepatocellular, ovarian, renal, colorectal and pancreatic cancers, but are usually resistant to induction of Fas-

mediated apoptosis. Additionally, Legembre *et al.*, showed that not only were tumor cells not sensitive to Fas-induced cell death, but Fas stimulation increased their motility and invasiveness due to the activation of NF- $\kappa$ B, ERK, and caspase-8 [155]. Fas could actually promote the growth of tumors through its non-apoptotic activities [81]. Another evidence that Fas overexpression can be favorable to tumor growth was established in a mouse model of Lewis lung carcinoma, where deregulated Fas expression failed to sensitize toward apoptosis but enhanced tumor formation [156]. Ever since additional studies have provided evidence that Fas induces the migration/invasion of multiple cancer cell types [93]. Even more, higher expression of Fas in human breast cancer tissue has been significantly correlated with poorer prognosis in breast cancer patients. It was defined that blocking Fas signaling can inhibit breast cancer progression by preventing proinflammatory cytokine production by disrupting NF- $\kappa$ B, signifying that Fas signaling-initiated cancer-related inflammation in breast cancer cells which may be a potential target for treatment of breast cancer [157].

Additionally, we demonstrated high levels of Fas tyrosine phosphorylation, which is a direct molecular indicator of Fas pro-survival signal output, in malignant tissues from some types of cancer such as colon, breast, and ovarian cancers, proposing that the pro-survival signal of Fas may govern in these cancers [126].

The cells that are apoptosis-resistant and pro-inflammatory, are found to upregulate Bcl-2 or Bcl-XL in type II cells. Indeed, Fas-FasL expression was found to increase with increasing grades of tumor severity, which correlated with increased expression of anti-apoptotic Bcl-2, suggesting a role for Bcl-2 family proteins in selecting for apoptosis-resistant, Fas-positive tumors [158]. Another mechanism of resistance may be through inhibition of caspase-8 pro-apoptotic activity through upregulation of c-FLIP<sub>L</sub>, which may be facilitated through Fas-dependent NF- $\kappa$ B activation. Interestingly, c-FLIP<sub>L</sub> expression has also been associated with inflammatory signaling in response to Fas. Fas-dependent liver regeneration after partial hepatectomy in mice correlated with increased c-FLIP<sub>L</sub> expression [159].

Interestingly, Fas is found frequently down-regulated during cancer progression, raising the possibility that loss of Fas is part of a mechanism for tumor evasion. However, complete loss of Fas is hardly observed in human cancers and numerous cancer cells express large amounts of Fas and are highly sensitive to Fas-mediated apoptosis *in vitro*. A study in a mouse model

displayed that in general Fas/FasL system is necessary for optimal growth cancer cells. Actually, loss of Fas reduced cancer occurrence as well as the size of the tumors, in mouse models of ovarian cancer and liver cancer [160].

Furthermore, FasL upregulation has been found in many types of malignancy, including melanoma, lung cancer, breast cancer, cholangiocarcinoma and ovarian cancer [112]. It was elucidated that FasL induces migration and invasion of glioblastoma and other apoptosis-resistant tumor cells, through activation of PI3K, ERK, NF- $\kappa$ B, and caspases, and also leads to the secretion of inflammatory cytokines[118]. Moreover, numerous cancer cell lines express FasL and kill Fas-expressing T-cell by Fas-mediated apoptosis *in vitro*. Thus, the expression of FasL may play an important role in tumor cell immune escape by creating a tumor immune privilege mechanism [161].

All together these studies demonstrate that Fas plays a growth promoting role in tumorigenesis and propose that efforts to limit its activity rather than to enhance its activation should be taken in consideration throughout cancer therapy. Indeed, knockdown of endogenous FasL reduced the invasive phenotype in glioblastoma model cell lines. Upon the addition of soluble recombinant FasL in FasL knockdown cells, invasion was restored, and this effect was inhibited by APG101 [162, 163].

#### I.B.c.2.2.3 Role of Fas/FasL system in colon cancer

##### I.B.c.2.2.3.1 Colon Cancer

Colorectal cancer (CRC) is the third most common type of cancer worldwide and is the third leading cause cancer death in women and men. The majority of colorectal cancer cases display no apparent evidence of having inherited disorders, about 75 %, also known as sporadic, suggesting contribution of genetic and environmental factors, whereas only 25 % of the patients have family histories of the disease [164].

Most colon cancers start as a polyps, noncancerous growths, on the inner lining of the colon or rectum and consequently grow into blood vessels or lymph vessels, increasing the chance of metastasis [165]. During colorectal adenocarcinoma development, epithelial cells from gastrointestinal tract acquire successive genetic and epigenetic mutations in specific oncogenes and/or tumor suppressor genes, presenting them a selective advantage on

proliferation and self-renewal. So, normal epithelium becomes hyperproliferative mucosa and successively gives rise to a benign adenoma that evolves into carcinoma and metastasis.

CRC is a heterogeneous disease both in the biological behavior and at molecular level, in which various patterns of mutations contribute to the commencement and progression. Loss of genomic integrity facilitates the accumulation of multiple mutations during the development of CRCs. CRCs and their pathological precursors display specific molecular signatures, specific pathological features and specific natural histories. The molecular features and mechanisms involved in colorectal epithelial cell transformation and play a significant role in CRCs can be divided into 3 groups [166]:

- i. The most common is the chromosomal instability (CIN), which represent approximately 70%–85% of CRCs. This group is characterized by accumulation of mutations in specific oncogenes and tumor suppressors. For example, the CIN pathway is associated with mutation in *APC* gene, *KRAS* oncogene and tumor suppressor *TP53*.
- ii. The microsatellite instability (MSI) group regroups the dysfunction of DNA mismatch repair genes leading to genetic hypermethylation.
- iii. The CpG island methylator phenotype (CIMP) group that silence gene function with aberrant DNA methylation.

#### *1.B.c.2.2.3.2 Fas/FasL expression in normal intestine*

Fas is found expressed in a large variety of tissues, including the intestinal tract. Along the intestinal tract, Fas is uniformly expressed at the basolateral side of all epithelial cells. By contrast, FasL is mainly expressed on lymphoid cells including T, B, and NK cells and on myeloid cells, such as activated neutrophils. Epithelial cells of the intestinal tract do not express FasL, except for the differentiated Paneth cells which reside at the bottom of the crypts in the small intestine. In the colon, Paneth cells are rare, and expression of FasL is restricted to a few lamina propria cells [167].

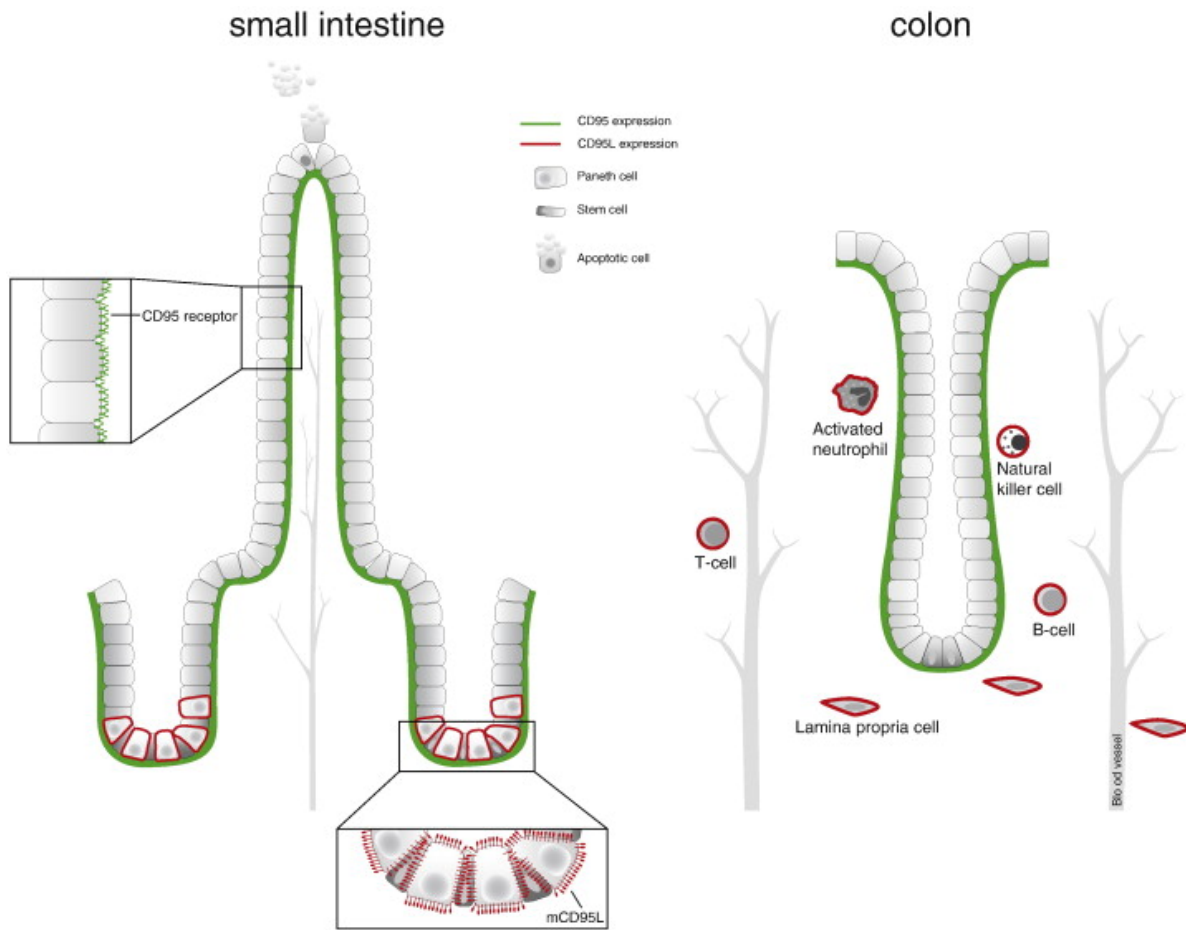


Figure 11: Fas and FasL expression in small intestine and colon (Hoogwater, Steller et al. 2012)

#### 1.B.c.2.2.3.3 Fas/FasL as a tumor suppressor in colon cancer

Fas can activate cell death by apoptosis in different colorectal tumor cell lines. Actually, CH-11, an anti-Fas monoclonal antibody, exhibited antitumor activity in colon cancer cells with elevated Fas level expression. This death inducing outcome was efficiently avoided by overexpression of Bcl-2, indicating a crucial function of mitochondria for Fas signaling in CRC in type II cells [168]. Moreover, FasL had a strong inhibitory effect on the clone-forming capacity of colon cancer cells characterized by microsatellite instability (MSI) and deficient mismatch repair with wild-type *KRAS*. Where Fas stimulation caused an irreversible cell cycle arrest, which was associated with cytokine secretion [169]. Furthermore, FasL is a potent chemoattractant and activation signal for neutrophils *in vitro* and *in vivo*. Overexpression of FasL in colorectal tumor xenografts in mice attract neutrophils that infiltrate the tumor and result in total tumor elimination [170].

#### *I.B.c.2.2.3.4 Fas/FasL as a tumor promotor in colon cancer*

The consequence of Fas activation in colon cancer is context-dependent. Most human tumor cells express low levels of Fas and as a consequence are resistant to Fas-mediated apoptosis. Nevertheless the expression is never null and suggesting that Fas low expression may be considered as an advantage by the tumor [171].

Along with this idea, it has been shown that colon cancer cells use Fas signaling to stimulate both local growth and metastatic spreading, and to induce hepatocyte apoptosis and thereby create a favorable niche for tumor cell growth [172]. Additionally, Zheng *et al.* demonstrated that Fas signaling downregulated epithelial markers such as E-cadherin, upregulated mesenchymal markers such as N-cadherin and promoted motility in gastrointestinal (GI) cancer cells. FasL treatment also amplified the expression of epithelial–mesenchymal transition (EMT) transcriptional factors in the nucleus and induced a spindle shape cell morphology in these cells. FasL-induced motility was decreased significantly by the knockdown of Snail or Twist expression [173].

The expression of FasL is progressively increased during evolution from adenoma to colorectal carcinoma and metastasis. Fas and FasL are co-expressed in the majority of adenomas and carcinomas but this does not result in apoptosis spontaneously. Studies in human colon cancer biopsies have shown that infiltration of primary tumors by memory and cytotoxic T cells is associated with reduced metastatic spread and a better prognosis [174]. This suggests that tumor progression and metastasis formation are suppressed by the adaptive immune system, in which Fas plays a central role. Hence, colon cancer cells may escape immune surveillance and killing via decreasing Fas expression and counterattack the immune system via increasing FasL expression [175].

#### *I.B.d Potential Fas/FasL targeted therapies*

The dual role of Fas/FasL in death and non-death is currently well accepted. As presented before, Fas/FasL can act as a tissue protector in some cases, whereas, in other cases it can act as tissue dismantler. Therefore, it is essential to understand better the switch between death and non-death signaling of Fas, which will help in explaining its pathological roles and help identify the appropriate therapeutic choices. There are several approaches in targeting of Fas/FasL signaling therapies including i) activation Fas death signaling using Fas/mouse chimeric monoclonal antibody such as ARG098 [176],

ii) inhibiting the Fas signaling by targeting FasL using drugs consisting of the extracellular domain of the receptor Fas including APG101 which is a soluble Fas-Fc fusion protein [177]. Additional drugs targeting Fas/FasL signaling are presented in table 3.

*Table 3: Table of Fas targeting drugs.[178]*

Intervention	Tumor type	Mouse model	Outcome	References
<b>Activation of Fas</b>				
<b>Pharmacological activation of Fas</b>	Burkitt-like lymphoma	Xenotransplantation of BJAB cells	Tumor regression	[47]
	T cell leukemia	Xenotransplantation of Jurkat cells	Tumor growth delay	[179]
	Colorectal adenocarcinoma	Xenotransplantation of HCT116 cells	Tumor growth delay	[179]
	Glioblastoma	Xenotransplantation of U87MG cells	Increased tumor cell apoptosis and overall survival	[180]
<b>Forced FasL expression in tumor cells</b>	Colorectal adenocarcinoma	Syngeneic transplantation of MC38 cells	Enhanced tumor incidence, growth, and incidence of metastasis	[172]
<b>Overexpression of FasL</b>	Lewis lung carcinoma	Syngeneic transplantation of 3LL cells	Accelerated tumor growth and decreased overall survival	[156]
<b>Inhibition of Fas/FasL</b>				
<b>Fas deletion in hepatocytes</b>	Hepatocellular carcinoma	Drug-induced (DEN)	Reduced tumor incidence and size, and reduced angiogenesis	[160]
<b>Mutated mFasL</b> <i>FasL<sup>Δm/Δm</sup></i>	Histiocytic sarcoma	Spontaneous	Spontaneous development of histiocytic sarcoma	[71]
<b>Fas knockdown in tumor cells in combination with erlotinib</b>	Non-small cell lung carcinoma	Xenotransplantation of H1650 cells	Overcome erlotinib resistance and reduced tumor volume	[181]
<b>Fas knockdown in tumor cells</b>	Ovarian carcinoma	Xenotransplantation of SKOV3.ip1 cells	Reduced tumor size, incidence, and angiogenesis	[160]
<b>Fas deletion in tumor cells</b>	Ovarian carcinoma	Genetic (LSL-Kras <sup>G12D</sup> ; Pten <sup>loxp/loxp</sup> ; Fas <sup>loxp/loxp</sup> )	Reduced tumor size and incidence	[160]
<b>Pharmacological block of FasL</b>	Glioblastoma	Syngeneic transplantation of SMA-560 cells	Reduced invasion/migration	[118]
	Hepatocellular carcinoma	Induction of chronic hepatitis	Reduced tumor incidence	[182]



## I.C Epithelial tissue architecture and organization

### I.C.a Organization and functions of epithelial tissues

Epithelium belongs to one of the four basic types of animal tissues, along with connective, muscle and nervous tissues. The epitheliums may be the first tissues that emerged during the evolution of multicellular animals, and is the first differentiated structure to emerge during embryogenesis [183]. The appearance of epithelia in evolution reflects the requirement of Metazoan for a tissue structure that is capable of segregating their internal medium from the outside environment. These functions are assured by polarized cells consisting of a continuous sheet of tightly connected cells that line the outside surfaces of organs and tissues.

Epithelial tissues are derived from all three major embryonic germ layers. From the ectoderm originates the epithelia lining the skin on the mouth, nose and the anus. Epithelium cells that are found covering most of the digestive system and the airways are developed from the endoderm. From the mesoderm derives the endothelium, which is the epithelium that lines vessels in the cardiovascular and lymphatic system.

Both during development and adult life in animal, epithelial cells undergo intensive cell proliferation that alter their position, shape and size in stereotypical ways. This results in ordered robust structure, which ultimately leads to the formation of mature organs with cellular organization suited to their specialized functions, despite being a highly dynamic process. These properties are regulated by various types of cell-cell junctions that connect epithelial cells together into a structural and functional continuum, and apical-basal polarity to distinguish the outside surface from the inside surface of the sheet. Abnormalities in the organization of these characteristics are typical in metabolic and genetic disorders of epithelia [184].

Hence, epithelial tissues play a pivotal role in providing the body's first line of protection from physical, chemical, and biological deterioration. Moreover, epithelium cells act as gatekeepers of the body regulating permeability and permitting selective transfer of materials across a physical barrier. All substances are compelled to cross an epithelium to enter the body. Certain epithelia often comprise structural features that permit the selective transport of molecules and ions throughout their cell membranes [184]. Here are some examples of surfaces that are lined by an epithelium and face the outside environment; skin, the digestive tract, the respiratory tract, the urinary and reproductive systems. Other epithelial cells may have acquired an additional specific function. Many epithelial cells have the capacity to secret

and release mucous and specific chemical compounds onto their apical surfaces. For instance, the release of digestive enzymes by the small intestine epithelium, trapping entering microorganisms and particles by secreting mucous by the cells lining the respiratory tract.

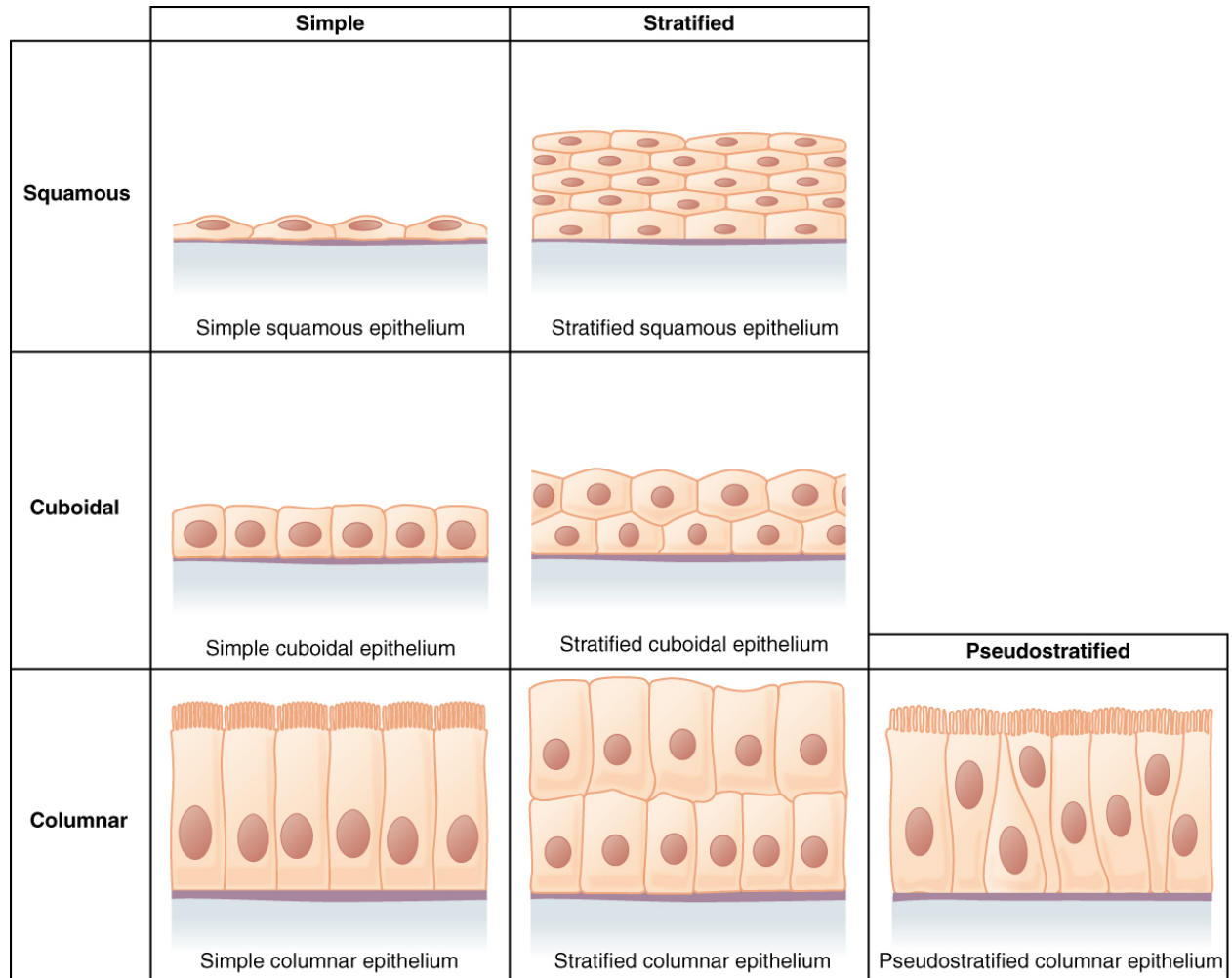

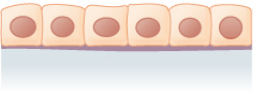
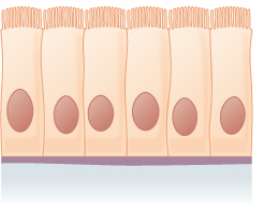
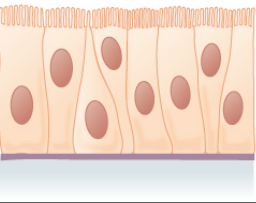
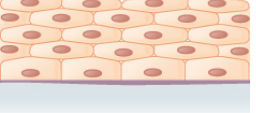
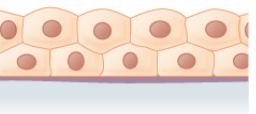
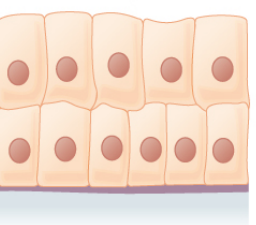
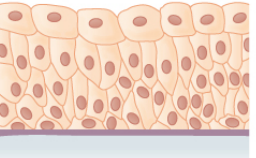


Figure 12: Cells of Epithelial Tissue. Simple epithelial tissue is organized as a single layer of cells and stratified epithelial tissue is formed by several layers of cells.[185]

Epithelial tissues are classified depending on the number of the cell layers formed and the shape of the cells (Figure 12). Cell shapes can be squamous (flattened and thin), cuboidal (cube-like shape cell that is as wide as it is tall), or columnar (rectangular, taller than it is wide). Moreover, epithelial tissues can be arranged in a single layer of cells where every cell rests on the basal lamina, known as simple epithelium, or in layers of two or more in which only the basal layer of cells rests on the basal lamina, known as stratified epithelium. All three shapes can form either simple or stratified epithelium [186]. The morphology of the epithelia is

representative of the function they perform, the different epithelial functions are summarized in (Table 4).

Table 4: Summary of epithelial tissues function and localization [185]

Cells	Location	Function
<b>Simple squamous epithelium</b> 	Air sacs of lungs and the lining of the heart, blood vessels, and lymphatic vessels	Allows materials to pass through by diffusion and filtration, and secretes lubricating substance
<b>Simple cuboidal epithelium</b> 	In ducts and secretory portions of small glands and in kidney tubules	Secretes and absorbs
<b>Simple columnar epithelium</b> 	Ciliated tissues are in bronchi, uterine tubes, and uterus; smooth (nonciliated tissues) are in the digestive tract, bladder	Absorbs; it also secretes mucous and enzymes
<b>Pseudostratified columnar epithelium</b> 	Ciliated tissue lines the trachea and much of the upper respiratory tract	Secretes mucus; ciliated tissue moves mucus
<b>Stratified squamous epithelium</b> 	Lines the esophagus, mouth, and vagina	Protects against abrasion
<b>Stratified cuboidal epithelium</b> 	Sweat glands, salivary glands, and the mammary glands	Protective tissue
<b>Stratified columnar epithelium</b> 	The male urethra and the ducts of some glands	Secretes and protects
<b>Transitional epithelium</b> 	Lines the bladder, urethra, and the ureters	Allows the urinary organs to expand and stretch

## I.C.b Polarity and cell-cell adhesion function in epithelia

### I.C.b.1 Polarity in epithelia

Cell polarity involves the asymmetric distribution of the components of the cell, such as plasma membrane proteins, organelles, and cytoskeletal, which is essential for normal cellular function and morphogenesis during development in unicellular and multicellular organisms. Polarity in cells can be divided into three main types. First, the apico-basal cell polarity that is responsible for asymmetric distribution of cell junction and polarity protein between the apex and the base in epithelial cells. Secondly, the planar cell polarity in epithelial cells display polarization along the orthogonal axis within the plane of the epithelium [187, 188]. Lastly, the front-rear cell polarity involved in redistributing cytoskeletal and membranous proteins directing migrating cells in response to chemotactic signals [189].

Epithelial cells are characterized by the polarization of their plasma membrane at the apico-basal domain, known as the apico-basal polarity. This is defined by the presence of at least two plasma membrane, facing the external medium at the apical surface, connected to adjacent cell at lateral surface and connected to the to the extracellular matrix (ECM) at basal surface. The protein and lipid composition of these domains are different, displaying their specific functions. Moreover, epithelial cells display polarization along the orthogonal axis within the plane of the epithelium, known as planar cell polarity. It is also known as the collective alignment of cell polarity across the tissue plane, which is represented by the uniform orientation of bristles covering the insect epidermis or of the hairs covering the mammalian body surface and the organization of the hair cells within the cochlea [187, 190].

Polarity is critical for the function of epithelial cells that demonstrate definite apico-basal polarity, in contrast to motile cells that acquire front-rear polarity. In epithelial cell, the change of the cuboidal or columnar shape through the loss of apico-basal polarity is essential process in morphogenesis in development and during wound healing. However, deregulation in polarity signaling pathway lead to invasiveness. In cancer development, the transition from epithelial to migrating polarity by is seen as a hallmark in cancer progression to invasiveness or metastasis [191, 192]. Consequently, altered polarity leads to disrupted cell-cell adhesion, cytoskeleton rearrangements and loss of epithelial homeostasis overall. In the further **chapter**

I.E, I will develop in more details the organization of polarity in epithelium and its function in maintaining epithelium homeostasis.

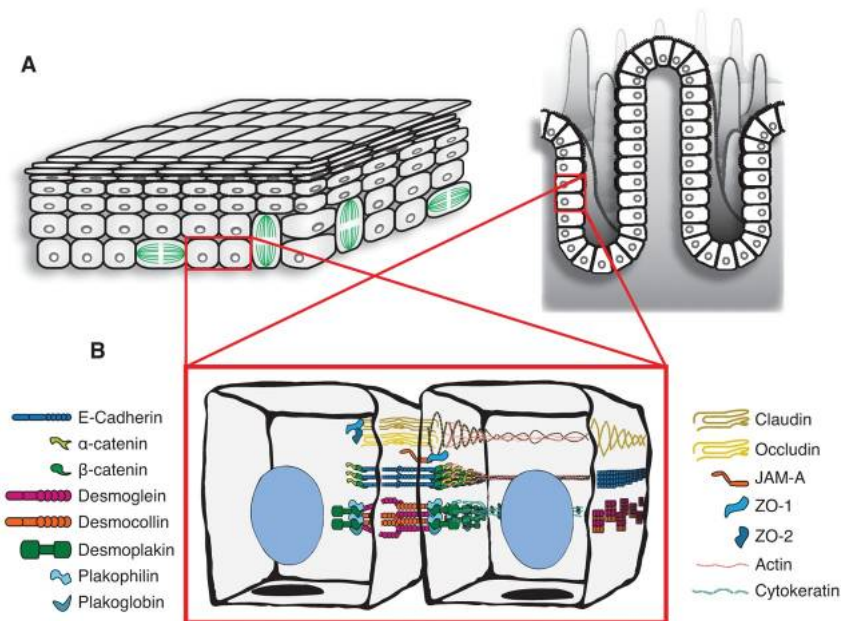


Figure 13: Tissue organization and cell junction composition in two different epithelia (intestine and epidermis).[4]

#### I.C.b.2 Intracellular junction types in epithelia

Cohesion of epithelial cells within an epithelium requires a close connection between neighboring cells. This cohesion is ensured by the formation of various types of cell-cell junctions.

These junctions maintain tissue homeostasis by controlling the structural integrity of the epithelia. However, beside this structural function, cell-cell junction participates also in the regulation of, cell proliferation, cell migration and the diffusion of ions, solutes and microbes across the tissue. Cell–cell contacts are far from being static structures despite being essential to maintain a barrier function by binding cells together. During the turnover of growing tissues including the intestine or the skin, they are continuously remodeled to permit the extrusion of old cells and to include young cells derived from stem cells without being accompanied with loss of the barrier function [193]. Likewise, during leukocyte extravasation at sites of an ongoing immune response or in secondary lymphoid organs, the homotypic interactions between endothelial cells must be disrupted to allow the passage of the leukocytes without disturbing the barrier of the endothelium [194]. Cell–cell junctions are also implicated in most developmental processes, which also necessitate intense remodeling of these structures.

These diverse demands in different cell types and different physiological situations necessitate a complex regulatory network which allows a partial disassembling and re-establishment of cell– cell contacts.

As mention above, within each epithelium it exists different types of intercellular adhesion complexes that ensured specific functions. Cell-cell junction complexes are divided in three basic types of connections: tight junctions, anchoring junctions that include adherens junctions and desmosomes, and gap junctions (Figure 13). I will now briefly detail the different types of intercellular junctions present in epithelia, as well as their contributions to epithelial adhesion.

#### I.C.b.2.1 Tight junction

Tight junctions (TJ) are required to separate tissue spaces and regulate selective permeability barrier across the epithelium, forming the continuous intercellular barrier between epithelial cells. In conventional electron micrographs, tight junctions appear as regions of tight apposition of neighboring cells, where the plasma membranes of adjacent cells appear to fuse together, which are called kissing points. Tight junction are composed of different transmembrane proteins, known as claudins, occludin, tricellulin and the junctional adhesion molecules (JAM) that form homophilic and heterophilic interactions with their the extracellular domains [195]. These transmembrane proteins are connected with a large number of cytoplasmic proteins known as zonula occludens (ZO-1, ZO-2, ZO-3) that in turn associate with the actin cytoskeleton. These proteins interact with each other and form a complex protein network in a dense, cytoplasmic plaque as observed by freeze-fracture electron micrographs. These proteins belong to the MAGUK family and contain PDZ domains that help them to act as scaffold proteins to recruit other proteins to the tight junction plaque. The tightness of the barrier to ion and molecules is affected by the precise composition of the junction. This composition varies with tissue type and can be regulated to allow the passage of certain molecules or ions depending on the local environmental conditions. When the TJ barrier fails due to alteration of TJ's proteins, material can cross the epithelia in an unregulated fashion. Indeed, trans-epithelial absorption and secretion stop, and antigens and microbes can breach the barrier, which leads to several diseases in human. Alteration of TJ leads to autoimmune diseases (such as inflammatory bowel diseases) and loss of sensory hair cells in the hearing organs leading to deafness [196].

Beside its essential barrier function, tight junctions have been also involved in the regulation of other cellular functions such as proliferation, differentiation and cell polarity (see polarity section).

#### I.C.b.2.2 Adherens junction

Adherens junctions (AJ) are cell-cell adhesion complexes that make important contribution to embryogenesis and tissue homeostasis. AJ are ubiquitous structures that act primarily to hold adjacent cells together through interactions between the extracellular domains of the transmembrane proteins of the adjacent cells. The intracellular domain of the transmembrane proteins, which are mainly cadherins, is associated with catenin family members, which in turn interact with actin that forms an anchoring cytoskeleton at the site of the junction. By their interaction with the actin cytoskeleton, they also fulfill a role of diffusion barrier for proteins between the apical domain and the basolateral domain. The AJ performs multiple functions including initiation and stabilization of cell-cell adhesion, regulation of the actin cytoskeleton, intracellular signaling including transcriptional regulation [197]. Disruption of the AJs or defects in their different components are associated with a variety of diseases including inflammatory bowel disease [198], disorders of the skin [199] and cancer [200]. Since my thesis focus on the importance of AJ in the regulation of cell surface receptors, I will go through in detail the organization and function of AJ with the focus on the role of AJ in mediating and regulating multiple signaling pathways in the section below.

#### I.C.b.2.3 Desmosomes

Like AJ, Desmosomes are intercellular junctional complexes that strengthen cell-cell adhesion. Their function is especially important in tissues under extensive mechanical strain, such as the cardiac muscle or skin epithelia. Desmosomes are calcium-dependent junctions, composed of desmosomal cadherins. Desmosomal cadherins interact with Plakin proteins which act as anchors of the intermediate filaments between neighboring cells. The desmosomal adhesion complexes establish an integrated mechanically resistant unit throughout the tissue [201]. Desmosomes are found compromised in several human diseases including: blistering skin diseases, cardiomyopathies and some cancers [202]. These pathological conditions are caused by both reduced intercellular adhesion and aberrant signaling pathways.

#### I.C.b.2.4 Gap junction

Gap junctions are cell-to-cell channels that allow diffusion of small metabolites, second messengers, ions and other small molecules between the cytoplasm of adjacent cells. Gap junctional communication is essential for electrical transduction, signaling and nutrition. Gap junction channels can be open or closed. This regulatory process is highly dynamic and regulated at multiple levels [203]. Gap junctional channels are composed of hexamers of medium-sized families of integral proteins: connexins in mammals. Similarly, to other cell–cell junctions, at gap junctions' cytoskeletal interactions with microtubules and indirectly with actin have been described. Mutations in specific connexin genes are associated with a variety of disorders such as inherited hearing loss, hyperproliferation of skin known as erythrokeratoderma, atrial fibrillation and cancer. These disorders are caused by defects in intercellular communication, signaling via hemichannels or non-junctional effects through interactions of connexins with other molecular signaling systems [204].

#### I.D Adherens junction

In this section, I will introduce the main organization of AJ in epithelial cells, presenting the structure of the different molecules implicated in the formation of AJ. I will start to describe the cadherin family and their evolution within species. I will then detail the mechanisms leading to AJ assembly which starts with cadherin clustering and assembly. I will detail the importance of AJ in the physiopathology of the epithelial tissue. Finally, in order to understand the functions of AJ in epithelial tissues, I will describe the molecular involvement of AJ in the regulation of different signaling pathways

##### I.D.a Organization of Adherens junctions

AJ consists of two basic adhesive units: the nectin–afadin complex and the cadherin–catenin complex. Both nectin and cadherin are multimembers families that have a cell-specific expression, which determine the strength and adhesive specificity of the AJ.

Nectins and cadherins have been localized in a specific area of the lateral domain of the cell called *zonula adherens* (ZA) in simple epithelium [205]. The ZA was described by Farquhar and Palade as a belt like attachment between epithelial cells supported by cytoplasm filaments, which are usually located between the apical tips of most simple epithelia [206]. The nectin/afadin complex and cadherin/catenin complex are found in distinct clusters and don't



intermix, in fact both of nectins and cadherins are found in mosaic clusters in ZA [207]. In contrast to nectin/afadin complex, cadherin/catenin complex are also found stretched at the entire length of the epithelial lateral membrane. Several studies indicated the ability of cadherin receptors to aggregate into lateral clusters. Indeed, studies in *Drosophila* embryonic epithelia [208] and cultured mammalian cells [209] found that E-cadherin organized into nanoscale clusters that distributed extensively throughout the lateral AJ (LAJ) below the ZA. The particular localization of AJ in simple epithelium is necessary for proper modulation of various signaling pathways.

However, the organization of AJ differ in basal keratinocytes, which lacks ZA and cadherin is found all around the cell membrane in cell-cell adhesions [9] where nectin is found colocalized with cadherin [210]. This different organization suggest that the mechanisms by which cadherin/catenin regulate signaling pathways in these cells may differ from simple epithelia.

#### *I.D.a.1 Cadherin-Catenin complex*

##### *I.D.a.1.1 Classical cadherins and the cadherin superfamily*

###### *I.D.a.1.1.1 Structure and function of cadherins*

Cadherins were first recognized by the labs of Takeichi, Kemler and Jacob as membrane proteins that maintained calcium-dependent cell-cell adhesion [211-213]. The large superfamily of cell surface glycoproteins was identified using molecular cloning, based on sequence homology with the unique domain first discovered in the extracellular regions of the classical cadherins E- and N-cadherin, known as the cadherin superfamily. The cadherins consists of more than 100 members, in vertebrates, which are grouped into distinct subfamilies [214]. Cadherin subfamilies comprise: classical cadherins, desmosomal cadherins, flamingo cadherins, proto-cadherins as well as FAT, *table 5*. This diversity is the result of multiple cadherin encoding genes as well as alternative RNA splicing mechanisms [215]. Only classical cadherins are adhesion molecules and are the core components of AJ. Their expression seems to be tightly controlled during development. Indeed, each tissue or cell type shows a characteristic pattern of cadherin molecules, which is often linked with changes in cellular morphology and tissue architecture, since cadherins play different roles in the cell.

Table 5: Members of the cadherin superfamily [5]

Subtype	Examples	Common features
Classic cadherins	Type I: E-cadherin, N-cadherin, R-cadherin Type II: VE-cadherin	Well established Ca <sup>2+</sup> -dependent homophilic adhesion function Cytoplasmic domains interact with $\beta$ -catenin, $\alpha$ -catenin and p120 Linked to the actin cytoskeleton Associated with adherens junctions
Desmosomal cadherins	Desmocollins, Desmosgleins	Adhesion proteins of desmosomal junctions Similar in structure to classic cadherins, but have distinct structural differences Cytoplasmic domains interact with plakoglobin ( $\gamma$ -catenin) and desmoplakin Linked to intermediate filament cytoskeleton
Atypical cadherins	T-cadherin, LI-cadherin	Function as homophilic adhesion proteins without interaction with catenins or link to actin cytoskeleton
Proto-cadherins	CNRs ( $\alpha$ -Pcdhs), $\beta$ -Pcdhs, $\gamma$ -Pcdhs, PAPC, AXPC	Variable EC domains Many proto-cadherin genes (such as $\alpha$ -, $\beta$ -, $\gamma$ -pcdhs) often present in large gene clusters with complex splicing Others, such as PAPC and AXPC, are present as single genes Highly expressed in nervous system Cytoplasmic domains do not interact with catenins — some interact with Fyn-kinase, other interactions unknown
Cadherin-related signalling proteins	FAT, Daschous, Flamingo (seven membrane spanning), RET tyrosine kinase	Variable number of cadherin-type repeats in extracellular domains Roles in tumour suppression, embryonic patterning and establishment of cell polarity (especially in <i>Drosophila melanogaster</i> ) Not known if these proteins function as homophilic adhesion molecules

The members of this superfamily are transmembrane glycoproteins characterized by a repetitive sequence homology in their extracellular region (amino acid sequence of about 110 residues), known as cadherin repeats (also called extracellular cadherin domains (EC) or cadherin motifs). The EC harbor negatively charged conserved sequence motifs (DXD, DRE, and DXND- NAPXF), which is involved in calcium-binding [216]. Binding of calcium to each EC domain is required for the correct conformational organization of the cadherin extracellular domain. The number of the EC domains varies considerably within the members [217]. By contrast, the intracellular domain is not conserved among the members of this subfamilies, thereby leading to diverse functions ranging from cell–cell adhesion to signaling.

The biological functions of the family members have diverged: some of them are well defined as adhesion molecules, including classic cadherins and desmosomal cadherins, whereas the other members do not necessarily show strong adhesive activities [218]. For instance; Flamingo functions in planar cell polarity, Fat cadherin and its binding partner Daschous regulate cell proliferation and planar cell polarity, cadherin 23 and protocadherin 15 regulate the formation of apical cell protrusions [219].

In my thesis, I will concentrate on the classical cadherins since they form AJ. The given name of classical cadherins were originated from the tissue in which they are essentially expressed.

However, most cadherins can be expressed in many different tissues and that these expression patterns are not exclusive. The classical cadherins include E-cadherin (E- for epithelium), N-cadherin (N- for neural), P-cadherin (P- for placenta) and VE-cadherin (VE- for vascular endothelial).

Expression of classical cadherin at the cell surface is critical for cell-cell adhesion and accordingly for development and for integrity of solid tissues [5]. The capacity for exogenous expression of specific classical cadherins to increases the adhesiveness of cadherin-deficient cells that primarily adhere poorly to one another, demonstrated that classical cadherins support cell-cell adhesion. This was shown for *Drosophila* Schneider cells, mouse fibroblastic L cells, and Chinese hamster ovary cells [220-222]. The capacity of newly isolated cells to aggregate in agitated suspensions also permitted the evaluation of cell-cell adhesion. The advantage of this method is to investigate the capacity of cells to adhere to one another independent of cell-matrix adhesive interactions [223].

Classical cadherins are encoded as an inactive precursor proteins containing a signal sequence and a pro-domain that immediately precedes EC1 [224]. After protein translation, the pro-domain is cleaved from the cadherin protein precursor. The cleavage of the pro-domain by proteolysis is essential for cadherin's adhesive activity [225]. The mature protein is composed of five extracellular cadherin repeats (EC1-EC5), a transmembrane region, and a highly conserved cytoplasmic tail. The cytoplasmic domain of classical cadherin is divided into two domains, the juxtamembrane domain (JMD) that binds p120-catenin, and the  $\beta$ -catenin binding domain (CBD) that can also bind  $\gamma$ -catenin (plakoglobin). Sequences homologous to the  $\beta$ -catenin/plakoglobin-binding region exist also in the desmosomal cadherins.

However, catenins are not the only proteins interacting functionally with cadherin. Certainly, other cytoplasmic proteins including signaling molecules [226], cytoskeletal regulators [227] and determinants of epithelial polarity [228] associate directly or indirectly to cadherin.

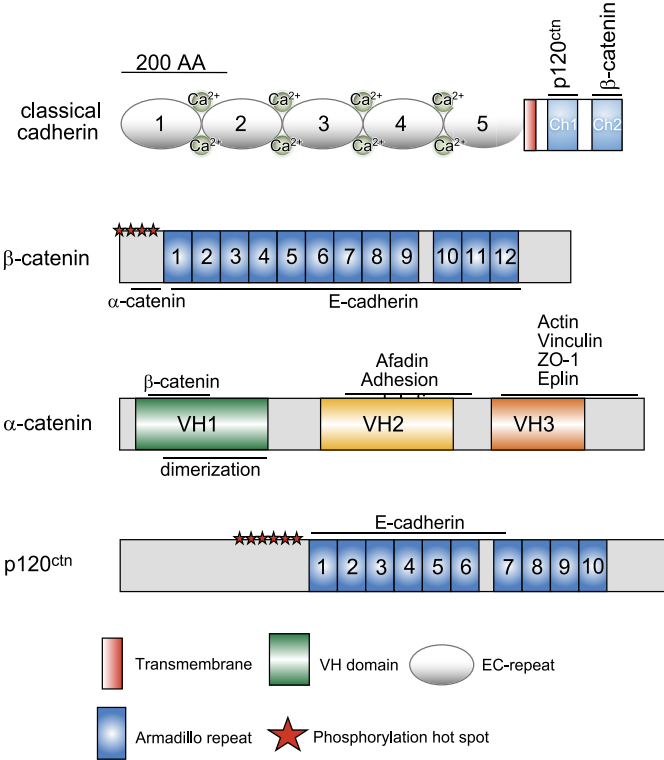


Figure 14: Domain structure of the cadherin-catenin complex components. Schematic overview of the domain structure of a classical cadherin and its three associated catenins:  $\alpha$ -catenin,  $\beta$ -catenin, and p120. (Niessen et al., 2011)

Classical cadherins structure is highly conserved during evolution. Successive studies found EC domain-containing proteins in invertebrates, remarkably the *Drosophila* homologs DE- and DN-cadherin. While DE- and DN-cadherins cytoplasmic regions are very comparable to their vertebrate counterparts, their ectodomains differ significantly, containing up to 15 EC repeats rather than five. Moreover, between the EC cluster and the transmembrane region, a primitive classical cadherin proteolytic site domain (PCPS, previously termed the non-chordate cadherin domain), an EGF-like domain (EGF), and a laminin globular domain (LmG) are present. AJ are also present in the nematode *Caenorhabditis elegans*, which expresses the homologues HMR-1 (cadherin), HMP-1 ( $\alpha$ -catenin) and HMP-2 ( $\beta$ -catenin) [229].

#### I.D.a.1.1.2 Expression in adult and during development of classical cadherins

Classical cadherin superfamily has distinct expression patterns during embryonic development and in the mammalian adult. Changes in cadherin expression are frequently accompanied with changes in cellular morphology and tissue architecture. Here, I will describe the expression of some of the well-known classical cadherins including E-, N-, P- and VE-cadherins.

**E-cadherin**, for epithelial-cadherin, is generally considered the prototype of all cadherins because of its early identification and its thorough characterization, both in normal and in pathological conditions. The human E-cadherin is encoded by the *CDH1* gene, which is located on chromosome 16q22.1. E-cadherin is expressed in all mammalian epithelia. The spatiotemporal control of E-cadherin expression throughout embryonic development permits cell migration and morphogenesis. The first zygotic E-cadherin gene expression in the mouse starts at the two-cell stage. Throughout gastrulation this dynamic regulation is demonstrated by decreased E-cadherin expression in delaminating epiblast cells at the primitive streak so that mesoderm is formed. Similarly, E-cadherin expression in the ectoderm is switched off at neurulation but remains strong at the ectoderm-neuro-ectoderm borders, where it plays an important role in neural tube closure. Reciprocally, E-cadherin transcription is re-initiated in cells undergoing mesenchymal-epithelial transitions (MET) during kidney organogenesis [230]. This E-cadherin switching is also accompanied by the switching of the neural cadherin, N-cadherin as explained below.

**N-cadherin** protein is encoded by the *CDH2* gene, which is positioned on chromosome 18q11.2. N-cadherin is detected in the mesoderm and the notochord in the early embryo, while in the late embryo it is found in neural tissue, lens and some other epithelial tissues, nephric primordial, cardiac and skeletal muscles, mesothelium, primordial germ cells some mesenchymal tissue. N-cadherin, in the adult, is limited to neural tissue, retina, endothelial cells, osteoblasts, fibroblasts, mesothelium, myocytes, limb cartilage, oocytes, spermatids and Sertoli cells [231]. N-cadherin expression is spatiotemporally regulated during neural development, which is important during a number of processes.

The so-called “cadherin switch” happens throughout embryonic development, tissue morphogenesis and cancer progression. In fact, loss of E-cadherin expression in cell lines and tumor tissues is often associated with induced expression of mesenchymal cadherins, such as N-cadherin and cadherin-11, a phenomenon generally referred to as cadherin switching [232,

233]. E-cadherin repressors, such as Snail and SIP1, can induce N-cadherin expression during EMT, suggesting that this cadherin switch is part of a transcriptional reprogramming of dedifferentiating epithelial cells [234]. Carcinoma cells, during EMT, shed their differentiated epithelial characteristics that includes cell-cell adhesion, polarity and lack of motility and develop mesenchymal traits such as motility and invasiveness. These invasive cells would infiltrate to the circulating system, till it reaches a secondary site where the cells go through a reverse mechanism, known as mesenchymal-epithelial transitions (MET). These two mechanisms were observed in breast cancer [235].

**P-cadherin** was described for the first time by Hirai *et al.*, in 1986, where it was found in the placenta and was characterized as part of new class of cadherins that emerged in developing mouse embryos [236]. P-cadherin is encoded by the *CDH3* gene, which shares 66 % homology with *CDH1* encoding E-cadherin protein. P-cadherin is less characterized and differs from E-cadherin mostly in the extracellular portion [237]. P-cadherin expression is restricted to the extra-embryonic ectoderm and visceral endoderm, the structures creating the placenta in mice as shown by embryonic histogenesis [236]. P-cadherin is only expressed in certain tissues in the adult, typically co-expressed with E-cadherin, which includes the basal layer of the epidermis, the hair follicle, the breast and the prostate, the ovary, mesothelium, the cervix, as well as the corneal endothelium [238, 239].

**VE-cadherin**, vascular endothelial-cadherin, is encoded by the human gene *CDH5* located in a gene cluster in a region on the long arm of chromosome 16. VE-cadherin expression is tissue-specific and is found in endothelial tissues and in the brain, in a way that its promoter is repressed in other cell types [240]. Although VE-cadherin is specific to endothelial cells, endothelial cells express another major cadherin, N-cadherin [241]. VE-cadherin arises as the organizer of endothelial cell–cell junctions, as it governs the level of expression and/or the localization of other junctional molecules, such as claudin-5 and N-cadherin [242].

### I.D.a.1.2 Catenins

The catenins are vital linkers between cadherins and both actin and microtubules (Figure 15). I will present briefly the structure and the function of the different catenins implicated in AJ formation and stability.

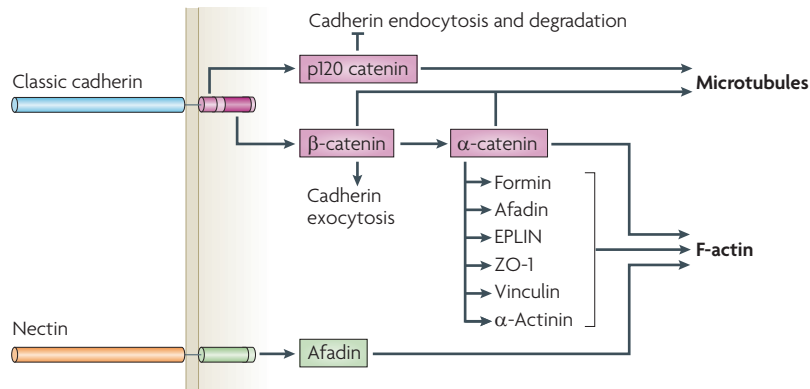


Figure 15: Organization and cytoskeletal relationships of cadherin–catenin and nectin–afadin complexes. [8]

#### I.D.a.1.2.1 $\beta$ -catenin

$\beta$ -catenin can bind at the same time to the cytoplasmic portion of classical cadherins and to  $\alpha$ -catenin. The protein  $\beta$ -catenin, encoded by *CTNNB1*, is extremely conserved with only six amino acids distinctive between the *Xenopus* and human proteins, and it is 67% identical to the *Drosophila* homolog armadillo.  $\beta$ -catenin exists as a single protein in vertebrates and insects, in contrast to the multitude of cadherins and various tissue-specific  $\alpha$ -catenin variants (see below).

The structure of  $\beta$ -catenin consists of a central armadillo domain (residues 141-664) composed of 12 armadillo repeats, known as armadillo repeat domain (ARM), an N-terminal domain that harbors the binding site for  $\alpha$ -catenin [243] as well as the glycogen synthase kinase-3 (GSK3) and casein kinase 1 (CK1) phosphorylation sites that are recognized by the  $\beta$ -TrCP ubiquitin ligase, and a C-terminal domain that functions as a transcriptional activator in WNT signaling. The ARM domain is an extended super-helical structure produced by the subsequent packing of helical ARM repeats. This superhelix is involved in the binding of most  $\beta$ -catenin ligands including E-cadherin, TCF family transcription factors, adenomatous polyposis coli (APC), and the transcription inhibitor ICAT [244]. Since the surface of the ARM

domain can typically accommodate only one peptide motif at a time, all these proteins compete for the same cellular pool of  $\beta$ -catenin molecules. In order to separate the adhesion and signaling functions of  $\beta$ -catenin a selective targeting of distinct molecular forms of  $\beta$ -catenin is specified in the cells [245].

#### I.D.a.1.2.2 $\gamma$ -catenin

Plakoglobin, also called  $\gamma$ -catenin, is encoded by the *CTNNG* gene. Plakoglobin belongs to the armadillo family of proteins along with  $\beta$ -catenin, shares nearly an 80% homology to  $\beta$ -catenin, and has a nearly identical interaction with E-cadherin. The N-terminal  $\beta$ -TrCP-binding motif is also conserved in plakoglobin, in addition to the resemblances of their ARM domain in both architecture and ligand binding capacity which implies a common ancestry and shared regulation with  $\beta$ -catenin [246].

Despite their structural similarities and their common interacting partners, plakoglobin and  $\beta$ -catenin seem to play opposite roles regarding cell signaling in tumorigenesis and metastasis. It is worth mentioning plakoglobin primarily associated with desmosomal cadherins, but it can substitute for  $\beta$ -catenin in the classical cadherin/catenin complex, and links cadherins to the actin cytoskeleton.

#### I.D.a.1.2.3 $\alpha$ -catenin

Three isoforms of  $\alpha$ -catenin have been described,  $\alpha$ -E-catenin that is prevalent in epithelial tissues,  $\alpha$ -N-catenin that is restricted to neural tissues, and  $\alpha$ -T-catenin that is found in heart and testis. Each one of the isoforms are encoded by different genes, *CTNNA1*, *CTNNA2*, *CTNNA3* respectively [247]. I will be concentrating on  $\alpha$ -E-catenin function in epithelial cells.  $\alpha$ -E-catenin is essential for AJ formation and function and operates at the interface of the cadherin–catenin complex and the actin cytoskeleton.  $\alpha$ -E-Catenin is a roughly 900-amino-acid protein that contains three regions with strong homology with the actin-binding protein vinculin, known as the vinculin-homology domain (VH).  $\beta$ -catenin binds to a region near the N-terminus (residues 57–146), and F-actin binds to the C-terminal region. In addition,  $\alpha$ -E-catenin binds to itself, using a domain that overlaps the  $\beta$ -catenin-binding site (residues 82–264).  $\alpha$ -E-catenin (the mammalian  $\alpha$ -catenin isoform with which these experiments were carried out) was found to preferentially binds to actin as a dimer. The interactions between  $\alpha$ -catenin and F-actin might also be mediated by other actin-binding proteins (Figure 15). Indeed,  $\alpha$ -catenin is a known binding partner of an increasing number of actin-associated



proteins, including vinculin,  $\alpha$ -actinin, formin, zonula occludens protein 1 (ZO1) and afadin [247].

#### I.D.a.1.2.4 p120-catenin

p120-Catenin (p120-ctn) is encoded by CTNND1 and consists of 21 exons encoding 968 amino acids. p120-Catenin is an armadillo-family protein whose ARM repeat domain interact with the juxtamembrane region of the cadherin cytoplasmic tail. An amino-terminal sequence, prior the ARM domain, differs in length in the four splice variants of the protein [248]. Multiple p120-catenin isoforms can be produced by alternative usage of translation initiation codons and through differential splicing events, proposing extra possibilities for additional diversification in expression and functions in mammalian cells. Isoforms 1 and 3 are the most broadly and abundantly expressed isoforms. Isoform 1 is preferentially expressed in more motile mesenchymal-type cells, whereas isoform 3 characterizes epithelial cells [249].

#### I.D.a.2 Nectin-Afadin complex

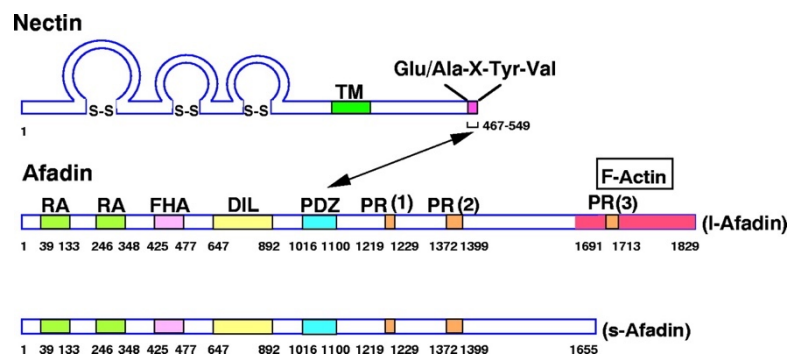


Figure 16: Molecular structures of nectin and afadin. [12]

#### I.D.a.2.1 Nectin

The nectin family is calcium independent IgG-like adhesion receptors consists of four members, nectin-1 to -4, (Table 6). Several splice variants have been showed for each nectin. Nectins assemble lateral homodimers that can be involved in both homophilic and heterophilic adhesion with other nectins or nectin-like receptors, although with variable specificity and affinity. Nectins constitute of an extracellular (EC) domain containing three IgG-like loops, a single transmembrane region and a cytoplasmic domain with a C-terminal PDZ binding motif existing in most splice variants [250] (Figure 16). Nectins can offer the first scaffold for adherens and tight junction formation. Utilizing many different *in vitro* assays

Takai and co-workers exhibited that cadherin mediated cell-cell adhesion and tight junction development was dependent on nectins [251].

Table 6: Nectin and Nectin-like family members [252]

Member	Old nomenclature	Function
Nectin-1	PRR1, HVEC	Cell-cell adhesion molecule Receptor for $\alpha$ -herpes virus (HSV-1, HSV-2 and pseudorabies virus) entry into cells. (Defects in humans: cleft lip/palate-ectodermal dysplasia syndrome, also known as Zlotogora–Ogur syndrome)
Nectin-2	PRR2, HVEB	Cell-cell adhesion molecule Receptor for $\alpha$ -herpesvirus entry into cells
Nectin-3	PRR3	Cell-cell adhesion molecule
Nectin-4	–	Cell-cell adhesion molecule Overexpressed in breast carcinoma
NECL-1	TSLL1, SynCAM3	Cell-cell adhesion molecule with neural tissue-specific expression: localized at contact sites between axons and glial cells or Schwann cells but not at synaptic junctions
NECL-2	IGSF4, RA175, SgIGSF, TSLC1, SynCAM1	Cell-cell adhesion molecule that is localized on the basolateral membranes in epithelial cells Involved in spermatogenesis and synapse formation Tumour suppressor in lung carcinoma
NECL-3	Similar to NECL3, SynCAM2	Putative cell-cell adhesion molecule
NECL-4	TSLL2, SynCAM4	Cell-cell adhesion molecule Mediates axo-glial interaction, Schwann cell differentiation and myelination Possible involvement in tumour suppression
NECL-5	TAGE4, PVR, CD155	Receptor for poliovirus Overexpressed in various carcinomas Enhancement of cell movement and proliferation (in cooperation with integrin $\alpha$ v $\beta$ 3 and PDGF receptor)

#### I.D.a.2.2 Afadin

The actin-binding protein afadin, also known as “AF-6,” forms a structural adhesive unit with nectins, providing these adhesion molecules with a direct link to the cytoskeleton. Afadin consists of a PDZ domain (to which nectins bind), two Ras/Rap binding domains, a dilute domain, a forkhead-associated domain, and three proline rich regions (two in humans), suggesting the potential to function as a signal integrator at AJs (Figure 16). A smaller variant of afadin also exist, S-Afadin, lacking the actin-binding region (Figure 16). The knockdown of the longer variant resulted in decrease in E-cadherin-catenin association with actin cytoskeleton which affected intercellular adhesion, however knockdown of smaller variant did not have this effect on intercellular adhesion [253].

#### I.D.a.3 Mechanisms of AJ assembly and disassembly

The lifecycle of a cadherin-mediated adhesion can be separated into three stages: formation, maintenance, and disassembly. The formation of cadherin contacts starts with an initial step involving the establishment of primordial contacts (puncta formation). Followed by fast

presentation of more nascent contacts lateral to the initial puncta, leading to maturation/expansion of nascent contacts via clustering of cadherins. I will discuss these different steps in the next paragraphs.

### I.D.a.3.1 Cadherin clustering and AJ formation

Cadherin clustering could be promoted by lateral interactions between cadherin extracellular regions or through cytoplasmic interactions that link cadherins into a supramolecular complex. I will briefly explain how both of these interactions lead to cadherin clustering and AJ formation.

#### I.D.a.3.1.1 Clustering driven by cadherin ectodomains

There is conserved pattern of adhesive interactions between classical cadherins ectodomains, which was revealed by their crystal structures. Two types of interaction between these ectodomains have been described: the *trans*-interactions that are formed between ectodomains presented on opposite cell surfaces and the lateral *cis*-interactions that are formed between cadherins presented on the same cell surface (Figure 17). Adhesive *trans*-interactions take place between the EC1 domains of neighboring cadherins, whereas *cis*-interactions include an interface between the base of the EC1 domain and the EC2 domain of its adjacent cadherin. *Trans*- and *cis*-interactions can be present simultaneously, yet, the *cis*-interactions are essentially weak and required strengthening by the formation of *trans*-dimers. Thus, productive adhesion is generated by both *trans*-interactions and *cis*-

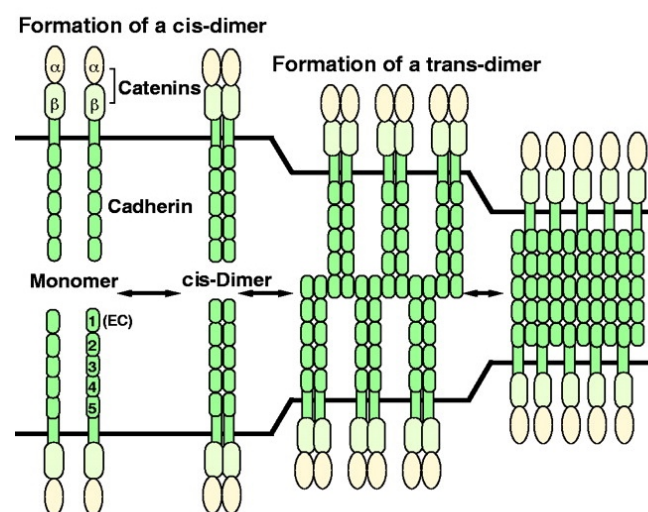


Figure 17: Models for the intercellular adhesion activities of cadherin [12]

interactions, which restricts the molecular motion of the ectodomains. This ability of ectodomains to simultaneously engage in both trans- and cis-interactions was predicted to generate a two-dimensional lattice with the capacity to aggregate cadherins into lateral clusters [254].

The ectodomain alone is not sufficient to support clustering, although lateral clustering may increase adhesion by regulating the local distribution of the ectodomain presented on the cell surface. Instead, multiple cytoplasmic factors contribute to cadherin clustering in cells, including the catenins and the actin cytoskeleton.

#### I.D.a.3.1.2 Cadherin-catenin clusters and actin

There is a mutual relationship between actin and cadherin-catenin clusters during AJ formation. The interplay between actin and the cadherin-catenin clusters is observed at different stages of AJ formation. Indeed, the role of actin in AJ formation is detected at the initiating, establishment and the later stages known as the matured AJ. First, actin create cell protrusions that initiate cadherin clustering. Second, actin polymerization and reorganization are coupled to cell contact development and the aggregation of cadherin–catenin clusters into AJs. Third, multiple actin networks associate with cadherin–catenin clusters at mature AJs to hold them in place [16]. Cadherins can stimulate actin assembly to generate protrusive force [255] by orchestrating cortical signaling and regulators of actin assembly [256]. The cadherin-actin cytoskeleton association is dynamic which undergoes turnover and assembly mutually during junction assembly and at steady state [257]. Disturbance in the assembly, stability, and/or disassembly can change the functional activity of cadherin-mediated AJs, which in turn can generally disrupt intercellular adhesion and tissue integrity. Some example of physiological consequences of AJ assembly alteration are described below (see paragraph adherens junctions: implication in disease, page 85).

At early stages of AJ establishment, cadherin associates with  $\beta$ -catenin, the latter in turn associates to  $\alpha$ -E-catenin.  $\beta$ -catenin-cadherin association is important for the targeting of cadherins to the plasma membrane and cadherin stability [8].  $\beta$ -catenin-cadherin association occurs in the endoplasmic reticulum and is needed for the effective surface transport of cadherin through the biosynthetic pathway. Failure of cadherins to interact with  $\beta$ -catenin results in retention in the endoplasmic reticulum and degradation of cadherin. Moreover,  $\beta$ -

catenin protect cadherin cytoplasmic domain from proteolysis *in vitro* [258]. Once at the plasma membrane, the cadherin- $\beta$ -catenin complex rapidly recruits  $\alpha$ -E-catenin.

Since  $\alpha$ -E-catenin was shown to independently bind F-actin [259], it was proposed that  $\alpha$ -E-catenin directly interlinked E-cadherin/ $\beta$ -catenin complex to F-actin. Although early *in-vitro* experiments with purified proteins contradicted this model, since they failed to demonstrate a quaternary complex between cadherin,  $\beta$ -catenin,  $\alpha$ -E-catenin and actin [260, 261]. This was explained by the fact that  $\alpha$ -E-catenin can exist both as monomers and homodimers in the cytosol [260] and  $\alpha$ -E-catenin was found to preferentially binds to actin as a homodimer. Accordingly,  $\alpha$ -E-catenin may not be able to bind  $\beta$ -catenin and F-actin simultaneously [262]. Accordingly, mammalian  $\alpha$ -E-catenin exists in distinctive cellular pools including cytoplasmic monomer and homodimer and membrane-tethered, monomeric  $\alpha$ -E-catenin bound directly to E-cadherin/ $\beta$ -catenin.

An *in vivo* study by Buckley *et al.* demonstrated that tension forces applied to the  $\alpha$ -E-catenin/F-actin interface is necessary to strengthen the interaction between E-cadherin/ $\beta$ -catenin/ $\alpha$ -E-catenin complex and F-actin. Explaining that the reason why the previous studies have failed to observe the quaternary complex is due to the lack of tension force in the *in vitro* model that is only observable *in vivo* [263]. Moreover another study by Bianchini *et al.*, demonstrated that the cytosolic homodimer pools  $\alpha$ -E-catenin and monomeric junctional  $\alpha$ -E-catenin work together to induce strong cell-cell adhesion [262].

In early stages of AJs assembly, cadherin-catenin clusters are found at the nascent adhesion, also known as punctum adherens. These nascent adhesions are promoted by filopodial and lamellipodial extensions. These dynamic membrane protrusive activity implicate the actin cytoskeleton and Rho GTPases including Rac1, Cdc42, and RhoA [264]. Alongside the formation of nascent contacts, E-cadherin ligation recruits and activates Rac1 to these sites, in a phosphoinositide 3-kinase (PI3K) dependent manner. As a result, Arp2/3 is recruited and activated at cadherin-mediated contacts, which promotes the formation of actin branched networks [265, 266]. Rac1 may also contribute to the stable formation of nascent contacts through facilitating the interaction of  $\alpha$ -catenin with  $\beta$ -catenin [16].

When more nascent contacts begin to form, the densities of AJ-associated proteins, including  $\alpha$ -catenin increases. The formation of AJ promotes the formation of  $\alpha$ -E-catenin homodimers. Homodimers of  $\alpha$ -E-catenin compete with the actin-related protein 2/3 (ARP2/3) complex, a nucleator of branched actin, for actin interaction. This competition serves as a feedback

mechanism to reduce lamellipodial movements as cell-cell junction formation progresses [260, 267, 268]. This is consistent with the loss-of-function and mutagenesis studies that involve  $\alpha$ -E-catenins in radial cable formation [16]. Since the loss-of-function mutations in  $\alpha$ -E-catenin produce cells that fail to dynamically bring themselves together, stabilize cell-cell junctions and integrate actin networks throughout an epithelial sheet. This was further confirmed by drugs that impair actin polymerization weaken cell-cell contacts and reduce adhesion [269]. Thus,  $\alpha$ -E-catenin plays an integral role in remodeling the actin cytoskeleton into radial actin cables once recruited to the nascent contacts. In addition, several proteins essential for actin organization at filopodia protrusion, in keratinocytes, are recruited in a  $\alpha$ -catenin-dependent manner. These proteins include vinculin, zyxin, VASP, and Mena. In the absence of  $\alpha$ -catenin or when VASP/Mena function is stopped, keratinocytes still send out filopodia, but they fail to reorganize the actin cytoskeleton at AJs and do not seal cell borders [269].

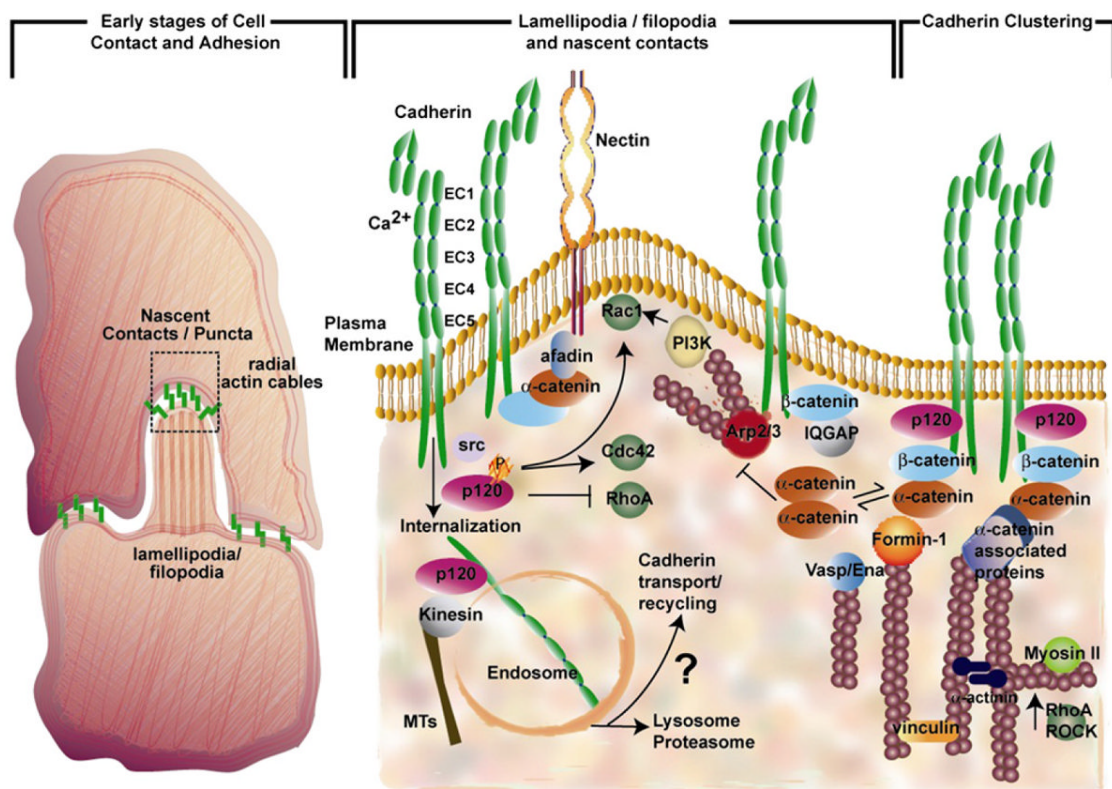


Figure 18: Schematic representation of cadherin-mediated adhesion stages in epithelial cells [16]

Several studies have demonstrated that cadherins-catenin produce signals that influence the cytoskeleton through the catenins. Indeed, both  $\alpha$ -catenin and p120-catenin bind to Rho

GTPases and coordinate cell adhesion and actin dynamics. p120-catenin controls small GTPases such as RhoA, Cdc42 and Rac and their effectors, including Rho-associated protein kinase 1 (ROCK1) PAK (p21-activated kinase), that regulate the actin cytoskeleton. The interaction with cadherin is thought to localize the small GTPase regulatory activity of p120-catenin at AJs to affect actin organization. Rho signaling is required for cell-cell integrity and this is likely to largely reflected by local regulation of the actomyosin cytoskeleton. Both loss- and gain-of Rho function disturb junctional integrity, demonstrating that rigorous expression of Rho signaling at junctions plays a crucial role in cell-cell cohesion [256]. During the early steps of cell adhesion in epidermal cultures, cells extend lamellipodia and filopodia in response to elevated Rac1/Cdc42 activity and other signal transducers including phosphatidylinositol 3-kinase (PI3K) and growth factor ligands for transmembrane receptor tyrosine kinases (RTK). The recruitment of both p120-catenin and  $\alpha$ -catenin at the contact sites with Rac1 collectively contribute to the extension of the contact surface and maturation of cadherin-mediated AJs [16, 248].

