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## In vivo study of MAIT cell tissue repair function

Anastasia de Poulpiquet Du Halgouet

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**THÈSE DE DOCTORAT**  
**DE L'UNIVERSITÉ PSL**

Préparée à l'Institut Curie

***In vivo* study of MAIT cell tissue repair function**  
Étude in vivo de la fonction réparatrice des cellules MAIT

Soutenue par

**Anastasia de Poulpiquet  
du Halgouet**

Le 23 septembre 2022

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**Bio Sorbonne Paris Cité**

Spécialité

**Immunologie**

Composition du jury :

Sophie, CAILLAT-ZUCMAN Professeur PU-PH, Université Paris Cité	<i>Président</i>
Paul, KLENERMAN Professeur, Oxford University	<i>Rapporteur</i>
Christophe, PAGET Chargé de recherche, HDR Université de Tour	<i>Rapporteur</i>
Marie, MALISSEN Directeur de recherche, Université de Marseille	<i>Examineur</i>
Marc-Henri, STERN Chargé de recherche, HDR Université PSL	<i>Examineur</i>
Benedicte, OULES Medecin, praticien hospitalier Université Paris Cité	<i>Examineur</i>
Olivier, LANTZ Medecin spécialiste, HDR Université PSL	<i>Directeur de thèse</i>



## ABSTRACT

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The mechanical, chemical, or infectious mediated tissue damages affect all epithelial barriers including the skin. The rapid resolution of these damages is key to recover their integrity and function, it involves the joint action of several immune cells that infiltrate the barrier tissues. The interest surrounding the study of MAIT (mucosal-associated immune T) cells in repair stems from their high frequency in the skin (up to 10% of T cells) and their capacity to rapidly secrete various effector molecules. Recently, it has been shown both in vitro and in vivo that MAIT cells are involved in wound healing. However, the mechanisms that underly this pro-repair function remain elusive. Using the congenic B6-MAITCAST strain (10 times more MAIT cells than the C57BL/6 mice) and an full thickness excision model mimicking the human repair process (Dunn et al., 2013), we aimed at unravelling these mechanisms. With these tools, we first show that MAIT cells accelerates wound closure. Because MAIT cell numbers and frequency increased following injury without increased proliferation, we hypothesized that MAIT cells were recruited to the wound site. Using skin graft experiments we evidenced that, contrary to the resident  $\gamma\delta$ -T cells, MAIT cells are rapidly recruited into the inflamed site. The Kaedes mouse (in which local ultraviolet light induces a shift in fluorescence) strain and the use of FTY720 treatment demonstrated that recruited cells transited through the lymph nodes rather than from the surrounding skin. We next investigated whether TCR signaling, via MR1 the MAIT cell antigen presenting molecule, could be necessary both for the recruitment and the tissue repair function. To investigate MAIT cells reliance on TCR signaling, we performed parabiosis of MR1+ and MR1- animals and MR1- skin grafted onto MR1+ animals, and we showed that MAIT cell recruitment was MR1-independent. Instead, the recruitment relied on the CXCL16-CXCR6 signaling, as shown by blocking CXCL16 in vivo and deleting CXCR6 on MAIT cells by CRISPR-Cas9 genetic modification. Adoptive transfer experiments further demonstrated that wound closure does not depend on a continuous TCR signaling.

Concerning their effector function our scRNAseq analysis suggested that MAIT cell repair program was acquired already at steady state in the skin. Additionally, we observed an increase in epithelial tongue length and proliferation within these tongues in the presence of MAIT cells as compared to their absence. Thus, we presupposed that MAIT cells could play a direct effector function on keratinocyte growth. This theory was supported by scRNAseq that showed that signatures associated to amphiregulin-producing pro-repair cells were up-regulated by skin MAIT cells. We observed that amphiregulin produced by PLZF cells was necessary in our model and that MAIT cells increased its production at the protein level following wounding. Transfer experiments of Areg deficient MAIT cells formally demonstrated that Areg produced by MAIT cells was instrumental for promoting wound repair.

Overall, our study describes the dynamics of MAIT cells within the skin, the signals they rely on for recruitment and how they promote tissue repair in an MR1 independent manner. It also provides evidence to an Areg mediated mechanism supporting this repair function. These additional insights on MAIT cell biology could provide key therapeutic axes that could help alleviate the pain, discomfort and complications caused by unhealed or poorly healed wounds.

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## KEYWORDS

MAIT cells – Skin – in vivo – repair



## Remerciements

*Je dédie mes travaux de thèse à mon Bon-Papa, mon Grand-Papa et mon Oncle Jacques. Trois grands hommes partis trop tôt et dont la culture, l'amour et l'humour m'accompagnent chaque jour.*

*« Science sans conscience n'est que ruine de l'âme » – Rabelais*

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# List of abbreviations

## **All entries in alphabetical order:**

**Ac-6-FP:** Acetyl-6-formylpterin

**Areg:** Amphiregulin

**APC:** Antigen presenting cells

**CAR:** Coxsackie and Adenovirus receptor

**CE:** Cornified envelope

**COVID-19:** Coronavirus Disease 2019

**DAMP:** Damage-associated molecular patterns

**DETC:** Dendritic epidermal T cell

**dDC:** Dermal dendritic cell

**DEJ:** Dermal-epidermal junction

**DN:** Double negative

**DP:** Double positive

**ECM:** Extracellular matrix

**GF:** Germ-free

**HBV:** Hepatitis B virus

**HCV:** Hepatitis C virus

**HIV:** Immunodeficiency virus

**ILC:** Innate lymphoid cell

**IGF-1:** Insulin-like growth factor 1

**iTCR $\alpha$ :** TCR with an invariant TCR $\alpha$ -chain

**KGF-1-2:** Keratinocyte growth factors 1 and 2

**LBs:** lamellar bodies

**LC:** Langerhans cell

**LN:** Lymph node

**MHC:** Major histocompatibility complex

**MMPs:** Matric metalloproteinases

**MR1:** monomorphic major histocompatibility complex class I-related molecule

**MAIT cell:** Mucosal-associated invariant T cell  
**MDR1:** Multidrug-resistance-1  
**NKT cell:** Natural killer T cell  
**NETs:** Neutrophil extracellular traps  
**PAMP:** Pathogen-associated molecular pattern  
**PLZF:** Promyelocytic leukemia zinc finger  
**ROR $\gamma$ t:** RAR-related orphan receptor gamma thymic  
**ROCO:** Reaggregated thymic organ  
**Treg:** Regulatory T cell  
**SAP:** SLAM-associated protein  
**scRNA-seq:** Single-cell transcriptomic sequencing  
**SLAM:** Signaling lymphocytic activation molecule  
**SPF:** Specific-pathogen free  
**TCR:** T cell receptor  
**TEM:** T effector memory cell  
**Tbet:** T-box transcription factor  
**TEC:** Thymic epithelial cell  
**TRM:** Tissue-resident memory T cell  
**TLR:** Toll-like receptor  
**UV:** Ultraviolet  
**PBMC:** peripheral blood mononuclear cells  
**WT:** wild-type

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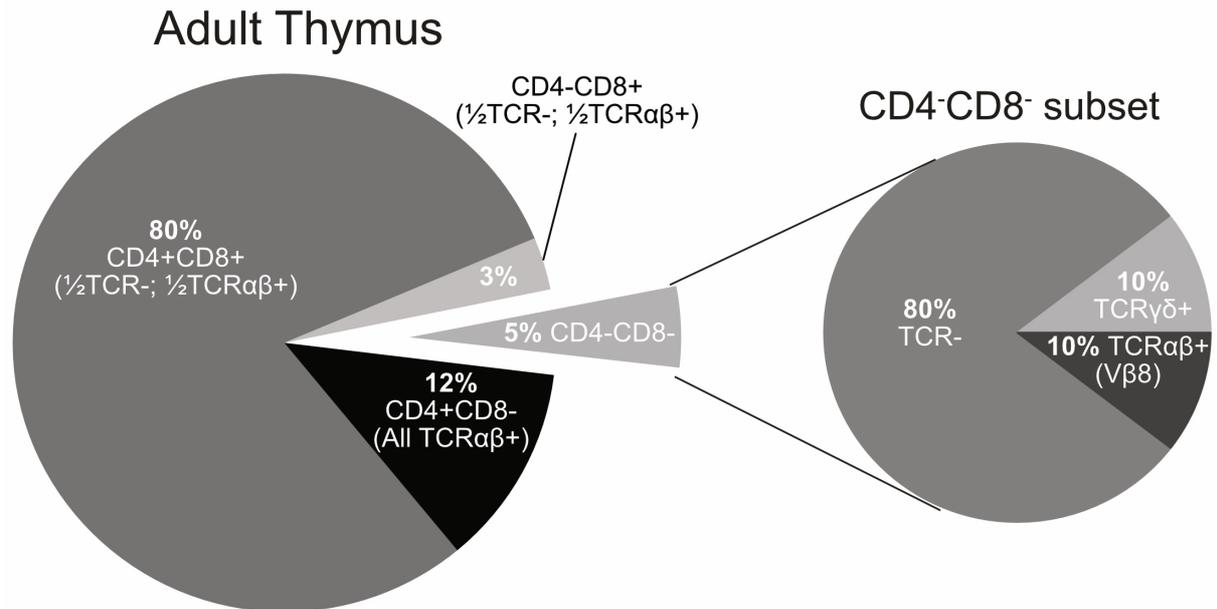
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# INTRODUCTION

## Chapter I : MAIT cells

### A. Discovery

In the 80s, studies portraying intra-thymic differentiation uncovered a minor CD4 CD8 double negative (DN) subset amongst thymic cells. Within the DN subset, 80% of the cells were TCR<sup>neg</sup> cells, 10% were TCR $\gamma\delta^+$ , and 10% were TCR $\alpha\beta^+$  cells, with a substantial proportion of those last cells using the V $\beta$ 8 TCR chain (Fowlkes and Pardoll, 1989) (Fig 1).



**Fig 1: Pie chart of relative proportions of the major thymocyte subpopulations in the mouse thymus.** Cells were defined by CD4, CD8, and T cell receptor (TCR) usage. Adapted diagram and percentages are from (Fowlkes and Pardoll, 1989).

As CD4 and CD8 molecules are involved in the selection of major histocompatibility complex (MHC) type 1 and 2 restricted T cells within the thymus (Zúñiga-Pflücker et al., 1989, 1990) the existence of cells lacking these receptors was puzzling. It raised the question of their development, repertoire, MHC restriction, and specific function.

The question in the early 90s was the relationship of the double negative TCR $\alpha\beta^+$  cells with the mainstream single positive (CD4<sup>+</sup> or CD8<sup>+</sup>) T cells. More precisely, the nature of the TCR repertoire these DN TCR $\alpha\beta^+$  cells used, as compared to their mainstream CD4<sup>+</sup> or CD8<sup>+</sup> counterparts (Porcelli et al., 1993) (Fig 1).

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Cloning and sequencing the TCR of these DN CD4 CD8 cells uncovered an invariant Va14-Ja18 chain known today as the invariant Natural killer T cells (iNKT cells) receptor. This Va14-Ja18 subtype, initially identified within the DN CD4 CD8 compartment was also found in the CD4<sup>+</sup> cell compartment and expressed high levels of the CD44 memory marker within the thymus (Lantz and Bendelac, 1994).

Following this finding, RT-qPCR on sorted T cells was used to identify a second recurrent and invariant TCR chain: the hv7S2 and J33 elements in humans and the ortholog V19 and J33 elements in mice and cattle. These cells are known today as Mucosal associated invariant T cells (MAIT cells) (Treiner et al., 2003). MAIT cells, found amongst DN CD4 CD8 cells and having a restricted repertoire, were reminiscent of dendritic epidermal  $\gamma\delta$  T-cells (DETC). Similarly to DETC cells (Havran et al., 1991), MAIT cells were proposed to play a role in wound healing (Tilloy et al., 1999). This thesis work set more than 20 years later aimed at exploring the potential tissue repair function of MAIT cells.

### **B. Snapshot of MAIT cell characteristics**

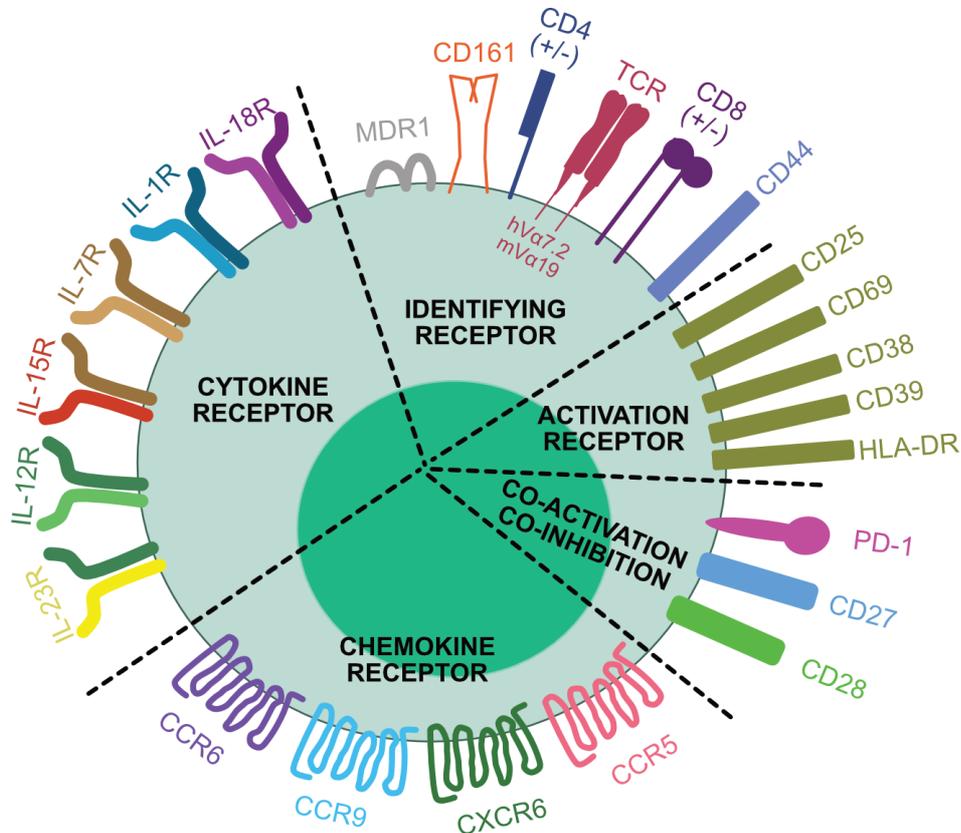
#### **Surface receptors**

The surface receptor which defines MAIT cell identity is their semi-invariant TCR. Although the TCR $\alpha$  is invariant some heterogeneity is observed within the TCR $\beta$  chain usage by the human MAIT cell TCR. In fact, the TRAV1-2-TRAJ33 (Va7.2-Ja33) MAIT cell  $\alpha$ -chains pairs primarily with the TRBV6-4 and TRBV20 chain but also with others such as TRBV28, TRBV25, TRBV24, TRBV19, TRBV15, TRBV11-2, TRBV6-5, TRBV6-1, TRBV4-3, and TRBV4-2 (Reantragoon et al., 2013; Tilloy et al., 1999). On top of their invariant TCR, in humans MAIT cells express high levels of CD161. Thus, the first flow-cytometry strategy to specifically identify MAIT cells utilized the anti-Va7.2 and anti-CD161 antibodies (Martin et al., 2009a).

Besides their TCR, MAIT cells express various receptors at their surface (Fig 2). These include cytokine binding receptors both in humans and in mice, such as IL-7R, IL-18R, IL-23R, and IL-12R (Billerbeck et al., 2010; Cui et al., 2015; Martin et al., 2009a; Rahimpour et al., 2015; Ussher et al., 2014), indicative of a potential cytokine response that will be further discussed below. MAIT cells also express tissue chemokine/homing receptors at their surface: CXCR6 (e.g., liver tropism), CCR6 (e.g., skin, gut tropism) CCR9

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(e.g., small intestine tropism), CCR2 and CCR5 (e.g., migration to inflammatory tissues) (Provine and Klenerman, 2020a; Salou et al., 2019a; Trivett et al., 2019; Tse et al., 2014; Woodward Davis et al., 2019).



**Fig 2: Schematic representation of known surface markers for MAIT cells.** Markers have been classified in four distinct categories: Identifying, activating, co-activation/co-inhibition, cytokine, and chemokine receptors. Scheme is based on (Cogswell et al., 2021; Kurioka et al., 2016).

Variable levels of the co-stimulatory molecules CD4 and CD8 have also been described for human MAIT cells. The most frequent subset is CD8<sup>+</sup> and CD8<sup>-</sup>CD4<sup>-</sup>, although small populations of CD8<sup>-</sup>CD4<sup>+</sup> and CD8<sup>+</sup>CD4<sup>+</sup> are also detected (Koay et al., 2016; Lee et al., 2014; Reantragoon et al., 2013). In mice, most MAIT cells are CD4<sup>-</sup>CD8<sup>-</sup>.

MAIT cells also express the ATP-dependent efflux pump Multidrug-resistance-1 (MDR1) at their surface (Fergusson et al., 2018). MDR1 is an efflux pump that protects MAIT cells from drug-induced death, including chemotherapy medication such as daunorubicin (Chen et al., 2020; Fergusson et al., 2018). Other elements at the surface of human (and some in murine) MAIT cells have a varied expression depending on the

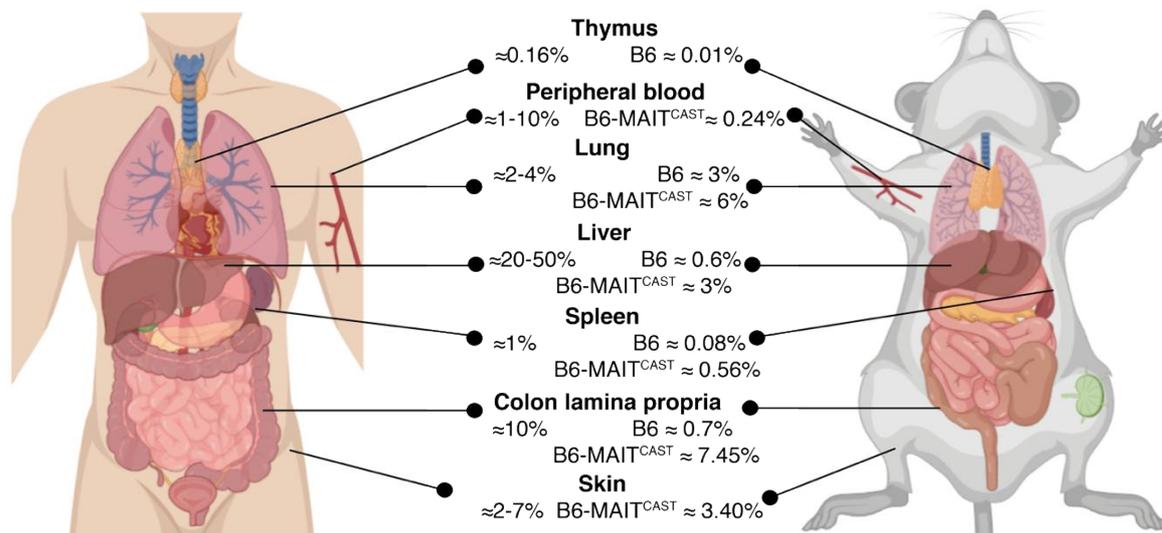
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activation status, inflammatory context, and location; these surface molecules are found in other T-cell and include activation markers (CD25, CD69, CD38, CD39, HLA-DR) and co-stimulatory/co-inhibitory molecules (PD-1, CD27, CD28) (Fig 2).

### Prepositioned memory cells

Mucosal-associated invariant T cells were named because these semi-invariant T cells are enriched in the gut lamina propria (Treiner et al., 2003). MAIT cells constitutes the largest mono-specific T cell in the human body whereas in the classical laboratory strains (e.g., B6) NKT cells are the most frequent subset (Garner et al., 2018; Slauenwhite and Johnston, 2015). In the murine peripheral blood, MAIT cells are at low frequency (0.01% of T cells), while in humans, MAIT cells are abundant (1-10% of circulating T cells) (Martin et al., 2009a)(Fig 3).

Extensive characterization of the different organs show that MAIT cells, both in humans and mice, are most frequent in the liver and barrier organs such as the lung, intestine, and skin, reaching up to 45% of the T lymphocytes within the human liver (frequencies are described in figure 3).



**Fig 3: Frequencies of MAIT cells amongst T cells in human and B6 or B6-MAIT<sup>CAST</sup> mice.** MAIT cells were defined either with MR1:5-OP-RU Tet staining (for mice and humans) or as CD161hi Va7.2+ T cells (for humans), frequencies given are amongst total T cells within the given organs. Frequencies for B6-MAIT<sup>CAST</sup> mice are from (Salou et al., 2019a) and frequencies for human and B6 mice from (Cui et al., 2015; Dusseaux et al., 2011a; Kurioka et al., 2016; Rahimpour et al., 2015). Scheme realized using the Bio-Render online tool.

## INTRODUCTION

### **MR1 restricted cells**

A key characteristic of all T cells is their restriction to a specific MHC allele. In the case of MAIT cells, this MHC is the monomorphic major histocompatibility complex class I-related molecule (MR1). MAIT cells rely on MR1 expression by hematopoietic cells for thymic selection, detailed in the development part of this introduction (Treiner et al., 2003a).

Interestingly, the MR1 transcript is found highly conserved in several different mammalian species: Human, mouse, rat, cow, opossum, elephant, non-human primates (Boudinot et al., 2016; Lantz and Legoux, 2018). In these same species this high conservation is also observed for the invariant TCR $\alpha$  (iTCR $\alpha$ ) chain of MAIT cells (Boudinot et al., 2016; Tilloy et al., 1999). The MR1-antigen complex and iTCR $\alpha$  are so conserved between these species that cross-reactivity between them (e.g., MR1<sub>cow</sub>-MAIT<sub>mouse</sub>; MR1<sub>rat</sub>-MAIT<sub>mouse</sub>; MR1<sub>human</sub>-MAIT<sub>mouse</sub>; MR1<sub>mouse</sub>-MAIT<sub>human</sub>) is possible (Huang et al., 2009; Le Bourhis et al., 2010). This is indicative of a strong selection pressure against diversification, suggesting important functions for MR1 and the iTCR $\alpha$  MAIT cell TCR (Boudinot et al., 2016).

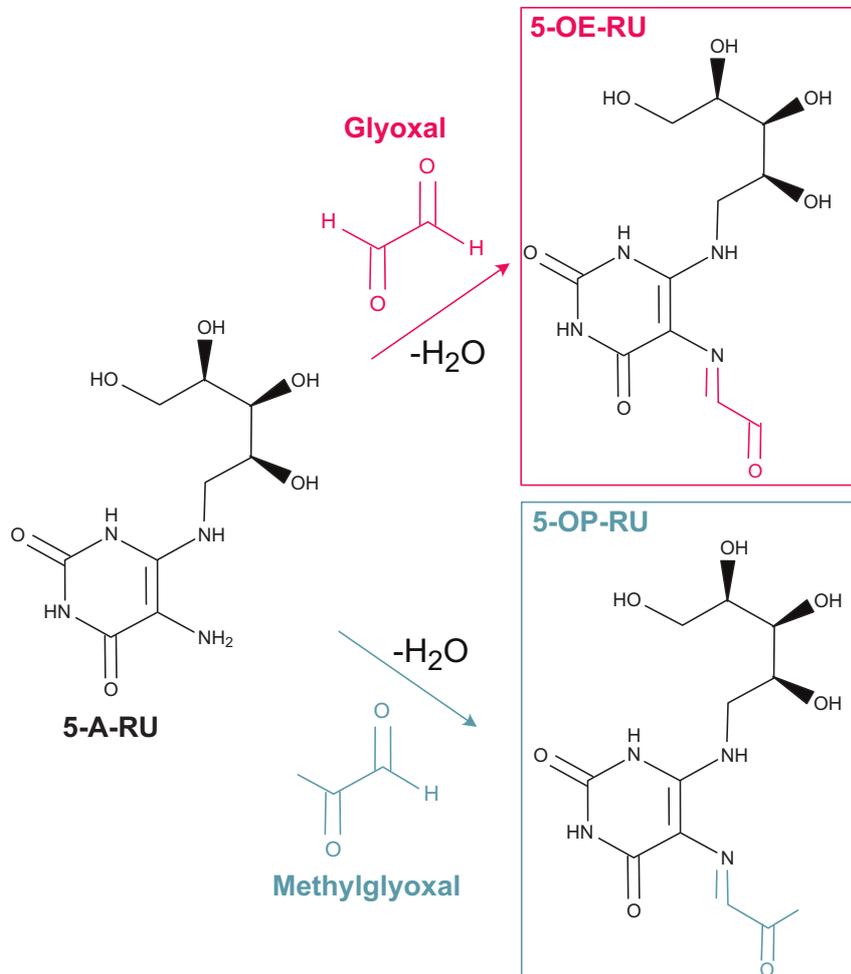
To note, in the species that have lost MR1 such as lagomorphs another highly conserved MHC molecule is found: MHX along with another probable invariant iTCR $\alpha$  chain (TRAV41-TRAJ38) (Boudinot et al., 2016). This other purifying selection could be indicative of an evolutionary need for these cells possibly reflecting a common conserved function (Lantz and Legoux, 2018).

### **Recognition of bacterial or fungal metabolites**

Evidence of MAIT cell activation through their TCR, as given by CD69 expression, was demonstrated *in vitro* using MR1 expressing antigen presenting cells (APC) infected by bacteria or exposed to bacteria supernatant. The bacteria capable of activating MAIT cells included amongst others the *Escherichia*, *Staphylococcus*, *Mycobacteria*, *Salmonella*, *Pseudomonas*, and *Lactobacillus* strains (Gold et al., 2010; Le Bourhis et al., 2010). To find the source and properties of the antigen(s) responsible, Le Bourhis and colleagues showed that while live bacterial strains were the most efficient, paraformaldehyde-fixed

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bacteria but not heat-killed bacteria were also capable of activating MAIT cells (Le Bourhis et al., 2010), indicating that MAIT cell activation relied on heat-labile molecule(s). Furthermore, MAIT cell activating bacterial strains had the riboflavin (Vitamin B2) synthesis pathway (Gold et al., 2010; Kjer-Nielsen et al., 2012; Le Bourhis et al., 2010).



**Fig 4: Formation of MAIT cell activating riboflavin precursor-derived metabolites.** The 5-A-RU generated in the riboflavin biosynthesis pathway can adduct with glyoxal and methylglyoxal in a non-enzymatic reaction leading to the synthesis of 5-OE-RU and 5-OP-RU. This process generates unstable adducts that spontaneously cyclize. Scheme was made using Affinity Publisher.

Surprisingly, when riboflavin was added to the bacterial culture media, MAIT cell activation did not occur (Corbett et al., 2014). The loss of MAIT cell activation could be caused by riboswitch, which inhibits bacterial biosynthesis of metabolites such as riboflavin when these metabolites are present in the environment (Vitreschak, 2004).

Loss of activation indicated that active riboflavin biosynthesis was necessary for MAIT cell activation, inferring that the cognate ligand of MAIT cells originated from the

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riboflavin synthesis pathway (Corbett et al., 2014; Eckle et al., 2014; Kjer-Nielsen et al., 2012; Soudais et al., 2015). Importantly, this biosynthesis riboflavin pathway necessitates an enzymatic machinery absent from mammals, but found in plants, fungi and bacteria (Bacher et al., 2000). The subsequent formation of the MAIT cell ligand is the result of a non-enzymatic condensation of 5-A-RU (a product of the riboflavin pathway) and methylglyoxal or glyoxal, two metabolic molecules found in both eukaryotic and bacterial cells (Corbett et al., 2014; Soudais et al., 2015) (Fig 4). These riboflavin-derived ligands are, however, extremely unstable when not bound to MR1, cyclizing spontaneously to produce molecules that are unable to bind to MR1 and become weak MAIT cell agonists (Kjer-Nielsen et al., 2012; Mak et al., 2017).

The discovery of MR1-binding ligands with sufficient avidity to activate MAIT cell via their semi-invariant TCR enabled the development of a tetramer that is now distributed by the NIH platform (Kjer-Nielsen et al., 2012; Reantragoon et al., 2013). The newly developed tetramer confirmed that the CD161<sup>hi</sup>TRAV1-2<sup>+</sup>IL-18Ra<sup>+</sup> cells, previously identified as MAIT cells in human tissues and blood, were indeed MR1:5-OP-RU-restricted cells. Tetramer synthesis also allowed for the facilitated detection of murine MAIT cells that was complex as no specific V $\alpha$ 19-specific mAbs were available.

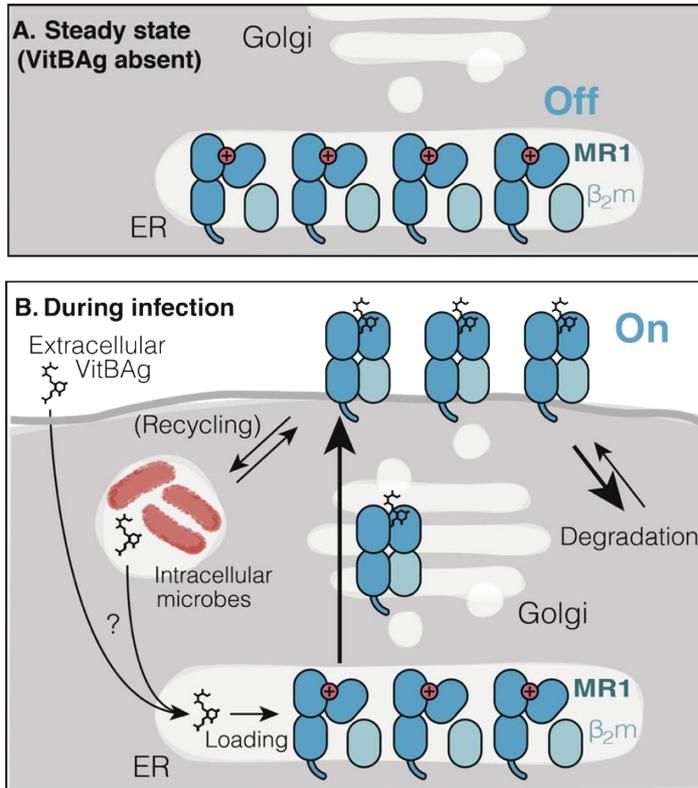
### C. Development

Since the 90s', several studies have been conducted to understand the requirements for MAIT cell development which, like T lymphocytes, occurs in the thymus before migration to the periphery. Contrary to mainstream CD4 and CD8 T cells that exit the thymus with a naïve phenotype and recirculate in the secondary lymphoid organs, in mice, MAIT cells exit the thymus as memory cells targeted to peripheral tissues (Salou et al., 2019a). Although human MAIT cells acquire a specific differentiation program in the thymus, they become memory through an additional extrathymic maturation step after birth (Ben Youssef et al., 2018; Koay et al., 2016; Salou et al., 2019a). The current chapter will describe the mechanisms involved in intra-thymic MAIT cell development and the acquisition of some of the MAIT cell defining characteristics.

T-lymphocyte thymic selection occurs to check for TCR functionality without allowing self-reactivity. In the case of MAIT cells, thymic selection appeared independent

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of both the classical MHC and the CD1 presenting molecules, but relied on the  $\beta_2$ -microglobulin essential for the acquisition of the tertiary structure of MHC class I molecules (Tilloy et al., 1999). Another MHC class I molecule, MR1, was suggested to restrict MAIT cell development. The unequivocal demonstration was provided by the total absence of MAIT cells within the MR1<sup>-/-</sup> mice (Treiner et al., 2003).



**Fig 5: Intracellular MR1 presentation pathway.** (A) in the absence of Vitamin B2-derived antigens, MR1 is located in the endoplasmic reticulum (ER), antigen mediated MR1 presentation is thus OFF (B) Microbial-derived antigens are loaded onto MR1 molecules in the ER leading to association with  $\beta_2m$  and transition through the Golgi apparatus to the cell surface. MR1 presentation is thus ON. Scheme is from (McWilliam and Villadangos, 2017)

Importantly, MR1 expression at the cell surface requires the loading of a molecule into the antigen binding groove (McWilliam and Villadangos, 2017). As seen fig 5, in the absence of molecules to bind to the antigen-binding groove, MR1 remains in an unfolded conformation within the endoplasmic reticulum. Antigen binding is therefore necessary for acquisition of a folded conformation and surface expression (McWilliam and Villadangos, 2017). As stated previously, one molecule capable of binding to MR1 and activating MAIT cells is 5-OP/OE-RU. Since this molecule cannot be produced by mammalian cells but is of non-eucaryote origin (e.g., fungi and bacteria) it raises the question of the reliance of MAIT cells on the microbiota for their development.

Partial reliance was demonstrated using germ-free (GF) mice which have much lower numbers of MAIT cells than specific-pathogen free (SPF) mice (Treiner et al., 2003).

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Topical application on the skin of 5-OP/OE-RU showed that the ligand was capable of diffusing, reaching the thymus and activating thymic MAIT cells (Legoux et al., 2019a).

Although much fewer in numbers in the germ-free mice some MAIT cells remain as compared to MR1<sup>-/-</sup> mice where no MAIT cells can be found at all (Treiner et al., 2003). These results raised the possibility of the existence of an endogenous ligand (Lantz and Legoux, 2018; Legoux et al., 2019b; Treiner et al., 2003) that is attested by unpublished data (Ito et al, 2022 – EMBO conference).

Mainstream T cells (non-innate like T cells) require the expression of MHC by the thymic epithelial cells (TEC) for development (Anderson et al., 1994). For MAIT cells however, injecting the bone-marrow of a B6 mouse in a  $\beta 2m^{-/-}$  recipient was sufficient to find MAIT cells in the mesenteric lymph nodes. In this context, only the hematopoietic compartment would express  $\beta 2m^{-/-}$  and consequently MR1, showing that MAIT cells relied on the hematopoietic compartment for their development (Treiner et al., 2003). *In vivo* while the transfer of wild-type (WT) bone marrow into MR1<sup>-/-</sup> mice elicits mature MAIT cell development, the transfer of MR1<sup>-/-</sup> bone marrow into WT mice only led to naïve CD4<sup>+</sup> mainstream-like MR1:5-OP-RU tetramer positive cell to develop (Legoux et al., 2019a). Therefore, the hematopoietic compartment is both sufficient and necessary for the thymic maturation of MAIT cells.

The possible origin of the cells necessary for the selection of MAIT cells was assessed using reaggregated thymic organ (RTOC) *in vitro* cultures and assessing iV $\alpha$ 19 transgenic MAIT cell maturation (Guerra et al., 2013; Seach et al., 2013). It showed that double positive (DP) thymocytes were the subpopulation most capable of eliciting iV $\alpha$ 19 transgenic MAIT cell maturation at levels comparable to the whole thymus culture (Seach et al., 2013). Furthermore, transfer of MR1<sup>+/+</sup> DP thymocytes into RAG<sup>-/-</sup> MR1<sup>-/-</sup> (devoid of T cells, no MR1 expression) led to an expansion of MAIT cells reaching the levels of a RAG<sup>-/-</sup> MR1<sup>-/-</sup> injected with a complete MR1<sup>+/+</sup> thymus (Seach et al., 2013). However, when injecting MR1<sup>+/+</sup> bone-marrow devoid of thymocytes (CD3<sup>-/-</sup>) into MR1<sup>-/-</sup> mice this expansion of MAIT cells did not occur (Seach et al., 2013). Demonstrating that DP thymocytes are required for MAIT cell thymic maturation, as it had been shown previously for NKT cells (Legoux et al., 2019a; Seach et al., 2013; Wei et al., 2005).

As SLAM/SAP signaling (SLAM: signaling lymphocytic activation molecule; SAP: SLAM-associated protein) is necessary for the development of NKT cells, its involvement

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in MAIT cell development was investigated (Chung et al., 2005; Nichols et al., 2005; Pasquier et al., 2005). Using SAP-deficient animals SLAM-SAP signaling appeared required for the positive selection of MAIT cells by hematopoietic cells, but not for the selection of naive MR1:5-OP-RU tet<sup>+</sup> cells on TECs (Legoux et al., 2019a). Importantly thymocytes but not TEC cells express SLAM (Seach et al., 2013). It seems that selection on DP thymocytes induces robust TCR signaling which could be reduced by Slam interaction (Lu et al., 2019). Conversely, Slam stimulation is also associated with a strong activation of Egr1/2 downstream of TCR signaling, which could enhance TCR signaling (Dutta et al., 2013). Therefore, SLAM/SAP pathway may either provide additional signaling to the TCR and/or on the contrary decrease the signal strength allowing for the survival of selected cells (Dutta et al., 2013; Legoux et al., 2019a; Lu et al., 2019).

As for NKT cells, SLAM pathway activation in MAIT cells induces the expression of the transcription factor *Zbtb16* (PLZF: promyelocytic leukemia zinc finger) (Legoux et al., 2019a; Savage et al., 2008) (Fig 6). The importance of intrinsic expression of PLZF by NKT cells was demonstrated using mixed-bone marrow transfers from PLZF-WT and PLZF-deficient mice (Savage et al., 2008). Briefly, analysing the transferred cells showed that bone marrow of PLZF-WT mice developed into mature NKT cells, while PLZF-deficient cells (despite being in the presence of PLZF producing cells) did not (Savage et al., 2008). In PLZF-null mice (lacking PLZF), MAIT cell intra-thymic maturation is stopped before the acquisition of the CD44<sup>hi</sup> memory phenotype and MAIT cells are absent from the peripheral organs (Koay et al., 2016; Legoux et al., 2019a; Savage et al., 2008). By contrast to murine MAIT cells, human MAIT cells with a low PLZF expression are found in the cord blood, indicating that PLZF is not necessary for MAIT cell thymic egress. Whilst human cord blood MAIT cells are naïve (CD45RA<sup>+</sup>/RO<sup>-</sup>) cells, in the adult blood MAIT cells express high levels of PLZF and are memory cells (Ben Youssef et al., 2018). Indicating that for both human and mice PLZF is linked with the acquisition of a memory phenotype by MAIT cells (Fig 6).

Besides PLZF, other transcription factors are crucial for the acquisition of effector characteristics by MAIT cells. These include RAR-related orphan receptor gamma thymic (ROR $\gamma$ t) and T-box transcription factor (Tbet) (Fig 6). In mice, mature MAIT cells start expressing these transcription factors within the thymus in the last stage of their development (Koay et al., 2016). While the expression of these transcription factors is

## INTRODUCTION

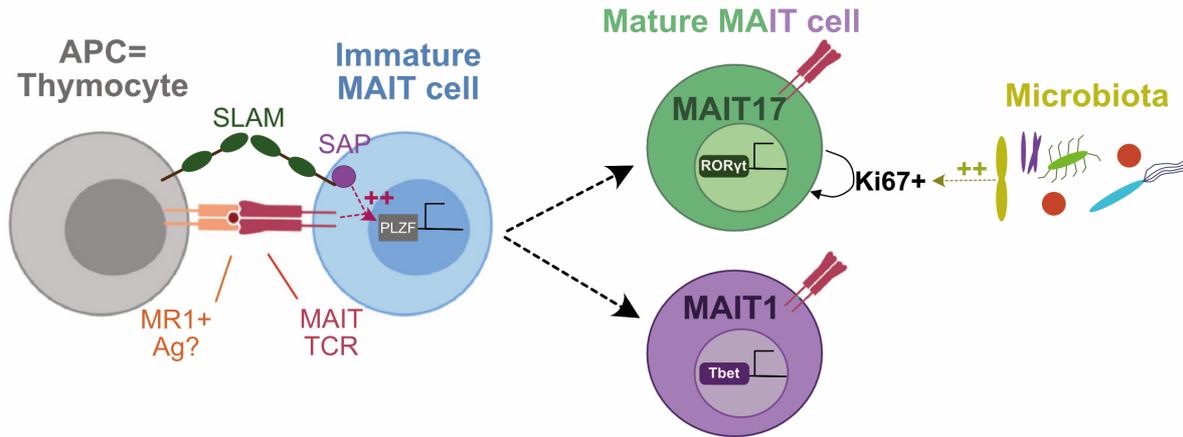
mutually exclusive in mice, in humans they are co-expressed by mature thymic MAIT cells (Koay et al., 2016). Furthermore, mature human MAIT cells also co-express Eomes and Helios (Leeansyah et al., 2015). Importantly, this transcriptional factor profile contrasts with mainstream CD4<sup>+</sup> and CD8<sup>+</sup> T cells and suggests a functional heterogeneity (Dias et al., 2017).

Because of the mutually exclusive expression of ROR $\gamma$ t and Tbet in mice, murine MAIT cells are divided into two distinct subsets: type 1 (Tbet<sup>+</sup>) and type 17 (ROR $\gamma$ t<sup>+</sup>) (Koay et al., 2016; Salou et al., 2019a). This subtyping of mice MAIT cells is reminiscent of other immune cells such as NKT cells (NKT1, NKT17, and NKT2); with the subset distinction in NKT based on PLZF, Tbet, and ROR $\gamma$ t expression (Lee et al., 2013). Tonic TCR signaling could impact MAIT 1 *versus* MAIT17 differentiation in mice, as MAIT1 have a lower Nur77 expression than MAIT17 indicative of a milder TCR-stimulation (Legoux et al., 2019b).

Single cell analysis of sorted MAIT cells unraveled transcriptomic differences in thymic MAIT cells expressing the ROR $\gamma$ t or Tbet factors. It revealed that ROR $\gamma$ t but not Tbet was consistently detected in cycling MAIT cells (Legoux et al., 2019a). Surprisingly, while the microbiota had a small but significant impact on the early stages of MAIT cell development (i.e., more immature MAIT cells in the thymus), it appears also required for MAIT17 proliferation. This reliance is evidenced by a reduced Ki67 expression (a proliferation marker), lower frequency of thymic and peripheral MAIT17 cells in germ-free mice compared to SPF mice (Constantinides et al., 2019a; Legoux et al., 2019b; Tastan et al., 2018) (Fig 6).

Figure 6 depicts a summary of all major steps and interactions required for MAIT cell thymic development and maturation.

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**Fig 6: Summary of the major steps required for murine MAIT cell maturation in the thymus.** MAIT cells are selected on double thymocytes expressing MR1, probably loaded with an endogenous ligand. Survival signals are provided by the SLAM-SLAM homotypic interactions. Consequently, MAIT cell upregulate PLZF. This then leads to the maturation of MAIT cells into type 1 or 17 cells with a microbiota dependent MAIT17 proliferation.

### D. Residency

#### Tissue targeting

PLZF is essential for the residency of NKT cells in tissues (Koay et al., 2016; Salou et al., 2019a; Thomas et al., 2011a). Indeed, PLZF represses the transcription factor *Klf2*, resulting in a decrease in the expression of CD62L (*Sell*) a pivotal molecule for the control of T cell recirculation in secondary lymphoid organs (Mao et al., 2016). Accordingly, thymic mature MAIT cells express lower levels of a circulating signature than the mainstream single-positive T cells, while up-regulating genes associated with tissue residency (e.g. *Cd69*, *Itgae*) (Salou et al., 2019a), suggesting that they might be targeted to tissues. Indeed, when transferring thymic cells, MAIT cells appear preprogrammed to locate in the periphery as their recovery yield is higher than their mainstream T cell counterparts in the peripheral organs (lung, liver) (Salou et al., 2019a). Within the thymus, MAIT cells also express higher levels of chemokine and homing receptors as compared to single positive mainstream T cells, such as CXCR6, CXCR3, CCR6, CCR9 and CCR5 that are involved in tissue targeting and retention (Provine and Klenerman, 2020a; Salou et al., 2019a) (Fig 2).

A critical aspect of MAIT cell establishment in the periphery is the developmental window. Colonization of GF mice early in life with commensal bacteria (before week 3) leads to an adequate development of MAIT cells reaching the levels of SPF mice

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(Constantinides et al., 2019a). However, if this colonization is performed in adult mice (after week 7), while MAIT cells develop normally within the thymus, their numbers remain lower throughout life in the peripheral organs (e.g., lung, skin, spleen) (Constantinides et al., 2019a; Legoux et al., 2019b). These results reveal two fundamental concepts of MAIT cell biology: A partial reliance on the microbiota for peripheral seeding of MAIT cells and a time constraint for said seeding (before week 3). It has been suggested that this time constraint could be the result of a defined microbial exposure that would occur in the first weeks of life with a change in microbiota composition at weaning (Al Nabhani et al., 2019; Constantinides et al., 2019a). Alternatively, the peripheral tissue niches may be receptive only at early ages or be completely filled by mainstream T cells after week 3 (Alferink et al., 1998).

### **Tissue residency**

The expression of memory markers and loss of CD62L in the periphery by MAIT cells appears reminiscent of tissue-resident memory T cells (TRM) that do not recirculate throughout the organism (Fan and Rudensky, 2016). In humans, despite being frequent in the blood, MAIT cells are also found in the barrier tissues and do express tissue residency signatures (with a loss of CD62L), suggesting that they are poised towards a tissue-resident phenotype (Salou et al., 2019a).

Formal demonstration of MAIT cell tissue-residency properties in mice was achieved by parabiotic experiments, previously employed to demonstrate residency of TRM cells (Steinert et al., 2015). Parabiosis joins the vascular system of two mice with distinct congenic markers for about 5 weeks, allowing circulating cells to be distinguished from resident cells (Beura and Masopust, 2014; Bunster and Meyer, 1933). MAIT cells from the peripheral organs (e.g., lung, liver) were found to exchange very little between parabionts (less than 20%), except for MAIT1 cells in the lung ( $\approx 30 - 40\%$ ) as compared to the mainstream T-cells ( $\approx 50\%$ ) (Salou et al., 2019a). Based on the evidence presented, with the exemption of MAIT1 in the lung, murine MAIT cells are retained in tissues where they reside.

While TRM cells acquire their tissue-resident phenotype following systemic or local infection, in the case of MAIT cells and NKT cells no obvious priming has been

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demonstrated as necessary to become tissue resident (Gebhardt et al., 2009; Salou et al., 2019a). However, further research is warranted to assess factors that restrain MAIT cells to the different peripheral tissues in the absence of infection. These factors could include signals from the microbiota and/or the implication of adhesion retention molecules as MAIT cells do express various chemokine and homing receptors (Fig 2). The LFA1/ICAM interaction, for example, previously shown to be induced by PLZF and essential for NKT retention in the liver, is critical for MAIT1 cell retention in the liver and in the spleen but unnecessary for MAIT17 (Salou et al., 2019a; Thomas et al., 2011a). Further works will be necessary to better understand the basis for organ retention of MAIT cells.

Diving into the tissues themselves, an additional complexity is observed regarding the exact location of MAIT cells in the tissues. Indeed, lung MAIT1 are inside the vasculature while MAIT17 are located in the parenchyma, as shown by intravascular staining before sacrifice (Salou et al., 2019a). Because there are many differences in chemokine receptor expression between MAIT1 and MAIT17 (e.g., *Ccr6*:MAIT17 and *Cxcr3*:MAIT1) in the thymus (Salou et al., 2019a), we could hypothesize that this different localization is the result of MAIT cell type 1 or 17 programming.

Demonstrating tissue residency and non-recirculating properties in humans is not as straightforward as in mice. Some clues are provided by the collection of donor matched blood and peripheral samples and subsequent assessment of TCR diversity. One such study collected donor matched blood and thoracic duct lymph (Voillet et al., 2018). In this study MAIT cells, despite being CCR7<sup>-</sup> (necessary for the entry in lymphoid tissues), were present in the lymph and presented an overlapping clonotype usage between the blood and lymph (Voillet et al., 2018). This suggests that contrary to the parabiosis demonstration for murine MAIT cells, a significant proportion of human MAIT cells undergoes tissue egress from the organs and can re-enter the lymph.

### **“Tissue niches”**

One area of debate around MAIT cell and their establishment in the periphery is the notion of “tissue niches”. This is based on the idea that some cells with similar or redundant functions compete within a specific location for survival signals (growth factors or membrane ligands for receptors) (Zaid et al., 2014). This active competition has

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been demonstrated for other immune cells such as TRM cells in the skin, were TRM cells compete for TGF $\beta$ , leading to the preferential retention of antigen-specific TRM cells that express higher levels of the TGF $\beta$  receptor as compared to bystander TRM cells (Hirai et al., 2021a). However when removing this limiting factor by expressing a constitutively active form of the TGF $\beta$  receptor on bystander TRM cells, the preferential retention does not occur (Hirai et al., 2021a).

MAIT cells are very close to NKT cells. This proximity is visible at the transcriptomic level, where these cells cluster very close to each other based on their type1/17 subtype, indicating a highly similar if not identical transcriptomic profile (Salou et al., 2019a) (Fig 9). Concerning the existence of a niche competition between MAIT and NKT cells, conflicting reports have been published. While in NKT deficient mice (CD1d<sup>-/-</sup>) MAIT cells are more frequent than in B6 mice (Koay et al., 2018), the numbers of MAIT and NKT cells is positively correlated in the peripheral organs of the B6-CAST<sup>MAIT</sup> mice (Salou et al., 2019a). The first observation supports the idea that MAIT cells and NKT cells are mutually exclusive, and the second that MAIT cells and NKT cells have common requirements for tissue seeding and/or tissue population establishment as they locate within the same organs. The origin of these conflicting results is unclear, but as external factors such as the microbiota can impact MAIT cell frequency in the periphery it would be conceivable that other external factors could influence these observations (Constantinides et al., 2019a). In another study MAIT cell frequency and numbers were increased in the absence of another innate-like-T cell subset, the  $\gamma\delta$ -T cells (Constantinides et al., 2019a). However, a caveat in this study is the  $\gamma\delta$  deficient mouse model used which encompasses a large deletion of the delta locus and an additional prokaryote sequence (TK promoter driving a neomycin resistance gene), that could both modify distal TRAV usage. Indeed, deletion of the TCR $\delta$  chain leads to an increased usage of the distal Va chains, which includes the MAIT cells TCR $\alpha$  chain (Dauphars et al., 2022; Tilloy et al., 1999).

Unravelling the specific location of each of these innate-like subtypes could help prove or disprove the “competitive niche” theory. *In situ* tetramer staining recently showed that MAIT and NKT cells have distinct intra-organ location in the spleen and the LN (Johnson et al., 2022). *In situ* staining also allows to preserve more surface epitopes than classical isolation methods involving single cell extraction after tissue digestion.

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These classical methods can lead to underestimation or loss of some subsets of prepositioned cells because of incomplete cell extraction and/or loss of surface markers (Masopust and Soerens, 2019a). As such histology could be a useful tool to better understand the inter- and intra-organ properties of innate-like cells, including but not limited to MAIT cells.

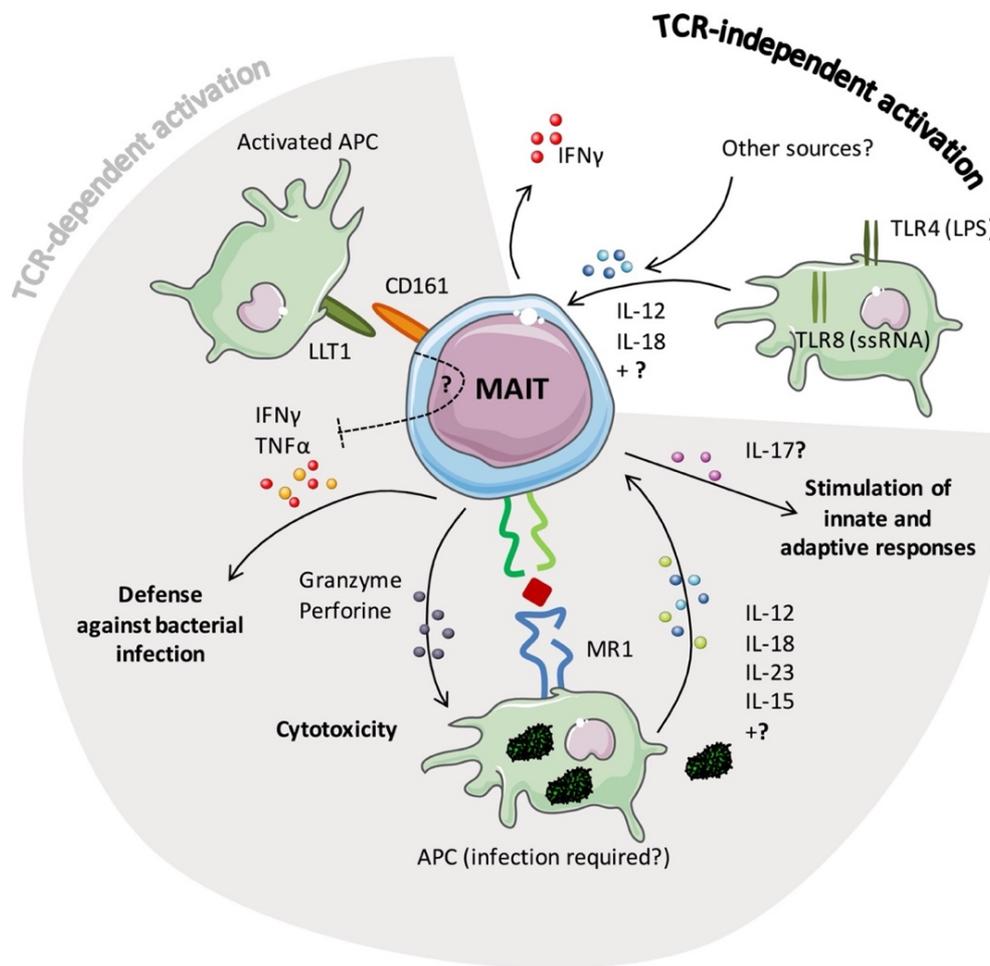
### **Remobilization induced by physiological or pathogenic stimuli**

As mentioned previously, MAIT cells express different chemokines receptors which could lead to their retention in the peripheral tissues (e.g., CXCR6: liver), and others that can drive T cell recruitment into inflamed tissue (e.g., CCR2: extravasation) (Provine and Klenerman, 2020a; Salou et al., 2019a; Trivett et al., 2019; Tse et al., 2014; Woodward Davis et al., 2019) (Fig 2). This surface expression on top of MAIT cell high frequency in the human peripheral blood suggests that when needed these cells could be mobilized and migrate within the tissues (Fig 2, 3).

In various types of viral or bacterial infections or autoimmune diseases, blood MAIT cell frequency is decreased (Cosgrove et al., 2013; Fernandez et al., 2015; Lee et al., 2013; Leeansyah et al., 2015; Leung et al., 2014; Magalhaes et al., 2015; Ussher et al., 2015; Wong et al., 2013; Yong et al., 2018). In some studies this increase in the blood is also linked with a subsequent increase of MAIT cells in the inflamed tissues (as compared to healthy controls) (Cho et al., 2014; Illes, 2004; Serriari et al., 2014). This supports the idea that in humans the blood may constitute a reservoir of mobilizable MAIT cells.

Accordingly, *in vivo* in a model of ear skin inflammation human MAIT cells are recruited into the ear in a C/EBP $\delta$  (CCAAT/enhancer-binding protein delta) dependent manner after only 10 minutes. In mice, following intranasal 5-OP-RU antigen exposure coupled with recombinant chemokine CXCL16 administration, MAIT cells are also recruited into the lung (Yu et al., 2020a). Both findings demonstrate the migratory capacity of human and murine MAIT cells *in vivo*.