AJ formation at later stages involves the maturation and extension of nascent contacts through clustering of cadherins. At this stage, membrane sealing is increased and ultimately radial actin cables are rearranged and get stabilized by myosin II [270],  $\alpha$ -actinin, and possibly  $\alpha$ -E-catenin homodimers. The actin cytoskeleton is reorganized to be in a static state by the formation of mature cell contacts.

#### I.D.a.3.2 Regulation of cadherin stability at the plasma membrane

Although AJs may appear to be in a static structure in mature tissues, cadherin junctions are dynamic structures that display molecular turnover. Moreover, cadherins surface levels can be controlled by various mechanisms.

Cells disassembly and remodeling of AJs is essential during different physiological events such as: tissue remodeling during developmental process and wound healing, angiogenesis, transmigration of immune cells within an epithelium. AJ disruption can be induced by a plethora of signaling events. There are four main routes that are involved in cadherin disassembly:

Endocytosis is one of the mechanisms regulating cadherin-adhesion remodeling. For example, studies in mammalian cell culture have shown that cadherins are continually internalized

[271]. The endocytosis rate of E-cadherin in mature contacts in epithelial cell lines stably transfected with E-cadherin-GFP were studied by De Beco *et al.* [272]. They studied the dynamics of E-cadherin by combining the 2-photon FRAP technique with fast 3D microscope. The study demonstrated that E-cadherin endocytosis and recycling to the plasma membrane in mature contacts happens in the scale of tens of seconds. Also they showed that the diffusion of E-cadherin at the plasma membrane is extremely decreased in mature contacts in contrast to immature contacts [272]. Hence, cadherin redistribution at mature AJs seems to be due primarily to endocytosis and recycling rather than lateral diffusion [272]. Interestingly, a study using cadherin constructs with mutated endocytic elements showed that these mutants can still be recruited to AJs and form stable contacts but were not endocytosed [273], which shows that E-cadherin can be recruited to the cell membrane and by only its extracellular region. Studies in mammalian cell culture propose that cadherins are mostly internalized by clathrin-mediated endocytosis, although other mechanisms have also been recognized. Clathrin adaptor proteins often bind to diLeu motifs, and a diLeu motif in the juxtamembrane region of E-cadherin promotes its endocytosis [274]. Dynamin functions in the fission of clathrin-coated pits and is involved in cadherin endocytosis in both mammalian cell culture and *D. melanogaster* [8].

The internalization into clathrin-coated vesicles (CCV) depends on p120-catenin. There appear to be two principal roles for p120-catenin in AJ. The first is regulating the actin cytoskeleton as mentioned above. The second regulates cadherin levels by controlling the rate of cadherin turnover. The cadherin–p120-catenin interaction counteracts cadherin endocytosis and degradation, and thus enhances the surface abundance of cadherin [275]. p120-catenin play an essential role in maintaining AJs stability [276]. p120-catenin interacts with the juxtamembrane domain of cadherins, a domain that comprises docking sites for the endocytic machinery and sites for cadherin ubiquitylation by the E3 ubiquitin ligase hakai, which can trigger cadherin endocytosis and degradation [277]. Loss of p120-catenin increases cadherin turnover and endocytosis, suggesting that it normally blocks the endocytic machinery from acting on cadherins. This idea is supported by the fact that mutation of the diLeu motif in the juxtamembrane domain of E-cadherin reverses the internalization seen with mutation of the p120 catenin-binding site [274].

Cadherin recycling back to the cell surface could be facilitated by p120-catenin. Examination of living cells after AJ-disruption induced by calcium depletion show that p120-catenin



accelerates the delivery of N-cadherin to the plasma membrane via an interaction with kinesin motors [278]. These studies suggest that p120 catenin transport cadherins back to the cell surface and away from the degradative pathway.

Moreover, mechanical forces and tension exerted on cell adhesion, notably through the actin cytoskeleton and Rho GTPases. The Rho GTPases Cdc42 and Rac1 were found to also regulate AJs endocytosis by regulating cadherin levels at the plasma membrane through Dynamin-dependent E-cadherin endocytosis process. Two other small GTPases, namely Rap1 and Arf6 (ADP-Ribosylation Factor 6) were also implicated in the regulation of E-cadherin endocytosis, although additional studies are required to clarify their exact contribution for E-cadherin stabilization at the membrane [8].

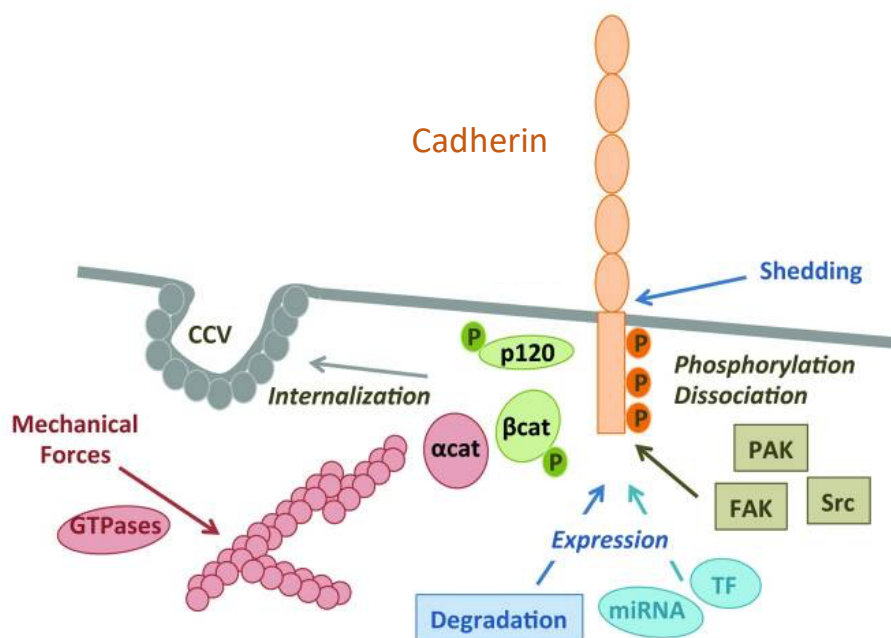


Figure 19: Schema presenting mechanisms regulating cadherin stability at cell plasma [19]

Regulation of cadherin expression at the plasma membrane may also be transcriptionally regulated. A variety of transcription factors target E-cadherin promoter [279]. These include transcriptional repressors of the Snail/slugg family [280], and the transcriptional repressors SIP1 and ZEB1, the aberrant expression of which may, in turn, reflect loss of inhibitory microRNAs that preserve cadherin expression [281].

Functional levels of cadherins at the plasma membrane can also be regulated by proteolytic processes. E-cadherin and N-cadherin can also be cleaved in both the extracellular and

intracellular domains by ADAM10 and other metalloproteases. The extracellular domain cleavage of E-cadherin lead to the generation of a soluble biologically active ectodomain fragment [282]. This fragment acts as an adhesive substrate for other cells to attach to or migrate upon. This ectodomain can compete with full length cadherin binding and intervene with intercellular adhesion as well as inducing cell signals. This can reduce cell aggregation and increase migration and invasion [283]. Moreover, the cytoplasmic tail of cadherin is cleaved after the sheddase, which gives rise to a cytoplasmic tail that translocate to the nucleus and regulate transcription of targeted genes [11, 284].

In summary, various mechanisms regulate the pool of functional cadherin present at the plasma membrane and that are able to participate to AJ. These different mechanisms (cadherin endocytosis, transcriptional regulation, degradation and sheddase) facilitate AJ turn-over and the remodeling of AJ required in various physiological situations.

## I.D.b AJ functions in physiology and pathology

### I.D.b.1 AJ in physiology

The cadherin-catenin-cytoskeleton complex has an essential function in diverse cellular processes including cell-cell adhesion which is essential for maintaining tissue integrity in adult and during embryogenesis, controlling proliferation, cell sorting, apico-basal polarity, migration, inflammation, and cell survival.

#### I.D.b.1.1 Cell-cell adhesion: maintenance of tissue integrity in adult and during embryogenesis

The most commonly understood function of classical cadherin lies in their involvement in the preservation of cell-to-cell cohesion in solid tissues of the body. This adhesive function is required as soon as the first steps of embryo development and therefore it is not surprising that loss of AJ has a strong impact on epithelial tissue organization in adult as well as during the early development. Here, I will introduce the diverse morphogenetic impacts associated with the loss of AJ components in mice development and in conditional KO in specific adult tissue, followed by the role exhibited in mammalian cell lines.

Knockout of any classical cadherins compromised murine development and leads to an embryonic lethality (excepting in the case of P-cadherin KO mice) which are viable (explained below page 75). Like cadherins, the knockout of any of the other AJ components ( $\beta$ -catenin,

$\alpha$ E-catenin, p120-catenin and plakoglobin) have a similar consequence to cadherin KO and leads to major developmental defects associated with embryonic lethality.

E-cadherin is crucial for embryonic development and for epithelial integrity in adult. Mice knockout for E-cadherin (*Cdh1<sup>-/-</sup>*) die at the time of implantation, embryonic day 4 (E4), and have defects in cell junctional and cytoskeletal organization, resulting in failure to form trophectoderm, the first polarized epithelial layer in the mouse embryo [285]. Several studies leave little doubt that cadherin function is necessary for cell-cell cohesion. However, it is noteworthy to mention that in other contexts disruption of cadherins has more subtle effects on tissue integrity [286-288]. Interestingly, conditional disruption of E-cadherin in the mouse skin was associated with mild adhesion defects, or defective tight junctions, but did not disrupt epidermal integrity [289]. However, combining the deletion of P-cadherin as well as E-cadherin disrupted cell-cell cohesion in the mouse epidermis [290, 291]. Hence individual cadherin species may not be completely accountable for cell-cell cohesion since they can be compensated by other classical cadherins or other adhesion molecules. The reason of the modest effect is demonstrated in an *in vitro* study. Reduction of E-cadherin levels in confluent MDCK cell monolayers had little effect on the localization or function of AJs or tight junctions, in contrast to  $\alpha$ -E-catenin silencing [292] since E-cadherin is compensated by other cadherins. However, the effects of E-cadherin silencing on establishment of cell adhesion became evident during reassembly of junctions in a calcium switch experiment, which suggest the E-cadherin mainly play a role in the establishment but not in the maintenance of AJ in epithelial cell lines [292].

Beside E-cadherin, other classical cadherin has been shown to be essential for tissue structuration such as N-cadherin, which is needed for structural integrity of the neural tube and in cortical structures in mice [293]. Knockout N-cadherin in mice embryos exhibit major heart defects and malformed neural tubes and somites and die at day 10 of gestation [294]. At this stage all tissues expected to present seem to be normally differentiated. N-cadherin re-expression rescues partially N-cadherin null embryos, which is exhibited by an augmentation in number of somites, branchial arches and the presence of forelimb buds, nonetheless, brain development was still impaired [295]. Multiple *in vitro* and *in vivo* studies, in the adult, demonstrated that N-cadherin overexpression is associated mostly with a

morphological change towards a more fibroblastic phenotype, with cells becoming more motile, invasive and metastatic [231]. However, there are invasive tumors where N-cadherin is downregulated and where it may play the role of a tumor suppressor molecule [231].

P-cadherin knockout mice are viable and fertile in contrast to E-cadherin and N-cadherin. Still, P-cadherin-deficient females display early differentiation of the mammary gland and present mammary hyperplasia later in life. These observations demonstrate the necessity of P-cadherin in cell-cell adhesion in order to limit growth of the mature luminal epithelial cells. This indicates the role P-cadherin play in the biology of stem cells by maintaining the normal mammary gland in undifferentiated state.

Disrupting VE-cadherin in embryos and adult mice alters vascular integrity. VE-cadherin gene knockout mouse embryos exhibit severe angiogenic defects which is lethal. VE-cadherin adhesive function is important for the control of vascular permeability and leukocyte extravasation. Silencing VE-cadherin expression and blocking its adhesive function *in vitro* provided evidence that this adhesion molecule is essential for AJ formation and endothelial barrier maintenance [296-298]. VE-cadherin expression defects, leading to vascular leakage is observed in many human diseases such as ischemic disease and cancer [299, 300].

$\beta$ -catenin knockout mouse display defects in the formation of embryonic ectodermal layer. At E7.5,  $\beta$ -catenin knockout embryos were half as large as wild-type and heterozygous embryos due to an absence of formation of the three germ cell layers. Most notably, the normal epithelial organization of the ectodermal layer was completely absent [301]. In fact,  $\beta$ -catenin was shown to be needed for anterior-posterior axis formation in mice, since its absence anterior markers were not induced [302]. Huelsken et al shows that  $\beta$ -catenin deletion did not disrupt AJ *in vivo*, since plakoglobin was able to substitute  $\beta$ -catenin [302]. Which reflects  $\beta$ -catenin involvement in mediating signaling, since  $\beta$ -catenin has a central role in the WNT pathway.

The mutation of *Ctnna1*, encoding for  $\alpha$ -E-catenin, at the actin-binding domain in mice disturbs development of the trophoblastic epithelium resulting in mutant blastocysts incapable of hatching from the zona pellucida and implanting in the uterus [303]. Regardless of the presence of E-cadherin/ $\alpha$ -E-catenin complex at the plasma membrane, the mutant embryos are not able to generate a blastocoelic cavity, which confirms the importance of

regulation of actin cytoskeleton by  $\alpha$ -E-catenin in AJ. E-cadherin-null embryos present the same trophectoderm defect [285]. These data establish that  $\alpha$ -E-catenin is essential for E-cadherin-mediated adhesion in the early pre-implantation embryo. Moreover, tissue-specific deletion of  $\alpha$ -catenin in mouse epidermis leads to dramatic alteration of this epithelium associated with loss of intercellular adhesion [304, 305].

Conditional knockout studies have been used to study the impact of p120 deletion in specific mouse epithelial tissues. The severity of p120 KO with respect to cell adhesion and alteration of epithelial morphology differs markedly from one tissue to another [306].

The function of each member of the nectin family in cell-cell adhesion is redundant or compensated by other cell adhesion proteins, and consequently, mice knockout lacking only one member of family are viable. However, phenotypes are observed in specific tissue, revealing their importance in cell-cell adhesion. For instance, knockout studies of nectin-1 and nectin-3 revealed an heterophilic trans-interactions between nectin isoforms, which plays significant role in morphogenesis [307]. Both nectin-1 and nectin-3-deficient mice show several developmental eye abnormalities which include the malfunction of the ciliary body and a separation between the pigment and non-pigment epithelial cells of the ciliary body. This developmental defects are due to a decrease of the adhesive property of the apical region of these cells [308].

Knockout in mice of Afadin, the other member of the afadin-nectin complex is also associated with developmental defects. However, these defects do not phenocopy Nectin knockout, suggesting afadin is indispensable during embryogenesis. Indeed, Afadin KO mice display during the gastrulation stage at (E7.5) disorganization of the ectoderm, an impaired migration of the mesoderm, and the loss of somites and structures normally derived from both the ectoderm and the mesoderm [309].

In general, the members of the AJ exhibit specific role in AJ formation and hence have different implication in tissue integrity. For instance,  $\alpha$ -catenin is indispensable for AJ formation and maintenance since it links the cadherins to the actin cytoskeleton and no other catenin can compensate for this role, whereas cadherins for example have a functional redundancy in the adult tissue.

### I.D.b.1.2 Cell-cell recognition and cell sorting

Since cadherin interacts in an homophilic dependent manner, the expression of different types of cadherins mediates selective cell recognition events that are responsible for the sorting of different groups of cells in developing tissues. This was first recognized by the classic experiments done by Townes and Holtfreter in 1955 showing that cells dissociated from gastrula stage embryos would rearrange to link again with those from the same germ layer when allowed to re-aggregate. Additionally, these cell populations relative position within the reformed aggregate reflected what was established in the embryo [310]. This sorting behavior was depends on distinction adhesion between different cell populations [11].

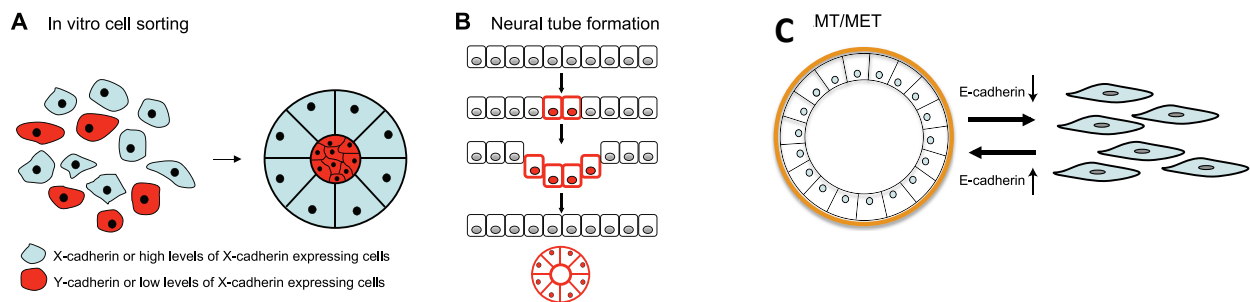


Figure 20: Role of cadherin in cell-cell recognition and cell sorting. [11]

A classic example is exhibited by neural crest cells that undergo a series of cadherin switches during neurulation. E-cadherin is downregulated by neural crest precursors during their initial induction, N-cadherin and cadherin-6b are expressed when they reside in the neuroepithelium and then downregulate the latter when they delaminate. E-cadherin downregulation by transcriptional repression appears to be a critical early stage in the epithelial-mesenchymal transition (EMT) that this cell population undergoes, while N-cadherin and cadherin-6b are necessary at later stages [311]. It is also worth mentioning, reciprocally, E-cadherin transcription is re-initiated in cells undergoing mesenchymal-epithelial transitions (MET) during kidney organogenesis [312]. Such cadherin switching is commonly seen in many other forms of EMT [279].

Cell sorting seems to be due in part to the homophilic binding specificity of the extracellular domain, as the identity of the EC1 domain determines cell-sorting specificity when experimentally transferred from one cadherin to another. Another characteristic found to strongly influence cell-sorting behavior, is the level of cadherin expression, and presumably therefore the overall strength of adhesion, independently of the type of cadherin expressed. Consequently, the mechanisms regulating cadherin adhesiveness, can also contribute to cell-sorting behavior. This includes cadherin-mediated signaling events, quantitative differences in adhesion, or other cellular consequences of adhesion that are mediated by different cadherins, for example, cytoskeletal organization.

#### I.D.b.1.3 Inflammation

Components of the AJ were found also involved in protecting against inflammation in the skin and intestine. The absence of p120 or  $\alpha$ -catenin in the skin of mice resulted in increase of NF $\kappa$ B activity and inflammation in mice epidermis [304, 313]. Additional study in the intestine demonstrated that conditional ablation of p120 in the epithelium of the small and large intestines of mice lead to inflammation and mucosal damage, resulting in bleeding and death within the first 3 weeks of life. This last study demonstrates the essential role of p120 and AJ in the intestine barrier function and epithelial homeostasis [306].

#### I.D.b.1.4 Apico-basal polarity

The AJ assembly occur simultaneously with the establishment of cell polarity and the recruitment at cell-cell junctions of several protein complexes known to be essential for polarity, suggesting a close relationship between junction formation and polarity [314].

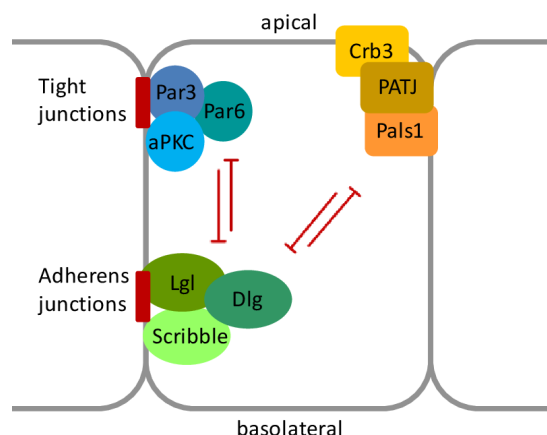


Figure 21: Polarity complexes localization at cell-cell junction in simple epithelia

In particular, AJ has been found associated with the members of the Scrib polarity complex. SCRIB co-localize with E-cadherin on lateral membranes and a recent study has demonstrated the direct interaction between SCRIB and E-cadherin [315]. DLG1 is also found to co-localize at the lateral membrane with E-cadherin and immunoprecipitation experiments demonstrate that they belong to the same macromolecular-complex [228, 316].

Several early studies have demonstrated that AJ formation is required for the establishment of polarity in mammalian epithelial cell lines, and both mice and *Drosophila* animal models [317-319].

In mammalian epithelial cells, deletion of E-cadherin resulted in the mis-localization of SCRIB [320]. The re-expression of E-cadherin in *cadherin*-null cells lead to the re-localization of SCRIB and DLG1 to the basolateral membrane [316, 320]. In mammalian cells, the effects of E-cadherin silencing became evident on cell polarity when cell-cell junctions are disrupted by calcium-depletion. In this case cells fails to reestablished a normal apico-basal polarity [292]. Studies in mice epidermis exhibited the role of AJ in regulating the proper localization of polarity protein. The conditional KO of  $\alpha$ -catenin resulted the mis-localization of aPKC, LGN and PAR3 [321]. Another study demonstrated that conditional *E-cadherin* KO in luminal cells of the prostate epithelium trigger the mis-localization of both apical (PAR3 and aPKC) and basolateral polarity proteins (DLG1 and SCRIB), resulting the loss of polarity of prostate luminal cells [315].

In *Drosophila*, mutation in *armadillo* lead the displacement of Dlg in the epithelial cells [322]. In general, AJ might be implicated directly in to the polarization of epithelium through modulating the subcellular localization of members of the apico-basal polarity complexes. In addition, several studies shows that loss of AJ affect also TJ formation, which is involved also in the recruitment of polarity proteins to cell-cell junction in simple epithelia [323].

Moreover, another level of complexity is added to the interrelationship function of AJ and polarity factors by the fact that polarity factors can feedback on AJ function [324](explained in page 125).

#### I.D.b.1.5 Cell migration

Although cadherins are often thought to mediate stable cell interactions, they also have dynamic roles in mediating cell rearrangements and cell migration. Indeed, association of cadherin with the actin cytoskeleton allow rearrangement at cell-cell borders and a



synchronized cell movement within an epithelium. This process is called collective cell migration (CCM). CCM play a major role in physiological process in embryonic development, tissue repair as well as in pathological processes including tumor invasion and metastasis [325].

Cadherins were suspected to play a role in CCM by maintaining cell-cell junction. This well-characterized form of cadherin-dependent morphogenetic movement, occurs in the *Drosophila* egg chamber, known as border cell migration. In this process a small group of cells derived from the follicular epithelium migrate through the nurse cells within the egg chamber to the anterior border of the oocyte. *DE*-cadherin was found necessary in both the border cells and in their neighboring nurse cells, for the border cell cluster to migrate [326]. Moreover, Cai et al., were able to uncover multiple critical functions of E-cadherin-mediated adhesion in different subcellular location within the border cell migration system. They demonstrate that *DE*-cadherin functions in a feedback loop with Rac that act downstream of receptor tyrosine kinase (RTK) signaling guideline in order to preserve a forward-directed protrusion and develop a stable front. Also, they show that the leading cell communicates direction to side and back cells through the border cell/border cell adhesion that transmits directional information within the group. The expression of *DE*-cadherin is important to hold the cluster together guaranteeing collective behavior [255].

During tissue turnover in the adult tissue many circumstances occur where cell-cell interactions must be dynamically remodeled. This is observed in the gut epithelium that exhibits continuous and rapid turnover [327]. Throughout the life cycle of gut epithelial cells, cells move gradually and consistently up the crypt-villus axis where they undergo apoptosis and are shed at the tips of the villi. Cells must maintain the intestinal epithelial barrier even with constant rearrangement during this migration. This indicated that maintenance of stable AJ is important for epithelial barrier function during cell migration and cell extrusion in order to avoid entry points to pathogens that could disrupt homeostasis. The consistent patterning of migratory cells was disrupted leading to disturbed epithelial barrier by the expression of a dominant-negative cadherin [328]. Unexpectedly, overexpression of E-cadherin delayed also the rate of epithelial cell migration [329].

Plutoni *et al* was able to demonstrate the role of P-cadherin in mediating CCM, [330]. The study used a 2D system to analyze P-cadherin's role in cell migration in response to the sudden removal of boundaries. This *in vitro* system simulates tumor cells collective cell movement

proceeding as 2D monolayers migrating along tissue clefts [331]. They used myoblast cells lines that are of mesenchymal origin, which express only N- and M-cadherins, and form and maintain cell–cell contacts. These cells in contrast to other epithelial cells (MCF10A or MDCK) do not endogenously display collective cell movement. Interestingly, overexpression of P-cadherin in myoblast cells, induces a very effective collective cell movement phenotype with typical features of a collective migration model. Principally, this study illustrated that during collective cell movement, P-cadherin specifically activates Cdc42 through the guanine nucleotide exchange factor (GEF)  $\beta$ PIX. Moreover, they showed that the partnership of P-cadherin/ $\beta$ -PIX/Cdc42 is crucial for P-cadherin–mediated collective cell movement. Collective cell movement was not observed upon E- or R-cadherin expression in this mesenchymal cell model, suggesting the specificity of P-cadherin to ensure this function. Accordingly, P-cadherin is involved in collective cell movement in epithelial cells as well as in mesenchymal cells. Interestingly, P-cadherin expression is found in invasive carcinoma and sarcoma, plus is correlated with poor prognosis [332], which can explain P-cadherin role in invasive cancer. Other AJ components were also involved in cell migration. For instance, a study has revealed that p120-catenin plays a role in mediating collective invasion, since the deletion of p120 in squamous cell carcinoma cells inhibited invasion in collagen gels and Matrigel [333]. Another study has demonstrated the importance of cadherin and  $\alpha$ -catenin in activating myosin-II–mediated contractility at large distances from the wound edge [334]. These different examples suggest that, cadherins with the help of catenins actively participate in morphogenetic cell-upon-cell movements in adult life and in the embryo.

#### I.D.b.1.6 Cell proliferation

E-cadherin plays an vital role in suppression of motility and in contact inhibition of proliferation (CIP) upon establishment of cell-cell contact, through the regulation of growth inhibitory signaling pathways including the growth factor receptor tyrosine kinase signaling and Hippo pathway [335, 336] (signaling explained further in page 90).

I will present some examples exhibiting the involvement of AJ components in mediating cell proliferation.

It was observed, a long time ago, that formation of a confluent cell monolayer leads to cell cycle withdrawal [337], which is now known as **CIP**. Different studies have elucidated the function of E-cadherin in mediating cell proliferation. Overexpression of E-cadherin in cancer epithelial cells lacking E-cadherin or the inhibition of E-cadherin ligation with neutralizing antibodies, exhibited that cadherin-mediated adhesion plays a fundamental role in implementing contact inhibition of cell proliferation [338]. The ability of E-cadherin to prevent proliferation includes upregulation of the cyclin-dependent kinase inhibitor p27, which binds to cyclin E–Cdk2 complex to reduce its activity [338, 339].

Since E-cadherin was shown to also regulate cell morphology and the re-establishment of apico-basal polarity, it was not clear whether E-cadherin indirectly or directly mediated negative regulation of cell proliferation. Therefore, a study carried out in Gumbiner's lab addressed this question and demonstrated that the ligation of E-cadherin by E-cadherin-coated beads in isolated epithelial cells decreased the occurrence of cells to enter S phase [340]. Inhibition of proliferation mediated by E-cadherin is based on E-cadherin bound  $\beta$ -catenin [340, 341]. These studies demonstrated that E-cadherin ligation was sufficient to induce proliferation inhibitory signaling.

Conditional Knockout of  $\alpha$ -catenin in the skin induced proliferation and developed squamous cell carcinoma through mediating Hippo pathway [304, 305]. In fact,  $\alpha$ -catenin overexpression restricted cell proliferation in myeloid leukemia cell line lacking  $\alpha$ -catenin expression HL-60 cells [342] and in lung carcinoma cells lacking endogenous  $\alpha$ -catenin expression [343]. Overexpression of  $\alpha$ -catenin in glioma cells via inhibiting  $\beta$ -Catenin nuclear translocation and consequently inhibiting the expression of downstream genes *c-Myc* and *CCND1 (Cyclin D)* [344].

Noticeably, loss of p120 in several epithelia is associated with cell hyperproliferation and tumor development. Loss of p120 in the salivary gland induces dysplasia indistinguishable from high-grade intraepithelial neoplasia whereas p120 ablation in the epidermis induces hyperproliferation and inflammation [313, 345].

In general, cadherins and catenins have vital roles in regulating negatively cell proliferation.

#### I.D.b.1.7 [Cell survival](#)

Several studies have shown that loss of AJ in various epithelia has been associated with increased cell death in different animal models and *in vitro* studies. These studies suggest that

intercellular junctions, like cell-extracellular matrix (ECM) contacts, induce signals necessary for cell survival. These works show that both establishment of cell-ECM and cell-cell contacts participate in the establishment of survival signals. Cell death mechanisms following loss of cell adhesion to ECM (a phenomenon known as anoikis) have been studied and largely described (for review [346, 347]). However, the mechanisms at the origin of the involvement of AJ in the control of cell survival have been much less studied. I will summarize here the different example of the literature that assigned a pro-survival function to AJ.

The conditional ablation of *E-cadherin* in mice lead to loss of adhesion and increase apoptosis in diverse tissues including intestine [348], lactating mammary gland [349] and ovaries epithelia [350]. The importance of E-cadherin in maintain mammary epithelium cell integrity and cell survival was observed also in the goat [351].

When both P- and E-cadherin KO are suppressed in skin, different types of cell-cell junctions are lost including, AJ but also TJ and desmosome. Loss of cell-cell junctions correlates with defect in tissue integrity and increase apoptosis. Apoptotic cells are mostly supra-basal and therefore not anymore in contact with the ECM, suggesting that cell-ECM contact may protect the epidermal cells from cell-death and only cells that relies on cell-cell contact initiate a cell-death program [291].

E-cadherin is not the only cadherin showing importance in cell survival, even the deletion of VE-Cadherin in endothelial cell induced increase in apoptosis (due to disruption of the survival pathway of VEGFR-2) in mice [19, 352, 353]. Another early study demonstrated the function N-cadherin in activating fibroblast growth factor (FGF) receptor in order to prevent a rise in intracellular free calcium consequently inhibiting apoptosis in ovarian cells [354].

Ablation of  $\alpha$ -catenins in epithelial cells has been also accompanied with an augmented sensitivity to apoptosis. Analysis of conditional KO of  $\alpha$ -catenin in epidermis demonstrate that the cells coming from conditional  *$\alpha$ -catenin* knockout skin degenerated and was replaced by  *$\alpha$ -catenin* positive cells, suggesting that  *$\alpha$ -catenin* KO cells are more sensitive to apoptosis in comparison to their counterpart WT cells [304]. Participation of  $\alpha$ -catenin in the control of apoptosis has been established in  *$\alpha$ -catenin* KO epidermis [355]. Livshits et al. determine that KO of  *$\alpha$ -catenin* epidermis in murine epidermis is accompanied with a significant apoptosis of keratinocytes situated in the supra-basal compartment of the epidermis, that do not interact

with the ECM [355]. These data confirmed the results obtained in E/P-cadherin double KO animals (see above) and support the theory that cell-ECM contact provide additional survival signals in absence of formed AJs. Furthermore, the expression of  $\alpha$ -catenin in  $\alpha$ -catenin-deficient colon carcinoma cells reconstruct AJ in these cells and reduced their sensitivity to apoptosis induced by sphingosine, an endogenous inducer of apoptosis [356].

Alongside  $\alpha$ -catenin, the capability of  $\beta$ -catenin to control WNT-signaling has been also determined to be needed to maintain survival of mammary epithelial cells [357]. Transgenic mice expressing in mammary epithelial cells a mutant form of  $\beta$ -catenin lacking the C-terminal regulatory domain, which is enabled to active the transcription of WNT-target genes, mice induced apoptosis of mammary stem cells impairing the development of the mammary gland in these animals [357].

These results display the importance of cadherins in maintaining cell integrity and regulating cell survival by inhibiting apoptosis. I will elucidate some of the mechanism by which AJ mediate cell survival.

AJs are involved in the regulation of phosphoinositide 3-kinase (PI3K) intracellular signaling pathway. PI3Ks are heterodimers consisting of a catalytic subunit (p110) in complex with a regulatory subunit (p85), that catalyze the conversion of phosphatidylinositol (4,5)-bisphosphate (PI (4,5) P<sub>2</sub>) into phosphatidylinositol (3,4,5)-trisphosphate (PI (3,4,5) P<sub>3</sub>). PI3K-mediated activation of protein kinase B or Akt is followed by a down-regulation of the growth-promoting MEK/ERK signaling, causing cell cycle arrest and differentiation in keratinocytes [358], epithelial cells, and carcinoma cells. Several studies described that E-cadherin engagement result in PI3K/Akt recruitment and activation, this activation is required for E-cadherin and  $\beta$ -catenin association to actin cytoskeleton [226, 359, 360].

Another study by Carmeliet et al has showed that VE-cadherin/ $\beta$ -catenin is needed to induce survival signals in endothelial cells, since truncated or absence of VE-cadherin induced apoptosis [352]. VE-cadherin mediates survival signaling through the formation of a complex consisting of  $\beta$ -catenin, PI3-kinase, and VEGFR-2. VE-cadherin promotes and mediates VEGF-A activation of AKT and Bcl-2 (both mediate anti-apoptotic signaling) and endothelial survival [352].

VE-Cadherin regulate cell survival signaling through mediating RTK, Tie2, localization within

the endothelial cell. Tie2 is a RTK which is required for vascular formation [361]. A study by Fukuhara et al. demonstrated that Tie2 can induce two opposing signaling either survival and cell integrity or proliferation depending on its localization in endothelial cells. Tie2 is activated by Angiopoietin-1 (Ang1) which results in cell survival signaling and cell integrity through activation of PI3K and consequently AKT when is recruited to cell-cell junction. Interestingly, when cell-cell junction is disrupted by VEGF, Tie2 is found at the ECM adhesion where it induced ERK activation that leads to migration and proliferation [362].

AJ allows the cells to adapt to external mechanical forces including compaction, tension, stretching and shear stress, which is necessary for cell survival. Cells response to these forces control cell survival, and the disturbance of this process results in cell death. This kind of external forces is detected by cadherins. In response to force, E-cadherin promotes a signaling cascade that leads to elevated cell stiffening through reinforcing actin cytoskeleton. A study by Frank et al., has demonstrated that p21-activated kinase 2 (PAK2), the serine/threonine kinase, is recruited at AJ [363] where it promotes cytoskeletal rearrangements that are essential for cells to increase contractility [363]. A more recent study have shown that PAK2 is able to sense the force that the cell is exposed to and protects cells under force from cell death [364]. Therefore, PAK2 is able to link force-induced mechano-transduction to cell survival [364].

In conclusion, AJ components play a role in cell survival through regulation of different signaling pathways including regulation of RTK localization and signaling

#### *1.D.b.2 Adherens junction role in human disease*

Dysregulation of AJs or defects in the associated proteins leads to various human disorders which are induced by somatic or germline mutations, epigenetic silencing, and dysregulation of protein processing. Here I will give some examples of non-cancer and cancer diseases caused by a non-functional AJ.

##### *1.D.b.2.1 Adherens junction in non-cancer diseases*

Many studies have shown the importance of AJs in maintaining tissue integrity and their disruption have been associated with some genetic diseases (For review see[365]). Defect in inherited diseases is limited if we compare to the implication of AJ in tumor formation. This can be explained either by a compensatory mechanism that exist in some tissues expressing

various cadherins or by the fact that mutation in AJ component caused an early lethality as it is observed in animal models.

A loss-of-function mutations in *CDH3*, which encodes P-cadherin, result in two allelic autosomal recessive disorders known as hypotrichosis with juvenile macular dystrophy (HJMD) and ectodermal dysplasia, ectrodactyly, and macular dystrophy (EEM) syndromes. Both syndromes display sparse hair showing signs of progressive macular dystrophy. EEM syndrome is characterized in addition by ectodermal and limb defects. Several studies have established that P-cadherin plays an important role in regulating cell signaling, malignant transformation, and other major intercellular processes together with its involvement in cell-cell adhesion. The strong P-cadherin gene expression detected in the hair follicle matrix, the apical ridge of limb buds, and the retinal pigment epithelium (RPE) associate with the affected sites in humans carrying mutations in this gene [199, 366].

Another effects of cadherin mutation, mirrored closely the characteristics of inflammatory bowel disease (IBD) pathology. Interestingly, genome-wide association studies have identified multiple susceptibility loci for both Crohn's disease (CD) and ulcerative colitis (UC), which include members of the cadherin families, *CDH1* and *CDH3*, that encode E-cadherin and P-cadherin, respectively [367]. Elding and colleagues have confirmed this causal variant link to *CDH1* and *CDH3* [368], indicating that alterations in these genes may be important for the development of inflammatory bowel disease (IBD). Moreover several studies illustrated that other catenin proteins associated with AJ such as  $\alpha$ -catenin, p120-catenin and  $\beta$ -catenin were found to be decreased around regions of ulceration in inflammatory bowel disease (IBD) mucosa compared to control samples [198]. In agreement with these results, a chimeric mouse that expressed a dominant-negative cadherin in intestinal epithelial stem cells, by Hermiston and Gordon in 1995, has shown that disruption of AJ in these cells triggers a phenotype resembling human IBD, confirming the involvement of dysregulation of cadherins in this pathology.

#### I.D.b.2.2 Adherens junction in cancers

Numerous studies in mice or in culture cells demonstrated that alteration of AJ component function is associated with tumor formation in different epithelial tissues [200, 336, 369]. In agreement with this, mutations or altered expression of AJ components has been observed in various human epithelial cancer. I present here an overview of the main observations both *in*

*vitro* and *in vivo* demonstrating the involvement of E-cadherin and some catenins ( $\alpha$ -catenin,  $\beta$ -catenin and p120) in tumor progression.

Abnormal E-cadherin expression or loss of its normal localization at cell–cell contacts has been often observed in advanced cancers. Moreover, the loss of E-cadherin is also a key feature in epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells lose their epithelial phenotype and acquire a more mesenchymal phenotype that favors cell migration and invasion. This gives additional weight to the overall concept that physiological processes controlling cadherin expression throughout development may be pathogenetically active to disturb E-cadherin expression in carcinomas. The loss of E-cadherin expression can be due to different molecular mechanisms. Loss of E-cadherin expression is frequently associated in different types of human carcinoma with genetic alteration (loss of heterozygosity (LOH)) in the region of the *CDH1* gene [230].

During tumor invasion and EMT E-cadherin expression is reduced by transcriptional silencing, notably through a variety of transcription factors that target the E-cadherin promoter [279]. These include transcriptional repressors that are overexpressed in advanced carcinomas [280, 370].

Many studies both in cellular and animal models demonstrate that suppression of AJ components expression favors tumor progression from a non-invasive tumor to an invasive and metastatic tumor. One example was the demonstration that the expression of a dominant-negative E-cadherin mutant in a mouse pancreatic  $\beta$ -cell tumor model accelerated the rate at which adenomas converted into carcinomas [371]. Conversely, over-expression of E-cadherin in those cells delayed tumor progression. Another example by Derksen et al. exhibited that conditional ablation of E-cadherin in p53-deficient mouse mammary epithelium stimulated tumor initiation and progression to invasion and metastasis. However, it is important to emphasize that in mouse model's loss of E-cadherin alone is not sufficient to induce tumor formation [372]. This demonstrate the need of a supplementary oncogene activation in order for E-cadherin to promote tumor formation.

Beside E-cadherin, catenin and in particular  $\beta$ -catenin, by its ability to regulate the WNT signaling pathway, has been associated with tumorigenesis in several cancer types including colon carcinomas.  $\beta$ -catenin is known as a central transcriptional co-activator of the WNT signaling pathway [373]. This pathway determines cell fate during embryogenesis and tissue renewal in the adult. Activation of WNT signaling pathway inhibits the degradation of  $\beta$ -



catenin and permits its translocation to the nucleus, where it interacts with TCF/LEF family of DNA-binding proteins and induce the activation of transcription factors leading to cellular proliferation. Without WNT signaling,  $\beta$ -catenin binds adenomatous polyposis coli (APC) that associate  $\beta$ -catenin to a destruction complex resulting in its degradation. Mutations of *CTNNB1* gene are commonly found in a variety of cancers such as in primary hepatocellular carcinoma, colorectal cancer, ovarian carcinoma, breast cancer, lung cancer and glioblastoma. Most of these mutations are grouped on a tiny area of the N-terminal segment of  $\beta$ -catenin that contains several Ser/Thr phosphorylation sites. Phosphorylation in this motif, called the  $\beta$ -TrCP binding motif, regulates the destabilization of the protein. Mutations in this motif prevents the ubiquitinylation and degradation of  $\beta$ -catenin since it blocks  $\beta$ -catenin phosphorylation and therefore lead to increased stability of the  $\beta$ -catenin. This will lead to the translocation of  $\beta$ -catenin to the nucleus without any external stimulation and to constantly drive transcription of its target genes [374].

Involvement of  $\alpha$ -catenin in cancer has been less studied in comparison to E-cadherin and  $\beta$ -catenin, but it has been documented that  $\alpha$ -catenin expression is downregulated in numerous cancers. The loss of  $\alpha$ -catenin in tumor cells was primarily reported in a human lung cancer cell line in the early 1990s. This cell line displayed impaired cell-cell adhesion even though E-cadherin expression is abundant, this is likely to be a consequence of homozygous deletion of the  $\alpha$ -E-catenin gene, *CTNNA1* [375]. Re-establishing  $\alpha$ -catenin expression induced cell-cell adhesion and delayed cell proliferation. Successively, many other cancer cell lines or primary tumor tissues were found to lack or to downregulate  $\alpha$ -catenin, including myeloid leukemia, breast cancer, colon cancer, lung cancer, and prostate cancer. In these cancers, alteration of  $\alpha$ -E-catenin correlates with high tumor grade, metastasis and poor survival [376]. Additionally, tissue-specific deletion of  $\alpha$ -E-catenin in the epidermis developed squamous cell carcinoma in mice [304, 305]. In these mice, ablation of  $\alpha$ -catenin in keratinocyte not only altered AJ, but also lead to loss of epidermal cell polarity and hyper-proliferation [377], demonstrating that E-cadherin- $\alpha$ -E-Catenin functional adhesion participate in contact inhibition of growth.

Multiple findings suggest that  $\alpha$ -E-catenin has a tumor-suppressing role. For instance, overexpression of  $\alpha$ -E-catenin inhibited EGF-promoted glioblastoma proliferation, migration and invasion, through impairing  $\beta$ -catenin nuclear translocation [344]. Restoration of  $\alpha$ -E-catenin in a myeloid leukemia cell line lacking  $\alpha$ -E-catenin expression, resulted in reduced

proliferation and apoptotic cell death [342]. Through loss-of-function and gain-of-function analyses, a study identified  $\alpha$ -E-catenin as a tumor suppressor in basal-like breast cancer [378].

Finally, the p120-catenin has received increasing attention in the context of cancer progression. p120-catenin is frequently altered and/or lost in tumors of the colon, bladder, stomach, breast, prostate, lung, and pancreas [379]. The catenin p120 gene, *CTNND1*, is rarely found mutated, suggesting that other mechanisms such as epigenetic silencing by DNA methylation and protein processing dysregulation may downregulate p120-catenin in tumors. The role of p120-catenin in cancer progression is complex. Indeed, p120-catenin has been demonstrated to function as either a tumor suppressor or a metastasis promoter depending on the presence of E-cadherin. E-cadherin loss leads to degradation of  $\alpha$  and  $\beta$ -catenins, but not p120-catenin which remains stranded in the cytoplasm. The resulting accumulation of p120-catenin in the cytoplasm induces cell migration by activating Rac1 and Cdc42 Rho GTPases [380]. Suggesting that p120-catenin may function as a metastasis promoter. In other cases, p120-catenin loss may be the initial event promoting eventually to inactivation of the cadherin complex. As I mentioned above, p120-catenin is required to stabilize plasma membrane E-cadherin. Furthermore, p120-catenin is capable of associating with and stabilizing mesenchymal cadherins that do not establish stable AJs such as N-cadherin [381]. When cancer cells undergo an EMT, N-cadherin is expressed, and E-cadherin is repressed, a phenomenon known as cadherin switch. It has been shown that in these EMT conditions p120-catenin is required to stabilize N-cadherin and to trigger Rac1 activation, thereby inducing an increase in motility and invasion as well as the anchorage-independent growth of these breast cancer cell line [382, 383].

These data show that catenins acts as a regulator that endorses either epithelial integrity when it is bound to E-cadherin, or motility when it is associated with mesenchymal cadherins.

#### I.D.c Regulation of intracellular signaling pathways by AJ

As we discuss above the role of AJ and AJ components goes far behind a unique role in cell-cell adhesion, since AJ modulate various cellular functions including proliferation, migration, survival and polarity. Indeed, AJ are required to interpret and integrate signals from the surrounding microenvironment in order to modulate these different cellular functions.

Several molecular mechanisms have been proposed to explain the cellular responses controlled by AJ and involve AJ in the regulation of various important signaling pathway including: WNT, Hippo, NFκB, and receptor Tyrosine kinase (RTK), PI3K, and Rho proteins. I will review here several of these most relevant regulatory mechanisms including transcriptional regulation of catenins, YAP/TAZ and NF-κB by AJ, regulation of cell surface receptors such as EGFR, FGFR, VEGFR and TGF-β receptor.

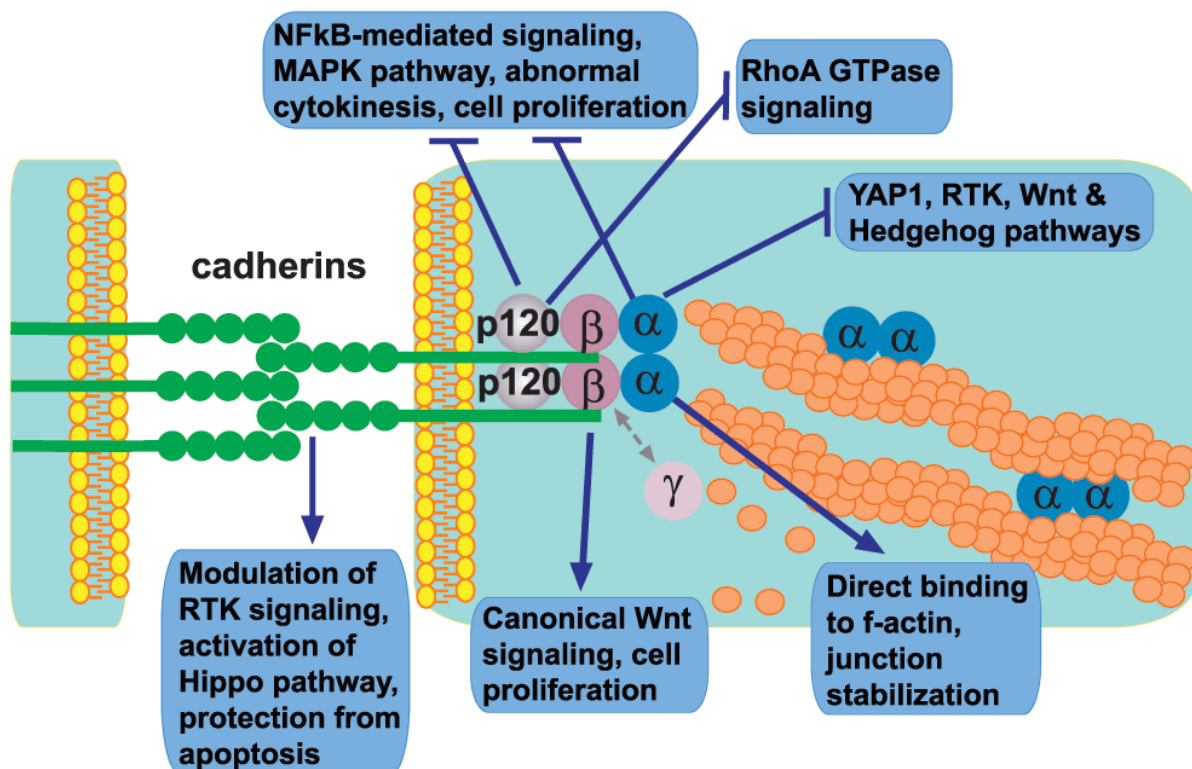


Figure 22: Scheme displaying the regulation of major intracellular signaling pathways by Cadherin-catenin complexes. [6]

### I.D.c.1.1 Role of AJ in “transcriptional regulation”

#### I.D.c.1.1.1 Catenins

As mentioned above (page 88), β-Catenin plays fundamental roles in both cell–cell adhesion and WNT-mediated transcriptional activation. Since β-Catenin has this dual role in cells, it is intriguing to understand how the same molecule can switch from AJ or DNA-binding factor. Several studies have demonstrated the presence of a tight coordination between signaling and adhesion through the competition for the same pool of β-catenin. This was confirmed by the similarity in the binding mechanism between β-catenin–cadherin and β-catenin–TCF

complexes [384]. A study by Gottardi *et al.*, show that E-cadherin suppress WNT/ $\beta$ -catenin pathway by sequestering  $\beta$ -catenin at AJ [385]. Indeed, overexpression of cadherin suppressed the cellular transformation independently of cell adhesion, through sequestration of  $\beta$ -catenin by the cadherin cytoplasmic domain. Another way E-cadherin was found to suppress WNT/ $\beta$ -catenin pathway is by increasing the interaction of  $\beta$ -catenin with caveolin-1 [386]. Caveolin-1 controls cell proliferation and viability by inhibiting expression of survivin via a transcriptional mechanism involving the  $\beta$ -catenin -TCF/LEF pathway in several cellular settings. Caveolin-1 targets  $\beta$ -catenin to a Triton X-100-insoluble protein complex apparently at the plasma membrane, accordingly inhibiting TCF/LEF-dependent transcription. In fact, E-cadherin is found in the soluble and insoluble fraction in complex with  $\alpha$  and  $\beta$ -catenins [387]. Caveolin-1/ $\beta$ -catenin interaction enhances  $\beta$ -catenin turnover leading to its reduced nuclear translocation and consequently transcriptional activity [386].

In addition, a study by Maher *et al.*, demonstrated that cadherin-based cell adhesion enhanced the phosphorylation and destruction of  $\beta$ -catenin by the  $\beta$ -catenin phospho-destruction complex composed of N-terminal phosphorylated  $\beta$ -catenin and Axin, APC2, GSK-3 $\beta$  located in proximity of AJ. This mechanism increased  $\beta$ -catenin cytosolic turn over in a density dependent manner [388]. However, although these findings clearly demonstrate that cadherin levels can affect  $\beta$ -catenin signaling, the notion that cadherin loss is enough to promote  $\beta$ -catenin signaling by releasing it from the cadherin-bound pool is mostly incorrect. Since cancer cell lines that lack E-cadherin do not manifest a corresponding up-regulation of  $\beta$ -catenin signaling [389, 390]. Moreover, in a mouse model for pancreatic cancer depletion of E-cadherin described no role for  $\beta$ -catenin signaling in the progression of these tumors [391].

$\alpha$ -catenin directly binds to  $\beta$ -catenin which makes it tempting to hypothesize that  $\alpha$ -catenin controls  $\beta$ -catenin signaling. Several experiments reveal that  $\alpha$ -catenin can suppress WNT signaling by different molecular processes: 1) by stimulating  $\beta$ -catenin degradation [392]; 2) by obstructing nuclear translocation of  $\beta$ -catenin [344]; 3) by inhibiting formation of the  $\beta$ -catenin-TCF-DNA complex [393]; 4) by recruiting APC to the  $\beta$ -catenin-TCF complex [392]. Further investigation is needed to see if the tumor-suppressing effect of  $\alpha$ -E-catenin *in vivo* is mediated by inhibition of  $\beta$ -catenin signaling [376, 392, 393]. However, KO of  $\alpha$ -E-catenin in

the epidermis is not sufficient to increase  $\beta$ -catenin nuclear signaling [377].

Similarly,  $\gamma$ -catenin (Plakoglobin) can also interact with TCF family DNA binding proteins and impact gene expression, although  $\gamma$ -catenin appears to bind a distinct region on TCF that may differentially impact target gene expression involved in cell-cycle control, apoptosis, cell proliferation, and invasion [394]. Plakoglobin is a very weak transactivator when bound to DNA, which is probably caused by the divergence of their C-terminal sequences [394]. Plakoglobin can activate TCF-dependent transcription *in vitro* in the absence of  $\beta$ -catenin [395]. Unlike  $\beta$ -catenin that has well-documented oncogenic potential through its involvement in the WNT signaling pathway,  $\gamma$ -catenin generally acts as a tumor/metastasis suppressor [396]. Another studies have shown that plakoglobin binds to the tumor suppressor p53 in the nucleus, where p53 and  $\gamma$ -catenin association decreased significantly cell migration and invasion in carcinoma cell lines through most probably activating targeted genes [397]. Indeed, Aktary et al. demonstrated that the transcriptional activity of p53 increased in the presence of  $\gamma$ -catenin [398].

p120-catenin can directly influence gene expression as well as AJ stability similarly to its close cousin  $\beta$ -catenin. p120-catenin interacts directly with the transcription inhibitor Kaiso. Kaiso belongs to a large family of BTB/ZF proteins, which are important in development and cancer. p120-catenin binding to Kaiso weakens its association with DNA that results in de-repression of Kaiso target genes which have been linked to WNT-dependent developmental processes [399]. This suggests that p120 can indirectly modulate WNT signaling through localizing to the nucleus and releasing the repression of Kaiso by binding to it [400].

All in all,  $\beta$ -catenin but also the other catenins can regulate gene transcription. Catenins are able to act as transcription factors and/or through binding to transcription factors in order to promote or repress gene expression in order to influence cell behavior.

### I.D.c.1.1.2 AJ control the YAP/TAZ in Hippo signaling pathway

The Hippo signaling pathway is well-known for controlling organ size through the regulation of cell growth and proliferation in *Drosophila* and vertebrates. The heart of the Hippo pathway consists of a core kinase cascade that consists of the Ser/Thr kinase Mst 1 and Mst2 (corresponding to Hippo in flies), and the Large tumor suppressor 1 and 2 (Lats 1/2) (corresponding to Warts in flies) together with the scaffolding proteins Salvador homologue 1 (Sav1) and MOB kinase activator 1 (MOB1). Lats1 and Lats2 phosphorylates the transcriptional co-activators YES-associated protein 1 (YAP1, York1 in flies) and the transcriptional co-activator with PDZ-binding motif TAZ, causing it to be excluded from the nucleus and retained in the cytoplasm. These transcription co-activators will translocate to the nucleus to induce gene expression that will activate cell proliferation and prevent apoptosis [401]. Elevated YAP/TAZ expression or nuclear enrichment has been observed in many types of cancers, including liver, breast, lung, colon, ovary and others [402].

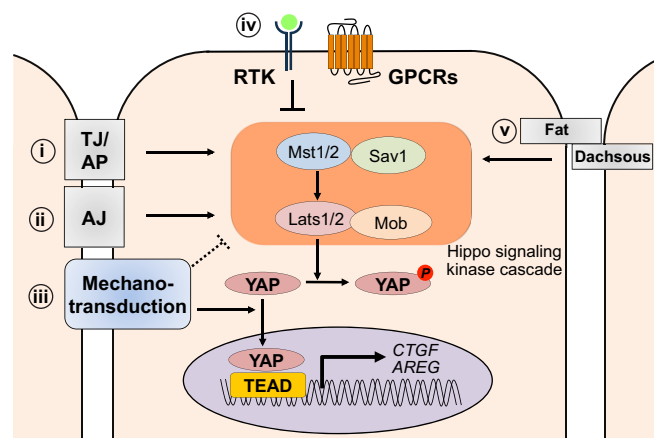


Figure 23: Hippo signaling pathway regulated by various mechanisms . [18]

Several mechanisms modulate Hippo-YAP signaling by sensing the spatial and physical organization of cells including epithelial polarity and tight junction complexes, the FAT-Dachsous morphogen pathway, cell shape, or mechano-transduction (Figure 23).

Beside these mechanisms, AJ has been found to participate to the control of the Hippo signaling pathway and inhibit cell growth [18]. Kim et al demonstrated that E-cadherin controlled the sub-localization of YAP in response to cell density [341]. E-cadherin homophilic ligation was enough to induce YAP subcellular localization. E-cadherin inhibition of cell

proliferation is dependent on  $\alpha$ -catenin and  $\beta$ -catenin. This was confirmed by the observation of YAP redistribution from the nucleus to the cytoplasm in dense cells overexpressing E-cadherin in contrast to the wild-type cells lacking E-cadherin or  $\alpha$ -catenin and  $\beta$ -catenin [341].

$\alpha$ -E-catenin can regulate YAP in a cadherin-independent manner. A study by Silvis et al. has demonstrated that  $\alpha$ -E-catenin mediates YAP cellular sub-localization, where  $\alpha$ -E-catenin triggers YAP cytoplasmic retention through direct interaction [305]. Schlegelmilch et al proposed that  $\alpha$ -E-catenin interacts with YAP and promotes its cytoplasmic localization by preventing its de-phosphorylation [403].  $\alpha$ -E-catenin form a multimolecular complex with 14-3-3 and phosphorylated-YAP1, which prevents YAP1 de-phosphorylation by PP2A and YAP1 subsequent nuclear localization and transcriptional activity [403].

The cadherin-mediated adhesion and cell polarity regulate Hippo pathway through different mechanism during the early blastocyst by mediating angiomin (AMOT). At the pre-implantation stage compaction of mouse embryo lead to the formation of two different lineages, the trophectoderm, the first polarized epithelium that generate the extra-embryonic tissues and the placenta, and the inner cell mass, non-polarized cells that develop to embryo and is the origin of embryonic stem cells. Both lineages have cadherin-mediated adhesion. In the inner non-polarized cells AMOT localize to AJ and activate the Hippo pathway, whereas the outer polarized cells (the trophectoderm) AMOT is sequestered by cell polarity from basolateral domain which leads to suppressing Hippo pathway [404].

This regulatory mechanism of Hippo pathway by AJ explains, at least in part, the role of AJ formation in the process of contact inhibition of proliferation observed in epithelial tissues.

#### I.D.c.1.1.3 NF- $\kappa$ B activity

Hyperactivation of NF- $\kappa$ B signaling is frequently found in cancer [405]. Genes controlled by the NF- $\kappa$ B pathway are involved in numerous hallmarks of cancer, including survival, cell death, proliferation, invasion, angiogenesis and inflammation. The five subunits of the NF- $\kappa$ B transcription factor family, include RelA (p65), RelB, cRel, NF- $\kappa$ B1 (p50 and its precursor p105) and NF- $\kappa$ B2 (p52 and its precursor p100), assemble homodimers or heterodimers. The classical form is constituted of RelA (p65) and p50.

Formation of the E-cadherin/catenin complex have the ability to down-modulate NF- $\kappa$ B

activity. It was indicated that an overexpression of E-cadherin, in distinct cell lines, decreases NF- $\kappa$ B activation while loss of E-cadherin leads to an increased activity of this transcription factor [406]. Additional studies have proposed that presence of  $\alpha$ -catenin is required to prevent NF- $\kappa$ B activation. Mechanistically,  $\alpha$ -catenin binds to the I $\kappa$ B $\alpha$  protein, and stabilizes I $\kappa$ B $\alpha$  by preventing its ubiquitylation and its association with the proteasome. This stabilization, in turn, prevents nuclear localization of RelA and p50, leading to decreased expression of various target genes induced by a NF- $\kappa$ B dependent-mechanism such as TNF- $\alpha$ , IL-8 and RelB [304, 378].

In apparent contradiction with these data, p120-catenin deletion in various epithelia has been associated with a strong inflammatory response associated with cancer progression [306, 313, 407, 408]. This strong inflammation of epithelial tissue has been associated with an activation of the NF- $\kappa$ B pathways in the epidermis and the derived tumors, suggesting that p120-could directly regulate NF- $\kappa$ B in the skin [313, 407]. However, cultures of keratinocytes isolated from p120 KO mice do not display NF- $\kappa$ B activation, suggesting that this effect is indirect and due to the underlying microenvironment [313].

#### I.D.c.1.2 Regulation of cell surface receptors

Another way AJ regulate cytoplasmic signaling is by associating and modulating the signaling activity of different cell surface receptors. Such regulation has been exemplified with several classical cadherins expressed in different tissues. Here, I will report the molecular mechanisms that underlie this regulation.

##### I.D.c.1.2.1 EGFR

The epidermal growth factor receptor (EGFR) plays an important role in the regulation of epithelial cell growth and differentiation of many epithelia [409]. It is well established that deregulation of EGFR signaling is associated with tumorigenesis. Indeed, EGFR upregulation or mutations leading to its constitutive activation have been observed in numerous epithelial tumors [410], resulting in tumor overgrowth.

Therefore, one-way AJ may have a growth inhibitory function is link to their capacity to control EGFR signaling. Qian et al., initially shows that formation of E-cadherin-catenins complex can



inhibit cell responsiveness to EGF [411]. This study demonstrates that the mitogenic response associated with EGF stimulation declined when cells reach confluency. By contrast this inhibition is overcome when E-cadherin function is blocked by addition of inhibitory antibodies. In agreement with this study, Perrais *et al.* show that E-cadherin engagement obtained by microspheres coated with recombinant E-cadherin is sufficient to inhibit EGFR cell growth signaling, confirming the inhibitory function of AJ formation on EGFR. [340].

Various studies proposed that inhibition of EGFR signaling by cadherin required a molecular proximity of these molecules that are found co-localized at cell-cell contacts. Various molecular interactions have been proposed to participate to this association. Qian *et al.* suggests that the E-cadherin extracellular domain is required since this domain is needed to co-immunoprecipitate the two molecules when they are co-expressed in 293T cells [411]. The team of McClatchey demonstrate that EGFR and E-cadherin interaction requires two adaptor molecules: Merlin (an ezrin radixin and moesin (ERM) family of membrane/cytoskeleton linking proteins) and NHERF1 (Na<sup>+</sup>-H<sup>+</sup> exchanger regulatory factor 1 (also called EBP50) [412]. NHERF1 binds both the cytoplasmic domain of EGFR and Merlin. Merlin is known to localize with AJ and to interact with AJ components such as  $\alpha$ -catenin [413]. Moreover, Merlin is required to establish stable cadherin-mediated AJs between cells in dense culture [414] and to undergo contact-dependent inhibition of proliferation at confluence.

Therefore, the interaction of EGFR with AJ and Merlin/NHERF1 in confluent cell culture allows the association of EGFR with the actomyosin network present at cell-cell junction, which blocks the endocytosis of the ligand-bound EGFR [415]. Since EGFR internalization is needed for a full EGFR signaling response [416], the recruitment of EGFR to the E-cadherin/catenin complex inhibits EGFR internalization into the endocytic compartment and consequently its signaling.

A study by Pece e Gutkind demonstrated an inverse finding, where E-cadherin promotes an EGFR dependent signaling instead of inhibiting it. Using a non-tumorigenic keratinocyte cell line (HaCat), they show that E-cadherin-catenin complex induces a ligand-independent activation of EGF receptors (EGFRs), and the consequent activation of a biochemical route leading to the stimulation of MAPKs [417].

These controversial findings could be explained by the level of E-cadherin expressed at the cell surface. Low cell surface expression of E-cadherin would promote EGFR activity, whereas

high surface expression of E-cadherin and the AJs induce an important co-aggregation of EGFR together with E-cadherin, which alters the rate of activated EGFR endocytosis, resulting in a decreased responsiveness of the RTK.

#### I.D.c.1.2.2 FGFR

The fibroblast growth factor (FGF) ligands are involved in early embryo patterning and development. In the adult, FGFs are considered as key players in tumorigenesis. FGFs and their cognate receptors (FGFRs) are also implicated in epithelial to mesenchymal transitions (EMTs) [279]. FGFRs trigger numerous intracellular signaling pathways orchestrating key cellular events including cell migration.

A study by Bryant et al. has demonstrated that FGFR1 activation by its ligand FGF induces the co-internalization of FGFR1 and E-cadherin into early endosomes [418]. This is followed by nuclear translocation of FGFR1 resulting in the activation of mitogen-activated protein kinase (MAPK) pathway. In addition, they established that reciprocal presence of high-quantities of E-cadherin at the plasma membrane blocks FGFR1 endocytosis and signaling. Thus, E-cadherin is a negative regulator of FGFR1 by limiting its endocytosis but at the same time E-cadherin can be co-endocytosed with activated FGFR1, revealing a complex co-regulation between these two receptors.

It is worth mentioning that E-cadherin is not the only cadherin found to mediate FGFR signaling. Since N-cadherin and VE-cadherin also modulate FGFR signaling in endothelial cells. Although N- and VE-cadherin have a similar structure and bind the same intracellular partners, they seem to display different functions in the endothelium. Remarkably, both cadherins have opposite impact on FGFR signaling. N-cadherin expression in endothelial cells or in tumor cells lead to FGFR signaling activation and increased migratory signals [419, 420]. Whereas, VE-cadherin decreased the migratory effects of FGFR by forming a multiprotein complex with FGFR1 and the phosphatase Dep1[421].

Therefore, the modulatory function of cadherins on FGFR is dependent of the type of cadherin considered and the level of engagement of the cadherin.

#### I.D.c.1.2.3 VEGFR

VEGF is a major growth factor for endothelial cells which binds to two tyrosine kinase receptors, VEGFR-1 and -2. VEGFR activation by VEGF triggers a proliferative response important during formation of new vessels. However, VEGFR activity need to be strictly

controlled to avoid endothelial cells overgrowth. In this respect, Dejana group shows that association of VEGFR with VE-cadherin at cell-cell junctions in confluent culture play a crucial role to inhibit VEGFR pro-proliferative signaling [352]. This group also elucidated the mechanism by which VE-cadherin expression and clustering regulates the growth factor receptor VEGFR2 signaling. They demonstrated that the association of VE-cadherin with VEGFR-2 allows the co-clustering of the phosphatase DEP-1 which is located at cell-cell junction together with VE-cadherin and that DEP-1 dephosphorylates VEGFR-2 to limit its internalization and signaling. When VE-cadherin is absent or not engaged at cell-cell contacts, VEGFR2 is endocytosed more rapidly and remains in endosomal compartments for a longer time where the receptor signals [422]. The regulation of VEGFR2 signaling by VE-cadherin is probably required to control vascular stabilization when new vessel is formed.

#### I.D.c.1.2.4 TGF- $\beta$ receptor

TGF- $\beta$  belongs to the members of the TGF- $\beta$  superfamily that include activins, BMPs and GDFs. TGF- $\beta$  induce signaling via the heteromeric serine/threonine kinase transmembrane receptor complexes T $\beta$ RII/T $\beta$ RI. The activation of T $\beta$ RI results in the phosphorylation of receptor-associated members of the SMAD family, which will dissociate from the receptor to form a complex with the mediator Smad-4. This complex will translocate to the nucleus to regulate transcription which will lead to biological outputs depending on the context. For instance, TGF- $\beta$  family members have the ability to both promote and inhibit proliferation, induce stem cell renewal as well as stem cell differentiation, and serve to suppress as well as promote tumorigenic events [423].

TGF- $\beta$  receptor complex, is another receptor identified to be regulated by VE-cadherin. A study by Dejana's group reported that VE-cadherin expression and junctional clustering are required for optimal transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling in endothelial cells [424]. VE-cadherin knockdown inhibited Smad1/5 and Smad2/3 phosphorylation induced by TGF- $\beta$ , leading to a reduced expression of Smad target genes and preventing the inhibitory effect of TGF- $\beta$  on endothelial cells proliferation and migration.

#### I.D.c.1.2.5 Overview on receptor regulation by AJ

All in all, these studies elucidate the mechanisms by which cadherins control signaling pathways emanating from various cell surface receptors (summarized in Table 7). It is interesting to notice that this mechanism of regulation is not unique and depends of the

specific cadherin and RTK considered and the cellular context. RTK can associate with one cadherin only in a specific cellular context as it is the case for EGFR, whereas other receptors, such as FGFR1, can be regulated by different cadherins in different cell types leading to different signaling. This observation supports the hypothesis that it exists specific functional interactions between cadherin subtypes and receptor tyrosine kinase (RTK) members. However, depending on the cellular context, the same cadherin may regulate in a different manner different cell surface receptors. For example, VE-cadherin promotes or inhibits the cytoplasmic signaling respectively of TGF- $\beta$  receptor and VEGFR. To regulate cell surface receptors, AJ serve as molecular organizer by recruiting several adaptors such as actin binding protein (the ERM Merlin), scaffold molecule (NHERF1) and signaling regulator (the phosphatase DEP-1) indispensable to the regulation of the growth factor receptors. Finally, inhibition of RTK activity in confluent cells relies on two general rules: first, the physical association of the RTK with a cadherin; second, this association inhibits the endocytosis of the receptor in order to blocks its signaling capacity.