**E. Stimulation and effector potential of MAIT cells**



**Fig 7: MAIT cell effector functions and triggers.** Detailed scheme of the activation pathways and subsequent effector capabilities of MAIT cells. Figure is from (Franciszkievicz et al., 2016).

TCR- and/or cytokine-mediated triggering are two types of afferent signals that can activate MAIT cells. Once activated, these cells secrete various mediators and participate in cytotoxicity, anti-bacterial defenses, immune cell stimulation, and other effector functions (Franciszkievicz et al., 2016) (Fig 7). These efferent and afferent signals will be introduced in the following chapter.

**i. Effector capabilities**

As MAIT cells are prepositioned memory cells in barrier tissues and acquire some pleiotropic function in the thymus without overt infection, it was proposed to name them “preset” cells along with other immune cells that share these characteristics, such as NKT

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cells and  $\gamma\delta$ -T cells (Legoux et al., 2017). This term suggests that these cells are “ready to go” immune cells that do not require priming to enact their function and are able to respond quickly. Accordingly, MAIT cells have been found capable of a direct cytotoxic effect against infected cells, tumor cell lines, can be directly or indirectly anti-viral, anti-bacterial and can play a pro-repair role in barrier tissues in which they are found (Gherardin et al., 2018; Klenerman et al., 2021; Legoux et al., 2020a; Ling et al., 2016; Won et al., 2016).

The mediators required for MAIT cells to enact these various functions are under the control of different transcription factors. In humans, as previously mentioned MAIT cells express ROR $\gamma$ t along with other transcription factors such as Tbet, Eomes, and Helios (Gibbs et al., 2017; Koay et al., 2016; Leeansyah et al., 2015). Consequently these human MAIT cells are capable of secreting cytotoxic molecules (e.g. granzyme and perforin), as well as pro-inflammatory cytokines (e.g. IFN- $\gamma$ , TNF- $\alpha$ , IL-17, CSF2/GM-CSF), and chemokines (e.g. XCL1, CCL3, CCL4, and CXCL16) (Franciszkievicz et al., 2016; Hinks and Zhang, 2020).

In mice, as MAIT cells express either ROR $\gamma$ t or Tbet, two subtypes with distinct effector capabilities (Koay et al., 2016; Salou et al., 2019a). Murine MAIT1 cells harbor more NK receptors than MAIT17, produce more cytotoxic molecules such as IFN- $\gamma$  and TNF- $\alpha$  (Kumar, 2018; Legoux et al., 2019b, 2019a, 2020a). Murine type 17 cells that have been thoroughly studied secrete inflammatory molecules such as IL-17 (Legoux et al., 2019b, 2019a; Salou et al., 2019a). Interestingly, under some stimulatory conditions, MAIT17 upregulate Tbet while maintaining a ROR $\gamma$ t expression (Wang et al., 2018). Hence following *Legionella in vivo* infection those MAIT17/1 (ROR $\gamma$ t<sup>+</sup> Tbet<sup>+</sup>) cells secrete type 1-associated effector molecules such as IFN- $\gamma$  and TNF- $\alpha$  (Wang et al., 2018).

Recently the expression of a pro-repair effector signature has also been described following TCR-mediated stimulation *in vitro* for both mice and human MAIT cells. Upon TCR-mediated stimulation, MAIT cells up-regulate genes such as *Areg*, *TGF $\alpha$* , *Il-22*, and others that are found up-regulated by other pro-repair cells (Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a).

Numerous studies have demonstrated a pleiotropic effector capability for MAIT cells. To better understand the effector function of MAIT cells it is paramount to first comprehend the afferent signals that lead to their activation.

### ii. MAIT cell TCR as a microbial sensor

#### ***In vitro* evidence of TCR-mediated activation**

As previously mentioned, the well-described MAIT cell cognate ligands, 5-OP/OE-RU, are derived from the bacterial 5-A-RU. Therefore, the TCR specificity of MAIT cells suggest that they could be activated following bacterial infections. This is the case for *Salmonella typhimurium* which activates human MAIT cells *ex vivo*. However, when deficient for RibD, an enzyme of the Riboflavin synthesis pathway necessary for 5-A-RU production, these bacteria are incapable of activating MAIT cells, indicating TCR-dependent triggering for this bacterial-mediated activation (Corbett et al., 2014). Additionally, when co-culturing MAIT cells with bacterially infected MR1<sup>-/-</sup> antigen presenting cells, CD69 up-regulation by MAIT cells was lost as compared to co-cultures with MR1<sup>+/+</sup> APCs (Le Bourhis et al., 2010). MR1 blocking with an anti-MR1 antibody also prevented MAIT cell activation in the presence of riboflavin-producing bacteria (Kjer-Nielsen et al., 2012; Le Bourhis et al., 2010; Magalhaes et al., 2015). Altogether, these data show that MAIT cells are reactive to riboflavin-producing bacteria through TCR recognition.

#### ***In vivo* evidence of anti-bacterial function**

The TCR specificity of MAIT cells suggested that they could play protective roles in bacterial infections *in vivo*. Indeed, when looking at the capacity of different bacterial strains to synthesize 5-A-RU (a 5-OP/OE-RU precursor), out of 1077 16S sequenced prokaryote genomes from the KEGG database, 77% (829) displayed the riboflavin pathway (Mondot et al., 2016). Accordingly, MAIT cells have a protective role in *Mycobacterium abscessus*, BCG (*Bacillus Calmette-Guérin*), *Francisella tularensis*, and *Legionella longbeachae* infections, as their absence leads to a delay in the clearance of these intracellular bacteria (Chua et al., 2012; Le Bourhis et al., 2010; Meierovics and Cowley, 2016; Meierovics et al., 2013; Wang et al., 2018). Whether this holds true in humans is difficult to assess but suggested by the fact that MAIT cells are activated and decreased in numbers in the blood of bacterially infected patients, such as in children suffering from *Vibrio Cholerea* (Leung et al., 2014). In humans, to date, only one study

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evidenced a human single nucleotide polymorphism in MR1 linked to a higher susceptibility to bacterial illness: tuberculosis (Seshadri et al., 2017).

Many other examples have been detailed thoroughly and show the impact of these bacterial-mediated diseases on MAIT cell frequencies in the blood (Grimaldi et al., 2014; Le Bourhis et al., 2010). Consistently, MAIT cell number is increased at the site of infection, suggesting specific migration to the inflamed site. The early blood decrease following *Salmonella enteric* challenge, local accumulation following gram-negative peritonitis (following peritoneal dialysis) and the up-regulation of gut and inflamed tissue “homing” molecules (CCR9 and CCR6) all suggest an increased recruitment of these cells (Howson et al., 2018; Liuzzi et al., 2016; Salerno-Goncalves et al., 2017). It is also noteworthy to mention that pathologies not directly linked to bacterial infections such as psoriasis, multiple sclerosis, rheumatoid arthritis, and Crohn’s disease lead to a blood frequency decrease of MAIT cell and an increase in the inflamed organ (Cho et al., 2014; Illes, 2004; Serriari et al., 2014). In some of these pathologies, a dysbiosis characterized by an altered microbiota composition or location is observed (Drago et al., 2016; Fahlén et al., 2012; Walker et al., 2011; Wright et al., 2015). Given that 65% of analyzed gut microbes possess the riboflavin synthesis pathway (Magnúsdóttir et al., 2015), MAIT cells could react against the dysbiosed microbiota in these pathologies.

However, whether TCR signaling explains these variations in the aforementioned human infections remains unclear. In mice, following intranasal *Salmonella* infection, MAIT cells are activated and accumulate in the lung, which is not the case when using a *Salmonella* strain deficient for RibD or after in vivo MR1-TCR blockade (via an anti-MR1 antibody) (Corbett et al., 2014). These results indicate that MAIT cell recruitment and activation in this infectious setting rely on TCR-mediated stimulation *in vivo*. Additionally, *in vivo* protection against *Legionella Longbeachae* infection is also provided by TCR-triggered MAIT cell activation, as MR1 blocking leads to increased mortality and bacterial load within the lung (Wang et al., 2018).

The next question is, how do MAIT cells protect *in vivo* against bacterial pathogens? In bacterial infections, MAIT cells could play a role directly (e.g., killing of infected cells) as shown *in vitro* using epithelial cells lines (Hela) infected with *Esherichia coli*. In this setting, MAIT cells achieved lysis of the infected cells (Le Bourhis et al., 2013). Interestingly, after *F. Tularensis* infection, MAIT cells act indirectly by promoting

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monocyte differentiation into dendritic cells through early production of GM-CSF in the lungs (Meierovics and Cowley, 2016). Whether these mechanisms always rely on TCR activation warrants further investigation.

### **Other factors that could help to formally demonstrate cognate activation.**

Several tools can be used to assess whether MAIT cells are activated *in vivo* through their TCR. The first one is **Nur77 expression**. Nur77 (*Nr4a1*) is a transcription factor induced rapidly following TCR-mediated stimulation of T lymphocytes and constitutes a valuable readout to evaluate if T lymphocytes undergo cognate activation (Osborne et al., 1994). Thanks to knock-in Nur77-gfp reporter mice, Nur77 has been used to study the TCR triggering of MAIT cells in the thymus during development (Legoux et al., 2019b) and could also be used to assess if MAIT cell cognate stimulation occurs in the periphery in various settings.

Another way of assessing MAIT cell reliance on cognate-mediated stimulation is to assess MAIT cell function in the absence of MR1 expression *in vivo*. However, as MR1 expression is necessary for MAIT cell development, two ways are available: **1) *in vivo* transfer of MAIT cells into MR1<sup>-/-</sup> mice; 2) the use of an MR1 inducible knockout mice (*Mr1f/f*).** **1) MAIT cell transfer** can be done by sorting and injecting MAIT cells in MR1 sufficient or deficient mice and assessing if MAIT cells become activated following *in vivo* infection. To transfer MAIT cells in sufficient quantity, previous expansion (either *in vivo* (van Wilgenburg et al., 2018) or *in vitro*) may be required, which could artificially skew the MAIT cell phenotype. This possible skewing is evidenced in the *Legionella longbeachea* infection where MAIT cells initially type 17 and IL-17 producing cells become type 1/17 and IL-17, IFN- $\gamma$  producing cells (Wang et al., 2018). Furthermore, because MAIT cells themselves express MR1 on their surface, MAIT cells may present their cognate ligand to each other. However, MAIT-cell mediated cognate triggering is unlikely as there would be a dilution of MAIT cells within the recipient following injection, but it could theoretically occur. **2) In the MR1 conditional knockout mice (*Mr1f/f*)**, a LoxP site leads to the elimination of the  $\alpha$ 2-helix necessary for the structure of the MR1 ligand binding cleft (Constantinides et al., 2019a). This mouse crossed onto the CD4<sup>CRE</sup> mouse (resulting in the deletion of MR1 in all cells that expressed CD4) showed a substantial

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reduction of MAIT cells and confirmed that MAIT cells are selected on thymocytes (Constantinides et al., 2019a). Crossing this mouse onto a Tamoxifen-inducible Cre (Metzger et al., 1995) would allow MR1 deletion on all cells and thus demonstrate the importance of cognate mediated activation *in vivo* while still allowing the *in vivo* MAIT cell development (as MR1 is necessary for thymic MAIT cell development). This conditional knockout mouse could also help assess the specific cellular subtypes that would be implicated in cognate activation by crossing the *Mr1<sup>f/f</sup>* mouse to mice with the Cre recombinase under the control of different cell-specific gene promoters.

MAIT cell cognate-depend function could also be assessed by **deleting the MAIT cell TCR** after MAIT cell thymic development and before *in vivo* infection. Inducible TCR-ablation method on mature T cells has been previously used for studying NKT cells, another preset immune cell, and showed no deleterious impact on NKT numbers as far as 45 weeks post deletion (Vahl et al., 2013).

### **Possible checkpoint for TCR-mediated activation of MAIT cells**

As the microbiota produces the MAIT cell ligand precursor and as this ligand diffuses through the epithelial barrier (Legoux et al., 2019a), fine tuning MAIT cell activation is necessary to avoid deleterious functions during steady state, but also following infections.

The necessity of **co-stimulation** for TCR-dependent MAIT cell activation, as demonstrated for other mainstream and innate-like T cells could be one possible checkpoint (Chen et al., 2017; Slichter et al., 2016, 2016; Turtle et al., 2011; Wang et al., 2019). Accordingly, intranasal administration of 5-OP-RU *in vivo* induces CD69 upregulation but fails to induce MAIT cell accumulation in the lung. MAIT cells effectively accumulated within the lung only in the presence of both 5-OP-RU and TLR (toll-like receptors) ligands, supporting a partial reliance of MAIT cells on co-stimulation for cognate recognition (Chen et al., 2017). Additionally, injection of 5-OP-RU alone induces deletion of MAIT cells (Legoux et al., 2019a).

**Surface levels of MR1** may also be a limiting factor and, as such, another method of controlling MAIT cell activation. In fact, in the periphery, MR1 expression by splenocytes cells is up to six times lower as compared to thymocytes (Legoux et al.,

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2020a; The Immunological Genome Project Consortium et al., 2008). Following TLR9 signaling however MR1 can be upregulated by B lymphocytes (Liu and Brutkiewicz, 2017). This would mean that in an infectious context, MR1 could be up-regulate to elicit cognate activation of MAIT cells, while at steady state, its low expression would prevent unnecessary activation. Additionally, in some viral infections (e.g., HSV-1) surface expression of MR1 is prevented (e.g., degraded by the proteasome) (McSharry et al., 2020). This downregulation of MR1 expression could constitute an immunomodulatory control by the virus to prevent MAIT cell cognate-activation.

**Antagonist molecules** may also prevent MAIT cell cognate activation. The vitamin B9 derivatives, 6-FP and its acetylated form Acetyl-6-formylpterin (Ac-6-FP) effectively inhibit MAIT cell activation by up-regulating and stabilizing MR1 at the surface without activating MAIT cells, thereby preventing the activating ligand(s) from being presented (Eckle et al., 2014; McWilliam and Villadangos, 2017).

### iii. Cytokine mediated activation

The expression of numerous cytokine receptors such as IL-1R, IL-15R IL-7R, IL-18R, IL-23R, and IL-12R by mature MAIT cells both in humans and mice suggest that MAIT cells could be activated via cytokine signaling (Billerbeck et al., 2010; Cui et al., 2015; Martin et al., 2009a; Rahimpour et al., 2015; Ussher et al., 2014) (Fig 2).

#### ***In vitro* and *in vivo* evidence of a cytokine dependent activation**

Blocking MR1 via anti-MR1 antibody effectively decreased the cytokine production in the *Escherichia coli* mediated stimulation of human blood MAIT cells (whole peripheral blood mononuclear cells: PBMC stimulation), indicating a cognate activation of MAIT cells. Anti-MR1 blocking during IL-18 and IL-12 PBMC stimulation did not alter the secretion of cytokines (e.g., IFN $\gamma$ ) by MAIT cells (Ussher et al., 2014), indicating that effector molecule production by MAIT cells can also be elicited by cytokines independently of TCR stimulation.

**IL-18** has been described as a potent MAIT cell activator in the absence of TCR-triggering (Ussher et al., 2018). In a co-culture of human PBMCs with an

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influenza-infected line of human lung epithelial cells MAIT cells activate (CD69<sup>+</sup>) and up-regulate IL-18Ra. In this setting were the pathogen (influenza) does not provide the cognate ligand, MAIT cell activation relies on IL-18 as the use of anti-IL-18 neutralizing antibodies prevents activation (Loh et al., 2016). Monocytes constitute a potential source for IL-18 as they produce IL-18 following cytokine triggering (e.g., IL-15) or direct viral sensing (e.g., TLR8) and subsequently are capable of MAIT cell activation (Jo et al., 2014; Sattler et al., 2015). **IL-12** can also be sufficient for MAIT cell activation. *In vitro*, *Mycobacterium bovis* infected macrophages can activate MAIT cells and while this activation is unaltered by MR1 blocking, it is lost if macrophages are IL-12 deficient or if anti-IL-12 neutralizing antibodies are present in the culture medium (Chua et al., 2012). To a lesser extent, other cytokines such as **IL-2 and IL-7** elicit human MAIT activation as shown by CD69 expression and/or cytokine expression following whole PBMC stimulation and despite anti-MR1 blocking (Sattler et al., 2015). Interestingly, cytokines such as **type 1 interferons (IFN- $\alpha/\beta$ )** and **TL1A** have a synergic effect with IL-12 and IL-18 and lead to an increase in MAIT cell cytokine production (e.g., IFN $\gamma$ ) (Pavlovic et al., 2020; van Wilgenburg et al., 2018).

It is possible that MAIT cells are activated in circumstances where pathogens do not produce the cognate ligand because they can directly respond to cytokines *in vitro* in a TCR-independent manner. This would present an alternate route where MAIT cells would not just respond to bacteria and fungi that produce 5-OP/OE-RU, but also to viruses that lack 5-A-RU and its derivatives. *In vivo* in mice, MAIT cells are found to be protective during influenza infection (H1N1) as more fatal outcomes were observed in the group devoid of these cells. In this influenza *in vivo* setting, MAIT cells accumulated and had an activated phenotype (CD69<sup>+</sup>CD25<sup>+</sup>) in the infected lungs of mice. TCR independent activation of MAIT cells following *in vivo* influenza infection was demonstrated by the injection of pulmonary MAIT cells within MR1<sup>-/-</sup> infected. This MAIT cell transfer led to increased survival and decreased weight loss compared to non-transferred MAIT deficient MR1<sup>-/-</sup> mice (van Wilgenburg et al., 2018). This seems to indicate that MAIT cell protective role occurs in a TCR-independent manner. Yet, prior to MAIT cell transfer these cells were expanded *in vivo* using *Salmonella Typhimurium* intranasal infection which provided a cognate-dependent activation in a Th1 context, that may bias the response. Additionally, as stated earlier, MAIT cells could trigger each other's TCR despite being diluted by the host's immune cells as they would accumulate locally in the infected lungs.

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Therefore, we can only ascertain that sustained cognate stimulation by circulating cells and local epithelium (excluding MAIT cells) is not required for this protective phenotype.

In infected IL-18 deficient mice (IL-18<sup>-/-</sup>) accumulation of MAIT cells appeared altered during an experimental viral infection (van Wilgenburg et al., 2018). This strongly suggests that IL-18 signaling is necessary for MAIT cell activation. However, IL-18 deficiency also impairs thymic MAIT cell development and results in fewer mature MAIT cells in the periphery (Koay et al., 2016). As such, the impaired recruitment of MAIT cells within the IL-18<sup>-/-</sup> influenza-infected lungs could be the result of an impeded development of MAIT cells.

### **MAIT cells in viral pathologies.**

Several human viral-based pathologies such as *Hepatitis C virus* (HCV), *Human Immunodeficiency virus* (HIV), *dengue virus*, *influenza*, *Hepatitis B virus* (HBV) have shown alteration in MAIT cell phenotype, functionality and/or frequencies (Ussher et al., 2018). In HCV infected patients, MAIT cell frequencies amongst T cells were significantly lower in the blood and liver samples of patients compared to healthy donors (Bolte et al., 2017). In another study, MAIT cells decreased in the blood and presented an impaired phenotype with high expression of PD-1 in patients with the chronic HBV virus (Yong et al., 2018). In 2016, a study of hospitalized patients with H7N9 avian influenza showed a correlation between fewer MAIT cells and fatal outcomes (Loh et al., 2016).

In the recent COVID-19 pandemic, MAIT cells were found to decrease significantly in frequency and numbers in the blood of COVID-19 infected patients as compared to uninfected donors (Flament et al., 2021; Parrot et al., 2020; Youngs et al., 2021). Projections of patient clinical outcomes (i.e., alive/deceased; mild/severe pathology) on unsupervised transcriptomic analysis and flow-cytometry phenotyping show that activated MAIT cells (e.g., CD69 up-regulation, CXCR3 downregulation) are associated to worst disease outcomes (Flament et al., 2021; Parrot et al., 2020; Youngs et al., 2021). This emerging evidence points towards a pathogenic effect of MAIT cells in the COVID-19 pandemic as they probably deleterious inflammatory response (e.g., increased secretion of: IFN $\gamma$ , TNF- $\alpha$ , and IL-17) (Flament et al., 2021; Parrot et al., 2020; Youngs et al., 2021).

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Interestingly in studies on MAIT cell involvement in HIV, MAIT cells declined numerically in the peripheral blood of HIV-1 infected subjects (Cosgrove et al., 2013; Fernandez et al., 2015; Leeansyah et al., 2013, 2015; Ussher et al., 2015; Wong et al., 2013).

As MAIT cells in nonhuman primates are phenotypically and transcriptionally very similar to human MAIT cells, a simian HIV (SHIV) infection was used to see if MAIT cells could be recruited in an HIV setting. These studies showed that like human MAIT cells, simian MAIT cells decrease in the circulating blood following SHIV infection. Moreover, they upregulate the  $\alpha 4\beta 7$ -gut integrin and accumulate in the gut mucosa indicating a recruitment of these cells (Juno et al., 2019). This latest study also reveals nonhuman primates as a promising model to help understand MAIT cell function in human pathologies.

### **Is the antiviral response only mediated by cytokines in a cognate independent manner?**

During viral infection, **dysbiosis** occurs in the form of altered microbial composition, metabolic activities, and/or spatial distribution (Deriu et al., 2016; Harper et al., 2021; Li et al., 2019). As mentioned previously, bacterial strains within the local microbiota can produce MAIT cells cognate ligand (Mondot et al., 2016). If this dysbiosis results in an increased of the riboflavin biosynthesis pathway, hence an increase in MAIT cell ligand precursor, MAIT cells could be activated by their ligand following viral infection despite the absence of riboflavin biosynthesis pathway in viruses or rib<sup>neg</sup> bacteria.

As discussed above, an endogenous ligand responsible for MAIT cell intra-thymic selection may also exist. Such **endogenous ligand(s)** could also be up-regulated/secreted/available during a viral infection and could also constitute a potential source for indirect TCR-mediated viral activation of MAIT cells.

Overall, the data mentioned in this part shows that the identification of the afferent signaling for MAIT cells *in vivo* following bacterial and viral infections doesn't only occur in a dichotomic manner cytokine versus TCR mediated stimulation but is highly complex. This complexity brings us to the importance of understanding the context of stimulation in the study of MAIT cell biology, which will be the subject of the next chapter.

#### iv. Context dependent activation and effector functions of MAIT cells.

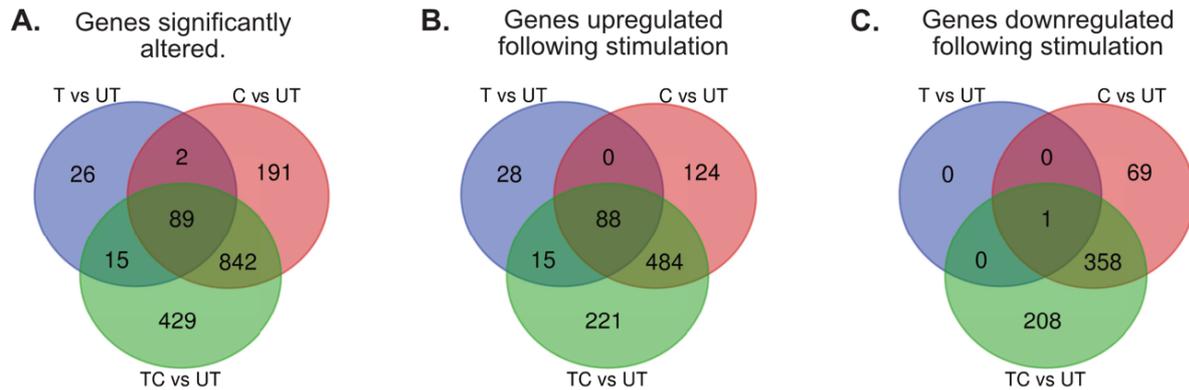
On top of the TCR and cytokine-mediated activation pathways, different combinations of these signals and specific organ location could impact MAIT cell effector functions. For instance, IL-15-triggered IL-18 and sub-optimal TCR triggering of MAIT cells potentiate IFN $\gamma$  secretion (Sattler et al., 2015). Other combinatory signaling, such as TL1A (Tumor necrosis factor (TNF)-like protein 1A) and IL-15, along with suboptimal doses of IL-12 and IL-18, were also shown to lead to a higher IFN $\gamma$  secretion as compared to TL1A or IL-15 stimulation alone (Leng et al., 2019a). Recently, it has been demonstrated that IL-12 co-administered with 5-OP-RU was necessary for sustained T-bet expression (MAIT1) and that the IL-12-to-IL-23 ratio dictated the MAIT1-to-MAIT17 ratio following antigen exposure or bacterial infection (Wang et al., 2022).

In bacterial pathologies, cytokine-mediated stimulation could also contribute to the maintenance of MAIT cell activation (Legoux et al., 2020; Ussher et al., 2018). In a co-culture between CD8<sup>+</sup> T cells and antigen-presenting cells (THP-1) exposed to fixed *Escherichia coli*, cytokine production by human MAIT cells relied on cognate stimulation alone (5h) but required IL-12 and IL-18 stimulation after 20h (Ussher et al., 2014). As such, cytokines could synergize with other cytokines and/or the cognate ligand(s) and impact MAIT cell effector functions (Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a).

To evaluate the impact of this synergic effect on MAIT cell effector function, RNA and/or protein analysis of MAIT cells was done following various stimulatory settings (e.g., cytokine, TCR-mediated, cytokine+TCR). In all three reports as previously demonstrated, both human and murine MAIT cells quickly mounted proinflammatory responses following TCR-mediated stimulation (Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a). On top of this observation, the MAIT cell effector response was tailored to the activation signal(s) (i.e., nature of the signal: cognate/non-cognate; combination of signals). One example reflecting this additional complexity can be seen in figure 8 (Leng et al., 2019a). Briefly, gene expression by MAIT cells was examined following TCR stimulation alone (T); IL-12, IL-18, IL-15, and TL1A cytokine stimulation

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(C) and a combination of both types of signaling (TC) as compared to the untreated group (UT) (Fig 8). Following these different types of activations a visible alteration in the expression levels of MAIT cell transcripts was observed with the upregulation (Fig 8 B) or downregulation (Fig 8 C) of numerous genes (Leng et al., 2019a). These results demonstrate that, depending on the type of stimulation the MAIT cell effector profile is adapted to the stimulatory stimuli indicative of a great adaptability of these cells.



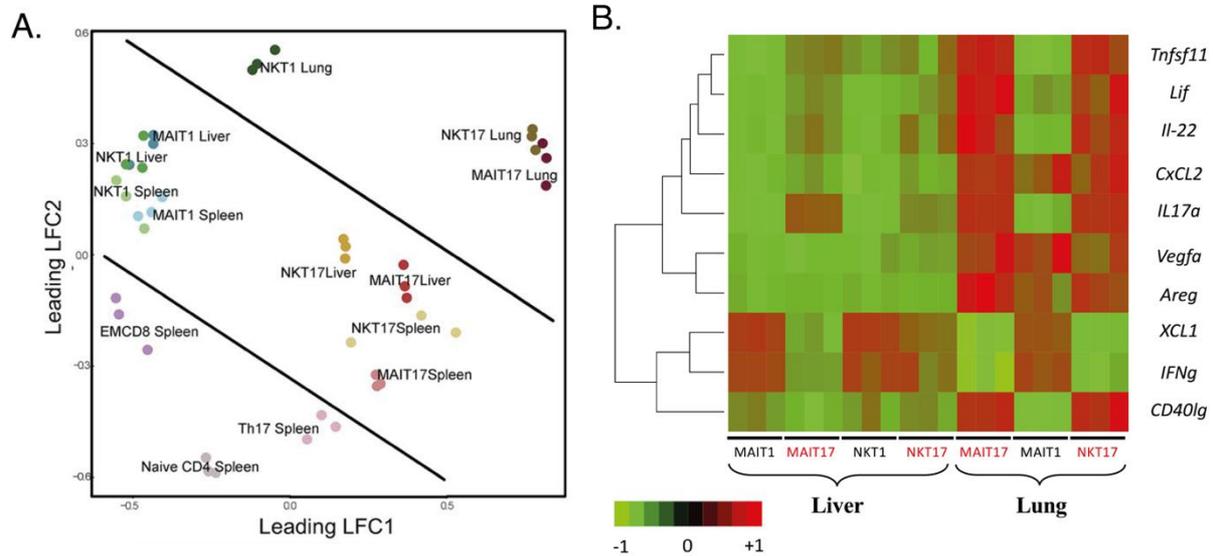
**Fig 8: Venn diagrams showing differentially modulated genes ( $p < 0.05$ , fold change  $> 4$ ) in CD8+ MAIT cells following TCR (T)-, cytokine (C)-, TCR and cytokine (TC)-treatment as compared to untreated (UT) MAIT cells. MAIT cells were from three healthy donors. Diagrams and corresponding legends are from (Leng et al., 2019a).**

Another factor that could influence MAIT cell effector function is tissue location. Human MAIT cells from the female genital tract (FGT) display a distinct effector profile as compared to other locations: a large proportion of MAIT cells from the FGT produce IL-17 following TCR-triggering. Additionally, they secrete higher levels of IFN $\gamma$ , TNF, and Granzyme B after *Escherichia coli*-mediated stimulation (Gibbs et al., 2017). The distinct functional properties based on the tissue origin of human MAIT cells indicate that tissue origin impacts the effector capabilities of these cells.

In mice, to study organ specificity, affymetrix data from sorted MAIT (CD44<sup>hi</sup>TetMR1<sup>+</sup>) and NKT (CD44<sup>hi</sup>TetCD1d<sup>+</sup>) cells from the lung, liver, and spleen was generated in my host lab (Salou et al., 2019a). Analysis of these datasets revealed distinct transcriptional profiles leading to an organ specific clustering (Fig 9A). Analysis of DEG (differentially expressed genes) between MAIT and NKT subsets from the lung and the liver showed a specific upregulation of genes associated with pro-repair mediators as compared to their liver equivalents (Fig 9B). Amongst the overexpressed genes are the epithelium growth factors (EGF), Vascular endothelial growth factor A (VEGF-a), and

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Amphiregulin (Areg) that are all implicated in T cell-mediated tissue repair (Arpaia et al., 2015). The expression of Areg by MAIT cells and NKTs from the lung in the B6-MAIT<sup>CAST</sup> was also confirmed in human affymetrix data on sorted blood MAIT cells (OL unpublished).



**Fig 9: Lung MAIT cells present a specific gene expression profile with tissue repair properties at steady state.** MAIT cells and NKTs were FACS-sorted based on their tetramer (TetMR1<sup>+</sup> or TetCD1d<sup>+</sup>) and ROR $\gamma$ t status (ROR $\gamma$ t-GFP<sup>+</sup> or ROR $\gamma$ t-GFP<sup>-</sup>). The mRNA of these cells were isolated, hybridized, and amplified data represented in (a) Multidimensional scaling (MDS) plot based on the 10% most variable genes between the samples, the distances (root-mean-square log-fold change (lfc)) calculated according to the differentially expressed genes between each pair of samples (b) Heat-map representation of differentially expressed effector molecules sampled from total affymetrix data (ANOVA liver vs lung MAIT17) after a gene ontology analysis. Data and MDS plot is from (Salou et al., 2019a).

Aside from multiple mediators working together to elicit diverse effector qualities, signal duration should also be considered when studying context dependent effector functions. When performing a time-course stimulation of human MAIT cells through their TCR, different cytokine profiles were observed. Short TCR-stimulation (48h) led to the secretion of IFN- $\gamma$  and TNF- $\alpha$  while prolonged stimulation (4 to 14 days) led to a gradual increase of type 2 cytokines such as IL-13 and IL-5 (Kelly et al., 2019). This time-dependent secretion of effector molecules also occurs in murine MAIT cells. While short *Escherichia coli* stimulation (6h) led to the co-production of IFN- $\gamma$  and TNF- $\alpha$  by murine MAIT cells, prolonged *Escherichia coli* stimulation (24h) led to an almost-exclusive production of

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IFN- $\gamma$  (Lamichhane et al., 2019). This gradual increase of exclusive IFN- $\gamma$  producing-MAIT cells was also observed after cytokine mediated stimulation (involving IL-18 and IL-12) but not after cognate stimulation (Lamichhane et al., 2019a).

Overall, depending on the activation context, tissue location, and stimulation duration, MAIT cells can present different effector profiles ranging from pro-inflammatory to pro-repair functions.

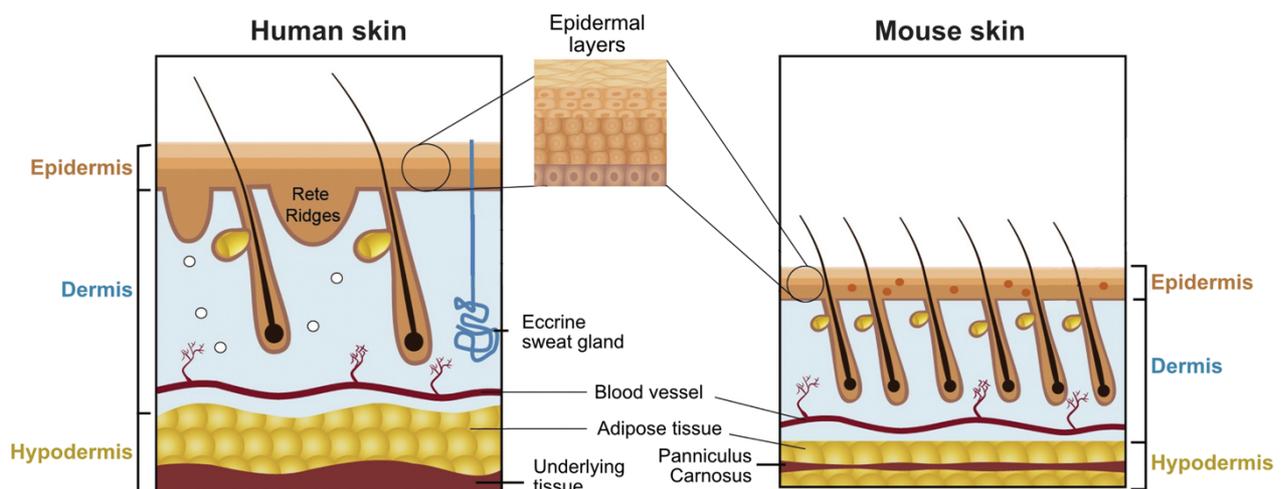
## Chapter II : The skin barrier

Epithelial barrier protective function can be subdivided into two main roles, the first one inside-out (by preventing the loss of essential elements) and the second one outside-in (by preventing the entry of harmful elements) (de Souza Carvalho et al., 2014). The skin is implicated in various functions including, but not limited to, water retention, energy storage, ultraviolet (UV) filter and acts as first line barrier to infection and other physical or chemical assaults. First, I will introduce the physical structure and main components of the skin at steady state. Second, I will provide a brief description of the sequence of events that follow skin injury. Finally, the function of some specific populations of T cells will be introduced.

### A. Description and function

The skin is the biggest organ, covering on average 2m<sup>2</sup> in an adult human. It is divided into three major layers: the epidermis, the dermis, and the hypodermis (Fig 10).

Although some differences exist between mice and human skin (detailed in each part) — as seen in Fig. 10 — the main structural properties of the skin are common between both species.



**Fig 10: Structural properties of the human and mouse skin.** Representation is simplified from (Zomer and Trentin, 2018).

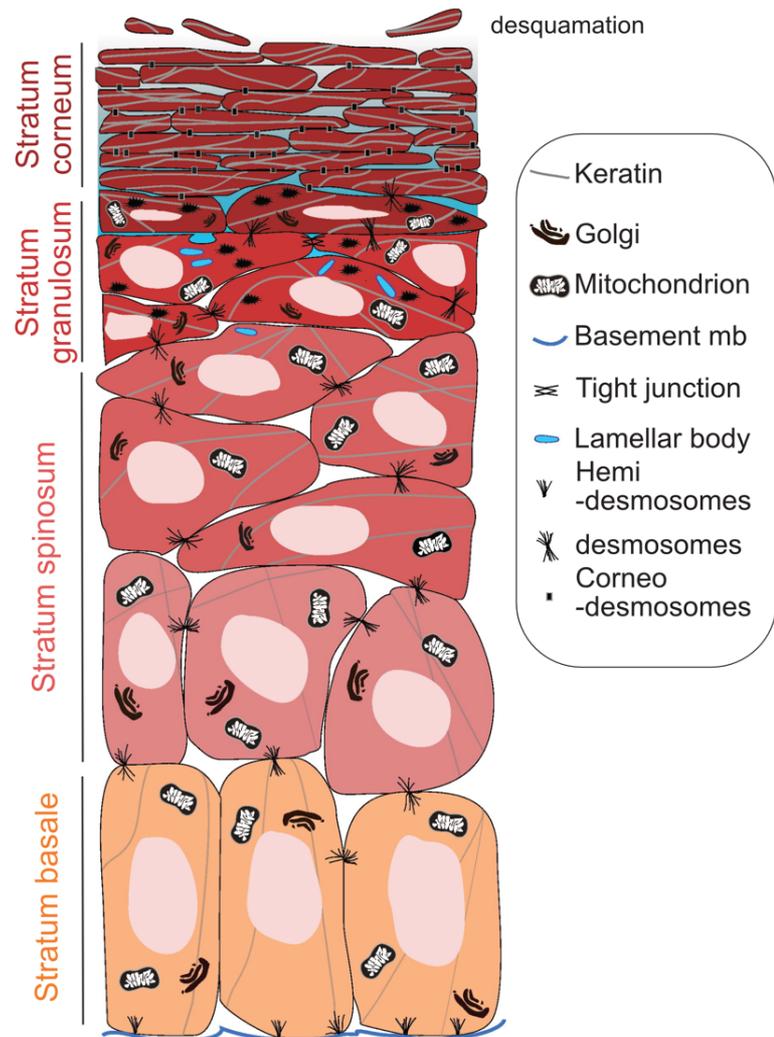
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### i. Epidermis

The epidermis is a keratinocyte-based multilayered epithelium, made of four layers: **Stratum basale**, **stratum spinosum**, **stratum granulosum** and **stratum corneum** (Fig 11).

Within the epithelium new cells are produced constantly at the bottom of the epithelium near the basal membrane and are pushed up in a process called keratinization or cornification. Keratinization/Cornification is the gradual accumulation of keratin by epidermal cells that leads to the formation of a hydrophobic cornified cellular envelope beneath the plasma membrane making the skin impermeable (Eckhart et al., 2013).

**Fig 11: Epidermal layers resulting from the cornification/keratinization process.** Represented are the Stratum basale, spinosum, granulosum and corneum. Representation is simplified from (Eckhart et al., 2013).



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### **Stratum basale**

The stratum basale is the first layer of keratinocyte cells, it sits right above the basal membrane and is linked to the dermis underneath by a complex network of macromolecules called the dermal-epidermal junction (DEJ) (Brody, 1969) (Fig 11). The keratinocytes that make up this layer are cuboidal or columnar, and represent the stem cells of the epidermis (Tumbar, 2004). This layer also contains Merkel cells, also known as "touch" cells, that are close/in touch to sensory nerve endings (Merkel, 1875). This proximity signals to the central nervous system thus contributing to the somatosensation of the skin (Munde et al., 2013). Melanocytes are another cell type found in this layer; they produce melanosomes which are organelles that contain the melanin pigment. Melanocytes have dendrite like protrusions that extend to the keratinocyte of the layer above: the stratum spinosum.

### **Stratum spinosum**

This epidermal sheet is named after the spine-like aspect of the keratinocytes, which are linked together by desmosomes (Barbieri et al., 2014) (Fig 11). It is made up of several layers of living mature keratinocytes that continue keratin production (Brody, 1969). The keratinocytes within this layer also engulf the melanosomes produced by the basal layer melanocytes, their melanin content is released and forms a perinuclear melanin cap that protects keratinocyte DNA from UV rays (Randolph Byers et al., 2003). This sheet also includes immune cells like Langerhans cells, which will be discussed further below.

### **Stratum granulosum**

This sheet's keratinocytes are flattened and have a granular appearance (Fig 11). The granular appearance results from the concentration of the keratin protein in hydrophobic keratohyalin granules in the cytoplasm (Freeman and Sonthalia, 2022). This is the final sheet of the epidermis that contains nucleated cells. The gradual loss of the nuclei and organelles within this layer is part of the cornification programmed cell death in which living keratinocytes become dead cells called corneocytes (Eckhart et al., 2013). As the nuclei and organelles are lost the keratin that accumulates within the cytoplasm

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forms a complex network. This complex network called the cornified envelope (CE), fills the entire cellular cytoplasm. Moreover, intracellular lamellar bodies (LB) made of phospholipids, glucosylceramides, sphingomyelin, cholesterol, and enzymes drastically increase in numbers within the keratinocytes of this layer (Feingold, 2007)(Fig 11).

### **Stratum corneum**

This layer is entirely composed of corneocytes with a CE, that are tightly linked to each other by corneodesmosomes made by cross-linking between the CEs of the neighboring corneocytes (Fig 11). Corneocytes are also surrounded by the contents of the LBs at this stage, contents that are metabolized by enzymes co-secreted by the LBs (Feingold, 2007). These intracellular and extracellular networks (e.g., CE cross-linking and LB secretion and metabolism) result in a keratin barrier, which confers a mechanical strength, makes the skin impermeable to water thereby protecting the organism from dehydration (Irvine et al., 2011; Mao-Qiang et al., 1995; Matsuki et al., 1998). The use of genetically modified mice demonstrated that this feature of the skin is essential, as defects in the formation of the stratum granulosum and corneum results in nonviable animals (Matsuki et al., 1998).

Furthermore, within this layer, a process known as epidermal shedding or desquamation occurs. Desquamation is the result degradation of the corneodesmosome junctions by proteases which leads to the shearing of the top layer of the stratum corneum (Eckhart et al., 2013). This process enables the epidermis to maintain a constant thickness while allowing the renewal of the corneocytes of the stratum corneum layer. Although no conclusive evidence has been presented, some speculate that this shedding may be required as a first line of defense against physical, chemical, and infectious insults (Milstone, 2004).

The “acid mantle”, an acidic layer (pH≈5) on the surface of the epidermis, reinforces this first line of defense (Lambers et al., 2006; Surber et al., 2018). The exact origin of this “acid mantle” is not fully elucidated but appears in humans to be a combination of sweat derivatives (e.g., lactic acid), sebum and filaggrin (monomers that contribute to the aggregation of the keratin fibers in the corneocytes) (Öhman and Vahlquist, 1998). The acidic property of this mantle is crucial for the control of the skin

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microbiota, as it regulates the growth of several strains and establishes electrostatic interactions that allow binding of the skin bacteria (Arnold, 1942; Lambers et al., 2006). The surface of the stratum corneum also contains antimicrobial peptides that can be stored within secretory granules in keratinocytes (Gallo and Hooper, 2012; Schmid-Wendtner and Korting, 2006).

It is worth noting that, a distinct layer between the stratum granulosum and corneum known as the **stratum lucidum** can be distinguished. This layer is flatter than the stratum granulosum, and the cells lack organelles and nuclei, instead having a keratinized wall beneath the plasma membrane (Ranvier, 1880). This additional layer is visible by histology in thicker skins (sole of the feet and palm of the hands) and provides additional protection against friction (Yousef et al., 2022).

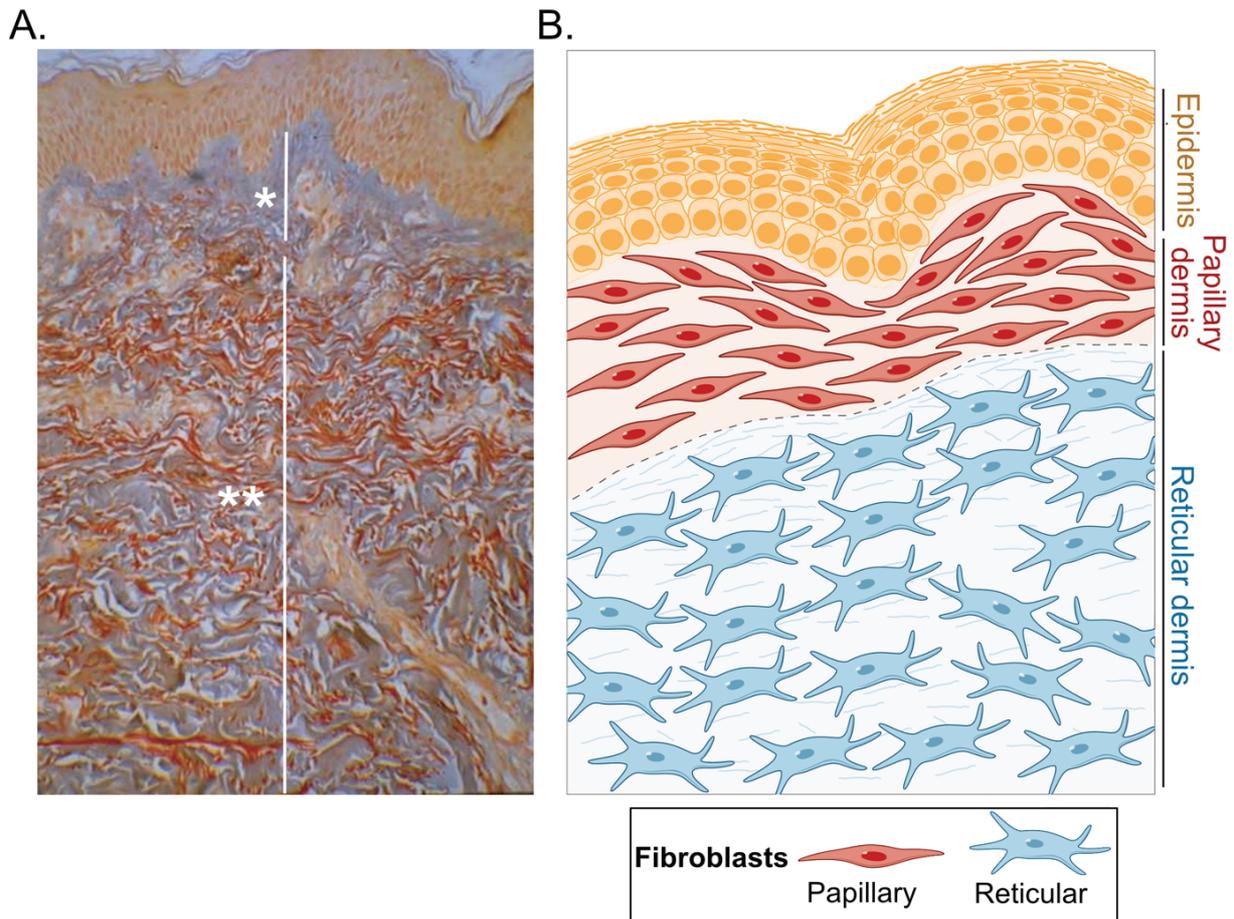
### **Some of the main differences between human and mice**

While very similar in structure, the human epidermis is thicker (60-90 $\mu\text{m}$ ) as compared to the mouse (10 $\mu\text{m}$ ) (Zomer and Trentin, 2018). In both mice and humans the DEJ allows for the adhesion of the dermis to the epidermis, to increase the adhesion to the dermis, in human skin there are also additional interdigitations called rete ridges (Roig-Rosello and Rousselle, 2020) (Fig 10).

## ii. Dermis

The dermis is a fibroblast-based structure composed primarily of fibroblast-produced collagen-rich extracellular matrix.

Two main layers can be observed by histology in the dermis (Fig 12A). The **papillary dermis** lies below the epidermis. This thin layer is made of papillary fibroblasts that have proliferative properties. The **reticular dermis** is thick and made of reticular fibroblast that have a more fibrogenic potential (Capolupo et al., 2022). Fibroblast within these layers have distinct morphological characteristics: while reticular fibroblasts are flattened and express the myofibroblast marker ( $\alpha$ -smooth muscle actin), papillary fibroblasts have a more of a “spindle-shaped” morphology (Schafer et al., 1985) (Fig 12B).



**Fig 12: Dermal architecture.** (A) Representative histology slide of the skin with indicators of: (\*) the papillary dermis and (\*\*) the reticular dermis, histology slide is from (Prost-squarcioni et al., 2008). (B) schematic representation of the dermal layers and the subtypes of fibroblast commonly found within those layers, scheme is from (Capolupo et al., 2022).

One of the most important characteristics of this skin layer is the vascularization, present throughout the dermis although denser within the papillary dermis. This vascularization allows for the continuous supply of blood and thus nutrients to the living epidermal cells (Brown and Krishnamurthy, 2021). Several appendages are also present within this layer: the **hair follicles**, which contain some of the stem cells required for the generation of the epidermal layers; **the arrector pili muscle**, which is attached to those follicles and allows for the erection of body hair; and the **sebaceous glands** which produce sebum, which helps lubricates and waterproofs the skin (Driskell et al., 2011; Sennett and Rendl, 2012; Song et al., 2006) (Fig 10).

### **Main differences between human and mice**

As for the epidermis, the dermis in mice is thinner than in the human skin (Fig 10). Another difference is the density of the hair follicles as it is higher in mice (1000/mm<sup>2</sup>) than in humans (25/mm<sup>2</sup>) (Zomer and Trentin, 2018). Humans have an additional appendage as compared to mice (with the exception of the paw pads): the sweat glands in the dermis that allows for temperature control and contribute to the acid mantle (Hoover et al., 2022; Öhman and Vahlquist, 1998).

### **iii. Hypodermis**

The hypodermis, also known as subcutaneous tissue, is the deepest and, in some areas such as the human abdomen the thickest skin compartment. This loose connective tissue is primarily made up of adipose cells, which are responsible for fat storage. It is essential in the body for water storage and energy storage, as well as thermoregulation (McKnight et al., 2022).

### **iv. Microbiota**

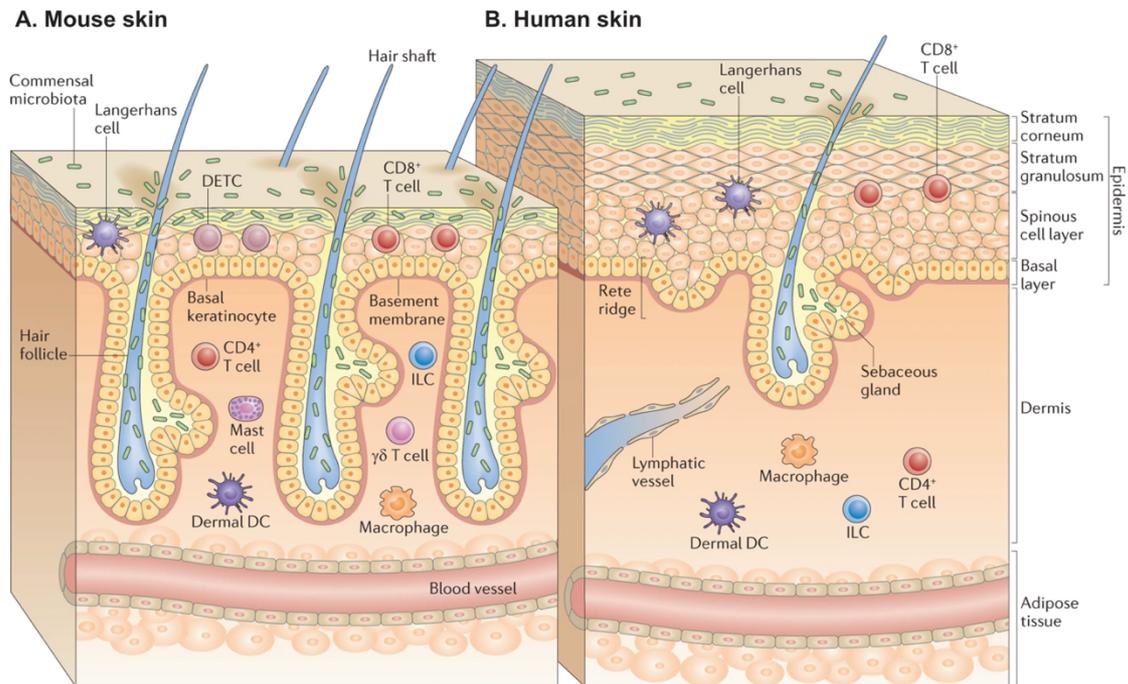
Another key component of the skin barrier is the microbiota located at steady-state at both the surface and inside the corneocyte layers of the *stratum corneum* (Malcolm and Hughes, 1980). Microbial colonization occurs in newborns right after birth (Dominguez-Bello et al., 2010). The microbiota composition varies both in biomass and diversity from one organ to another and also between individuals of the same species (O'Dwyer et al., 2016). However, core features (e.g. shared microbial genes, related strains) are conserved in the bacterial communities that colonize the body (Belheouane et al., 2020; Ding and Schloss, 2014).

Despite no differences reported in the keratinocyte structure of the skin, several lines of evidence using germ-free (GF) mice point towards an active crosstalk between the microbiota and the skin actors: epidermal cells and immune response (Kobayashi and Imanishi, 2021; Meisel et al., 2018). This crosstalk is revealed with the up-regulation of

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genes associated with epidermal development and differentiation in GF as compared to SPF skin biopsies (Meisel et al., 2018). Additionally, in the absence of the microbiota, a decreased expression of genes associated with TLRs, antimicrobial peptides and diverse chemokines and cytokines was observed (Meisel et al., 2018). Altogether, these data show that the microbiota can modulate skin actors, by increasing/inducing the sensing of microbes (e.g., TLR surface expression), effector responses (e.g., cytokines production), recruitment capabilities (e.g., chemokine secretion) and restorative capabilities (e.g., increase in epidermal growth) of the skin.

Importantly, microbial communities also induce immune tolerance by promoting the accumulation of regulatory cells: Tregs (Scharschmidt et al., 2015). Crucially, this induced tolerance develops only with early microbial colonization of the skin, as it is not present GF mice colonized as adults (Scharschmidt et al., 2015). This demonstrates the existence of a specific temporal window in which immune cells require signaling from microbiota for their development and function. Other T lymphocytes also rely on this specific temporal window to develop and/or perform their protective role within the skin (Constantinides et al., 2019a; Kerksiek et al., 1999). This will be covered in greater details in the section on T cells and repair.

v. Immune component

**Fig 13: Immune cell populations in the steady-state mouse and human skin.** Cells represented are the most frequently observed and are represented in the skin layers at steady state. Scheme is from (Pasparakis et al., 2014).

Several types of immune cells coexist in the epidermal and dermal layers. I will focus on the specialized immune cells that reside or patrol in steady-state mouse skin, excluding cells that require pathogen priming, such as tissue resident memory T cells (TRMs).