These findings offer an attractive mechanism whereby cell-cell adhesion molecules can affect cell fate decision upon cell-cell contact formation. These observations suggest that cadherin mediated cell surface receptor signaling is very complex and as to be studied in a context-dependent manner since no prediction of the outcome of this regulation can be proposed.

Table 7: Cadherin regulating growth factor receptor signaling

RTK	Cadherin	Cell	Cellular response	Interrelation	References
FGFR1	N-cadherin	mouse epiblast stem cells	Pluripotency maintenance	Stabilization of FGFR by N-cadherin at cell membrane	[425]
FGFRs/FGFR1		mouse cerebellar neurons ventral spinal cord	Neurite outgrowth	co-clustering, N-cadherin-dependent activation of FGFR	[426] [427]
FGFRs/FGFR1		zebrafish, xenopus	RGC extension	N-cadherin-dependent activation of FGFR	[428]

RTK	Cadherin	Cell	Cellular response	Interrelation	References
		RGC (Retina Ganglion Cells)	and guidance		
FGFRs		poliovirus middle T-antigen (PvVMT)-induced mouse mammary cancer cells	Increase in invasion and pulmonary metastasis	N-cadherin-dependent activation of FGFR	[429]
FGFR1		human breast cancer cells	Increased invasion and metastasis	co-clustering, N-cadherin –dependent-increase of FGFR membranous stability	[419]
FGFR		mouse breast cancer cells	Increase in tumor invasion, proliferation and metastasis	N-cadherin –dependent increase of FGFR 1-4 expression and phosphorylation N-cadherin-dependent increase of FGFR1 and 2 mRNA expression clustering in complex with NCAM	[420]
FGFR1	E-cadherin	Human breast adenocarcinoma	Blocks mitogen-activated protein kinase pathway	Overexpression of E-cadherin blocks both the co-endocytosis of E-cadherin and FGFR1	[418]
EGFR		Liver-derived epithelial cells (LDCs) from mice	Inhibit mitogenic signaling in response to cell contact.	Merlin immobilizes EGFR at the plasma membrane and stabilizes associated cell junctions	[415]

RTK	Cadherin	Cell	Cellular response	Interrelation	References
EGFR		keratinocyte cell line	Activation of MAPK pathway	E-cadherins stimulate the MAPK pathway through the ligand-independent activation of epidermal growth factor receptors	[417]
VEGFR2	VE-cadherin	human endothelial cells	Increases contact inhibition of cell growth, limitation of endothelial cell proliferation	E-cadherin binds to VEGFR2, preventing its endocytosis and promoting its inactivation at the cell surface	[422]
VEGFR		VEGF-expressing murine colon tumor cancer	Increase in tumor metastasis to lung and liver	VEGF uncouples VE-cadherin junctions creating endothelial gaps	[430]
VEGFR2, 3		human endothelial cells	Enhanced endothelial response to flow shear stress	co-clustering facilitates ligand-independent transactivation of VEGFR 2,3	[431]
FGFR		human endothelial cells	Preventing cell motility and apoptosis and	VE-cadherin associate with FGF receptor	[421]

RTK	Cadherin	Cell	Cellular response	Interrelation	References
			controlling permeability		

## I.E Apico-basal Polarity

### I.E.a Definition of polarity

Cell polarity is the asymmetrical organization within the cell that aim to control the localization and distribution of a variety of membrane proteins, organelles and cytoskeletal components. The ability of the cell to maintain and establish functionally distinct domains involves complex interactions between many protein complexes, which regulate different signaling pathways. Organization of molecules and organelles within a cell is fundamentally important to ensure cellular function in a tissue such as transmitting of signals across neurons and preserving a barrier within an epithelium. Therefore, cell polarity control a number of critical cellular processes, including cell migration, differentiation, proliferation, and morphogenesis in both unicellular and multicellular organisms [432]. Consequently, the deregulation of mechanisms regulating cell shape can lead to developmental disorders, tissue degeneration or cancer [433, 434].

Several different types of cell polarity exist to assure the proper embryo development and tissue homeostasis in the adult, including apico-basal, planar and front–rear, polarity [190, 435, 436].

The **apico-basal cell polarity (ABCP)** is a form of polarity specific to epithelial cells, which is characterized by a separating axis between the apical and basal domains within epithelial cell. The apical domain faces the outside of body or the internal lumen cavity, the lateral domain is linked to the adjacent cell and the basal domain is connected to extracellular matrix (ECM) in simple epithelia. Apico-basal polarity is essential for the asymmetrical positioning of proteins, lipids and organelles in epithelia, which is necessary for organization of various signaling pathways, cell–cell contact and cell communication in order to establish coherent epithelial tissue and to regulate tissue growth differentiation and apoptosis. The establishment and maintenance of defined specialized domains along the apico-basal axis of a cell is achieved via both antagonistic and cooperative relationships between evolutionary conserved polarity modules known as the **Par**, **Crumbs** and **Scribble** modules [437] (Their function in ABCP is described in details in the section below).



In addition to apico-basal cell polarity there are other forms of cell polarity in epithelia, known as **planar cell polarity (PCP)**. **PCP** forms domains orthogonally to apico-basal polarity along the X and Y axes (across the plane of an epithelium) through signaling, in order to orient cells or tissues in a given direction. This is displayed in the organization of the hair cells within the cochlea [190]. PCP is a consequent of a mutual antagonistic between two complexes, the proximal cassette (including vang-like protein (Vangl) and Prickle (pk)) and the distal complex (including Frizzled (Fz), Dishevelled (Dsh), and Diego (Dgo)) [438]. The ABCP contribute in PCP, for instance, the Par and Scrib complexes physically interact with core PCP components [439, 440], which displays an interconnection between ABCP and PCP in maintaining epithelial integrity.

Migrating cells adopt a **front–rear polarity** in response to environmental cues (including chemotactic), which is required to orientate cell during the migratory step. Migration concerns in individual cells, single-cell migration, or cluster of cells interconnected by cell-cell junctions which migrate collectively, known as collective migration. Adoption of front-rear polarity in migrating cells requires epithelial polarity complexes, but the molecular mechanism that leads to the establishment of the front rear polarity is still under debates. The Rho-GTPases including Cdc42, Rac and RhoA, play an important role in mediating front-rear polarity by having different distribution within the cell. RhoA is activated at the rear of the cell to facilitate retraction, whereas Rac and Cdc42 are activated at the leading edge of the migrating cell to drive directed protrusions [441]. The activation the Rho-GTPases are mediated by the Scrib complex and Par complex at the leading edge (explained page 124).

Here, I will concentrate on the mechanisms leading to the establishment of the apico-basal polarity in epithelial cells and its functional importance.

#### I.E.b Molecular complexes involved in establishment of Apico-basal polarity

Genetic and biochemical studies in *Caenorhabditis elegans*, *Drosophila melanogaster* and monolayer or three-dimensional (3D) cultures of mammalian epithelial cells have generated a large scale of knowledge on a network of polarity proteins and lipids that specify identity to the apical and basolateral domains of epithelial cells.

Genetic studies in different organisms have shown the existence of evolutionarily conserved and ubiquitously expressed proteins that regulate ABCP (Table 8). These cell polarity proteins belong to three main polarity complexes: the **Scribble polarity module**, which is composed of Scribble (Scrib), discs large (Dlg) and lethal giant larvae (Lgl); the **Par complex** which is composed of Par-3, Par-6 and aPKC (atypical protein kinase C); the **Crumbs complex** which is composed of the transmembrane protein Crumbs (Crb), Pals1 and Patj (Pals1-associated tight junction protein)[14]. These polarity protein complexes serve as scaffolds engaging other proteins in response to both intracellular and extracellular cues, which leads to the reorganization of the cytoskeleton and vesicular trafficking that results in the establishment and maintenance of cellular asymmetry [442].

The localization of each of these modules is distinct in epithelial cells and is not similar in all epithelia. In simple epithelia, the CRB and PAR complexes are restricted to the apical region of the lateral membrane whereas SCRIB complex is concentrated along the lateral membrane.

In contrast to simple epithelia cells, the apical domain of stratified epithelia (such as basal keratinocyte of the epidermis) do not face the interior of a lumen but this domain is linked to supra-basal cells with cell-cell contacts identical to the one developed with lateral cells. It is important to point out that basal keratinocytes own the same polarity components of simple epithelial cells but the organization of these polarity proteins within the epithelial cells can differ [9].

Therefore, it is noteworthy to point out that the mechanisms involved in the establishment

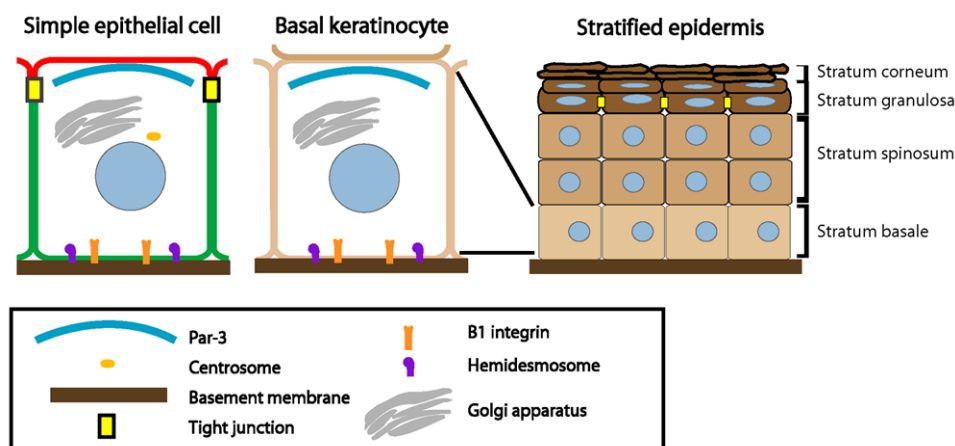


Figure 24: Simple epithelia vs basal keratinocyte molecular organization [9]

and the maintenance of ABCP have been obtained in experiments carried out using simple epithelia model systems. The mechanisms by which polarity complexes regulate stratified epithelium homeostasis is still under investigation.

Here I will briefly describe the different components of two of these polarity complexes, the Par complex and the Crumb complex and I will define with more details the composition of the Scrib polarity complex. I will discuss how these three complex work together to form and maintain the homeostasis of apico-basal polarity in epithelium. I will also mention the role of

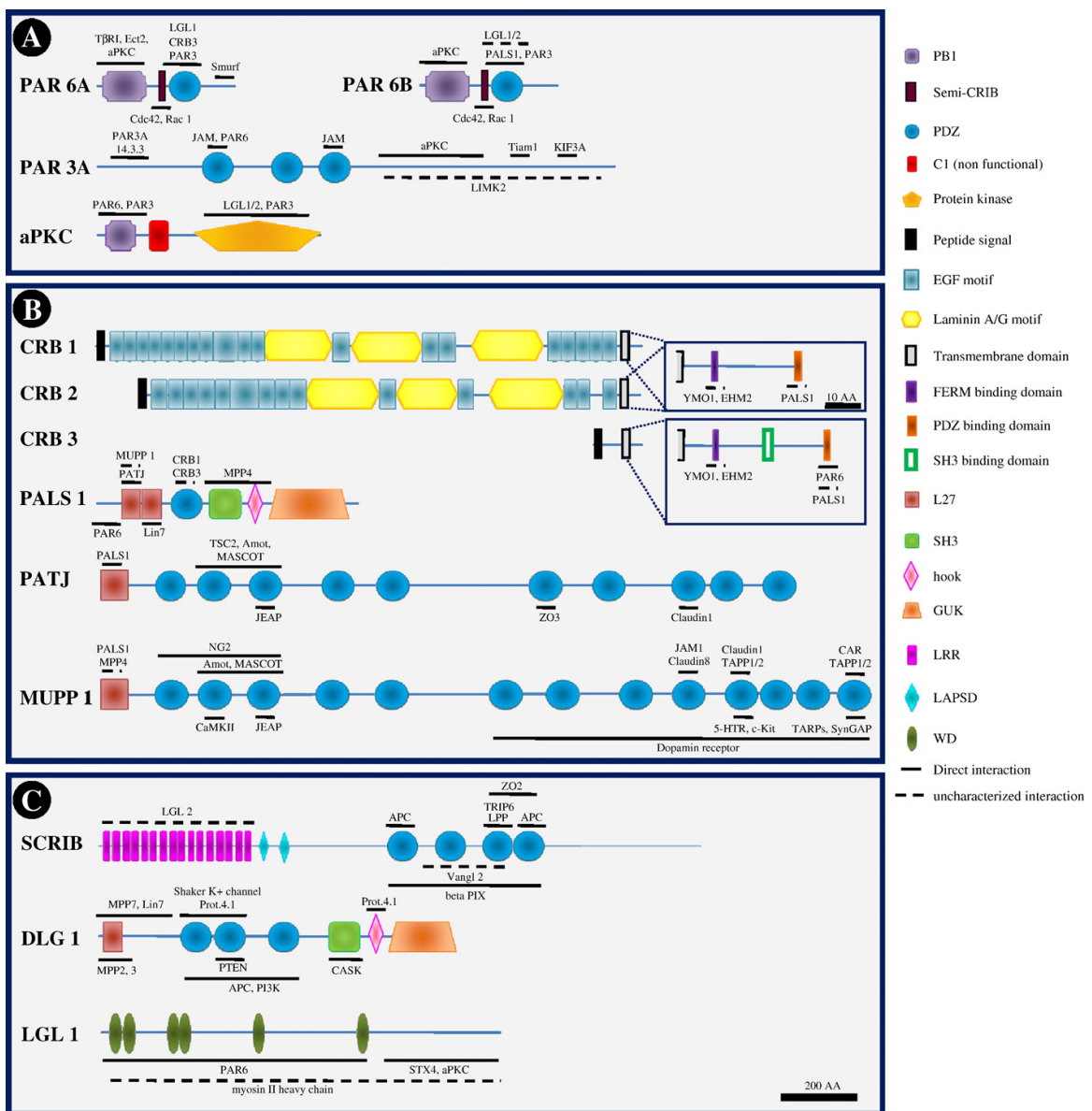


Figure 25: Structure and interactions of mammalian polarity complex proteins. [14]

several proteins that do not directly belong to these complexes and that have been demonstrated to regulate ABCP.

#### *I.E.b.1 The Par complex*

The *Par* genes were the first genes identified by Kemphues *et al.* in 1988 to be involved in cell polarization and asymmetric divisions arising in the *C. elegans* zygote [443]. They identified six genes controlling asymmetric division and called them *par* (for partition defective) (from Par1 to Par6) [443]. These 6 genes encode for proteins of different structural features. Par1 and Par4 are two protein kinases, Par5 is a member of the 14.3.3 family protein, Par3 and Par6 are two scaffold and adapter proteins [14]. Subsequently, Tabuse *et al.* in 1998 [444] identified atypical protein kinase C (aPKC/PKC3 in *C.elegans*) protein coded by the seventh *par* gene. *C.elegans* embryos lacking PKC3 as the result of RNA interference were lethal, displaying *par3*-like phenotypes. These studies discovered the Par complex as an apical protein complex consisting of the proteins PAR3, PAR6 and aPKC [445]. The other proteins (Par1, Par4 (Lkb1 in mammals) and Par5 (14-3-3 in mammals)) also play a role in regulating apico-basal polarity [13] (described in page 119).

Mainly, the Par complex is involved in the establishment and conservation of TJ and apical domain [446]. PAR6 and PAR3 are two scaffold proteins containing PDZ domains that mediate interaction with the other proteins of the complex. The major role of these two proteins is to localize and regulate the serine/threonine kinase aPKC, which is the core effector of this complex. PAR3 and PAR6 have also several interactions with the small G proteins of the Rho family, Cdc42 and Rac, that are also important effectors in the apical-basal polarity pathway by regulating the actin cytoskeleton [447].

The Par complex is conserved from worms to vertebrates. In fact, after being originally characterized in the nematode *C. elegans* as essential proteins for asymmetric division, it was later illustrated in the fruit fly *D. melanogaster* and vertebrates. Although only single copies of the Par genes exist in nematodes and flies, this gene family has expanded in vertebrates, for instance, there are three genes encoding *PAR6* (*PAR6A/C*, *PAR6B* and *PAR6D/G*), two genes encoding *PAR3* (*PAR3A* and *PAR3B*) and two genes encoding aPKC (aPKC $\lambda/\iota$  and aPKC $\zeta$ ). Studies have shown that various isoforms have different roles. For instance, PAR6 proteins display diverse patterns of localization in epithelial MDCK cells [448]. PAR6A is localized both at tight junctions and in the cytosol, while PAR6B is localized in the cytosol, whereas PAR6G

mostly co-localizes with tight junction markers [448]. The specific spatiotemporal localizations for each PAR6 proteins strongly advocate diverse functions. This was confirmed by a study demonstrating that substitution of the zebrafish *par6* gene with a different isoform did not rescue the wild type phenotype [449].

PAR6 contains conserved domains mediating its interactions with the other members of the complex, including a Phox/Bem 1 (PB1) domain at the N-terminal that binds to other PB1-domain-containing proteins such as aPKC, and an adjacent Cdc42/Rac interaction binding (CRIB) motif binds to the Cdc42 or Rac GTPases only in their activated GTP-bound state [450]. Lastly, PAR6 have a PDZ-domain that binds to other proteins including PAR3 and CRB3 (Figure 25). PAR6 proteins do not hold enzymatic domains, therefore they function by bringing together several proteins at a specific localization such as tight junctions, the leading edge of a migrating cell, the tip of the growing axon, etc. [451]. The key function of PAR6 is to allow the interaction between aPKC and its downstream effectors such as PAR3 and LGL (described below) [452].

PAR3A forms a homodimer via its N-terminal region and this self-association seems to be required for the correct targeting of PAR3A at the apical side of the cell–cell contact region during polarization [453]. It seems likely that the starting point required for PAR3A to target the tight junctions is its ability to form self-associations and to bind to the junctional adhesion molecules (JAMs). At this point PAR3A play a role of a scaffold by the recruitment of the other proteins involved in the development of TJ such as PAR6 [454] and aPKC [455]. Several studies have exposed that overexpression or depletion of PAR3A in epithelial cells leads to the disruption of tight junctions, as well as the mis-localization of PAR6, aPKC and tight junction markers [453, 454, 456]. PAR3A can also interact with other proteins depending on its phosphorylation state, which is dependent on aPKC. These molecular partners to PAR3A include 14.3.3 (Par5) and the Rac exchange factor Tiam1. These interactions display the role PAR3A can play in regulating cytoskeleton dynamics [14](explained below).

aPKC is composed of an N-terminal PB1 domain which allows its interaction with PAR6 [457], and a PKC catalytic domain within its C-terminal region [458] (Figure. 25). aPKC is known to phosphorylate several proteins such as PAR3 [458] and LGL [459]. In addition, the complex aPKC/PAR6 can interact independently with LGL and PAR3, therefore able to form

(LGL/aPKC/PAR6 or PAR3/aPKC/PAR6) to regulate epithelial cell polarity [452](explained below page 117). aPKCs are the only members of the Par complex showing catalytic activity. The main function of aPKCs is to prevent lateral proteins from localizing to the apical domain. In MDCK epithelial cells, aPKCs localize with the other members of the PAR complex at tight junctions [460]. This kinase activity is required for the formation of tight junctions, since overexpression of aPKC mutant lacking kinase activity blocks TJ formation and leads to mis-localization of PAR6 and PAR3 [455].

### *I.E.b.2 Crb complex*

In 1990, Tepass et al. recognized the presence of a genetic link between epithelial polarity and crumbs (*crb*), in *Drosophila*. Indeed, *Crb* mutations fail to form or maintain a zonula adherens, which results in the loss of cell polarity and adhesion, and increased cell death [461].

The Crumbs complex is an apical polarity protein consisting of the transmembrane protein Crumbs, and cytoplasmic scaffolding proteins PALS1 (Stardust in *Drosophila*), PATJ (formerly known as Discs Lost in *Drosophila*) and PATJ homologue in mammals MUPP1 (Multi-PDZ domain protein 1).

The Crumbs complex was first identified in the epithelia of *Drosophila* and subsequently in vertebrates (**Table 8**). Although *D. melanogaster* has only one Crumbs gene in, three genes have been identified in mammals (denoted *CRB1*, 2, and 3). *CRB1* and *CRB2* are primarily present in the retina and brain [462] and *CRB2* is as well expressed in the kidney [463]. *CRB3* is present in all epithelial tissues including retina [464], and in skeletal muscles [465].

*CRBs* proteins may have a compensatory effect in certain tissues like the retina, but also that can have more specific roles in other tissues. For instance, in mice retina *CRBs* proteins can be found expressed in the same cell type. In mice, both mutations in *CRB1* and *CRB2* lead to defects in the retina. Moreover a study has shown a compensatory effect of *CRB2* on *CRB1* mutants in mice retina [466]. However, *CRB3* was not linked to retinal diseases [467].

*CRB3* is a transmembrane protein that localizes to the apical membrane of epithelial cells and is concentrated in TJ, where it interacts with PALS1. Interaction of *CRB3* with the PDZ domain of PALS1 is mediated by an evolutionarily conserved C-terminal PDZ-binding motif, the amino acid sequence ERLI [14]. *CRB3* complex is crucial to define the apical domain. Overexpression of *CRB3* in MDCKII cells leads to the expansion of the apical domain resulting in the decreased of the basolateral domain, delayed formation of TJ and disruption of cell polarity [465, 468].

This function depends on two conserved domains in the cytoplasmic tail of Crumbs, the membrane proximal FERM-binding domain and a C-terminal ERL1 motif [465, 468]. Moreover, CRB3 contributes to primary cilia formation, which are cell surface microtubule-based organelles that play a role as mechano-sensor and chemo-sensor to mediate signaling cues. Small interfering RNA experiments showed that CRB3 is required for ciliogenesis in kidney tubules epithelial cells [469, 470].

PALS1 is a scaffold protein, also known as MPP5 (for membrane-associated palmitoylated protein 5)[471]. PALS1 Knockdown in MDCK cells leads to TJ and polarity defects, including increase in permeability and disrupted development of luminal cysts, result from a failure of recruitment of the Par6/Par3/aPKC complex to the tight junction [472] and to the mis-targeting of E-cadherin to the cell membrane [473]. PALS1 is needed for the proper localization of Par6/Par3/aPKC complex to the tight junction and correct targeting of E-cadherin at cell-cell junction.

PATJ interacts with different TJ components including ZO-3 (via its sixth PDZ domain), claudin-1 (via its eighth PDZ domain) [474], as well as members of the Crumbs complex such as PALS1 and CRB3 [475]. Overexpression or downregulation of PATJ in epithelial cells disrupts localization of TJ proteins ZO1, ZO3 and Occludin. This observation suggests that PATJ is required for TJ formation [474-476]. The downregulation of PATJ also affects the distribution of the CRB complex proteins CRB3 and PALS1, which were no longer accumulated at the apical membrane and in TJ, suggesting that PATJ is involved in apical stabilization of the Crumb complex [475].

MUPP1 is a paralog of PATJ, that contains PDZ domains (13 and 10 respectively) and a L27 domain. MUPP1 and PATJ share numerous binding partners ZO-3, PALS1, PAR6, and nectins [477, 478]. MUPP1 and PATJ display similar subcellular distributions, where they localize to TJs. However, they may have different functions since PATJ is necessary in establishing of TJs and epithelial polarization, while MUPP1 is not [478].

Table 8: Components of the polarity complexes in different species, adapted from [14]

<b>Polarity complex</b>	<b><i>Drosophila</i></b>	<b><i>C.elegans</i></b>	<b>Mammalian</b>	
			Protein	
			Gene symbol	
<i>Par complex</i>	DmPar6	Par-6	PARD6A	<i>PARD6A</i>
			PARD6B	<i>PARD6B</i>
			PARD6G	<i>PARD6G</i>
	Bazooka	Par-3	PARD3	<i>PARD3</i>
			PARD3B	<i>PARD3B</i>
	DmaPkc	PKC-3	aPKC $\iota$ / $\lambda$	<i>PRKCi</i>
PKC $\zeta$			<i>PRKCz</i>	
<i>Crumbs complex</i>	Crb	Crb1	CRB1	<i>CRB1</i>
		Eat-20	CRB2	<i>CRB2</i>
			CRB3	<i>CRB3</i>
	Sdt	TAG-117	Pals1	<i>MPP5</i>
	Dpatj		PATJ	<i>INADL</i>
<i>Scrib complex</i>	Scrib	LET-413	SCRIB	<i>SCRIB</i>
	Dlg	dlg-1	DLG1/SAP97	<i>DLG1</i>
			PSD-93; Chapsyn110	<i>DLG2</i>
			SAP102; NE-DLG	<i>DLG3</i>
			PSD95; SAP90	<i>DLG4</i>
			P-DLG	<i>DLG5</i>
	Lgl		LGL1	<i>LLGL1</i>
LGL2			<i>LLGL2</i>	

### I.E.b.3 *Scrib complex*

The *scribble (scrib)* gene was identified in *Drosophila* using a screen for maternal mutations disrupting aspects of epithelial morphogenesis such as cell adhesion, shape and polarity [479]. The *scrib* mutation lead to defects in the in the epithelial organization as a result of irregularly shaped cells that piled upon each other. In addition to *scrib*, two other genes were found to establish the same phenotype when mutated which are known as *lethal giant larvae (lgl)* and *lethal discs large (dlg)*. In *Drosophila* development, Dlg, Lgl, and Scrib, show common patterns of distribution at the lateral domain of epithelial cells and loss-of-function phenotypes, and may form a biochemical complex that controls and refines the segregation of apical and



basolateral membrane domains through promoting the proper localization of apical proteins at the apical domain [480, 481].

The *Drosophila* homologues of Scrib module in mammals are evolutionary conserved in sequence and function. In mammals, only one gene of *SCRIB* exists, which also known as *VARTUL*, whereas there are five genes of *DLG* including *DLG1* (*hDlg1*, *SAP97*), *DLG2* (*Chapsyn-110*, *PSD-93*), *DLG3* (*NE-Dlg*, *SAP102*), *DLG4* (*PSD-95*, *SAP90*), and *DLG5* (*LP-DLG*) and two *LGLs*, *LGL1* and 2 (*Hugl1* and 2 in human).

The diverse genes of *DLG* and *LGL* in mammals are involved in specific functions but may also have a redundancy effect (explained further in the Scrib complex function section below page 120).

#### I.E.b.3.1 Structure

Although the *SCRIB*, *DLG* and *LGL* proteins do not have intrinsic enzymatic activity, they all contain a number of well-characterized protein–protein interaction domains (**described in more detail below**) that allows them to bind to signaling proteins, such as kinases and phosphatases. Essentially, their function looks to be absolutely dependent on their cellular sub-localization. Hence, much of the currently known functions of the Scribble module proteins can be attributed to their role as signaling scaffold proteins that regulate site-specific signaling within the cell.

In this section, I will introduce the structure of the members of the Scrib module and how the organization enables them to function as scaffold that coordinate the assembly of large signal transduction networks at specific sites of the cell and in particular in define area of the plasma membrane.

##### I.E.b.3.1.1 SCRIB

*SCRIB* is a large cytoplasmic multi-domain protein of 175 kDa that plays many roles in flies and mammals. *SCRIB* belongs to the LAP protein family, which contain leucine-rich repeats (LRR) and PDZ domains, and function in controlling cell shape, size and subcellular protein localization [482]. The LAP protein family also includes Erbin and Densin180 [483, 484]. *SCRIB* contains 16 LRR at its amino terminus, followed by 2 LAP-specific domains (LAPSD), a linker region, 4 PDZ domains and a C-terminal lacking any identifiable motives [482]. The *SCRIB* PDZ domains, are regions of 80–90 amino acids that are found ubiquitously through the animal

kingdom and ensure protein–protein interactions. The PDZ and LRR domains of SCRIB are needed for effective activity, for example in proper localization and targeting to the membrane. SCRIB LRR repeats regulate its ability to target the basolateral epithelial membranes. However, this membrane localization of Scrib required first E-cadherin engagement [320]. The LRR domain of Scrib is critical for to organize a polarized epithelial monolayer and are involved in the control of cell proliferation. *Drosophila* Scrib LRR domain mutants have similar phenotype to *scrib*<sup>-/-</sup> mutants, however, when overexpressed, a *scrib* mutant lacking the PDZ domains function as the wild-type gene *in vivo* [485], suggesting that the PDZ domain are insufficient to support Scrib function.

SCRIB interacts directly to the C-terminal motif of ZO-2 through its PDZ domains 3 and 4. ZO-2 interacts with several components of the tight junctions, including ZO1, Occludin, Cingulin and Claudins. SCRIB/ZO-2 do not co-localize at tight junction but are found co-localized in non-polarized cells. Thus, SCRIB/ZO-2 interaction most probably happens before ZO-2 segregation at tight junctions [486].

The PDZ domain of SCRIB also binds the GEF βPix protein [487]. The Scrib/βPix complex was demonstrated to be central for Scrib-dependent employment of Rac1/Cdc42 to the leading edge of migrating cells, vesicle trafficking in neurons, oriented directional migration, receptor recycling in thyroid cells [488].

#### I.E.b.3.1.2 LGL

In mammals two genes encode Lgl *Drosophila* homologue protein, that are named LGL1 (115 kDa) and LGL2 (113 kDa). Two extra related LGL proteins have been described in mammals: syntaxin-binding protein 5 (LGL3) and syntaxin-binding protein-5-like (LGL4), which are homologous to the Tomosyn *Drosophila* protein. LGL proteins contains several WD-40 repeats, also known as WD or β-transduction repeats, and conserved phosphorylation sites targeted by aPKC and Aurora kinases. WD-40 repeats are structural motifs composed of approximately 40 amino acids that usually terminate with a tryptophan–aspartic acid (WD) dipeptide and are known to form β-propellers that act as protein interacting modules. In human cell line, LGL2 WD40 motif interacts with SCRIB [489]. This interaction has yet not been confirmed in in any other organism.

LGL1 was found to bind specifically with syntaxin-4 (STX4) (a t-SNARE specific for the lateral

plasma membrane), which suggests that LGL may contribute to apico-basal polarity by regulating basolateral exocytosis in mammalian epithelial cells [490].

#### I.E.b.3.1.3 DLG

In mammals, five *DLGs* exist, including *DLG1* (*hDlg1*, *SAP97*), *DLG2* (*Chapsyn-110*, *PSD-93*), *DLG3* (*NE-Dlg*, *SAP102*), *DLG4* (*PSD-95*, *SAP90*), and *DLG5* (*LP-DLG*). These five proteins have the same structure belonging to the MAGUK superfamily. The existence of multiple *DLG* genes in mammals with a similar structure suggests that they have a certain functional redundancy (detailed in page 120).

In the following paragraph, I will focus on the biological roles of Dlg1 in human epithelial cells, considering that the high level of conservation among the different Dlg suggest a possible redundancy among these different proteins.

*Drosophila* discs large 1 (DLG1) is the mammalian homologue of *Drosophila's* Dlg and is the most closely related to *Drosophila* Dlg and the most frequently studied DLG in epithelial cells [491]. The disc large homologue 1 (DLG1) protein, also named synapse-associated protein-97 (SAP-97). DLG1 belongs to the membrane-associated guanylate kinase (MAGUK) superfamily, which are characterized by the presence of PSD-95/Dlg-A/ZO-1 (PDZ) domains, a Src homology 3 (SH3) domain that mediate critical protein–protein interactions and a guanylate kinase-like (GUK) domain (see Figure 25). That is catalytically inactive, thus DLG1 is a protein lacking intrinsic enzymatic activity. The family of MAGUKS include also other cell-cell adhesion or polarity molecules such as ZO proteins and PALS1 [492].

DLG1 contain also a L27 (Lin-2, Lin-7) domain located at its N-terminus and a HOOK domain located in between the SH3 and GUK domains.

In *Drosophila* the alternative splicing gives rise to two main Dlg isoforms, DlgS97 that contains L27 domain and DlgA lacking the L27 domain. The DlgA, is found specifically expressed in *Drosophila* epithelial tissues and localize via its PDZ2 at the septate junctions (the counterpart of TJ in vertebrates) [493-495]. DlgS97 is localized at the neuromuscular junction. DlgS97 was reported to be the main isoform in neurons and muscles during *Drosophila* development [493, 496]. Dlg PDZ1-2 domain is required for the targeting and clustering of its partners, the adhesion protein Fasciclin II and the Shaker potassium channel at the glutamatergic synapse [497, 498].

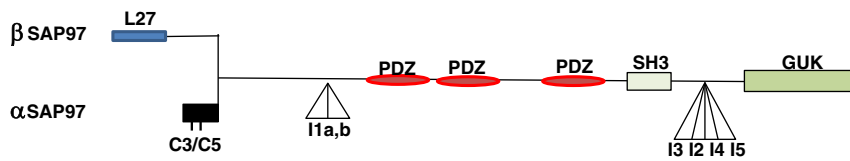


Figure 26: Schematic diagram of the domain organization of SAP97 isoforms. [17]

Mammalian Dlg1 contains two regions of alternative splicing. The first region is at the N-terminus, containing two alternative splicing exons termed I<sub>1A</sub> and I<sub>1B</sub>, and the second region is located between the SH3 and GUK domain (also termed the HOOK domain), contains four alternative splicing exons I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub> and I<sub>5</sub>. This alternatively splicing insertions results in different alternative spliced isoforms of Dlg1, which gives Dlg1 specific function and different intracellular distribution within the cell [491] (Figure 26).

The N-terminal alternatively spliced region of Dlg1 can bind SH3 domains from multiple proteins and also moderate Dlg1 self-association [491]. Moreover, N-terminus of Dlg1 also called the MAGUK recruitment (MRE) domain binds to the Lin2-Lin7 (L27) domain of calmodulin-associated Ser/Thr kinase (Cask) and mediates the localization of Dlg1 to the membrane of epithelial cells [499]. Firestein and Rongo et al., demonstrated that the conserved N-terminus of Dlg1 targets Dlg1 to the adherens junction in *C.elegans* [500]. Which demonstrate the importance of the conserved domains of Dlg1 in the functionality and localization of the protein.

The I<sub>2</sub> and I<sub>3</sub> insertions, that are located between SH3 domain and GUK domain (Known also as HOOK domain), have been shown to contribute to the subcellular localization of DLG1. DLG1 I<sub>3</sub> directs membrane targeting, in addition to the N-terminus domain of Dlg1, probably through interaction with the cytoskeleton protein 4.1/ERM family members at an early stage of cell-cell formation, via PDZ-1 and PDZ-2 on Dlg1 and a HOOK domain [501]. Which associate a fundamental role of the conserved HOOK domain of MAGUKs in mediating direct binding to the FERM domain of protein 4.1, thus regulating the membrane targeting properties of the scaffolding complex in epithelial cells [502]. The HOOK domains provide a link with the cortical actin cytoskeleton after binding to members of the FERM superfamily [503]. Whereas I<sub>2</sub> promotes a nuclear confinement [491]. Hence, this gives rise to the possibility that alternative

splicing creates hDlg1 isoforms with discrete functional properties.

Whereas the interaction between the different proteins of the Crumb and Par complexes is clearly demonstrated and illustrated by many studies, there is only small evidences of physical interactions between the members of the Scrib module. Studies in *Drosophila* and in human demonstrated a direct interaction between Scrib and Dlg1 through the GUK domain [504], [505]. SCRIB physical interaction with LGL2 was also characterized in polarized mammalian cells [489]. Moreover, a recent study has demonstrated that a RhoG-specific GEF, SGEF form a ternary complex linking indirectly SCRIB and DLG1 at cell- cell junctions in epithelial mammalian cells [506].

#### I.E.c Polarity complexes in establishment and maintenance of epithelial polarity

Establishment of apico-basal polarity is based on the spatiotemporal coordination of the activity of the three main polarity complexes. Between each of these complexes, there are finely tuned antagonistic interactions that determines a definitive role of each complex in establishing apico-basal polarity in simple epithelia. Here I will discuss how the proteins of the polarity complexes and their interactions with other molecules are involved in the initiation, formation and maintenance of ABCP.

##### Establishment ABCP:

Epithelial cells orient their basal surface through the adhesion of integrin receptors to the extracellular matrix, and they extend membrane protrusions, known as filopodia, to contact neighboring cells which allow the formation of cohesive layers of cells [507]. The establishment of intercellular adhesion facilities mammalian epithelial polarization, partly by targeting protein sorting, targeting and distribution of basolateral and apical proteins to their proper membrane localization [508].

At the initial stages of intercellular adhesion, E-cadherin and junctional adhesion molecule A (JAMA) are recruited to the cell cortex, which cluster these primordial adhesion sites, known as puncta [269], into adhesive homophilic interactions. These primordial adhesion sites contain a mixture of AJ and TJ components and extend the interface of adhesion along the basolateral domain through the recruitment of the actin cytoskeleton and its remodeling by regulating **Rho-GTPase Rac1 and Cdc42** [509].

The maturation of primordial adhesion is assured by Rho-GTPase activity of Rac1 and Cdc42, which are essential in activating aPKC. aPKC mediates LGL1/2 phosphorylation during the epithelial polarity establishment phase [452], resulting in the detachment of LGL1/2 from the PAR6/aPKC dimer which re-localize to the basolateral membrane, where it can interact with DLG and SCRIB and exclude apical proteins [452, 459]. Following this, aPKC interacts with and phosphorylates PAR3, favoring the formation of the active PAR complex at the apical junctions [460, 510].

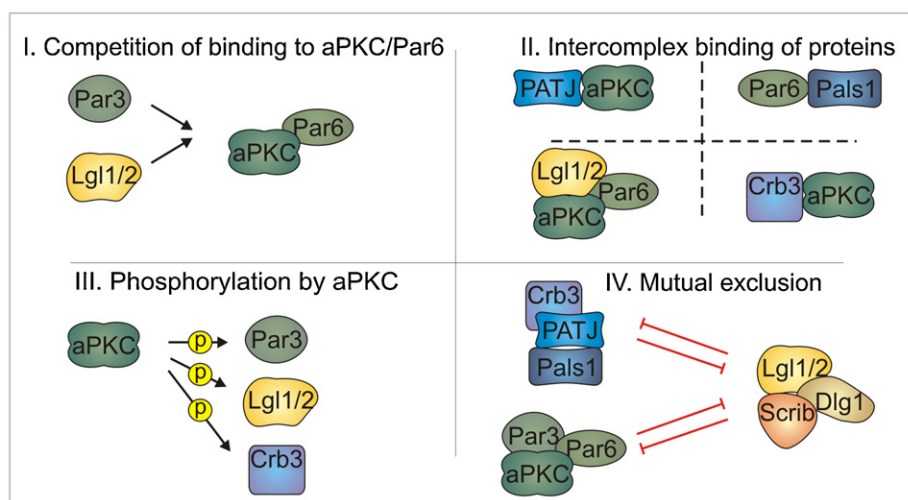


Figure 27: Interchange between polarity proteins of the different complexes is possible in multiple ways, (I) involving competition of binding of Lgl1/2 and Par3 to Par6/aPKC, (II) binding of proteins from different complexes, (III) phosphorylation of Par3, Lgl1/2 and Crb3 by aPKC and (IV) mutual exclusion of the Scrib complex and the apically located Par and Crumbs complex.[20]

CRB3 interacts directly to PAR6 (Lemmers, Michel et al. 2004) or via PALS1 (Hurd, Gao et al. 2003) to induce the differentiation of the premature junctional structure into mature epithelial structures.

In general, the establishment of epithelial polarity is induced by signaling cues emanating from the cell-cell junctions which leads to the activation of Rho GTPases activates aPKC. Activated aPKC leads to phosphorylation of several polarity proteins to assure the correct sub-localization in the cell, which consequently maintain cell junction.

Epithelial polarity maintenance:

The polarity complexes Par, Crumbs and Scrib complexes are involved in a mutual and antagonistic relationship that maintain their polarized localization in simple epithelia.

The Par complex mediates the basolateral localization of the Scrib complex protein member LGL in an antagonistic manner. At the apical domain aPKC phosphorylates LGL in the aPKC/PAR6/LGL complex. Once phosphorylated, LGL detach from this complex and re-localize to the lateral domain of mammalian cells [459].

The Par complex works in cooperation with the Crumbs complex, in contrast to the Scrib complex, to mediate Par localization to TJ, which is facilitated by the interaction between PAR6 and PALS1 in mammalian epithelial cells [511]. The association between CRB complex and PAR complex has been further reinforced by the discovery that PALS1 regulate localization of aPKC to tight junctions in mammalian epithelial cells [472]. This possibly occurs via PATJ that interacts directly with members of tight junction, recruits PALS1 to the tight junction region with PAR6 that interacts to aPKC.

In *Drosophila*, Dpatj is able to modulate the phosphorylation of Crb by aPkc that is required for the proper localization of Crb and Dpatj to the apical membrane. This study provided a mechanistic framework for the functional interaction between the Par and Crumb complexes based in the post-translational modification of Crb by DaPKC [512].

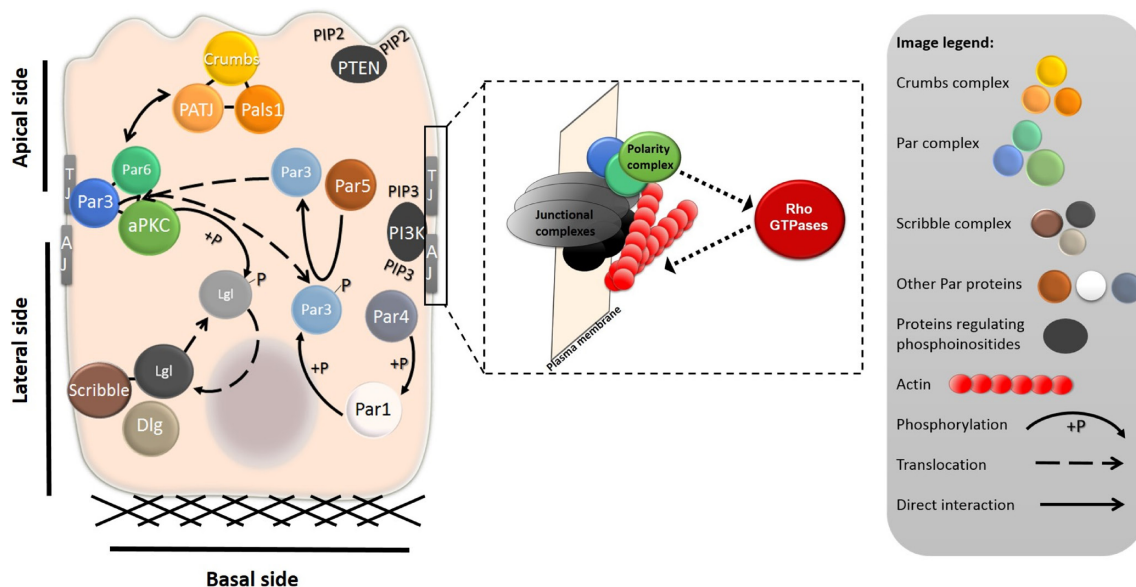


Figure 28: The crosstalk between different polarity complex in differentiated epithelium cell. [13]

The cooperative and antagonistic relationships between the polarity complexes are essential in maintaining the apico-basal polarity in epithelial cells (Figure 28). The disruption of any of the complexes lead to the disruption of the sub-localization of the other polarity proteins leading to a disorganized epithelia.

#### I.E.d Other molecules involved in apico-basal polarity

In addition to the three polarity complexes, other molecules are found essential for polarity regulation. Here, I will briefly introduce these polarity proteins and their function.

Another complex, known as the Yurt/Coracle complex, was discovered in *Drosophila* and involved in the apico-basal establishment. Yurt/Coracle complex displays antagonistic interactions with Crb. This complex consists of FERM-domain proteins, Coracle and Yurt and the membrane proteins, Neurexin IV and the Na<sup>+</sup>/K<sup>+</sup>-ATPase that form a complex at basal lateral junctions (Laprise et al., 2009). An antagonist interaction was also exhibited between Yurt and aPKC which is dependent on Yurt phosphorylation by aPKC [513]. The mammalian orthologue of Yurt (Ymo1) also binds CRB, where Ymo1 is also involved in the formation of lateral membrane in epithelial cell, which can imply that regulation of polarity by Yurt is conserved in the mammalian epithelial cells.

The other Par proteins (PAR1, PAR4 and PAR5) are involved in the exclusion of PAR complex role in apico-basal polarity. In mammalian cells and *Drosophila*, the kinase PAR1 has been exhibited to localize to the lateral membrane of epithelia and regulate polarization through controlling the localization of PAR3 polarity protein [514, 515]. aPKC phosphorylates PAR1 in order to prevent its apical targeting [516, 517]. PAR1 phosphorylates PAR3 which allows the binding of PAR5, a protein of the 14-3-3 family [518, 519]. The recruitment of PAR5 on PAR3 excludes Par3 from the lateral membrane, insuring the restriction of PAR3 of the apical domain [13].

In mammals and *Drosophila*, PAR4 act upstream PAR1, PAR1 is phosphorylated and activated by PAR4 [520, 521].

Another important family of proteins implicated in the epithelial polarity are the Rho GTPases family. Cdc42 play an significant role in regulating the cell polarity [522], which seems to be highly conserved from yeast to man [523]. Cdc42 has been shown to be required for apical polarization in various *in vivo* and *in vitro* models [524]. Cdc42 binds to PAR6 and which increases the activity of aPKC within the PAR6-PAR3-aPKC complex, in mammalian and



*Drosophila* systems. The other GTPase Rac1 is also required for polarization and tight junction formation. TIAM1, an exchange factor of Rac1, is associated with the PAR3/PAR6/aPKC polarity complex. aPKC phosphorylation of TIAM1 triggers its activation resulting in the recruitment and activation of Rac1 at the PAR complex [525].

It exists a close interconnection between polarity complexes and Rho GTPases where polarity complexes promote the sub-localization and activity of Rho GTPases within the cell in order to regulate the cytoskeleton organization in order to maintain ABCP and the epithelial structure.

## I.E.e The Scrib complex

### *I.E.e.1 The Scrib module function in physiology and in physiopathology*

#### *I.E.e.1.1 Scrib module function in physiology*

The Scrib module is required in the establishment of apico-basal polarity and planar cell polarity and participates also the regulation of various cellular processes including proliferation, migration, apoptosis and differentiation of epithelial tissues [433, 446, 488, 526]. Here I will describe the role each member of the Scrib module play in different physiological process.

##### *I.E.e.1.1.1 In polarity*

Divers studies in *Drosophila*, mice and mammalian cells analyzed the function of Scrib complex components in establishing and maintaining Apico-basal polarity but also front-rear and PCP. Here, I will describe the role Scrib complex members play in these different types of polarity.

##### *I.E.e.1.1.1.1 In apico-basal polarity*

In *Drosophila*, the Lgl and Dlg proteins localize at septate junctions (occludin junction in invertebrate, homologue to TJ) in the apical belt of the lateral cell membrane. Scrib and Dlg co-localize with Lgl in epithelial cells [481]. The mutants *scrib*, *dlg* and *lgl* in *Drosophila* has demonstrated that the localization at plasma membrane of each of these three proteins relies on the presence of the others [479]. In *Drosophila*, *scrib* mutants defects in epithelial cells leads to the misdistribution of apical proteins, such as Crb, Dpatj, E-cadherin and Armadillo that are found dispersed throughout the plasma membrane of epithelial cells [479]. These findings indicate that one main Scrib function in epithelial polarity is to exclude proteins (such

as Crb complex) from the basolateral domain [479]. Likewise, *dlg* and *lgl* mutation in *Drosophila* displayed the same phenotype as *scrib* mutants, which results in disruption of septate junction, defects in apico-basal polarity, and mis-localization of apical junctions and AJs [481, 494, 527].

In mammals, the role played by DLG1 and SCRIB in establishment of epithelial polarity is still poorly understood and is still under investigation. *SCRIB* null mice, did not show any apico-basal polarity defects, but rather defects in planar cell polarity (PCP) [528]. In mammalian epithelial cells, the knockdown of SCRIB did not display defects in apico-basal polarity [324, 529, 530]. *DLG1* knockout mice also show no obvious epithelial polarity defects [531, 532]. Similarly, DLG1 knockdown in mammalian epithelium cells has only a weak effect on polarity [228, 533]. These *in vivo* and *in vitro* data on DLG1 can be explained by the redundancy with one or several of the five mammalian DLG family members [534, 535]. For example, DLG5 and DLG3 displayed a similar function in apico-basal polarity [534, 536], which suggests the redundancy in between these two DLG proteins.

The different DLG members can be found co-expressed in the same tissues. For instance, in epithelial neuroectodermal cells, all DLGs were found expressed in the head region in mice [534]. In addition, DLG2, DLG3, and DLG4 were expressed in the epithelial lining of the gut along the anterior-posterior axis [534]. Interestingly, the DLG proteins show different subcellular localizations in epithelial cell types, in mice embryos and in polarized mammalian epithelial cell cultures [534, 537]. DLG1 had the expected restricted distribution to the basolateral membrane where it colocalized with the AJ component  $\beta$ -catenin, whereas DLG2 and DLG3 were present in the cytoplasm and at different membrane localization than Dlg1. Indeed, DLG3's is found at the apical regions where it co-localizes with TJs. DLG4 is targeted to the membrane but shows no restriction to any particular domain [534]. Finally, DLG5 is found localized at AJ and in cytoplasmic vesicles containing cadherin [535]. Taken together, these data suggest that the different DLG family members can have possible functional redundancy but can also have a more specific function.

*LGL-1* knockout mice exhibit defects in the distribution of  $\beta$ -catenin, F-actin, and myosin II-B that are normally localized at the apical junctional complex in neuroepithelia, displaying defects in cell polarity [538]. Moreover, the absence of LGL-1 disrupted the asymmetric localization of Numb [538]. Numb play an essential role in the regulation of cell differentiation in neuroepithelia [539, 540]. *LGL-1* knockout mice results in defects in asymmetrical cell

division (ACD) where neural progenitor cells is not able to exist cell cycle and differentiate leading to brain dysplasia and mice lethality [538]. LGL-1 is essential for Numb asymmetrical localization during ACD. However, the knockout of *LGL-2* display no defects in apico-basal polarity and are viable [541]. In mammalian cells, overexpression of LGL1/2 during the polarity formation phase disturbs the establishment of junctional complexes, through competing with PAR3 for its interaction with the complex aPKC/PAR6 [452]. Other studies demonstrate that the depletion of LGL1/2, in mammalian epithelial cells, results in an increase of the activity amount of PAR3/aPKC/PAR6 complex that result in the inhibition of apical protein disassembly after calcium withdraw [542]. LGL1/2 is needed for disassembly of apical proteins by regulating the activity of PAR3/aPKC/PAR6 complex to prompt the orientation of apical membrane polarity. Moreover, 3D-organotypic cultures made with human cells deficient for LGL demonstrate that LGL is needed for the proper orientation of the apical membrane and lumen formation [542]. These studies demonstrate the importance of LGL in the establishment and maintenance of ABCP in reconstructed mammalian epithelial generated *in vitro* with mammalian epithelial cells.

#### *I.E.e.1.1.1.2 In planar cell polarity*

The Scrib module proteins plays an important role in the planar cell polarity (PCP) pathway in epithelium tissue, in addition to its function in ABCP. PCP controls global or local patterning events in the epithelium through the propagation and restriction of polarization signals along the surface of an epithelium. PCP disruption lead to various development defects in mammals including neural tube formation, lung and heart defects, deafness and polycystic kidney disease [543, 544].

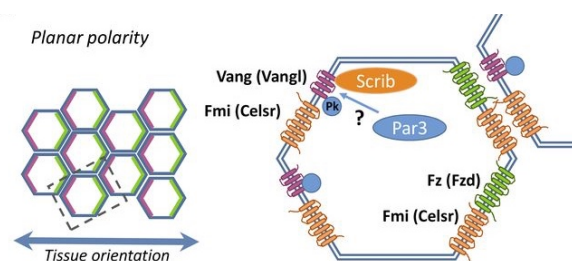


Figure 29: Planar cell polarity

In *Drosophila* Scrib and Lgl exhibit genetic and physical interactions with core PCP regulators [190], which demonstrate their involvement in PCP [545, 546].

SCRIB was shown to be necessary in the PCP pathway during mammalian development. For instance, *SCRIB* null mice display typical phenotype resulting from an abnormal PCP. *SCRIB* null mice result in a lethal phenotype after birth accompanied by defects in neural migration [547] and neural tube defects (NTD) (known as craniorachischisis (explained page 130)), resulting in the failure to initiate neural tube closure [528, 548, 549]. Indeed, SCRIB was found to interact with the core PCP protein Vangl2 [489] (Figure 29). In mice, SCRIB is needed for lung morphogenesis by signaling through PCP in developing lung, where PCP core proteins Vangl2 and Celsr1 are found mis-localized in the absence of SCRIB in the lung [550]. Moreover, SCRIB is found to regulate the activity of RhoA and therefore the localized regulation of the actin cytoskeleton needed in the establishment and growth of epithelial branches. SCRIB homologue in zebrafish (Scribble1) was also shown to be involved in PCP and cell migration during development [547, 551].

#### *1.E.e.1.1.1.3 In front rear polarity*

Activation of cell migration is induced in response to extracellular cues such as chemokines or growth factors gradients, mechanical stimulation or electric field. The cell migration process requires that migratory cells adopt a front-rear polarity in order to be able to have a persistent and directional migration. At the leading edge, polarized migratory cells display extension of an actin-rich protrusion to allow formation of novel focal adhesion sites in the protrusion, which pull the cell forward in the direction of new attachment sites. At the rear of the cell, adhesion sites are disrupted to induce detachment and tail retraction [552]. Cell migration plays an important function in development, immune response, angiogenesis, wound healing and cancer metastasis.

Lgl, Scrib and Dlg have opposing roles in cell migration of the *Drosophila* ovarian border cells. *lgl* or *dlg* mutants enhance border cell migration, whereas, *scrib* mutants reduce it [99, 553, 554], suggesting that Lgl, Scrib and Dlg can work independently to control collective migration in *Drosophila*.

The components of the Scrib complex, SCRIB, DLG1 and LGL-1 are all found at the leading edge of mammalian migrating cells, where they promote cytoskeleton organization, promoting the protrusion of lamellipodia through different mechanisms described below [446](Figure 30).

The Scrib module participates in the regulation of cell migration during embryonic

development and the wound healing process [555]. SCRIB is necessary for epithelial cell sheet migration as well as abdominal wall closure during murine embryonic development [528, 530]. The involvement of Scrib in epithelial cell migration has been also demonstrated both *in vitro* on mammary derived cells and *in vivo* in skin wound healing experiments on *SCRIB* knockout mice [435]. SCRIB control cell migration by regulating the localization and activity of several Rho GTPase (Rac and cdc42) in adhesion sites located at the leading edge of migrating cells. SCRIB regulates Rac and cdc42 activity through its association with  $\beta$ Pix, a GEF of both Rac and cdc42 [487], which activates Rac and cdc42 to specifically activate actin polymerization and cytoskeleton organization at the leading edge of migratory cells.

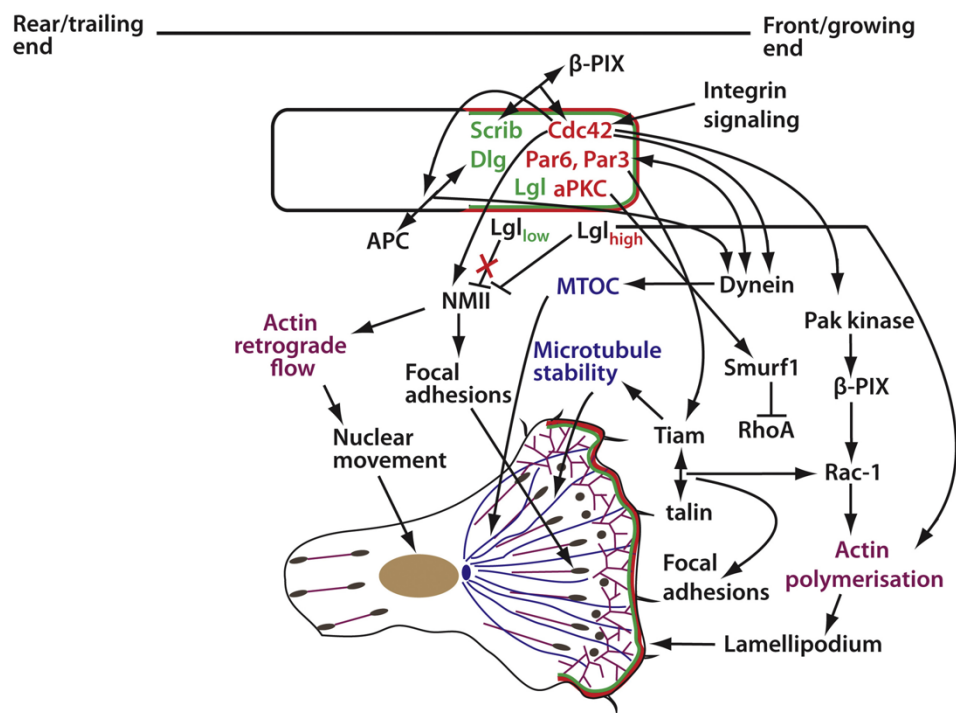


Figure 30: Regulation of the cytoskeleton by Scrib complex during cell polarity [15]

DLG1 is involved in cell migration during development of mice embryo, *DLG1* knockout leads to disturbance in cell migration resulting in craniofacial defects [556], and epithelial duct formation and morphogenesis abnormalities in urogenital development [531]. In astrocytes, DLG1 regulates directed cell migration at the leading edge in a PAR6/aPKC-dependent manner by re-orientating the microtubule network. PAR6/aPKC activation by Cdc42 at the leading edge leads to APC and DLG1 recruitment which allows anchoring of the microtubule plus end

at the plasma membrane and centrosome reorientation [557].

LGL-1 is also found to be involved in cell migration, where it is found localized at the leading edge of cell [552]. LGL-1 was found to bind to Non-muscle myosin II (NMII). NMII is known as an actin-based motor protein that plays an important role in cell migration via modulation of lamellar protrusion and rear retraction. LGL-1 binds to NMII at the leading edge of migrating cell which allows F-actin polymerization a key step to initiate cell to movement. At the rear of the cell LGL-1 is absent permitting NMII to assemble into filaments to allow cell retraction [552].

Altogether these studies demonstrate that the Scrib module play an important role in the epithelium integrity during development not only by assuring establishment of ABCP but also by participating to both PCP and front-rear polarity, two important steps required for the correct morphogenesis of tissues.

#### I.E.e.1.1.2 Cell-cell junction formation

The Scrib polarity proteins play critical role in cell-cell adhesion by influencing the correct positioning of AJ and TJ components in specific compartment of the plasma membrane. The role Scrib polarity module plays in preserving the AJ and TJ is important for the cohesion of the epithelium and its function.

The first experiments depleting *scrib*, *dlg* or *lgl* in *Drosophila*, allow to observe the mis-localization of apical proteins to the basolateral domain and the disruption of the AJ formation [479, 481].

In the worm *C. elegans*, Scrib homologue (called Let-413) is required for AJ formation and ABCP [558, 559].

In mammals, SCRIB localizes to the basolateral domain together with cell-cell junction. In particular, SCRIB is found co-localized with  $\beta$ -catenin and E-cadherin at AJ in mammalian epithelial cells [320, 324]. SCRIB is needed in the lumen morphogenesis in mice lung, since *SCRIB* mutant (conditional KO mice) displayed disturbance in the localization of AJ and TJ proteins leading to disturbance of epithelial integrity [550]. Moreover, conditional knockout of *SCRIB* in the mice lens leads to the loss of epithelial proteins including the loss of E-cadherin and ZO-1 of the lens cell, and the abnormal expression of a mesenchymal markers such as

$\alpha$ SMA. These features could be explained by a sustained activation of the TGF $\beta$  signaling in these cells, which promotes EMT process to occur EMT [560]. This study propose that SCRIB is needed for the establishment and maintenance of specialized traits that define epithelial cells, which are needed for epithelial integrity *in vivo* in the developing embryo [560].

The targeting of E-cadherin and SCRIB to AJ is mutually dependent [99, 320, 324]. The absence of SCRIB in the mammalian epithelial cells (MDCK), displayed disorganization in E-cadherin localization leading to decrease in cell-cell adhesion and increased migration [324]. Later on, it has been demonstrated that SCRIB decreases E-cadherin endocytosis by stabilizing p120catenin-E-cadherin association. Moreover, SCRIB controls vesical sorting of E-cadherin by blocking retromer-mediated retrieval of E-cadherin to the Golgi. This observation supports a model in which SCRIB regulate AJ trafficking [561]. In contrast, in mammary epithelial cells (MCF10A), SCRIB displayed no effect on AJ structure but is involved in cell migration [530]. An additional study has demonstrated the role of SCRIB in the assembly of TJ in intestinal epithelial cells (SK-CO15) [529]. Altogether, these studies propose that the role of SCRIB in AJ establishment is context-dependent in mammalian epithelial cells.

In *C. elegans* Dlg-1-deficient embryos that are unable to recruit an AJ protein known as apical junction molecule-1 (AJM-1) but was not necessary for the apico-basal polarity preservation [562, 563]. This suggests that Dlg-1 function in paracellular barrier formation is conserved but not in the maintenance of epithelial polarity. The human homologue of Dlg, hDLG1, colocalizes with E-cadherin and found to expand on the cell-cell contact in intestinal and renal epithelial cells [228]. The conditional mutation of *DLG1* in mice lens leads to an alteration in the distribution of polarity and cell–cell adhesion markers such as E-cadherin, N-cadherin and  $\alpha$ -catenin/ $\beta$ -catenin in epithelial cells [564]. Similarly in mammalian cells, deletion of hDLG1 disorganized the actin cytoskeleton in epithelial cells [564]. This was shown also in intestinal epithelial cells where the knockdown of DLG1 disrupted the localization of E-cadherin and F-actin at cell junctions [228].

LGL1/2 localization is restricted to the basolateral domain through its phosphorylation, since non-phosphorylatable LGL1/2 escaped into the apical domain of mammalian cell epithelial cell line [490]. The knockout of *LGL-1* lead to mis-localization of  $\beta$ -catenin in the neuroepithelia, which propose that LGL-1 is needed in the establishment of cell-cell adhesion [538].

The overall conclusion is that the Scrib module members participate in different extents to the organization of cell-cell junctions by influencing their proper asymmetric localization and stabilization.

#### I.E.e.1.1.3 Cell proliferation

The polarity is established by asymmetric distribution of proteins modulating intracellular polarity and cell–cell junctions and cell–extracellular matrix contacts. Therefore, the characteristic organization of epithelial cells offers a scaffold for proper positioning of the cell–cell junctions, directional secretion and spatially regulated signaling pathways. The establishment of polarity in epithelium, among the different signaling pathway, controls cell proliferation, which is a key event linked to polarity.

Some genetic studies in *Drosophila* provided evidence that the genes coupled with loss of cell polarity phenotype can in addition regulate cell cycle progression. The Scrib module components were initially discovered for their role in regulating proliferation in *Drosophila*. Mutations of *scrib*, *dlg* or *lgl* in *Drosophila* imaginal discs lead to over-proliferation and tumor formation [481, 527, 565]. All three genes were identified to suppress cell proliferation through regulating negatively cell entry to phase S by the increase of cyclin E levels [566, 567]. However, the regulation of cell proliferation by SCRIB, DLG1 and LGL in mammals is less characterized.

The knockdown of SCRIB in a non-tumorigenic human keratinocyte cell line (HaCaT) is enough to increase cell growth, reduce cell-cell contacts and increase cell invasion [568].

It has been proposed that SCRIB directly regulate cell proliferation by controlling MAPK signaling pathways. The mitogen-activated protein kinase (MAPK) pathways that activate extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK) and p38 kinases have important roles in regulating cell fate in response to growth, proliferation, differentiation and survival signals [569]. Indeed, SCRIB play a role in inhibiting cell growth through interacting with PH domain leucine-rich-repeats protein phosphatase (PHLPP1) that negatively regulate Akt. Scrib binds PHLPP1 and target it to the plasma membrane where it can mediate Akt dephosphorylation [570]. SCRIB can also directly regulate the Erk signaling pathway, through direct protein-protein interaction between SCRIB and Erk [571]. The loss of



SCRIB in cooperation with the oncogene Myc increase tumor cell growth in mammary epithelial cells [572].

DLG1 is found to interact with tumor suppressors like adenomatous polyposis coli (APC) protein through an interaction between the PDZ domain of Dlg1 and the C-terminal S/TXV motif of APC. APC is mutated in familial adenomatous polyposis and in sporadic colorectal tumors [573]. The APC-DLG1 complex formation plays an important role in transducing the APC cell cycle blocking signal [574]. Another tumor suppressor involved in the control of cell proliferation and known to be a binding partner of DLG1 PDZ is the phosphatase PTEN. PTEN is the lipid phosphatase that dephosphorylates the D3 position of phosphatidylinositol (3,4,5)-triphosphate (PIP3), antagonizing the action of the oncogenic phosphatidylinositol 3-kinase (PI3K) and counteracting the pro-survival and cell growth activity of the protein kinase Akt (or PKB) [575, 576]. The growth of cancer cells is repressed when DLG1 and PTEN are co-expressed [577, 578]. The mechanism underlying the effect on cell growth is unclear, but DLG1 rises PTEN stability and augments PTEN's inhibitory activity upon the AKT kinase (or PKB), a potent stimulator of cell proliferation and survival [578]. DLG1's anti-proliferative function maybe linked to DLG1's ability to recruit PI3K to E-cadherin complexes at the cell membrane of epithelial cells where the formation of this macromolecular complex is required for their differentiation [228]. Moreover, DLG1 can bind to APC and PTEN together, which forms a ternary complex that could act as a platform for the assembly of a network of signal transduction factors that regulate cell growth [578].

*LGL-1* knockout mice have been described to display hyperproliferation of neuroepithelial cells in the developing brain, which is caused by defects in cell cycle exit [538]. *LGL-1* knockout mice do not have a significant increase of neural progenitor cells [538].

Moreover in epithelial cells, the Scrib complex members regulated cell growth through mediating hippo signaling pathway(explained further page 133) [18].

In general, these studies demonstrate a clear involvement of the Scrib polarity complex in the regulation of cell growth. Nevertheless, even though various mechanisms have been proposed to explain the role of this polarity module in control of proliferation, these regulatory mechanisms are still under investigation.

#### I.E.e.1.1.4 Cell survival

Several studies shown that cell survival, another major cellular process, is influenced by the Scrib complex. Apoptosis is an important mechanism that removes cells during embryogenesis, eliminates potentially cancerous and virus-infected cells, and maintains balance in the adult tissues such as the intestine. The regulation of cell survival is crucial in the organism since the failure of cells to undergo cell death via apoptosis can result in a range of effects from embryonic death to neoplasia or autoimmune disease [579]. Indeed, several studies have provided evidence that loss of apico-basal polarity is an important cue to induce apoptosis. Moreover, Scrib complex components have been directly involved in this mechanism of cell elimination.

In this section I will discuss how Scrib module polarity proteins can influence cell death.

Mutation of the basolateral protein complex genes, *scrib*, *dlg* and *lgl* induced neoplastic growth that lead to large larva in *Drosophila* [481]. However, clonal deletion of these genes in the imaginal disc are eliminated by apoptosis, a molecular mechanism involving Jun N-terminal kinases JNK-mediated activation [580-584]. JNK can mediate apoptosis via upregulation pro-apoptotic genes through the transactivation of certain transcription factors or by directly regulating the activities of mitochondrial pro- and anti-apoptotic proteins via defined phosphorylation events [585].

Indeed, several studies describe that clones mutant for *scrib* were eliminated by surrounding wild-type cells in the imaginal disc eye [584]. In mammalian MDCK cells, the absence of SCRIB in cells surrounded by wild type cells, also induced apoptosis and apical extrusion of *SCRIB*<sup>-/-</sup> cells through the activation of p38 mitogen-activated protein kinase (MAPK) [586].

However, the apoptotic signaling is inhibited in *scrib*<sup>-/-</sup>, *dlg*<sup>-/-</sup>, *lgl*<sup>-/-</sup> mutant clones in the eye disc expressing an activated form of Ras (Ras<sup>V12</sup>), which display an intense tumor-like overgrowth and metastatic behavior [582, 584]. The loss of SCRIB increased primary tumor size in Myc-driven mammary tumors through blocking Myc-induced apoptosis. Here, SCRIB mediates Rac/JNK/c-Jun pathway that regulates the pro-apoptotic protein Bim expression [572].

These studies demonstrate that the Scrib module is able to modulate cell survival.

### I.E.e.1.2 Scrib module role in disease

Disrupting the regulation of epithelial polarization results in abnormal embryonic morphogenesis that lead to diverse diseases [587] and can lead to tumor formation [433, 488].

Since the original identification of Scrib, Dlg and Lgl as neoplastic tumor suppressors in *Drosophila*, much effort has concentrated on understanding their contribution in mammalian cancer progression and metastasis. Here I will describe below evidence demonstrating an important role for the Scribble polarity module in mammalian cancer but also in various human diseases such as some mental disorders including Alzheimer disease and schizophrenia.

#### I.E.e.1.2.1 In non-cancer

In human, mutation in *SCRIB* were found in two different pathologies resulted from neural tube defects known as craniorachischisis [548] and spina bifida [588]. Neural tube defects are the consequence from a failure of embryonic neural tube closure, which leads to a class of human birth defects such as spina bifida. Spina bifida results from failure of the complete low spinal closure. Craniorachischisis is the consequence from failure to initiate the closure process, resulting in the absence of complete closure between the hindbrain and spinal neural tube. The neural tube defects are known to be caused by defects in the planar cell polarity pathway in mice and human [588]. These results confirm the potential of the involvement of SCRIB in the planar cell polarity pathway also in human development.

A recent study by Xu *et al.*, [589] demonstrated that Scribble is down-regulated in autosomal-dominant polycystic kidney disease (ADPKD). The autosomal-dominant polycystic kidney disease (ADPKD) is characterized by the gradual expansion of countless renal cysts in bilateral kidneys, which may lead to end-stage renal disease [590]. Knockdown of Scrib in zebrafish results in the same phenotype characterized by an abnormal pronephric cyst formation [591]. Until recently this disease has been associated with germline mutations in PKD1 or PKD2, which encode for the proteins, polycystin (PC)-1 and PC2, respectively. PC1 and PC2 are expressed in the apical mono-cilia, cytoplasmic endoplasmic reticulum/Golgi membranes, and the basolateral cell surface, and were found to be essential in regulating the function of cell membranes [592]. The study of Xu *et al.* revealed that deregulation of Scribble during kidney development affects the Hippo signaling pathway. Similarly, PKD1 and PKD2 mutations result also in deregulation of the Hippo pathway in the kidney. Thus, these works demonstrate that

Scribble is required for normal kidney development by playing a key role in regulating the Hippo signaling pathway [589].