### Langerhans cells

In the epidermis at steady state, the only myeloid cell type are **Langerhans cells (LCs)** which account for 2% of the human epidermal cell population (Bauer et al., 2001). At steady state, LCs located in the stratum spinosum sample the environmental antigens in all layers of the skin via their dendritic like protrusions (Deckers et al., 2018). These LCs along with **dermal dendritic cells (dDCs)** (located in the dermis) induce tolerance to self-antigens by migrating to the draining lymph nodes and regulating commensal specific T cells (Deckers et al., 2018; Haniffa et al., 2015). In fact, LCs promote skin resident **regulatory T cell (Treg)** expansion while inhibiting **T effector memory cells (TEMs)** in

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*vitro* (Seneschal et al., 2012). Additionally, Tregs located near LCs in the epidermis express higher levels of Ki67 (proliferative marker) than those located in the dermis (Seneschal et al., 2012).

dDCs are derived from the peripheral migratory myeloid dendritic cells. This is evidenced by the complete loss of dDCs in human patients that have monocyte deficiency in the blood (Bigley et al., 2011). LCs develop from yolk sac progenitors and are not able to repopulate the skin following full body irradiation and bone marrow transplantation (Gentek et al., 2018; Yu et al., 1994). LCs are maintained in time through clonal proliferation (Yu et al., 1994). Both LCs and DETCs (discussed below) are found in contact with nearly all the keratinocytes of the *stratum basale*. Within this layer, immune cell density is tightly regulated, as a decrease in keratinocyte density directly correlates with a decrease in LC and DETC density (Lim and Ito, 2021). This suggests a possible cross-talk between keratinocytes and resident immune cells such as LCs. This is also supported by the near complete loss of LCs in the absence of hair-follicle keratinocytes (e.g. hairless mice or in patients suffering from alopecia) (Nagao et al., 2012). Additionally, hair-follicle express chemokines (CCL1, CCL2, CCL8, and CCL20) that can initiate LCs recruitment in inflammatory settings (Nagao et al., 2012).

## DETC

In the human epidermis, LCs are the dominant immune population; while in mice, the main immune cell subset is composed of T lymphocytes called **dendritic epidermal T cells (DETC)** because of their dendrites, that are absent from the human skin (Fig 13). At steady-state, DETC cells regulate keratinocyte population by constitutively producing IGF-1 (insulin-like growth factor 1) a critical keratinocyte growth and survival factor (Sharp et al., 2005). DETC also act as sentinels, detecting precancerous changes (e.g., Rae-1 upregulation by keratinocytes via the DETC NKG2D receptor) and enhancing adaptive immune responses in draining lymph nodes (e.g., induction of IgG production by B lymphocytes) (Jung et al., 2012; Shimura et al., 2010). Like LCs, DETC develop from yolk sac progenitors and are unable to replenish the skin from circulating cells, they also self-renew within the skin through clonal proliferation (Gentek et al., 2018).

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### **Innate lymphoid cells**

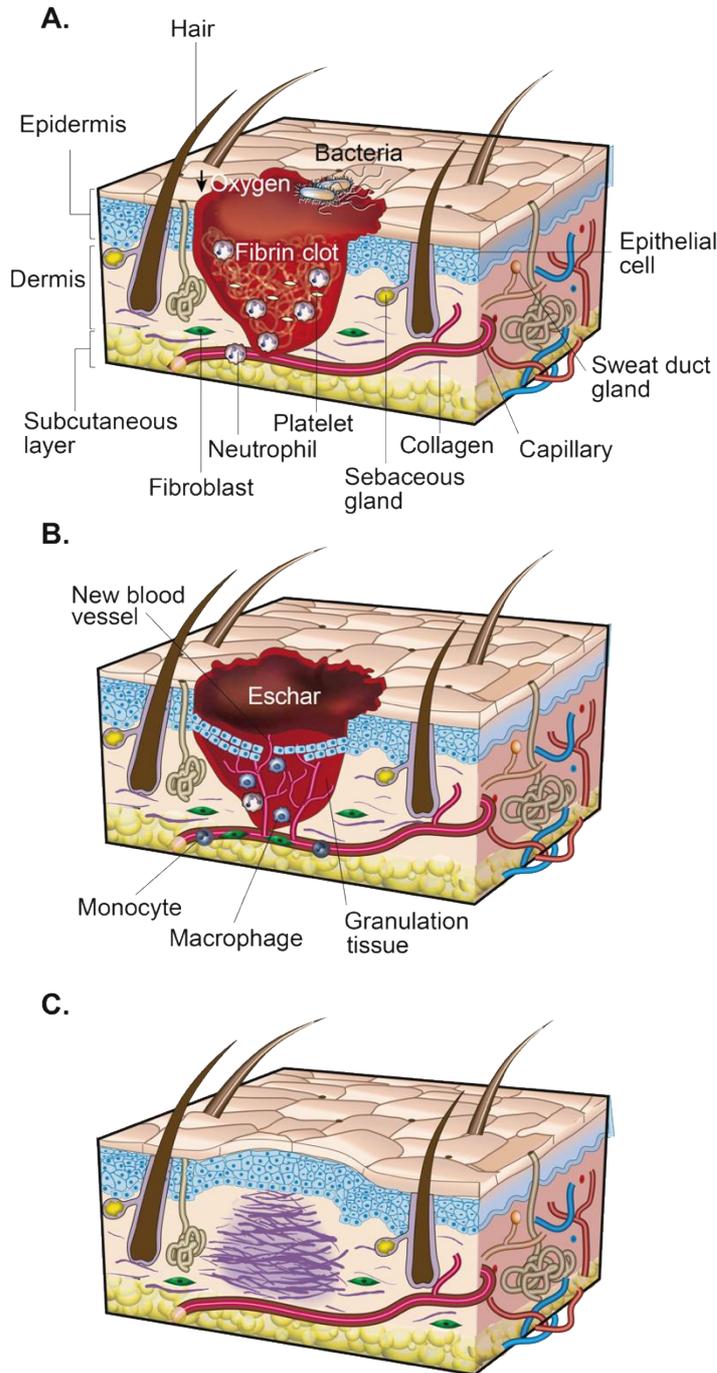
**Innate lymphoid cells (ILCs)** are also described in the healthy dermis, with the ROR $\gamma$ t<sup>+</sup>CCR6<sup>+</sup> ILC subset found near the sebaceous gland. Interestingly, ILCs appear critical in maintaining a healthy balanced microbiota by influencing sebaceous gland production of antimicrobial molecules (Kobayashi et al., 2019).

### **Macrophages**

Present in both the papillary and reticular dermis are **macrophages**, that phagocytose cellular debris and regulate hair growth (Amberg et al., 2016; Yanez et al., 2017). At steady-state, the major macrophage population is composed of skin resident macrophages originating from fetal progenitors (e.g. fetal yolk sac) and maintained by self-renewal (Bertrand et al., 2005; Davies et al., 2013; Ginhoux and Jung, 2014). However during inflammation, a large part of the macrophage population originates from the circulating monocytes (Martin and Leibovich, 2005).

Other myeloid cells, such as **neutrophils**, **mast cells**, and **eosinophils**, can be found in the uninflamed reticular dermis, though at a lower frequency. To date, the function of these cells within the steady state skin has not been described (Nguyen and Soulika, 2019). However, like monocyte-derived macrophages these cells play diverse roles during skin inflammation and particularly during skin-repair their dynamics and roles will be discussed in the corresponding repair chapter.

**B. Tissue repair**



**Fig 14: The three main stages of wound repair in the skin. (A) Inflammation**, a fibrin clot is formed and bacteria, neutrophils, and platelets infiltrate the wound. **(B) New tissue formation** (about 2-10days following injury), a scab (eschar) forms on the surface of the wounds. New blood vessels form and both migration and proliferation of epithelial cells occurs under the eschar. **(C) Remodeling** (lasts up to a year), Disorganized collagen fibers present in the wound area are replaced by new ordered and thicker collagen fibers. Scheme and legend are slightly adapted from (Gurtner et al., 2008).

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The steps that follow any type of injury are highly similar across different organs and different types of injuries (e.g., a burn, a cut, a myocardial infarction...). The sequence of events restoring tissue integrity constitute a highly conserved function (Aarabi et al., 2007; Gurtner et al., 2008; Klein et al., 2003; Singer and Clark, 1999). Tissue repair occurs in 4 sequential yet overlapping phases. The process starts with vascular constriction, platelet aggregation and formation of a fibrin clot. This is followed by a rapid inflammatory response to prevent infection via the recruitment/activation of immune cells (Fig 14A). A third phase, called the proliferative phase aims at reforming and/or restructuring the tissue, with re-epithelialization, angiogenesis, collagen synthesis and extracellular matrix formation (Fig 14B). Lastly, the remodeling phase restores as much tissue strength and function as possible, through collagen remodeling, vascular maturation and regression (Gurtner et al., 2008; Krafts, 2010) (Fig 14C).

### i. Hemostasis phase

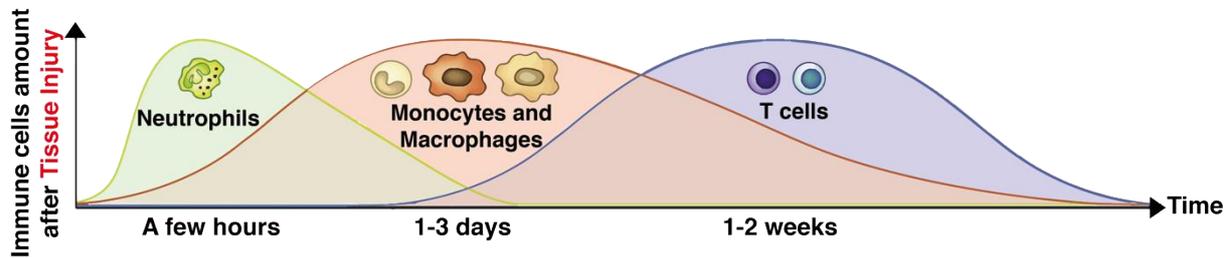
This step begins immediately after a blood vessel rupture: platelets stick together by interacting with extracellular matrix proteins (e.g., fibronectin, collagen) to seal the vessel wall, reducing blood flow. Fibrin threads reinforce the platelet plug, resulting in the formation of an insoluble blood clot (Eming et al., 2014). The clot and damaged cells release pro-inflammatory cytokines that signal the presence of the wound and induce growth factors (transforming growth factor- $\beta$ : TGF- $\beta$ , fibroblast growth factor: FGF, epidermal growth factor: EGF) that act on fibroblasts and keratinocytes to induce their proliferation (Scully et al., 2020). Additionally, activated platelets release chemokines such as platelet factor 4 (PF4), CXCL4, and others that recruit immune cells such as neutrophils (e.g., CXCL4-CXCR4) required for the inflammatory step (Anitua et al., 2004) (Table 1, 2).

### ii. Inflammatory step

The immune actors arrive and act in the wound following several “waves”, as depicted Fig 15. Briefly, in the first few hours following tissue injury neutrophils are recruited, quickly followed by innate immune cells such as monocytes and macrophages that peak at 1-2days. Later on, the adaptive mainstream T cells are amongst the last

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immune cells to enter the wound site, reaching their peak 1-2 weeks post-injury (Fig 15) (Julier et al., 2017).



**Fig 15: Scheme of the immune cell mobilization kinetics.** Scheme is from (Julier et al., 2017).

Here I will concentrate on the most prominent innate immune cells which play a role during repair, the available data on the adaptive and innate like T cell compartment will be discussed in the next chapter.

### **Early inflammation: Neutrophils**

Neutrophils are the first leucocytes recruited to the wound from the circulation via diapedesis from adjacent vessels, reaching their maximum density 24-48 hours after wounding (Julier et al., 2017). Neutrophil recruitment in the wound involves chemoattractants, including DAMPs (damage-associated molecular patterns), PAMPs (pathogen-associated molecular patterns) and chemokines such as PF4 or CXCL8 released at the injury site (Anitua et al., 2004; Kukulski et al., 2009; Ng et al., 2011). The recruited neutrophils start the process of debris removal and pathogen clearance in the wound site.

This cleaning involves phagocytosis, degranulation (e.g., proinflammatory, toxic molecules), reactive oxygen species production and the release of NETs (Neutrophil extracellular traps) that immobilize and neutralize micro-organisms (Borregaard, 2010; Kolaczowska and Kubes, 2013) (Table 1). Neutrophils also secrete cytokines (e.g., IL-17) and vascular growth factors (e.g., VEGF-A), which initiate the angiogenic process and promote the recruitment and activation of other neutrophils and inflammatory cells (Kolaczowska and Kubes, 2013). However, if maintained, the NET secretion and neutrophil overall inflammatory function impairs wound closure. Therefore, tissue repair is a tightly regulated process (Dovi et al., 2003).

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Other immune cells, such as mast cells, which are partly resident in the skin and partly recruited from the blood, appear to play an early role in regulating neutrophil recruitment. This is evidenced by a significant higher number of neutrophils infiltrating the wounds of mast cell deficient mice as compared to wild-type animals (Egozi et al., 2003). Furthermore, neutrophils secrete "find me" signals (e.g. low levels of ATP), which, in conjunction with neutrophil and monocyte secretion of effector molecules, leads to monocyte recruitment (Ravichandran, 2010).

### **Late-inflammation: Monocytes and macrophages**

An important macrophages infiltration occurs at the wound site in the late inflammatory stage. There are still several pending questions on the origin of these different macrophages and their phenotypical characteristics (Krzyszczyk et al., 2018). For sake of clarity, macrophages will be classified here according to their function: pro-inflammatory, pro-wound healing and pro-resolving.

Macrophages increase in numbers during the inflammatory phase however, contrary to neutrophils, because of their pleiotropic characteristics macrophages persist beyond the inflammatory stage. Accordingly, macrophage numbers peak during the proliferative and remodeling stages, some of these macrophages being tissue resident but the vast majority being monocyte-derived (Julier et al., 2017; Martin and Leibovich, 2005).

During the inflammatory stage, blood-born monocytes, stimulated by wound-derived  $\text{TNF}\alpha$  and  $\text{IFN}\gamma$ , differentiate into pro-inflammatory macrophages. These macrophages phagocytose infiltrating pathogens, necrotic tissue, and wound debris (Table 1). They also produce various pro-inflammatory mediators including cytokines (e.g.,  $\text{TNF}$ , IL-6, IL-12) and chemokines (e.g., PDGF) therefore promoting activation and recruitment of other immune cells. Following the debridement of the wound, macrophages adopt a non-inflammatory pro-healing phenotype which helps initiating the proliferative step of wound healing (Boyette et al., 2017).

Cell type	Function related to wound healing
<b>Platelets</b>	Involved in thrombus formation
	$\alpha$ granules are a rich source of inflammatory mediators including cytokines (e.g. TGF- $\beta$ , PDGF, $\beta$ -thromboglobulin, platelet factor-4)
	Major initial stimulus for inflammation
<b>Neutrophils</b>	First cells to infiltrate site of injury
	Phagocytosis and intracellular killing of invading bacteria
<b>Monocytes (macrophages)</b>	Phagocytose and destroy invading bacteria
	Clear debris and necrotic tissue
	Rich source of inflammatory mediators including cytokines
	Stimulate fibroblast division, collagen synthesis and angiogenesis

**Table 1: Function of the most important cells involved in the inflammatory wound healing step.** Table is from (Enoch and Leaper, 2008).

### iii. Proliferative step

The proliferative phase starts day 3 post-wounding and lasts up to 2-4 weeks and involves several different growth factors (Table 2) produced by different immunological (e.g. neutrophils, macrophages, lymphocytes, mast cells) or structural (e.g. keratinocytes, fibroblasts, endothelial cells) cells (Enoch and Leaper, 2008) (Table 2). These growth factors promote fibroblast migration and proliferation, extracellular matrix (ECM) deposition, angiogenesis as well as recruitment of immune actors (Table 2).

During this step, the fibrin clot of the inflammatory phase is replaced by a granular tissue made of ECM, vascularized and infiltrated by immune cells (Gurtner et al., 2008; Krafts, 2010). Fibroblasts are the key structural players in this repair stage. Briefly, they migrate within the wound and synthesize collagens (mainly type III) which, along with proteoglycans and adhesive glycoproteins lead to the formation of a temporary ECM (Xue and Jackson, 2015). The ECM provides a substrate for cell adhesion and stabilizes the wound structure. Epithelial cells then proliferate and migrate from the wound edges, forming a thin superficial layer that is then thickened with additional keratinocyte layers in a process called re-epithelization (Gurtner et al., 2008).

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Growth factor	Major sources in the wound	Function related to wound healing
<b>VEGF</b>	Platelets, neutrophils	Stimulates angiogenesis in granulation tissue Stimulates formation of collateral blood vessels in peripheral vascular disease
<b>FGFs</b>	Fibroblasts, endothelial cells, smooth muscle cells, macrophages	Proliferation of fibroblasts and epithelial cells; matrix deposition; wound contraction; angiogenesis Accelerates formation of granulation tissue
<b>KGFs</b>	Fibroblasts	Proliferation and migration of keratinocytes
<b>EGF</b>	Platelets, macrophages, keratinocytes; also plasma	Differentiation, proliferation, migration and adhesion of keratinocytes Formation of granulation tissue
<b>PDGF</b>	Platelets, fibroblasts, macrophages, endothelial cells	Mitogenic for smooth muscle cells, endothelial cells and Chemoattractant for neutrophils and fibroblasts Fibroblast proliferation and collagen metabolism
<b>G-CSF</b>	Monocytes, fibroblasts, lymphocytes	Stimulates production of neutrophils Enhances function of neutrophils and monocytes Promotes proliferation of keratinocytes
<b>GM-CSF</b>	Keratinocytes, macrophages, lymphocytes, fibroblasts	Mediates proliferation of epidermal cells
<b>TGF-<math>\alpha</math></b>	Activated macrophages, platelets, epithelial cells	Stimulates proliferation of epithelial cells and fibroblast Formation of granulation tissue
<b>TGF-<math>\beta</math></b>	Platelets, macrophages, fibroblasts, neutrophils, keratinocytes	Mitogenic for fibroblasts and smooth muscle cells Chemotactic for macrophages Stimulates angiogenesis (indirect) and collagen
<b>IL-1</b>	Macrophages, lymphocytes, many other cells	Neutrophil chemotaxis Fibroblast proliferation
<b>TNF</b>	Macrophages, mast cells, T-lymphocytes	Fibroblast proliferation
<b>IGF-1</b>	Fibroblasts, plasma.	Fibroblast proliferation Stimulates synthesis of sulphated proteoglycans and
<b>HGF</b>	Fibroblasts, keratinocytes, endothelial cells.	Re-epithelialization Neovascularization Formation of granulation tissue

**Table 2: Important growth factors with a demonstrated involvement in wound healing.** Table is from (Enoch and Leaper, 2008).

During this stage, neovascularization occurs to restore blood flow to the newly synthesized skin. New vessels are formed by endothelial proliferation and migration promoted by pro-angiogenic factors such as VEGF produced by mast cells and other infiltrating immune cells (Christoffersson et al., 2012; Enoch and Leaper, 2008; Gong and Koh, 2010).

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Wound contraction also occurs during this stage, resulting from the contraction of myofibroblasts that have differentiated from dermal fibroblasts, allowing the wound area to be reduced by bringing wound edges closer together (Wipff et al., 2007). The TGF- $\beta$  growth factor (produced by platelets, macrophages, fibroblasts, neutrophils, and keratinocytes) is involved in this process by promoting fibroblast and smooth muscle cell proliferation. Contrary to human skin the dermis of mice is loose and not attached to the underlying organs (Fig 10). The looseness of the murine skin leads to more wound contraction within the murine wounds as compared to human wounds, resulting in faster wound closure (Masson-Meyers et al., 2020).

### iv. Remodeling phase

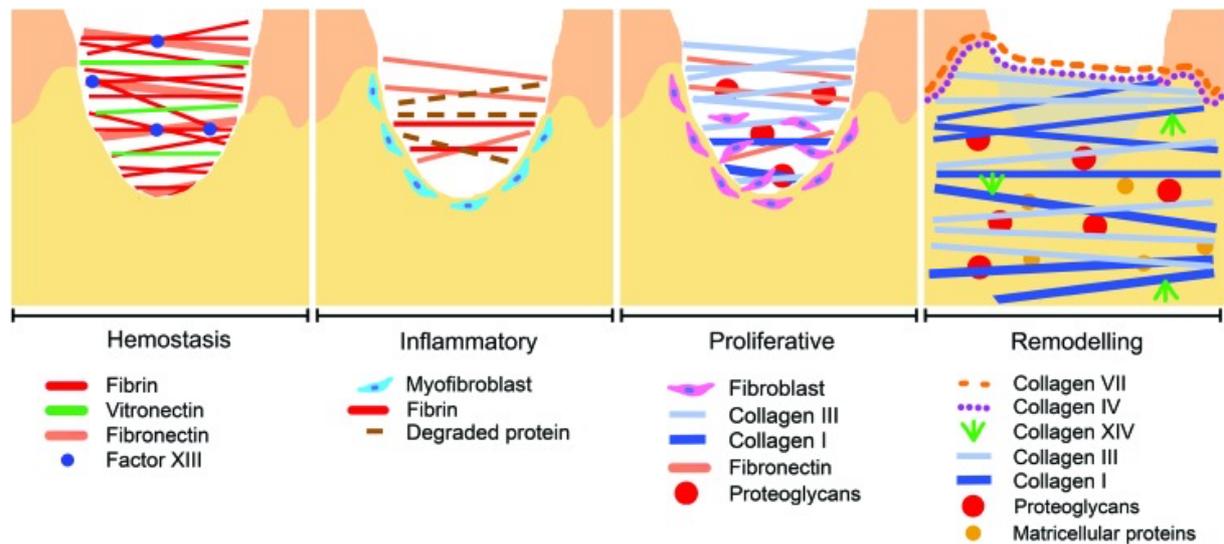
The maturation or remodeling phase is the final stage of wound healing (Fig 14), which begins around week 3 and can last up to 12 months in the human skin.

This step aims at restoring as much tissue strength and function as possible, through remodeling, vascular maturation, and regression of collagen (Gurtner et al., 2008; Krafts, 2010) (Fig 16). During this phase, signals (e.g. IL-10, pentraxin-10) drive macrophages reprogramming into pro-resolving macrophages (Lichtnekert et al., 2013). These pro-resolving macrophages contribute to decreasing the inflammation within the closed wound via the production of anti-inflammatory cytokines such as IL-10 (Morhardt et al., 2019). This anti-inflammatory signaling leads to a steep decrease of the leucocytes infiltrating the skin (Fig 15).

Throughout the wound healing process, the composition of the ECM evolves, from the fibrin-based structure (hemostasis phase) to its reinforcement by proteoglycans, adhesive glycoproteins and mostly type III collagen fibers (proliferative step) (Tracy et al., 2016; Xue and Jackson, 2015). During the resolving phase, pro-resolving macrophages produce MMPs (matrix metalloproteinases) that contribute to the replacement and/or addition of collagen fibers in the ECM. Type III collagen is replaced by thicker type I collagen also mainly produced by fibroblasts. Non-fiber forming collagens type VII and IV are also produced at this stage and contribute to the reformation of the basement membrane that supports the newly synthesized epithelium (Mak and Mei, 2017; Tracy et al., 2016). Meanwhile, the non-fiber forming collagen XIV helps to decrease both

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fibroblast proliferation and myofibroblast-mediated contraction (Tracy et al., 2016) (Fig 16).



**Fig 16: Changes in the ECM structure during the four stages of wound healing.** Figure is from (Tracy et al., 2016).

Collagen fibers that were originally laid down in a disorganized manner are aligned and cross-linked. This reorganization lasts up to a year and reinforces the skin tensile strength (Xue and Jackson, 2015). Around week 2 post-wound when collagen type III fibers are the dominant form, the tensile strength of the wound is at 10% of its final strength (Ireton et al., 2013). The increase in type I collagen fibers, their rearrangement, and cross-linkage within the wound bed all contribute to the increase in tensile strength of the newly closed wound, reaching up to 80% of intact-skin resistance (Levenson et al., 1965).

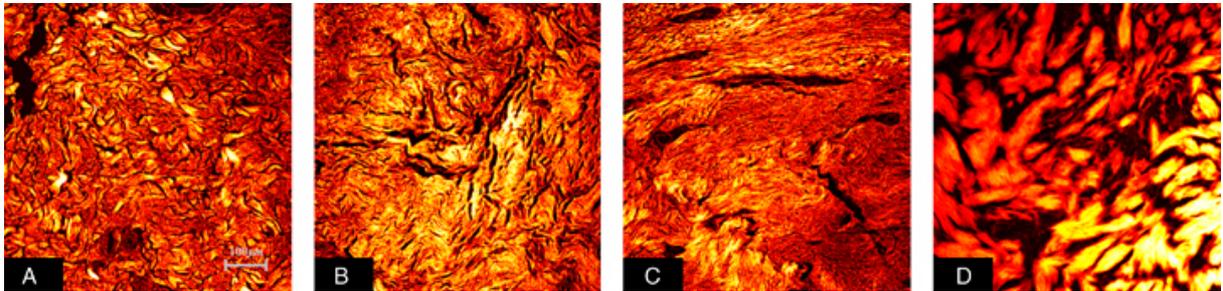
## v. Defects in wound healing

### **Hypertrophic scars and keloid scars**

At the end of the repair process the skin does not regain its full strength nor its original architecture as some permanent scar tissue remains (Ireton et al., 2013). While scar tissue formation itself is a normal part of the healing process, genetic or environmental factors can lead to excessive scarring and loss of function of the damaged tissue (Corr and Hart, 2013). The different types of scars with distinct collagen structures

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are classified based on clinical behavior as well as macroscopic and microscopic observations (Liu et al., 2012; Verhaegen et al., 2009) (Fig 17).



**Fig 17: Differences in collagen architecture between (A) normal skin, (B) normotrophic scar, (C) hypertrophic scar and (D) keloid scar.** Confocal microscopy images and corresponding legend are from (Verhaegen et al., 2009).

Considered to be the least deleterious type, **normotrophic scars** are characterized by a higher collagen deposition as compared to normal skin (Fig 17B). On the other hand, **hypertrophic scars** (Fig 17C) have at least twice the amount of collagen deposition as normotrophic scars, as well as an abnormal amount of infiltrating myofibroblasts. This infiltration of myofibroblasts leads to excessive tensions which cause the skin to rise creating a “bump” at the surface of the skin (Schmieder and Ferrer-Bruker, 2022).

**Keloid scars** are another type of abnormal scarring; they are caused by abnormal hyperproliferative growth of dermal fibroblasts, which results in excessive type III collagen fiber deposition (Kelly, 2004) (Fig 17D). Because of these excessive and abnormally thick deposits the keloid scar extends beyond the original wound and a mass is visible on the surface of the skin (Sadiq et al., 2020). Interestingly, because keloid scars are shared among similar ethnic groups, families, and twins, this abnormal scarring process may have a genetic component (Téot et al., 2020). TGF- $\beta$ , which promotes proliferation and collagen deposition by dermal fibroblasts, is upregulated in both hypertrophic and keloid scars, indicating a potential defect in the regulation of its production during the wound healing process (Liu et al., 2016).

### Chronic wounds

Chronic wounds are defined as wounds that do not follow the sequence of wound healing stages and thus take an unusually long time to heal. Frequently, the inflammatory

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phase is prolonged caused by pro-inflammatory stimuli such as bacterial infiltration, necrotic tissue, and excessive wound exudate (Zhao et al., 2016). Chronic wounds affect over 6 million people in the United States and cost up to 10 billion USD per year, making them a major public health concern (Powers et al., 2016). Ulcers of the foot or leg are a well-known type of chronic wound; this pathology lasts on average 12 months and costs up to 3% of the healthcare budget in developed countries (Frykberg and Banks, 2015). The unhealing wound has several negative consequences, including sepsis, as infections of the tissue are common, and in extreme cases can cause death (Richmond et al., 2013). Chronic venous insufficiency, arterial diseases, elevated blood pressure, neuropathy, and diabetes are all factors that can cause or aggravate ulcers (Frykberg and Banks, 2015; Richmond et al., 2013). Analysis of patient profiles around the world has revealed that older people appear to be more at risk of developing these chronic wounds (Gould et al., 2015; Yao et al., 2020).

As a result, chronic wounds pose a serious threat in our aging societies. Therefore it is paramount to develop new therapies to alleviate the societal, financial and personal (e.g., pain, discomfort, and health issues) caused by unhealed or incorrectly healed wounds (Eming et al., 2014). Therapeutical development however relies on comprehensive studies of the cellular and molecular actors at play in wound healing, as such this work aims at understanding one of these actors: MAIT cells.

### **C. T cells and repair**

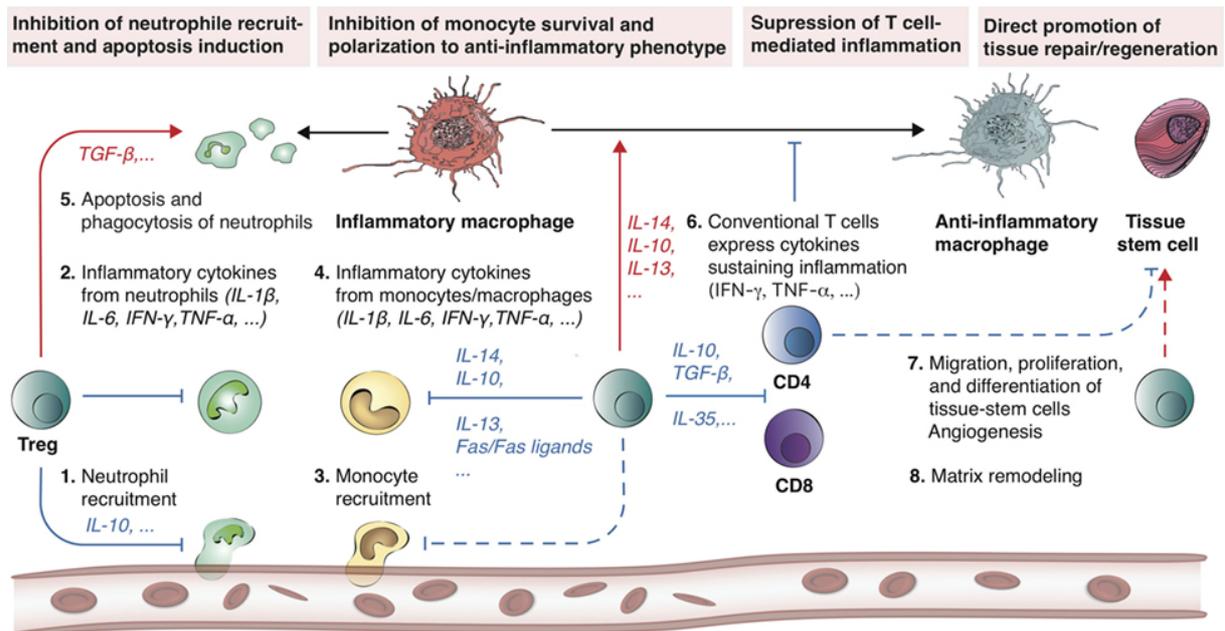
The previous chapter described key immune cells that participate in and regulate wound repair steps. In this next and final introductory chapter, I will detail the literature on major pro-healing T-lymphocytes as they help to clarify MAIT cells potential function within the wound.

## INTRODUCTION

### i. Tregs

#### Pro-repair functions

Both in humans and mice, in different organs and tissue-damage models, Tregs have been shown as potent tissue repair mediators (Zhang et al., 2017). In the skin, Tregs are crucial players as their absence leads to a delay in wound closure (Nosbaum et al., 2016)



**Fig 18: Tregs direct and indirect pro-repair functions.** Red arrows: Induction; Blue arrows: inhibition; Dashed lines: hypothetical mechanism. Scheme is from (Li et al., 2018).

Because of their immunomodulatory and suppressive properties, Tregs can have an indirect function by acting on other immune cells during the wound healing process. In the inflammatory phase Tregs secrete anti-inflammatory cytokines (e.g., IL-10, TGF-β, IL-35), which prevent the recruitment and repress the inflammatory function of other immune cells (e.g., neutrophils, CD4/CD8 T cells). Treg anti-inflammatory cytokines (e.g., TGF-β), also induce the apoptosis and subsequent phagocytosis of neutrophils as well as the conversion of macrophages from pro-inflammatory to pro-healing. As such via the secretion of these anti-inflammatory mediators Tregs influence the immune landscape within the wound tilting it from an inflammatory towards a remodeling/resolving phenotype (Li et al., 2018) (Fig 18).

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Besides this indirect modulation of other immune cells, Tregs are also capable of directly promoting tissue repair and regeneration through their production and secretion of growth factors such as amphiregulin (Areg) (Li et al., 2018) (Fig 18).

### **Afferent signals necessary for Treg repair function**

TCR signaling is essential in regulating Treg differentiation, homeostasis, and suppressive function in many contexts (Li and Rudensky, 2016). However, in tissue repair, TCR stimulation is not always considered paramount to elicit tissue-repair, as it is not required for Tregs to produce repair mediators (e.g., Areg). On the contrary, TCR stimulation leads to the decrease of Areg production by Tregs but also to the increase in Areg<sup>neg</sup> Tregs. Indicating that TCR-stimulation can be detrimental for the production of key repair mediators (e.g., Areg) by Tregs in the damaged site (Arpaia et al., 2015).

Tregs also express surface receptors such as ST2, EGFR and IL-18R (Zhang et al., 2017). ST2 recognizes the IL-33 alarmin which is released by necrotic cells, while the ligand of IL-18R; IL-18 is first released in a non-active form by immune cells (e.g., macrophages, DCs) and cleaved by the alarmin-induced NLRP3 inflammasome (Chen and Nuñez, 2010; Yang et al., 2017; Zhang et al., 2017). As such Treg appear as capable of recognizing direct potent signals produced/cleaved following barrier disruption and subsequent cell death.

The EGF receptor is up-regulated by Tregs following full-thickness skin excision (Nosbaum et al., 2016). The lineage-specific deletion of EGFR on Tregs in the context of skin injury leads to a loss of Treg accumulation and activation, resulting in an increase pro-inflammatory macrophage presence and a delayed wound closure (Nosbaum et al., 2016). These results, demonstrate that, in the skin, following injury, EGFR is essential for the recruitment and repair/anti-inflammatory function of Tregs (Nosbaum et al., 2016). The ligand of EGFR is the epidermal growth factor Areg which promotes keratinocyte proliferation and wound healing. Importantly, the stimulation of Tregs via Areg could be autocrine as they secrete it themselves, but also exocrine as other immune cells can secrete it (e.g., dendritic-cells, mast cells) (Berasain and Avila, 2014).

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### **Dynamics in the skin**

Tregs numbers peaks around 1 to 2 weeks post-wounding and mainly originate from the secondary lymphoid organs (as shown by FTY720-blocking) (Nosbaum et al., 2016). Crucially, Tregs also represent up to 50% of the CD4+ T cell population in the steady-state skin of both human and mice (Gratz et al., 2013; Sanchez Rodriguez et al., 2014), suggesting that, as prepositioned cells they could act on the early stages of wound closure.

To assess the phase in which Tregs would play a role, Tregs were depleted either in the early (inflammatory phase) or late (resolution phase) healing stages. While deletion in the early stages (day 2 →5 post wounding) led to a significant delay in wound closure visible at day 5 until day 11, deletion in the late stages (days 5→10 post wounding) showed no differences as compared to the undeleted mice (Nosbaum et al., 2016). This shows that Tregs to play a role in the early wound repair process, it also raises the question: Which cells play the pro-repair role the resident or recruited Tregs?

### ii. $\gamma\delta$ T cells

#### **Different subsets**

In both humans and mice  $\gamma\delta$  T cells encompass several different subsets.  $\gamma\delta$  T cells are identified according to the expression of the  $\gamma\delta$  TCR.  $\gamma\delta$  T cell subsets are defined according to their specific V $\delta$  (for humans)/V $\gamma$  (for mice) chain usage, location (i.e., dermis *versus* epidermis), antigen-reactivity and cytokine production (Godfrey et al., 2015; Hu et al., 2022).

Here I will present the dynamics, activation, and functions of  $\gamma\delta$  T cells in wound healing; focusing on the most frequent  $\gamma\delta$  subsets in the skin of mice (V $\gamma$ 5, V $\gamma$ 4, V $\gamma$ 6) and mentioning some of the most relevant data for this work on human subsets (V $\delta$ 1, V $\delta$ 2) (Adams et al., 2015; Muñoz-Ruiz et al., 2017).

## INTRODUCTION

### **Dynamics in the skin**

According to their subsets,  $\gamma\delta$  T cells can be either resident at steady state (e.g., V $\gamma$ 5  $\rightarrow$   $\gamma\delta$ -DETC) or recruited following injury (e.g., V $\gamma$ 4) (Hu et al., 2022; Li et al., 2018b).

As stated previously,  $\gamma\delta$ -DETC (V $\gamma$ 5) are in the murine epidermis and present a “dendrite-like” aspect in the steady state skin. Following wounding, DETC “round-up” and proliferate within the damaged epidermis (Jameson et al., 2002). In the murine dermis, V $\gamma$ 4 and V $\gamma$ 6 can be found at steady state but the vast majority of these subsets are recruited to the wound site (Li et al., 2018b; Mabuchi et al., 2013). Recruitment to the wound site of V $\gamma$ 4 and V $\gamma$ 6 involves the binding of CCR6 to the CCL20 ligand produced by keratinocytes in the wound (Li et al., 2018b; Mabuchi et al., 2013). For these dermal  $\gamma\delta$  T cells an exchange between the skin and its draining lymph-nodes occurs, with a skin  $\rightarrow$  lymph-node migration of the V $\gamma$ 6 subset (mechanism not resolved) and a lymph-node  $\rightarrow$  circulation  $\rightarrow$  skin migration of the V $\gamma$ 4 subset (dependent on S1P1 and CCR2 signaling) (Maeda et al., 2015; Ramírez-Valle et al., 2015). Altogether, the dynamics of  $\gamma\delta$  T cells within the skin are dependent on their subtypes as well as the inflammatory status of the skin.

### **Role in the skin wound**

The absence of  $\gamma\delta$  T cells (TCR $\delta^{-/-}$  mice) leads to a 2–3 day delay in skin wound closure indicating a pro-repair role for these cells (Jameson et al., 2002).

In the wound, V $\gamma$ 5 and V $\gamma$ 4  $\gamma\delta$  T cells can enhance inflammation by promoting the recruitment of other immune cells (e.g., CCL-3/CCL-4/CCL-5: Macrophages, neutrophils,  $\alpha\beta$  T lymphocytes; M $\phi$ -1: monocytes; XCL1:  $\alpha\beta$  T lymphocytes) (Hu et al., 2022). In response to IL-1 $\beta$  and IL-23 produced by pro-inflammatory macrophages and neutrophils, the V $\gamma$ 4 subset also secrete IL-17A which further amplifies the inflammation within the skin (Sutton et al., 2009, 2012). Importantly, IL-17 produced by V $\gamma$ 4 cells can both promote and inhibit wound closure (MacLeod et al., 2013; Rodero et al., 2013). Although no experimental proof has yet emerged, it has been hypothesized that this dual effect for IL-17 may be related to the levels of IL-17 production (hypothesis: moderate accumulation in the wound periphery  $\rightarrow$  beneficial; important accumulation in the wound

## INTRODUCTION

center → detrimental) (Li et al., 2018b). This further emphasizes the importance of a controlled inflammatory response in the wound healing process.

Furthermore, in both human and mice  $\gamma\delta$  T cells have been shown to directly produce pro-repair mediators such as the insulin-like growth factor 1 (IGF-1) which promotes repair by stimulating cellular proliferation, differentiation and migration (e.g. keratinocytes, fibroblasts, endothelial cells) (Garoufalia et al., 2021; Jameson et al., 2002; Sadagurski et al., 2006; Toulon et al., 2009). In the wounds, the DETC  $\gamma\delta$  T cells subset also produce keratinocyte growth factors 1 and 2 (KGF-1-2) that can also directly promotes the proliferation and migration of epidermal keratinocytes (Werner et al., 1992).

### **Afferent signaling**

The different subsets of  $\gamma\delta$  T cells possess different TCR receptors at their surface and as such could potentially recognize different cognate ligands, although to date the nature of some of these ligands is still unknown (e.g., Human V $\delta$ 1; Murine V $\gamma$ 4) (Godfrey et al., 2015).

Despite DETCs being mainly  $\gamma\delta$  T cells in the skin some  $\alpha\beta$ -DETCs have also been described (Jameson et al., 2004). Since the  $\alpha\beta$ -DETCs lack the  $\gamma\delta$ -DETC TCR the TCR $\delta^{-/-}$  mice (devoid of  $\gamma\delta$ -DETC, with  $\alpha\beta$ -DETCs) provides clues to address if wound repair enacted by DETC relies on the V $\gamma$ 5 TCR. Interestingly,  $\alpha\beta$ -DETCs from TCR $\delta^{-/-}$  are incapable of damage-keratinocyte recognition as they fail to activate and produce cytokines while  $\gamma\delta$ -DETC up-regulate CD25 at 1 day post-wounding (Jameson et al., 2004). To assess if TCR-ligand interactions were imperative for the  $\gamma\delta$ -DETC repair function (without knowing the exact  $\gamma\delta$ -DETC ligand)  $\gamma\delta$ -DETC TCR-tetramers were used. Blocking the TCR-ligand interaction using  $\gamma\delta$ -DETC TCR-tetramers effectively blocked the healing process. Altogether this indicates that TCR-stimulation is required for  $\gamma\delta$ -DETC repair function (Komori et al., 2012).

Additionally, *in vivo* imaging revealed that whilst at steady state TCR-signaling of  $\gamma\delta$ -DETC was polarized towards the *stratum granulosum*, following tissue disruption it became polarized towards the *stratum basale* and surrounding Langerhans cells (Chodaczek et al., 2012). Without providing the exact ligand, *in vivo* imaging of the  $\gamma\delta$ -DETC strongly supports that these cells interact in a TCR dependent manner with the

## INTRODUCTION

epidermal cells at steady-state near the keratinocyte tight-junctions (Chodaczek et al., 2012). This supports the existence of a steady state ligand produced by the epidermis allowing for monitoring the skin integrity by these  $\gamma\delta$ -DETC. However, it does not exclude the existence of a stress-induced ligand(s) which, according to the authors could provide a different signal for the activation of  $\gamma\delta$ -DETC (Chodaczek et al., 2012). Additional research is required to define the nature of the  $\gamma\delta$ -DETC ligand(s) and the potential different functions/programs they induce.

Beside TCR-ligand interactions, other receptors provide stimulatory signals to  $\gamma\delta$ -DETC. These include NKG2D that recognized keratinocyte stress-receptors (Rae1/H60c) and JAML that binds to CAR (Coxsackie and Adenovirus receptor) expressed by keratinocytes (member of the Junctional Adhesion Molecule). These additional stimulatory signals act on  $\gamma\delta$ -DETC and can increase their proliferation, degranulation and cytokine and growth factor production. Preventing JAML-CAR interactions (using a soluble competitor) leads to a decrease activation and secretion of  $\text{TNF}\alpha$  by  $\gamma\delta$ -DETC and results in a delay of wound closure similar to a complete  $\gamma\delta$ -DETC deletion (Witherden et al., 2010). As such, this JAML-CaR interaction appears necessary for  $\gamma\delta$ -DETC pro-repair phenotype and possibly their repair function. Indicating that like Tregs DETC can recognize “danger-signals” provided by the damaged epithelium.

### iii. H2M3 T cells

#### **A microbial induced T-cell**

H2-M3-restricted T cells recognize bacterial ligands (that contain fMet: f-MIIINA and f-MFLLVN) presented by the non-classical MHC-Ib (H2-M3) molecule. Following *Staphylococcus epidermidis* association H2-M3 T-cells activate and expand within the lymph nodes, H2-M3 T-cells then colonize the skin (Linehan et al., 2018).

#### **H2-M3 and wound-healing**

Transcription analysis of H2-M3 T cells shows that *Staphylococcus epidermidis* topical application induces type 17 (*RORc*) and type 1 (*Tbx21*) cells. Detailed analysis reveals that type 17 induced H2-M3 T cells appear poised towards tissue repair with an

## INTRODUCTION

expression of immune-regulatory (e.g., *Il-10*, *Tnfsf4*, *Ctla4*) and tissue-repair associated genes (e.g., *Areg*, *Csf2*, *Furin*). The transcriptomic analysis therefore suggests that H2-M3 T cells could play various effector functions including wound repair. (Linehan et al., 2018)

Assessment of the repair function of H2-M3 function was done using H2-M3<sup>-/-</sup> (without H2-M3 T cells) or wild-type mice. H2-M3 T cells promote skin wound healing as H2-M3<sup>-/-</sup> mice have smaller epidermal tongues as compared to WT mice during skin wound healing. To address if the defect in wound closure was linked to the *Staphylococcus epidermidis* topical application (which leads to the activation and expansion of H2-M3 T cells) wound closure was assessed with or without *Staphylococcus epidermidis* application. WT mice without topical also presented significantly smaller epidermal tongues as compared to WT-*Staphylococcus epidermidis* mice. Indicating that the H2-M3 T cells and *Staphylococcus epidermidis* addition improve wound closure (readout: epithelial tongue length) (Linehan et al., 2018).

Deletion of the IL-13 expression (IL-13<sup>-/-</sup>) in mice resulted in an impaired skin wound healing *Staphylococcus epidermidis* colonized mice. The transfer of Bowie<sup>Tg</sup> CD8<sup>+</sup> T-cells (Bowie<sup>Tg</sup>: *Staphylococcus epidermidis* specific TCR-transgenic CD8<sup>+</sup> T-cells) could partially rescue this phenotype in IL-13<sup>-/-</sup> mice. However, no rescue was observed if Bowie<sup>Tg</sup> CD8<sup>+</sup> T-cells transfer was accompanied by IL-13 blocking (anti-IL-13), indicating that commensal-specific T cell mediated-repair (i.e., H2-M3, Bowie<sup>Tg</sup>) is associated with secretion of IL-13 within the wound. (Harrison et al., 2019)

### **Afferent signaling**

TCR signaling following *Staphylococcus epidermidis* application appears necessary for the expansion and acquisition of the tissue repair transcriptional program by H2-M3 T cells (Linehan et al., 2018). In the absence of tissue injury, type 17 H2-M3 T-cells co-express the ROR $\gamma$ t and Gata-3 transcription factors indicative of type 17 and type 2 programming respectively (Harrison et al., 2019). The co-expression of these transcription factor appears surprising as their expression was found previously to be mutually exclusive (e.g., ILCs) (Hsu et al., 2020; Zhong et al., 2016). Furthermore, these type 17 H2-M3 T-cells express the IL-17 protein (associated with ROR $\gamma$ t) and the IL-13

## INTRODUCTION

mRNA (associated with Gata-3) without IL-13 protein expression (Ivanov et al., 2006; Lavenu-Bombled et al., 2002).

As previously mentioned, following injury several mediators are released including alarmins such as IL-18. As IL-18 had been previously described as capable of eliciting type 2 responses; the authors hypothesized that IL-18 signaling would induce protein expression of IL-13 by the H2-M3 T cells (Arpaia et al., 2015; Harrison et al., 2015; Nakanishi et al., 2001). Accordingly, when injecting IL-18 *in vivo* these commensal specific H2-M3 T cells were found to produce type 2 mediators such as IL-5 and IL-13 while decreasing others such as the type 17 IL-17 (Harrison et al., 2019a).

These data show that H2-M3 cells are transcriptionally pre-poised at steady-state towards a pro-repair function (IL-13 mRNA) but require additional signaling to initiate translation and secrete their pro-repair mediators.

### iv. MAIT cells and skin repair

As introduced in this chapter: T cells (Tregs, H2M3, DETC) are capable of enacting tissue repair indirectly via the modulation of other immune cells (e.g., recruitment, induction of pro-repair phenotype) or directly via the secretion of pro-repair mediators (e.g., Areg). Reminiscent of these other immune T-cells, MAIT cells also express a pleiotropic arsenal of effector molecules including tissue repair mediators (e.g., Areg, TGF $\alpha$ , and IL-22) (Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019; Salou et al., 2019).

In addition to the transcriptomic pro-repair profile of MAIT cells, their pro-repair role is supported by *in vivo* and *in vitro* data that have recently been published. Using a scratch test (*in vitro*: monolayer of cell scratched to mimic a wound) as a tissue repair model human MAIT cells were shown capable of restoring the cellular mono-layer (Leng et al., 2019a). *In vivo* evidence was also provided by performing a full thickness wound on the back of either TCR $\delta^{-/-}$  (devoid of  $\gamma\delta$ -T cells) or TCR $\delta^{-/-}$ MR1 $^{-/-}$  (devoid of  $\gamma\delta$ -T cells and MAIT cells) mice as wound repair (readout: epidermal tongue length) was significantly lower in the mice devoid of MAIT cells. However, due to the low numbers of MAIT cells in the classical B6 laboratory strain, this pro-repair function was only evidenced in the absence of  $\gamma\delta$ -T cells and after *Staphylococcus epidermis* mediated expansion of MAIT cells

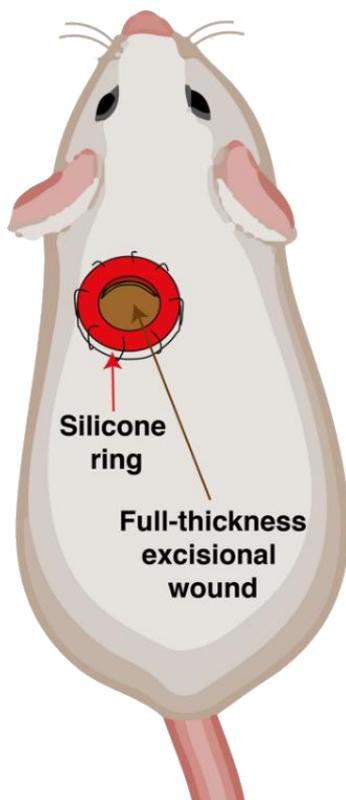
## INTRODUCTION

(Constantinides et al., 2019). As bacterial exposure has been shown to skew T-cell effector programming (e.g., H2-M3 via *Staphylococcus epidermis*) including MAIT cells (e.g., *Legionella longbeachea* type 1/17 MAIT cell phenotype), it is possible that skewing also occurred in this *in vivo* repair-setting (Harrison et al., 2019; Linehan et al., 2018).

Based on this strong evidence of a pro-repair function of MAIT cells, my work aimed at answering some of the pending questions on the mechanisms involved in this skin repair function. These questions and the skin damage model used throughout this work will be detailed in the objective and methodology sections.

# METHODOLOGY

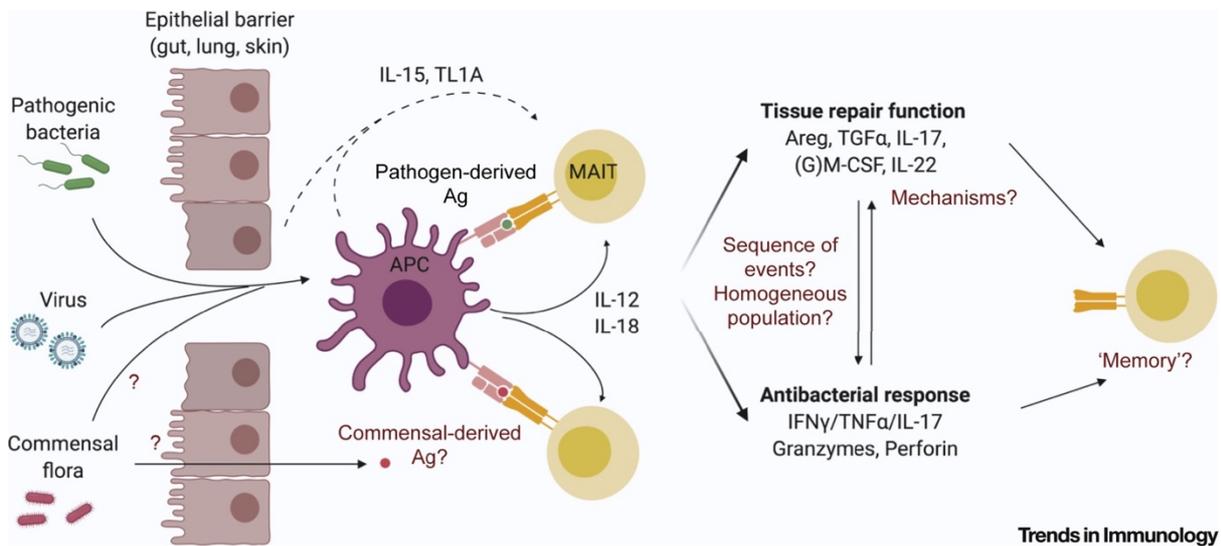
Although *in vitro* techniques such as the scratch test provide some very useful information on the capabilities of immune cells to induce reconstruction via cell proliferation, they do not provide clues on the cellular dynamics that occur at the whole organism level. To study tissue repair while maintaining this complexity, several *in vivo* techniques are currently used. One of these models is skin excision, a piece of the back skin including the whole skin up to the *panniculosus carnosus* is excised using a biopsy punch. This allows for a precise measurement of wound closure. One limitation is wound margin epithelial contraction that occurs in animals that have a “loose skin”, a phenotype due to the heightened skin elasticity and lack of strong adherence to underlying structures. This phenomenon also occurs in humans but to a lower extent and at a slower rate (Cross et al., 1995). While loose skin animals such as rabbits and mice mostly rely on epithelial contraction, human tissue repair is dependent on re-epithelization through proliferation and granulation. In order to tilt skin repair in mice towards a more “human-like” process one method is the placement of a silicone around the wound to inhibit epithelial contraction (Dorsett-Martin, 2004; Wong et al., 2011) (Fig 24).



On top of this skin excision model, I also used throughout this study the B6-CAST<sup>MAIT</sup> (B6 mouse with a 0.6Mb Castaneus genomic region of chromosome 14 in the TRAV-TRAD-TRAC locus) mouse, which harbors 6-10-fold more MAITs than the C57Bl/6 strain (Cui et al., 2015). This model provides an adequate readout of MAIT cell function.

**Fig 19: Scheme of the tissue repair skin excision silicone ring model.**

# THESIS OBJECTIVES



**Fig 20: Remaining questions on MAIT cell pro-repair role.** Figure is from (Salou and Lantz, 2019).

Fig 20 — Recently published *in vitro* and *in vivo* works provide strong evidence for the repair function of MAIT cells. However, several questions remain on the dynamics, afferent signals, and efferent functions of MAIT cells. The questions I will address in this work are listed below.

## Repair function of MAIT cells in an unmanipulated model.

Using the skin excision model with a silicone ring and the B6-CAST<sup>MAIT</sup> mice either on a MR1+ or MR1- background (with or without MAIT cells), I first assessed the impact of MAIT cells on skin wound healing in the presence of a full immune system and without preemptive amplification by *Staphylococcus epidermis*. This unmanipulated “human-like” repair model enables to assess the mechanisms involved in the repair function of MAIT cells.

### **Dynamics of MAIT cells in the skin: recruitment versus proliferation.**

As mentioned previously MAIT cells are prepositioned at steady state in the mucosal sites. Following *Staphylococcus epidermis* association MAIT cells were found to not recirculate between parabiotic mice indicating tissue residency of these cells within the skin (Constantinides et al., 2019a). Using complementary models (parabiosis, graft and Kaede mice), we set out to understand if this residency was also observed in the absence of bacterial skewing, at steady state and in the context of a tissue insult.