The role of DLG1 in human pathologies varies depending on its function in the tissue. DLG1 is indeed involved in many pathologies in human, which can be explained by the different roles of DLG1 in the regulation of cell polarity and synapse assembly, and as an organizer of the signal transduction in diverse cellular systems. The function of DLG1 in vesicular trafficking has been shown to be involved in neuropathology. Indeed, DLG1 expression was found augmented in the dorsolateral prefrontal cortex of post-mortem brains from patients with schizophrenia [593, 594]. In addition, in Alzheimer patients, DLG1 ability to bind and regulate the trafficking of several surface proteins including the disintegrin and metalloproteinase 10 (ADAM10) and GluR1 is altered [595]. These results suggest that DLG1 is indispensable for proper neuronal functioning possibly by regulating the intracellular trafficking of various receptors.

#### *I.E.e.1.2.2 In cancer*

In addition to the role of the Scrib complex in the maintenance of tissue homeostasis, this polarity complex has been found altered in various cancer. Indeed, loss of ABCP is an early event in epithelial cancers and can occur at pre-invasive stages [596]. Furthermore, several polarity proteins have altered expression owing to gene amplification, deletion or epigenetic regulation, which frequently associates with disease progression and strongly correlates with occurrence of metastasis.

##### *I.E.e.1.2.2.1 SCRIB and DLG are targets of viral oncoproteins*

High-risk strains of human papillomavirus (HPVs) play a contributing role in the development of cervical cancer. Over 80% of cervical cancers are initiated by the high-risk human papilloma viruses HPV16 and HPV18. HPV16 and HPV18 target PDZ-domain-containing proteins for degradation via the E6 oncoprotein [597]. Here are some examples of polarity proteins contain PDZ domains identified as targets of the E6 oncoprotein; SCRIB, DLG1, MAGI-1 (membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1) and MUPP1 (multi-PDZ- domain protein 1) [598-600]. Since the regulation of cell polarity depends on the correct spatiotemporal expression of the cell polarity complexes, modifications in expression levels or mis-localization of any of these components disturbs the function of the

complex as a whole and result in abnormal signaling that can lead to neoplastic transformation [183].

Interestingly, while most studies revealed the involvement of human virus oncoproteins to inactivate the tumor suppressor role of hDLG1, Frese and co-workers revealed an oncogenic function to hDLG1 upon binding to the adenovirus 9 E4-ORF1[601]. Indeed, they reveal the importance of hDLG1 in promoting Ras mediated PI3K activation that mediated cellular transformation. Hence, they reveal an additional function of oncoprotein E4-ORF1 that oppose to inactivating hDLG1 tumor suppressor, but rather in using hDLG1 in activating PI3K that gives it an oncogenic function. The contradictory roles seem to be related to differences in the precise pattern of expression.

These studies demonstrate the importance of the proper localization and expression of the Scrib module members in order to maintain the homeostasis of the tissue.

#### *1.E.e.1.2.2.2 Abnormal expression of Scrib, Dlg and LGL in human cancer*

An important mechanism by which polarity proteins function is by spatially restraining signal transduction components. Consequently, mutations that alter the localization or protein–protein interactions of polarity proteins with other oncogenic proteins may also affect downstream signaling [596].

Each member of the Scrib polarity module was found mis-localized in several human cancers. Scrib was found mis-localized in half of the ductal carcinoma *in situ* (DCIS) breast cancer lesions [572]. SCRIB and DLG1 has been shown to be mis-localized in colon and cervical cancer, whereas Lgl2 is mis-localized in gastric adenocarcinomas [602-604]. The Scrib polarity module members often display abnormal expression throughout different types of tumor such as colon, cervical, endometrial, breast, prostate and lung [587, 602-606].

Furthermore, overexpression of SCRIB, LGL-1 in transformed cell lines, lead to an increase in cell adhesion that reversed the malignant phenotype, which suggest a direct function of Scrib polarity complex in tumor progression [607, 608]. Generally, these studies approve the idea that abnormal expression of polarity proteins is associated with malignancy and invasion.

Tissue specific knockouts of *SCRIB* in the adult mouse leads to hyperproliferation and cell

morphology changes, and predisposes cells to oncogenic-mediated tumorigenesis in the prostate [587], breast [572], lung [609] and skin epithelial tissues [610], demonstrating a direct link between alteration of Scrib and tumor progression.

#### *I.E.e.2 Role of the Scrib complex in modulation of Signaling Cascades*

In order to establish and maintain tissue integrity, the members of Scrib module localize at cell-cell junctions where and interacts with protein complexes to modulate signaling pathways that play critical roles in cell growth, proliferation and survival [433]. SCRIB, DLG1 and LGL have been involved in the regulation of many different pathways including RTK, WNT, Hippo, thyroid hormone receptor and glutamate receptor signaling. Here, I limit my review on the molecular mechanisms by which the Scrib complex regulates: i- the Hippo pathway; ii- cell surface receptor signaling, since these signaling pathways control cell proliferation and survival in respond to cell polarity.

##### *I.E.e.2.1 Hippo signaling*

Cells that are unable to perceive density failed to regulate growth and over-proliferate, which lead to the development of hyperproliferative diseases including tumorigenesis [611, 612]. The Hippo pathway (described in detail page 93) is well known to regulate organ size in mammals and *Drosophila*. The Hippo/Yap pathway acts through the actin cytoskeleton, cell-cell junctions and apical-basal polarity to sense cell's density. The apical-basal polarity complex play a role in regulating of epithelial cell-density and proliferation, in addition its deregulation is exhibited in various cancer types [18]. Several studies linked the Scrib complex to the regulation of the Hippo pathway.

In *Drosophila* epithelial tissues, the depletion of Lgl or upregulation of aPKC blocks Hippo pathway signaling, resulting in the activation of York1 (Yap1 in mammals), which activates cell proliferation and survival [613]. Lgl/aPKC regulate the Hippo pathway by controlling the localization of Hippo (Mst1/2 in mammalians) and therefore its activity [613]. Indeed, Hippo is activated when recruited to specific membrane regions where it constitute a complex with Expanded, and with its downstream kinase, Warts (LATS1/ 2 in mammalians) [614]. The molecular mechanism explaining how Lgl/aPKC regulates Hippo localization in *Drosophila* is currently unclear. In mammalian cells, overexpression of aPKC results in its binding to the MST1/2 (Hippo orthologs), which leads to uncouple the binding of LATS1/ 2 (Warts ortholog), thus inhibiting LATS1/2 phosphorylation and inactivation of YAP (York1) [615].

Scrib mutation results in abnormal regulation of the Hippo pathway in mammalian, zebrafish and *Drosophila* systems [616-618]. In breast cancer stem cells, SCRIB interacts with TAZ, Mst1/2 and Lats1/2 acting as an adaptor protein, which inhibits TAZ activity [619]. Either loss or mis-localization of SCRIB blocks the ability of Mst1/2 to activate Lats1/2, resulting in constant TAZ activation [619]. However, it is uncertain if the same regulation happens in other organisms. In fact, Scrib regulates Hippo signaling by another mechanism in zebrafish. Scrib interacts with FAT1, an upstream regulator of Hippo signaling, and together with FAT1 blocks YAP activity [618]. In *Drosophila scrib* mutants exhibited high activity of Yorkie [620]. Scrib acts downstream of the Fat (Ft) receptor in *Drosophila*, demonstrating that Scrib regulates cell growth through Hippo signaling [621].

In *Drosophila*, however, no direct interaction between Hippo pathway members and Scrib or Dlg have been defined. Actually, *scrib* and *dlg* mutants block Hippo signaling only when cell polarity is lost, and cell death is also inhibited [620].

Some membrane-associated proteins, including the Merlin/NF2 tumor suppressor and Kibra, also interact with the core kinase components and act as common upstream activators of the Hippo pathway [622]. Merlin/Nf2 tumor suppressor and Kibra appear to connect cadherin ligation to the Hippo cascade [623-625]. Merlin directly associates with  $\alpha$ -catenin to promote maturation of the adherens junction. In both *Drosophila* and mammals, Merlin promotes Hippo signaling by targeting LATS (Warts ortholog) to the cell membrane. Furthermore, Merlin is able to physically link  $\alpha$ -catenin to the apical polarity complex protein PAR3. This interaction is required during early stages of junction formation and polarity establishment. Thus, during junctional maturation, Merlin is found to be specifically essential for the organization of adhesion and polarity [413]. This data suggests a mechanism whereby cadherin junction and polarity complexes may function together to sense the integrity of the epithelium and regulate this pathway in certain cells.

The ability of numerous components of cell adhesion and polarity complexes to bind YAP1/TAZ could assure its sequestration out of the nucleus in cells only after junction formation and when apical–basal polarity is entirely established. This is beneficial by sensitizing the cell to a variety of changes to the epithelial state, which allow a graded activation of YAP1. For instance, the ablation of cell polarity can activate certain YAP1/TAZ

pools, while more severe polarity and adhesion disruption can generate a stronger growth response through the release of several YAP1/TAZ pools [596].

#### I.E.e.2.2 Role in regulating cell surface receptors

Different studies have shown Scrib module polarity members modulating the signaling of various cell surface receptors in order to modulate diverse cellular process. Scrib module control growth and polarity of epithelial cells through the regulation of receptor aggregation and trafficking. These receptors include receptor tyrosine kinase (RTK), thyroid hormone receptor, glutamate receptor. Here, I will describe some of the receptors that are regulated by the Scrib module.

##### I.E.e.2.2.1 Receptor tyrosine kinase

Receptors tyrosine kinase (RTK) are transmembrane receptors that contain a tyrosine kinase activity in their cytoplasmic domain. Many growth factors, cytokines and hormones receptors belong to the family of RTK. Activation of these receptors by the fixation of their ligand in their external domains leads to the activation of the Tyrosine kinase. The RTK activity is crucial in various processes such as cell growth, cell survival, differentiation and motility. Hence, RTK should be tightly regulated, since deregulation in the signaling pathway can lead to cancer progression.

Studies have demonstrated the involvement of the Scrib complex proteins in the regulation of RTK [488].

Lgl2 is involved in the negative regulation of the ErbB2-Ras signaling pathway in the zebrafish epidermal cells. The study showed that *lgl2* (*pen* in zebrafish) mutants displayed enhanced ErbB2 signaling, which result in alteration of E-Cadherin localization and induction of EMT [626]. The absence of Lgl2 induce ErbB2 signaling activation which resulted in Sgk1 upregulation. Sgk1 is a molecules regulating E-cadherin function at the cell membrane through phosphorylating NdrG1, a protein implicated in E-cadherin vesicular recycling [626]. This suggest the Lgl2 modulate ErbB2-Ras signaling, by decreasing its activity, in order to maintain epithelial phenotype and integrity.

DLG1 was found to regulate the signaling FGFR2 and fiber cell differentiation in the mouse lens [627]. FGF stimulation upstream Erk activation is essential for lens proliferation and differentiation [628]. Conditional knockout of *DLG1* in mice lens reduced FGFR2 levels and therefore its activity resulting in a strong decrease of Erk activation in the epithelial cells. DLG1 is necessary to stabilize and localize FGFR2 in epithelia cells. DLG1 is found to co-localize with



FGFR at the cell membrane in the fiber lens. However, a direct interaction between the two molecules was not demonstrated [627]. This study demonstrates the importance of DLG1 in modulating cell fiber differentiation through the regulation of FGFR2 activity.

SCRIB regulates the Hepatocyte Growth Factor (HGF) signaling in the MDCK cysts. HGF-treatment of 3D-cultured MDCK cysts results in partial EMT, associated with formation of actin-rich extensions from the basal surface of the cells. These extensions are observed during the initial step of tubulogenesis. Depletion of SCRIB decreases HGF-mediated cell morphogenesis [629]. Mechanistically, SCRIB interacts with the Rho family GEF ( $\beta$ PIX) and p21 activated kinase (PAK) downstream of HGF signaling. Studies has shown that SCRIB/ $\beta$ PIX interacts with PAK at the leading edge of migrating cells to promote actin polymerization and cytoskeleton organization [630]. SCRIB/ $\beta$ PIX is needed for the correct localization of phosphorylated active PAK during tubulogenesis in MDCK cysts [629]. These studies demonstrate the requirement of SCRIB/ $\beta$ PIX in modulating PAK localization and activation downstream HGF-mediated signaling for proper tubulogenesis.

Altogether these different studies suggest that the Scrib complex proteins regulate RTK activity by regulating the receptor trafficking or localization

#### I.E.e.2.2.2 Thyroid stimulating hormone receptor

TSHR physiological role is mainly to regulate thyroid gland growth in embryos and homeostasis in the adult. TSHRs localization and density on thyroid membranes are critical for TSH responsiveness and thyroid function. Studies have shown that, upon TSH stimulation, TSHR is rapidly internalized by clathrin-coated pits by in various thyroid and non-thyroid cells [631]. SCRIB regulates the G protein-coupled thyroid stimulating hormone receptor (TSHR) recycling process following receptor activation by TSH. Lahuna *et al.*, demonstrated that SCRIB bind directly to TSHR, via one of its PDZ binding domains, in order to recruit the  $\beta$ PIX-GIT1-ARF6 complex that is involved in vesicle trafficking [487] and is required for TSHR recycling to plasma membrane in mammalian cells [632].

#### I.E.e.2.2.3 Glutamate receptor

The proper trafficking of glutamate receptors to synapses is fundamental for synaptic function and plasticity. The postsynaptic density (PSD) is a postsynaptic membrane where synaptic scaffold proteins assemble high concentration of glutamate receptors (composed of N-methyl-d-aspartate receptor (NMDAR) and  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPA)) in excitatory synapses, which are connected with signaling

proteins and cytoskeletal components. AMPA and NMDA receptors are tetrameric transmembrane ion channels which regulates Ca<sup>2+</sup> entry following activation of the neurotransmitter glutamate.

DLG1 plays a major role in the trafficking and targeting of membrane ion channels and cytosolic structural proteins in several cell types. DLG1 was found localized throughout the secretory trafficking pathway and at the postsynaptic density (PSD) in neurons [633]. DLG1 is found to be essential for NMDAR trafficking to the synapse, DLG1 is also involved in AMPAR trafficking to the synapse [634, 635]. Therefore, regulation of membrane targeting of these receptors by DLG1 controls neuronal excitability, synaptic transmission and plasticity, which is essential for normal brain function [17].

DLG1 regulates AMPARs in synaptic plasticity via targeting the AMPAR subunit GluA1 forward trafficking from the Golgi network to the plasma membrane. GluA1 recruitment is a crucial step for promoting and preserving forms of synaptic plasticity such as long-term potentiation (LTP). DLG1 interact directly with the AMPAR subunit GluA1 in contrast to other members of the MAGUK family [636]. The mechanism behind the transport of AMPA receptors to the postsynaptic plasma membrane by DLG1 is dependent of the tri-partite association of Dlg1-myosin VI (an actin-based motor) and GluA1, where DLG1 function as a molecular link between GluA1 and myosin VI [637].

DLG1 regulates NMDAR trafficking by interacting with GluN2A/2B subunits through one of its PDZ domain. Jeyifous, Waites et al., revealed that DLG1 uses the Golgi outpost secretory pathway when in a complex with NMDARs. CASK, a molecular partner of DLG1, is needed for the DLG1/NMDAR complex sorting. CASK distinguishes the sorting of NMDARs from AMPARs by DLG1 in the somatic ER, leading NMDARs to mainly avoid somatic Golgi and transport via dendritic Golgi outposts to synapses in hippocampal neurons [638]. Indeed, CASK requires an L27 domain-mediated interaction to associate with the DLG1, which stabilize DLG1 extended conformation that favorably interact with NMDAR complex [639].

However, it is important to point out that certain experiments *in vivo* were not able to show the necessity of DLG1 in the regulation of AMPAR and NMDAR. These results can be explained by the fact that mammalian neuronal tissue has, in addition to DLG1 (SAP-97), three homologous proteins named postsynaptic density-95 (PSD-95), postsynaptic density-93 (PSD-

93) and synapse-associated-protein-102 (SAP-102), which are thought to play a key role in synapse-assembly and plasticity [492, 640]. The *in vivo* importance of DLG1 for brain function is unclear, taking in consideration the high degree of functional redundancy among these four proteins.

In rats, Scrib1 is involved in the functional and spatial control of synaptic NMDAR. Scrib1 is found co-localized with NMDAR at excitatory synapses of the postsynaptic density in the stratum region of the hippocampus. Scrib1 interacts directly with GluN2 subunits. Scrib1 levels controlled the trafficking and recycling of NMDAR to the membrane via the direct interaction with the AP2 adaptor proteins and the help of the regulatory proteins ARF6, which is implicated in trafficking of biological membranes and transmembrane protein cargo. Moreover, synaptic NMDAR currents is decreased in depleted Scrib1 in hippocampal neurons [635].

In conclusion, the members of the Scrib complex establish and maintain tissue integrity through its implication in regulating the activity of several cell membrane receptors. Interestingly, the regulation of the signaling of these receptors was mainly modulated through trafficking and recycling to the cell membrane. These findings propose that the Scrib complex can regulate spatially the activity of several receptors, which reinforce the importance of the asymmetric distribution of polarity proteins within the cells.

## II. Rational and objectives of the thesis

The superfamily of TNF receptors (TNFRSF) plays a determinant role in the control of both inflammatory responses and apoptotic cell death. Among the TNFRs, Fas (CD95/TNFSFR6), one of the best-studied apoptosis inducers, is found ubiquitously expressed in human tissues including many epithelia such as in the intestine. Its activation by its ligand (FasL) can induce both death and non-death signaling pathways including secretion of pro-inflammatory cytokines, cell migration and proliferation. The pro-death role of Fas depends on the rapid recruitment to its cytoplasmic death domain (DD) of adaptor protein FADD and the initiator caspases-8 and -10 to form the death-inducing signaling complex (DISC), which leads to the cleavage and the activation of these caspases and ultimately to cell destruction [81]. It is only more recently that the non-apoptotic Fas signaling pathways have been more fully appreciated with the report that activated Fas is able to recruit different signaling modulators, which may not automatically leads to caspase-8/10 activation and cell death [93]. However, so far, the molecular mechanisms controlling the inhibition of cell death and the induction of pro-survival pathways of Fas remains largely unclear. This question plays a central role in a context of tumorigenesis since cancer cells (including CRC cells) repress Fas cell death pathways and activate non-cell death Fas signaling to increase their invasiveness [172, 641]. Therefore, the activation of Fas signaling in these tissues should be rigorously regulated in order to maintain the balance between cell death and non-death signaling.

The main aim of our team is to progress in the understanding of the molecular mechanism that regulate the pro- or non- death Fas signaling in order to better predict the outcome of Fas signaling in both normal and pathological conditions including tumor development.

On the way to decipher the role of the Fas signaling pathways, our lab met an apparent biological contradiction that puzzles the domain of the death receptor Fas since several years: whereas Fas receptor is highly expressed in most of the epithelia (including gut), the polarized epithelial cells are resistant to Fas induced cell death possibly induced by circulating or resident FasL presenting immune cells. In contrast, intestinal cells of patients suffering of inflammatory bowel diseases are characterized by an elevated percentage of apoptotic cells, which correlates with an altered formation of cell-cell junctions and a loss of cell polarity [642]. These observations suggest that maintenance of cell polarity and cell-cell adhesion may protect epithelial cells from cell death induction possibly due to the regulation of TNFR receptor members.

Programmed cell death should be tightly regulated during embryogenesis and the adult life in order to maintain tissue homeostasis and integrity. At a cellular level, the choice of life and death, is regulated by different environmental cues including cell-cell junctions and cell-ECM adhesion through the involvement of various sets of specialized macromolecules [643, 644]. Several studies demonstrated the involvement of AJ components, including cadherins and catenins, in mediating cell survival (see introduction page 83). In brief, several studies exhibited that conditional knockout of cadherins in different tissues including intestinal epithelia [348], lactating mammary gland [349] and in ovaries [350] resulted in loss of adhesion and induced apoptosis *in vivo*. The ablation of  $\alpha$ -catenin in mice epidermis is also associated with an important apoptosis of keratinocytes [355]. Mutation in  $\beta$ -catenin's gene resulted in apoptosis of mammary stem cells in mice [357]. However, the molecular mechanism by which AJ components control cell survival have been much less studied.

Based on these observations, our lab decided to investigate the possible involvement of cell-cell junction and role of cell polarity establishment in the regulation of the death receptor Fas/TNFR members signaling upon engagement by its (their) cognate ligand.

This study was initiated by my PhD co-director Dr. Laurent Gagnoux is part of my PhD studies who found that formation of cell-cell junction protected epithelial cells from Fas cell death signaling and that this protection is released when junctions are disrupted. My PhD studies were developed around the two following objectives:

## II.A My thesis objective 1: Characterization of the role of AJ and polarity in the regulation of Fas cell death signaling

L.Gagnoux found that formation of cell-cell junction, and in particular adherens junctions, protected epithelial cells from Fas cell death signaling. He demonstrated that in colon epithelia, the receptor Fas concentrates at cell-cell junctions with E-cadherin and that Fas-cadherin association protects cells from FasL-induced cell death. Additionally, thanks to a proteomic approach, it was found that the polarity molecule DLG1 interacts with the C-terminal PDZ-binding site of Fas, possibly regulating the localization of Fas at cell-cell junction and influencing Fas cell death signaling.

When I joined the team, I first continued the ongoing study on characterizing the role of AJ and DLG1 polarity protein in the regulation of Fas cell death signaling.

More specifically we were interested in understanding:

- If Fas signaling could be regulated by different cadherins.
- The molecular mechanism by which DLG1 influences Fas death and non-death signaling.
- If DLG1 is able to modulate Fas-signaling independently of the formation of AJ.

In order to answer these questions, we used the colon epithelial cells HCT15 that are known to form extensive cell-cell junctions and mature AJ when they reach confluency. Along the way I used both biochemical and molecular biology techniques such as transfection, infection of viruses and generating stable expressing mutants, PCR, RT –PCR, Oligo designing, siRNA designing and utilized spinning disc microscopy in order to examine AJ role in Fas signaling.

## II.B My thesis objective 2: Investigation of the role of novel interactants of Fas in the modulation of receptor signaling and trafficking

In the second part of my PhD, I was interested in deciphering the role of the newly identified Fas molecular partners, interacting with its C-terminal PDZ-binding site [645], in the regulation of Fas signaling. Indeed, besides DLG1, our proteomic analysis revealed that 53 new molecular partners are capable to bind the C-terminal region of Fas.

Among the proteins identified in the proteomic approach, we chose to study the effect on Fas signaling of the five following proteins: the actin-binding LIM (abLIM1), the E3 ubiquitin ligase Ligand Numb-protein X2 (LNX2), the endoplasmic reticulum chaperone GRP78, the polarity protein PATJ and the adaptor protein Beta-2-syntrophin (SNTB2). These proteins were chosen based on their known involvement in the regulation of cell surface receptor signaling, apoptosis, stress response or polarity.

My objective in this second part of my thesis studies was to understand how the chosen Fas partners may regulate Fas signaling. To examine this, I was determined to answer these questions.

- Does Fas chosen partners regulate Fas signaling?
- If yes, how do they modulate Fas signaling?

- Do they co-localize with Fas in the cell and influence Fas receptor localization or trafficking?

To answer these questions, I used the same techniques than the ones described above.



### III. Results

### III.A Project 1: Characterization of the role of AJ in the regulation of Fas cell death signaling

In this study, we were able to display that both cell polarity establishment and adherens junction formation control the pro-apoptotic signaling of the death receptor Fas. We demonstrate that:

- Fas receptors concentrate at cell-cell junctions together with E-cadherin and that Fas-cadherin association protects cells from FasL- induced cell death.
- The polarity/scaffold molecule DLG1 interact with the C-terminal PDZ-binding site of Fas and attenuates Fas cell death signaling.
- DLG1 inhibits Fas cell death signaling independently of the presence of mature AJ. Indeed, DLG1 interacts directly with Fas and decrease the death- inducing complex formation upon Fas activation, therefore, providing an additional mechanism to protect against cell death.

Taken together, our data show that inhibition of FasL-induced cell death by Fas-cadherin-DLG1 complex helps to maintain epithelial homeostasis by protecting normal epithelia from apoptosis and promotes elimination of compromised non-polarized cells to avoid development of pathological conditions such as cancer development.

[Article: Cell polarity and adherens junction formation inhibit epithelial Fas cell death receptor signaling](#)

Laurent Gagnoux-Palacios, **Hala Awina**, Stéphane Audebert, Aurélie Rossin, Magali Mondin, Franck Borgese, Carlota Planas-Botey<sup>1</sup>, Amel Mettouchi, Jean-Paul Borg, and Anne-Odile Hueber

*Journal of cell biology*, 2018.

REPORT

# Cell polarity and adherens junction formation inhibit epithelial Fas cell death receptor signaling

Laurent Gagnoux-Palacios<sup>1</sup>, Hala Awina<sup>1</sup>, Stéphane Audebert<sup>3</sup>, Aurélie Rossin<sup>1</sup>, Magali Mondin<sup>1</sup>, Franck Borgese<sup>1</sup>, Carlota Planas-Botey<sup>1</sup>, Amel Mettouchi<sup>2</sup>, Jean-Paul Borg<sup>3,4</sup>, and Anne-Odile Hueber<sup>1</sup>

**Finely tuned regulation of epithelial cell death maintains tissue integrity and homeostasis. At the cellular level, life and death decisions are controlled by environmental stimuli such as the activation of death receptors. We show that cell polarity and adherens junction formation prevent proapoptotic signals emanating from the Fas death receptor. Fas is sequestered in E-cadherin actin-based adhesion structures that are less able to induce downstream apoptosis signaling. Using a proteomic-based approach, we find that the polarity molecule Dlg1 interacts with the C-terminal PDZ-binding site in Fas and that this interaction decreases formation of the death-inducing complex upon engagement with Fas ligand (FasL), thus acting as an additional cell death protection mechanism. We propose that E-cadherin and Dlg1 inhibit FasL-induced cell death by two complementary but partially independent mechanisms that help to maintain epithelial homeostasis by protecting normal polarized epithelia from apoptosis. When polarity is lost, the Fas-cadherin-Dlg1 antiapoptotic complex is disrupted, and FasL can promote the elimination of compromised nonpolarized cells.**

## Introduction

Programmed cell death by apoptosis is a physiological process that leads to the elimination of damaged or potentially harmful cells to maintain tissue homeostasis. As a consequence, evading apoptosis is an integral part of tumor development and resistance to therapy (Hanahan and Weinberg, 2011). Cytokines of the TNF receptor family including the Fas receptor (CD95; TNFRSF6) are among the best-characterized apoptosis inducers. The apoptotic signaling induced by Fas upon activation by its ligand (FasL; TNFSF6) depends on the recruitment to the receptor of adaptor proteins and caspases forming a death-inducing signaling complex (DISC). This complex triggers caspase activation, responsible for the proteolytic cleavage of a broad spectrum of cellular targets, leading ultimately to cell death (Kischkel et al., 1995). Beside this prodeath function, Fas activation can also promote alternative nondeath signaling pathways leading to cell survival, proliferation, motility, cancer growth, and metastasis, depending on the cellular context (Peter et al., 2007). Invalidation and mutations of the Fas/FasL system in animal models and in human pathologies mainly demonstrate that Fas regulates tissue homeostasis in the immune system through the induction of apoptosis (Ramaswamy et al., 2009). Nevertheless, previous

work reports that Fas is expressed in almost all human tissues including many epithelia such as the intestine (Chen et al., 2010). Therefore activation of Fas in these tissues has to be strictly controlled in order to maintain an equilibrium between survival and cell death signals.

At a cellular level, the life-and-death decision is controlled by different environmental cues including cell anchorage, which guarantees both cell-ECM adhesion and cell-cell adhesion thanks to different sets of specialized macrocomplexes. Both types of adhesion complexes have been shown to protect from cell death, but the exact mechanisms underlying these observations are still debated (Grossmann, 2002; Stupack and Chersesh, 2002). Cell-cell contacts are mediated by specialized cell surface receptors including E-cadherin, the main cell-cell adhesion molecule expressed in epithelial tissues. The cytosolic domain of E-cadherin interacts with catenins ( $\alpha$ -,  $\beta$ -, and p-120), which are adaptor molecules that connect the receptor to the actin cytoskeleton to form intercellular adhesion complexes called adherens junctions (AJs; Niessen et al., 2011). AJs play a master organizer role in the establishment of apical-basal polarity and are found functionally associated with the Scrib-Dlg1-Hugl1 polarity complex (Reuver

<sup>1</sup>Université Côte d'Azur, Centre National de la Recherche Scientifique, Inserm, Institute de Biologie Valrose, Nice, France; <sup>2</sup>Institut Pasteur, Département de Microbiologie, Unité des Toxines Bactériennes, Université Paris Descartes, Paris, France; <sup>3</sup>Aix Marseille Université, Centre National de la Recherche Scientifique, Inserm, Institut Paoli-Calmettes, Centre de Recherche en Cancérologie de Marseille, Marseille Proteomics, Marseille, France; <sup>4</sup>Centre de Recherche en Cancérologie de Marseille, Cell Polarity, Cell Signaling, and Cancer, Equipe Labellisée Ligue Contre le Cancer, Aix Marseille Université, Centre National de la Recherche Scientifique, Inserm, Institut Paoli-Calmettes, Marseille, France.

Correspondence to Laurent Gagnoux-Palacios: [gagnoux@unice.fr](mailto:gagnoux@unice.fr); Anne-Odile Hueber: [hueber@unice.fr](mailto:hueber@unice.fr).

© 2018 Gagnoux-Palacios et al. This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

and Garner, 1998; Qin et al., 2005). In addition, AJs act as signaling platforms and are directly involved in the control of various pathways (Kobiela and Fuchs, 2006; McCrea et al., 2015). Their signaling capacities are at least in part due to their ability to associate with some cell surface receptors and to modulate their activities (Qian et al., 2004; Curto et al., 2007; Fukuhara et al., 2008; Lu et al., 2014). Alteration of AJs in epithelia is associated with loss of cell–cell contacts and cell polarity, which leads to uncontrolled proliferation and carcinoma formation (Jeanes et al., 2008; Stairs et al., 2011). However, loss of AJs in various animal models or in in vitro studies has been also associated with increased cell death (Carmeliet et al., 1999; Hofmann et al., 2007; Schneider et al., 2010), which is in agreement with a role of cell–cell junctions in the regulation of cell survival.

The possible role of cell–cell contacts and cell polarity in the control of Fas signaling has been largely unexplored. In this study, we report that E-cadherin plays a critical role in the regulation of Fas cell death signaling. Indeed, we demonstrate that in polarized colon epithelial cells, Fas is sequestered in E-cadherin actin-based adhesion structures, preventing its cell death signaling function. Moreover, we identify that the C-terminal PDZ domain of Fas interacts with the polarity molecule Dlg1, which accumulates together with AJs at cell–cell junctions. The interaction of Dlg1 with Fas inhibits Fas cell death signaling by impairing efficient formation of the DISC. Therefore, our findings uncover an important mechanistic link between cell–cell contacts and cell polarity along with the protection of polarized cohesive epithelial tissues from the Fas-induced death signal.

## Results and discussion

### Cell–cell contacts protect epithelial colon cells from FasL-induced cell death

To evaluate the importance of human epithelial tissue organization, and in particular cell–cell junctions, in the regulation of Fas proapoptotic signaling, we first analyzed the FasL-induced sensitivity of HCT15 cells, a human epithelial cell line of colon origin, which forms extensive cell–cell junctions and grows as independent islets (Fig. 1 A). Using time-lapse video microscopy experiments, we showed that induction of cell death by addition of FasL in HCT15 cells occurs preferentially in the cells located in the islet periphery that do not form mature junctions or in cells that do not form a cohesive islet (Fig. 1 A and Video 1). In contrast, cells that form extensive cell–cell junctions (i.e., located in the center of the islet) are protected from cell death. Treatment of HCT15 with staurosporine, a widely used chemical promotor of intracellular stress apoptosis, did not reveal a protective effect of cell–cell junctions (Fig. 1 A and Video 2), suggesting that cell–cell contacts specifically affect Fas signaling rather than having a general antiapoptotic effect. To rule out the possibility that the cell death resistance of cells located in the center of the islet was due to a limited diffusion of FasL through tight junction assembly, HCT15 cells were grown on permeable culture inserts. Video microscopy experiments showed that FasL treatment in both apical and basal reservoirs did not sensitize the central cells of the islet to FasL, demonstrating that this protective effect cannot be explained by a restriction of FasL diffusion at the basal side of the cells (Video 3).

Since maturation of cell–cell contacts including tight junctions and AJs requires the presence of extracellular calcium (Takeichi, 1988), we then analyzed the FasL-mediated cell death induction in HCT15 cells in which cell junctions were disrupted by growing cells in low-calcium conditions (Fig. 1 A and Video 4). Depletion of calcium resulted in a strong increase of FasL-induced cell death (Fig. 1, B–D and I), confirming the protective effect of cell–cell contacts in the regulation of Fas proapoptotic signaling.

To further characterize the nature of the cell–cell adhesion complex involved in the regulation of Fas signaling, we evaluated the role of AJs, the major cell–cell contact organizers (Halbleib and Nelson, 2006; Niessen et al., 2011), by specifically targeting their assembly. This was achieved by the transfection of HCT15 cells with siRNAs directed against either  $\alpha$ -catenin, which is essential to nucleate mature AJs (Vasioukhin et al., 2001), or E-cadherin. We showed that the partial knockdown of AJ components alters cell–cell contact formation and results in a strong increase in the number of cells sensitive to FasL-mediated cell death (Fig. 1, E–H and J). Altogether, these experiments suggest that the formation of AJs in human epithelial cells inhibits the proapoptotic function of the Fas receptor.

### Fas associates and forms a stable complex with AJs

Since the formation of AJs regulates Fas cell death signaling, we analyzed the localization of Fas at the border and the center of the cell islets. We found that at cell–cell contacts of confluent cells, Fas strongly colocalizes with E-cadherin (Fig. 2, A–C) and  $\alpha$ -catenin (Fig. 2 F; Pearson coefficient of 0.815 and 0.655 for E-cadherin and  $\alpha$ -catenin, respectively). Fas colocalized with E-cadherin together with actin in the zona adherens located at the apical end of lateral cell–cell contacts (Fig. 2, A and B, arrows) but also in cell contacts formed along the lateral surface of HCT15 epithelial cells (Fig. 2 B, arrowheads). Interestingly, Fas was less enriched in AJs in cells located at the periphery of the islet (Fig. 2, A and D), suggesting that maturation of cell–cell contacts favors Fas–E-cadherin association. Using proximity ligation assays (PLAs), we confirmed the molecular proximity of Fas and AJ components in HCT15 cells (Fig. 2 E). A similar colocalization of Fas and AJs was observed in human colon epithelial tissues, showing that Fas undergoes a similar polarized expression in vivo (Fig. 2 G).

To finely evaluate the role of AJs in the polarized expression of the Fas receptor, its localization was analyzed by immunofluorescence (IF) in HCT15, in which cell–cell contacts were disrupted by inhibiting  $\alpha$ -catenin expression with siRNA. As shown in Fig. 2 F, in the absence of cell–cell junctions, Fas is no longer limited to a specific domain of the plasma membrane but rather becomes expressed all over the plasma membrane.

The formation of mature AJs corresponds with the assembly of cadherins and various cytoplasmic molecules together with the actin cytoskeleton into a macromolecular complex characterized by its detergent insolubility (Kinch et al., 1995). Since Fas associates with AJs in confluent cells, we analyzed whether the detergent solubility of Fas was dependent on AJ formation. Detergent solubility assays demonstrated that the proportion of Fas molecules associated with the detergent-insoluble cytoskeletal fraction decreased in HCT15 cells transfected with either  $\alpha$ -catenin or E-cadherin siRNAs compared with cells transfected

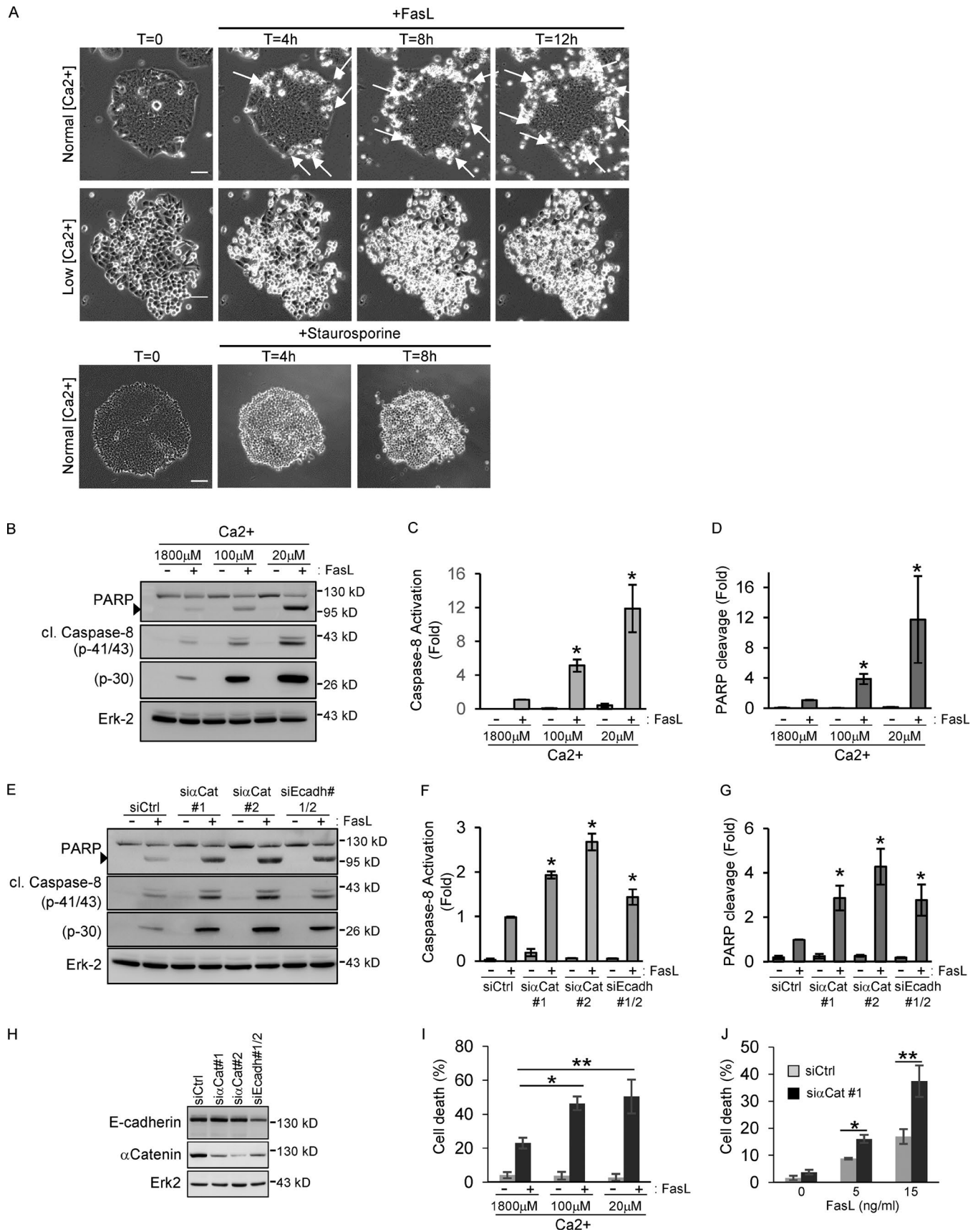


Figure 1. **Ajs protect epithelial colon cells from FasL-induced cell death.** (A) HCT15 cells were grown in normal media or in low-calcium conditions (20 μM) before FasL (20 ng/ml + M2) or staurosporine (20 μM) treatment. Cell death was analyzed by phase-contrast time-lapse microscopy. Arrows indicate the localization of dead cells. Bar, 50 μm. (B–D) HCT15 cells were grown in media containing various concentrations of calcium (from 1,800 μM [normal

with a control siRNA (Fig. 2, H and I). Altogether, our data suggest that maturation of AJs controls the presence of Fas at sites of cell–cell contacts and its molecular association with this adhesion complex.

### Sequestration of Fas in AJs inhibits FasL binding to the plasma membrane and consequently cell death signaling

To determine whether the inhibition of FasL-mediated cell death signaling found in confluent cells was due to a less-efficient binding of FasL to the cell surface, we developed a FasL-binding assay using FasL-coated beads. FasL-coated beads and noncoated beads were added to HCT15 cells forming mature cell–cell contacts or in which AJs were disrupted by transfecting cells with an  $\alpha$ -catenin siRNA. Following 30 min incubation, FasL binding efficiency was determined using microscopic observations, bead counting, and IF studies (Fig. 3, A–E).

We showed that FasL-coated beads bound efficiently to HCT15 cells compared with noncoated control beads (Fig. 3 B). Moreover, IF studies followed by quantitative analyses revealed that FasL-coated beads induced a progressive accumulation of Fas and caspase-8 to small patches located beneath the area of bead–cell contacts (Fig. 3, C–E). As expected, a longer activation time (90 min) led to a strong cytosolic accumulation of activated caspase-8 associated with cell shrinkage (Fig. 3 C). Fas and caspase-8 recruitments were mostly observed beneath the beads attached to cells located at the border of the islet, suggesting that the efficiency of FasL binding is improved in bordering cells in which Fas does not accumulate in AJs. We next used the same FasL-coated bead assay to isolate Fas receptors together with DISC components. In agreement with our IF results, the quantity of Fas immunoprecipitated at each time point strongly increases during activation time, suggesting that the aggregation of Fas underneath each bead is a dynamic process (Fig. 3 F). These results demonstrated that FasL-coated beads preferentially bind to epithelial cells located at the margin of the islet, which do not form extensive cell–cell junctions.

We then compared the FasL-coated bead-binding efficiency and the caspase activation of HCT15 cells in which AJs were disrupted by knocking down  $\alpha$ -catenin expression using specific siRNA. In the absence of AJs, FasL-coated beads were able to bind efficiently to the cells within the islet independently of their position and not only to the cells located at the periphery (Fig. 3 A), suggesting that the absence of AJs sensitizes cells within the islet to FasL binding. Counting the beads bound in each culture condition confirmed that the absence of AJs strikingly increased the binding efficiency of FasL-coated beads compared with control cells (siCtrl; Fig. 3 B). In agreement with these observations, more DISC components (Fas, caspase-8, and FADD) were pulled down by FasL-coated beads in  $\alpha$ -catenin–knockdown cells com-

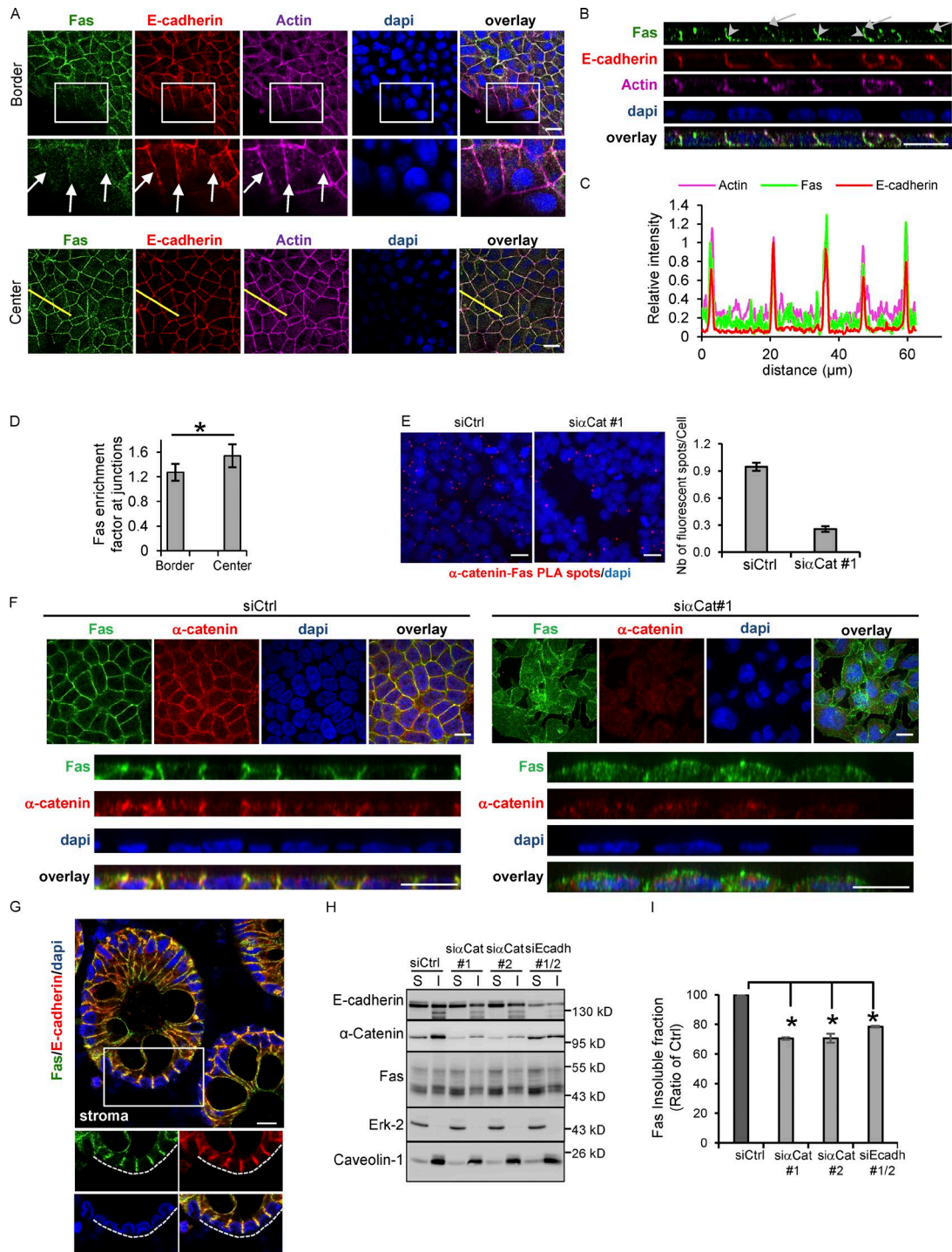
pared with control cells (Fig. 3, F and G). Altogether, our results demonstrate that the disruption of AJs increases the availability of the Fas receptor for ligand binding, which strongly increases epithelial cell sensitivity to FasL-induced cell death.

### The Fas C-terminal PDZ binding site interacts with various PDZ proteins and is required to stabilize and localize Fas at cell–cell contact

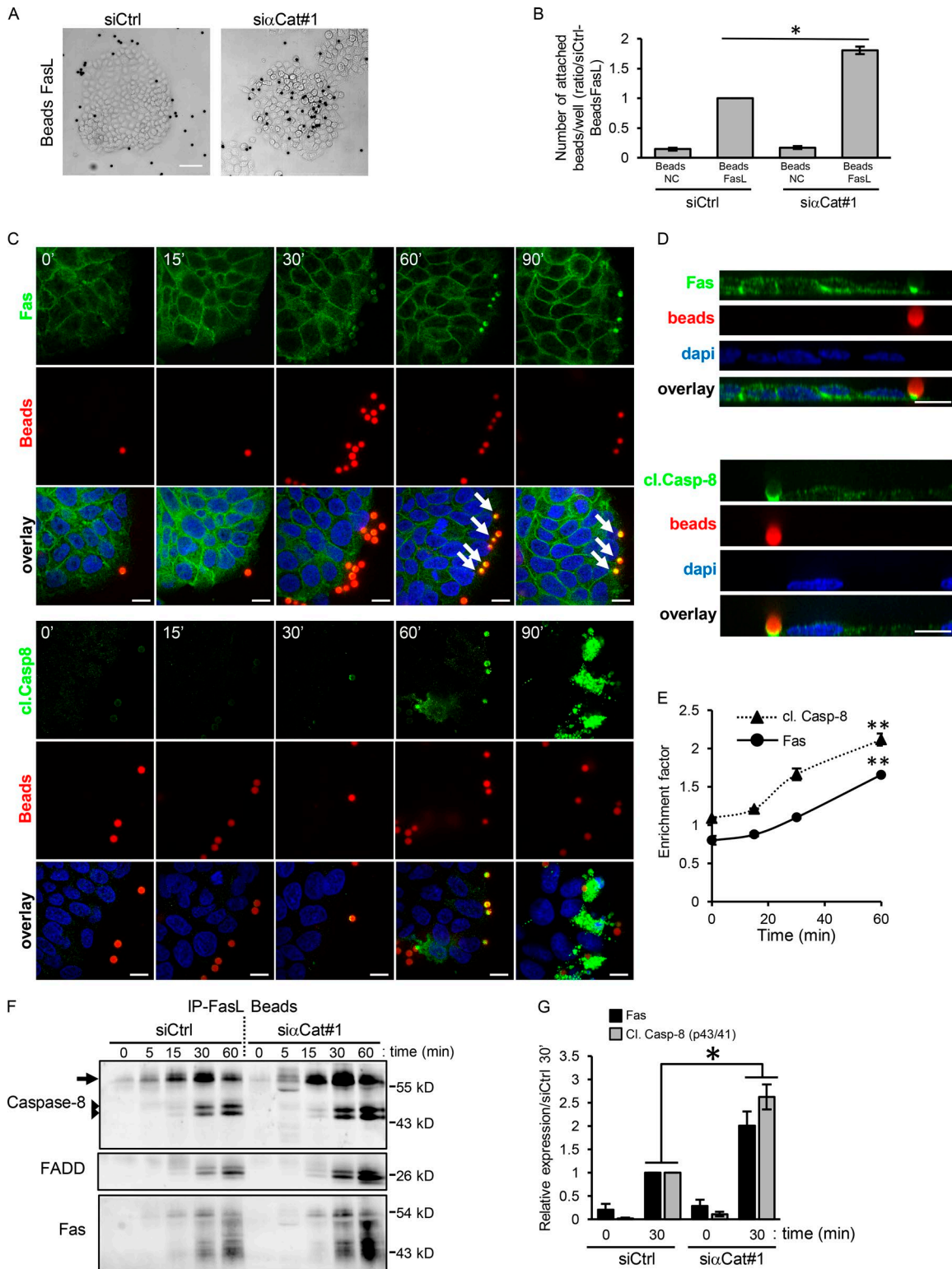
Recent examples demonstrate that PDZ domain-containing molecules interact directly with various cell surface receptors through a PDZ-binding site to target them to specific membrane domains including cell–cell contacts (Borg et al., 2000; Curto et al., 2007; Theisen et al., 2007; Chiasson-MacKenzie et al., 2015). Since Fas has been reported to contain a PDZ C-terminal binding motif (Yanagisawa et al., 1997), we tested the possibility that this domain was involved in the subcellular localization of Fas at cell–cell contacts. We generated HCT15 cells stably expressing a GFP-tagged version of the WT or a mutant form of Fas in which the last three amino acids (SLV), which compose a class I PDZ-binding motif (FasGFP $\Delta$ SLV), were deleted (Fig. 4 A). Confocal analysis of the subcellular distribution of the FasGFPWT and FasGFP $\Delta$ SLV ectopic receptors was performed on HCT15 cells stably expressing these constructs and forming extensive cell–cell junctions. This experiment showed that whereas FasGFPWT mainly colocalizes with E-cadherin at the lateral cell–cell junctions, FasGFP $\Delta$ SLV not only does not efficiently associate with cell–cell junctions but also displays a cytosolic localization (Fig. 4, B and C). Cell surface biotinylation assays confirmed that the FasGFP $\Delta$ SLV mutant is significantly less expressed at the cell surface compared with the WT form of Fas (Fig. 4, D and E). Detergent solubility assays demonstrated that the FasGFP $\Delta$ SLV mutant is more soluble than its WT counterpart (Fig. S1A), which correlates with a reduced association of the Fas PDZ mutant to cell–cell junctions. Thus, these data suggest that the Fas C-terminal PDZ binding site is required to target and/or to sequester Fas at cell–cell junctions.

We then used a previously described proteomic approach (Belotti et al., 2013) to identify the Fas molecular partner(s) able to associate with the PDZ-binding site of Fas and possibly be involved in its subcellular localization. Two peptides corresponding respectively with the last 20 amino acids of WT Fas and a mutant form lacking the PDZ-binding motif (Fas $\Delta$ SLV; Fig. 4 A) were bound to agarose beads and used as baits to pull down Fas molecular partners from HCT15 cell extracts. Following pull-down experiments, proteomic analysis allowed us to identify 53 molecules that were significantly pulled down with the FasWT peptide but not with the Fas peptide lacking the PDZ-binding motif (Fig. 4 F and Table S1). Among these proteins, 19 are PDZ domain-containing proteins, which have been associated with

conditions] to 20  $\mu$ M [low conditions]) before FasL treatment (15 ng/ml + M2), and cell death was measured by monitoring caspase-8 activation (presence of p43/41 and p30 cleaved fragments) and PARP cleavage by IB. Relative caspase-8 activation (p41/43) and PARP cleavage were measured by quantitative densitometry ( $n = 3$ ). (E–G) Cell death was induced by FasL treatment (15 ng/ml + M2) in HCT15 cells transfected with the indicated siRNAs and analyzed by IB as above. IBs were quantified by densitometry ( $n = 3$ ). (H) The efficiency of each siRNA was verified by IB. (I and J) Cell death was analyzed by sub-G1 quantification (PI incorporation) in HCT15 grown in low-calcium conditions or transfected by the indicated siRNAs. Arrowheads in B and E indicate the cleaved forms of PARP. In all graphs, error bars represent means  $\pm$  SEM. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; Student's  $t$  test.

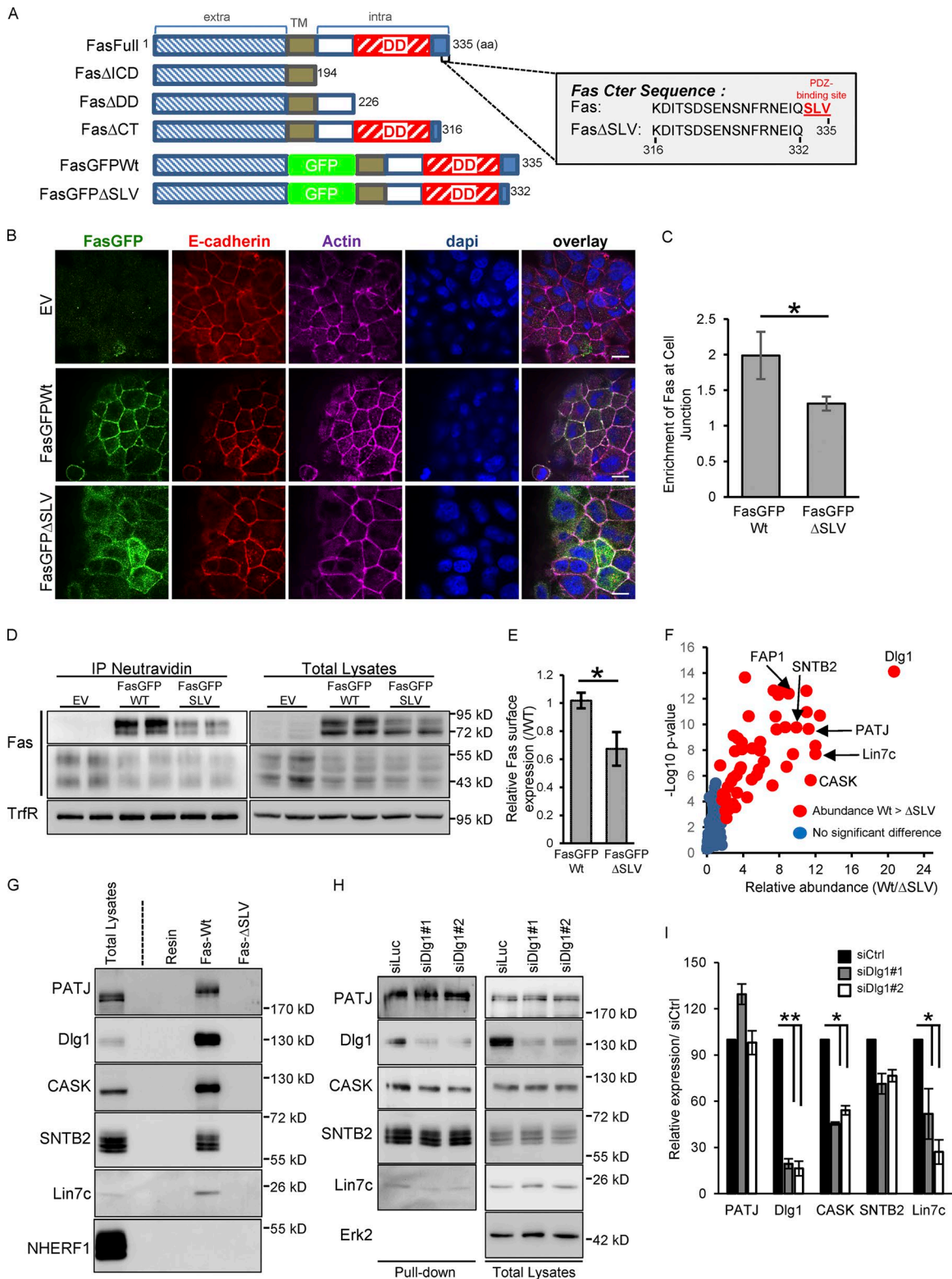


**Figure 2. Fas accumulates at cell–cell junctions and colocalizes with AJ components in both colorectal cell lines and colon tissue sections. (A)** Detection of E-cadherin, actin, and Fas by IF on HCT15 cells in border or center regions of the cell islet. An enlarged part of images from bordering cells is shown. Arrows indicate the diffuse localization of Fas at the plasma membrane in bordering cells. **(B)** XZ projection of Z stack acquired in A. Arrows and arrowheads indicate Fas–E-cadherin colocalization both at the zona adherens and along the lateral surface, respectively. **(C)** Line scan (yellow line in A) profile of fluorescence intensity of Fas, E-cadherin, and actin obtained at the center of the cell islet. **(D)** Quantification of the specific enrichment of Fas at cell–cell junctions in cells located at the center (confluent area) compared with the border of the HCT15 cell islets. Bars in graphs represent means  $\pm$  SEM ( $n = 5$ ). **(E)** PLA was used to determine the interaction of Fas and E-cadherin in HCT15 cells. Red fluorescent spots indicate colocalization of Fas and E-cadherin. PLA dots/cells in each condition were counted, and the right panel shows the mean of dots/cells  $\pm$  SEM. One representative experiment is shown out of three. **(F)** Detection of Fas and  $\alpha$ -catenin in HCT15 invalidated for  $\alpha$ -catenin expression by using a siRNA approach. A XZ projection of Z stack is shown. **(G)** IF localization of Fas and E-cadherin in human colon tissue sections using confocal microscopy. **(H and I)** Fas detergent solubility in HCT15 cells transfected by the indicated siRNAs was analyzed by IB. S, soluble fraction; I, insoluble cytoskeletal fraction. Erk-2 and caveolin-1 were used as controls of soluble protein and membrane protein, respectively. Relative percentage of solubility of Fas in each condition was quantified by densitometry ( $n = 3$ ). Bars in graphs represent means  $\pm$  SEM ( $n = 5$ ). Bars: 10  $\mu$ M (A, B, E, and F); 20  $\mu$ M (G). \*,  $P < 0.05$ ; Student's  $t$  test.



**Figure 3. AJs sequester Fas at site of cell–cell junction, and this impairs efficient FasL binding and therefore Fas cell death signaling. (A)** Binding assays of latex beads coated with FasL (Beads FasL) on HCT15 transfected with siRNAs against  $\alpha$ -catenin and followed by phase-contrast microscopy. **(B)** FasL-coated or -noncoated (NC) beads attached to cells were counted using flow cytometry after cell lysis. Graphs represent means  $\pm$  SEM ( $n = 3$ ). **(C and D)** Detection of Fas and cleaved caspase-8 in HCT15 cells incubated with FasL-coated beads was done by IF at different time points. Beads appear in red (by autofluorescence). An XZ projection of the Z stack is shown. Arrows indicate the accumulation of Fas beneath the beads. **(E)** Accumulation of Fas and activated caspase-8 under FasL-coated beads was quantified. Graph represents mean  $\pm$  SEM ( $n > 3$ ). **(F)** FasL-coated beads were used to immunoprecipitate activated Fas from cell lysates of HCT15 cells transfected with indicated siRNAs. Fas, caspase-8, and FADD were detected by IB. Arrows and arrowheads indicate native caspase-8 and cleaved (activated) caspase-8, respectively. **(G)** Relative caspase-8 and Fas were quantified by densitometry at  $t = 0$  or after 30 min FasL-coated bead activation ( $n = 3$ ). Bars: 10  $\mu$ M (C and D); 50  $\mu$ M (A). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; Student's  $t$  test.





**Figure 4. Fas PDZ binding domain regulates Fas cell–cell junction localization and interacts with the polarity molecule Dlg1. (A)** Schematic representation of human Fas constructs and of the C-terminal sequence of Fas. The SLV C-terminal PDZ-binding motif is shown in red. TM, transmembrane domain. **(B)** Detection of E-cadherin and GFP by IF in HCT15 cells transduced with empty vector (EV), GFP-Fas WT, and ΔSLV mutant. **(C)** Quantification of the specific enrichment of GFP-Fas WT and ΔSLV mutant at cell–cell junctions. Error bars in graphs represent means ± SEM ( $n = 5$ ). **(D)** Cell surface expression of GFP-Fas WT and ΔSLV mutant were compared using biotinylation assays. **(E)** Relative cell surface expression of GFP-Fas WT and of the ΔSLV mutant was quantified by densitometry. Error bars in graphs represent means ± SEM ( $n = 3$ ). **(F)** Volcano plot summarizing comparison between the protein interacting with Fas C-terminal (Cter) peptide of WT and the ΔSLV mutant. The  $\log_2$  ratio of protein intensities was plotted against negative  $\log_{10}$  P values. Red circles correspond with proteins significantly more abundant in the WT than in the ΔSLV mutant ( $P < 0.05$ ), and blue circles correspond with proteins without significant changes.

cell–cell junctions, cell polarity, receptor trafficking, and actin cytoskeleton interaction/regulation. FAP1 is the only protein already identified as a Fas interactor (Yanagisawa et al., 1997).

The most abundant protein found to interact with the Fas C-terminal part is the polarity molecule Dlg1. Dlg1 is a scaffold molecule containing four PDZ domains, and it belongs to the Scrib polarity complex. Dlg1 has been found to be tightly associated with AJs in various animal and cellular models (Reuver and Garner, 1998; Guo et al., 2014). Moreover, Dlg1 has been found to interact with several molecular partners also identified in our proteomic analysis such as CASK, Lin7c, and MPP7 (Fig. 4 F). Pull-down experiments followed by immunoblotting (IB) were used to verify the interaction of the Fas C-terminal domain with Dlg1 as well as with other abundant PDZ domain-containing proteins identified in our screen such as CASK, Lin7c, SNTB2, and PATJ but not with NHERF1, used as a negative control (Fig. 4 G). Using pulldown experiments, we also showed that the knockdown of Dlg1 expression by siRNAs affects the recruitment of several Fas molecular partners known to associate with Dlg1, such as CASK and Lin7c, but has only a minor effect on other PDZ-binding proteins (Fig. 4, H and I), supporting the idea that Dlg1 could organize a complex structural network composed of several proteins linked to the Fas C-terminal domain and connected to the actin cytoskeleton.

To further demonstrate the molecular interaction between Fas and Dlg1, Fas–Dlg1 association was verified by using both Fas immunoprecipitation (IP) experiments in 293T cells transiently transfected with Dlg1 and various Fas-deletion mutants (Fig. S1 B) as well as PLA on HCT15 cells (Fig. 5 D). Our results support the idea of a direct interaction between Fas and Dlg1 in epithelial cells.

### The Dlg1 polarity molecule colocalizes with Fas and inhibits FasL-induced cell death signaling

As we mentioned, the Dlg1 polarity molecule has been found tightly associated with E-cadherin (Guo et al., 2014). As expected, IF experiments demonstrate that Dlg1 colocalizes with Fas at cell–cell junctions (Pearson's correlation coefficient of 0.73) in HCT15 cells, both at the border and in the center of the cell islets (Fig. 5, A and B).

We then investigated the importance of the Fas–Dlg1 association on Fas plasma membrane stability, localization, and signaling using a loss-of-function approach. IF experiments demonstrated that the knockdown of Dlg1 in HCT15 cells only slightly impacts Fas localization at the cell junctions (Fig. 5, A and C). In agreement with this result, solubilization assays revealed that siRNA silencing of Dlg1 in HCT15 has only a modest effect on Fas and  $\alpha$ -catenin solubility (Fig. S2 A), suggesting that in HCT15 cells, AJ formation and Fas recruitment in AJs do not absolutely require Dlg1.

We then compared the binding efficiency of FasL-coated beads on HCT15 cells in which Dlg1 expression was knocked

down by siRNAs. Dlg1 silencing in HCT15 did not statistically modify either the binding efficiency of FasL-coated beads or the preferential binding on peripheral cells observed in control cells (Figs. 3 A and S2 B). This suggests that the absence of Dlg1 is not sufficient to affect the Fas basal–lateral localization and to sensitize cells within islets to FasL binding as it occurs when cell–cell junctions are disrupted.

The potential role of Dlg1 in the modulation of Fas proapoptotic signaling was then studied by comparing FasL death sensitivity of HCT15 cells transfected with either control (siCtrl) or two different Dlg1 (siDlg1#1 and 2) siRNAs. The knockdown of Dlg1 significantly increased Fas-induced cell death as judged by IB using anti-cleaved caspase-8 and poly-ADP ribose polymerase (PARP) antibodies (Fig. 5, E and F). The increase of Fas cell death correlates with a slight increase of Fas total and cell surface expression in cells transfected by siRNAs against Dlg1 compared with a control receiving the control siRNA (siCtrl; Fig. S2 C). To analyze whether the role of Dlg1 in the regulation of Fas cell death signaling was dependent on AJ formation, we took advantage of another colorectal cell line named SW480 that does not form AJs due to a very low level of E-cadherin expression (Fig. S2 D; Conacci-Sorrell et al., 2003). The absence of Dlg1 in SW480 cells strongly increased FasL-induced cell death (Fig. 5, E and F) without significantly affecting Fas total or cell surface expression (Fig. S2 E).

Altogether, these data show that Dlg1 does not play an essential role in the sequestration of Fas in AJs but inhibits Fas cell death signaling by modulating the ability to optimally form a DISC upon FasL engagement. Since the Fas PDZ mutant is unable to efficiently localize in AJs, we hypothesize that other PDZ molecules may participate or compensate for the absence of Dlg1 to target/stabilize Fas at cell–cell junctions. Indeed, several PDZ proteins identified in our screen, such as MAGI-1/3,  $\beta$ 2-syntrophin, MPP7, and CASK, have been found associated with cell–cell junctions. Further studies would be necessary to determine the exact mechanism by which Dlg1 interferes with cell death signaling. Indeed, several examples have shown that Dlg1 modulates receptor signaling by influencing their trafficking, localization, and molecular environment by recruiting adaptor molecules (Round et al., 2005; Fourie et al., 2014).

In this study, we uncover a new role of AJs as key regulators of Fas proapoptotic signaling in polarized epithelial cells. We clearly demonstrate that AJ formation is accompanied by the accumulation of Fas receptor within these adhesion complexes. This results in a strong inhibition of Fas-mediated cell death by impairing efficient FasL binding, thereby preventing the activation of the receptor. Moreover, we unravel that Fas accumulation in these lateral cellular domains requires the PDZ-binding domain of Fas. Using a proteomics approach, we reveal that this domain of Fas interacts preferentially with Dlg1, a scaffold molecule involved in the maintenance of cell polarity. We demonstrate that the Fas–Dlg1 interaction inhibits Fas cell death signaling,

(C) Pulldown experiment done with the C-terminal peptide of Fas (WT or Fas $\Delta$ SLV) followed by IB. (H) Pulldown experiment done with beads coupled to the C-terminal peptide of WT Fas on cell lysates of HCT15 transfected with the indicated siRNAs and followed by IB. (I) Relative expression of each protein pulldown with WT Fas C-terminal peptide was quantified by densitometry ( $n = 3$ ). Bars, 10  $\mu$ M. \*,  $P < 0.05$ ; Student's *t* test.



suggesting that Dlg1 interferes with the formation/stability of the death signaling complex. Therefore, accumulation of Fas at cell–cell junctions inhibits Fas cell death signaling by at least two different complementary mechanisms (Fig. 5 G).

Our study supports for the first time in human cells a molecular link between a scaffold protein involved in the maintenance of cell polarity and a cell death receptor. This result is in agreement with previous studies revealing the importance of cell polarity in the control of cell death (Weaver et al., 2002; Brumby and Richardson, 2003; Igaki et al., 2009; Frank et al., 2012). This cell death–regulatory mechanism is crucial to protect normal cells from apoptotic signals and to sense and eliminate abnormal cells from epithelial tissues in pathological conditions such as tumor development. Our data reveal a striking similarity with data obtained in *Drosophila melanogaster* in which activation of the fly TNF receptor Grnd by its ligand Eiger leads to the apoptotic elimination of mispolarized clonal epithelial cells due to siRNA silencing of Scrib or Dlg1 (Igaki et al., 2009; Andersen et al., 2015). Moreover, Grnd is found associated with the Crumbs polarity complex and Veli (lin7), which regulates the fly TNF receptor signaling capacity. Our proteomics analysis demonstrates that Lin7c, the mammalian homologue of Veli, also interacts with Fas and that its association with Fas is dependent on Dlg1, suggesting that the control of TNF receptor signaling by polarity molecules has been conserved during evolution.

Finally, since Fas activation also leads to nonapoptotic signaling, in particular in the context of tumor development (Peter et al., 2007), it is possible that recruitment of Dlg1 and/or E-cadherin by Fas blocks cell death signaling while alternatively promoting nondeath pathways. Therefore, our study opens the door for further investigations to unravel the molecular mechanisms underlying the versatile regulation of Fas signaling by the cadherin–Dlg1 complex.