### ***In vivo* afferent signals required for MAIT cell functions**

TCR-mediated stimulation leads to the up regulation of pro-repair mediators by MAIT cells *in vitro* (Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a). Additionally, the local microbiota in our SPF mice could potentially provide the cognate ligand. Therefore, we sought to assess if cognate-mediated activation was required for MAIT cells repair function and for their dynamics *in vivo* within the skin.

### **Effector functions of MAIT cells.**

The final objective of this thesis was understanding how MAIT cells exert their tissue repair function *in vivo*. Would it be indirectly via the recruitment or activation of other immune cells? MAIT cells have been previously described as capable of modulating other immune cells such as monocyte differentiation into dendritic cells in the lungs (Meierovics and Cowley, 2016).

Given the pleiotropic effector capabilities of MAIT cells: could this function be directly exerted? What would be the mediators? antibacterial or tissue repair effector molecules or both?

The use of transcriptomic analysis of single MAIT cells within the skin both at steady state and after wounding was used to assess

- whether MAIT cells represent a homogenous or heterogenous population within the skin at steady state and during wound healing;
- whether a specific differentiation program is expressed within the skin and to identify the mediators necessary for MAIT cell repair function.

# Results

# MECHANISMS UNDERLYING MAIT CELL ABILITY TO PROMOTE SKIN WOUND REPAIR

Anastasia du Halgouët<sup>1</sup>, Aurélie Darbois<sup>1</sup>, Mansour Alkobtawi<sup>2</sup>, Martin Mestdagh<sup>1</sup>, Aurélie Alphonse<sup>1</sup>, Virginie Premel<sup>1</sup>, Thomas Yvorra<sup>3</sup>, Ludovic Colombeau<sup>3</sup>, Dietmar Zaiss<sup>4</sup>, Yara El Morr<sup>1</sup>, Hélène Bugaut<sup>1</sup>, François Legoux<sup>1</sup>, Laetitia Perrin<sup>1</sup>, Selim Aractingi<sup>2</sup>, Rachel Golub<sup>5</sup>, Olivier Lantz<sup>1,6,7\*</sup>, Marion Salou<sup>1\*</sup>

\*equal contribution

## Affiliations

- 1) INSERM U932, PSL University, Institut Curie, 75005 Paris, France.
- 2) Cutaneous Biology, Institut Cochin, Inserm 1016 and Université de Paris Cité, 75014 Paris, France
- 3) Department of Immune Medicine, University of Regensburg, Regensburg, Germany
- 4) Pasteur Institute, Université de Paris, INSERM U1223, 75724 Paris, France.
- 5) Laboratoire d'Immunologie Clinique, Institut Curie, Paris, 75005, France.
- 6) Centre d'investigation Clinique en Biothérapie Gustave-Roussy Institut Curie (CIC-BT1428) Institut Curie, Paris, 75005, France.

## Abstract

Tissue repair processes maintain proper organ function following mechanical or infection-related damage. In addition to anti-bacterial properties, MAIT cells express a tissue repair transcriptomic program and promote skin wound healing when expanded. Herein, we use a human-like full-thickness skin excision mouse model to assess the underlying mechanisms of MAIT cell tissue repair function. Single-cell RNAseq analysis suggests that skin MAIT cells already express a repair program at steady state. Following skin excision, MAIT cells promote keratinocyte proliferation thereby accelerating healing. Using skin grafts, parabiosis and adoptive transfer experiments, we show that MAIT cells migrate into the wound from other tissues in a TCR-independent but CXCR6-dependent manner. Amphiregulin secreted by MAIT cells following excision promotes wound healing. Expression of the repair function is probably maintained independently of sustained TCR stimulation. Overall, our study provides mechanistic insights into MAIT cell wound healing function in the skin.

## MAIN MANUSCRIPT

### Introduction

Restoring skin barrier following damage is key to maintain its function. The first step of skin healing is an inflammatory phase preventing infection and promoting debris clearance. Then, proliferation and migration of keratinocytes, endothelial cells and fibroblasts creates new tissue. The final remodeling/reorganization phase lasts for months (Gurtner et al., 2008b). Delayed or improper healing may result in pain and infection, up to cutaneous carcinogenesis and limb amputations. Understanding the fine tuning of skin healing is therefore crucial.

Anti-infectious and pro-inflammatory functions of T cells are well described, but several T cell subsets are also involved in skin homeostasis. Upon skin injury, type 17 commensal-specific CD8<sup>+</sup> T cells express type 2 cytokines leading to tissue repair (Harrison et al., 2019b). Skin  $\gamma\delta$  T cells also promote wound healing through secretion of various molecules including keratinocyte growth factors and IL-17 (Jameson et al., 2002; MacLeod et al., 2013). Recently, Mucosal Associated Invariant T (MAIT) cells were shown to have tissue repair potential (Constantinides et al., 2019b; Hinks et al., 2019b; Lamichhane et al., 2019b; Leng et al., 2019b), but the *in vivo* mechanisms involved are unclear.

In humans, MAIT cells represent the most abundant T cell subset with a single specificity (Franciszkiewicz et al., 2016; Martin et al., 2009b). MAIT cells recognize an unstable compound, 5-OP-RU, stabilized and presented by the MH1b molecule MR1 (Corbett et al., 2014). 5-OP-RU derives from the riboflavin (Vitamin B2) synthesis pathway present in most bacteria and yeasts but not in animal cells (Kjer-Nielsen et al., 2012). In mice, MAIT cells encompass MAIT1 (expressing Tbet) and MAIT17 (expressing ROR $\gamma$ t) cell subsets (Rahimpour et al., 2015). These subsets secrete different effector molecules, from IFN- $\gamma$  and cytotoxic molecules for MAIT1 cells, to IL-17, (G)M-CSF and tissue repair mediators for MAIT17 cells (reviewed in Legoux et al., 2020).

MAIT cells are numerous in human tissues, representing 2-10% of T cells in the gut, around 4% in the lungs and up to 15% in the liver (Provine and Klenerman, 2020b). Tissue homing is driven by the master transcription factor Promyelocytic Leukemia Zinc Finger (PLZF), which downregulates Klf2 and its target CD62L (Mao et al., 2016).

## Results

Parabiosis experiments, which join the vascular systems of two mice, demonstrated that MAIT cells reside in lungs, liver and spleen at steady state, similar to invariant Natural Killer T (iNKT) cells (Salou et al., 2019b; Thomas et al., 2011b). Altogether, these innate-like T cell populations resemble mainstream tissue resident memory (TRM) cells that remain in tissues following resolution of infection and confer protection upon reinfection (Masopust and Soerens, 2019b).

MAIT cells represent 0.5-2% of T cells in the human skin (Provine and Klenerman, 2020b), reaching up to 40% in the mouse skin with a high variability range (1-40% depending on the cage) (Constantinides et al., 2019b; Salou et al., 2019b). The increase of MAIT cell numbers in *Tcrd*<sup>-/-</sup> mice (Constantinides et al., 2019b) suggests competition for peripheral niches, but deletion of the TCR delta locus also influences the TCR alpha chain rearrangement process (Dauphars et al., 2022). *In vivo*, after antigen-driven expansion, MAIT cells promote skin wound healing in C57BL/6 mice (Constantinides et al., 2019b). *In vitro*, MAIT cell culture supernatant promotes proliferation of an intestinal epithelial cell line (Leng et al., 2019b). Whether this holds true *in vivo* remains unknown. Moreover, the mechanisms triggering MAIT cell repair function have not been determined. *In vitro* analyses suggest that MAIT cells acquire tissue repair program following TCR triggering (Hinks et al., 2019b; Lamichhane et al., 2019b; Leng et al., 2019b). *In vivo*, whether recognition of microbiota-derived 5-OP-RU or any endogenous ligand occurs during wound healing is unknown. Additional signals may be required for eliciting MAIT cell tissue repair function, such as cytokines, tissue cues, or activating signal duration (Legoux et al., 2020b).

To address the mechanisms of MAIT cell repair function, we turned to a human-like model of full thickness skin excision in mice. We showed that wound closure was accelerated in the presence of MAIT cells and analyzed the mechanisms leading to MAIT cell accumulation at the wound site. We tested whether TCR triggering was necessary for either MAIT cell accumulation or tissue repair function. Finally, we explored the mechanisms favoring wound healing and showed a key role of MAIT cell-derived amphiregulin (Areg). Thus, our work unraveled migration and effector mechanisms leading to MAIT cell-dependent skin repair.

## Results

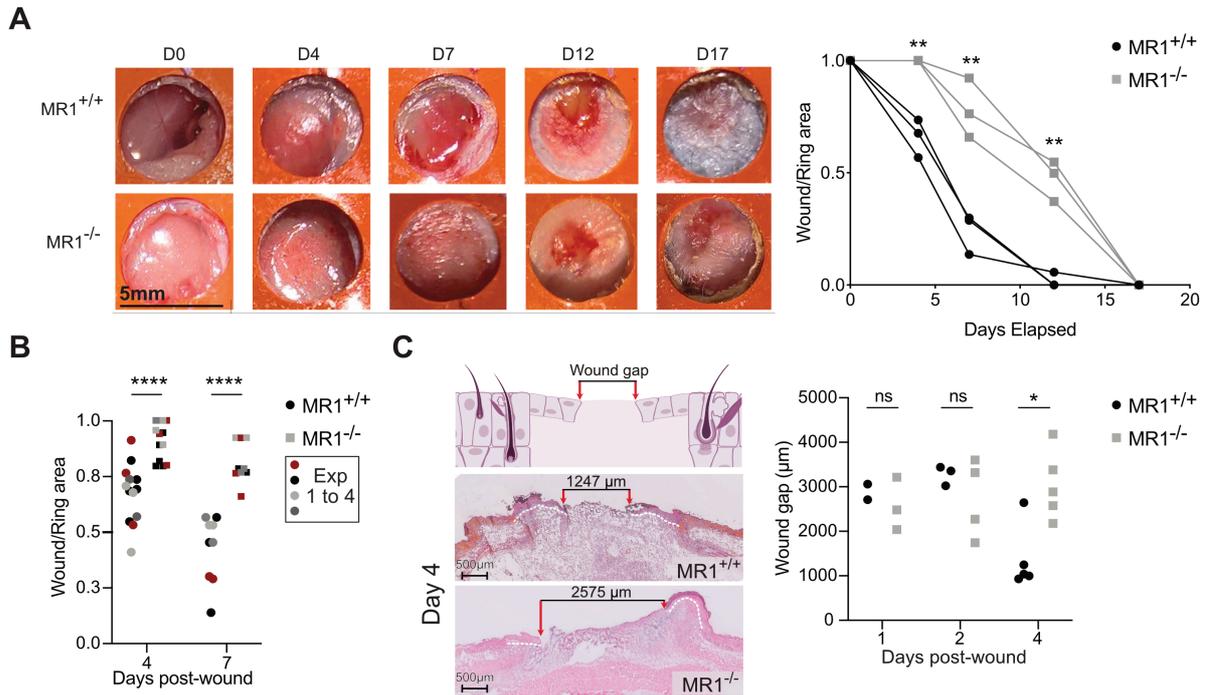
### **MAIT cells accelerate wound closure.**

Until now, the pro-repair functions of MAIT cells *in vivo* were observed after increasing their numbers in the skin, either in *S. epidermidis*-associated *Tcrd*<sup>-/-</sup> animals or after topical application of 5-OP-RU in C57BL/6 mice (Constantinides et al., 2019b). To dissect the mechanisms by which MAIT cells improve skin wound healing in immunocompetent non-manipulated animals, we took advantage of the B6-MAIT<sup>CAST</sup> mouse strain with higher MAIT cell numbers than the C57BL/6 mice (Cui et al., 2015). Of note, in the skin, MAIT cell numbers depend rather on the housing cages than on the strain used (Constantinides et al., 2019b; Salou et al., 2019b). To prevent skin contraction occurring in mice but not in humans, full-thickness excision punches were splinted using a silicon ring (Dunn et al., 2013). We compared wound healing in the presence or absence of MAIT cells using MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice, respectively. Wound closure (assessed by the wound-on-ring area ratio) was faster in MR1<sup>+/+</sup> as compared to MR1<sup>-/-</sup> animals, as early as day 4 (Fig. 1A, 1B). Analysis of Haematoxylin-Eosin-Safran-colored tissue sections evidenced smaller wound gaps at day 4 in MR1<sup>+/+</sup> as compared to MR1<sup>-/-</sup> animals (Fig. 1C).

To test whether the MR1 molecule itself was involved in wound healing, we assessed wound closure in MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice devoid of T cells (CD3<sup>-/-</sup> mice). As expected in the absence of T cells, wound closure was delayed (50% closure at day 14 instead of day 6 in B6-MAIT<sup>Cast</sup> strain), but no difference was observed between MR1<sup>+/+</sup> and MR1<sup>-/-</sup> backgrounds (Fig. S1A). In the T cell compartment, MR1 deficiency only affects MAIT cells (Franciszkiewicz et al., 2016; Treiner et al., 2003). Hence, MAIT cells accelerate wound healing early on in this human-like excision model.

Fig 1

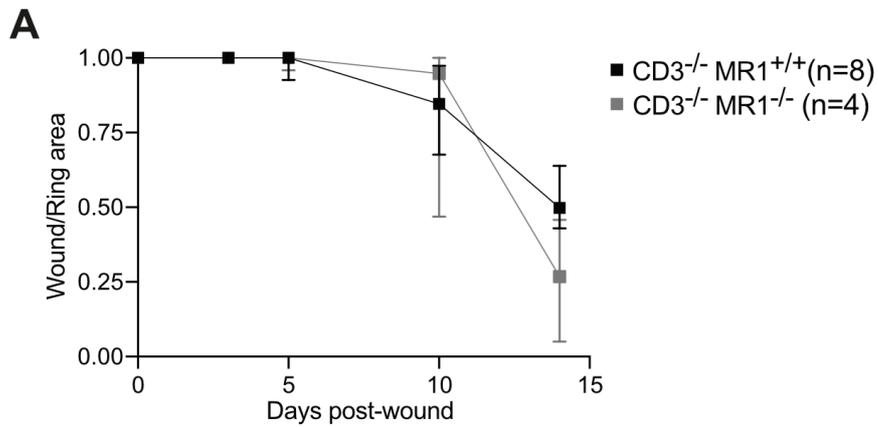
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**Fig 1: MAIT cells accelerate wound closure.**

Full thickness wounds were performed on B6-MAITCAST mice and splinted with a silicone ring (orange) to prevent epithelial contraction.

- (A)** Representative pictures and longitudinal of wound surface (ratio Wound/Ring area) for MR1<sup>+/+</sup> (black circle) or MR1<sup>-/-</sup> (grey square) littermates. *Blind experiment. t-test.*
- (B)** Wound surface at days 4 and 7. *Pooled data from 4 independent experiments (n<sub>4</sub>=13; n<sub>7</sub>=9). Mann-Whitney test.*
- (C)** Haematoxylin-Eosin-Safron staining of MR1<sup>+/+</sup> and MR1<sup>-/-</sup> wounds four days after excision and longitudinal follow-up of wound gap (distance between the epithelial tongues). *Pooled data from 2 independent experiments analyzed blindly (n<sub>1</sub>=2/3; n<sub>2</sub>=3/4; n<sub>4</sub>=5). Mann-Whitney test.*



**Fig S1 related to figure 1: MR1 deficiency alone is not sufficient to delay wound closure.**

**(A)** Wound surface follow-up in CD3<sup>-/-</sup> MR1<sup>+/+</sup> (black square) or MR1<sup>-/-</sup> (light grey square) mice. Pooled data from 2 independent (n=8/4) experiments.

## **Skin MAIT cells are type 17, express a tissue repair program, and increase in numbers at wound site.**

To better understand MAIT cell involvement in wound healing, we analyzed their number and phenotype in the skin. Four days after excision, the percentage of TCR $\beta$ <sup>+</sup>MR1:5-OP-RU Tet<sup>+</sup> MAIT cells (Fig. S2A) was significantly increased at the wound site as compared to contra-lateral control skin (Fig. 2A left and middle panels). To account for the variability of MAIT cell numbers between animals (Constantinides et al., 2019b), MAIT cell number at the wound site was normalized to the number from the same surface of the control site. MAIT cell numbers at the wound site increased up to 10-fold at days 4 and 6 and returned to basal levels by day 35 (Fig. 2A right panel). Thus, skin repair is associated with a large and early increase of MAIT cell numbers following excision.

To provide an unbiased view of MAIT cell functions in the skin, we characterized their transcriptome at steady state and four days after excision using single cell RNA sequencing (scRNAseq) from B6-MAIT<sup>CAST</sup> mice. The two datasets were integrated together with a thymic dataset (Legoux et al., 2019c) to provide a reference (Fig. 2B top panel). Twelve clusters were defined. Based on expression of *Zbtb16* and *Rorc* (Fig. 2B bottom panel) as well as gene sets from the literature (Table S1 and Fig. 2C), MAIT0 (clusters 0 and 1), MAIT1 (cluster 2), cycling MAIT (cluster 3, mainly thymus) and MAIT17 (clusters 4 to 12) cells were identified. Although expressing the MAIT17 signature at a low level, cluster 3 belonged to the MAIT17 cell subtype, as seen by *Rorc* expression (Fig. 2B). Importantly, MAIT1, MAIT17 and cycling cells from the 3 datasets merged, which demonstrated successful integration.

Analyzing the differentially expressed genes between all clusters (Table S2, adjusted p-value<0.05) allowed detailed description of MAIT17 cell clusters. The thymus-specific cluster 5 (Fig. 2D and Table S2) expressed the ribosomal *Rpl* and *Rps* genes, indicating active protein synthesis. On the contrary, the ribosomal genes were downregulated in the skin-specific cluster 6, suggesting resting cells. Many interferon-related genes (including *Isg15*, *Cxcl10*, *Ifit1*, *Stat1*, *Bst2*) were expressed in cluster 4, defining interferon stimulated gene (ISG)-MAIT cells both in the thymus and skin, as described for thymic iNKT cells (Baranek et al., 2020) (Fig. 2C). *Gzmb* and *Gzmc* had the highest fold change in cluster 7 (Table S2), suggesting cytotoxic capacities. Interestingly, the skin-specific cluster 8 (Fig. 2D) overexpressed genes associated with tissue repair

## Results

such as *Il17a*, *Il17f*, *Areg*, the hypoxia-induced factor 1- $\alpha$  *Hif1a*, *Itgav* and *Fgl2* (Table S2). No specific function was identified in the remaining MAIT17 cell clusters (9 to 12) which represent variations of a common program. Thus, MAIT17 cells span various transcriptional states and effector functions, including a skin-specific subset expressing repair mediators.

In the skin, most MAIT cells were type 17 both before and after excision (Fig. 2B and 2D), as confirmed at the protein level using a *Rorc*-GFP reporter mouse (Fig. 2E) and intracellular staining for ROR $\gamma$ t and Tbet (Fig. S2B). Strikingly, scRNAseq datasets from wound and steady state skins fully overlapped and were evenly distributed in the different clusters (Fig. 2D and S2C). Moreover, gene expression was highly correlated between the two datasets (Fig. 2F), suggesting that the functional program responsible for accelerated wound closure was already expressed at steady state. To better understand skin MAIT17 cell specificities, we compared skin and thymic MAIT17 cell clusters (Table S3). Skin MAIT17 cells were more activated with overexpression of *Cd69*, *Cd44*, *Nr4a1/3* as well as *Jun/Fos* and *Nfkb* pathways (Fig. 2G and Table S3). TCR signaling in MAIT cells from the skin was confirmed by flow cytometry using the *Nr4a1*-GFP reporter mouse (Fig. 2H). Additionally, skin MAIT17 cells overexpressed the TGF $\beta$  induced factor homeobox *Tgif1* (Fig. 2G). Accordingly, skin MAIT17 cells overexpressed genes identified in skin TRM cells expressing the TGFBR2 receptor (Christo et al., 2021a) (Fig. S2D). These results suggest that skin MAIT cells rely on TGF $\beta$  for retention and functions, similarly to mainstream TRM cells (Casey et al., 2012; Christo et al., 2021a; Hirai et al., 2021b; Mackay et al., 2015). Interestingly, *Zfp36*, *Zfp36l1*, and *Zfp36l2* were also upregulated in skin MAIT17 cells (Table S3). The corresponding proteins regulate the stability of mRNAs encoding cytokines and other immune mediators (Makita et al., 2021).

Finally, overexpression of *Hif1a* (Fig. 2G) suggested that skin MAIT17 cells have tissue repair capacity (Rezvani et al., 2011). To formally assess this hypothesis, we tested the enrichment of a tissue repair signature from commensal-specific skin CD8<sup>+</sup> T cells (Linehan et al., 2018b) previously used to assess MAIT cell tissue repair potential (Hinks et al., 2019b). Strikingly, all MAIT17 cells from the skin overexpressed the repair signature as compared to thymic cells (Fig. 2I). This was confirmed using three other tissue repair gene sets expressed by *Areg*-producing regulatory T cells responsible for muscle (Burzyn et al., 2013) or lung repair (Arpaia et al., 2015), or demonstrated to have

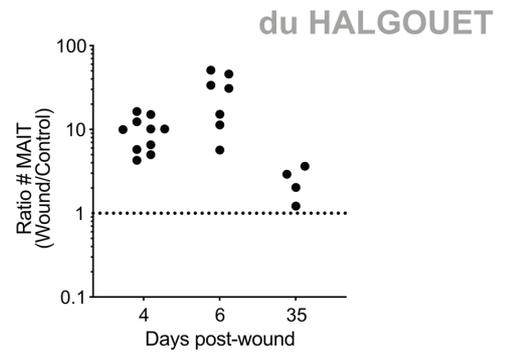
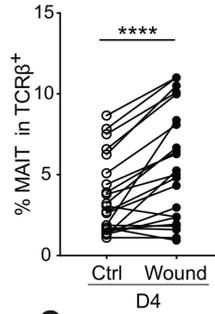
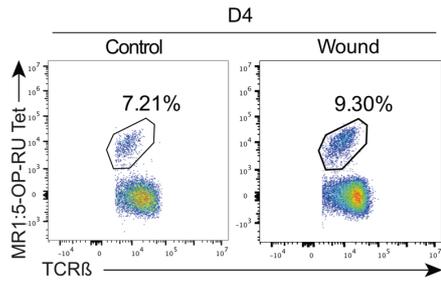
## Results

an *in vivo* repair function in full-thickness wounds (identified in the TiRe database (Yanai et al., 2016)) (Fig. S2E). On average, MAIT17 cells from cluster 8 expressed high levels of all 4 tissue repair signatures, as did the skin-specific clusters 6, 9, 10 and 12 (Fig. 2I and S2F). MAIT17 cells from the thymus (cluster 5) slightly overexpressed the tissue repair signatures as compared to immature MAIT0 cells (Fig. 2I and S2F). Thus, the MAIT17 cell program was associated with tissue repair functions, and skin location reinforced this program.

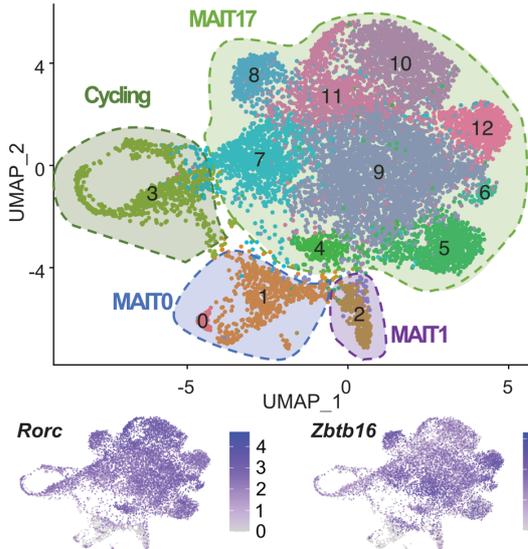
Altogether, the number of MAIT cells increased in the skin after excision but their transcriptome was not modified. Notably, wound closure was correlated ( $R^2=0.40$ ) with the increase in T cell numbers (Fig. S2G). The correlation was slightly higher ( $R^2=0.44$ ) with the increase of MAIT cell numbers in the wound (but not with the normalized percent of MAIT cells within T cells) (Fig. S2H), further suggesting the involvement of MAIT cells in wound healing.