## Materials and methods

### Reagents and antibodies

For IB or IF, antibodies against anti-Fas (B10), Dlg1/SAP97 (2D11 and S19), Erk-2, Lin7 (C15), and CASK (H107) were purchased from Santa Cruz Biotechnology; anti-caspase-8 and Fadd were from MBL; anti-cleaved caspase-8 (cl. caspase-8) and PARP were from Cell Signaling Technology; anti-caspase-10 was from BioVision; anti- $\beta$ -catenin, N-cadherin, and NHERF1 were from BD; anti-HA was from Covance; anti-E-cadherin was from Invitrogen; anti-Fas (EPR5700), p120, E-cadherin, and  $\alpha$ -catenin were from Epitomics; anti-GAPDH was from EMD Millipore; anti-PATJ was from Gene-Tex; and anti- $\beta$ 2-syntrophin (SNTB2) was from Thermo Fisher Scientific. Fluorescent and HRP-conjugated secondary antibodies were from Molecular Probes and Jackson ImmunoResearch Laboratories, respectively, and staurosporine was from Sigma-Aldrich. For FasL treatment, Flag-recombinant human FasL from Alexis was cross-linked with anti-Flag M2 from Sigma-Aldrich.

### Constructs

Human Fas  $\Delta$ Cter (pCR3.hFas $\Delta$ Cter) constructs were obtained using the QuikChange site-directed mutagenesis kit (Invitrogen) with pCR3-hFas as template and using 5'-CAGACTATCATCCTC

TAGGACATTACTAGTGACTCAG-3' and 5'-CTGAGTCACTAGTAA TGTCCTAGAGGATGATAGTCTG-3' oligonucleotides, and they corresponded with the deletion of the last 20 aa of human Fas. pCR3-hFas, pCR3-hFas $\Delta$ ICD, and pCR3-hFas $\Delta$ DD were kind gifts from P. Schneider (University of Lausanne, Epalinges, Switzerland) and correspond respectively with Fas full-length cDNA (FasFull), deleted of the entire cytoplasmic domain (Fas $\Delta$ ICD), and deleted of the C-terminal domain of Fas including the death domain (DD; Fas $\Delta$ DD). Fas GFP WT was a kind gift from J. Lane (University of Bristol, Bristol, UK). Fas GFP  $\Delta$ SLV was obtained by deleting the SLV tripeptide by PCR. Both GFP constructs (WT and mutant) were cloned in the retroviral vector LXSN. Rat Dlg1-expressing vector was a gift from L. Banks (International Centre for Genetic Engineering and Biotechnology, Trieste, Italy).

### Cell Culture, treatment, transfection, and viral infection

All cell lines were grown in DMEM supplemented with 10% FBS supplemented with nonessential amino acids (Gibco; Thermo Fischer Scientific). Transient transfection of plasmids in 293T was obtained by using the JetPRIME transfection reagent (Polyplus). For siRNA transfection, cells were transiently transfected using RNAiMAX lipofectamine reagent (Invitrogen) according to the manufacturer's procedure.  $\alpha$ -Catenin, E-cadherin, and Dlg1 silencing were obtained using individual sequences: for  $\alpha$ -catenin #1, 5'-GAAGAGAGGUCGUUCUUAAG-3'; and #2, 5'-GAU GGUAUCUUGAAGUUGA-3'; and for Dlg1 #1, 5'-CCAUAGAAC GGGUUAUUA-3'; and #2, 5'-GGAAGACAUUACAACCAAGU-3'. E-cadherin silencing was obtained using a mix of two individual siRNAs (#1, 5'-GGCCUGAAGUGACUCGUAA-3'; #2, 5'-GGGACA ACGUUUAUUACUA-3'), and siRNA targeting luciferase was used as control (siCtrl, 5'-CGUACGCGGAUACUUCGA-3').

Infection of HCT15 with LXSN retroviral vector expressing Fas GFP WT and mutant was performed as described (Gagnoux-Palacios et al., 2005). Briefly, infectious supernatants generated using phoenix retroviral producer cell line supplemented with polybrene were added to actively growing cells at high MOI followed by spinoculation procedure. Expressing cells were selected for neoresistance to geneticin (500  $\mu$ g/ml; Sigma-Aldrich) 48 h after infection.

### Preparation of FasL-coated beads

400  $\mu$ l magnetic beads linked to human anti-mouse IgG (Dynabeads; Invitrogen) were incubated with 40  $\mu$ g M2 antibody for 1 h at RT in DMEM and 0.1% BSA on a wheel. M2-coated beads were washed and incubated one more hour with 2  $\mu$ g recombinant FasL in the same conditions. After washing, beads were kept at 4°C in sterile conditions or used immediately for Fas activation. The same amount of uncoated beads was used as a control in these experiments.

### Cell death assays

For cell death experiments, cells were incubated with indicated doses of recombinant human Flag-FasL (FasL) cross-linked with 1  $\mu$ g/ml M2 antibody or with FasL-coated beads. After the indicated incubation time at 37°C and 5% CO<sub>2</sub>, cells were collected and fixed in ice-cold 70% ethanol. Cells were washed in 38 mM sodium citrate, pH 7.4, stained for 20 min at 37°C with 50  $\mu$ g/ml propidium

iodide (PI; Sigma-Aldrich) and 5  $\mu\text{g}/\text{ml}$  RNaseA (Sigma-Aldrich) in 38 mM sodium citrate, and analyzed by flow cytometry using the Fortessa analyzer (BD). The proportion of apoptotic cells as represented by the sub-G1 peak was determined. Alternatively, cell death induced by FasL/M2 cotreatment or staurosporine (20  $\mu\text{M}$ ) was analyzed by video microscopy using an inverted ZEISS Axiovert200 microscope equipped with a Zeiss 10 $\times$  Plan-Neofluar PH1 dry 0.3 NA objective lens. Images were acquired with a monochrome Neo sCMOS camera (Andor Technology) controlled with MetaMorph 7.7 software (Molecular Devices).

### IF and FasL-binding assay

For immunolabeling, cells attached to glass coverslips were fixed with 3.7% formaldehyde for 15 min at RT and either subsequently permeabilized with 0.1% Triton X-100 or incubated on ice in cold methanol for 5 min. After 1-h saturation in PBS containing 1% BSA and overnight incubation at 4 $^{\circ}\text{C}$  with the indicated primary antibodies, cells were washed with PBS and incubated with fluorescent-conjugated secondary antibodies (Molecular Probes/Invitrogen) and DAPI (Sigma-Aldrich). Samples were analyzed using an inverted IX81 Olympus microscope (Olympus) equipped with a motorized XY stage (Prior Scientific), a CSU-X1 confocal head (Yokogawa Electric Corporation), and a sensitive iXon DU-897-BV electron-multiplying charge-coupled device (EMCCD) camera (Andor Technology). Z stacks were acquired using a piezo stage NanoScan Z100 (Prior Scientific). The system was controlled using MetaMorph software. Fresh frozen sections of human colon were immunolabeled using a similar procedure and analyzed on an LSM780 confocal microscope (Zeiss) using a 40 $\times$  1.1 NA water objective. All experimental and control images of the IF or phase-contrast data were collected using identical imaging settings, and images were merged using Fiji software tools (ImageJ; National Institutes of Health; Schindelin et al., 2012). For FasL-binding assay, cells were incubated with FasL-coated beads or uncoated beads (as a control) in DMEM and 0.1% BSA for 30 min at 37 $^{\circ}\text{C}$  and 5%  $\text{CO}_2$ . After extensive washing to remove unbound beads, cells were fixed with a solution of 3.7% formaldehyde in PBS, and phase-contrast pictures of each well were performed using a 10 $\times$  objective. Alternatively, cells were lysed, and lysates were subjected to flow cytometry using the HTS Plate Manager (BD) to calculate the number of cell-fixed beads in each condition.

### Specific enrichment quantification

Fas specific enrichment at cell-cell junctions or Fas and caspase recruitment at FasL-coated beads were quantified using the same the process with Fiji software. Briefly, reference regions, either cell-cell junctions (labeled by E-cadherin, actin, and Dlg1) or bead contours were defined using intensity-based thresholds. The intensity of the marker of interest, endogenous Fas, FasGFP, or cleaved caspase-8, was measured inside those predefined contours (cell-cell junctions or beads). Then, the intensity of the marker of interest, endogenous Fas, FasGFP, or cleaved caspase-8, was measured outside those predefined contours to measure the basal level of expression. Finally, the enrichment factor was calculated by the ratio of the signal of interest inside/outside of the junctions or beads. An enrichment factor value  $>1$  meant that the

protein of interest was specifically enriched either in the cell-cell junction or at the FasL-coated beads.

### DuoLink in situ PLA

To detect the interaction between Fas and Dlg1 or  $\alpha$ -catenin, we used the DuoLink in situ PLA (Olink Bioscience) according to the manufacturer's protocol. HCT15 or SW480 cells were seeded on poly-L-lysine (40  $\mu\text{g}/\text{ml}$ )-coated microscope slides. Cells were fixed with 4% PFA and permeabilized with 0.5% Triton X-100. Cells were immunolabeled with primary antibodies: anti-Fas, anti-Dlg1, or anti- $\alpha$ -catenin overnight at 8 $^{\circ}\text{C}$ . The secondary antibodies (PLA probes supplied in the DuoLink kit) were incubated for 1 h at 37 $^{\circ}\text{C}$ . Images were collected on an inverted Axio-Observer Z1 microscope (Zeiss) equipped with a 63 $\times$  1.4 NA Plan Apochromat DICIII oil objective lens. Images were acquired with a monochrome EMCCD iXON+897 camera (Andor Technology) controlled with MetaMorph 7.8 software. A fluorescence signal indicated that two proteins were separated by  $<40$  nm. PLA dots and nuclei were calculated in each field, and results were expressed as the mean number of count/cells.

### Peptide pulldown, IP, and IB

Peptides corresponding with carboxyl terminals of human Fas (316–335 aa [Fas] or 316–332 aa [Fas $\Delta$ SLV]) were synthesized (GenScript) and cross-linked to NHS-activated Sepharose 4 Fast Flow beads according to the manufacturer's instructions (GE Healthcare). Pulldown experiments were performed using HCT15 cell lysates, and interacting proteins were identified by IB. Briefly, peptide-coated beads were added to cell lysates and incubated for 2 h at 4 $^{\circ}\text{C}$ . Beads were washed in lysis buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 0.2 mM EDTA, 0.5% Triton X-100, 2 mM  $\text{MgCl}_2$ , 2 mM DTT, 10 mM NaF, 20 mM  $\beta$ -glycerophosphate, and a mix of protease inhibitors), and proteins bound to beads were separated by SDS-PAGE classical procedure. For IP, 293 cells transfected with Fas and rat Dlg1-encoding plasmids were lysed at 4 $^{\circ}\text{C}$  in IP buffer: 20 mM Hepes, pH 7.5, 150 mM NaCl, 0.2 mM EDTA, 0.5% Triton X-100, 10% glycerol, 2 mM  $\text{MgCl}_2$ , 10 mM NaF, 20 mM  $\beta$ -glycerophosphate, and a mix of protease inhibitors. The postnuclear supernatants obtained after centrifugation at 10,000 g for 10 min at 4 $^{\circ}\text{C}$  were incubated with protein G Sepharose beads (Invitrogen) coupled to Fas antibody (Apo1.3) for 2 h. After washes with lysis buffer, beads were eluted with sample buffer at 95 $^{\circ}\text{C}$  for 5 min and subjected to SDS-PAGE and IB. To analyze DISC formation, cells were incubated with 20  $\mu\text{l}$  FasL-coated beads at 4 $^{\circ}\text{C}$  for 30 min to allow bead deposition and initial attachment. Cell plates were lysed directly ( $t = 0$ ) or transferred at 37 $^{\circ}\text{C}$  for various periods of time (from 15 to 60 min) to trigger Fas activation and DISC formation before cell lysis in IP buffer. Cell lysates were sonicated, and protein concentration was evaluated. The same quantity of protein (1 mg) was used in the IP for each condition. Lysates were incubated for 1 h at 4 $^{\circ}\text{C}$  under agitation. FasL-coated beads were then isolated using a magnet and washed four times with the lysis buffer. Magnetic beads were resuspended in Laemmli buffer, and protein extracts were separated by SDS-PAGE followed by classical IB procedures. IB analysis of total proteins was performed according to standard protocols. Briefly, cells were lysed in 50 mM Tris, pH 8, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.5% deoxycholate,

and 0.1% SDS containing phosphatase and protease inhibitors. After preclearing by centrifugation and quantification, the solubilized denatured proteins were resolved by SDS-PAGE. IB was performed using the indicated primary antibody and HRP-coupled secondary antibody (Jackson ImmunoResearch Laboratories). Quantitative densitometry was performed using ImageJ.

### Cell surface biotinylation

The presence of Fas at the cell surface was assessed by biotinylation assays followed by IP with agarose beads coupled with avidin according to the manufacturer's instructions. Briefly, cells were washed three times in PBS, pH 8, 1 mM MgCl<sub>2</sub>, and 0.5 mM CaCl<sub>2</sub> at 4°C, and then sulfo-NHS-SS-biotin (Pierce) was added to a final concentration of 0.2 mg/ml for 30 min at 4°C. The monolayers were washed once in ice-cold PBS, pH 8, 1 mM MgCl<sub>2</sub>, and 0.5 mM CaCl<sub>2</sub> and incubated with PBS solution containing 100 mM glycine for 10 min to block free biotin. After incubation, cells were washed once with ice-cold PBS and lysed in lysis buffer. The same amount of biotinylated proteins were precipitated with Neutravidin-conjugated agarose (Pierce) in each condition for 2 h. The beads were washed three times in lysis buffer for 5 min each and resuspended in Laemmli buffer. Protein extracts were separated by SDS-PAGE followed by classical IB procedures.

### Mass spectroscopy analysis

Following peptide pulldown (see above), protein extracts were loaded and stacked on a NuPAGE gel (Life Technologies). Stained bands were submitted to an in-gel trypsin digestion. Peptide extracts were reconstituted with 0.1% trifluoroacetic acid in 4% acetonitrile and analyzed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific) online with an Ultimate 3000RSLCnano chromatography system (Thermo Fisher Scientific). Protein identification and quantification were processed using the MaxQuant computational proteomics platform (version 1.5.3.8) using the human subset of the SwissProt database (date 2018.01; 20,244 entries). The statistical analysis was done with Perseus program (version 1.5.6.0). Differential proteins were detected using a two-sample *t* test at 0.01 and 0.05 permutation-based false discovery rate. The mass spectrometry proteomics data, including search results, have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009659.

### Detergent solubility assay

The detergent solubility assay was derived from Gimond et al. (1999). Cell cultures were lysed 20 min on ice in 300 µl of 0.5% Brij58, 50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 5 mM NaF, 20 mM β-glycerophosphate, and 10 mM sodium pyrophosphate in the presence of protease inhibitors, and cell lysates were centrifuged at 14,000 rpm for 10 min to obtain the soluble fraction of protein. The pellet represents the cytoskeleton-associated protein (insoluble fraction) and was resuspended in 400 µl Laemmli buffer. To compare the solubility of each protein, 1:15 soluble fraction (representing ~20 µg soluble protein) and 1:5 insoluble protein (80 µl) for each

condition were separated in SDS-PAGE followed by classical IB procedures. Quantitation of protein expression was performed using ImageJ and was used to determine the proportion of each protein in soluble versus insoluble fractions. The solubility index for each protein represents the ratio of total amount of soluble versus insoluble. One representative experiment was shown out of three.

### Statistical analysis

Data were analyzed using a homoscedastic two-tailed *t* test. *P* < 0.05 was considered statistically significant. \*, *P* < 0.05; \*\*, *P* < 0.005.

### Online supplemental material

Fig. S1 describes the role of the Fas C-terminal PDZ-binding site in the regulation of Fas detergent solubility and Fas-Dlg1 binding. Fig. S2 shows the impact of the Dlg1 polarity molecule on Fas detergent solubility, FasL binding, and both total and cell surface Fas expression. Video 1 shows the effects of FasL treatment on an islet of HCT15 cells. Video 2 shows the effects of the treatment of the same cell line by staurosporine, a common chemical inducer of apoptosis. Video 3 and 4 are filmed in similar conditions to Video 1 except that HCT15 cells were grown on a semipermeable cell culture or in culture media depleted of calcium, respectively. Table S1 shows the comprehensive listing of all Fas interactors identified by pulldown assay and LC-MS/MS analysis.

### Acknowledgments

We are grateful to Lawrence Banks, Jon Lane, and Pascal Schneider for providing us with expression vectors (human rat Dlg1, human GFP-Fas, and human Fas, respectively), to the Institute de Biologie Valrose facilities (Histology-S. Rekima and Cytometry-A. loubat), and to Sébastien Huault for technical assistance.

This work was supported by institutional funding from Inserm, Centre National de la Recherche Scientifique, and by grants from the Association pour la Recherche sur le Cancer and the LABEX SIGNALIFE program (ANR-11-LABX-0028-01). J.-P. Borg's laboratory is supported by La Ligue Nationale Contre le Cancer (Label Ligue) and Ruban Rose Award. J.-P. Borg is a scholar of Institut Universitaire de France. Proteomic analyses were done using the mass spectrometry facility of Aix Marseille Université Proteomics labeled "Plateforme technologique de l'Université Aix-Marseille," supported by Infrastructures Biologie Santé et Agronomie, the Cancéropôle PACA, the Provence-Alpes-Côte d'Azur Region, the Institut Paoli-Calmettes, and the Centre de Recherche en Cancérologie de Marseille.

The authors declare no competing financial interests.

Author contributions: L. Gagnoux-Palacios and A.O. Hueber conceived the study and wrote the manuscript; L. Gagnoux-Palacios designed, performed, and analyzed most experiments; H. Awina, A. Rossin, C. Planas-Botey, and A. Mettouchi performed experiments; S. Audebert performed and analyzed MS/MS experiments; M. Mondin performed, analyzed, and quantified IF experiments; F. Borgese performed and analyzed PLA experiments; J.-P. Borg conceived and analyzed MS/MS experiments.

Submitted: 14 May 2018  
 Revised: 27 June 2018  
 Accepted: 24 August 2018

## References

- Andersen, D.S., J. Colombani, V. Palmerini, K. Chakrabandhu, E. Boone, M. Röthlisberger, J. Toggweiler, K. Basler, M. Mapelli, A.-O. Hueber, and P. Léopold. 2015. The *Drosophila* TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. *Nature*. 522:482–486. <https://doi.org/10.1038/nature14298>
- Belotti, E., J. Polanowska, A.M. Daulat, S. Audebert, V. Thomé, J.C. Lissitzky, F. Lembo, K. Blibek, S. Omi, N. Lenfant, et al. 2013. The human PDZome: a gateway to PSD95-Disc large-zonula occludens (PDZ)-mediated functions. *Mol. Cell. Proteomics*. 12:2587–2603. <https://doi.org/10.1074/mcp.0112.021022>
- Borg, J.P., S. Marchetto, A. Le Bivic, V. Ollendorff, F. Jaulin-Bastard, H. Saito, E. Fournier, J. Adélaïde, B. Margolis, and D. Birnbaum. 2000. ERBIN: a basolateral PDZ protein that interacts with the mammalian ERBB2/HER2 receptor. *Nat. Cell Biol.* 2:407–414. <https://doi.org/10.1038/35017038>
- Brumby, A.M., and H.E. Richardson. 2003. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in *Drosophila*. *EMBO J.* 22:5769–5779. <https://doi.org/10.1093/emboj/cdg548>
- Carmeliet, P., M.G. Lampugnani, L. Moons, F. Brevario, V. Compernelle, F. Bono, G. Balconi, R. Spagnuolo, B. Oosthuysse, M. Dewerchin, et al. 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. *Cell*. 98:147–157. [https://doi.org/10.1016/S0092-8674\(00\)81010-7](https://doi.org/10.1016/S0092-8674(00)81010-7)
- Chen, L., S.M. Park, J.R. Turner, and M.E. Peter. 2010. Cell death in the colonic epithelium during inflammatory bowel diseases: CD95/Fas and beyond. *Inflamm. Bowel Dis.* 16:1071–1076. <https://doi.org/10.1002/ibd.21191>
- Chiasson-MacKenzie, C., Z.S. Morris, Q. Baca, B. Morris, J.K. Coker, R. Mirchev, A.E. Jensen, T. Carey, S.L. Stott, D.E. Golan, and A.I. McClatchey. 2015. NF2/Merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin. *J. Cell Biol.* 211:391–405. <https://doi.org/10.1083/jcb.201503081>
- Conacci-Sorrentelli, M., I. Simcha, T. Ben-Yedidia, J. Blechman, P. Savagner, and A. Ben-Ze'ev. 2003. Autoregulation of E-cadherin expression by cadherin-cadherin interactions. *J. Cell Biol.* 163:847–857. <https://doi.org/10.1083/jcb.200308162>
- Curto, M., B.K. Cole, D. Lallemand, C.H. Liu, and A.I. McClatchey. 2007. Contact-dependent inhibition of EGFR signaling by NF2/Merlin. *J. Cell Biol.* 177:893–903. <https://doi.org/10.1083/jcb.200703010>
- Fourie, C., D. Li, and J.M. Montgomery. 2014. The anchoring protein SAP97 influences the trafficking and localisation of multiple membrane channels. *Biochim. Biophys. Acta*. 1838:589–594. <https://doi.org/10.1016/j.bbame.2013.03.015>
- Frank, S.R., J.H. Bell, M. Frödin, and S.H. Hansen. 2012. A βPIX-PAK2 complex confers protection against Scrib-dependent and cadherin-mediated apoptosis. *Curr. Biol.* 22:1747–1754. <https://doi.org/10.1016/j.cub.2012.07.011>
- Fukuhara, S., K. Sako, T. Minami, K. Noda, H.Z. Kim, T. Kodama, M. Shibuya, N. Takakura, G.Y. Koh, and N. Mochizuki. 2008. Differential function of Tie2 at cell-cell contacts and cell-substratum contacts regulated by angiopoietin-1. *Nat. Cell Biol.* 10:513–526. <https://doi.org/10.1038/ncb1714>
- Gagnoux-Palacios, L., C. Hervouet, F. Spirito, S. Roques, M. Mezzina, O. Danos, and G. Meneguzzi. 2005. Assessment of optimal transduction of primary human skin keratinocytes by viral vectors. *J. Gene Med.* 7:1178–1186. <https://doi.org/10.1002/jgm.768>
- Gimond, C., A. van Der Flier, S. van Delft, C. Brakebusch, I. Kuikman, J.G. Collard, R. Fässler, and A. Sonnenberg. 1999. Induction of cell scattering by expression of beta1 integrins in beta1-deficient epithelial cells requires activation of members of the rho family of GTPases and downregulation of cadherin and catenin function. *J. Cell Biol.* 147:1325–1340. <https://doi.org/10.1083/jcb.147.6.1325>
- Grossmann, J. 2002. Molecular mechanisms of “detachment-induced apoptosis--Anoikis”. *Apoptosis*. 7:247–260. <https://doi.org/10.1023/A:1015312119693>
- Guo, Z., L.J. Neilson, H. Zhong, P.S. Murray, S. Zanivan, and R. Zaidel-Bar. 2014. E-cadherin interactome complexity and robustness resolved by quantitative proteomics. *Sci. Signal.* 7:rs7. <https://doi.org/10.1126/scisignal.2005473>
- Halbleib, J.M., and W.J. Nelson. 2006. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev.* 20:3199–3214. <https://doi.org/10.1101/gad.1486806>
- Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell*. 144:646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Hofmann, C., F. Obermeier, M. Artinger, M. Hausmann, W. Falk, J. Schoelmerich, G. Rogler, and J. Grossmann. 2007. Cell-cell contacts prevent anoikis in primary human colonic epithelial cells. *Gastroenterology*. 132:587–600. <https://doi.org/10.1053/j.gastro.2006.11.017>
- Igaki, T., J.C. Pastor-Pareja, H. Aonuma, M. Miura, and T. Xu. 2009. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in *Drosophila*. *Dev. Cell*. 16:458–465. <https://doi.org/10.1016/j.devcel.2009.01.002>
- Jeanes, A., C.J. Gottardi, and A.S. Yap. 2008. Cadherins and cancer: how does cadherin dysfunction promote tumor progression? *Oncogene*. 27:6920–6929. <https://doi.org/10.1038/onc.2008.343>
- Kinch, M.S., G.J. Clark, C.J. Der, and K. Burridge. 1995. Tyrosine phosphorylation regulates the adhesions of ras-transformed breast epithelia. *J. Cell Biol.* 130:461–471. <https://doi.org/10.1083/jcb.130.2.461>
- Kischkel, F.C., S. Hellbardt, I. Behrmann, M. Germer, M. Pawlita, P.H. Kramer, and M.E. Peter. 1995. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J.* 14:5579–5588. <https://doi.org/10.1002/j.1460-2075.1995.tb00245.x>
- Kobiela, A., and E. Fuchs. 2006. Links between alpha-catenin, NF-kappaB, and squamous cell carcinoma in skin. *Proc. Natl. Acad. Sci. USA*. 103:2322–2327. <https://doi.org/10.1073/pnas.0510422103>
- Lu, M., S. Marsters, X. Ye, E. Luis, L. Gonzalez, and A. Ashkenazi. 2014. E-cadherin couples death receptors to the cytoskeleton to regulate apoptosis. *Mol. Cell*. 54:987–998. <https://doi.org/10.1016/j.molcel.2014.04.029>
- McCrea, P.D., M.T. Maher, and C.J. Gottardi. 2015. Nuclear signaling from cadherin adhesion complexes. *Curr. Top. Dev. Biol.* 112:129–196. <https://doi.org/10.1016/bs.ctdb.2014.11.018>
- Niessen, C.M., D. Leckband, and A.S. Yap. 2011. Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. *Physiol. Rev.* 91:691–731. <https://doi.org/10.1152/physrev.00004.2010>
- Peter, M.E., R.C. Budd, J. Desbarats, S.M. Hedrick, A.O. Hueber, M.K. Newell, L.B. Owen, R.M. Pope, J. Tschopp, H. Wajant, et al. 2007. The CD95 receptor: apoptosis revisited. *Cell*. 129:447–450. <https://doi.org/10.1016/j.cell.2007.04.031>
- Qian, X., T. Karpova, A.M. Sheppard, J. McNally, and D.R. Lowy. 2004. E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. *EMBO J.* 23:1739–1784. <https://doi.org/10.1038/sj.emboj.7600136>
- Qin, Y., C. Capaldo, B.M. Gumbiner, and I.G. Macara. 2005. The mammalian Scribble polarity protein regulates epithelial cell adhesion and migration through E-cadherin. *J. Cell Biol.* 171:1061–1071. <https://doi.org/10.1083/jcb.200506094>
- Ramaswamy, M., S.Y. Cleland, A.C. Cruz, and R.M. Siegel. 2009. Many checkpoints on the road to cell death: regulation of Fas-FasL interactions and Fas signaling in peripheral immune responses. *Results Probl. Cell Differ.* 49:17–47. [https://doi.org/10.1007/400\\_2008\\_24](https://doi.org/10.1007/400_2008_24)
- Reuver, S.M., and C.C. Garner. 1998. E-cadherin mediated cell adhesion recruits SAP97 into the cortical cytoskeleton. *J. Cell Sci.* 111:1071–1080.
- Round, J.L., T. Tomassian, M. Zhang, V. Patel, S.P. Schoenberger, and M.C. Miceli. 2005. Dlg1 coordinates actin polymerization, synaptic T cell receptor and lipid raft aggregation, and effector function in T cells. *J. Exp. Med.* 201:419–430. <https://doi.org/10.1084/jem.20041428>
- Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, et al. 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods*. 9:676–682. <https://doi.org/10.1038/nmeth.2019>
- Schneider, M.R., M. Dahlhoff, D. Horst, B. Hirschi, K. Trülzsch, J. Müller-Höcker, R. Vogelmann, M. Allgäuer, M. Gerhard, S. Steininger, et al. 2010. A key role for E-cadherin in intestinal homeostasis and Paneth cell maturation. *PLoS One*. 5:e14325. <https://doi.org/10.1371/journal.pone.0014325>
- Stairs, D.B., L.J. Bayne, B. Rhoades, M.E. Vega, T.J. Waldron, J. Kalabis, A. Klein-Szanto, J.S. Lee, J.P. Katz, J.A. Diehl, et al. 2011. Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. *Cancer Cell*. 19:470–483. <https://doi.org/10.1016/j.ccr.2011.02.007>

- Stupack, D.G., and D.A. Cheresch. 2002. Get a ligand, get a life: integrins, signaling and cell survival. *J. Cell Sci.* 115:3729–3738. <https://doi.org/10.1242/jcs.00071>
- Takeichi, M. 1988. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development.* 102:639–655.
- Theisen, C.S., J.K. Wahl III, K.R. Johnson, and M.J. Wheelock. 2007. NHERF links the N-cadherin/catenin complex to the platelet-derived growth factor receptor to modulate the actin cytoskeleton and regulate cell motility. *Mol. Biol. Cell.* 18:1220–1232. <https://doi.org/10.1091/mbc.e06-10-0960>
- Vasioukhin, V., C. Bauer, L. Degenstein, B. Wise, and E. Fuchs. 2001. Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin. *Cell.* 104:605–617. [https://doi.org/10.1016/S0092-8674\(01\)00246-X](https://doi.org/10.1016/S0092-8674(01)00246-X)
- Weaver, V.M., S. Lelièvre, J.N. Lakins, M.A. Chrenek, J.C. Jones, F. Giancotti, Z. Werb, and M.J. Bissell. 2002. beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell.* 2:205–216. [https://doi.org/10.1016/S1535-6108\(02\)00125-3](https://doi.org/10.1016/S1535-6108(02)00125-3)
- Yanagisawa, J., M. Takahashi, H. Kanki, H. Yano-Yanagisawa, T. Tazunoki, E. Sawa, T. Nishitoba, M. Kamishohara, E. Kobayashi, S. Kataoka, and T. Sato. 1997. The molecular interaction of Fas and FAP-1. A tripeptide blocker of human Fas interaction with FAP-1 promotes Fas-induced apoptosis. *J. Biol. Chem.* 272:8539–8545. <https://doi.org/10.1074/jbc.272.13.8539>



## Supplementary material

Gagnoux-Palacios et al., <https://doi.org/10.1083/jcb.201805071>

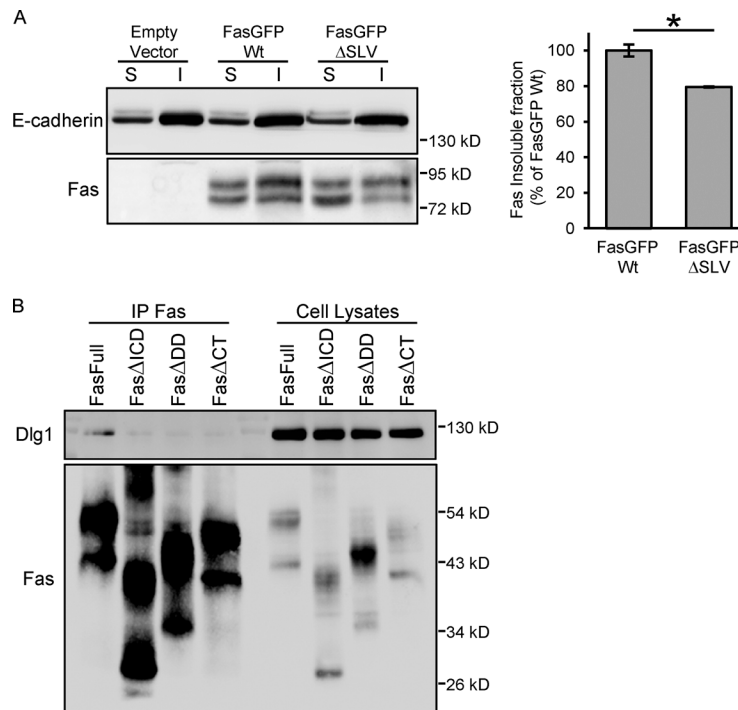
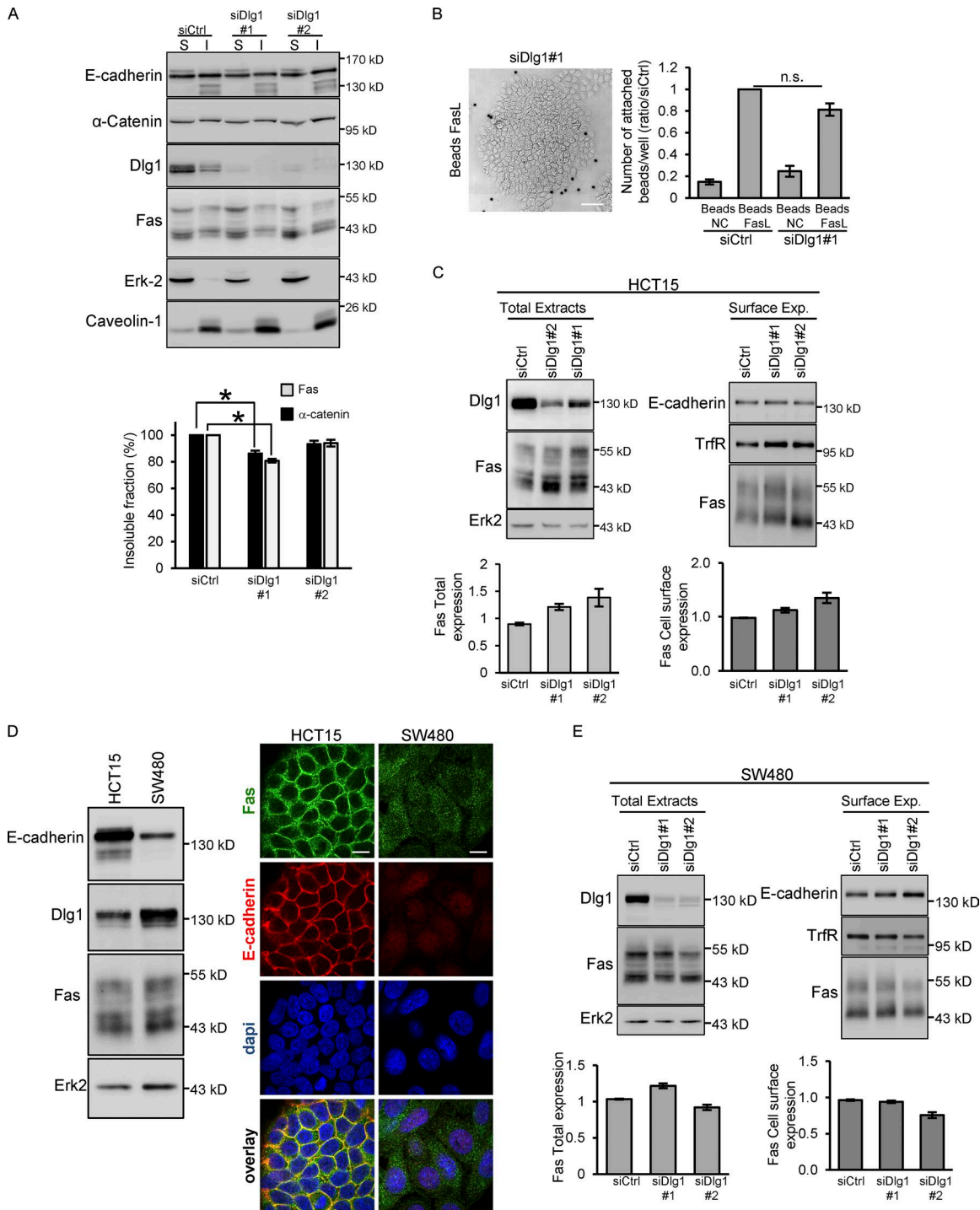
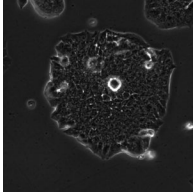


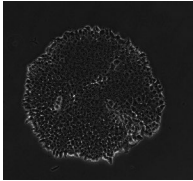
Figure S1. **Role of Fas PDZ binding domain in the regulation of Fas solubility and Dlg1 association.** **(A)** Detergent solubility of Fas-GFP (WT and ΔSLV mutant) stably expressed in HCT15 cells was analyzed by IB. S, soluble fraction; I, insoluble cytoskeletal fraction. E-cadherin was used as a control for membrane protein. The percentage of insolubility of WT and ΔSLV mutant Fas-GFP was quantified by densitometry and is shown on the right ( $n = 3$ ). Graphs represent means  $\pm$  SEM. \*,  $P < 0.05$ ; Student's  $t$  test. **(B)** IP experiments on 293T cell extracts expressing Dlg1 and either WT or Fas deletion mutant proteins. The presence of Dlg1 in the IP was detected by IB.



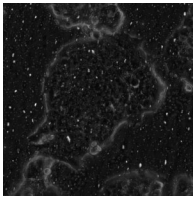
**Figure S2. Impact of Dlg1 polarity molecule on Fas expression.** (A) Detergent solubility of Fas, A) components, and Dlg1 in HCT15 cells transfected by Ctrl or Dlg1 siRNAs was analyzed by IB as described above (Fig. 2 H). \*,  $P < 0.05$ ; Student's  $t$  test. (B) Binding assay of latex beads coated with FasL (Beads FasL) on HCT15 transfected with Dlg1 siRNA and followed by phase-contrast microscopy. FasL-coated or -noncoated (NC) beads attached to cells were counted using flow cytometry after cell lysis. Graphs represent means  $\pm$  SEM ( $n = 3$ ). (C) Cell surface expression of Fas was compared in Dlg1-knockdown and control HCT15 cells using biotinylation assays. Relative cell surface expression of Fas in Dlg1-knockdown and control cells was quantified by densitometry. Bars in graphs represent means  $\pm$  SEM ( $n = 3$ ). (D) Comparison of A) components, Fas, and Dlg1 expression levels in HCT15 and SW480 cells by IB. Detection of E-cadherin and Fas localization by IF in HCT15 and SW480 cells. (E) Cell surface expression of Fas in Dlg1-knockdown and control SW480 cells was compared using biotinylation assays. Relative cell surface expression of Fas in Dlg1-knockdown and control cells was quantified by densitometry. Bars in graphs represent means  $\pm$  SEM ( $n = 3$ ). Bars: 50  $\mu$ m (B); 10  $\mu$ m (D).



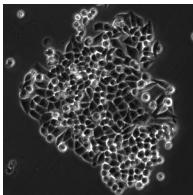
Video 1. **HCT15 cells forming cohesive islets were treated with 20 ng/ml of FasL in the presence of cross M2 antibody and immediately visualized by phase contrast using a video microscope for 12 h.** Frames were taken every 15 min for 12 h. Cell death is observed mainly in the periphery of the cell islet, suggesting that cells in the center of the islet are protected from Fas cell death signaling.



Video 2. **HCT15 cells were treated with staurosporine, a chemical inducer of apoptosis, and cells were filmed as described in Video 1.** Contrary to what happens with the FasL treatment, cells treated with staurosporine died with the same kinetic independently of their position within the cell islet. Frames were taken every 15 min for 12 h.



Video 3. **HCT15 were grown on a semipermeable cell culture insert and treated with FasL added apically or basolaterally.** Cell death is observed mainly in the periphery of the cell islet, demonstrating that the protective effect observed in cells located at the center of the islet cannot be explained by a restriction of FasL diffusion at the basal side of the cells. Frames were taken every 15 min for 12 h.



Video 4. **HCT15 were grown in culture media depleted of calcium before being treated with FasL and filmed in similar conditions to Video 1.** Absence of mature cell–cell contacts resulting from calcium depletion prevents the cell death–protective effect observed in control cells forming extensive cell–cell junctions. Indeed, FasL treatment induced cell death in all the cells independently of their position within the cell islet. Frames were taken every 15 min for 12 h.

**Table S1 is a separate Excel document showing identification of Fas interactors by pulldown assay and LC-MS/MS analysis.**

Table S1. Identification of Fas interactors by pulldown assay and LC-MS/MS analysis

Peptide pulldown using Fas WT or Fas DeltaSLV peptides was performed on HCT15 cell lysates to identify new molecular partners of Fas interacting with the C-terminal PDZ-binding motif of Fas. LC-MS/MS of the sample were processed as described in Materials and methods, and Fas interactome data presented in this table resulted from the relative intensity-based label-free quantification analysis (LFQ) processed using the MaxLFQ algorithm. For each identified and quantified protein, enrichment (log<sub>2</sub>) as well as P value (-log<sub>10</sub>) were indicated and used to plot the Volcano plot shown in Fig. 4 F. Protein name, protein ID, the gene name, the number of unique peptides identified (Peptides), the percent peptide coverage (Sequence coverage %), and the molecular weight of each protein are also indicated. Proteins significantly more abundant in the WT than in the DeltaSLV mutant (P < 0.05) are written in red.

-Log Student's T-test p-value FasWT/FasDelSLV	FDR FasWT/FasDelSLV	Difference FasWT/FasDelSLV	Student's T-test Significant FasWT/FasDelSLV	Gene names	Protein IDs	Majority protein IDs	Protein names	Peptides	Sequence coverage [%]	Mol. weight [kDa]	Score	Intensity	MS/MS Count	Protein with PDZ domain
14.1	0	20.7	+	DLG1	Q12959;P78.Q12959		Disks large homolog 1	55	64.9	100.45	323.31	55333000000	885	+
10.7	0	12.5	+	MPP7	Q57271 Q57271		MAGUK p55 subfamily member 7	30	52.3	65.523	323.31	57038000000	398	+
8.3	0	12.0	+	NSF	P46459;Q96.P46459		Vesicle-fusing ATPase	36	53.2	82.593	323.31	45129000000	288	+
7.7	0	12.0	+	LINC7	Q9NU99;Q1-Q9NU99		Protein lin-7 homolog C	13	65.5	21.834	123.59	23552000000	95	+
5.7	0	11.5	+	CASK	Q14936 Q14936		Peripheral plasma membrane protein CASK	39	41.5	105.12	323.31	17520000000	478	+
9.6	0	11.2	+	INADL	Q8N135;Q75.Q8N135		InoD-like protein	68	48.8	196.37	323.31	47185000000	598	+
11.0	0	11.0	+	MAG11	Q96Q27 Q96Q27		Membrane-associated guanylate kinase, class C	34	30.4	164.58	323.31	22732000000	291	+
12.6	0	11.0	+	MAG13	Q5TC09 Q5TC09		Membrane-associated guanylate kinase, class C	48	40.2	165.61	323.31	20589000000	312	+
9.8	0	9.9	+	SNTB2	Q13425 Q13425		Beta-2-syntrophin	19	44.4	57.949	304.1	12250000000	137	+
7.7	0	9.5	+	MPP5	Q8N3R9;P08.Q8N3R9		MAGUK p55 subfamily member 5	27	51.9	77.293	323.31	13601000000	189	+
12.4	0	9.0	+	PTPN13	Q12923;REV.Q12923		Tyrosine-protein phosphatase non-receptor type 13	57	31.5	276.9	323.31	70218000000	304	+
6.7	0	8.8	+	UTRN	P46939 P46939		Utrophin	74	26.3	394.46	323.31	99446000000	342	+
9.8	0	8.7	+	MPP6	Q9NZW5;Q1.Q9NZW5		MAGUK p55 subfamily member 6	22	51.5	61.116	221.18	59470000000	107	+
12.9	0.0	8.5	+	ABLIM1	Q14639 Q14639		Actin-binding LIM protein 1	21	33.7	87.687	166.4	38958000000	112	+
12.3	0	7.9	+	DTNB	Q60941;O9Y.Q60941		Dystrobrevin beta	15	31.9	71.355	323.31	30337000000	92	+
9.6	0	7.7	+	SEC23B	Q15437 Q15437		Protein transport protein Sec23B	19	31.9	86.478	323.31	25975000000	98	+
10.6	0	7.6	+	SNX27	Q96L92 Q96L92		Sorting nexin-27	12	24.8	61.264	94.948	17049000000	51	+
12.6	0	7.4	+	SNTB1	Q13884 Q13884		Beta-1-syntrophin	16	36.8	58.06	84.887	27619000000	65	+
5.2	0	7.2	+	LNK2	Q8N448 Q8N448		Ligand of Numb protein X2	14	27	76.003	323.31	81591000000	109	+
7.1	0	6.3	+	SEC24A	Q95486 Q95486		Protein transport protein Sec24A	11	12.9	119.75	131.85	11302000000	56	+
8.6	0	5.9	+	KIF26A	Q9ULI4;Q2K.Q9ULI4		Kinesin-like protein KIF26A	18	14.9	194.59	144.01	74679000000	67	+
8.0	0	5.9	+	SEC24B	Q95487 Q95487		Protein transport protein Sec24B	14	14.7	137.42	149.95	85766000000	67	+
6.5	0	5.8	+	HSPA8	P11142 P11142		Heat shock cognate 71 kDa protein	42	61.9	70.897	323.31	64702000000	1308	+
6.1	0	5.5	+	HSPA1L	P34931 P34931		Heat shock 70 kDa protein 1-like	12	24	70.374	8.1016	78633000000	10	+
8.1	0	5.0	+	CTNNA1	Q9UBT7 Q9UBT7		Alpha-catenin	8	12.7	81.895	45.096	39351000000	20	+
5.7	0	5.0	+	HSPA1B;HSPA	P0DMV9;P01.P0DMV9;P0DMV9;P0DMV9		Heat shock 70 kDa protein 18;Heat shock cognate 70 kDa protein 105 kDa	32	55.5	70.051	323.31	212190000000	623	+
10.6	0	4.6	+	ABLIM2	Q6H8Q1 Q6H8Q1		Actin-binding LIM protein 2	9	22.4	67.812	116.25	35682000000	29	+
4.3	0.000114286	4.4	+	FAS	P25445 P25445		Tumor necrosis factor receptor superfamily member 1	2	5.7	37.732	56.535	45159000000	12	+
13.7	0	4.2	+	HSPA9	P38646 P38646		Stress-70 protein, mitochondrial	44	58.3	73.68	323.31	257730000000	890	+
8.1	0	3.9	+	COL11A2	P13942 P13942		Collagen alpha-2(XI) chain	2	3	171.79	2.3443	17471000000	1	+
8.6	0	3.8	+	ARHGFB2	Q96DR7 Q96DR7		Rho guanine nucleotide exchange factor 2	9	11.7	97.344	27.27	16484000000	32	+
6.7	0	3.8	+	LNK1	Q8TB81 Q8TB81		E3 ubiquitin-protein ligase LNK1	5	9.2	80.628	15.813	16285000000	26	+
6.5	0	3.7	+	MPP3	Q13368 Q13368		MAGUK p55 subfamily member 3	4	9.9	66.151	96.623	14892000000	17	+
8.9	0	3.4	+	CCDC47	Q96A33 Q96A33		Coiled-coil domain-containing protein 47	2	5	55.873	3.6001	12389000000	4	+
4.7	0.000105263	3.3	+	HSPA6	P17066;P48.P17066		Heat shock 70 kDa protein 6	14	23.2	71.027	26.532	66901000000	74	+
6.0	0.000108108	3.3	+	HSPA4	P34932 P34932		Heat shock 70 kDa protein 4	12	21.8	94.33	90.558	37541000000	64	+
3.6	9.76E-05	3.1	+	KARS	Q15046 Q15046		Lysine-tRNA ligase	5	13.2	68.047	15.726	31806000000	22	+
7.9	0.000111111	3.1	+	DLG2	Q15700 Q15700		Disks large homolog 2	17	13.9	97.551	2.4884	87682000000	3	+
9.1	0.000117647	3.0	+	SDCBP	Q00560 Q00560		Syntenin-1	7	33.9	32.444	204.35	52187000000	103	+
5.8	0.000102564	2.9	+	RALGAPB	Q86X10 Q86X10		Ral GTPase-activating protein subunit beta	6	7	166.8	16.943	98579000000	14	+
3.8	9.52E-05	2.9	+	TJP1	Q07157 Q07157		Tight junction protein ZO-1	9	6.8	195.46	34.463	20882000000	25	+
5.6	0.0001	2.7	+	SPEG	Q15772 Q15772		Striated muscle preferentially expressed protein 1	2	0.6	354.28	3.0201	71818000000	2	+
3.4	0.000444444	2.4	+	UPF1	Q92900 Q92900		Regulator of nonsense transcripts 1	10	12.7	124.34	40.269	17833000000	26	+
3.5	0.000454545	2.4	+	PDIA4	P13667 P13667		Protein disulfide-isomerase A4	16	30.4	72.932	114.27	20527000000	70	+
5.1	9.30E-05	2.3	+	HSPH1	Q92598 Q92598		Heat shock protein 105 kDa	16	22.3	96.864	176.73	26057000000	129	+
3.5	0.000851064	2.2	+	DDX17	Q92841 Q92841		Probable ATP-dependent RNA helicase DDX17	9	18.8	80.272	121.08	24798000000	45	+
3.8	0.000521739	2.2	+	STAU1	Q95793 Q95793		Double-stranded RNA-binding protein Stau1	4	8.5	63.182	8.606	99031000000	9	+
2.7	0.00431373	2.2	+	QARS	P47897 P47897		Glutamine-tRNA ligase	7	11.5	87.798	73.231	28440000000	32	+
3.3	0.00367347	2.0	+	HSPAS	P11021 P11021		78 kDa glucose-regulated protein	42	54.9	72.332	323.31	45308000000	140	+
4.4	0.001	2.0	+	IARS	P41252 P41252		Isoleucine-tRNA ligase, cytoplasmic	14	14.7	144.5	144.67	61947000000	79	+
4.6	0.00036	1.8	+	SFPQ	P23246 P23246		Splicing factor, proline- and glutamine-rich	6	9.9	76.149	103.81	75825000000	48	+
4.1	0.00576923	1.7	+	SI00A7	P31151 P31151		Protein SI00A7	1	10.9	11.471	5.6645	13140000000	16	+
0.6	0.148361	1.7		DMD	P11532;Q13.P11532		Dystrophin	10	3	426.74	128.72	28263000000	69	+
3.2	0.016	1.6		EPRS	P07814 P07814		Bifunctional glutamate/proline-tRNA ligase	14	14.7	170.59	110.07	73820000000	114	+
2.4	0.0298246	1.6		ALYREF	Q86V81 Q86V81		THO complex subunit 4	6	32.3	26.888	124.76	39275000000	58	+
2.3	0.0311186	1.6		CLTC	Q00610;P53.Q00610		Claudin heavy chain 1	21	16.8	191.61	318.65	18917000000	127	+
1.2	0.0697838	1.5		VCL	P18206 P18206		Vinculin	17	20.4	123.8	45.852	94545000000	60	+
3.4	0.0191273	1.5		SEC24C	P53992 P53992		Protein transport protein Sec24C	2	2.7	118.32	4.5941	82950000000	4	+
6.8	0.00618868	1.5	+	UBA52;RPS27	P62987;P62.P62987;P6297		Ubiquitin-60S ribosomal protein L40;Ubiquitin-protein ligase E3 complex subunit epsilon	3	26.6	14.728	73.682	36560000000	39	+
1.6	0.0592	1.4		CC25	P48643 P48643		T-complex protein 1 subunit epsilon	5	10.9	59.67	10.957	83230000000	15	+
3.4	0.0266429	1.4		NONO	Q15233 Q15233		Non-POU domain-containing octamer-binding protein 1	4	14.4	54.231	11.021	12952000000	12	+
3.2	0.0293793	1.4		RP111	Q81WN7 Q81WN7		Retinitis pigmentosa 1-like 1 protein	1	1	261.2	3.5695	111600000000	3	+
1.3	0.0701053	1.4		HP;HPR	P00738;P00.P00738;P00738		Haptoglobin;Haptoglobin alpha chain;Haptoglobin beta chain	4	13.1	45.205	10.724	63427000000	14	+
1.4	0.0706301	1.4		MAP4K4	Q95819;Q8N.Q95819		Mitogen-activated protein kinase kinase kinase 4	15	13.8	142.1	58.295	11268000000	71	+
2.9	0.0350667	1.4		PRKDC	P78527 P78527		DNA-dependent protein kinase catalytic subunit	16	4.9	469.08	108.65	33352000000	71	+
1.0	0.088898	1.3		RALGAP2	Q2PPI7 Q2PPI7		Ral GTPase-activating protein subunit alpha	10	7.7	210.77	28.258	94818000000	22	+
1.7	0.0686667	1.3		ILF3	Q12906;Q96.Q12906		Interleukin enhancer-binding factor 3	8	11.6	95.337	48.038	21865000000	28	+
1.3	0.0753882	1.2		PROSER2	Q86WR7 Q86WR7		Proline and serine-rich protein 2	1	3	45.801	3.4221	46797000000	17	+
4.0	0.0358689	1.2		SRRM2	Q9UQ35 Q9UQ35		Serine/arginine repetitive matrix protein 2	8	4.3	299.61	20.997	49043000000	30	+
2.1	0.0610149	1.2		PRSS3	P35030 P35030		Trypsin-3	1	4	32.528	2.4031	75485000000	9	+
2.0	0.0636	1.2		IDE	P14735 P14735		Insulin-degrading enzyme	4	4.4	117.97	14.315	16428000000	10	+
1.5	0.0761446	1.1		BAG6	P46379 P46379		Large proline-rich protein BAG6	3	3.4	119.41	5.7456	97516000000	5	+
3.1	0.0563175	1.1		GEMIN5	Q8TEQ6 Q8TEQ6		Gem-associated protein 5	4	4.5	168.59	10.401	95474000000	7	+
3.2	0.057125	1.1		DDX5	P17844 P17844		Probable ATP-dependent RNA helicase DDX5	11	19.4	69.147	25.143	10589000000	65	+
3.5	0.0562581	1.1		MYO1C	Q00159 Q00159		Unconventional myosin-1c	11	13.5	121.68	149.32	61038000000	60	+
2.4	0.064507	1.1		CNN3;CNN2	Q15417;Q99.Q15417;Q99A1		Calponin-3;Calponin-2	2	6.7	36.413	11.063	14047000000	9	+
1.6	0.0769545	1.1		DRG1	Q9Y295 Q9Y295		Developmentally-regulated GTP-binding protein 1	5	17.7	40.542	107.42	69674000000	11	+
1.9	0.0724675	1.1		HSPA4L	Q95757 Q95757		Heat shock 70 kDa protein 4L	5	8.6	94.511	4.4961	30772000000	6	+
1.3	0.0846737	1.1		TRIM28	Q13263 Q13263		Transcription intermediary factor 1-beta	12	26.5	88.549	153.43	46251000000	74	+
1.1	0.0970385	1.1		TUFM	P49411 P49411		Elongation factor Tu, mitochondrial	10	30.5	49.541	54.628	68596000000	29	+
3.0	0.0618551	1.1		FUS	P35637;Q92.P35637		RNA-binding protein FUS	3	6.3	53.425	14.151	47178000000	30	+
1.9	0.0761463	1.1		FMNL3;FMNL	Q81VF7;Q96.Q81VF7;Q96GY		Formin-like protein 3;Formin-like protein 2	2	2.4	117.21	7.			

1,7	0,0967767	0,9	HMG2	P26583	P26583	High mobility group protein B2	2	12,9	24,033	4,884	15070000	8
1,5	0,102349	0,8	TBL2	Q9Y4P3	Q9Y4P3	Transducin beta-like protein 2	3	8,1	49,797	9,2767	13200000	9
3,4	0,0795217	0,8	TUBB	P07437	P07437	Tubulin beta chain	12	40,8	49,67	49,721	96270000	36
4,1	0,0788764	0,8	MCM5	P33992	P33992	DNA replication licensing factor MCM5	3	5,6	82,285	6,0725	13508000	7
1,6	0,1044	0,8	PKP2	Q99959	Q99959	Plakophilin-2	5	7,6	97,414	15,187	13543000	26
1,7	0,104036	0,8	MAT2A	P31153	P31153	S-adenosylmethionine synthase isoform 1	4	18,7	43,66	44,23	122750000	12
1,1	0,139831	0,8	PRMT1	Q99873;Q9N	Q99873	Protein arginine N-methyltransferase 1	10	30,2	41,515	77,925	57460000	30
3,2	0,0884444	0,8	CAPRIN1	Q14444	Q14444	Caprin-1	5	7,5	78,365	25,717	37363000	38
4,6	0,0828085	0,8	HNRNPU	Q00839	Q00839	Heterogeneous nuclear ribonucleoprotein	10	19,0	58,383	147,36	88646000	70
0,8	0,183324	0,8	CDSN	Q15517	Q15517	Corneodesmosin	2	11,9	51,522	4,7626	16947000	11
1,1	0,150952	0,8	ARL6IP4	Q66P13	Q66P13	ADP-ribosylation factor-like protein 6-int	4	7,6	44,915	101,63	32191000	48
3,2	0,0940392	0,7	EWSR1	Q01844	Q01844	RNA-binding protein EWS	3	7,2	68,477	76,831	53484000	51
2,2	0,103676	0,7	NACA;NACAP1	Q13765;E9P	Q13765;E9PAV	Nascent polypeptide-associated complex	2	13	23,384	84,203	13734000	20
4,1	0,08912	0,7	MYO1B	O43795;Q9L	O43795	Unconventional myosin-1b	18	20,5	131,98	323,31	173330000	170
1,9	0,113183	0,7	IPO5	O00410;O6O	O00410	Importin-5	10	13,9	123,63	44,627	38320000	48
2,6	0,101593	0,7	KPNA2	P52292	P52292	Importin subunit alpha-1	5	14	57,861	13,296	11738000	19
1,8	0,116138	0,7	PHB2	O99623	O99623	Prohibitin-2	3	11,4	33,296	20,76	20490000	19
2,3	0,109088	0,7	ATAD3B;ATAD	Q5T9A4;Q9H	Q5T9A4;Q9NVN	ATPase family AAA domain-containing pr	4	6,2	72,572	7,5502	14690000	13
1,2	0,151707	0,7	RRM1	P23921	P23921	Ribonucleoside-diphosphate reductase la	4	6,1	90,069	43,303	12883000	14
1,2	0,154333	0,7	MUT	P22033	P22033	Methylmalonyl-CoA mutase, mitochondr	2	2,8	83,134	2,7691	8625300	3
1,0	0,179045	0,7	ECM1	Q16610	Q16610	Extracellular matrix protein 1	2	5	60,673	31,015	16141000	20
1,4	0,15171	0,7	MYOF	Q9NZM1	Q9NZM1	Myoferlin	2	1,2	234,71	3,1109	3513600	5
3,8	0,138467	0,7	RUVBL2	Q9Y230	Q9Y230	RuvB-like 2	3	7,6	51,156	5,7901	10899000	14
3,8	0,109487	0,7	DCD	P81605	P81605	Dermcidin;Survival-promoting peptide;Di	3	32,7	11,284	5,3529	66171000	12
1,7	0,152064	0,6	POLDIP3	Q9BY77	Q9BY77	Polymerase delta-interacting protein 3	9	32,1	46,089	123,65	62614000	83
2,6	0,135248	0,6	ZNF622	Q96953	Q96953	Zinc finger protein 622	5	16,1	54,271	19,711	20516000	26
1,4	0,172489	0,6	CCT7	Q99832	Q99832	T-complex protein 1 subunit eta	12	27,3	59,366	87,339	71021000	50
1,0	0,200829	0,6	CSTA	P01040	P01040	Cystatin-A;Cystatin-A, N-terminally proce	3	5,2	11,006	56,61	30702000	33
2,8	0,145752	0,6	DDX21	Q9NR30;Q9I	Q9NR30	Nucleolar RNA helicase 2	9	14,8	87,343	100,78	81093000	52
1,7	0,165292	0,6	DPM1	O60762	O60762	Dolichol-phosphate mannosyltransferase	2	10,4	29,634	10,639	18602000	20
1,6	0,173909	0,6	ATP5B	P06576	P06576	ATP synthase subunit beta, mitochondrial	15	42,3	56,559	141,44	168410000	111
1,6	0,173714	0,6	DNAIC10	Q81X81	Q81X81	Dnal homolog subfamily C member 10	8	11,3	91,079	53,346	49682000	59
2,3	0,163375	0,6	TUBB2A;TUBB	Q13885;Q9B	Q13885;Q9BVV	Tubulin beta-2A chain;Tubulin beta-2B ct	13	44,5	49,906	37,599	58518000	28
0,9	0,243493	0,6	ACSS2	Q9NR19	Q9NR19	Acetyl-coenzyme A synthetase, cytoplasm	1	2,9	78,579	2,4985	10918000	15
0,9	0,242462	0,5	NCCRP1	Q6ZVX7	Q6ZVX7	F-box only protein 50	5	22,9	30,847	24,893	19379000	23
2,7	0,164124	0,5	SNRPD3	P62318	P62318	Small nuclear ribonucleoprotein Sm D3	2	23,8	13,916	4	10534000	13
1,4	0,201209	0,5	CCT8	P50990	P50990	T-complex protein 1 subunit theta	12	26,8	59,62	50,167	69410000	54
2,0	0,179941	0,5	GD12	P50395	P50395	Rab GDP dissociation inhibitor beta	2	22	50,663	124,23	33172000	38
0,5	0,395442	0,5	HSP90AB2P	Q58F88	Q58F88	Putative heat shock protein HSP 90-beta	6	16,3	44,348	37,818	83729000	18
2,4	0,184335	0,5	S100A9	P06702	P06702	Protein S100-A9	5	50,9	13,242	71,399	67447000	43
0,9	0,270531	0,5	HSPD1	P10809	P10809	60 kDa heat shock protein, mitochondria	18	38,7	61,054	323,31	458830000	180
2,3	0,191507	0,5	SYNCRIP	O60506	O60506	Heterogeneous nuclear ribonucleoprotein	9	22,3	69,602	36,031	70361000	38
0,8	0,297865	0,5	CCT4	P50991	P50991	T-complex protein 1 subunit delta	10	24,3	57,924	37,579	56413000	41
0,7	0,344748	0,5	LGALS3BP	Q08380	Q08380	Galectin-3-binding protein	5	11,3	65,33	37,041	31040000	24
2,3	0,217957	0,5	RCC2	Q9P258	Q9P258	Protein RCC2	7	18	56,084	17,514	70948000	61
1,6	0,244111	0,5	LSG1	Q9H089	Q9H089	Large subunit GTPase 1 homolog	3	5,3	75,225	18,756	26126000	29
1,7	0,250877	0,4	RPL38	P63173	P63173	60S ribosomal protein L38	3	32,9	8,2178	6,758	30578000	17
0,7	0,368604	0,4	SET;SETSIP	Q01105;POD	Q01105;PODM	Protein SET;Protein SETSIP	5	22,8	33,488	49,522	31310000	37
1,2	0,29702	0,4	ARF4	P18085	P18085	ADP-ribosylation factor 4	4	27,2	20,511	4,0888	8479700	5
0,7	0,367175	0,4	PPP2R1A	P30153;P301	P30153	Serine/threonine-protein phosphatase 2f	6	15,6	65,308	10,003	25161000	16
0,6	0,428786	0,4	ASS1	P00966	P00966	Argininosuccinate synthase	4	10,4	46,53	24,898	38357000	14
0,9	0,3395	0,4	IQGAP1	P46940	P46940	Ras GTPase-activating-like protein IQGAP	18	16,3	189,25	155,06	68239000	94
0,5	0,543775	0,4	GANAB	Q14697	Q14697	Neutral alpha-glucosidase AB	11	13,7	106,87	39,924	65038000	41
0,9	0,375975	0,4	HNRNPK	P61978	P61978	Heterogeneous nuclear ribonucleoprotein	8	23,5	50,976	241,64	159580000	70
1,5	0,334093	0,4	EIF3B	P55884	P55884	Eukaryotic translation initiation factor 3 : I	10	19,8	92,48	87,64	84426000	79
2,4	0,306587	0,4	HDAC10	Q96958	Q96958	Histone deacetylase 10	10	27,5	71,444	143,52	185350000	101
0,7	0,416886	0,4	PDIAG	Q15084	Q15084	Protein disulfide-isomerase A6	3	9,3	48,121	75,068	33414000	32
0,9	0,398964	0,4	MVP	Q14764	Q14764	Major vault protein	3	16,1	99,326	56,911	41756000	64
2,3	0,342013	0,3	TUBA1B	P68363;A6N	P68363	Tubulin alpha-1B chain	18	54,5	50,151	323,31	150230000	219
1,5	0,367745	0,3	RUVBL1	Q9Y265	Q9Y265	RuvB-like 1	7	21,7	50,227	45,931	49700000	53
1,5	0,367873	0,3	RPL22L1	Q6PSR6	Q6PSR6	60S ribosomal protein L22-like 1	2	19,7	14,606	2,8271	36116000	6
2,1	0,353154	0,3	TMPO	P42167	P42167	Lamina-associated polypeptide 2, isoform	6	24	50,67	2,6706	10582000	6
1,2	0,395509	0,3	EFTUD2	Q15029	Q15029	116 kDa US small nuclear ribonucleoprot	5	17,2	109,43	13,87	23681000	24
1,5	0,374395	0,3	KLHL22	Q53GT1	Q53GT1	Kelch-like protein 22	8	17,5	71,666	51,529	38063000	64
0,5	0,60713	0,3	RPL23	P62829	P62829	60S ribosomal protein L23	3	30,7	14,865	156,62	139580000	45
0,9	0,441893	0,3	PSMA4	P25789	P25789	Proteasome subunit alpha type-4	2	6,9	29,483	5,3656	22199000	14
0,5	0,585	0,3	CCT6A	Q40227;Q92	Q40227	T-complex protein 1 subunit zeta	6	13,6	58,024	52,76	62483000	37
0,7	0,471368	0,3	TCP1	P17987	P17987	T-complex protein 1 subunit alpha	12	29,1	60,343	108,14	56475000	75
0,7	0,482659	0,3	PRPF19	Q9JUM54	Q9JUM54	Pre-mRNA-processing factor 19	2	7,3	55,18	6,092	7283800	14
0,6	0,492323	0,3	CCT3	P49368	P49368	T-complex protein 1 subunit gamma	14	30,6	60,533	53,808	92690000	47
0,9	0,474953	0,3	GGCT	O75223	O75223	Gamma-glutamylcystotransferase	6	37,2	21,007	32,491	35196000	53
0,8	0,486491	0,3	C7orf50	Q98R16	Q98R16	Uncharacterized protein C7orf50	3	29,4	22,083	7,4133	10608000	26
4,3	0,397073	0,3	CFAP54	Q96N23	Q96N23	Cilia- and flagella-associated protein 54	2	0,9	35,197	3,4216	26651000	5
0,6	0,571448	0,3	PABPC1	P11940;Q9H	P11940	Polyadenylate-binding protein 1	18	27,8	70,767	165,92	485250000	169
0,9	0,496227	0,3	HNRNPDL	Q14979	Q14979	Heterogeneous nuclear ribonucleoprotein	3	7,6	46,437	5,6956	17481000	11
2,4	0,444494	0,3	NOLC1	Q14978	Q14978	Nucleolar and colled-body phosphoprotei	5	7,6	73,602	16,39	128930000	47
1,3	0,488828	0,3	RPS10;RPS10P	P46783;Q9N	P46783;Q9NQC	40S ribosomal protein S10;Putative 40S r	4	18,8	18,898	32,554	440210000	33
0,4	0,7098	0,3	RPL11	P62913	P62913	60S ribosomal protein L11	6	32,6	20,252	48,308	199050000	42
0,5	0,636127	0,3	CANX	P27824	P27824	Calnexin	10	20,8	67,567	43,643	111070000	41
0,9	0,547531	0,3	XRCC6	P12956	P12956	X-ray repair cross-complementing protei	11	24,1	69,842	111,58	74701000	63
0,6	0,615005	0,2	DDX3X;DDX3Y	Q00571;O15	Q00571;O1555	ATP-dependent RNA helicase DDX3X;ATP	9	17,2	73,243	166,98	87566000	63
0,5	0,646492	0,2	GNL3	Q9BV22	Q9BV22	Guanine nucleotide-binding protein-like 3	2	3,5	61,992	2,3004	4103900	3
0,5	0,660351	0,2	AZGP1	P25311	P25311	Zinc-alpha-2-glycoprotein	5	18,1	34,258	20,355	64418000	27
0,7	0,62385	0,2	GALNT3	Q14435	Q14435	Polypeptide N-acetylgalactosaminyltrans	2	3,6	72,609	4,7032	10123000	9
1,1	0,588879	0,2	SUB1	P53999	P53999	Activated RNA polymerase II transcriptio	6	44,9	14,395	116,64	421170000	71
0,7	0,628596	0,2	PABPC4	Q13310;POC	Q13310	Polyadenylate-binding protein 4	11	18,8	70,782	10,504	32960000	33
1,0	0,610208	0,2	EIF3L	Q9Y262	Q9Y262	Eukaryotic translation initiation factor 3 :	9	21,5	66,726	278,35	48774000	84
0,6	0,658213	0,2	YBX1	P67809;Q9Y	P67809	Nuclease-sensitive element-binding prot	11	54,3	35,924	114,45	45499000	82
1,1	0,622602	0,2	UBTF	P17480	P17480	Nucleolar transcription factor 1	12	19	89,405	170,83	358460000	142
0,7	0,65799	0,2	DHX15	Q43143	Q43143	Pre-mRNA-splicing factor ATP-dependent	5	7,3	90,932	16,774	39245000	36
0,4	0,751301	0,2	CAPN1	P07384	P07384	Calpain-1 catalytic subunit	10	16,1	88,889	53,242	56390000	38
0,6	0,686747	0,2	HSP90AB1	P08238;Q58	P08238	Heat shock protein HSP 90-beta	30	48,2	83,263	323,31	585990000	18

0,4	0,998987	0,1	ACTB;ACTG1	P60709;P632;P60709;P6326	Actin, cytoplasmic 1;Actin, cytoplasmic 1	16	56	41,736	323,31	2531800000	285	
0,4	0,994956	0,1	SNRPE	P62304	P62304	Small nuclear ribonucleoprotein E	1	12	10,803	3,2698	6913100	4
0,3	1	0,1	HSPA2	P54652	P54652	Heat shock-related 70 kDa protein 2	17	31,8	70,02	34,066	26857000	34
0,3	1	0,1	ILF2	Q12905	Q12905	Interleukin enhancer-binding factor 2	4	13,6	43,062	15,762	15631000	14
0,4	1	0,1	KRT18	P05783;CONP05783	Keratin, type I cytoskeletal 18	11	33,5	48,057	110,78	75456000	63	
0,4	1	0,0	EPB41L5	Q9HCM4	Q9HCM4	Band 4.1-like protein 5	7	13	81,855	60,821	39546000	45
0,4	1	0,0	GTF2F1	P35269	P35269	General transcription factor IIF subunit 1	3	7,9	58,24	43,844	11168000	14
0,3	1	0,0	PRMT5	O14744	O14744	Protein arginine N-methyltransferase 5;F	3	6,3	72,683	29,94	17529000	26
0,4	1	0,0	SLC25A5	P05141;Q9H P05141	ADP/ATP translocase 2;ADP/ATP transloc	5	16,3	32,852	3,2182	12366000	11	
0,3	1	0,0	SRP9	P49458	P49458	Signal recognition particle 9 kDa protein	3	36	10,112	10,714	29537000	20
0,4	1	0,0	S100A8	P05109	P05109	Protein S100-A8;Protein S100-A8, N-term	4	39,8	10,834	19,476	108490000	43
0,3	1	0,0	ATPSA1	P25705	P25705	ATP synthase subunit alpha, mitochondri	14	33,8	59,75	107,01	141810000	87
0,3	1	0,0	HNRNPH1;HNI	P31943;P557;P31943;P5579	Heterogeneous nuclear ribonucleoprotei	6	16,7	49,229	134,65	51424000	37	
0,4	1	0,0	GNB2L1	P63244	P63244	Guanine nucleotide-binding protein subu	13	60,3	35,076	182,1	620010000	199
0,4	1	0,0	RPS19	P39019	P39019	40S ribosomal protein S19	6	37,2	16,06	39,017	195510000	58
0,3	1	0,0	SYCP1	Q15431	Q15431	Synaptonemal complex protein 1	1	1,3	114,19	3,262	315730000	25
0,3	1	0,0	NEED8	Q15843	Q15843	NEED8	2	34,6	9,0714	9,0674	296160000	17
0,3	1	0,0	SERP1NB2	Q96P63	Q96P63	Serpin B12	8	26,7	46,276	90,188	149240000	67
0,3	1	0,0	BCLAF1	Q9NYF8	Q9NYF8	Bcl-2-associated transcription factor 1	3	4,2	106,12	7,4976	11624000	15
0,3	1	0,0	ESF1	Q9H501	Q9H501	ESF1 homolog	4	5,9	98,795	44,956	6018300	17
0,3	1	0,0	SLC25A1	P53007	P53007	Tricarboxylate transport protein, mitochr	3	11,3	34,012	8,1881	16777000	16
0,3	1	0,0	NAF1	Q96H88	Q96H88	H/ACA ribonucleoprotein complex non-cr	1	2	53,716	3,3808	6275000	2
0,3	1	0,0	TUBA1A;TUBA	Q71U36;Q9P Q71U36;Q9NY	Tubulin alpha-1A chain;Tubulin alpha-8 c	18	54,5	50,135	54,378	4710000	14	
0,3	1	0,0	TUBB4B;TUBB	P68371;P043;P68371;P0435	Tubulin beta-4B chain;Tubulin beta-4A ct	15	51	49,83	313,29	468340000	182	
0,2	1	0,0	TXNDC5	Q8N859	Q8N859	Thioredoxin domain-containing protein 5	2	5,8	47,628	5,5167	9742700	8
0,3	1	-0,1	RAN	P62826	P62826	GTP-binding nuclear protein Ran	5	26,9	24,423	12,105	63064000	33
0,3	1	-0,1	TOP1	P11387	P11387	DNA topoisomerase 1	10	16,2	90,725	77,503	98063000	84
0,2	1	-0,1	SUPT16H	Q9Y589	Q9Y589	FACT complex subunit SPT16	23	24,8	119,91	323,31	511160000	271
0,2	1	-0,1	CSNK2A1;CSNI	P68400;Q8N P68400;Q8NE	Casein kinase II subunit alpha;Casein kin	4	11	45,143	34,048	37780000	27	
0,1	1	-0,1	PCBP1	Q15365	Q15365	Poly(rC)-binding protein 1	6	25,6	37,497	43,424	85136000	49
0,1	1	-0,1	RPL23A	P62750	P62750	60S ribosomal protein L23a	6	32,7	17,695	14,552	144410000	31
0,3	1	-0,1	PDIA3	P30101	P30101	Protein disulfide-isomerase A3	14	34,1	56,782	94,139	152800000	45
0,3	1	-0,1	AGK	Q53H12	Q53H12	Acylglycerol kinase, mitochondrial	3	8,1	47,137	5,7039	12562000	14
0,2	1	-0,1	ARGLU1	Q9NWB6	Q9NWB6	Arginine and glutamate-rich protein 1	2	5,9	33,216	2,9215	21282000	6
0,2	1	-0,1	TKT	P29401	P29401	Transketolase	9	22	67,877	49,505	113870000	86
0,1	1	-0,1	RPS21	P63220	P63220	40S ribosomal protein S21	2	22,9	9,1113	4,6386	17178000	9
0,2	1	-0,1	SRSF3;SRSF7	P84103;Q16P P84103;Q1662	Serine/arginine-rich splicing factor 3;Ser	1	8,5	19,329	20,916	37814000	21	
0,2	1	-0,1	ZRANB2	O95218	O95218	Zinc finger Ran-binding domain-containi	8	24,8	37,404	83,273	90599000	81
0,0	1	-0,1	RPS3	P23396	P23396	40S ribosomal protein S3	12	59,7	26,688	123,4	528210000	139
0,1	1	-0,1	EIF3C;EIF3CL	Q99613;B5M Q99613;B5ME	Eukaryotic translation initiation factor 3 :	10	12,5	105,34	75,293	80606000	84	
0,1	1	-0,1	PCBP2;PCBP3	Q15366;P57 Q15366;P5772	Poly(rC)-binding protein 2;Poly(rC)-bindi	5	20,3	38,58	10,796	35999000	16	
0,1	1	-0,1	RPS14	P62263	P62263	40S ribosomal protein S14	5	36,4	16,273	13,847	138220000	41
0,2	1	-0,1	HDFGRP2	Q724V5	Q724V5	Hepatoma-derived growth factor-relatec	1	2,2	74,316	19,633	23755000	21
0,1	1	-0,1	RPS16	P62249	P62249	40S ribosomal protein S16	4	28,1	16,445	17,827	212740000	47
0,1	1	-0,1	PIIB	P23284	P23284	Peptidyl-prolyl cis-trans isomerase B	6	29,2	23,742	13,964	46057000	15
0,1	1	-0,1	EIF3G	O75821	O75821	Eukaryotic translation initiation factor 3 :	4	21,2	35,611	10,773	20657000	12
0,2	1	-0,2	RPN1	P04843	P04843	Dolichyl-diphosphooligosaccharide--prot	11	21,4	68,569	55,832	62026000	54
0,1	1	-0,2	PLAA	Q9Y263	Q9Y263	Phospholipase A-2-activating protein 1	1	1,5	87,156	2,2517	44229000	3
0,2	1	-0,2	KRT2	P35908	P35908	Keratin, type II cytoskeletal 2 epidermal	47	82,6	65,432	323,31	74860000000	1095
0,1	1	-0,2	RPL21	P46778	P46778	60S ribosomal protein L21	2	20,6	18,565	7,6034	71045000	31
0,1	1	-0,2	SNRNP200	O75643	O75643	U5 small nuclear ribonucleoprotein 200 l	7	4,9	244,5	30,467	13388000	15
0,2	1	-0,2	FLNA	P21333	P21333	Filamin-A	42	25,9	280,74	305,24	238640000	262
0,1	1	-0,2	PA2G4	Q9UQ80	Q9UQ80	Proliferation-associated protein 2G4	13	44,2	43,786	323,31	182010000	128
0,0	1	-0,2	RPS20	P60866	P60866	40S ribosomal protein S20	3	25,2	13,373	14,785	80541000	36
0,0	1	-0,2	RPL30	P62888	P62888	60S ribosomal protein L30	5	57,4	12,784	83,407	202740000	52
0,0	1	-0,2	EIF3E	P60228	P60228	Eukaryotic translation initiation factor 3 :	7	17,3	52,22	14,992	25605000	21
0,0	1	-0,2	RPL13	P26373	P26373	60S ribosomal protein L13	4	19,9	24,261	25,144	56944000	39
0,2	1	-0,2	EIF2S1	P05198	P05198	Eukaryotic translation initiation factor 2 :	12	41,9	36,112	79,301	57973000	72
0,2	1	-0,2	SSR4	P51571	P51571	Translocon-associated protein subunit de	2	12,1	18,998	15,008	9685400	14
0,2	1	-0,2	HSP90AA1	P07900;Q14 P07900	Heat shock protein HSP 90-alpha	33	50,5	84,659	323,31	130430000	252	
0,0	1	-0,2	RPL7	P18124	P18124	60S ribosomal protein L7	6	29,4	29,225	36,437	161560000	69
0,1	1	-0,2	RPL37A	P61513;A6NI P61513	60S ribosomal protein L37a	3	38	10,275	38,135	27520000	24	
0,1	1	-0,2	SRSF5	Q13243	Q13243	Serine/arginine-rich splicing factor 5	3	13,2	31,263	8,1491	51448000	22
0,1	1	-0,2	RPL9	P32969	P32969	60S ribosomal protein L9	2	15,1	21,863	28,067	35907000	19
0,1	1	-0,3	EZR	P15311;P26 P15311	Ezrin	9	17,4	69,412	46,399	91928000	53	
0,1	1	-0,3	SNRPN;SNRPB	P63162;P146 P63162;P1467	Small nuclear ribonucleoprotein-associat	1	3,3	24,614	2,5985	10888000	7	
0,1	1	-0,3	CKAP4	Q07065	Q07065	Cytoskeleton-associated protein 4	13	30,2	66,022	46,838	46719000	70
0,0	1	-0,3	RPL27A	P46776	P46776	60S ribosomal protein L27a	2	14,9	16,561	8,0917	71916000	24
0,1	1	-0,3	LUC7L2	Q9Y383	Q9Y383	Putative RNA-binding protein Luc7-like 2	2	7,7	46,513	64,373	20739000	36
0,0	1	-0,3	RPS5	P46782	P46782	60S ribosomal protein S5;40S ribosomal j	3	18,6	22,876	93,206	124240000	41
0,1	1	-0,3	L10L7	P27635;Q96 P27635	60S ribosomal protein L10	3	60,5	14,604	40,376	28677000	28	
0,1	1	-0,3	YWHAE	P62255	P62255	14-3-3 protein epsilon	8	36,1	29,174	65,1	87674000	61
0,2	1	-0,3	CLIC1	Q00299	Q00299	Chloride intracellular channel protein 1	6	42,7	26,922	148,17	50730000	37
0,0	1	-0,3	SPRR1A;SPRR	P35321;P225 P35321;P22521	Corniflin-A;Corniflin-B	3	42,7	9,8774	5,1882	16731000	28	
0,0	1	-0,3	YARS	P54577	P54577	Tyrosine-tRNA ligase, cytoplasmic;Tyros	7	15,2	59,143	19,035	37217000	39
0,1	1	-0,3	RPL24	P83731	P83731	60S ribosomal protein L24	2	13,4	17,779	17,744	41269000	44
0,1	1	-0,3	HACD3	Q9P035	Q9P035	Very-long-chain (3R)-3-hydroxyacyl-CoA	2	6,6	43,159	14,5	12070000	22
0,2	1	-0,3	GSTP1	P09211	P09211	Glutathione S-transferase P	8	51,9	23,356	323,31	356590000	85
0,2	1	-0,3	DEK	P35659	P35659	Protein DEK	8	27,7	42,674	69,007	89942000	71
0,0	1	-0,3	RPS18	P62269	P62269	40S ribosomal protein S18	6	32,9	17,718	19,544	209350000	87
0,0	1	-0,3	RPL5	P46777	P46777	60S ribosomal protein L5	5	23,2	34,362	182,32	97599000	47
0,0	1	-0,3	RPS25	P62851	P62851	40S ribosomal protein S25	3	16	13,742	6,7208	59223000	19
0,1	1	-0,3	MDH2	P40926	P40926	Malate dehydrogenase, mitochondrial	7	25,7	35,503	76,648	69254000	36
0,0	1	-0,3	RPS17	P08708	P08708	40S ribosomal protein S17	5	56,3	15,55	53,487	23668000	25
0,0	1	-0,3	RPS13	P62277	P62277	40S ribosomal protein S13	5	37,7	17,222	39,089	121210000	42
0,1	1	-0,3	CPNE8;CPNE3	Q86YQ8;O7 Q86YQ8;O751	Copine-8;Copine-3;Copine-2;Copine-9;Co	2	3,5	63,107	4,3523	6252700	17	
0,0	1	-0,3	RPL18A	Q02543	Q02543	60S ribosomal protein L18a	5	18,9	20,762	14,956	88848000	52
0,0	1	-0,3	G6PD	P11413	P11413	Glucose-6-phosphate 1-dehydrogenase	6	17,1	59,256	100,93	28759000	28
0,0	1	-0,3	EIF6	P56537	P56537	Eukaryotic translation initiation factor 6	5	29,4	26,599	22,449	40670000	36
0,0	1	-0,4	RPL22	P35268	P35268	60S ribosomal protein L22	3	30,5	14,787	112,77	88892000	38
0,0	1	-0,4	RPS12	P25398	P25398	40S ribosomal protein S12	4	39,4	14,515	7,5224	51202000	28
0,0	1	-0,4	EFP1A1P5;EEF	Q5VTE0;P68 Q5VTE0;P6810	Putative elongation factor 1-alpha-like 3;	13	35,9	50,184	87,331	1811900000	206	
0,0	1	-0,4	RPS11	P62280	P62280	40S ribosomal protein S11	6	33,5	18,431	25,466	133240000	32
0,1	1	-0,4	HNRNPA1;HNI	P09651;Q32 P09651;Q32P5	Heterogeneous nuclear ribonucleoprotei	8	28,5	38,746	38,288	20804000	33	
0,0	1	-0,4	RPL10A	P62906	P62906	60S ribosomal protein L10a	8	35,9	24,831	44,937	164310000	103
0,1	1	-0,4	SRP14	P37108	P37108	Signal recognition particle 14 kDa protei	5	33,1	14,57	167,55	340	

0,1	1	-0,5	HSP90B1	P14625;Q58I	P14625	Endoplasmic	20	30,6	92,468	274,66	298960000	95
0,0	1	-0,5	RPS28	P62857	P62857	40S ribosomal protein S28	2	30,4	7,8409	69,318	77988000	21
0,0	1	-0,5	PRDX2	P32119	P32119	Peroxiredoxin-2	4	36,4	21,892	9,1734	47733000	20
0,0	1	-0,6	RPL14	P50914	P50914	60S ribosomal protein L14	3	15,8	23,432	11,664	72516000	26
0,0	1	-0,6	RPL31	P62899	P62899	60S ribosomal protein L31	4	33,6	14,463	121,28	127930000	54
0,0	1	-0,6	FARSB	Q9NSD9	Q9NSD9	Phenylalanine--tRNA ligase beta subunit	2	3,2	66,115	5,486	7867600	7
0,1	1	-0,6	TCOF1	Q13428	Q13428	Treacle protein	2	1,5	152,1	3,8257	6389400	8
0,2	1	-0,6	MYH9	P35579;P357	P35579	Myosin-9	31	20,9	226,53	323,31	144430000	152
0,0	1	-0,6	SRSF1	Q07955	Q07955	Serine/arginine-rich splicing factor 1	5	21,8	27,744	21,2	24022000	28
0,0	1	-0,6	LARS	Q9P2J5	Q9P2J5	Leucine--tRNA ligase, cytoplasmic	7	8,2	134,46	40,325	21116000	37
0,1	1	-0,6	EIF5	P55010	P55010	Eukaryotic translation initiation factor 5	3	10,7	49,222	11,027	30382000	32
0,0	1	-0,6	ENO1	P06733;P13P	P06733	Alpha-enolase	16	48,4	47,168	323,31	837090000	174
0,0	1	-0,6	RTF1	Q92541	Q92541	RNA polymerase-associated protein RTF1	4	6,6	80,313	21,407	13322000	28
0,1	1	-0,6	PARP1	P09874	P09874	Poly [ADP-ribose] polymerase 1	22	25,7	113,08	323,31	407480000	187
0,0	1	-0,6	RPL4	P36578	P36578	60S ribosomal protein L4	10	28,3	47,697	119,55	196500000	85
0,1	1	-0,6	PKM	P14618;P30P	P14618	Pyruvate kinase PKM	23	51,2	57,936	323,31	728320000	206
0,0	1	-0,6	TXN	P10599	P10599	Thioredoxin	1	10,5	11,737	4,4081	14943000	16
0,0	1	-0,6	ANXA2;ANXA2	P07355;A6N	P07355;A6NM	Annexin A2;Putative annexin A2-like prot	18	48,1	38,604	256,17	338980000	193
0,0	1	-0,7	HAL	P42357	P42357	Histidine ammonia-lyase	6	11,7	62,697	47,915	31428000	27
0,1	1	-0,7	RBM25	P49756	P49756	RNA-binding protein 25	5	9,1	100,18	8,2052	19535000	18
0,0	1	-0,7	EIF4A1	P60842	P60842	Eukaryotic initiation factor 4A-1	11	29,6	46,153	38,778	90671000	80
0,1	1	-0,7	YWHAQ	P27348	P27348	14-3-3 protein theta	4	17,6	27,764	57,35	10327000	21
0,1	1	-0,7	PIGR	P01833	P01833	Polymeric immunoglobulin receptor;Secr	12	19,6	83,283	166,97	128650000	54
0,0	1	-0,7	RPL8	P62917	P62917	60S ribosomal protein L8	3	13,2	28,024	29,521	128560000	42
0,1	1	-0,7	FLNB	Q75369;Q14O	Q75369	Filamin-B	14	8,3	278,16	38,753	34929000	36
0,0	1	-0,7	RPL19	P84098	P84098	60S ribosomal protein L19	2	13,3	23,466	31,397	26411000	20
0,0	1	-0,7	PHLD82	Q8650Q	Q8650Q	Pleckstrin homology-like domain family 8	5	5,4	142,16	78,689	83894000	22
0,0	1	-0,7	HIST1H2BL;H1	Q99880;Q99	Q99880;Q99880	Histone H2B type 1-L;Histone H2B type 1	3	27,7	13,952	67,171	125060000	27
0,0	1	-0,7	EIF3M	Q7L2H7	Q7L2H7	Eukaryotic translation initiation factor 3 : M	4	15	42,502	21,26	21348000	11
0,0	1	-0,7	PSMA6	P60900	P60900	Proteasome subunit alpha type-6	2	10,2	27,399	36,193	21010000	18
0,0	1	-0,7	DSG1	Q02413	Q02413	Desmoglein-1	23	29	113,75	323,31	948410000	378
0,0	1	-0,8	RPS2	P15880	P15880	40S ribosomal protein S2	4	15,4	31,324	9,5845	68151000	24
0,1	1	-0,8	GGH	Q92820	Q92820	Gamma-glutamyl hydrolase	3	12,6	35,964	11,731	18687000	14
0,1	1	-0,8	KPNB1	Q14974	Q14974	Importin subunit beta-1	13	19,6	97,169	240,44	53074000	40
0,0	1	-0,8	RPL29	P47914	P47914	60S ribosomal protein L29	1	9,4	17,752	3,1103	11579000	15
0,1	1	-0,8	SGPL1	O95470	O95470	Sphingosine-1-phosphate lyase 1	6	13,7	63,523	11,007	13959000	12
0,0	1	-0,8	PSMB5	P28074	P28074	Proteasome subunit beta type-5	4	16,3	28,48	12,172	10124000	20
0,1	1	-0,9	CAP1	Q01518	Q01518	Adenylyl cyclase-associated protein 1	4	11,8	51,901	132,25	28794000	36
0,0	1	-0,9	VDAC2	P45880	P45880	Voltage-dependent anion-selective chan	6	24,8	31,566	42,347	17190000	31
0,0	1	-0,9	S100A14	Q9HCY8	Q9HCY8	Protein S100-A14	3	35,6	11,662	56,867	25823000	30
0,1	1	-0,9	LRRCS9	Q96AG4	Q96AG4	Leucine-rich repeat-containing protein 5	7	30	34,93	81,99	206350000	58
0,0	1	-0,9	PPP2R4	Q12527	Q12527	Serine/threonine-protein phosphatase 2	2	7,3	40,667	3,8727	2680100	3
0,0	1	-1,0	DMBT1	Q9UGM3	Q9UGM3	Deleted in malignant brain tumors 1 pro	7	18,2	260,73	31,134	28734000	26
0,0	1	-1,0	RPS26P11;RPS	Q5JN25;P62I	Q5JN25;P62I	Putative 40S ribosomal protein S26-like 1	3	24,3	13,002	6,6554	61814000	34
0,1	1	-1,0	ALOX12B	O75342	O75342	Arachidonate 12-lipoxygenase, 12R-type	6	10,1	80,355	76,005	29628000	27
0,1	1	-1,1	FASN	P49327	P49327	Fatty acid synthase;[Acyl-carrier-protein]	32	19,3	273,42	290,68	162260000	149
0,0	1	-1,1	PSMD3	O43242	O43242	26S proteasome non-ATPase regulatory s	5	12,7	60,977	11,063	16364000	22
0,0	1	-1,1	RPS6	P62753	P62753	40S ribosomal protein S6	3	14,1	28,68	31,264	40261000	21
0,0	1	-1,1	PSMB6	P28072	P28072	Proteasome subunit beta type-6	3	13	25,357	6,0645	11705000	8
0,0	1	-1,1	PLEC	Q15149	Q15149	Plectin	8	2,3	531,78	15,705	5236600	18
0,0	1	-1,1	VPS35	Q96QK1	Q96QK1	Vacuolar protein sorting-associated prot	5	8,8	91,706	109,63	18491000	30
0,1	1	-1,1	EIF2S3;EIF2S3	P41091;Q2V	P41091;Q2VIR	Eukaryotic translation initiation factor 2 :	12	35	51,109	129,28	158000000	84
0,1	1	-1,1	TAPBP1	Q9BX59	Q9BX59	Tapasin-related protein	1	3,6	50,182	2,3302	240160000	2
0,0	1	-1,2	RPL15	P61313	P61313	60S ribosomal protein L15	4	22,5	24,146	13,287	48382000	30
0,0	1	-1,2	LYZ	P61626	P61626	Lysozyme C	4	39,9	16,537	51,378	62011000	34
0,0	1	-1,2	EEF1D	P29692	P29692	Elongation factor 1-delta	2	13,2	31,121	12,98	14905000	8
0,0	1	-1,2	AHCY	P23526	P23526	Adenosylhomocysteinase	3	8,1	47,716	5,9556	8340100	11
0,0	1	-1,2	EIF3F	O00303	O00303	Eukaryotic translation initiation factor 3 :	3	12	37,563	15,825	19205000	24
0,0	1	-1,2	PFN1	P07737;CONP	P07737	Profilin-1	6	57,1	15,054	115,22	54659000	40
0,0	1	-1,2	TALDO1	P37837	P37837	Transaldolase	3	10,7	37,54	12,866	13783000	20
0,0	1	-1,3	RNPEP	Q9H4A4	Q9H4A4	Aminopeptidase B	8	17,2	72,595	22,148	18584000	22
0,0	1	-1,3	RAB6B;RAB6A	Q9NRW1;P2Q	Q9NRW1;P2Q3	Ras-related protein Rab-6B;Ras-related p	1	5,3	23,461	12,432	22469000	12
0,1	1	-1,3	RPL35A	P18077	P18077	60S ribosomal protein L35a	2	19,1	12,538	4,0796	34125000	11
0,0	1	-1,3	DSC1	Q08554	Q08554	Desmocollin-1	13	20,8	99,986	323,31	513470000	182
0,0	1	-1,3	H2AFV;H2AFZ;	Q71U09;P0CQ	Q71U09;P0CQ	Histone H2A.V;Histone H2A.Z;Histone H2	3	20,3	13,509	5,4803	141490000	21
0,1	1	-1,4	ACTN4	Q43707;Q08O	Q43707	Alpha-actinin-4	27	36	104,85	311,4	151950000	128
0,0	1	-1,4	RPL2	P05387	P05387	60S acidic ribosomal protein P2	5	81,7	11,665	127,56	38489000	61
0,0	1	-1,4	SERPINB8	P50452;P504	P50452	Serpin B8	4	12,3	42,766	27,101	6656600	14
0,0	1	-1,4	VCP	P55072	P55072	Transitional endoplasmic reticulum ATPa	21	36,8	89,321	323,31	159790000	133
0,0	1	-1,4	ALDOA	P04075	P04075	Fructose-bisphosphate aldolase A	13	44,5	39,42	79,683	14250000	8
0,0	1	-1,4	PPP1CB;PPP1	P62140;P366	P62140;P3687	Serine/threonine-protein phosphatase PF	4	11,6	37,186	18,19	15717000	26
0,0	1	-1,4	PSMB1	P20618	P20618	Proteasome subunit beta type-1	3	19,9	26,489	8,4491	11672000	47
0,0	1	-1,4	TARS	P26639;A2R	P26639	Threonine--tRNA ligase, cytoplasmic	12	21,4	83,434	29,899	29823000	47
0,0	1	-1,4	ZG16B	Q96DA0	Q96DA0	Zylogen granule protein 16 homolog B	3	22,6	22,739	56,956	15242000	37
0,0	1	-1,5	CFL1	P23528;Q9Y	P23528	Cofilin-1	4	30,7	18,502	323,31	50856000	30
0,0	1	-1,5	RPL18	Q07020	Q07020	60S ribosomal protein L18	4	25,5	21,634	55,481	69162000	46
0,0	1	-1,5	P4HB	P07237	P07237	Protein disulfide-isomerase	11	24,2	57,116	74,219	171350000	80
0,0	1	-1,6	RPL3	P39023;Q92	P39023	60S ribosomal protein L3	8	23,1	46,108	26,673	76903000	32
0,0	1	-1,6	TGM3	Q08188	Q08188	Protein-glutamine gamma-glutamyl/trans	15	27,3	76,631	323,31	294290000	137
0,0	1	-1,6	ARHGDI1A	P52565	P52565	Rho GDP-dissociation inhibitor 1	2	23	23,207	16,106	13334000	22
0,0	1	-1,6	PSMA7;PSMAI	Q14818;Q8T	Q14818;Q8TA	Proteasome subunit alpha type-7;Proteas	5	30,6	27,887	119,89	38304000	32
0,0	1	-1,7	PPA1	Q15181	Q15181	Inorganic pyrophosphatase	5	26,3	32,66	59,056	16878000	21
0,0	1	-1,7	RPL35	P42766	P42766	60S ribosomal protein L35	1	8,1	14,551	3,5739	12386000	11
0,0	1	-1,7	RAB7A	P51149	P51149	Ras-related protein Rab-7a	4	26,1	23,489	130,54	30127000	24
0,0	1	-1,7	RPL6	Q02878	Q02878	60S ribosomal protein L6	4	13,5	32,728	5,7086	30559000	9
0,0	1	-1,7	ALDOC	P09972	P09972	Fructose-bisphosphate aldolase C	5	21,2	39,455	106,67	38431000	44
0,0	1	-1,7	RAB2A;RAB2B	P61019;Q8W	P61019;Q8WU	Ras-related protein Rab-2A;Ras-related p	4	25,5	23,545	15,398	8394600	19
0,0	1	-1,7	MTDH	Q86UE4	Q86UE4	Protein LYRIC	5	12,9	63,836	55,03	43086000	51
0,0	1	-1,8	EEF1G	P26641	P26641	Elongation factor 1-gamma	7	17,6	50,118	83,04	103600000	47
0,0	1	-1,8	PIP	P12273	P12273	Prolactin-inducible protein	5	35,6	16,572	101,78	46888000	30
0,0	1	-1,8	THRAP3	Q9Y2W1	Q9Y2W1	Thyroid hormone receptor-associated pr	3	3,7	108,66	4,2076	12657000	5
0,0	1	-1,8	DSC3	Q14574	Q14574	Desmocollin-3	8	13,1	99,968	59,459	84807000	38
0,0	1	-1,8	CALR	P27797	P27797	Calreticulin	8	35,3	48,141	193,97	65541000	42
0,0	1	-1,8	HSD17B4	P51659	P51659	Peroxisomal multifunctional enzyme type	5	11,1	79,685	35,91	17160000	41
0,0	1	-1,8	JUP	P14923	P14923	Junction plakoglobin	28	53,4	81,744	282,36	100270000	349
0,0	1	-1,9	RPS9	P46781	P46781	40S ribosomal protein S9	2	10,3	22,591	5,9093	41240000	15
0,0	1	-1,9	ARF1;ARF3	P84077;P612	P84077;P6120	ADP-ribosylation factor 1;ADP-ribosylat	5	37,6				





In addition to these findings that were published in the JCB journal, there are some unpublished data that try to answer some of the questions mentioned above.

**Additional results :(Unpublished data):**

**Possible association of DLG1 with Fas at the initial step following Fas activation by its ligand.**

Since DLG1 associates directly with Fas and inhibits Fas cell death signaling, we were interested to analyze whether DLG1 could be associated with Fas following its activation. To demonstrate the possible recruitment of DLG1 together with Fas activated receptors, HCT15 cells were activated with FasL-coated beads and activated Fas receptors recruited underneath FasL-coated beads were isolated after cell lysis at different time points. Western-blot analysis revealed that at early points, DLG1 is found associated with Fas, when the DISC components (Caspase-8 and FADD) are not fully recruited. However, at later time points DLG1 seems to dissociate from the DISC. These data suggest that DLG1 may be recruited at the initial step of DISC formation where it may play a negative role in either the recruitment or the activation of caspases 8 or 10 (*data not shown*). However, these results were difficult to reproduce possibly because the unstable association of DLG1 with Fas in the DISC. Other techniques would be required in order to prove the possible role of DLG1 has a negative regulator of DISC formation.

**Pro-invasive N-cadherin also associates with Fas and DLG1 in cancer cells and inhibit Fas cell death signaling.**

We examined whether N-cadherin could regulate Fas cell death signaling in a similar manner to E-cadherin, since EMT has been associated with acquisition of resistance to apoptosis [646]. To do this, we used the melanoma cell line MDA-MB-435 in which N-cadherin expression promotes invasive capacity. Since the parental MDA-MB-435 express small amount of Fas receptor (Figure 31. A), MDA-MB-435 cells were transduced by retroviral vectors containing the WT form of human Fas (MDA-MB-435-Fas) or an empty vector (MDA-MB-435-zeo) (Figure 31. B). Stable expression of Fas sensitized MDA-MB-435-Fas cells to FasL-induced cell death, demonstrating that transfected Fas is functionally active in these cells (Figure. 31 C). IF experiments demonstrated that, in MDA-MB-435-Fas cells, Fas is mainly found associated with N-cadherin and DLG1 at cell-cell contacts (Figure 31. D and E). Invalidation of N-cadherin

expression in MDA-MB-435-Fas cells abrogated the formation of AJs, and sensitized N-cadherin-deficient cells to Fas-cell death signaling (Figure 31. D and F). Knockdown of DLG1 in MDA-MB-435-Fas cells using siRNA reduced N-cadherin and Fas accumulation at cell-cell junctions compared to control cells (siCtrl), which affects FasL-induced cell death sensitivity (Figure 31. E and F). All together these data suggest that N-cadherin expression in invasive cancer cells protects individual cells from FasL-induced-cell death by promoting the formation

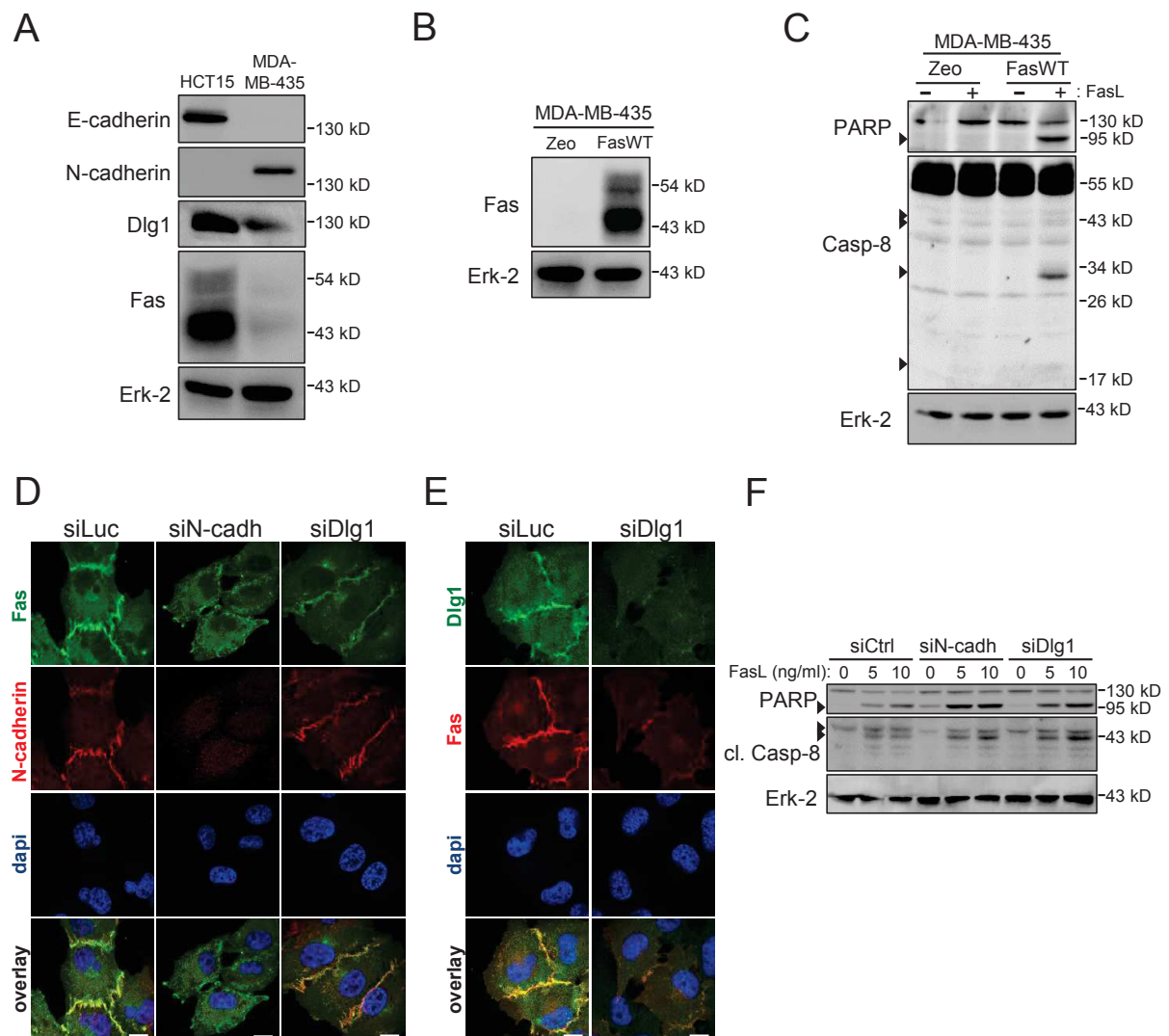


Figure 31: N-cadherin expression in invasive cancer cells associates with Fas and Dlg1 and restrains Fas cell death signaling

(A and B) IB procedure was used to compare Fas and AJ components expression in HCT15, parental (A) or transduced MDA-MB-435 (MDA-MB-435-zeo and MDA-MB-435-Fas) (B). (C) Cell death was induced in MDA-MB-435-Fas and MDA-MB-435-zeo cells by FasL treatment and measured by IB as described above. (D and E) IF of Fas, N-cadherin and Dlg1 in MDA-MB-435-FasWT transfected by Ctrl, N-cadherin or Dlg1 siRNAs. (F) Cell death was induced in MDA-MB-435-FasWT cells transfected with control (siCtrl), N-cadherin (siN-cadh) or Dlg1 (siDlg1) siRNA by IB as described above.

of an N-cadherin-Dlg1-Fas complex at the plasma membrane that restrains FasL-induced cell death.

### III.B Project 2: Investigating Fas novel interactants and their role in the receptor signaling

(Hala AWINA., et al, unpublished data):

#### **Analysis of proteomic results and selection of five *protein of interest*:**

Our proteomic approach revealed that, beside Dlg1, Fas C-terminal PDZ binding domain possibly interacts with 53 different proteins, suggesting an extreme complexity and variety of the molecular complexes formed at the C-terminal region of Fas. In order to analyze the rationale that underlie such complexity, we chose to study the effect of a limited number of proteins on Fas signaling. These proteins belong to different protein families and contain or not a PDZ-domain. All these proteins are known to modulate cell surface receptor signaling, apoptosis, stress response or polarity. The five selected proteins are: the actin-binding LIM (abLIM1), the E3 ubiquitin ligase Ligand Numb-protein X2 (LNX2), the endoplasmic reticulum chaperone GRP78, the polarity protein PATJ and the adaptor protein Beta-2-syntrophin (SNTB2).

The identified functions of these proteins are summarized below and help to reveal their possible involvement in the modulation of Fas signaling and/or trafficking.

abLIM1 is a multi-domain protein constituted of N-terminal LIM domains (cysteine-rich zinc finger motifs), dematin- like domain/villin domain that facilitated its interaction with F-actin [647]. abLIM1 was found to colocalizes in zones of high actin concentration, which is consistent with the presence of a recognized actin-binding domain in this protein. Which suggests ablim1's role in facilitating interactions between cytoplasmic targets and actin filaments[647, 648]. abLIM1 was shown to play a role in axon guidance in in the retina by mediating actin rearrangement, however the exact mechanism of this regulation is unclear [649, 650]. Recently it has been demonstrated that abLIM1 regulates osteoclast differentiation through the modulation of the signaling cascade of RANKL, a member of the TNF ligand family [651, 652], suggesting that abLIM1 may be an important regulator of various TNFR signaling including Fas. abLIM1 does not contain neither a PDZ domain nor a consensus PDZ-C-terminal binding site, suggesting does it is recruited to Fas through an indirect

interaction. Interestingly, data from the BIOGRID data bank suggest an interaction with CASK and Lin7c, two Dlg1 co-interactants.

GRP78, also known as BiP or HSP5a is protein involved in endoplasmic plasma stress and implicated in the folding and assembly of nascent polypeptides preventing misfolding and aggregation of proteins while targeting misfolded proteins to degradation via proteasome [653]. The role of GRP78 in cell survival was demonstrated thanks to the generation of *Grp78* knockout of mice that were found lethal [654]. GRP78 interacts directly or indirectly with several proteins and is able to modify the function or activity of these proteins upon stress or other stimuli. Overexpression of GRP78 maintain mitochondrial permeability and thus protect cells from ER stress-induced apoptosis through stabilizing proto-oncogene Raf-1 [655], and suppressing Caspase-7 activity [656]. Moreover, GRP78 is translocated to the plasma membrane where it signals to modulate survival signals in cells under stress [657]. GRP78 at the plasma membrane can induce apoptosis through the ligation of prostate apoptosis response-4 (Par-4), a proapoptotic protein found secreted in cells under stress [658]. Furthermore, it was exhibited that GRP-78-Par-4 are translocated to the plasma membrane upon TRAIL treatment. GRP78 is found overexpressed in several cancer types and is associated with poor prognosis. Therefore, it would be interesting to study whether apoptotic function mediated by GRP78 may be related to its association with Fas receptor.

SNTB2 a protein that belongs to the members of dystrophin-associated complex ( $\alpha$ 1-,  $\beta$ 1-, and  $\beta$ 2-syntrophin, dystrophin, and dystrobrevin). SNTB2 domain structure contain a pleckstrin homology domains, PDZ domain which mediates interaction with proteins and certain receptors, and a syntrophin-unique domain that mediates its interaction with dystrophin complex. SNTB2 can be found at cell-cell junction and can act as an adaptor protein that recruits and modulates different signaling proteins through building a signaling complex [659]. Hence, it will be interesting to examine if SNTB2 act as a scaffold protein and participate in Fas signaling. Intriguingly, data from the BIOGRID data bank reveal that SNTB2 can interact with Dlg1's partner CASK and LIN7.

PATJ is a polarity protein that belongs to the apical polarity complex Crumbs (as described page 110). Since PATJ belongs to a different polarity complex than Dlg1, we wanted to examine whether PATJ collaborate with Dlg1 in the regulation of Fas signaling. Indeed, the involvement of different polarity complexes in regulation of signaling pathways (such as the

Hippo pathway) has been already observed.

LN2, known also as PDZRN1, is an E3 ubiquitin ligase containing a RING finger domain and four PDZ domains. LN2 is a member of the LNX family, which includes five members LN1-LN5. Several studies demonstrate that LN2 plays an important role in the modulation of pro-apoptotic signals and proliferation in the physiological and cancerous context [660]. For instance, LN2 was reported to interact with Numb, promoting its degradation through ubiquitination and as a consequence altering NOTCH1 levels and WNT signaling leading to an increase of cell death by apoptosis [660, 661]. In addition, previous studies illustrated that LN2 was found overexpressed in colon cancer cells due to a chromosomal amplification [660]. Therefore, it was crucial for us to understand to test the possible role of LN2 in the regulation of Fas signaling in colon cell lines.

***Influence of Fas partners on Fas cell death signaling*** and its association with the cytoskeleton

To investigate the effect of each of the five selected proteins abLIM1, LN2, GRP78, PATJ and SNTB2 on Fas cell death signaling, I used a siRNA approach in colon epithelial cells HCT15 treated or not with FasL. We observed that the decrease expression of LN2 and GRP78 had the most significant effect on caspase-8 (Figure 32. D) and PARP cleavage (Figure 32 C). Interestingly, the siLN2 and siGRP78 augmented total Fas expression (Figure 32 E). As the siabLIM1, siSNTB2 and siPATJ have a subtle effect on Fas cell death signaling upon FasL-treatment, we decide to not continue the study on these proteins.

Moreover, the absence of GRP78 augmented cell death prior FasL treatment, which is not displayed by PARP or caspase cleavage, since the cells are washed with fresh medium before treatment. Next, we examined if the detergent solubility of Fas is dependent on one of the

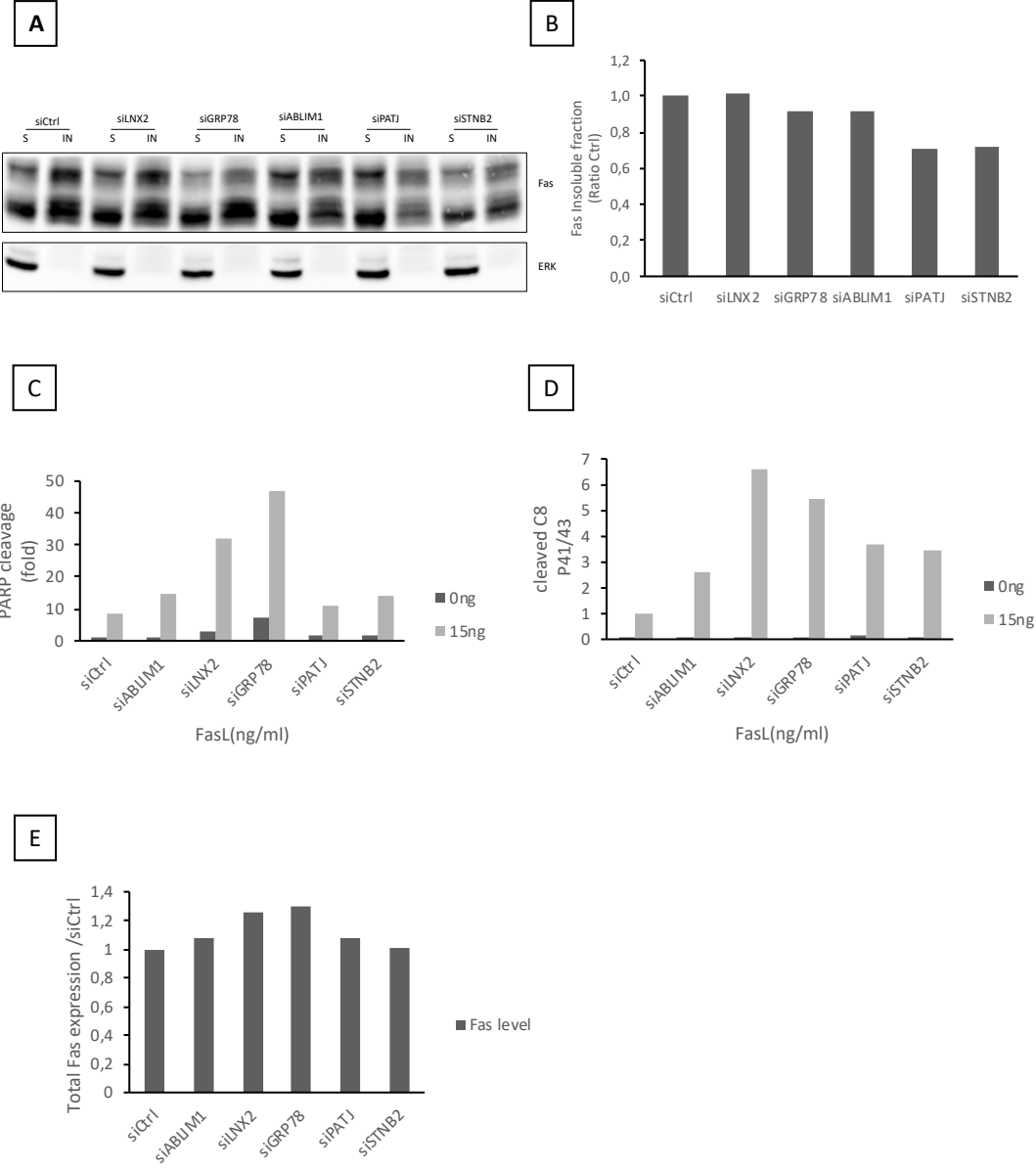


Figure 32: Screening the effect of Fas partners on Fas signaling

(A) Fas detergent solubility in HCT15 cells transfected with indicated siRNAs was determined by IB. S for soluble fraction and IN for insoluble cytoskeleton fraction. ERK is a control for the soluble fraction. (B) Bar chart representing Fas relative solubility percentage in each condition quantified via densitometry. (C, D) HCT15 cells were transiently transfected with control or indicated siRNAs for 3 days before FasL treatment (15 ng/ml + M2). Cell death was measured by monitoring caspase-8 activation (presence of p43/41 cleaved fragments) and PARP cleavage by IB. Relative caspase-8 activation (p41/43) and PARP cleavage were measured by quantitative densitometry. (E) Total Fas expression was observed in HCT15 cells transfected with indicated siRNAs by IB.

five proteins in the HCT15 cells, since certain of the chosen proteins interact with cytoskeleton. The detergent solubility assay displayed that the fraction of Fas associated to the detergent-insoluble cytoskeletal fraction decreased in the absence of PATJ and SNTB2 (Figure 32 A and B), however, no difference was demonstrated in the absence of LNX2, GRP78 or abLIM1. This suggest that PATJ and SNTB2 might connect Fas to the cytoskeleton.

We then choose to concentrate our studies on the LNX2 protein. By focusing our efforts on answering the following questions:

- How is LNX2 recruited to Fas?
- Does LNX2 regulate cell death signaling of Fas?
- Does LNX2 target Fas ubiquitination?
- Does LNX2 induce Fas degradation?

***LNX2 interacts with the C-terminal PDZ-binding domain of Fas***

In order to validate the interaction of LNX2 to Fas, a peptide pool-down assay was carried out, using two peptides corresponding respectively to the last 20 amino acids of human wild type Fas (FasWT) and a mutant form lacking the PDZ-binding motif (Fas $\Delta$ SLV)) coupled to agarose beads and used as bait in HCT15 lysate, followed by immunoblotting. We observe the immunoprecipitation of LNX2 with FasWT peptide but not with the mutant Fas $\Delta$ SLV. The protein NHERF1 was used as a negative control (Figure 33.A). We then confirm the direct specific interaction of LNX2 to the PDZ-binding domain of Fas.

In the proteomic analysis, another protein from the LNX family was found, LNX1. LNX1 and LNX2 present 50 % of sequence homology and has the same domain architecture [662, 663]. In order to investigate the dynamic of interaction between LNX1 and/or LNX2 with Fas, HEK293 cells were transfected with control GFP, LNX1 or/and LNX2 coupled GFP plasmids and carried out a peptide pool-down as previously described (Figure 33.B). We observe that LNX1 interacts with Fas in a weaker manner than LNX2 and that overexpressing LNX1 with LNX2 did not disturb the association between LNX2 and Fas, even though both proteins were overexpressed in the same manner (Figure 33.C). These results demonstrate that Fas interacts with higher affinity to LNX2 rather than LNX1.

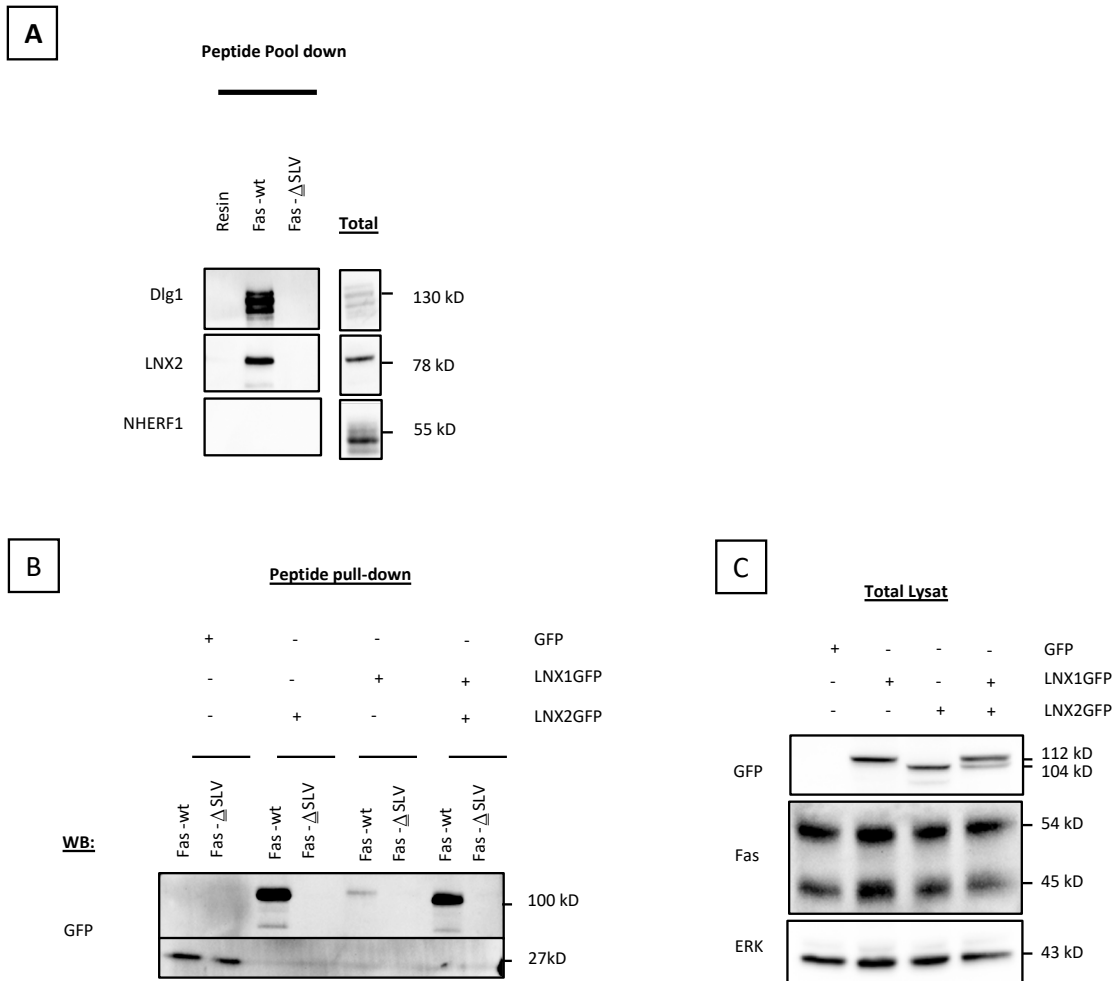


Figure 33: RING ub ligase LNX2 binds to Fas receptor through its c-ter PDZ binding domain (SLV)

**A)** Pool-down experiment done with the C-terminal peptide of Fas (WT or Fas $\Delta$ SLV) followed by IB. **(B)** GFP (lane 1), LNK1-GFP (lane 2) or LNK2-GFP (lane 3) was overexpressed in HEK293 cells. After 24 hours of transfection, Pool-down experiment using the C-terminal peptide of Fas (WT or Fas $\Delta$ SLV) was carried out and visualized by immunoblotting. The presence of LNX2 in the immunoprecipitants was revealed with anti-GFP antibody. **(C)** Total lysates were also analyzed by immunoblotting with anti-ERK antibody as loading control.

### LNK2 knock-down increases FasL-induced cell death

Knocking down LNK2 in epithelial colon line HCT15, increased significantly PARP cleavage and Caspase-8 activation after FasL-treatment (Figure 34. A-C). This data was confirmed using flow cytometry approach, by analyzing the sub G1 peak upon propidium iodide staining that reveal the nuclear DNA fragmentation characteristic of apoptotic cells (Figure 34.D).

We concluded that LNK2 knockdown intensify FasL-induced apoptosis.



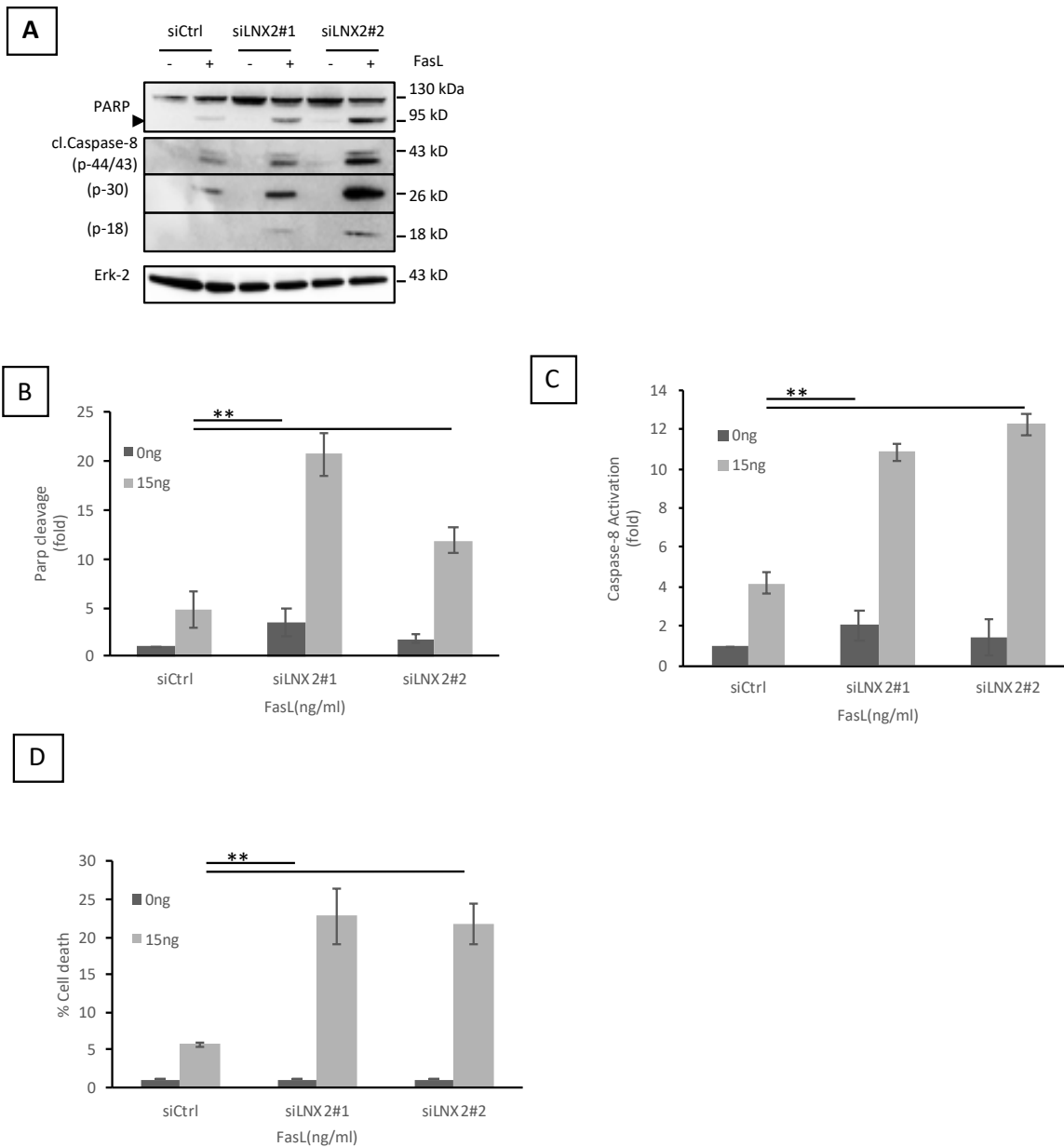


Figure 34: LNX2 silencing increase cell death induced by Fas-L

**(A-C)** HCT15 cells were transiently transfected with control, LNX2#1 or LNX2#2 siRNA for 3 days before FasL treatment (15 ng/ml + M2). Cell death was measured by monitoring caspase-8 activation (presence of p43/41 and p30 cleaved fragments) and PARP cleavage by IB. Relative caspase-8 activation (p41/43) and PARP cleavage were measured by quantitative densitometry ( $n = 3$ ). **(D)** Cell death was read out by PI incorporation (sub-G1 quantification) in HCT15 transfected with control, LNX2#1 or LNX2#2 siRNA for 3 days before FasL treatment (15 ng/ml + M2). Arrowheads in A indicate the cleaved forms of PARP. In all graphs, error bars represent means  $\pm$  SEM. \*\*, \*\*,  $P < 0.01$ ; Student's  $t$  test.

***LNX2 could regulates total Fas expression but does not affect Fas kinetics at cell surface.***

*Several studies have exhibited that LNX2 controlled the expression level of several receptors at cell surface and regulated their degradation including Numb and human glycoprotein CD8 $\alpha$  [661, 664], we questioned whether LNX2 regulates Fas expression at cell surface. To investigate this function, we performed a cell surface biotinylation assay to observe Fas surface kinetics in epithelial colon cell line HCT15 (Figure 35.E). We could observe, in the absence of FasL-treatment, that 50% and 80% of Fas is degraded after 3 and 8 hours respectively. We also observe that silencing LNX2 did not affect Fas kinetics at cell surface (Figure 35.F). However, silencing LNX2 increased Fas total expression (Figure 35.B). Therefore, LNX2 silencing could up-regulates Fas expression but does not affect Fas kinetics at cell surface. Therefore, we hypothesize that LNX2 might down regulates Fas expression through degradation.*

Since LNX2 is an E3 ubiquitin ligase, we hypothesized that LNX2 might regulate Fas expression through ubiquitination. An ubiquitin assay was carried out in HEK293 cells overexpressing GFP as control, LNX2-GFP with or without Ub-His, followed by immunoprecipitating His via Talon beads (data not shown). We could not observe any difference in Fas ubiquitination upon LNX2 overexpression LNX2 in comparison to control.

***LNX2 involved in Fas degradation***

In order to examine Fas targeted degradation via LNX2, we performed an endocytosis experiment in the colon cell line SW480 stably overexpressing FasWT, to increase immunoprecipitated Fas levels. Cells were incubated in the presence or not of FasL and the lysosome inhibitor (leupeptin) to stop Fas degradation. We could observe that 4% of cell surface Fas was internalized within one hour (Figure 36.B). Internalized non-activated Fas receptors did not go through degradation, as the lysosome inhibitor (leupeptin) did not increase the percentage of internalized Fas levels as displayed by IB (Figure 36.A). This suggests that the internalized non-activated Fas receptors were not targeted for degradation. Meanwhile, FasL engagement increased Fas internalization, and targeted Fas to degradation since leupeptin treatment increased Fas level in comparison to FasL treatment alone (Figure 36.A, B and C). These results lead us to propose that FasL treatment targets Fas endocytosis and degradation. On the other hand, silencing LNX2, did not change the level of Fas internalized after leupeptin treatment, which proves the necessity of LNX2 for Fas degradation

(Figure 36.A, B and C). Although absence of LNX2 did not affect the endocytosis rate of Fas. These findings propose that only activated Fas is targeted to degradation after 1 hour of treatment, and that LNX2 can be essential in targeting Fas to lysosome degradation.

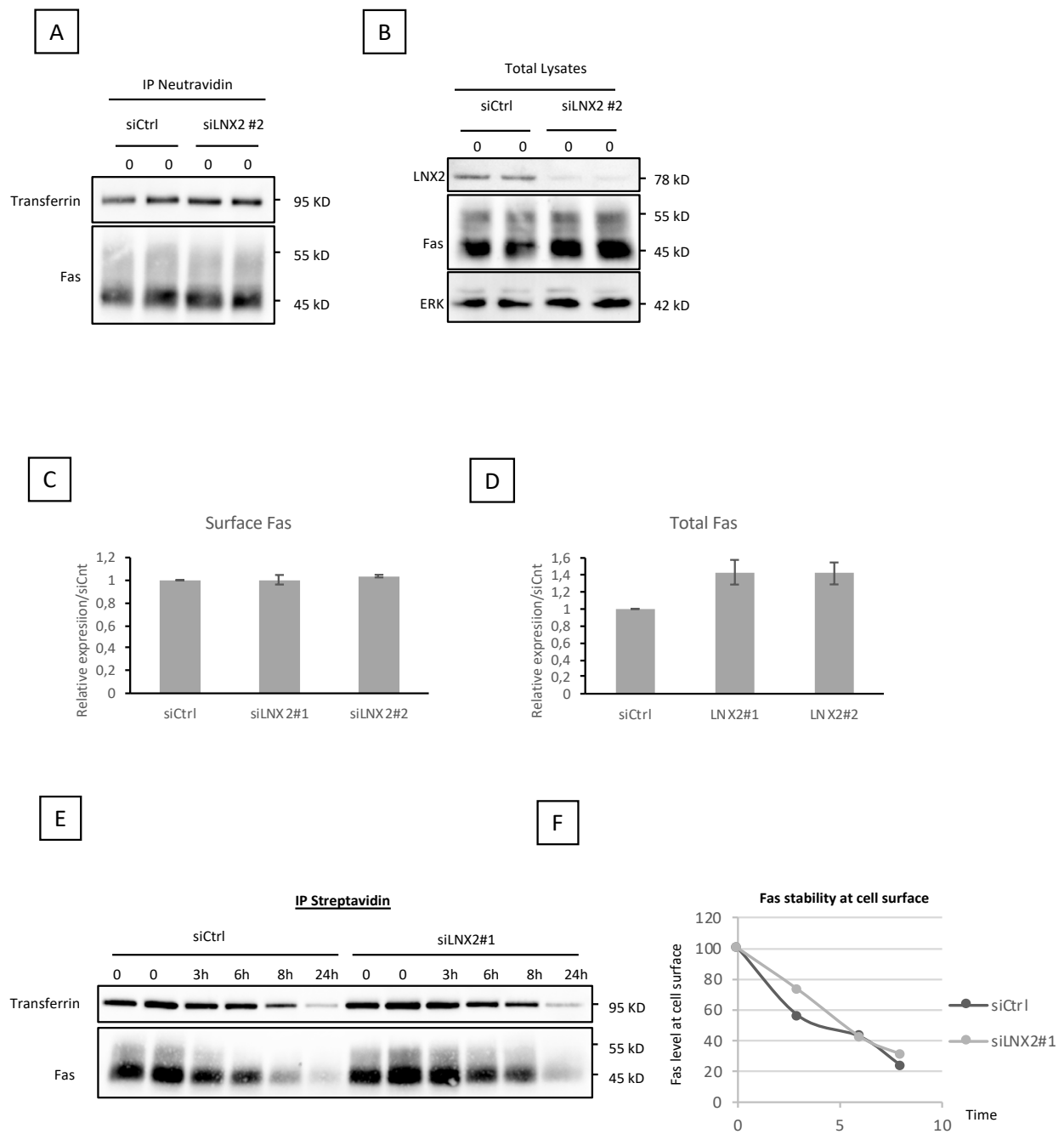


Figure 35: LNX2 regulates Fas total expression

**(A)** Cell surface biotinylation were performed with (HCT15) cells and membrane-impermeable SH-cleavable sulfo-NHS-SS-biotin. Biotinylated proteins were isolated and cell surface level of Fas was detected by SDS-PAGE and immunoblotting. **(B)** Total lysates. **(C)** Histograms representing Fas at cell surface at time point zero, depletion of LNX2 does not affect Fas cell surface expression. **(D)** Total Fas expression. **(E)** Cell surface biotinylation and kinetic of Fas at cell surface. **(F)** Line chart representing relative amount of Fas at cell surface in time.

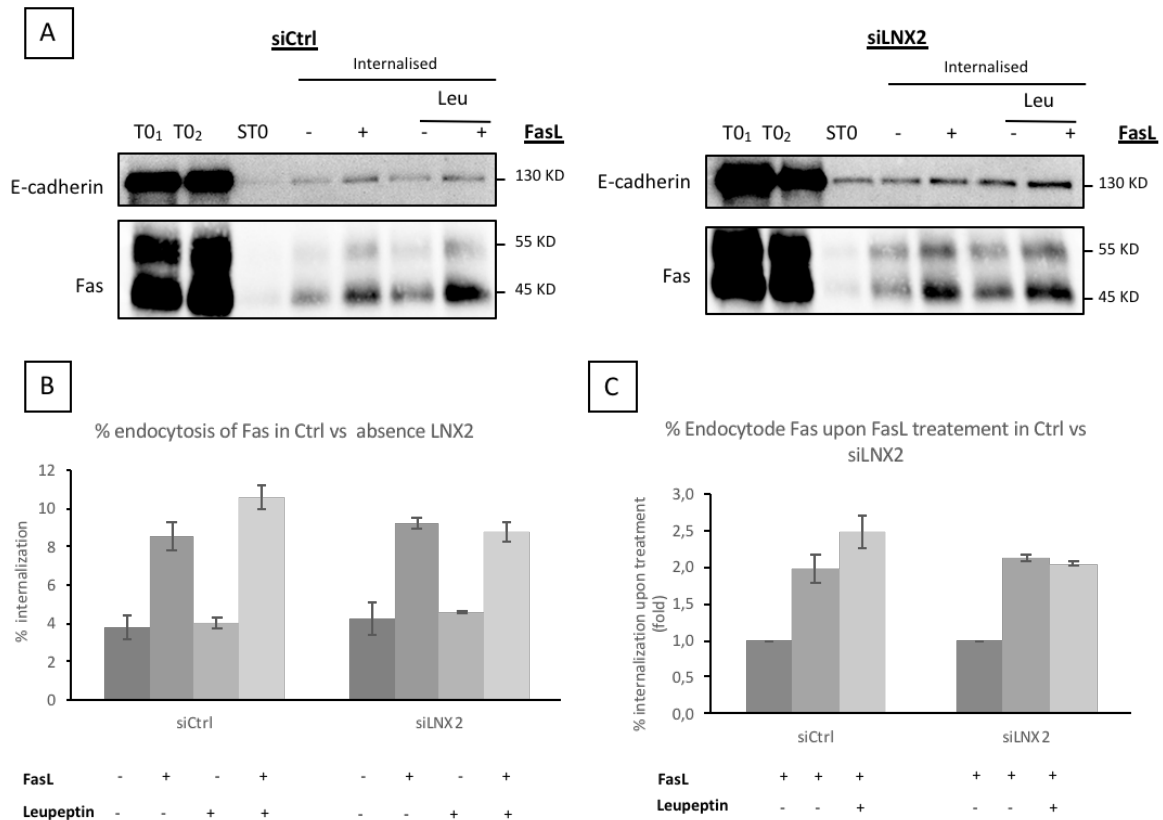


Figure 36: Fas degradation requires LNX2

**(A)** Internalization assay were performed with (SW480FasWT) cells as described in material and methods. **(B)** Average internalization data. The bars represent the percentage of internalized Fas with and without FasL (100ng/ml) and with and without Leupeptin treatment for 1 hour at 37°C, compared with total surface Fas at time zero. **(C)** The bars represent the percentage fold of internalized Fas upon treatment/FasL-independent internalization of Fas.

### LNX2 co-localize with endosome proteins

In order to further understand the mechanism of LNX2 regulating Fas, we wanted to look at LNX2 localization in SW480 colon cells. Immunofluorescence studies show that LNX2 did not co-localize with Fas at adherens junction. LNX2 proteins were rather found in defined compartments. Since we previously assessed that LNX2 targets Fas to degradation, we hypothesized that LNX2 can be localized in the endosome compartment. Double staining of both Rab5 an early endosome protein and Rab7 a late endosome protein with LNX2, confirmed the localization of LNX2 within the endosomes (Figure 37).

In conclusion, I propose a model where Fas degradation is regulated by an E3 ubiquitin ligase, LNX2. LNX2 might play a role in protecting against Fas-dependent apoptosis. Moreover, LNX2 can regulate Fas expression by targeting it to lysosomal degradation (Figure 38).

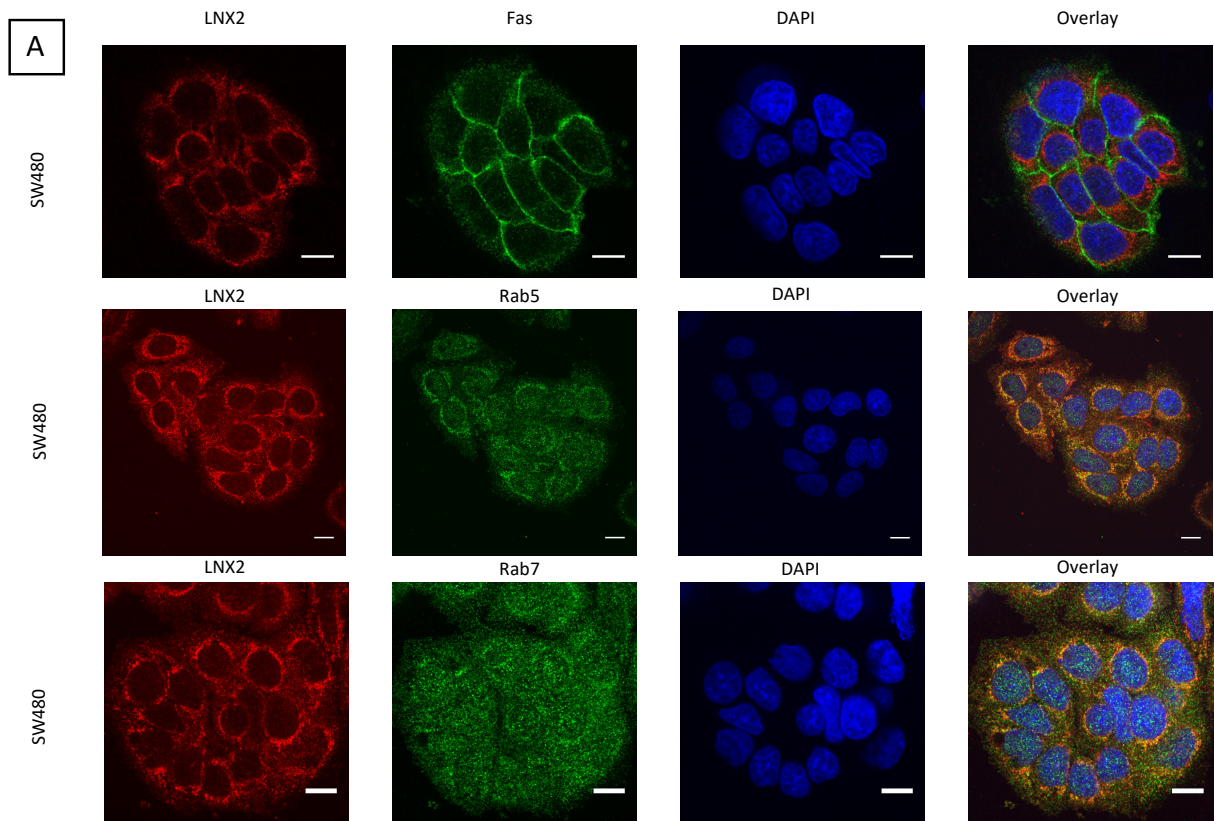


Figure 37: Localization of LNX2 in colon cell line

(A) SW480 cells were fixed and stained for Fas in (green) and LNX2 (red) and nuclei (blue). (B) Cells fixed and stained for Rab5 in (green) and LNX2 (red) and nuclei (blue). (C) fixed and stained for Rab7 in (green) and LNX2 (red) and nuclei (blue).

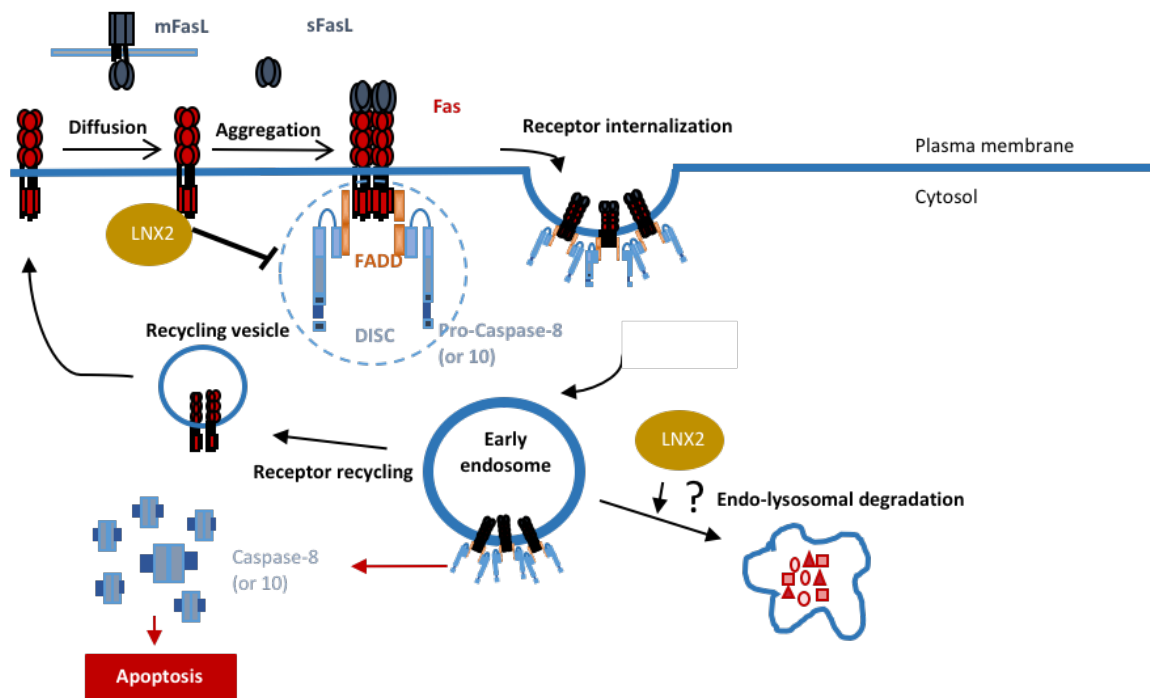


Figure 38: Schematic model proposing the inhibitory role of LNX2 on Fas cell-death signaling.

## IV. Discussion

### ***Regulation of cell death receptors signaling by AJ***

In our study, we were able to demonstrate that AJ protects epithelial colon cells from cell death. We show that the knockdown of AJ components E-cadherin or  $\alpha$ -catenin, using siRNA approach in epithelial colon cells HCT15, increases significantly FasL-induced apoptosis. In fact, Fas associates and form a stable complex with AJ, where E-cadherin and  $\alpha$ -catenin are needed for Fas sequestration at the plasma membrane. Interestingly, another study by Ashkenazi Lab has demonstrated a different mechanism by which E-cadherin regulate cell death receptor signaling [665]. The study by the group of Ashkenazi demonstrates that E-cadherin physically interacts with the cell death receptors DR4 and DR5 by their ectodomain. Ashkenazi's study establishes that complete loss of E-cadherin confers cells with resistance to TRAIL-induced apoptosis via both DR4 and DR5, which confers to cells undergoing EMT to become resistant to apoptosis. They demonstrate that E-cadherin association with DR4/DR5 receptor facilitates death receptors clustering at the plasma membrane. This aggregation step is mediated by the recruitment of the actin cytoskeleton, which leads to the activation of the pro-apoptotic signaling cascade [665]. However, Ashkenazi's group failed to explain the absence of DR4 or DR5 activation observed in normal epithelial tissue where the level of E-cadherin and TRAIL receptors are high. Therefore, it is possible that E-cadherin association with death receptors may play two different roles in the regulation of these receptors depending of the level of E-cadherin expression: low E-cadherin level facilitate receptor aggregation and DISC formation, whereas, high level of E-cadherin might confer an inhibitory effect on cell death receptor activity. Of note, the dual function of E-cadherin was also observed in the regulation of the EGFR, where E-cadherin display either an inhibitory or an activating role depending on the cell context [415, 417] (described above page 96).

### ***Loss of polarity trigger an apoptotic signaling in epithelial cells: an important fail-safe mechanism***

In *Drosophila*, Eiger was identified as the first invertebrate tumor necrosis factor (TNF) superfamily ligand that can prompt cell death [666]. Eiger is a type II transmembrane protein generally localized to the plasma membrane harboring a TNF homology domain at its C-terminal that displayed significant homology with all known TNF superfamily members including RANKL, CD40L, FasL, APRIL, TWEAK, TNF- $\alpha$  and TRAIL. Eiger. Genetic evidence illustrates that Eiger prompts cell death by stimulating the *Drosophila* JNK pathway [666].



Eiger was found responsible for the elimination of *scrib*<sup>-/-</sup> clones in the imaginal disc through the activation of JNK signaling pathway [582, 584, 667]. It is worth mentioning that Eiger mutant flies are viable and display no obvious developmental defects, which suggests that Eiger participates in a stress response to eliminate damaged cells in the organism [29, 666]. More recently, Andersen and co-workers discovered the receptor of Eiger that they called , Grindelwald (GrnD). Inhibition of Grnd expression using RNAi is sufficient to block Eiger-mediated pro-apoptotic signaling. Our lab participate to these studies notably by reporting that Grindelwald possess a cysteine-rich domain (CRD) in the extracellular part with homology to vertebrate TNFRs containing a glycosphingolipid- binding motif (GBM) which is a characteristic of many TNFRs including Fas [86].

These studies suggest that TNFR signaling participate to a tumor suppressive mechanism by eliminating abnormal cells which have lost their polarity through activation of pro-apoptotic signaling.

***Oncogene activation transforms the pro-apoptotic TNFR signaling into a pro-invasive and proliferative signaling- a switch from an anti- to a pro-tumoral function***

Interestingly, genetic studies in *Drosophila* reveal that loss of polarity, due to *scrib* or *dlg* mutations, cooperate with oncogenic activations to promote tumor development. Indeed, *scrib*<sup>-/-</sup>, *dlg*<sup>-/-</sup>, *lgl*<sup>-/-</sup> mutant clones in the eye disc expressing an activated form of Ras (Ras<sup>V12</sup>) exhibit a dramatic tumor-like overgrowth and metastatic behavior [582, 584].

This overgrowth process is due to the cooperation between an oncogenic activation and the loss of a member of Scrib polarity complex has been shown to rely on the expression of Eiger and its receptor Grindelwald [29, 668]. Diminishing Grindelwald levels in *Ras*<sup>V12</sup>/*scrib*<sup>-/-</sup> or *Ras*<sup>V12</sup>/*dlg*<sup>-/-</sup> clones is enough to reestablish normal levels of Mmp1 and eliminate invasiveness, which was also observed in an *Eiger*<sup>-/-</sup> background [29, 668].

These different works demonstrate that loss of polarity due to alteration of components of the Scrib complex leads to the elimination of these “abnormal” cells through a JNK dependent mechanism. Moreover, it has been shown that an additional genetic insult in Scrib complex deficient cells is sufficient to promote tissue overgrowth. Therefore, this mechanism of cell elimination can be considering as an efficient security system in order to avoid pathological conditions such as tumor development.

These data support a model where fly TNFR crosstalk with polarity factors to induce JNK-dependent apoptosis or neoplastic growth in a context-dependent manner. Such connection between cell polarity and tumor progression remained unclear in vertebrates. However, the conserved nature of the Grindelwald receptor advocates that particular TNFRs might achieve similar functions in vertebrates. In fact, our studies provide evidence of crosstalk between the TNFR, Fas, and polarity protein DLG1, which to our knowledge was demonstrated for the first time (See Results).

***A still unresolved molecular interplay between Fas and Dlg1 in the regulation of Fas signaling.***

We demonstrated the inhibitory effect of DLG1 on Fas cell death signaling pathway. Our results demonstrated that Dlg1 mediated Fas signaling independently of AJ which propose that DLG1 allows an additional layer of Fas regulation and contributes to the tight regulation of Fas activity within the cell.

We hypothesized that DLG1 might regulate Fas trafficking since Dlg1 has been described to associate and to target several receptors in specific area of the plasma membrane [633]. However, absence of DLG1 in colon epithelial cells have only a modest effect on Fas localization and stability at cell-cell junction (see JCB).

In this study, we were not able to demonstrate the molecular mechanism of DLG1's inhibitory effect on FasL-induced apoptosis. We propose that DLG1 can inhibit Fas-cell death signaling by conveying specific cytosolic proteins that would modulate Fas signaling. Several examples have demonstrated the association of DLG1 with adaptor proteins to regulate receptor trafficking and localization [17]. Indeed, DLG1 was shown to bind to a number of binding partners via its protein-protein interaction domains. For example, CASK interacts with DLG1 through the L27 domain [499]. CASK association with DLG1 is importance in the regulation of NMDAR sorting in hippocampal neurons [638]. CASK can distinguish NMDAR and AMPAR sorting by DLG1, through regulating DLG1 conformation [639]. Interestingly, CASK interaction with DLG1 favors DLG1's binding to NMDAR on AMPAR. This study provides a mechanism by which DLG1 conformation can mediate NMDAR and AMPAR sorting [639]. Another study has demonstrated that DLG1–AKAP79 complex can be essential for the employment of kinases and phosphatases to synaptic AMPARs [669]. DLG1 binds A-kinase-anchoring protein 79/150

(AKAP79/150) which acts as a scaffold protein for PKA, PKC and the calcium/calmodulin dependent protein phosphatase calcineurin (also known as PP2B) [669]. DLG1–AKAP79 enables PKA to phosphorylate the glutamate receptor subunit GluA1 [669]. These previous studies propose that DLG1 might use another molecule in order to modulate Fas signaling. Consequently, for the future we need to investigate the role of DLG1 partners including, LIN7, MPP7 and CASK which are also found in the proteomic analysis in Fas cell death signaling.

### ***LN2 a new ubiquitin ligase involved in the regulation of Fas***

In our preliminary data, we identify a novel possible interactant of human Fas receptor the E3 ubiquitin ligase LNX2. Our data shows that LNX2 interacts with Fas through a PDZ binding domain and could down-regulate Fas expression level and therefore protect colon cancer cells from Fas-dependent apoptosis.

Regulation of TNFR signaling by an ubiquitin ligase is not unique since many ubiquitin ligases has been involved in the regulation of both death and non-death signaling of Fas but also of various others TNFRs. The first obvious function of these ligase is to regulate the stability of the receptor itself. So far, there are already three E3 ubiquitin ligases that have been defined to regulate cell death receptors stability, including c-Cbl [670, 671], MARCH-8 [672], and Hrd1 [673].

- The E3 ubiquitin ligase c-Cbl, a member of the Casitas B-lineage Lymphoma protein family, was found to directly bind DR4/5, consequently inducing the mono-ubiquitination of DR4/5 leading to their degradation through the lysosomal pathway [671]. Furthermore, the decrease in c-Cbl expression increased the presence of DR4/5 at the cell surface and therefore their capacity to induce apoptosis [670].
- MARCH-8 targets DR4 polyubiquitination which is followed by DR4 degradation in lysosomal-dependent manner [672]. Interestingly, MARCH-8 did not degrade DR5 which suggest the specificity of this E3 ubiquitin ligase to DR4 [672].
- Another E3 ubiquitin ligase, Hrd1 was demonstrated to protect B-cells against activation-induced apoptosis via Fas degradation, however the distinct molecular mechanism is not clear [673].

Beside this role in the degradation of the receptor itself, the role of E3-ubiquitin ligase in the regulation of TNFR signaling has been largely illustrated and is well-known to be an important process. One of these ligases is the TRAF family protein TRAF2.

Upon TRAIL induction, caspase-8 and FADD are recruited to the TRAIL receptors DR4 and DR5 forming the DISC, which targets the recruitment of the E3 ubiquitin ligase TNF receptor-associated factor 2 (TRAF2). TRAF2 directly binds caspase-8 to induce its polyubiquitination, consequently targeting activated caspase-8 to proteasomal degradation, which attenuates cell death signaling [674]. The deletion of TRAF2 inhibited caspase-8 polyubiquitination which was enough to overcome resistance to TRAIL-induced apoptosis in gastric cancer cells [675]. Upon TRAIL induction, FADD was also found targeted to proteasomal degradation through the ubiquitination by Makorin Ring Finger Protein 1 (MKRN1) [676]. Another complex known as the linear ubiquitin chain assembly complex (LUBAC) constituted of HOIP, HOIL-1 and SHARPIN, is also found to negatively regulate TRAIL-R cell death signaling through ubiquitination [677, 678]. LUBAC attenuates cell death signaling through targeting caspase-8 ubiquitination limiting caspase-8 activity [678].

In addition to the role of TRAF-2 and LUBAC in modulating cell death signaling, they are implicated in promoting survival signals. TRAF2 act as a mediator of TNFR1 signaling and known to be necessary for TNF $\alpha$  induced activation of and stimulation NF- $\kappa$ B and MAPK pathways. TRAF2 induce NF- $\kappa$ B signaling independently of its role in inhibiting the extrinsic pathway [679]. The stimulation of TNFR1 by TNF $\alpha$  induce the sequential formation of two complexes; complex I that mediate cell survival, and complex II that mediate cell death [680]. TNFR1 activation leads to the assembly of TNFR1-associated signaling complex I, complex I is triggered, that is composed of TNF receptor-associated death domain (TRADD), TRAF2, receptor-interacting protein kinase 1 (RIP1), and cIAP1 and cIAP2 [680]. TNF $\alpha$  stimulation results in RIP1 ubiquitination by the E3 ubiquitin ligase cIAP1 and cIAP2, which facilitates the recruitment and activation of TAK1 in addition to the recruitment of LUBAC. LUBAC induce RIP1 ubiquitination that induce IKK kinase complexes recruitment resulting in activation of NF- $\kappa$ B and generation of survival genes [681-684]. Subsequently complex I dissociates from TNFR1 leading to formation of the cytoplasmic complex IIa which is constituted of TRAF2, RIP1, TRADD, FADD, and procaspase-8, leading to apoptosis. This latter signaling occurs if the NF- $\kappa$ B activation fails, which acts as a checkpoint mechanism [680].

In our study, we demonstrated that the E3 ubiquitin ligase LNX2 can modulate apoptosis through targeting Fas to degradation. However, we did not investigate if LNX2 could have non-death regulatory roles as it has been described for TRAF2 and the LUBAC complex. Therefore,

for the future it will be interesting to examine the ubiquitination cascade triggered by LNX2 upon FasL treatment and its consequence on Fas-signaling. It is also possible that LNX2 can play a more general role on other TNFRs.

### ***Future perspective***

Our proteomic analysis revealed a large number of new Fas molecular partners. This made us question how could all these proteins interact with the C-terminal part of Fas? Do these proteins bind Fas simultaneously or dependent on each other?

The above examples suggest that the establishment of multi-protein complexes might inflict limitation to the accessibility of binding sites. This call into question of how does the multi-protein structures bind and exploit other complexes to form huge axis in normal cellular homeostasis and disease state of Fas.

In order to answer these questions several molecular analyses should be carried out including co-immunoprecipitation, pull-down assays, ELISA, mass spectrometry and large peptide phage-display screens, to identify direct interactants and associated proteins that are conveyed via adaptor molecules.

## V. References

1. Sabine Pietkiewicz, C.W., Jörn H. Buchbinder Inna N. Lavrik, *Measuring Procaspase-8 and -10 Processing upon Apoptosis Induction*. 2017.
2. Bruce Alberts , A.J., Julian Lewis , Martin Raff , Keith Roberts , and Peter Walter . *Molecular biology of the cell*. 4th edition ed. 2002, New York: Garland Science.
3. Voss, M., et al., *Posttranslational regulation of Fas ligand function*. Cell Commun Signal, 2008. **6**: p. 11.
4. Garcia, M.A., W.J. Nelson, and N. Chavez, *Cell-Cell Junctions Organize Structural and Signaling Networks*. Cold Spring Harb Perspect Biol, 2018. **10**(4).
5. Gumbiner, B.M., *Regulation of cadherin-mediated adhesion in morphogenesis*. Nat Rev Mol Cell Biol, 2005. **6**(8): p. 622-34.
6. Klezovitch, O. and V. Vasioukhin, *Cadherin signaling: keeping cells in touch*. F1000Res, 2015. **4**(F1000 Faculty Rev): p. 550.
7. Guégan, J.P., et al., *CD95/Fas and metastatic disease: What does not kill you makes you stronger*. Semin Cancer Biol, 2019.
8. Harris, T.J. and U. Tepass, *Adherens junctions: from molecules to morphogenesis*. Nat Rev Mol Cell Biol, 2010. **11**(7): p. 502-14.
9. Muroyama, A. and T. Lechler, *Polarity and stratification of the epidermis*. Semin Cell Dev Biol, 2012. **23**(8): p. 890-6.
10. Askenasy, N., et al., *Induction of tolerance using Fas ligand: a double-edged immunomodulator*. Blood, 2005. **105**(4): p. 1396-404.
11. Niessen, C.M., D. Leckband, and A.S. Yap, *Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation*. Physiol Rev, 2011. **91**(2): p. 691-731.
12. Takai, Y. and H. Nakanishi, *Nectin and afadin: novel organizers of intercellular junctions*. J Cell Sci, 2003. **116**(Pt 1): p. 17-27.
13. Gandalovičová, A., et al., *Cell polarity signaling in the plasticity of cancer cell invasiveness*. Oncotarget, 2016. **7**(18): p. 25022-49.
14. Assémat, E., et al., *Polarity complex proteins*. Biochim Biophys Acta, 2008. **1778**(3): p. 614-30.
15. Raman, R., C.S. Pinto, and M. Sonawane, *Polarized Organization of the Cytoskeleton: Regulation by Cell Polarity Proteins*. J Mol Biol, 2018. **430**(19): p. 3565-3584.
16. Perez-Moreno, M. and E. Fuchs, *Catenins: keeping cells from getting their signals crossed*. Dev Cell, 2006. **11**(5): p. 601-12.
17. Fourie, C., D. Li, and J.M. Montgomery, *The anchoring protein SAP97 influences the trafficking and localisation of multiple membrane channels*. Biochim Biophys Acta, 2014. **1838**(2): p. 589-94.
18. Gumbiner, B.M. and N.G. Kim, *The Hippo-YAP signaling pathway and contact inhibition of growth*. J Cell Sci, 2014. **127**(Pt 4): p. 709-17.
19. Gavard, J., *Endothelial permeability and VE-cadherin: a wacky comradeship*. Cell Adh Migr, 2013. **7**(6): p. 455-61.
20. Ellenbroek, S.I., S. Iden, and J.G. Collard, *Cell polarity proteins and cancer*. Semin Cancer Biol, 2012. **22**(3): p. 208-15.
21. Scott, F.L., et al., *The Fas-FADD death domain complex structure unravels signalling by receptor clustering*. Nature, 2009. **457**(7232): p. 1019-22.
22. Wang, L., et al., *The Fas-FADD death domain complex structure reveals the basis of DISC assembly and disease mutations*. Nat Struct Mol Biol, 2010. **17**(11): p. 1324-9.

23. Croft, M. and R.M. Siegel, *Beyond TNF: TNF superfamily cytokines as targets for the treatment of rheumatic diseases*. Nat Rev Rheumatol, 2017. **13**(4): p. 217-233.
24. Carswell, E.A., et al., *An endotoxin-induced serum factor that causes necrosis of tumors*. Proc Natl Acad Sci U S A, 1975. **72**(9): p. 3666-70.
25. Aggarwal, B.B., S.C. Gupta, and J.H. Kim, *Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey*. Blood, 2012. **119**(3): p. 651-65.
26. Locksley, R.M., N. Killeen, and M.J. Lenardo, *The TNF and TNF receptor superfamilies: integrating mammalian biology*. Cell, 2001. **104**(4): p. 487-501.
27. Quistad, S.D. and N. Traylor-Knowles, *Precambrian origins of the TNFR superfamily*. Cell Death Discov, 2016. **2**: p. 16058.
28. Igaki, T. and M. Miura, *The Drosophila TNF ortholog Eiger: emerging physiological roles and evolution of the TNF system*. Semin Immunol, 2014. **26**(3): p. 267-74.
29. Andersen, D.S., et al., *The Drosophila TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth*. Nature, 2015. **522**(7557): p. 482-6.
30. Wiens, G.D. and G.W. Glenney, *Origin and evolution of TNF and TNF receptor superfamilies*. Dev Comp Immunol, 2011. **35**(12): p. 1324-35.
31. Black, R.A., et al., *A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells*. Nature, 1997. **385**(6618): p. 729-33.
32. Ware, C.F., *The TNF receptor super family in immune regulation*. Immunol Rev, 2011. **244**(1): p. 5-8.
33. Dostert, C., et al., *The TNF Family of Ligands and Receptors: Communication Modules in the Immune System and Beyond*. Physiol Rev, 2019. **99**(1): p. 115-160.
34. Schneider-Brachert, W., et al., *Compartmentalization of TNF receptor 1 signaling: internalized TNF receptors as death signaling vesicles*. Immunity, 2004. **21**(3): p. 415-28.
35. Banner, D.W., et al., *Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation*. Cell, 1993. **73**(3): p. 431-45.
36. Hymowitz, S.G., et al., *Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5*. Mol Cell, 1999. **4**(4): p. 563-71.
37. Chan, F.K., et al., *A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling*. Science, 2000. **288**(5475): p. 2351-4.
38. Chan, F.K., *Three is better than one: pre-ligand receptor assembly in the regulation of TNF receptor signaling*. Cytokine, 2007. **37**(2): p. 101-7.
39. Lee, H.W., et al., *Homomeric and heteromeric interactions of the extracellular domains of death receptors and death decoy receptors*. Biochem Biophys Res Commun, 2005. **330**(4): p. 1205-12.
40. Wajant, H., *Death receptors*. Essays Biochem, 2003. **39**: p. 53-71.
41. Sessler, T., et al., *Structural determinants of DISC function: new insights into death receptor-mediated apoptosis signalling*. Pharmacol Ther, 2013. **140**(2): p. 186-99.
42. Park, S.M., R. Schickel, and M.E. Peter, *Nonapoptotic functions of FADD-binding death receptors and their signaling molecules*. Curr Opin Cell Biol, 2005. **17**(6): p. 610-6.
43. Xie, P., *TRAF molecules in cell signaling and in human diseases*. J Mol Signal, 2013. **8**(1): p. 7.



44. Lin, W.W. and S.L. Hsieh, *Decoy receptor 3: a pleiotropic immunomodulator and biomarker for inflammatory diseases, autoimmune diseases and cancer*. *Biochem Pharmacol*, 2011. **81**(7): p. 838-47.
45. Mantovani, A., et al., *Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines*. *Trends Immunol*, 2001. **22**(6): p. 328-36.
46. Vanamee, É. and D.L. Faustman, *Structural principles of tumor necrosis factor superfamily signaling*. *Sci Signal*, 2018. **11**(511).
47. Trauth, B.C., et al., *Monoclonal antibody-mediated tumor regression by induction of apoptosis*. *Science*, 1989. **245**(4915): p. 301-5.
48. Yonehara, S., A. Ishii, and M. Yonehara, *A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor*. *J Exp Med*, 1989. **169**(5): p. 1747-56.
49. Oehm, A., et al., *Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen*. *J Biol Chem*, 1992. **267**(15): p. 10709-15.
50. Itoh, N., et al., *The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis*. *Cell*, 1991. **66**(2): p. 233-43.
51. Suda, T., et al., *Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family*. *Cell*, 1993. **75**(6): p. 1169-78.
52. Montel, A.H., et al., *Fas involvement in cytotoxicity mediated by human NK cells*. *Cell Immunol*, 1995. **166**(2): p. 236-46.
53. Nagata, S., *Apoptosis by death factor*. *Cell*, 1997. **88**(3): p. 355-65.
54. Lettau, M., et al., *Insights into the molecular regulation of FasL (CD178) biology*. *Eur J Cell Biol*, 2011. **90**(6-7): p. 456-66.
55. Takahashi, T., et al., *Human Fas ligand: gene structure, chromosomal location and species specificity*. *Int Immunol*, 1994. **6**(10): p. 1567-74.
56. Orlinick, J.R., K.B. Elkon, and M.V. Chao, *Separate domains of the human fas ligand dictate self-association and receptor binding*. *J Biol Chem*, 1997. **272**(51): p. 32221-9.
57. Bodmer, J.L., P. Schneider, and J. Tschopp, *The molecular architecture of the TNF superfamily*. *Trends Biochem Sci*, 2002. **27**(1): p. 19-26.
58. Lee, W.H., et al., *Reverse Signaling of Tumor Necrosis Factor Superfamily Proteins in Macrophages and Microglia: Superfamily Portrait in the Neuroimmune Interface*. *Front Immunol*, 2019. **10**: p. 262.
59. Sun, M., et al., *Cutting edge: two distinct motifs within the Fas ligand tail regulate Fas ligand-mediated costimulation*. *J Immunol*, 2007. **179**(9): p. 5639-43.
60. Nachbur, U., et al., *Posttranscriptional regulation of Fas (CD95) ligand killing activity by lipid rafts*. *Blood*, 2006. **107**(7): p. 2790-6.
61. Cahuzac, N., et al., *Fas ligand is localized to membrane rafts, where it displays increased cell death-inducing activity*. *Blood*, 2006. **107**(6): p. 2384-91.
62. Trambas, C.M. and G.M. Griffiths, *Delivering the kiss of death*. *Nat Immunol*, 2003. **4**(5): p. 399-403.
63. Green, D.R. and T.A. Ferguson, *The role of Fas ligand in immune privilege*. *Nat Rev Mol Cell Biol*, 2001. **2**(12): p. 917-24.
64. Hammam, O., et al., *The role of fas/fas ligand system in the pathogenesis of liver cirrhosis and hepatocellular carcinoma*. *Hepat Mon*, 2012. **12**(11): p. e6132.

65. Fernandes, P., et al., *Intestinal expression of Fas and Fas ligand is upregulated by bacterial signaling through TLR4 and TLR5, with activation of Fas modulating intestinal TLR-mediated inflammation.* J Immunol, 2014. **193**(12): p. 6103-13.
66. Matsuno, H., et al., *Stromelysin-1 (MMP-3) in synovial fluid of patients with rheumatoid arthritis has potential to cleave membrane bound Fas ligand.* J Rheumatol, 2001. **28**(1): p. 22-8.
67. Vargo-Gogola, T., et al., *Identification of novel matrix metalloproteinase-7 (matrilysin) cleavage sites in murine and human Fas ligand.* Arch Biochem Biophys, 2002. **408**(2): p. 155-61.
68. Kiaei, M., et al., *Matrix metalloproteinase-9 regulates TNF-alpha and FasL expression in neuronal, glial cells and its absence extends life in a transgenic mouse model of amyotrophic lateral sclerosis.* Exp Neurol, 2007. **205**(1): p. 74-81.
69. Kirkin, V., et al., *The Fas ligand intracellular domain is released by ADAM10 and SPPL2a cleavage in T-cells.* Cell Death Differ, 2007. **14**(9): p. 1678-87.
70. Shudo, K., et al., *The membrane-bound but not the soluble form of human Fas ligand is responsible for its inflammatory activity.* Eur J Immunol, 2001. **31**(8): p. 2504-11.
71. O' Reilly, L.A., et al., *Membrane-bound Fas ligand only is essential for Fas-induced apoptosis.* Nature, 2009. **461**(7264): p. 659-63.
72. Suda, T., et al., *Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing.* J Exp Med, 1997. **186**(12): p. 2045-50.
73. Siegel, R.M., et al., *Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations.* Science, 2000. **288**(5475): p. 2354-7.
74. Dupont, P.J. and A.N. Warrens, *Fas ligand exerts its pro-inflammatory effects via neutrophil recruitment but not activation.* Immunology, 2007. **120**(1): p. 133-9.
75. Tauzin, S., et al., *The naturally processed CD95L elicits a c-jun/calcium/PI3K-driven cell migration pathway.* PLoS Biol, 2011. **9**(6): p. e1001090.
76. Lundy, S.K., M.W. Klinker, and D.A. Fox, *Killer B lymphocytes and their fas ligand positive exosomes as inducers of immune tolerance.* Front Immunol, 2015. **6**: p. 122.
77. Abusamra, A.J., et al., *Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis.* Blood Cells Mol Dis, 2005. **35**(2): p. 169-73.
78. Lückerrath, K., et al., *Immune modulation by Fas ligand reverse signaling: lymphocyte proliferation is attenuated by the intracellular Fas ligand domain.* Blood, 2011. **117**(2): p. 519-29.
79. Sun, M., et al., *The cytoplasmic domain of Fas ligand costimulates TCR signals.* J Immunol, 2006. **177**(3): p. 1481-91.
80. Strasser, A., P.J. Jost, and S. Nagata, *The many roles of FAS receptor signaling in the immune system.* Immunity, 2009. **30**(2): p. 180-92.
81. Peter, M.E., et al., *The CD95 receptor: apoptosis revisited.* Cell, 2007. **129**(3): p. 447-50.
82. Inazawa, J., et al., *Assignment of the human Fas antigen gene (Fas) to 10q24.1.* Genomics, 1992. **14**(3): p. 821-2.
83. Peter, M.E., et al., *CD95 (APO-1/Fas)-associating signalling proteins.* Cell Death Differ, 1996. **3**(2): p. 161-70.
84. Behrmann, I., H. Walczak, and P.H. Krammer, *Structure of the human APO-1 gene.* Eur J Immunol, 1994. **24**(12): p. 3057-62.
85. Schneider, P., et al., *Characterization of Fas (Apo-1, CD95)-Fas ligand interaction.* J Biol Chem, 1997. **272**(30): p. 18827-33.

86. Chakrabandhu, K., et al., *The extracellular glycosphingolipid-binding motif of Fas defines its internalization route, mode and outcome of signals upon activation by ligand*. Cell Death Differ, 2008. **15**(12): p. 1824-37.
87. Fu, Q., et al., *Structural Basis and Functional Role of Intramembrane Trimerization of the Fas/CD95 Death Receptor*. Mol Cell, 2016. **61**(4): p. 602-613.
88. Tejedor, J.R., P. Papasaikas, and J. Valcárcel, *Genome-wide identification of Fas/CD95 alternative splicing regulators reveals links with iron homeostasis*. Mol Cell, 2015. **57**(1): p. 23-38.
89. Muppidi, J.R., et al., *Homotypic FADD interactions through a conserved RXDLL motif are required for death receptor-induced apoptosis*. Cell Death Differ, 2006. **13**(10): p. 1641-50.
90. Sato, T., et al., *FAP-1: a protein tyrosine phosphatase that associates with Fas*. Science, 1995. **268**(5209): p. 411-5.
91. Cuppen, E., et al., *No evidence for involvement of mouse protein-tyrosine phosphatase-BAS-like Fas-associated phosphatase-1 in Fas-mediated apoptosis*. J Biol Chem, 1997. **272**(48): p. 30215-20.
92. Leithäuser, F., et al., *Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells*. Lab Invest, 1993. **69**(4): p. 415-29.
93. Peter, M.E., et al., *The role of CD95 and CD95 ligand in cancer*. Cell Death Differ, 2015. **22**(5): p. 885-6.
94. Kobayashi, N., et al., *Anti-Fas monoclonal antibody is cytotoxic to human immunodeficiency virus-infected cells without augmenting viral replication*. Proc Natl Acad Sci U S A, 1990. **87**(24): p. 9620-4.
95. Falk, M.H., et al., *Expression of the APO-1 antigen in Burkitt lymphoma cell lines correlates with a shift towards a lymphoblastoid phenotype*. Blood, 1992. **79**(12): p. 3300-6.
96. Debatin, K.M., et al., *High expression of APO-1 (CD95) on T lymphocytes from human immunodeficiency virus-1-infected children*. Blood, 1994. **83**(10): p. 3101-3.
97. Lee, S.J., et al., *Differential regulation and function of Fas expression on glial cells*. J Immunol, 2000. **164**(3): p. 1277-85.
98. Stahnke, K., et al., *CD95 (APO-1/FAS)-mediated apoptosis in cytokine-activated hematopoietic cells*. Exp Hematol, 1998. **26**(9): p. 844-50.
99. Abrams, S.I., *Positive and negative consequences of Fas/Fas ligand interactions in the antitumor response*. Front Biosci, 2005. **10**: p. 809-21.
100. McIlwain, D.R., T. Berger, and T.W. Mak, *Caspase functions in cell death and disease*. Cold Spring Harb Perspect Biol, 2015. **7**(4).
101. Koff, J.L., S. Ramachandiran, and L. Bernal-Mizrachi, *A time to kill: targeting apoptosis in cancer*. Int J Mol Sci, 2015. **16**(2): p. 2942-55.
102. Hengartner, M.O., *The biochemistry of apoptosis*. Nature, 2000. **407**(6805): p. 770-6.
103. Peter, M.E. and P.H. Krammer, *The CD95(APO-1/Fas) DISC and beyond*. Cell Death Differ, 2003. **10**(1): p. 26-35.
104. Krammer, P.H., *CD95's deadly mission in the immune system*. Nature, 2000. **407**(6805): p. 789-95.
105. Lavrik, I.N. and P.H. Krammer, *Regulation of CD95/Fas signaling at the DISC*. Cell Death Differ, 2012. **19**(1): p. 36-41.

106. Kischkel, F.C., et al., *Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8*. J Biol Chem, 2001. **276**(49): p. 46639-46.
107. Fischer, U., C. Stroh, and K. Schulze-Osthoff, *Unique and overlapping substrate specificities of caspase-8 and caspase-10*. Oncogene, 2006. **25**(1): p. 152-9.
108. Tait, S.W. and D.R. Green, *Mitochondria and cell signalling*. J Cell Sci, 2012. **125**(Pt 4): p. 807-15.
109. Alderson, M.R., et al., *Fas transduces activation signals in normal human T lymphocytes*. J Exp Med, 1993. **178**(6): p. 2231-5.
110. Aggarwal, B.B., et al., *Fas antigen signals proliferation of normal human diploid fibroblast and its mechanism is different from tumor necrosis factor receptor*. FEBS Lett, 1995. **364**(1): p. 5-8.
111. Freiberg, R.A., et al., *Fas signal transduction triggers either proliferation or apoptosis in human fibroblasts*. J Invest Dermatol, 1997. **108**(2): p. 215-9.
112. Barnhart, B.C., et al., *CD95 ligand induces motility and invasiveness of apoptosis-resistant tumor cells*. EMBO J, 2004. **23**(15): p. 3175-85.
113. Sancho-Martinez, I. and A. Martin-Villalba, *Tyrosine phosphorylation and CD95: a FAScinating switch*. Cell Cycle, 2009. **8**(6): p. 838-42.
114. Cullen, S.P. and S.J. Martin, *Fas and TRAIL 'death receptors' as initiators of inflammation: Implications for cancer*. Semin Cell Dev Biol, 2015. **39**: p. 26-34.
115. Desbarats, J., et al., *Fas engagement induces neurite growth through ERK activation and p35 upregulation*. Nat Cell Biol, 2003. **5**(2): p. 118-25.
116. Senft, J., B. Helfer, and S.M. Frisch, *Caspase-8 interacts with the p85 subunit of phosphatidylinositol 3-kinase to regulate cell adhesion and motility*. Cancer Res, 2007. **67**(24): p. 11505-9.
117. Khadra, N., et al., *CD95 triggers Orai1-mediated localized Ca<sup>2+</sup> entry, regulates recruitment of protein kinase C (PKC)  $\beta$ 2, and prevents death-inducing signaling complex formation*. Proc Natl Acad Sci U S A, 2011. **108**(47): p. 19072-7.
118. Kleber, S., et al., *Yes and PI3K bind CD95 to signal invasion of glioblastoma*. Cancer Cell, 2008. **13**(3): p. 235-48.
119. Schneider-Brachert, W., U. Heigl, and M. Ehrenschwender, *Membrane trafficking of death receptors: implications on signalling*. Int J Mol Sci, 2013. **14**(7): p. 14475-503.
120. Lee, K.H., et al., *The role of receptor internalization in CD95 signaling*. EMBO J, 2006. **25**(5): p. 1009-23.
121. Rossin, A., et al., *Identification of a lysine-rich region of Fas as a raft nanodomain targeting signal necessary for Fas-mediated cell death*. Exp Cell Res, 2010. **316**(9): p. 1513-22.
122. Hueber, A.O., et al., *An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes*. EMBO Rep, 2002. **3**(2): p. 190-6.
123. Muppidi, J.R. and R.M. Siegel, *Ligand-independent redistribution of Fas (CD95) into lipid rafts mediates clonotypic T cell death*. Nat Immunol, 2004. **5**(2): p. 182-9.
124. Sharma, S., et al., *Apoptotic signalling targets the post-endocytic sorting machinery of the death receptor Fas/CD95*. Nat Commun, 2019. **10**(1): p. 3105.
125. Rossin, A., et al., *Fas palmitoylation by the palmitoyl acyltransferase DHHC7 regulates Fas stability*. Cell Death Differ, 2015. **22**(4): p. 643-53.

126. Chakrabandhu, K., et al., *An Evolution-Guided Analysis Reveals a Multi-Signaling Regulation of Fas by Tyrosine Phosphorylation and its Implication in Human Cancers*. PLoS Biol, 2016. **14**(3): p. e1002401.
127. Chakrabandhu, K. and A.O. Hueber, *Fas Versatile Signaling and Beyond: Pivotal Role of Tyrosine Phosphorylation in Context-Dependent Signaling and Diseases*. Front Immunol, 2016. **7**: p. 429.
128. Ta, N.L., et al., *The tyrosine phosphorylated pro-survival form of Fas intensifies the EGF-induced signal in colorectal cancer cells through the nuclear EGFR/STAT3-mediated pathway*. Sci Rep, 2018. **8**(1): p. 12424.
129. Kataoka, T., et al., *The caspase-8 inhibitor FLIP promotes activation of NF-kappaB and Erk signaling pathways*. Curr Biol, 2000. **10**(11): p. 640-8.
130. Yamada, A., et al., *Dual Role of Fas/FasL-Mediated Signal in Peripheral Immune Tolerance*. Front Immunol, 2017. **8**: p. 403.
131. Lavrik, I.N., et al., *Analysis of CD95 threshold signaling: triggering of CD95 (FAS/APO-1) at low concentrations primarily results in survival signaling*. J Biol Chem, 2007. **282**(18): p. 13664-71.
132. Horn, S., et al., *Caspase-10 Negatively Regulates Caspase-8-Mediated Cell Death, Switching the Response to CD95L in Favor of NF-kB Activation and Cell Survival*. Cell Rep, 2017. **19**(4): p. 785-797.
133. Castro, J.E., et al., *Fas modulation of apoptosis during negative selection of thymocytes*. Immunity, 1996. **5**(6): p. 617-27.
134. Hassin, D., et al., *Cytotoxic T lymphocyte perforin and Fas ligand working in concert even when Fas ligand lytic action is still not detectable*. Immunology, 2011. **133**(2): p. 190-6.
135. van Rensburg, I.C., et al., *B-cells with a FasL expressing regulatory phenotype are induced following successful anti-tuberculosis treatment*. Immun Inflamm Dis, 2017. **5**(1): p. 57-67.
136. Hahne, M., et al., *Activated B cells express functional Fas ligand*. Eur J Immunol, 1996. **26**(3): p. 721-4.
137. Hao, Z., et al., *Fas receptor expression in germinal-center B cells is essential for T and B lymphocyte homeostasis*. Immunity, 2008. **29**(4): p. 615-27.
138. Yoon, J.H. and G.J. Gores, *Death receptor-mediated apoptosis and the liver*. J Hepatol, 2002. **37**(3): p. 400-10.
139. Rescigno, M., et al., *Fas engagement induces the maturation of dendritic cells (DCs), the release of interleukin (IL)-1beta, and the production of interferon gamma in the absence of IL-12 during DC-T cell cognate interaction: a new role for Fas ligand in inflammatory responses*. J Exp Med, 2000. **192**(11): p. 1661-8.
140. Guo, Z., et al., *Fas signal links innate and adaptive immunity by promoting dendritic-cell secretion of CC and CXC chemokines*. Blood, 2005. **106**(6): p. 2033-41.
141. Paulsen, M. and O. Janssen, *Pro- and anti-apoptotic CD95 signaling in T cells*. Cell Commun Signal, 2011. **9**: p. 7.
142. Zuliani, C., et al., *Control of neuronal branching by the death receptor CD95 (Fas/Apo-1)*. Cell Death Differ, 2006. **13**(1): p. 31-40.
143. Knight, J.C., E.L. Scharf, and Y. Mao-Draayer, *Fas activation increases neural progenitor cell survival*. J Neurosci Res, 2010. **88**(4): p. 746-57.
144. Bidère, N., H.C. Su, and M.J. Lenardo, *Genetic disorders of programmed cell death in the immune system*. Annu Rev Immunol, 2006. **24**: p. 321-52.

145. Watanabe-Fukunaga, R., et al., *Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis*. Nature, 1992. **356**(6367): p. 314-7.
146. Lynch, D.H., et al., *The mouse Fas-ligand gene is mutated in gld mice and is part of a TNF family gene cluster*. Immunity, 1994. **1**(2): p. 131-6.
147. Matsuzawa, A., et al., *A new allele of the lpr locus, lprcg, that complements the gld gene in induction of lymphadenopathy in the mouse*. J Exp Med, 1990. **171**(2): p. 519-31.
148. Tauzin, S., et al., *CD95-mediated cell signaling in cancer: mutations and post-translational modulations*. Cell Mol Life Sci, 2012. **69**(8): p. 1261-77.
149. Ramenghi, U., et al., *Deficiency of the Fas apoptosis pathway without Fas gene mutations is a familial trait predisposing to development of autoimmune diseases and cancer*. Blood, 2000. **95**(10): p. 3176-82.
150. de Souza, H.S. and C. Fiocchi, *Immunopathogenesis of IBD: current state of the art*. Nat Rev Gastroenterol Hepatol, 2016. **13**(1): p. 13-27.
151. Kokkonen, T.S. and T.J. Karttunen, *Endothelial Fas-Ligand in Inflammatory Bowel Diseases and in Acute Appendicitis*. J Histochem Cytochem, 2015. **63**(12): p. 931-42.
152. Volpe, E., et al., *Fas-Fas Ligand: Checkpoint of T Cell Functions in Multiple Sclerosis*. Front Immunol, 2016. **7**: p. 382.
153. Farhood, B., M. Najafi, and K. Mortezaee, *CD8*. J Cell Physiol, 2019. **234**(6): p. 8509-8521.
154. Ahrends, T., et al., *CD4*. Immunity, 2017. **47**(5): p. 848-861.e5.
155. Legembre, P., et al., *Induction of apoptosis and activation of NF-kappaB by CD95 require different signalling thresholds*. EMBO Rep, 2004. **5**(11): p. 1084-9.
156. Lee, J.K., et al., *Lack of FasL-mediated killing leads to in vivo tumor promotion in mouse Lewis lung cancer*. Apoptosis, 2003. **8**(2): p. 151-60.
157. Liu, Q., et al., *Blockade of Fas signaling in breast cancer cells suppresses tumor growth and metastasis via disruption of Fas signaling-initiated cancer-related inflammation*. J Biol Chem, 2014. **289**(16): p. 11522-35.
158. Frankel, B., S.L. Longo, and T.C. Ryken, *Human astrocytomas co-expressing Fas and Fas ligand also produce TGFbeta2 and Bcl-2*. J Neurooncol, 1999. **44**(3): p. 205-12.
159. Desbarats, J. and M.K. Newell, *Fas engagement accelerates liver regeneration after partial hepatectomy*. Nat Med, 2000. **6**(8): p. 920-3.
160. Chen, L., et al., *CD95 promotes tumour growth*. Nature, 2010. **465**(7297): p. 492-6.
161. Zhang, W., et al., *Fas ligand expression in colon cancer: a possible mechanism of tumor immune privilege*. World J Gastroenterol, 2005. **11**(23): p. 3632-5.
162. Merz, C., et al., *Neutralization of the CD95 ligand by APG101 inhibits invasion of glioma cells in vitro*. Anticancer Drugs, 2015. **26**(7): p. 716-27.
163. Blaes, J., et al., *Inhibition of CD95/CD95L (FAS/FASLG) Signaling with APG101 Prevents Invasion and Enhances Radiation Therapy for Glioblastoma*. Mol Cancer Res, 2018. **16**(5): p. 767-776.
164. De Rosa, M., et al., *Genetics, diagnosis and management of colorectal cancer (Review)*. Oncol Rep, 2015. **34**(3): p. 1087-96.
165. Levine, J.S. and D.J. Ahnen, *Clinical practice. Adenomatous polyps of the colon*. N Engl J Med, 2006. **355**(24): p. 2551-7.
166. Worthley, D.L. and B.A. Leggett, *Colorectal cancer: molecular features and clinical opportunities*. Clin Biochem Rev, 2010. **31**(2): p. 31-8.

167. Hoogwater, F.J., et al., *CD95 signaling in colorectal cancer*. *Biochim Biophys Acta*, 2012. **1826**(1): p. 189-98.
168. Koehler, B.C., D. Jäger, and H. Schulze-Bergkamen, *Targeting cell death signaling in colorectal cancer: current strategies and future perspectives*. *World J Gastroenterol*, 2014. **20**(8): p. 1923-34.
169. Raats, D.A., et al., *CD95 ligand induces senescence in mismatch repair-deficient human colon cancer via chronic caspase-mediated induction of DNA damage*. *Cell Death Dis*, 2017. **8**(3): p. e2669.
170. Barreiro, R., et al., *Termination of antigen-specific immunity by CD95 ligand (Fas ligand) and IL-10*. *J Immunol*, 2004. **173**(3): p. 1519-25.
171. Houston, A., et al., *Fas ligand expressed in colon cancer is not associated with increased apoptosis of tumor cells in vivo*. *Int J Cancer*, 2003. **107**(2): p. 209-14.
172. Li, H., et al., *Human and mouse colon cancer utilizes CD95 signaling for local growth and metastatic spread to liver*. *Gastroenterology*, 2009. **137**(3): p. 934-44, 944.e1-4.
173. Zheng, H.X., et al., *Fas signaling promotes motility and metastasis through epithelial-mesenchymal transition in gastrointestinal cancer*. *Oncogene*, 2013. **32**(9): p. 1183-92.
174. Galon, J., et al., *Type, density, and location of immune cells within human colorectal tumors predict clinical outcome*. *Science*, 2006. **313**(5795): p. 1960-4.
175. Zhu, Q., et al., *Mechanism of counterattack of colorectal cancer cell by Fas/Fas ligand system*. *World J Gastroenterol*, 2005. **11**(39): p. 6125-9.
176. Odani-Kawabata, N., et al., *ARG098, a novel anti-human Fas antibody, suppresses synovial hyperplasia and prevents cartilage destruction in a severe combined immunodeficient-HuRAG mouse model*. *BMC Musculoskelet Disord*, 2010. **11**: p. 221.
177. Wick, W., et al., *A phase II, randomized, study of weekly APG101+reirradiation versus reirradiation in progressive glioblastoma*. *Clin Cancer Res*, 2014. **20**(24): p. 6304-13.
178. Martin-Villalba, A., E. Llorens-Bobadilla, and D. Wollny, *CD95 in cancer: tool or target?* *Trends Mol Med*, 2013. **19**(6): p. 329-35.
179. Verbrugge, I., et al., *Combining radiotherapy with APO010 in cancer treatment*. *Clin Cancer Res*, 2009. **15**(6): p. 2031-8.
180. Eisele, G., et al., *APO010, a synthetic hexameric CD95 ligand, induces human glioma cell death in vitro and in vivo*. *Neuro Oncol*, 2011. **13**(2): p. 155-64.
181. Bivona, T.G., et al., *FAS and NF- $\kappa$ B signalling modulate dependence of lung cancers on mutant EGFR*. *Nature*, 2011. **471**(7339): p. 523-6.
182. Nakamoto, Y., et al., *Prevention of hepatocellular carcinoma development associated with chronic hepatitis by anti-fas ligand antibody therapy*. *J Exp Med*, 2002. **196**(8): p. 1105-11.
183. McCaffrey, L.M. and I.G. Macara, *Epithelial organization, cell polarity and tumorigenesis*. *Trends Cell Biol*, 2011. **21**(12): p. 727-35.
184. Marchiando, A.M., W.V. Graham, and J.R. Turner, *Epithelial barriers in homeostasis and disease*. *Annu Rev Pathol*, 2010. **5**: p. 119-44.
185. BCCAMPUS. *ANATOMY AND PHYSIOLOGY*. Available from: <https://opentextbc.ca/anatomyandphysiology/chapter/4-2-epithelial-tissue/>.
186. Lommel, A.T.L.V., *From Cells to Organs*. 2003.
187. Devenport, D., *The cell biology of planar cell polarity*. *J Cell Biol*, 2014. **207**(2): p. 171-9.

188. Gubb, D. and A. García-Bellido, *A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster*. *J Embryol Exp Morphol*, 1982. **68**: p. 37-57.
189. Ridley, A.J., et al., *Cell migration: integrating signals from front to back*. *Science*, 2003. **302**(5651): p. 1704-9.
190. Goodrich, L.V. and D. Strutt, *Principles of planar polarity in animal development*. *Development*, 2011. **138**(10): p. 1877-92.
191. Lim, J. and J.P. Thiery, *Epithelial-mesenchymal transitions: insights from development*. *Development*, 2012. **139**(19): p. 3471-86.
192. Muthuswamy, S.K. and B. Xue, *Cell polarity as a regulator of cancer cell behavior plasticity*. *Annu Rev Cell Dev Biol*, 2012. **28**: p. 599-625.
193. Fuchs, E., T. Tumber, and G. Guasch, *Socializing with the neighbors: stem cells and their niche*. *Cell*, 2004. **116**(6): p. 769-78.
194. Ley, K., et al., *Getting to the site of inflammation: the leukocyte adhesion cascade updated*. *Nat Rev Immunol*, 2007. **7**(9): p. 678-89.
195. Daugherty, B.L., et al., *Regulation of heterotypic claudin compatibility*. *J Biol Chem*, 2007. **282**(41): p. 30005-13.
196. Brandner, J.M. and J.D. Schulzke, *Hereditary barrier-related diseases involving the tight junction: lessons from skin and intestine*. *Cell Tissue Res*, 2015. **360**(3): p. 723-48.
197. Troyanovsky, S., *Adherens junction assembly*. *Subcell Biochem*, 2012. **60**: p. 89-108.
198. Mehta, S., et al., *Defects in the adherens junction complex (E-cadherin/  $\beta$ -catenin) in inflammatory bowel disease*. *Cell Tissue Res*, 2015. **360**(3): p. 749-60.
199. Samuelov, L., E. Sprecher, and R. Paus, *The role of P-cadherin in skin biology and skin pathology: lessons from the hair follicle*. *Cell Tissue Res*, 2015. **360**(3): p. 761-71.
200. Vite, A., J. Li, and G.L. Radice, *New functions for alpha-catenins in health and disease: from cancer to heart regeneration*. *Cell Tissue Res*, 2015. **360**(3): p. 773-83.
201. Garrod, D. and M. Chidgey, *Desmosome structure, composition and function*. *Biochim Biophys Acta*, 2008. **1778**(3): p. 572-87.
202. Broussard, J.A., S. Getsios, and K.J. Green, *Desmosome regulation and signaling in disease*. *Cell Tissue Res*, 2015. **360**(3): p. 501-12.
203. Goodenough, D.A. and D.L. Paul, *Gap junctions*. *Cold Spring Harb Perspect Biol*, 2009. **1**(1): p. a002576.
204. Getsios, S., D.P. Kelsell, and A. Forge, *Junctions in human health and inherited disease*. *Cell Tissue Res*, 2015. **360**(3): p. 435-8.
205. Okabe, N., et al., *Expression patterns of nectins and afadin during epithelial remodeling in the mouse embryo*. *Dev Dyn*, 2004. **230**(1): p. 174-86.
206. FARQUHAR, M.G. and G.E. PALADE, *Junctional complexes in various epithelia*. *J Cell Biol*, 1963. **17**: p. 375-412.
207. Indra, I., et al., *The adherens junction: a mosaic of cadherin and nectin clusters bundled by actin filaments*. *J Invest Dermatol*, 2013. **133**(11): p. 2546-2554.
208. Truong Quang, B.A., et al., *Principles of E-cadherin supramolecular organization in vivo*. *Curr Biol*, 2013. **23**(22): p. 2197-2207.
209. Wu, Y., P. Kanchanawong, and R. Zaidel-Bar, *Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions*. *Dev Cell*, 2015. **32**(2): p. 139-54.



210. Matsushima, H., et al., *The expression of nectin-1alpha in normal human skin and various skin tumours*. Br J Dermatol, 2003. **148**(4): p. 755-62.
211. Hyafil, F., C. Babinet, and F. Jacob, *Cell-cell interactions in early embryogenesis: a molecular approach to the role of calcium*. Cell, 1981. **26**(3 Pt 1): p. 447-54.
212. Takeichi, M., *Functional correlation between cell adhesive properties and some cell surface proteins*. J Cell Biol, 1977. **75**(2 Pt 1): p. 464-74.
213. Vestweber, D. and R. Kemler, *Rabbit antiserum against a purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues*. Exp Cell Res, 1984. **152**(1): p. 169-78.
214. van Roy, F., *Beyond E-cadherin: roles of other cadherin superfamily members in cancer*. Nat Rev Cancer, 2014. **14**(2): p. 121-34.
215. Lodish, H., et al., *Molecular Cell Biology*, ed. S. ed. 2013.
216. Yagi, T. and M. Takeichi, *Cadherin superfamily genes: functions, genomic organization, and neurologic diversity*. Genes Dev, 2000. **14**(10): p. 1169-80.
217. Shapiro, L. and W.I. Weis, *Structure and biochemistry of cadherins and catenins*. Cold Spring Harb Perspect Biol, 2009. **1**(3): p. a003053.
218. Takeichi, M., *The cadherin superfamily in neuronal connections and interactions*. Nat Rev Neurosci, 2007. **8**(1): p. 11-20.
219. Oda, H. and M. Takeichi, *Evolution: structural and functional diversity of cadherin at the adherens junction*. J Cell Biol, 2011. **193**(7): p. 1137-46.
220. Angres, B., A. Barth, and W.J. Nelson, *Mechanism for transition from initial to stable cell-cell adhesion: kinetic analysis of E-cadherin-mediated adhesion using a quantitative adhesion assay*. J Cell Biol, 1996. **134**(2): p. 549-57.
221. Matsuzaki, F., et al., *cDNAs of cell adhesion molecules of different specificity induce changes in cell shape and border formation in cultured S180 cells*. J Cell Biol, 1990. **110**(4): p. 1239-52.
222. Nagafuchi, A., et al., *Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA*. Nature, 1987. **329**(6137): p. 341-3.
223. Duguay, D., R.A. Foty, and M.S. Steinberg, *Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants*. Dev Biol, 2003. **253**(2): p. 309-23.
224. Nollet, F., P. Kools, and F. van Roy, *Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members*. J Mol Biol, 2000. **299**(3): p. 551-72.
225. Häussinger, D., et al., *Proteolytic E-cadherin activation followed by solution NMR and X-ray crystallography*. EMBO J, 2004. **23**(8): p. 1699-708.
226. Laprise, P., et al., *Down-regulation of MEK/ERK signaling by E-cadherin-dependent PI3K/Akt pathway in differentiating intestinal epithelial cells*. J Cell Physiol, 2004. **199**(1): p. 32-9.
227. Mège, R.M., J. Gavard, and M. Lambert, *Regulation of cell-cell junctions by the cytoskeleton*. Curr Opin Cell Biol, 2006. **18**(5): p. 541-8.
228. Laprise, P., A. Viel, and N. Rivard, *Human homolog of disc-large is required for adherens junction assembly and differentiation of human intestinal epithelial cells*. J Biol Chem, 2004. **279**(11): p. 10157-66.
229. Simske, J.S., et al., *The cell junction protein VAB-9 regulates adhesion and epidermal morphology in C. elegans*. Nat Cell Biol, 2003. **5**(7): p. 619-25.

230. van Roy, F. and G. Berx, *The cell-cell adhesion molecule E-cadherin*. Cell Mol Life Sci, 2008. **65**(23): p. 3756-88.
231. Derycke, L.D. and M.E. Bracke, *N-cadherin in the spotlight of cell-cell adhesion, differentiation, embryogenesis, invasion and signalling*. Int J Dev Biol, 2004. **48**(5-6): p. 463-76.
232. Wheelock, M.J., et al., *Cadherin switching*. J Cell Sci, 2008. **121**(Pt 6): p. 727-35.
233. Hazan, R.B., et al., *Cadherin switch in tumor progression*. Ann N Y Acad Sci, 2004. **1014**: p. 155-63.
234. Vandewalle, C., et al., *SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell-cell junctions*. Nucleic Acids Res, 2005. **33**(20): p. 6566-78.
235. Liu, F., et al., *Biomarkers for EMT and MET in breast cancer: An update*. Oncol Lett, 2016. **12**(6): p. 4869-4876.
236. Hirai, Y., et al., *Expression and role of E- and P-cadherin adhesion molecules in embryonic histogenesis. II. Skin morphogenesis*. Development, 1989. **105**(2): p. 271-7.
237. Albergaria, A., et al., *P-cadherin role in normal breast development and cancer*. Int J Dev Biol, 2011. **55**(7-9): p. 811-22.
238. Imai, K., et al., *Identification of a novel tumor-associated antigen, cadherin 3/P-cadherin, as a possible target for immunotherapy of pancreatic, gastric, and colorectal cancers*. Clin Cancer Res, 2008. **14**(20): p. 6487-95.
239. Nose, A. and M. Takeichi, *A novel cadherin cell adhesion molecule: its expression patterns associated with implantation and organogenesis of mouse embryos*. J Cell Biol, 1986. **103**(6 Pt 2): p. 2649-58.
240. Gory, S., et al., *The vascular endothelial-cadherin promoter directs endothelial-specific expression in transgenic mice*. Blood, 1999. **93**(1): p. 184-92.
241. Nyqvist, D., C. Giampietro, and E. Dejana, *Deciphering the functional role of endothelial junctions by using in vivo models*. EMBO Rep, 2008. **9**(8): p. 742-7.
242. Gavard, J., *Endothelial permeability and VE-cadherin: a wacky comradeship*. Cell Adh Migr, 2014. **8**(2): p. 158-64.
243. Huber, A.H. and W.I. Weis, *The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin*. Cell, 2001. **105**(3): p. 391-402.
244. Choi, H.J., A.H. Huber, and W.I. Weis, *Thermodynamics of beta-catenin-ligand interactions: the roles of the N- and C-terminal tails in modulating binding affinity*. J Biol Chem, 2006. **281**(2): p. 1027-38.
245. Gottardi, C.J. and B.M. Gumbiner, *Distinct molecular forms of beta-catenin are targeted to adhesive or transcriptional complexes*. J Cell Biol, 2004. **167**(2): p. 339-49.
246. Sadot, E., et al., *Differential interaction of plakoglobin and beta-catenin with the ubiquitin-proteasome system*. Oncogene, 2000. **19**(16): p. 1992-2001.
247. Kobiela, A. and E. Fuchs, *Alpha-catenin: at the junction of intercellular adhesion and actin dynamics*. Nat Rev Mol Cell Biol, 2004. **5**(8): p. 614-25.
248. Kourtidis, A., S.P. Ngok, and P.Z. Anastasiadis, *p120 catenin: an essential regulator of cadherin stability, adhesion-induced signaling, and cancer progression*. Prog Mol Biol Transl Sci, 2013. **116**: p. 409-32.
249. Aho, S., et al., *Specific sequences in p120ctn determine subcellular distribution of its multiple isoforms involved in cellular adhesion of normal and malignant epithelial cells*. J Cell Sci, 2002. **115**(Pt 7): p. 1391-402.

250. Irie, K., et al., *Roles and modes of action of nectins in cell-cell adhesion*. *Semin Cell Dev Biol*, 2004. **15**(6): p. 643-56.
251. Fukuhara, A., et al., *Role of nectin in organization of tight junctions in epithelial cells*. *Genes Cells*, 2002. **7**(10): p. 1059-72.
252. Takai, Y., et al., *Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation*. *Nat Rev Mol Cell Biol*, 2008. **9**(8): p. 603-15.
253. Lorger, M. and K. Moelling, *Regulation of epithelial wound closure and intercellular adhesion by interaction of AF6 with actin cytoskeleton*. *J Cell Sci*, 2006. **119**(Pt 16): p. 3385-98.
254. Wu, Y., et al., *Cooperativity between trans and cis interactions in cadherin-mediated junction formation*. *Proc Natl Acad Sci U S A*, 2010. **107**(41): p. 17592-7.
255. Cai, D., et al., *Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration*. *Cell*, 2014. **157**(5): p. 1146-59.
256. Ratheesh, A. and A.S. Yap, *A bigger picture: classical cadherins and the dynamic actin cytoskeleton*. *Nat Rev Mol Cell Biol*, 2012. **13**(10): p. 673-9.
257. Kovacs, E.M., et al., *N-WASP regulates the epithelial junctional actin cytoskeleton through a non-canonical post-nucleation pathway*. *Nat Cell Biol*, 2011. **13**(8): p. 934-43.
258. Huber, A.H., et al., *The cadherin cytoplasmic domain is unstructured in the absence of beta-catenin. A possible mechanism for regulating cadherin turnover*. *J Biol Chem*, 2001. **276**(15): p. 12301-9.
259. Rimm, D.L., et al., *Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex*. *Proc Natl Acad Sci U S A*, 1995. **92**(19): p. 8813-7.
260. Drees, F., et al., *Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly*. *Cell*, 2005. **123**(5): p. 903-15.
261. Yamada, S., et al., *Deconstructing the cadherin-catenin-actin complex*. *Cell*, 2005. **123**(5): p. 889-901.
262. Bianchini, J.M., et al., *Reevaluating  $\alpha$ E-catenin monomer and homodimer functions by characterizing E-cadherin/ $\alpha$ E-catenin chimeras*. *J Cell Biol*, 2015. **210**(7): p. 1065-74.
263. Buckley, C.D., et al., *Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force*. *Science*, 2014. **346**(6209): p. 1254211.
264. Etienne-Manneville, S. and A. Hall, *Rho GTPases in cell biology*. *Nature*, 2002. **420**(6916): p. 629-35.
265. Kovacs, E.M., et al., *E-cadherin homophilic ligation directly signals through Rac and phosphatidylinositol 3-kinase to regulate adhesive contacts*. *J Biol Chem*, 2002. **277**(8): p. 6708-18.
266. Du, W., et al., *From cell membrane to the nucleus: an emerging role of E-cadherin in gene transcriptional regulation*. *J Cell Mol Med*, 2014. **18**(9): p. 1712-9.
267. Benjamin, J.M., et al., *AlphaE-catenin regulates actin dynamics independently of cadherin-mediated cell-cell adhesion*. *J Cell Biol*, 2010. **189**(2): p. 339-52.
268. Hansen, S.D., et al.,  *$\alpha$ E-catenin actin-binding domain alters actin filament conformation and regulates binding of nucleation and disassembly factors*. *Mol Biol Cell*, 2013. **24**(23): p. 3710-20.
269. Vasioukhin, V., et al., *Directed actin polymerization is the driving force for epithelial cell-cell adhesion*. *Cell*, 2000. **100**(2): p. 209-19.

270. Smutny, M., et al., *Myosin II isoforms identify distinct functional modules that support integrity of the epithelial zonula adherens*. *Nat Cell Biol*, 2010. **12**(7): p. 696-702.
271. Brüser, L. and S. Bogdan, *Adherens Junctions on the Move-Membrane Trafficking of E-Cadherin*. *Cold Spring Harb Perspect Biol*, 2017. **9**(3).
272. de Beco, S., et al., *Endocytosis is required for E-cadherin redistribution at mature adherens junctions*. *Proc Natl Acad Sci U S A*, 2009. **106**(17): p. 7010-5.
273. Hong, S., R.B. Troyanovsky, and S.M. Troyanovsky, *Spontaneous assembly and active disassembly balance adherens junction homeostasis*. *Proc Natl Acad Sci U S A*, 2010. **107**(8): p. 3528-33.
274. Miyashita, Y. and M. Ozawa, *A dileucine motif in its cytoplasmic domain directs beta-catenin-uncoupled E-cadherin to the lysosome*. *J Cell Sci*, 2007. **120**(Pt 24): p. 4395-406.
275. Xiao, K., et al., *Role of p120-catenin in cadherin trafficking*. *Biochim Biophys Acta*, 2007. **1773**(1): p. 8-16.
276. Ishiyama, N., et al., *Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion*. *Cell*, 2010. **141**(1): p. 117-28.
277. Fujita, Y., et al., *Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex*. *Nat Cell Biol*, 2002. **4**(3): p. 222-31.
278. Chen, X., et al., *p120 catenin associates with kinesin and facilitates the transport of cadherin-catenin complexes to intercellular junctions*. *J Cell Biol*, 2003. **163**(3): p. 547-57.
279. Thiery, J.P., *Epithelial-mesenchymal transitions in tumour progression*. *Nat Rev Cancer*, 2002. **2**(6): p. 442-54.
280. Moreno-Bueno, G., F. Portillo, and A. Cano, *Transcriptional regulation of cell polarity in EMT and cancer*. *Oncogene*, 2008. **27**(55): p. 6958-69.
281. Gregory, P.A., et al., *The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1*. *Nat Cell Biol*, 2008. **10**(5): p. 593-601.
282. Maretzky, T., et al., *ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation*. *Proc Natl Acad Sci U S A*, 2005. **102**(26): p. 9182-7.
283. Grieve, A.G. and C. Rabouille, *Extracellular cleavage of E-cadherin promotes epithelial cell extrusion*. *J Cell Sci*, 2014. **127**(Pt 15): p. 3331-46.
284. Shoval, I., A. Ludwig, and C. Kalcheim, *Antagonistic roles of full-length N-cadherin and its soluble BMP cleavage product in neural crest delamination*. *Development*, 2007. **134**(3): p. 491-501.
285. Larue, L., et al., *E-cadherin null mutant embryos fail to form a trophectoderm epithelium*. *Proc Natl Acad Sci U S A*, 1994. **91**(17): p. 8263-7.
286. Kintner, C., *Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain*. *Cell*, 1992. **69**(2): p. 225-36.
287. Lee, C.H. and B.M. Gumbiner, *Disruption of gastrulation movements in Xenopus by a dominant-negative mutant for C-cadherin*. *Dev Biol*, 1995. **171**(2): p. 363-73.
288. Levine, E., et al., *Selective disruption of E-cadherin function in early Xenopus embryos by a dominant negative mutant*. *Development*, 1994. **120**(4): p. 901-9.
289. Tunggal, J.A., et al., *E-cadherin is essential for in vivo epidermal barrier function by regulating tight junctions*. *EMBO J*, 2005. **24**(6): p. 1146-56.

290. Michels, C., et al., *Classical cadherins regulate desmosome formation*. J Invest Dermatol, 2009. **129**(8): p. 2072-5.
291. Tinkle, C.L., et al., *New insights into cadherin function in epidermal sheet formation and maintenance of tissue integrity*. Proc Natl Acad Sci U S A, 2008. **105**(40): p. 15405-10.
292. Capaldo, C.T. and I.G. Macara, *Depletion of E-cadherin disrupts establishment but not maintenance of cell junctions in Madin-Darby canine kidney epithelial cells*. Mol Biol Cell, 2007. **18**(1): p. 189-200.
293. Paulson, A.F., et al., *Regulation of cadherin expression in nervous system development*. Cell Adh Migr, 2014. **8**(1): p. 19-28.
294. Radice, G.L., et al., *Developmental defects in mouse embryos lacking N-cadherin*. Dev Biol, 1997. **181**(1): p. 64-78.
295. Luo, Y., et al., *Rescuing the N-cadherin knockout by cardiac-specific expression of N- or E-cadherin*. Development, 2001. **128**(4): p. 459-69.
296. Gavard, J. and J.S. Gutkind, *VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin*. Nat Cell Biol, 2006. **8**(11): p. 1223-34.
297. Taddei, A., et al., *Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5*. Nat Cell Biol, 2008. **10**(8): p. 923-34.
298. Heupel, W.M., et al., *Endothelial barrier stabilization by a cyclic tandem peptide targeting VE-cadherin transinteraction in vitro and in vivo*. J Cell Sci, 2009. **122**(Pt 10): p. 1616-25.
299. Weis, S.M. and D.A. Cheresh, *Pathophysiological consequences of VEGF-induced vascular permeability*. Nature, 2005. **437**(7058): p. 497-504.
300. Le Guellec, A., J. Dwyer, and J. Gavard, *Jumping the barrier: VE-cadherin, VEGF and other angiogenic modifiers in cancer*. Biol Cell, 2011. **103**(12): p. 593-605.
301. Haegel, H., et al., *Lack of beta-catenin affects mouse development at gastrulation*. Development, 1995. **121**(11): p. 3529-37.
302. Huelsken, J., et al., *Requirement for beta-catenin in anterior-posterior axis formation in mice*. J Cell Biol, 2000. **148**(3): p. 567-78.
303. Torres, M., et al., *An alpha-E-catenin gene trap mutation defines its function in preimplantation development*. Proc Natl Acad Sci U S A, 1997. **94**(3): p. 901-6.
304. Kobiela, A. and E. Fuchs, *Links between alpha-catenin, NF-kappaB, and squamous cell carcinoma in skin*. Proc Natl Acad Sci U S A, 2006. **103**(7): p. 2322-7.
305. Silvis, M.R., et al., *alpha-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1*. Sci Signal, 2011. **4**(174): p. ra33.
306. Smalley-Freed, W.G., et al., *p120-catenin is essential for maintenance of barrier function and intestinal homeostasis in mice*. J Clin Invest, 2010. **120**(6): p. 1824-35.
307. Miyoshi, J. and Y. Takai, *Nectin and nectin-like molecules: biology and pathology*. Am J Nephrol, 2007. **27**(6): p. 590-604.
308. Inagaki, M., et al., *Roles of cell-adhesion molecules nectin 1 and nectin 3 in ciliary body development*. Development, 2005. **132**(7): p. 1525-37.
309. Ikeda, W., et al., *Afadin: A key molecule essential for structural organization of cell-cell junctions of polarized epithelia during embryogenesis*. J Cell Biol, 1999. **146**(5): p. 1117-32.

310. Steinberg, M.S. and S.F. Gilbert, *Townes and Holtfreter (1955): directed movements and selective adhesion of embryonic amphibian cells*. J Exp Zool A Comp Exp Biol, 2004. **301**(9): p. 701-6.
311. Sauka-Spengler, T. and M. Bronner-Fraser, *A gene regulatory network orchestrates neural crest formation*. Nat Rev Mol Cell Biol, 2008. **9**(7): p. 557-68.
312. Chaffer, C.L., E.W. Thompson, and E.D. Williams, *Mesenchymal to epithelial transition in development and disease*. Cells Tissues Organs, 2007. **185**(1-3): p. 7-19.
313. Perez-Moreno, M., et al., *p120-catenin mediates inflammatory responses in the skin*. Cell, 2006. **124**(3): p. 631-44.
314. Nelson, W.J., *Adaptation of core mechanisms to generate cell polarity*. Nature, 2003. **422**(6933): p. 766-74.
315. Wang, X., et al., *E-cadherin bridges cell polarity and spindle orientation to ensure prostate epithelial integrity and prevent carcinogenesis in vivo*. PLoS Genet, 2018. **14**(8): p. e1007609.
316. Reuver, S.M. and C.C. Garner, *E-cadherin mediated cell adhesion recruits SAP97 into the cortical cytoskeleton*. J Cell Sci, 1998. **111 ( Pt 8)**: p. 1071-80.
317. Gumbiner, B., B. Stevenson, and A. Grimaldi, *The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex*. J Cell Biol, 1988. **107**(4): p. 1575-87.
318. Wheelock, M.J. and P.J. Jensen, *Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin*. J Cell Biol, 1992. **117**(2): p. 415-25.
319. Tepass, U., et al., *shotgun encodes Drosophila E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia*. Genes Dev, 1996. **10**(6): p. 672-85.
320. Navarro, C., et al., *Junctional recruitment of mammalian Scribble relies on E-cadherin engagement*. Oncogene, 2005. **24**(27): p. 4330-9.
321. Lechler, T. and E. Fuchs, *Asymmetric cell divisions promote stratification and differentiation of mammalian skin*. Nature, 2005. **437**(7056): p. 275-80.
322. Harris, T.J. and M. Peifer, *Adherens junction-dependent and -independent steps in the establishment of epithelial cell polarity in Drosophila*. J Cell Biol, 2004. **167**(1): p. 135-47.
323. Ebnet, K., *Organization of multiprotein complexes at cell-cell junctions*. Histochem Cell Biol, 2008. **130**(1): p. 1-20.
324. Qin, Y., et al., *The mammalian Scribble polarity protein regulates epithelial cell adhesion and migration through E-cadherin*. J Cell Biol, 2005. **171**(6): p. 1061-71.
325. Prasad, M., et al., *Border Cell Migration: A Model System for Live Imaging and Genetic Analysis of Collective Cell Movement*. Methods Mol Biol, 2015. **1328**: p. 89-97.
326. Montell, D.J., *Border-cell migration: the race is on*. Nat Rev Mol Cell Biol, 2003. **4**(1): p. 13-24.
327. Barker, N., M. van de Wetering, and H. Clevers, *The intestinal stem cell*. Genes Dev, 2008. **22**(14): p. 1856-64.
328. Hermiston, M.L. and J.I. Gordon, *In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death*. J Cell Biol, 1995. **129**(2): p. 489-506.

329. Hermiston, M.L., M.H. Wong, and J.I. Gordon, *Forced expression of E-cadherin in the mouse intestinal epithelium slows cell migration and provides evidence for nonautonomous regulation of cell fate in a self-renewing system*. *Genes Dev*, 1996. **10**(8): p. 985-96.
330. Plutoni, C., et al., *P-cadherin promotes collective cell migration via a Cdc42-mediated increase in mechanical forces*. *J Cell Biol*, 2016. **212**(2): p. 199-217.
331. Alexander, S., et al., *Preclinical intravital microscopy of the tumour-stroma interface: invasion, metastasis, and therapy response*. *Curr Opin Cell Biol*, 2013. **25**(5): p. 659-71.
332. Paredes, J., et al., *Epithelial E- and P-cadherins: role and clinical significance in cancer*. *Biochim Biophys Acta*, 2012. **1826**(2): p. 297-311.
333. Macpherson, I.R., et al., *p120-catenin is required for the collective invasion of squamous cell carcinoma cells via a phosphorylation-independent mechanism*. *Oncogene*, 2007. **26**(36): p. 5214-28.
334. Ng, M.R., et al., *Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility*. *J Cell Biol*, 2012. **199**(3): p. 545-63.
335. Jeanes, A., C.J. Gottardi, and A.S. Yap, *Cadherins and cancer: how does cadherin dysfunction promote tumor progression?* *Oncogene*, 2008. **27**(55): p. 6920-9.
336. Mendonsa, A.M., T.Y. Na, and B.M. Gumbiner, *E-cadherin in contact inhibition and cancer*. *Oncogene*, 2018. **37**(35): p. 4769-4780.
337. Eagle, H. and E.M. Levine, *Growth regulatory effects of cellular interaction*. *Nature*, 1967. **213**(5081): p. 1102-6.
338. St Croix, B., et al., *E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1)*. *J Cell Biol*, 1998. **142**(2): p. 557-71.
339. Motti, M.L., et al., *Reduced E-cadherin expression contributes to the loss of p27kip1-mediated mechanism of contact inhibition in thyroid anaplastic carcinomas*. *Carcinogenesis*, 2005. **26**(6): p. 1021-34.
340. Perrais, M., et al., *E-cadherin homophilic ligation inhibits cell growth and epidermal growth factor receptor signaling independently of other cell interactions*. *Mol Biol Cell*, 2007. **18**(6): p. 2013-25.
341. Kim, N.G., et al., *E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components*. *Proc Natl Acad Sci U S A*, 2011. **108**(29): p. 11930-5.
342. Liu, T.X., et al., *Chromosome 5q deletion and epigenetic suppression of the gene encoding alpha-catenin (CTNNA1) in myeloid cell transformation*. *Nat Med*, 2007. **13**(1): p. 78-83.
343. Watabe, M., et al., *Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line*. *J Cell Biol*, 1994. **127**(1): p. 247-56.
344. Ji, H., et al.,  *$\alpha$ -Catenin inhibits glioma cell migration, invasion, and proliferation by suppression of  $\beta$ -catenin transactivation*. *J Neurooncol*, 2011. **103**(3): p. 445-51.
345. Davis, M.A. and A.B. Reynolds, *Blocked acinar development, E-cadherin reduction, and intraepithelial neoplasia upon ablation of p120-catenin in the mouse salivary gland*. *Dev Cell*, 2006. **10**(1): p. 21-31.
346. Horbinski, C., C. Mojesky, and N. Kyprianou, *Live free or die: tales of homeless (cells) in cancer*. *Am J Pathol*, 2010. **177**(3): p. 1044-52.

347. Reddig, P.J. and R.L. Juliano, *Clinging to life: cell to matrix adhesion and cell survival*. *Cancer Metastasis Rev*, 2005. **24**(3): p. 425-39.
348. Schneider, M.R., et al., *A key role for E-cadherin in intestinal homeostasis and Paneth cell maturation*. *PLoS One*, 2010. **5**(12): p. e14325.
349. Boussadia, O., et al., *E-cadherin is a survival factor for the lactating mouse mammary gland*. *Mech Dev*, 2002. **115**(1-2): p. 53-62.
350. Peluso, J.J., A. Pappalardo, and G. Fernandez, *E-cadherin-mediated cell contact prevents apoptosis of spontaneously immortalized granulosa cells by regulating Akt kinase activity*. *Biol Reprod*, 2001. **64**(4): p. 1183-90.
351. Ben Chedly, H., et al., *Disruption of cell junctions induces apoptosis and reduces synthetic activity in lactating goat mammary gland*. *J Dairy Sci*, 2010. **93**(7): p. 2938-51.
352. Carmeliet, P., et al., *Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis*. *Cell*, 1999. **98**(2): p. 147-57.
353. Vittet, D., et al., *Targeted null-mutation in the vascular endothelial-cadherin gene impairs the organization of vascular-like structures in embryoid bodies*. *Proc Natl Acad Sci U S A*, 1997. **94**(12): p. 6273-8.
354. Peluso, J.J., *Putative mechanism through which N-cadherin-mediated cell contact maintains calcium homeostasis and thereby prevents ovarian cells from undergoing apoptosis*. *Biochem Pharmacol*, 1997. **54**(8): p. 847-53.
355. Livshits, G., A. Kobiela, and E. Fuchs, *Governing epidermal homeostasis by coupling cell-cell adhesion to integrin and growth factor signaling, proliferation, and apoptosis*. *Proc Natl Acad Sci U S A*, 2012. **109**(13): p. 4886-91.
356. Matsubara, S. and M. Ozawa, *Expression of alpha-catenin in alpha-catenin-deficient cells increases resistance to sphingosine-induced apoptosis*. *J Cell Biol*, 2001. **154**(3): p. 573-84.
357. Tepera, S.B., P.D. McCrea, and J.M. Rosen, *A beta-catenin survival signal is required for normal lobular development in the mammary gland*. *J Cell Sci*, 2003. **116**(Pt 6): p. 1137-49.
358. Calautti, E., et al., *Phosphoinositide 3-kinase signaling to Akt promotes keratinocyte differentiation versus death*. *J Biol Chem*, 2005. **280**(38): p. 32856-65.
359. Laprise, P., et al., *Phosphatidylinositol 3-kinase controls human intestinal epithelial cell differentiation by promoting adherens junction assembly and p38 MAPK activation*. *J Biol Chem*, 2002. **277**(10): p. 8226-34.
360. De Santis, G., et al., *E-cadherin directly contributes to PI3K/AKT activation by engaging the PI3K-p85 regulatory subunit to adherens junctions of ovarian carcinoma cells*. *Oncogene*, 2009. **28**(9): p. 1206-17.
361. Adams, R.H. and K. Alitalo, *Molecular regulation of angiogenesis and lymphangiogenesis*. *Nat Rev Mol Cell Biol*, 2007. **8**(6): p. 464-78.
362. Fukuhara, S., et al., *Tie2 is tied at the cell-cell contacts and to extracellular matrix by angiopoietin-1*. *Exp Mol Med*, 2009. **41**(3): p. 133-9.
363. Frank, S.R., et al., *A  $\beta$ PIX-PAK2 complex confers protection against Scrib-dependent and cadherin-mediated apoptosis*. *Curr Biol*, 2012. **22**(19): p. 1747-54.
364. Campbell, H.K., et al., *PAK2 links cell survival to mechanotransduction and metabolism*. *J Cell Biol*, 2019. **218**(6): p. 1958-1971.



365. El-Amraoui, A. and C. Petit, *Cadherin defects in inherited human diseases*. Prog Mol Biol Transl Sci, 2013. **116**: p. 361-84.
366. El-Amraoui, A. and C. Petit, *Cadherins as targets for genetic diseases*. Cold Spring Harb Perspect Biol, 2010. **2**(1): p. a003095.
367. Barrett, J.C., et al., *Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region*. Nat Genet, 2009. **41**(12): p. 1330-4.
368. Elding, H., et al., *Dissecting the genetics of complex inheritance: linkage disequilibrium mapping provides insight into Crohn disease*. Am J Hum Genet, 2011. **89**(6): p. 798-805.
369. Vasioukhin, V., *Adherens junctions and cancer*. Subcell Biochem, 2012. **60**: p. 379-414.
370. Onder, T.T., et al., *Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways*. Cancer Res, 2008. **68**(10): p. 3645-54.
371. Perl, A.K., et al., *A causal role for E-cadherin in the transition from adenoma to carcinoma*. Nature, 1998. **392**(6672): p. 190-3.
372. Derksen, P.W., et al., *Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis*. Cancer Cell, 2006. **10**(5): p. 437-49.
373. Nusse, R. and H. Clevers, *Wnt/ $\beta$ -Catenin Signaling, Disease, and Emerging Therapeutic Modalities*. Cell, 2017. **169**(6): p. 985-999.
374. Dar, M.S., et al., *Beta-catenin N-terminal domain: An enigmatic region prone to cancer causing mutations*. Mutat Res, 2017. **773**: p. 122-133.
375. Shimoyama, Y., et al., *Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell-cell adhesiveness*. Cancer Res, 1992. **52**(20): p. 5770-4.
376. Sun, Y., J. Zhang, and L. Ma,  *$\alpha$ -catenin. A tumor suppressor beyond adherens junctions*. Cell Cycle, 2014. **13**(15): p. 2334-9.
377. Vasioukhin, V., et al., *Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin*. Cell, 2001. **104**(4): p. 605-17.
378. Piao, H.L., et al.,  *$\alpha$ -catenin acts as a tumour suppressor in E-cadherin-negative basal-like breast cancer by inhibiting NF- $\kappa$ B signalling*. Nat Cell Biol, 2014. **16**(3): p. 245-54.
379. Thoreson, M.A. and A.B. Reynolds, *Altered expression of the catenin p120 in human cancer: implications for tumor progression*. Differentiation, 2002. **70**(9-10): p. 583-9.
380. Noren, N.K., et al., *p120 catenin regulates the actin cytoskeleton via Rho family GTPases*. J Cell Biol, 2000. **150**(3): p. 567-80.
381. Reynolds, A.B., et al., *Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes*. Mol Cell Biol, 1994. **14**(12): p. 8333-42.
382. Yanagisawa, M. and P.Z. Anastasiadis, *p120 catenin is essential for mesenchymal cadherin-mediated regulation of cell motility and invasiveness*. J Cell Biol, 2006. **174**(7): p. 1087-96.
383. Soto, E., et al., *p120 catenin induces opposing effects on tumor cell growth depending on E-cadherin expression*. J Cell Biol, 2008. **183**(4): p. 737-49.
384. Gottardi, C.J. and B.M. Gumbiner, *Adhesion signaling: how beta-catenin interacts with its partners*. Curr Biol, 2001. **11**(19): p. R792-4.

385. Gottardi, C.J., E. Wong, and B.M. Gumbiner, *E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner*. J Cell Biol, 2001. **153**(5): p. 1049-60.
386. Torres, V.A., et al., *E-cadherin is required for caveolin-1-mediated down-regulation of the inhibitor of apoptosis protein survivin via reduced beta-catenin-Tcf/Lef-dependent transcription*. Mol Cell Biol, 2007. **27**(21): p. 7703-17.
387. Näthke, I.S., et al., *Defining interactions and distributions of cadherin and catenin complexes in polarized epithelial cells*. J Cell Biol, 1994. **125**(6): p. 1341-52.
388. Maher, M.T., et al., *Activity of the beta-catenin phosphodestruction complex at cell-cell contacts is enhanced by cadherin-based adhesion*. J Cell Biol, 2009. **186**(2): p. 219-28.
389. Caca, K., et al., *Beta- and gamma-catenin mutations, but not E-cadherin inactivation, underlie T-cell factor/lymphoid enhancer factor transcriptional deregulation in gastric and pancreatic cancer*. Cell Growth Differ, 1999. **10**(6): p. 369-76.
390. van de Wetering, M., et al., *The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells*. Cell, 2002. **111**(2): p. 241-50.
391. Herzig, M., et al., *Tumor progression induced by the loss of E-cadherin independent of beta-catenin/Tcf-mediated Wnt signaling*. Oncogene, 2007. **26**(16): p. 2290-8.
392. Choi, S.H., et al.,  *$\alpha$ -Catenin interacts with APC to regulate  $\beta$ -catenin proteolysis and transcriptional repression of Wnt target genes*. Genes Dev, 2013. **27**(22): p. 2473-88.
393. Giannini, A.L., M. Vivanco, and R.M. Kypta, *alpha-catenin inhibits beta-catenin signaling by preventing formation of a beta-catenin\*T-cell factor\*DNA complex*. J Biol Chem, 2000. **275**(29): p. 21883-8.
394. Aktary, Z. and M. Pasdar, *Plakoglobin: role in tumorigenesis and metastasis*. Int J Cell Biol, 2012. **2012**: p. 189521.
395. Miravet, S., et al., *The transcriptional factor Tcf-4 contains different binding sites for beta-catenin and plakoglobin*. J Biol Chem, 2002. **277**(3): p. 1884-91.
396. Aktary, Z., M. Alaei, and M. Pasdar, *Beyond cell-cell adhesion: Plakoglobin and the regulation of tumorigenesis and metastasis*. Oncotarget, 2017. **8**(19): p. 32270-32291.
397. Alaei, M., et al., *The physical interaction of p53 and plakoglobin is necessary for their synergistic inhibition of migration and invasion*. Oncotarget, 2016. **7**(18): p. 26898-915.
398. Aktary, Z., et al., *Plakoglobin interacts with the transcription factor p53 and regulates the expression of 14-3-3 $\sigma$* . J Cell Sci, 2013. **126**(Pt 14): p. 3031-42.
399. Carnahan, R.H., et al., *The molecular evolution of the p120-catenin subfamily and its functional associations*. PLoS One, 2010. **5**(12): p. e15747.
400. Schackmann, R.C., et al., *p120-catenin in cancer - mechanisms, models and opportunities for intervention*. J Cell Sci, 2013. **126**(Pt 16): p. 3515-25.
401. Badouel, C., A. Garg, and H. McNeill, *Herding Hippos: regulating growth in flies and man*. Curr Opin Cell Biol, 2009. **21**(6): p. 837-43.
402. Harvey, K.F., X. Zhang, and D.M. Thomas, *The Hippo pathway and human cancer*. Nat Rev Cancer, 2013. **13**(4): p. 246-57.
403. Schlegelmilch, K., et al., *Yap1 acts downstream of  $\alpha$ -catenin to control epidermal proliferation*. Cell, 2011. **144**(5): p. 782-95.
404. Hirate, Y., et al., *Polarity-dependent distribution of angiominin localizes Hippo signaling in preimplantation embryos*. Curr Biol, 2013. **23**(13): p. 1181-94.

405. Xia, Y., S. Shen, and I.M. Verma, *NF- $\kappa$ B, an active player in human cancers*. *Cancer Immunol Res*, 2014. **2**(9): p. 823-30.
406. Kuphal, S., et al., *Loss of E-cadherin leads to upregulation of NF $\kappa$ B activity in malignant melanoma*. *Oncogene*, 2004. **23**(52): p. 8509-19.
407. Perez-Moreno, M., et al., *Loss of p120 catenin and links to mitotic alterations, inflammation, and skin cancer*. *Proc Natl Acad Sci U S A*, 2008. **105**(40): p. 15399-404.
408. Stairs, D.B., et al., *Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene*. *Cancer Cell*, 2011. **19**(4): p. 470-83.
409. Miguel, J.C., et al., *Epidermal growth factor suppresses intestinal epithelial cell shedding through a MAPK-dependent pathway*. *J Cell Sci*, 2017. **130**(1): p. 90-96.
410. Bublil, E.M. and Y. Yarden, *The EGF receptor family: spearheading a merger of signaling and therapeutics*. *Curr Opin Cell Biol*, 2007. **19**(2): p. 124-34.
411. Qian, X., et al., *E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases*. *EMBO J*, 2004. **23**(8): p. 1739-48.
412. Curto, M., et al., *Contact-dependent inhibition of EGFR signaling by Nf2/Merlin*. *J Cell Biol*, 2007. **177**(5): p. 893-903.
413. Gladden, A.B., et al., *The NF2 tumor suppressor, Merlin, regulates epidermal development through the establishment of a junctional polarity complex*. *Dev Cell*, 2010. **19**(5): p. 727-39.
414. Lallemand, D., et al., *NF2 deficiency promotes tumorigenesis and metastasis by destabilizing adherens junctions*. *Genes Dev*, 2003. **17**(9): p. 1090-100.
415. Chiasson-MacKenzie, C., et al., *NF2/Merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin*. *J Cell Biol*, 2015. **211**(2): p. 391-405.
416. Sorkin, A. and M. von Zastrow, *Endocytosis and signalling: intertwining molecular networks*. *Nat Rev Mol Cell Biol*, 2009. **10**(9): p. 609-22.
417. Pece, S. and J.S. Gutkind, *Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell-cell contact formation*. *J Biol Chem*, 2000. **275**(52): p. 41227-33.
418. Bryant, D.M., F.G. Wylie, and J.L. Stow, *Regulation of endocytosis, nuclear translocation, and signaling of fibroblast growth factor receptor 1 by E-cadherin*. *Mol Biol Cell*, 2005. **16**(1): p. 14-23.
419. Suyama, K., et al., *A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor*. *Cancer Cell*, 2002. **2**(4): p. 301-14.
420. Qian, X., et al., *N-cadherin/FGFR promotes metastasis through epithelial-to-mesenchymal transition and stem/progenitor cell-like properties*. *Oncogene*, 2014. **33**(26): p. 3411-21.
421. Giampietro, C., et al., *Overlapping and divergent signaling pathways of N-cadherin and VE-cadherin in endothelial cells*. *Blood*, 2012. **119**(9): p. 2159-70.
422. Lampugnani, M.G., et al., *Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments*. *J Cell Biol*, 2006. **174**(4): p. 593-604.
423. Massagué, J., *TGF $\beta$  signalling in context*. *Nat Rev Mol Cell Biol*, 2012. **13**(10): p. 616-30.
424. Rudini, N., et al., *VE-cadherin is a critical endothelial regulator of TGF-beta signalling*. *EMBO J*, 2008. **27**(7): p. 993-1004.

425. Takehara, T., et al., *Cdh2 stabilizes FGFR1 and contributes to primed-state pluripotency in mouse epiblast stem cells*. *Sci Rep*, 2015. **5**: p. 14722.
426. Utton, M.A., et al., *Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells*. *J Neurochem*, 2001. **76**(5): p. 1421-30.
427. Boscher, C. and R.M. Mège, *Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling*. *Cell Signal*, 2008. **20**(6): p. 1061-72.
428. Masai, I., et al., *N-cadherin mediates retinal lamination, maintenance of forebrain compartments and patterning of retinal neurites*. *Development*, 2003. **130**(11): p. 2479-94.
429. Hult, J., et al., *N-cadherin signaling potentiates mammary tumor metastasis via enhanced extracellular signal-regulated kinase activation*. *Cancer Res*, 2007. **67**(7): p. 3106-16.
430. Weis, S., et al., *Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis*. *J Cell Biol*, 2004. **167**(2): p. 223-9.
431. Coon, B.G., et al., *Intramembrane binding of VE-cadherin to VEGFR2 and VEGFR3 assembles the endothelial mechanosensory complex*. *J Cell Biol*, 2015. **208**(7): p. 975-86.
432. Mellman, I. and W.J. Nelson, *Coordinated protein sorting, targeting and distribution in polarized cells*. *Nat Rev Mol Cell Biol*, 2008. **9**(11): p. 833-45.
433. Humbert, P.O., et al., *Control of tumorigenesis by the Scribble/Dlg/Lgl polarity module*. *Oncogene*, 2008. **27**(55): p. 6888-907.
434. Bulgakova, N.A. and E. Knust, *The Crumbs complex: from epithelial-cell polarity to retinal degeneration*. *J Cell Sci*, 2009. **122**(Pt 15): p. 2587-96.
435. Dow, L.E. and P.O. Humbert, *Polarity regulators and the control of epithelial architecture, cell migration, and tumorigenesis*. *Int Rev Cytol*, 2007. **262**: p. 253-302.
436. Knoblich, J.A., *Mechanisms of asymmetric stem cell division*. *Cell*, 2008. **132**(4): p. 583-97.
437. St Johnston, D. and B. Sanson, *Epithelial polarity and morphogenesis*. *Curr Opin Cell Biol*, 2011. **23**(5): p. 540-6.
438. Campanale, J.P., T.Y. Sun, and D.J. Montell, *Development and dynamics of cell polarity at a glance*. *J Cell Sci*, 2017. **130**(7): p. 1201-1207.
439. Wu, J. and M. Mlodzik, *A quest for the mechanism regulating global planar cell polarity of tissues*. *Trends Cell Biol*, 2009. **19**(7): p. 295-305.
440. Humbert P.O., R.S.M., Smith L., Richardson H.E., *The Scribble–Dlg–Lgl Module in Cell Polarity Regulation*. 2015.
441. Iden, S. and J.G. Collard, *Crosstalk between small GTPases and polarity proteins in cell polarization*. *Nat Rev Mol Cell Biol*, 2008. **9**(11): p. 846-59.
442. Rodriguez-Boulan, E. and I.G. Macara, *Organization and execution of the epithelial polarity programme*. *Nat Rev Mol Cell Biol*, 2014. **15**(4): p. 225-42.
443. Kemphues, K.J., et al., *Identification of genes required for cytoplasmic localization in early C. elegans embryos*. *Cell*, 1988. **52**(3): p. 311-20.
444. Tabuse, Y., et al., *Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in Caenorhabditis elegans*. *Development*, 1998. **125**(18): p. 3607-14.

445. Goldstein, B. and I.G. Macara, *The PAR proteins: fundamental players in animal cell polarization*. Dev Cell, 2007. **13**(5): p. 609-22.
446. Allam, A.H., M. Charnley, and S.M. Russell, *Context-Specific Mechanisms of Cell Polarity Regulation*. J Mol Biol, 2018. **430**(19): p. 3457-3471.
447. Ngok, S.P., W.H. Lin, and P.Z. Anastasiadis, *Establishment of epithelial polarity--GEF who's minding the GAP?* J Cell Sci, 2014. **127**(Pt 15): p. 3205-15.
448. Gao, L. and I.G. Macara, *Isoforms of the polarity protein par6 have distinct functions*. J Biol Chem, 2004. **279**(40): p. 41557-62.
449. Munson, C., et al., *Regulation of neurocoel morphogenesis by Pard6 gamma b*. Dev Biol, 2008. **324**(1): p. 41-54.
450. Bose, R. and J.L. Wrana, *Regulation of Par6 by extracellular signals*. Curr Opin Cell Biol, 2006. **18**(2): p. 206-12.
451. Suzuki, A. and S. Ohno, *The PAR-aPKC system: lessons in polarity*. J Cell Sci, 2006. **119**(Pt 6): p. 979-87.
452. Yamanaka, T., et al., *Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity*. Curr Biol, 2003. **13**(9): p. 734-43.
453. Mizuno, K., et al., *Self-association of PAR-3-mediated by the conserved N-terminal domain contributes to the development of epithelial tight junctions*. J Biol Chem, 2003. **278**(33): p. 31240-50.
454. Joberty, G., et al., *The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42*. Nat Cell Biol, 2000. **2**(8): p. 531-9.
455. Suzuki, A., et al., *Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures*. J Cell Biol, 2001. **152**(6): p. 1183-96.
456. Chen, X. and I.G. Macara, *RNA interference techniques to study epithelial cell adhesion and polarity*. Methods Enzymol, 2006. **406**: p. 362-74.
457. Noda, Y., et al., *Human homologues of the Caenorhabditis elegans cell polarity protein PAR6 as an adaptor that links the small GTPases Rac and Cdc42 to atypical protein kinase C*. Genes Cells, 2001. **6**(2): p. 107-19.
458. Nagai-Tamai, Y., et al., *Regulated protein-protein interaction between aPKC and PAR-3 plays an essential role in the polarization of epithelial cells*. Genes Cells, 2002. **7**(11): p. 1161-71.
459. Plant, P.J., et al., *A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl*. Nat Cell Biol, 2003. **5**(4): p. 301-8.
460. Izumi, Y., et al., *An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3*. J Cell Biol, 1998. **143**(1): p. 95-106.
461. Tepass, U., C. Theres, and E. Knust, *crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia*. Cell, 1990. **61**(5): p. 787-99.
462. den Hollander, A.I., et al., *Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumbs homologue 1 (CRB1) gene*. Am J Hum Genet, 2001. **69**(1): p. 198-203.
463. van den Hurk, J.A., et al., *Characterization of the Crumbs homolog 2 (CRB2) gene and analysis of its role in retinitis pigmentosa and Leber congenital amaurosis*. Mol Vis, 2005. **11**: p. 263-73.

464. Herranz-Martín, S., et al., *Immunocytochemical evidence of the localization of the Crumbs homologue 3 protein (CRB3) in the developing and mature mouse retina*. PLoS One, 2012. **7**(11): p. e50511.
465. Lemmers, C., et al., *CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells*. Mol Biol Cell, 2004. **15**(3): p. 1324-33.
466. Quinn, P.M., L.P. Pellissier, and J. Wijnholds, *The CRB1 Complex: Following the Trail of Crumbs to a Feasible Gene Therapy Strategy*. Front Neurosci, 2017. **11**: p. 175.
467. Pellissier, L.P., et al., *CRB2 acts as a modifying factor of CRB1-related retinal dystrophies in mice*. Hum Mol Genet, 2014. **23**(14): p. 3759-71.
468. Klebes, A. and E. Knust, *A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in Drosophila*. Curr Biol, 2000. **10**(2): p. 76-85.
469. Fan, S., et al., *Polarity proteins control ciliogenesis via kinesin motor interactions*. Curr Biol, 2004. **14**(16): p. 1451-61.
470. Fan, S., et al., *A novel Crumbs3 isoform regulates cell division and ciliogenesis via importin beta interactions*. J Cell Biol, 2007. **178**(3): p. 387-98.
471. Kamberov, E., et al., *Molecular cloning and characterization of Pals, proteins associated with mLin-7*. J Biol Chem, 2000. **275**(15): p. 11425-31.
472. Straight, S.W., et al., *Loss of PALS1 expression leads to tight junction and polarity defects*. Mol Biol Cell, 2004. **15**(4): p. 1981-90.
473. Wang, Q., X.W. Chen, and B. Margolis, *PALS1 regulates E-cadherin trafficking in mammalian epithelial cells*. Mol Biol Cell, 2007. **18**(3): p. 874-85.
474. Lemmers, C., et al., *hINAD1/PATJ, a homolog of discs lost, interacts with crumbs and localizes to tight junctions in human epithelial cells*. J Biol Chem, 2002. **277**(28): p. 25408-15.
475. Michel, D., et al., *PATJ connects and stabilizes apical and lateral components of tight junctions in human intestinal cells*. J Cell Sci, 2005. **118**(Pt 17): p. 4049-57.
476. Shin, K., S. Straight, and B. Margolis, *PATJ regulates tight junction formation and polarity in mammalian epithelial cells*. J Cell Biol, 2005. **168**(5): p. 705-11.
477. Hamazaki, Y., et al., *Multi-PDZ domain protein 1 (MUPP1) is concentrated at tight junctions through its possible interaction with claudin-1 and junctional adhesion molecule*. J Biol Chem, 2002. **277**(1): p. 455-61.
478. Adachi, M., et al., *Similar and distinct properties of MUPP1 and Patj, two homologous PDZ domain-containing tight-junction proteins*. Mol Cell Biol, 2009. **29**(9): p. 2372-89.
479. Bilder, D. and N. Perrimon, *Localization of apical epithelial determinants by the basolateral PDZ protein Scribble*. Nature, 2000. **403**(6770): p. 676-80.
480. Bilder, D., et al., *Collective nomenclature for LAP proteins*. Nat Cell Biol, 2000. **2**(7): p. E114.
481. Bilder, D., M. Li, and N. Perrimon, *Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors*. Science, 2000. **289**(5476): p. 113-6.
482. Bryant, P.J. and A. Huwe, *LAP proteins: what's up with epithelia?* Nat Cell Biol, 2000. **2**(8): p. E141-3.
483. Borg, J.P., et al., *ERBIN: a basolateral PDZ protein that interacts with the mammalian ERBB2/HER2 receptor*. Nat Cell Biol, 2000. **2**(7): p. 407-14.

484. Strack, S., et al., *Association of calcium/calmodulin-dependent kinase II with developmentally regulated splice variants of the postsynaptic density protein densin-180*. J Biol Chem, 2000. **275**(33): p. 25061-4.
485. Zeitler, J., et al., *Domains controlling cell polarity and proliferation in the Drosophila tumor suppressor Scribble*. J Cell Biol, 2004. **167**(6): p. 1137-46.
486. Métais, J.Y., et al., *hScrib interacts with ZO-2 at the cell-cell junctions of epithelial cells*. FEBS Lett, 2005. **579**(17): p. 3725-30.
487. Audebert, S., et al., *Mammalian Scribble forms a tight complex with the betaPIX exchange factor*. Curr Biol, 2004. **14**(11): p. 987-95.
488. Stephens, R., et al., *The Scribble Cell Polarity Module in the Regulation of Cell Signaling in Tissue Development and Tumorigenesis*. J Mol Biol, 2018. **430**(19): p. 3585-3612.
489. Kallay, L.M., et al., *Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains*. J Cell Biochem, 2006. **99**(2): p. 647-64.
490. Müsch, A., et al., *Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells*. Mol Biol Cell, 2002. **13**(1): p. 158-68.
491. McLaughlin, M., et al., *The distribution and function of alternatively spliced insertions in hDlg*. J Biol Chem, 2002. **277**(8): p. 6406-12.
492. Funke, L., S. Dakoji, and D.S. Brecht, *Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions*. Annu Rev Biochem, 2005. **74**: p. 219-45.
493. Mendoza, C., et al., *Novel isoforms of Dlg are fundamental for neuronal development in Drosophila*. J Neurosci, 2003. **23**(6): p. 2093-101.
494. Woods, D.F., et al., *Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia*. J Cell Biol, 1996. **134**(6): p. 1469-82.
495. Lue, R.A., et al., *Cloning and characterization of hdlg: the human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1*. Proc Natl Acad Sci U S A, 1994. **91**(21): p. 9818-22.
496. Mendoza-Topaz, C., et al., *DLGS97/SAP97 is developmentally upregulated and is required for complex adult behaviors and synapse morphology and function*. J Neurosci, 2008. **28**(1): p. 304-14.
497. Tejedor, F.J., et al., *Essential role for dlg in synaptic clustering of Shaker K<sup>+</sup> channels in vivo*. J Neurosci, 1997. **17**(1): p. 152-9.
498. Zito, K., et al., *Synaptic clustering of Fascilin II and Shaker: essential targeting sequences and role of Dlg*. Neuron, 1997. **19**(5): p. 1007-16.
499. Lee, S., et al., *A novel and conserved protein-protein interaction domain of mammalian Lin-2/CASK binds and recruits SAP97 to the lateral surface of epithelia*. Mol Cell Biol, 2002. **22**(6): p. 1778-91.
500. Firestein, B.L. and C. Rongo, *DLG-1 is a MAGUK similar to SAP97 and is required for adherens junction formation*. Mol Biol Cell, 2001. **12**(11): p. 3465-75.
501. Lue, R.A., et al., *Two independent domains of hDlg are sufficient for subcellular targeting: the PDZ1-2 conformational unit and an alternatively spliced domain*. J Cell Biol, 1996. **135**(4): p. 1125-37.
502. Hanada, T., et al., *Protein 4.1-mediated membrane targeting of human discs large in epithelial cells*. J Biol Chem, 2003. **278**(36): p. 34445-50.
503. Nunomura, W., et al., *Regulation of protein 4.1R, p55, and glycophorin C ternary complex in human erythrocyte membrane*. J Biol Chem, 2000. **275**(32): p. 24540-6.

504. Mathew, D., et al., *Recruitment of scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein*. *Curr Biol*, 2002. **12**(7): p. 531-9.
505. Katoh, M., *Identification and characterization of human GUKH2 gene in silico*. *Int J Oncol*, 2004. **24**(4): p. 1033-8.
506. Awadia, S., et al., *SGEF forms a complex with Scribble and Dlg1 and regulates epithelial junctions and contractility*. *J Cell Biol*, 2019. **218**(8): p. 2699-2725.
507. Weber, G.F., M.A. Bjerke, and D.W. DeSimone, *Integrins and cadherins join forces to form adhesive networks*. *J Cell Sci*, 2011. **124**(Pt 8): p. 1183-93.
508. Apodaca, G., L.I. Gallo, and D.M. Bryant, *Role of membrane traffic in the generation of epithelial cell asymmetry*. *Nat Cell Biol*, 2012. **14**(12): p. 1235-43.
509. Kovacs, E.M. and A.S. Yap, *Cell-cell contact: cooperating clusters of actin and cadherin*. *Curr Biol*, 2008. **18**(15): p. R667-R669.
510. Hirose, T., et al., *Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation*. *J Cell Sci*, 2002. **115**(Pt 12): p. 2485-95.
511. Hurd, T.W., et al., *Direct interaction of two polarity complexes implicated in epithelial tight junction assembly*. *Nat Cell Biol*, 2003. **5**(2): p. 137-42.
512. Sotillos, S., et al., *DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in Drosophila*. *J Cell Biol*, 2004. **166**(4): p. 549-57.
513. Gamblin, C.L., et al., *A bidirectional antagonism between aPKC and Yurt regulates epithelial cell polarity*. *J Cell Biol*, 2014. **204**(4): p. 487-95.
514. Doerflinger, H., et al., *The role of PAR-1 in regulating the polarised microtubule cytoskeleton in the Drosophila follicular epithelium*. *Development*, 2003. **130**(17): p. 3965-75.
515. Cohen, D., et al., *Mammalian PAR-1 determines epithelial lumen polarity by organizing the microtubule cytoskeleton*. *J Cell Biol*, 2004. **164**(5): p. 717-27.
516. Hurov, J.B., J.L. Watkins, and H. Piwnica-Worms, *Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity*. *Curr Biol*, 2004. **14**(8): p. 736-41.
517. Suzuki, A., et al., *aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity*. *Curr Biol*, 2004. **14**(16): p. 1425-35.
518. Benton, R. and D. St Johnston, *Drosophila PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells*. *Cell*, 2003. **115**(6): p. 691-704.
519. Hurd, T.W., et al., *Phosphorylation-dependent binding of 14-3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia*. *Curr Biol*, 2003. **13**(23): p. 2082-90.
520. Wang, J.W., Y. Imai, and B. Lu, *Activation of PAR-1 kinase and stimulation of tau phosphorylation by diverse signals require the tumor suppressor protein LKB1*. *J Neurosci*, 2007. **27**(3): p. 574-81.
521. Spicer, J., et al., *Regulation of the Wnt signalling component PAR1A by the Peutz-Jeghers syndrome kinase LKB1*. *Oncogene*, 2003. **22**(30): p. 4752-6.
522. Macara, I.G., *Parsing the polarity code*. *Nat Rev Mol Cell Biol*, 2004. **5**(3): p. 220-31.
523. Etienne-Manneville, S., *Cdc42--the centre of polarity*. *J Cell Sci*, 2004. **117**(Pt 8): p. 1291-300.
524. Mack, N.A. and M. Georgiou, *The interdependence of the Rho GTPases and apical-basal cell polarity*. *Small GTPases*, 2014. **5**(2): p. 10.
525. Matsuzawa, K., et al., *PAR3-aPKC regulates Tiam1 by modulating suppressive internal interactions*. *Mol Biol Cell*, 2016. **27**(9): p. 1511-23.



526. Gödde, N.J., et al., *Dissecting the role of polarity regulators in cancer through the use of mouse models*. *Exp Cell Res*, 2014. **328**(2): p. 249-57.
527. Woods, D.F., J.W. Wu, and P.J. Bryant, *Localization of proteins to the apico-lateral junctions of Drosophila epithelia*. *Dev Genet*, 1997. **20**(2): p. 111-8.
528. Murdoch, J.N., et al., *Disruption of scribble (Scrb1) causes severe neural tube defects in the circletail mouse*. *Hum Mol Genet*, 2003. **12**(2): p. 87-98.
529. Ivanov, A.I., et al., *Tumor suppressor scribble regulates assembly of tight junctions in the intestinal epithelium*. *Am J Pathol*, 2010. **176**(1): p. 134-45.
530. Dow, L.E., et al., *The tumour-suppressor Scribble dictates cell polarity during directed epithelial migration: regulation of Rho GTPase recruitment to the leading edge*. *Oncogene*, 2007. **26**(16): p. 2272-82.
531. Iizuka-Kogo, A., et al., *Abnormal development of urogenital organs in Dlg1-deficient mice*. *Development*, 2007. **134**(9): p. 1799-807.
532. Mahoney, Z.X., et al., *Discs-large homolog 1 regulates smooth muscle orientation in the mouse ureter*. *Proc Natl Acad Sci U S A*, 2006. **103**(52): p. 19872-7.
533. Stucke, V.M., et al., *The MAGUK protein MPP7 binds to the polarity protein hDlg1 and facilitates epithelial tight junction formation*. *Mol Biol Cell*, 2007. **18**(5): p. 1744-55.
534. Van Campenhout, C.A., et al., *Dlg3 trafficking and apical tight junction formation is regulated by nedd4 and nedd4-2 e3 ubiquitin ligases*. *Dev Cell*, 2011. **21**(3): p. 479-91.
535. Nechiporuk, T., T.E. Fernandez, and V. Vasioukhin, *Failure of epithelial tube maintenance causes hydrocephalus and renal cysts in Dlg5<sup>-/-</sup> mice*. *Dev Cell*, 2007. **13**(3): p. 338-50.
536. Nechiporuk, T., et al., *Dlg5 maintains apical aPKC and regulates progenitor differentiation during lung morphogenesis*. *Dev Biol*, 2013. **377**(2): p. 375-84.
537. Cho, C., et al., *Dlg1 activates beta-catenin signaling to regulate retinal angiogenesis and the blood-retina and blood-brain barriers*. *Elife*, 2019. **8**.
538. Klezovitch, O., et al., *Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice*. *Genes Dev*, 2004. **18**(5): p. 559-71.
539. Shen, Q., et al., *Asymmetric Numb distribution is critical for asymmetric cell division of mouse cerebral cortical stem cells and neuroblasts*. *Development*, 2002. **129**(20): p. 4843-53.
540. Lu, B., L. Jan, and Y.N. Jan, *Control of cell divisions in the nervous system: symmetry and asymmetry*. *Annu Rev Neurosci*, 2000. **23**: p. 531-56.
541. Sripathy, S., M. Lee, and V. Vasioukhin, *Mammalian Lgl2 is necessary for proper branching morphogenesis during placental development*. *Mol Cell Biol*, 2011. **31**(14): p. 2920-33.
542. Yamanaka, T., et al., *Lgl mediates apical domain disassembly by suppressing the PAR-3-aPKC-PAR-6 complex to orient apical membrane polarity*. *J Cell Sci*, 2006. **119**(Pt 10): p. 2107-18.
543. Simons, M. and M. Mlodzik, *Planar cell polarity signaling: from fly development to human disease*. *Annu Rev Genet*, 2008. **42**: p. 517-40.
544. Butler, M.T. and J.B. Wallingford, *Planar cell polarity in development and disease*. *Nat Rev Mol Cell Biol*, 2017. **18**(6): p. 375-388.
545. Dollar, G.L., et al., *Regulation of Lethal giant larvae by Dishevelled*. *Nature*, 2005. **437**(7063): p. 1376-80.
546. Kaplan, N.A. and N.S. Tolwinski, *Spatially defined Dsh-Lgl interaction contributes to directional tissue morphogenesis*. *J Cell Sci*, 2010. **123**(Pt 18): p. 3157-65.

547. Walsh, G.S., et al., *Planar polarity pathway and Nance-Horan syndrome-like 1b have essential cell-autonomous functions in neuronal migration*. *Development*, 2011. **138**(14): p. 3033-42.
548. Robinson, A., et al., *Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis*. *Hum Mutat*, 2012. **33**(2): p. 440-7.
549. Wansleben, C., et al., *An ENU-mutagenesis screen in the mouse: identification of novel developmental gene functions*. *PLoS One*, 2011. **6**(4): p. e19357.
550. Yates, L.L., et al., *Scribble is required for normal epithelial cell-cell contacts and lumen morphogenesis in the mammalian lung*. *Dev Biol*, 2013. **373**(2): p. 267-80.
551. Wada, H., et al., *Dual roles of zygotic and maternal Scribble1 in neural migration and convergent extension movements in zebrafish embryos*. *Development*, 2005. **132**(10): p. 2273-85.
552. Dahan, I., et al., *The tumor suppressor Lgl1 forms discrete complexes with NMII-A and Par6 $\alpha$ -aPKC $\zeta$  that are affected by Lgl1 phosphorylation*. *J Cell Sci*, 2014. **127**(Pt 2): p. 295-304.
553. Zhao, M., et al., *Basolateral junctions utilize warts signaling to control epithelial-mesenchymal transition and proliferation crucial for migration and invasion of Drosophila ovarian epithelial cells*. *Genetics*, 2008. **178**(4): p. 1947-71.
554. Sakisaka, T., et al., *The roles of nectins in cell adhesions: cooperation with other cell adhesion molecules and growth factor receptors*. *Curr Opin Cell Biol*, 2007. **19**(5): p. 593-602.
555. Etienne-Manneville, S., *Polarity proteins in migration and invasion*. *Oncogene*, 2008. **27**(55): p. 6970-80.
556. Caruana, G. and A. Bernstein, *Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene*. *Mol Cell Biol*, 2001. **21**(5): p. 1475-83.
557. Etienne-Manneville, S., et al., *Cdc42 and Par6-PKCzeta regulate the spatially localized association of Dlg1 and APC to control cell polarization*. *J Cell Biol*, 2005. **170**(6): p. 895-901.
558. Bossinger, O., et al., *Zonula adherens formation in Caenorhabditis elegans requires dlg-1, the homologue of the Drosophila gene discs large*. *Dev Biol*, 2001. **230**(1): p. 29-42.
559. Legouis, R., et al., *LET-413 is a basolateral protein required for the assembly of adherens junctions in Caenorhabditis elegans*. *Nat Cell Biol*, 2000. **2**(7): p. 415-22.
560. Yamben, I.F., et al., *Scrib is required for epithelial cell identity and prevents epithelial to mesenchymal transition in the mouse*. *Dev Biol*, 2013. **384**(1): p. 41-52.
561. Lohia, M., Y. Qin, and I.G. Macara, *The Scribble polarity protein stabilizes E-cadherin/p120-catenin binding and blocks retrieval of E-cadherin to the Golgi*. *PLoS One*, 2012. **7**(11): p. e51130.
562. Köppen, M., et al., *Cooperative regulation of AJM-1 controls junctional integrity in Caenorhabditis elegans epithelia*. *Nat Cell Biol*, 2001. **3**(11): p. 983-91.
563. McMahon, L., et al., *Assembly of C. elegans apical junctions involves positioning and compaction by LET-413 and protein aggregation by the MAGUK protein DLG-1*. *J Cell Sci*, 2001. **114**(Pt 12): p. 2265-77.
564. Rivera, C., et al., *Cell-autonomous requirements for Dlg-1 for lens epithelial cell structure and fiber cell morphogenesis*. *Dev Dyn*, 2009. **238**(9): p. 2292-308.

565. Gateff, E. and H.A. Schneiderman, *Developmental capacities of benign and malignant neoplasms of Drosophila*. Wilhelm Roux Arch Entwickl Mech Org, 1974. **176**(1): p. 23-65.
566. Brumby, A., et al., *A genetic screen for dominant modifiers of a cyclin E hypomorphic mutation identifies novel regulators of S-phase entry in Drosophila*. Genetics, 2004. **168**(1): p. 227-51.
567. Grzeschik, N.A., et al., *Abnormalities in cell proliferation and apico-basal cell polarity are separable in Drosophila lgl mutant clones in the developing eye*. Dev Biol, 2007. **311**(1): p. 106-23.
568. Massimi, P., et al., *Differential regulation of cell-cell contact, invasion and anoikis by hScrib and hDlg in keratinocytes*. PLoS One, 2012. **7**(7): p. e40279.
569. McCubrey, J.A., et al., *Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance*. Biochim Biophys Acta, 2007. **1773**(8): p. 1263-84.
570. Li, X., et al., *Scribble-mediated membrane targeting of PHLPP1 is required for its negative regulation of Akt*. EMBO Rep, 2011. **12**(8): p. 818-24.
571. Nagasaka, K., et al., *The mechanism and implications of hScrib regulation of ERK*. Small GTPases, 2010. **1**(2): p. 108-112.
572. Zhan, L., et al., *Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma*. Cell, 2008. **135**(5): p. 865-78.
573. Matsumine, A., et al., *Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein*. Science, 1996. **272**(5264): p. 1020-3.
574. Ishidate, T., et al., *The APC-hDLG complex negatively regulates cell cycle progression from the G0/G1 to S phase*. Oncogene, 2000. **19**(3): p. 365-72.
575. Maehama, T. and J.E. Dixon, *The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate*. J Biol Chem, 1998. **273**(22): p. 13375-8.
576. Stambolic, V., et al., *Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN*. Cell, 1998. **95**(1): p. 29-39.
577. Adey, N.B., et al., *Threonine phosphorylation of the MMAC1/PTEN PDZ binding domain both inhibits and stimulates PDZ binding*. Cancer Res, 2000. **60**(1): p. 35-7.
578. Sotelo, N.S., et al., *A functional network of the tumor suppressors APC, hDlg, and PTEN, that relies on recognition of specific PDZ-domains*. J Cell Biochem, 2012. **113**(8): p. 2661-70.
579. Ranger, A.M., B.A. Malynn, and S.J. Korsmeyer, *Mouse models of cell death*. Nat Genet, 2001. **28**(2): p. 113-8.
580. Woods, D.F. and P.J. Bryant, *The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions*. Cell, 1991. **66**(3): p. 451-64.
581. Agrawal, N., et al., *Epithelial hyperplasia of imaginal discs induced by mutations in Drosophila tumor suppressor genes: growth and pattern formation in genetic mosaics*. Dev Biol, 1995. **169**(2): p. 387-98.
582. Brumby, A.M. and H.E. Richardson, *scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila*. EMBO J, 2003. **22**(21): p. 5769-79.
583. Pagliarini, R.A. and T. Xu, *A genetic screen in Drosophila for metastatic behavior*. Science, 2003. **302**(5648): p. 1227-31.

584. Igaki, T., R.A. Pagliarini, and T. Xu, *Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila*. *Curr Biol*, 2006. **16**(11): p. 1139-46.
585. Dhanasekaran, D.N. and E.P. Reddy, *JNK signaling in apoptosis*. *Oncogene*, 2008. **27**(48): p. 6245-51.
586. Norman, M., et al., *Loss of Scribble causes cell competition in mammalian cells*. *J Cell Sci*, 2012. **125**(Pt 1): p. 59-66.
587. Pearson, H.B., et al., *SCRIB expression is deregulated in human prostate cancer, and its deficiency in mice promotes prostate neoplasia*. *J Clin Invest*, 2011. **121**(11): p. 4257-67.
588. Lei, Y., et al., *Mutations in planar cell polarity gene SCRIB are associated with spina bifida*. *PLoS One*, 2013. **8**(7): p. e69262.
589. Xu, D., et al., *Scribble influences cyst formation in autosomal-dominant polycystic kidney disease by regulating Hippo signaling pathway*. *FASEB J*, 2018. **32**(8): p. 4394-4407.
590. Fliegauf, M., T. Benzing, and H. Omran, *When cilia go bad: cilia defects and ciliopathies*. *Nat Rev Mol Cell Biol*, 2007. **8**(11): p. 880-93.
591. Saburi, S., et al., *Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease*. *Nat Genet*, 2008. **40**(8): p. 1010-5.
592. Fischer, E., et al., *Defective planar cell polarity in polycystic kidney disease*. *Nat Genet*, 2006. **38**(1): p. 21-3.
593. Hammond, J.C., et al., *Evidence for abnormal forward trafficking of AMPA receptors in frontal cortex of elderly patients with schizophrenia*. *Neuropsychopharmacology*, 2010. **35**(10): p. 2110-9.
594. Toyooka, K., et al., *Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia*. *J Neurochem*, 2002. **83**(4): p. 797-806.
595. Marcello, E., et al., *SAP97-mediated local trafficking is altered in Alzheimer disease patients' hippocampus*. *Neurobiol Aging*, 2012. **33**(2): p. 422.e1-10.
596. Halaoui, R. and L. McCaffrey, *Rewiring cell polarity signaling in cancer*. *Oncogene*, 2015. **34**(8): p. 939-50.
597. Thomas, M., et al., *Human papillomaviruses, cervical cancer and cell polarity*. *Oncogene*, 2008. **27**(55): p. 7018-30.
598. Elsum, I., et al., *The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man*. *Essays Biochem*, 2012. **53**: p. 141-68.
599. Nakagawa, S. and J.M. Huibregtse, *Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase*. *Mol Cell Biol*, 2000. **20**(21): p. 8244-53.
600. Lee, S.S., R.S. Weiss, and R.T. Javier, *Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein*. *Proc Natl Acad Sci U S A*, 1997. **94**(13): p. 6670-5.
601. Frese, K.K., et al., *Oncogenic function for the Dlg1 mammalian homolog of the Drosophila discs-large tumor suppressor*. *EMBO J*, 2006. **25**(6): p. 1406-17.
602. Gardiol, D., et al., *Human discs large and scrib are localized at the same regions in colon mucosa and changes in their expression patterns are correlated with loss of tissue architecture during malignant progression*. *Int J Cancer*, 2006. **119**(6): p. 1285-90.
603. Lin, H.T., et al., *Differential expression of human Dlg in cervical intraepithelial neoplasias*. *Gynecol Oncol*, 2004. **93**(2): p. 422-8.

604. Lisovsky, M., et al., *Cell polarity protein Lgl2 is lost or aberrantly localized in gastric dysplasia and adenocarcinoma: an immunohistochemical study*. *Mod Pathol*, 2009. **22**(7): p. 977-84.
605. Nakagawa, S., et al., *Analysis of the expression and localisation of a LAP protein, human scribble, in the normal and neoplastic epithelium of uterine cervix*. *Br J Cancer*, 2004. **90**(1): p. 194-9.
606. Ouyang, Z., W. Zhan, and L. Dan, *hScrib, a human homolog of Drosophila neoplastic tumor suppressor, is involved in the progress of endometrial cancer*. *Oncol Res*, 2010. **18**(11-12): p. 593-9.
607. Dow, L.E., et al., *Loss of human Scribble cooperates with H-Ras to promote cell invasion through deregulation of MAPK signalling*. *Oncogene*, 2008. **27**(46): p. 5988-6001.
608. Kuphal, S., et al., *Expression of Hugl-1 is strongly reduced in malignant melanoma*. *Oncogene*, 2006. **25**(1): p. 103-10.
609. Elsum, I.A., et al., *Scrib heterozygosity predisposes to lung cancer and cooperates with KRas hyperactivation to accelerate lung cancer progression in vivo*. *Oncogene*, 2014. **33**(48): p. 5523-33.
610. Pearson, H.B., et al., *The polarity protein Scrib mediates epidermal development and exerts a tumor suppressive function during skin carcinogenesis*. *Mol Cancer*, 2015. **14**: p. 169.
611. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer*. *Cell*, 2000. **100**(1): p. 57-70.
612. Aragona, M., et al., *A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors*. *Cell*, 2013. **154**(5): p. 1047-1059.
613. Grzeschik, N.A., et al., *Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms*. *Curr Biol*, 2010. **20**(7): p. 573-81.
614. Sun, S. and K.D. Irvine, *Cellular Organization and Cytoskeletal Regulation of the Hippo Signaling Network*. *Trends Cell Biol*, 2016. **26**(9): p. 694-704.
615. Archibald, A., et al., *Atypical protein kinase C induces cell transformation by disrupting Hippo/Yap signaling*. *Mol Biol Cell*, 2015. **26**(20): p. 3578-95.
616. Kapil, S., et al., *The cell polarity protein Scrib functions as a tumor suppressor in liver cancer*. *Oncotarget*, 2017. **8**(16): p. 26515-26531.
617. Richardson, H.E. and M. Portela, *Tissue growth and tumorigenesis in Drosophila: cell polarity and the Hippo pathway*. *Curr Opin Cell Biol*, 2017. **48**: p. 1-9.
618. Skouloudaki, K., et al., *Scribble participates in Hippo signaling and is required for normal zebrafish pronephros development*. *Proc Natl Acad Sci U S A*, 2009. **106**(21): p. 8579-84.
619. Cordenosi, M., et al., *The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells*. *Cell*, 2011. **147**(4): p. 759-72.
620. Doggett, K., et al., *Loss of the Drosophila cell polarity regulator Scribbled promotes epithelial tissue overgrowth and cooperation with oncogenic Ras-Raf through impaired Hippo pathway signaling*. *BMC Dev Biol*, 2011. **11**: p. 57.
621. Verghese, S., et al., *Scribble acts in the Drosophila fat-hippo pathway to regulate warts activity*. *PLoS One*, 2012. **7**(11): p. e47173.
622. Xiao, L., et al., *KIBRA protein phosphorylation is regulated by mitotic kinase aurora and protein phosphatase 1*. *J Biol Chem*, 2011. **286**(42): p. 36304-15.

623. Oka, T., et al., *Functional complexes between YAP2 and ZO-2 are PDZ domain-dependent, and regulate YAP2 nuclear localization and signalling*. *Biochem J*, 2010. **432**(3): p. 461-72.
624. Yi, C., et al., *A tight junction-associated Merlin-angiomin complex mediates Merlin's regulation of mitogenic signaling and tumor suppressive functions*. *Cancer Cell*, 2011. **19**(4): p. 527-40.
625. Cravo, A.S., et al., *Hippo pathway elements Co-localize with Occludin: A possible sensor system in pancreatic epithelial cells*. *Tissue Barriers*, 2015. **3**(3): p. e1037948.
626. Reischauer, S., et al., *Lgl2 executes its function as a tumor suppressor by regulating ErbB signaling in the zebrafish epidermis*. *PLoS Genet*, 2009. **5**(11): p. e1000720.
627. Lee, S. and A.E. Griep, *Loss of Dlg-1 in the mouse lens impairs fibroblast growth factor receptor signaling*. *PLoS One*, 2014. **9**(5): p. e97470.
628. Iyengar, L., et al., *Duration of ERK1/2 phosphorylation induced by FGF or ocular media determines lens cell fate*. *Differentiation*, 2007. **75**(7): p. 662-8.
629. Eastburn, D.J., M.M. Zegers, and K.E. Mostov, *Scrib regulates HGF-mediated epithelial morphogenesis and is stabilized by Sgt1-HSP90*. *J Cell Sci*, 2012. **125**(Pt 17): p. 4147-57.
630. Nola, S., et al., *Scrib regulates PAK activity during the cell migration process*. *Hum Mol Genet*, 2008. **17**(22): p. 3552-65.
631. Singh, S.P., et al., *Upon thyrotropin binding the thyrotropin receptor is internalized and localized to endosome*. *Endocrinology*, 2004. **145**(2): p. 1003-10.
632. Lahuna, O., et al., *Thyrotropin receptor trafficking relies on the hScrib-betaPIX-GIT1-ARF6 pathway*. *EMBO J*, 2005. **24**(7): p. 1364-74.
633. Walch, L., *Emerging role of the scaffolding protein Dlg1 in vesicle trafficking*. *Traffic*, 2013. **14**(9): p. 964-73.
634. Montgomery, J.M., P.L. Zamorano, and C.C. Garner, *MAGUKs in synapse assembly and function: an emerging view*. *Cell Mol Life Sci*, 2004. **61**(7-8): p. 911-29.
635. Piguel, N.H., et al., *Scribble1/AP2 complex coordinates NMDA receptor endocytic recycling*. *Cell Rep*, 2014. **9**(2): p. 712-27.
636. Leonard, A.S., et al., *SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit*. *J Biol Chem*, 1998. **273**(31): p. 19518-24.
637. Wu, H., et al., *Interaction of SAP97 with minus-end-directed actin motor myosin VI. Implications for AMPA receptor trafficking*. *J Biol Chem*, 2002. **277**(34): p. 30928-34.
638. Jeyifous, O., et al., *SAP97 and CASK mediate sorting of NMDA receptors through a previously unknown secretory pathway*. *Nat Neurosci*, 2009. **12**(8): p. 1011-9.
639. Lin, E.I., O. Jeyifous, and W.N. Green, *CASK regulates SAP97 conformation and its interactions with AMPA and NMDA receptors*. *J Neurosci*, 2013. **33**(29): p. 12067-76.
640. Elias, G.M. and R.A. Nicoll, *Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins*. *Trends Cell Biol*, 2007. **17**(7): p. 343-52.
641. Hoogwater, F.J., et al., *Oncogenic K-Ras turns death receptors into metastasis-promoting receptors in human and mouse colorectal cancer cells*. *Gastroenterology*, 2010. **138**(7): p. 2357-67.
642. Antoni, L., et al., *Intestinal barrier in inflammatory bowel disease*. *World J Gastroenterol*, 2014. **20**(5): p. 1165-79.
643. Stupack, D.G. and D.A. Cheresh, *Get a ligand, get a life: integrins, signaling and cell survival*. *J Cell Sci*, 2002. **115**(Pt 19): p. 3729-38.

644. Hofmann, C., et al., *Cell-cell contacts prevent anoikis in primary human colonic epithelial cells*. *Gastroenterology*, 2007. **132**(2): p. 587-600.
645. Gagnoux-Palacios, L., et al., *Cell polarity and adherens junction formation inhibit epithelial Fas cell death receptor signaling*. *J Cell Biol*, 2018. **217**(11): p. 3839-3852.
646. Klymkowsky, M.W. and P. Savagner, *Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe*. *Am J Pathol*, 2009. **174**(5): p. 1588-93.
647. Roof, D.J., et al., *Molecular characterization of abLIM, a novel actin-binding and double zinc finger protein*. *J Cell Biol*, 1997. **138**(3): p. 575-88.
648. Koch, B.J., J.F. Ryan, and A.D. Baxeavanis, *The diversification of the LIM superclass at the base of the metazoa increased subcellular complexity and promoted multicellular specialization*. *PLoS One*, 2012. **7**(3): p. e33261.
649. Lu, C., et al., *Normal retinal development and retinofugal projections in mice lacking the retina-specific variant of actin-binding LIM domain protein*. *Neuroscience*, 2003. **120**(1): p. 121-31.
650. Dent, E.W., S.L. Gupton, and F.B. Gertler, *The growth cone cytoskeleton in axon outgrowth and guidance*. *Cold Spring Harb Perspect Biol*, 2011. **3**(3).
651. Narahara, H., et al., *Actin binding LIM 1 (abLIM1) negatively controls osteoclastogenesis by regulating cell migration and fusion*. *J Cell Physiol*, 2018. **234**(1): p. 486-499.
652. Jin, S.H., et al., *Actin-binding LIM protein 1 regulates receptor activator of NF- $\kappa$ B ligand-mediated osteoclast differentiation and motility*. *BMB Rep*, 2018. **51**(7): p. 356-361.
653. Hetz, C., *The unfolded protein response: controlling cell fate decisions under ER stress and beyond*. *Nat Rev Mol Cell Biol*, 2012. **13**(2): p. 89-102.
654. Luo, S., et al., *GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development*. *Mol Cell Biol*, 2006. **26**(15): p. 5688-97.
655. Shu, C.W., et al., *GRP78 and Raf-1 cooperatively confer resistance to endoplasmic reticulum stress-induced apoptosis*. *J Cell Physiol*, 2008. **215**(3): p. 627-35.
656. Reddy, R.K., et al., *Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation*. *J Biol Chem*, 2003. **278**(23): p. 20915-24.
657. Ni, M., Y. Zhang, and A.S. Lee, *Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting*. *Biochem J*, 2011. **434**(2): p. 181-8.
658. Burikhanov, R., et al., *The tumor suppressor Par-4 activates an extrinsic pathway for apoptosis*. *Cell*, 2009. **138**(2): p. 377-88.
659. Constantin, B., *Dystrophin complex functions as a scaffold for signalling proteins*. *Biochim Biophys Acta*, 2014. **1838**(2): p. 635-42.
660. Camps, J., et al., *Genetic amplification of the NOTCH modulator LNX2 upregulates the WNT/ $\beta$ -catenin pathway in colorectal cancer*. *Cancer Res*, 2013. **73**(6): p. 2003-13.
661. Nie, J., et al., *LNx functions as a RING type E3 ubiquitin ligase that targets the cell fate determinant Numb for ubiquitin-dependent degradation*. *EMBO J*, 2002. **21**(1-2): p. 93-102.
662. Young, Paul W., *LNx1/LNx2 proteins: functions in neuronal signalling and beyond*. *Neuronal Signaling*, 2018. **2**(2).
663. Flynn, M., O. Saha, and P. Young, *Molecular evolution of the LNx gene family*. *BMC Evol Biol*, 2011. **11**: p. 235.

664. D'Agostino, M., et al., *Ligand of Numb proteins LNX1p80 and LNX2 interact with the human glycoprotein CD8 $\alpha$  and promote its ubiquitylation and endocytosis.* J Cell Sci, 2011. **124**(Pt 21): p. 3545-56.
665. Lu, M., et al., *E-cadherin couples death receptors to the cytoskeleton to regulate apoptosis.* Mol Cell, 2014. **54**(6): p. 987-98.
666. Igaki, T., et al., *Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway.* EMBO J, 2002. **21**(12): p. 3009-18.
667. Uhlirova, M., H. Jasper, and D. Bohmann, *Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model.* Proc Natl Acad Sci U S A, 2005. **102**(37): p. 13123-8.
668. Cordero, J.B., et al., *Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter.* Dev Cell, 2010. **18**(6): p. 999-1011.
669. Colledge, M., et al., *Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex.* Neuron, 2000. **27**(1): p. 107-19.
670. Kim, S.Y., J.H. Kim, and J.J. Song, *c-Cbl shRNA-expressing adenovirus sensitizes TRAIL-induced apoptosis in prostate cancer DU-145 through increases of DR4/5.* Cancer Gene Ther, 2013. **20**(2): p. 82-7.
671. Song, J.J., et al., *c-Cbl-mediated degradation of TRAIL receptors is responsible for the development of the early phase of TRAIL resistance.* Cell Signal, 2010. **22**(3): p. 553-63.
672. van de Kooij, B., et al., *Ubiquitination by the membrane-associated RING-CH-8 (MARCH-8) ligase controls steady-state cell surface expression of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) receptor 1.* J Biol Chem, 2013. **288**(9): p. 6617-28.
673. Kong, S., et al., *Endoplasmic reticulum-resident E3 ubiquitin ligase Hrd1 controls B-cell immunity through degradation of the death receptor CD95/Fas.* Proc Natl Acad Sci U S A, 2016. **113**(37): p. 10394-9.
674. Gonzalez, F., et al., *TRAF2 Sets a threshold for extrinsic apoptosis by tagging caspase-8 with a ubiquitin shutoff timer.* Mol Cell, 2012. **48**(6): p. 888-99.
675. Xu, L., et al., *DR5-Cbl-b/c-Cbl-TRAF2 complex inhibits TRAIL-induced apoptosis by promoting TRAF2-mediated polyubiquitination of caspase-8 in gastric cancer cells.* Mol Oncol, 2017. **11**(12): p. 1733-1751.
676. Lee, E.W., et al., *Ubiquitination and degradation of the FADD adaptor protein regulate death receptor-mediated apoptosis and necroptosis.* Nat Commun, 2012. **3**: p. 978.
677. Peltzer, N., et al., *LUBAC is essential for embryogenesis by preventing cell death and enabling haematopoiesis.* Nature, 2018. **557**(7703): p. 112-117.
678. Lafont, E., et al., *The linear ubiquitin chain assembly complex regulates TRAIL-induced gene activation and cell death.* EMBO J, 2017. **36**(9): p. 1147-1166.
679. Tada, K., et al., *Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF- $\kappa$ B activation and protection from cell death.* J Biol Chem, 2001. **276**(39): p. 36530-4.
680. Micheau, O. and J. Tschopp, *Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes.* Cell, 2003. **114**(2): p. 181-90.
681. Shi, J.H. and S.C. Sun, *Tumor Necrosis Factor Receptor-Associated Factor Regulation of Nuclear Factor  $\kappa$ B and Mitogen-Activated Protein Kinase Pathways.* Front Immunol, 2018. **9**: p. 1849.



682. Mahoney, D.J., et al., *Both cIAP1 and cIAP2 regulate TNFalpha-mediated NF-kappaB activation*. Proc Natl Acad Sci U S A, 2008. **105**(33): p. 11778-83.
683. Xu, M., et al., *A ubiquitin replacement strategy in human cells reveals distinct mechanisms of IKK activation by TNFalpha and IL-1beta*. Mol Cell, 2009. **36**(2): p. 302-14.
684. Bertrand, M.J., et al., *cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination*. Mol Cell, 2008. **30**(6): p. 689-700.

Filename: Hala AWINA Thesis.docx  
Directory: /Users/halawina/Library/Containers/com.microsoft.Word/Data/ Documents  
Template: /Users/halawina/Library/Group Containers/UBF8T346G9.Office/User Content.localized/Templates.localized/Normal.dotm  
Title:  
Subject:  
Author: Hala Awina  
Keywords:  
Comments:  
Creation Date: 11/20/19 4:30:00 AM  
Change Number: 2  
Last Saved On: 11/20/19 4:30:00 AM  
Last Saved By: Hala Awina  
Total Editing Time: 1 Minute  
Last Printed On: 11/20/19 4:30:00 AM  
As of Last Complete Printing  
Number of Pages: 202  
Number of Words: 208 395 (approx.)  
Number of Characters: 1 187 855 (approx.)