# Results

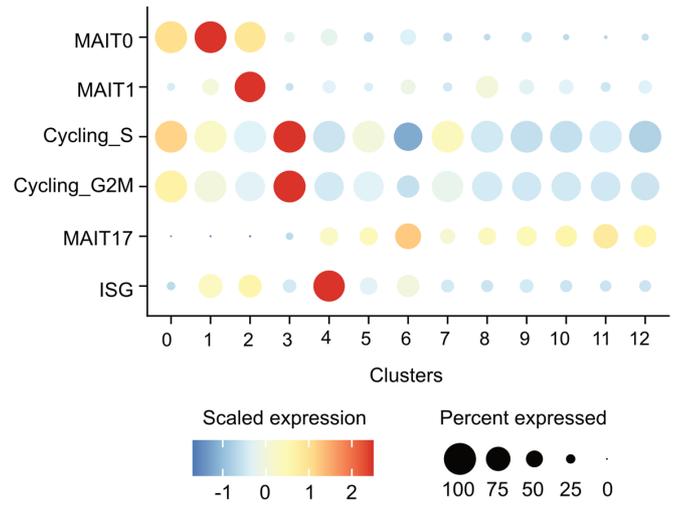
**Fig 2**  
**A**



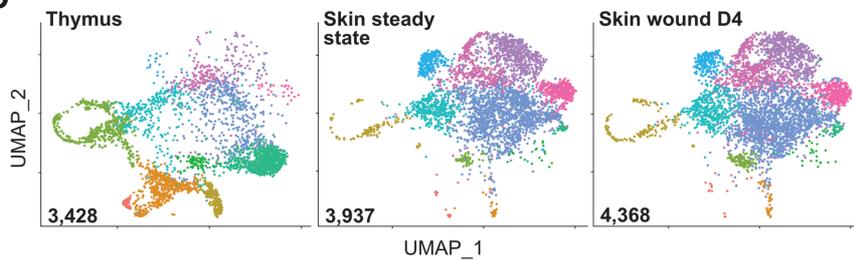
**B** Integration thymus, skin steady-state and wound (D4)



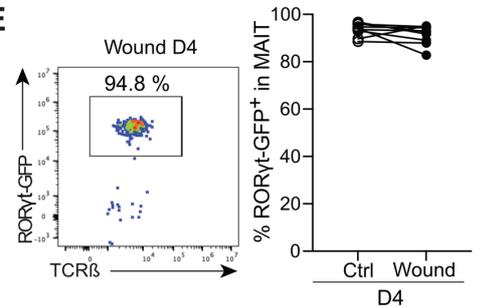
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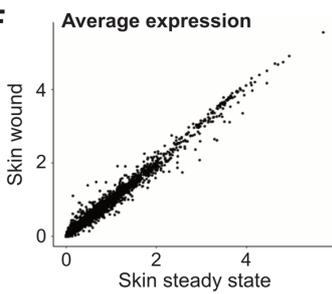
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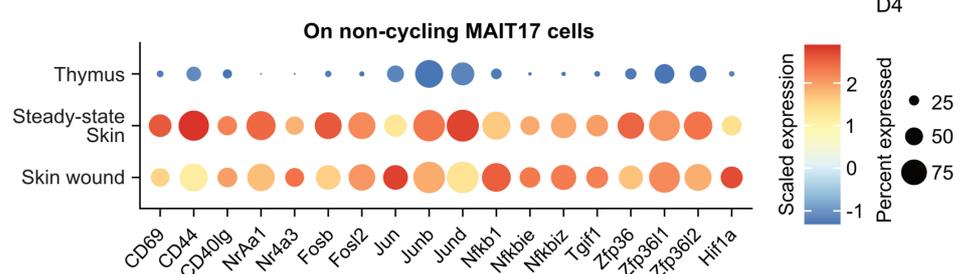
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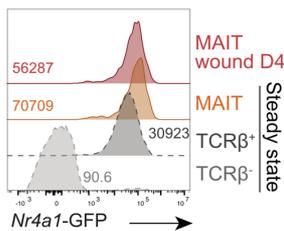
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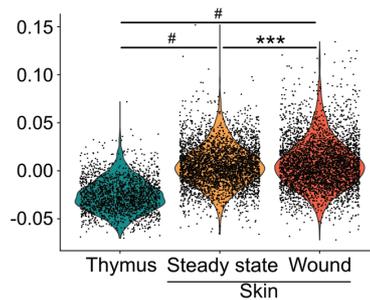
**G**



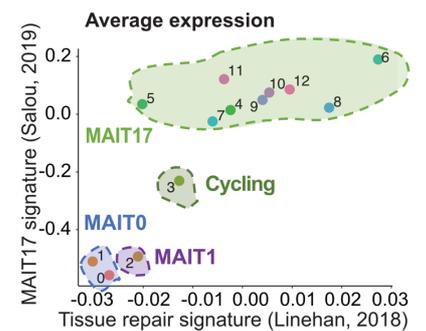
**H**



**I** Tissue repair signature (Linehan, 2018) on non-cycling MAIT17 cells



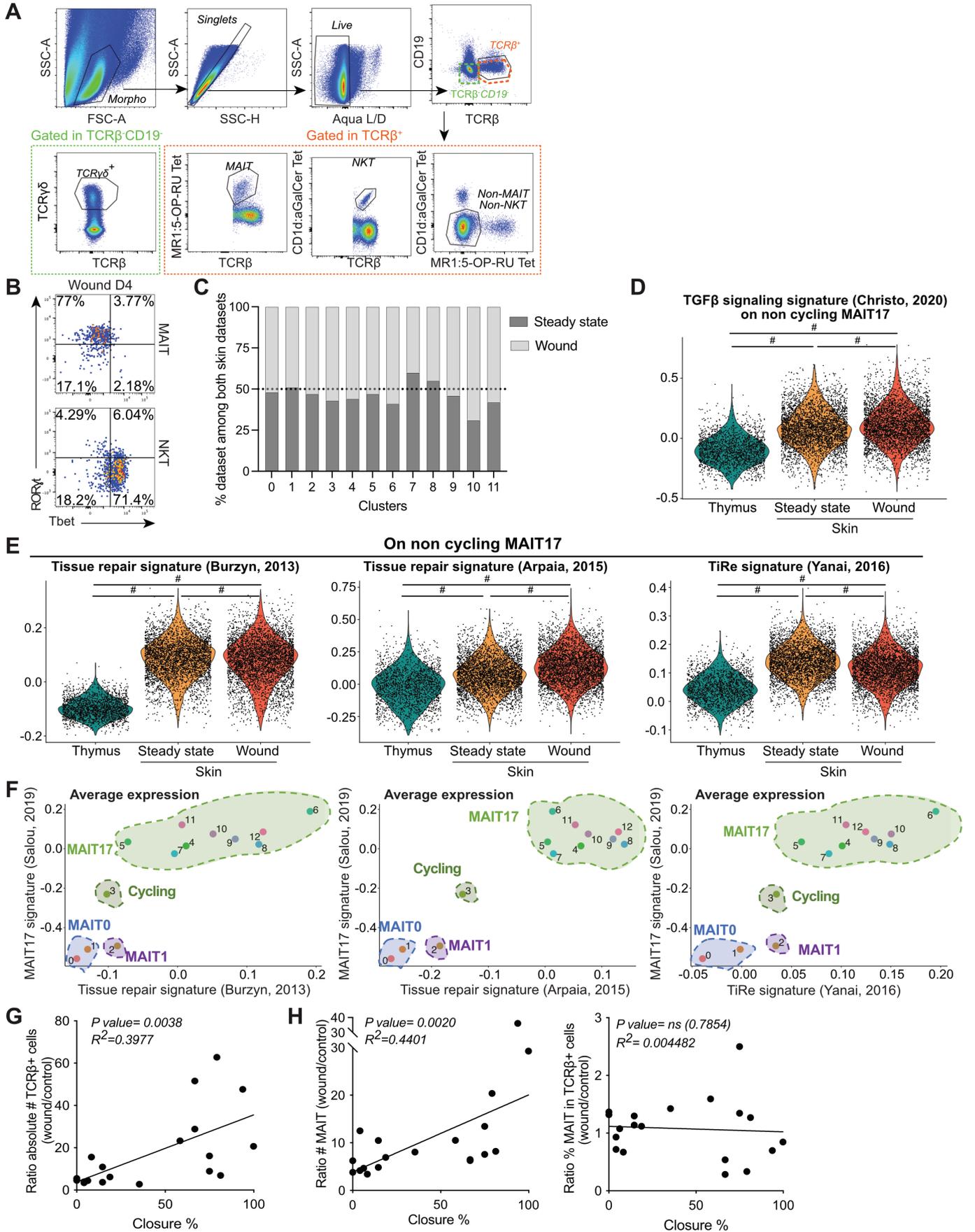
**J**



**Fig 2: Skin MAIT cells accumulate in the wound and constitute a homogeneous type 17 T cell population with a tissue repair program.**

- (A)** Flow cytometry staining (left), frequency (middle) and number (left, ratio wound/control numbers) of skin MAIT cells from wound and control sites at various time points. Pooled data from 7 (n=22) and 4 (nD4=10, nD6=7 and nD35=4) independent experiments for frequencies and numbers, respectively. *Wilcoxon Test*.
- (B)** MAIT cells sorted from thymus, wound (D4) and steady-state skin were analyzed by scRNA-seq and integrated. UMAP (top) and features plot for *Zbtb16* and *Rorc* expression (bottom) are displayed.
- (C)** Clusters were defined by signature enrichment (Table S1 and methods).
- (D)** UMAP from fig. 2B split according to dataset origin.
- (E)** ROR $\gamma$ t-GFP expression by MAIT cells from wound and control sites. *Pooled data from 3 independent experiments (n=8)*.
- (F)** Average gene expression from MAIT cells in wound site and steady state skin.
- (G)** Differentially expressed genes in non-cycling MAIT17 cells from skin (wound and steady state) as compared to thymus. The average expression was calculated on scaled data after subsetting MAIT17 clusters from (B).
- (H)** Nr4a1-GFP reporter expression by skin MAIT cells from wound (red) and control (orange) skin sites, by steady-state non-MAIT TCR $\alpha$ <sup>+</sup> (dark grey) and by TCR $\alpha$ <sup>-</sup> (light grey) cells. Data are representative of 2 independent experiments (n=5).
- (I)** Tissue repair (Linehan et al., 2018b) signature score on non-cycling MAIT17 cells. Tukey's multiple comparison test.
- (J)** Average expression of tissue repair (Linehan et al., 2018b) and MAIT17 (Salou et al., 2019c) signatures on clusters from fig. 2B.

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**Fig S2 related to figure 2**

- (A)** Gating strategy used to define MAIT, NKT, non-MAIT non-NKT (mainstream) and  $\gamma\delta$  T cells.
- (B)** Flow cytometry example of Tbet and ROR $\gamma$ t intracellular staining on MAIT and NKT cells. *Data are representative of 3 independent experiments (n=8).*
- (C)** Distribution of MAIT cells from the wound and steady-state skin in each single cell cluster, among the total number of skin MAIT cells.
- (D)** TGF $\beta$  signaling (Christo et al., 2021a) signature score on non-cycling MAIT17 cells. *Tukey's multiple comparison test.*
- (E)** Tissue repair signature score as in (D). The signatures are expressed by regulatory T cells producing Areg in the muscle (Burzyn et al., 2013) and in the lungs (Arpaia et al., 2015). The 3<sup>rd</sup> one was extracted from the TiRe database (Yanai et al., 2016). *Tukey's multiple comparison test.*
- (F)** Average expression of tissue repair (signatures presented in (E)) and MAIT17 (Salou et al., 2019c) signatures in all clusters of the integrated dataset of thymic, wound and steady state skin MAIT cells.
- (G)** Correlation between percent of closure and the increase in T cell number in the wound (wound/control site ratio). The line represents the linear regression. *Data are from 6 independent experiments (n=17).*
- (H)** Correlation between percent of closure and the increase in MAIT cell number or percent in the wound (wound/control ratio). The lines represent linear regressions. *Data are from 6 independent experiments (n=17).*

## MAIT cells are recruited to the inflamed skin

Increased MAIT cell number in the wound could result from either *in situ* proliferation or recruitment. Ki67 staining showed that MAIT cells proliferated similarly at wound and control sites (Fig. 3A), suggesting that proliferation alone was not responsible for the increased number of MAIT cells in the wound. To determine whether MAIT cells were recruited, we performed skin excision on parabiotic pairs (Fig. 3B left panel). By contrast to lung MAIT cells which were mostly of host origin (Fig. S3A), skin MAIT cells had exchanged between the two parabionts after 5 weeks of parabiosis: up to 50% of MAIT cells were partner-derived in steady state, control or wound skins (Fig. 3B). This high exchange rate was not a technical artifact as skin  $\gamma\delta$  T cells remained in their original parabiont as expected (Tan et al., 2019). Still, MAIT17 cells expressed higher levels of a residency signature (and slightly lower levels of a circulating signature) (Milner et al., 2017) in skin as compared to thymus (Fig. 3C). The shorter retention time of MAIT cells in the skin as compared to other organs could be related to their variable CD103/CD69 expression pattern: while CD69 and CD103 are both necessary for virus-specific skin TRM persistence (Mackay et al., 2013), MAIT cells were mainly CD103<sup>+</sup> but expressed low to medium levels of CD69 (Fig. 3D).

As skin MAIT cells exchanged at steady state, parabiotic pairs were not suitable to study their trafficking after excision. We turned to a skin graft model (Fig. 3E left panel). Six days after graft, all MAIT cells within the graft originated from the recipient, while around 70% of  $\gamma\delta$  T cells remained of graft origin (Fig. 3E middle panel), demonstrating possible MAIT cell recruitment into the skin. Moreover, MAIT cell numbers increased over time in the graft (Fig. 3E right panel), confirming their influx into the healing skin.

To assess whether MAIT cells were recruited from other organs or surrounding skin similarly to cutaneous  $\gamma\delta$  T cells (O'Brien and Born, 2015; Sumaria et al., 2011), we used the Kaede photo-convertible mouse. This mouse ubiquitously expresses the Kaede GFP, which shifts from green to red light emission after violet light illumination (Tomura et al., 2008). Following whole-body illumination (D0), all T cells including  $\gamma\delta$  T and MAIT cells were photoconverted (Green<sup>+</sup>Red<sup>+</sup>) in the skin (Fig. 3F).  $\gamma\delta$  T cells remained largely photo-converted at day 2, confirming their residency in the skin. In contrast, skin MAIT cells were replaced by non-converted ones as soon as day 2 (Fig. 3F). These results

## Results

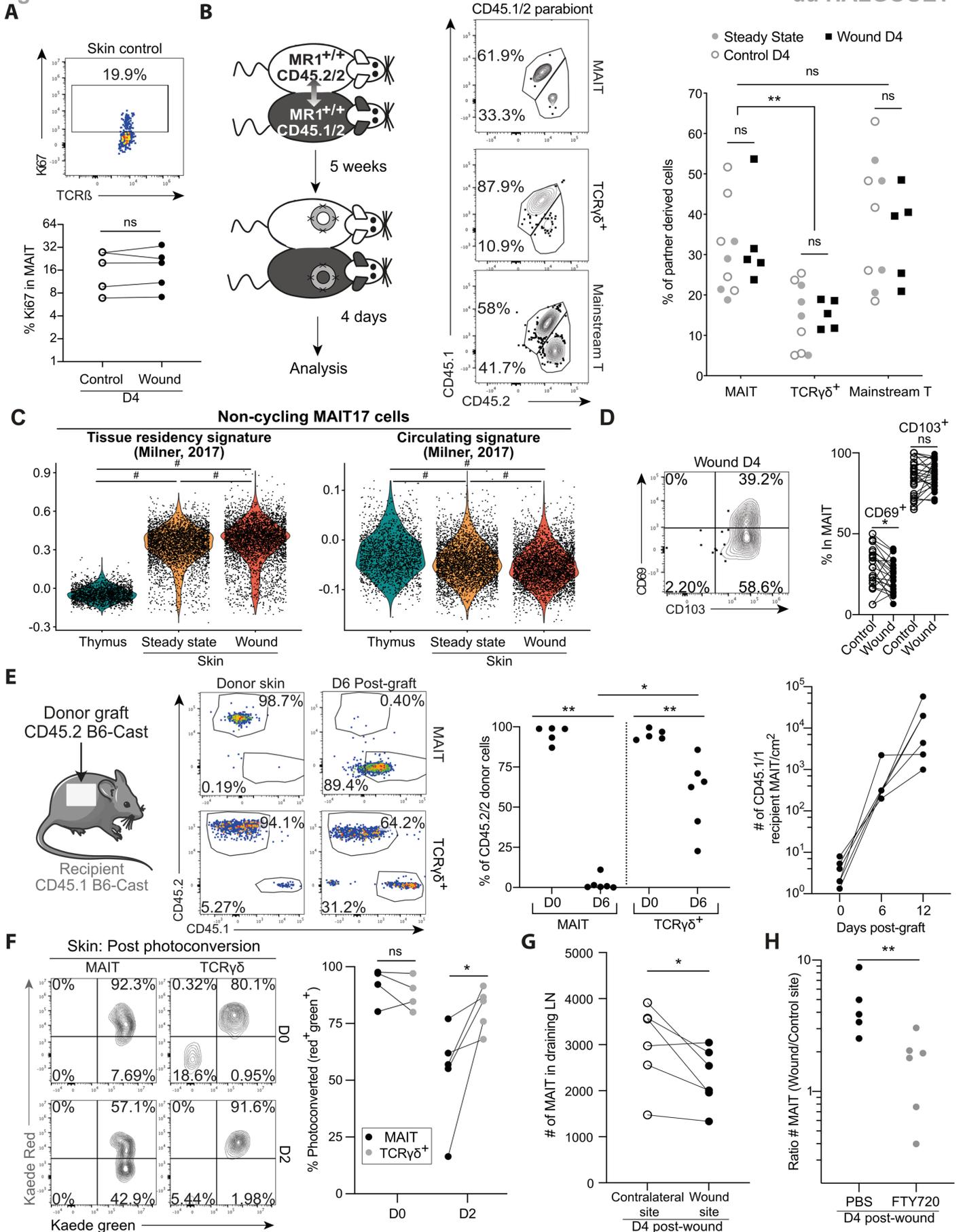
indicate recruitment from outside the skin as full thickness skin comprising dermis and epidermis was photoconverted at day 0.

Interestingly, 1 to 10% of photoconverted MAIT and  $\gamma\delta$  T cells were recovered from the draining inguinal and brachial lymph nodes (LN) at day 2 (Fig. S3B), suggesting migration from the skin to the draining LNs, as described for  $\gamma\delta$  T cells (Nakamizo et al., 2015). Additionally, MAIT cell numbers were lower in the LNs draining the excision as compared to the contra-lateral sites (Fig. 3G) and the LN of non-excised mice (Fig. S3C). To test whether MAIT cells were recruited from the LNs to the wound, we treated excised mice with FTY720. This S1P receptor agonist blocks T cell egress from the LNs (Brinkmann et al., 2002), resulting in strong T cell decrease in the blood (Fig. S3D). FTY720 blockade inhibited the increase of MAIT cell numbers in the wound (Fig. 3H), suggesting traffic through the LNs, despite the expression of a tissue residency transcriptional program in the skin.

# Results

**Fig 3**

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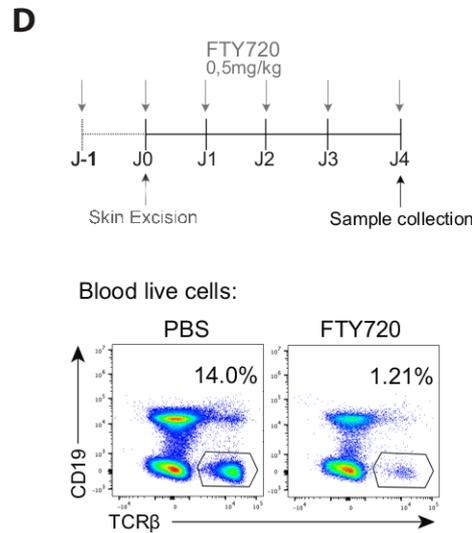
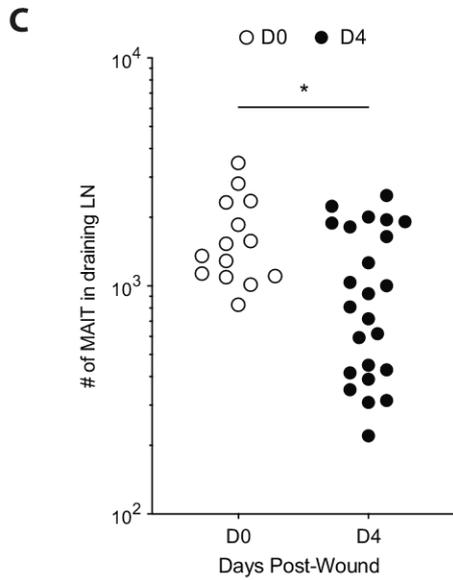
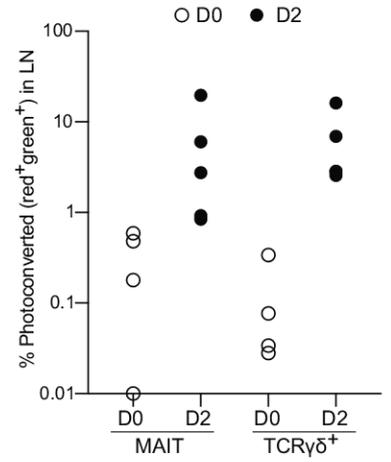
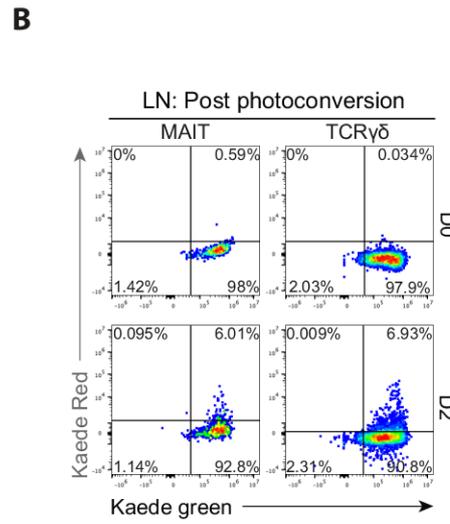
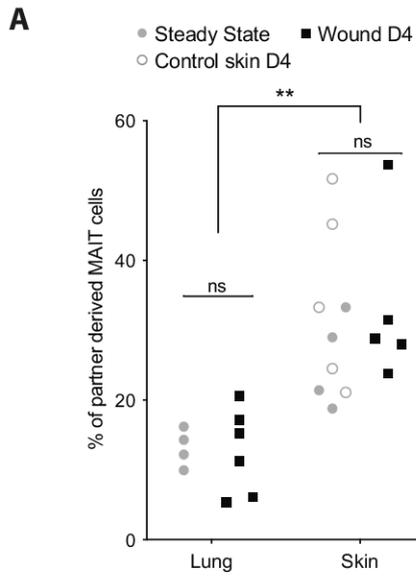
**Fig 3: MAIT cells are recruited into the inflamed skin.**

- (A)** Ki67 expression by MAIT cells. Data are from 2 (n=5) independent experiments. *Wilcoxon Test.*
- (B)** Parabiosis protocol (left) and CD45.2/2 and CD45.1/2 staining (middle). Percentage of partner-derived MAIT,  $\gamma\delta$  T and mainstream T cells in the skin (right) at steady state and in the wound and control sites four days after excision. *Data are from 3 independent experiments ( $n_{\text{steady state+control}}=9$ ;  $n_{\text{excision}}=5$ ). Sidák multiple comparison test.*
- (C)** Tissue residency and circulating (Milner et al., 2017) signature scores on non-cycling MAIT17 cells. *Tukey's multiple comparison test.*
- (D)** CD69 and CD103 expression by MAIT cells. *Pooled data from 6 independent experiments ( $n_{\text{CD69}}=23$ ;  $n_{\text{CD103}}=27$ ). Wilcoxon test.*
- (E)** Graft protocol and CD45.2 and CD45.1 staining (left). CD45.2 donor cell frequency in MAIT and  $\gamma\delta$  T cells from the donor skin (D0) and after six days in the graft (middle). Absolute number of recipient CD45.1+ MAIT cells in grafts from D0, D6 and D12 (right, grafts from same donor are linked). *Pooled data from 2 independent experiments ( $n_{\text{D0}}=5$ ;  $n_{\text{D6}}=3/6$ ;  $n_{\text{D12}}=6$ ). Mann Whitney and Wilcoxon tests as appropriate.*
- (F)** Example of Kaede Green and Red expression (left) and frequency of photoconverted cell (right) in skin MAIT and  $\gamma\delta$  T cells. *Pooled data from 2 independent experiments ( $n_{\text{D0}}=4$ ,  $n_{\text{D2}}=5$ ). Paired t-test.*
- (G)** Number of MAIT cells in the inguinal and brachial LNs draining the wound or the control sites. *Pooled data from 2 independent experiments (n=6). Paired t-test.*
- (H)** Numbers of MAIT cells (ratio wound/control site) four days after excision in FTY720- or PBS-treated mice. *Pooled data from 2 independent experiments ( $n_{\text{PBS}}=5$ ;  $n_{\text{FTY720}}=6$ ). Mann Whitney test.*

# Results

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**Fig S3 related to figure 3: MAIT cells exchange are less resident in the skin than in the lungs and exchange between the skin and the LN.**

- (A)** Quantitation of partner-derived MAIT cells in lung and skin, at steady state and four days after excision. *Pooled data from 3 ( $n_{\text{steady state+control}}=4/9$ ;  $n_{\text{excision}}=6/5$ ) independent experiments. Sidák's multiple comparison test.*
- (B)** Example of Kaede Green and Red expression (left) and frequency of photoconverted cells (right) in MAIT and  $\gamma\delta$  T cells from the draining LNs. *Pooled data from 2 independent experiments ( $n=5$ ).*
- (C)** Number of MAIT cells in the draining LNs at steady-state (D0) or four days following excision. *Pooled data from 10 independent experiments ( $n_{D0}=14$ ;  $n_{D4}=24$ ). Mann Whitney test.*
- (D)** Experimental setup for FTY720 administration (top). Representative T cell staining in the blood after PBS or FTY720 administration (bottom). *Data are representative of 2 independent experiments.*

## **Cognate stimulation is not required for MAIT cell recruitment and pro-repair functions**

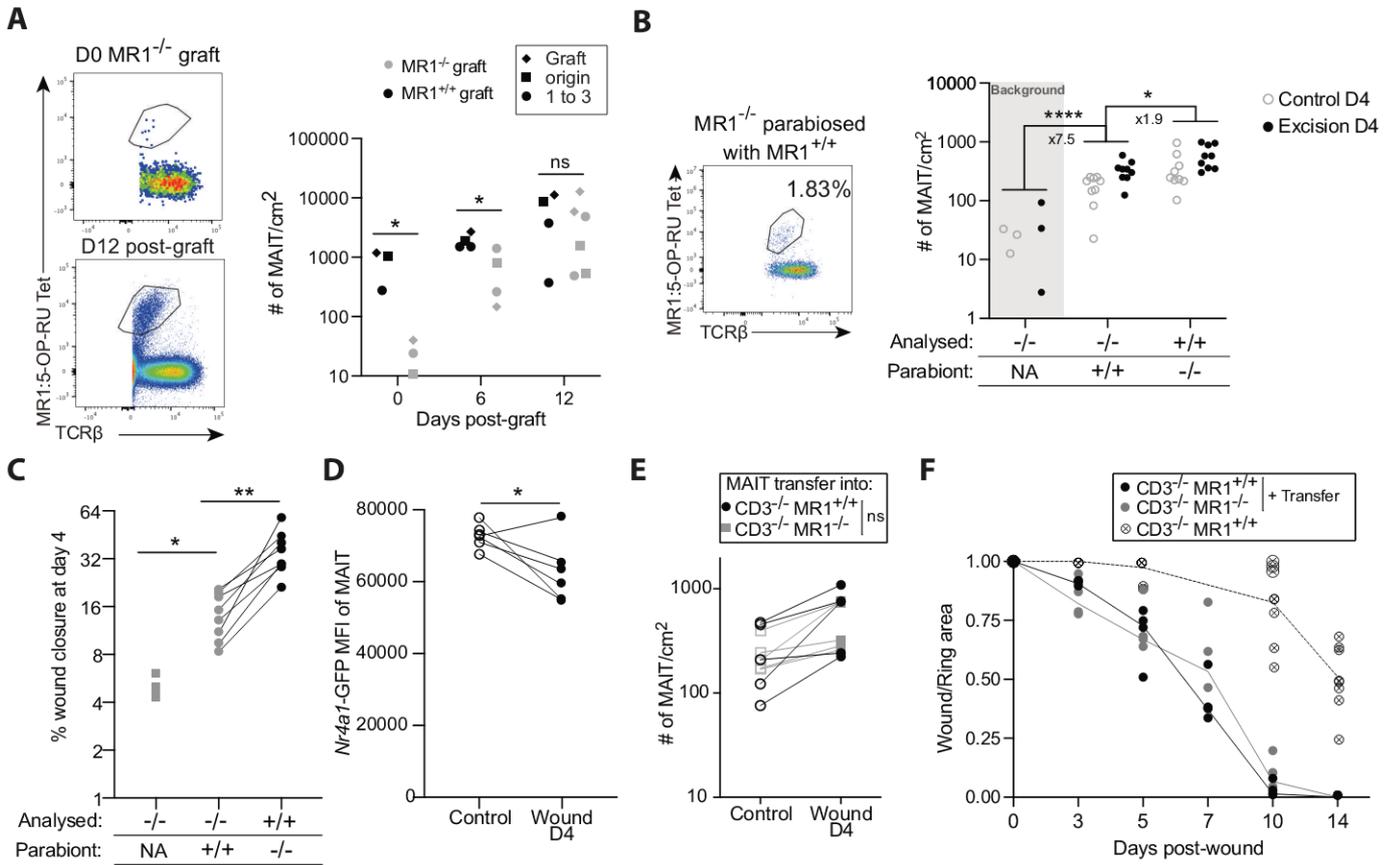
The full thickness-excision punch puts skin microbiota in direct contact with the wound. As the MAIT cell cognate ligand is produced by most skin bacteria (Constantinides et al., 2019b; Mondot et al., 2016), we studied whether MAIT cell recruitment to the skin relied on ligand recognition. As a surrogate, we used MR1<sup>-/-</sup> mice which do not present antigen to MAIT cells. MAIT cells infiltrated MR1<sup>-/-</sup> skin grafted onto a MR1<sup>+/+</sup> mouse, reaching similar levels to those of MR1<sup>+/+</sup> grafts (Fig. 4A). In parabiosis experiments linking MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice, MAIT cell numbers in the skin of the MR1<sup>-/-</sup> parabiont almost reached those of the MR1<sup>+/+</sup> parabionts, both at control and wound sites (Fig. 4B). Thus, MR1 expression on skin resident cells is not necessary for MAIT cell migration into the skin.

MAIT cell infiltration in the MR1<sup>-/-</sup> parabiont was associated with increased wound closure as compared to mice devoid of MAIT cells (Fig. 4C), suggesting that MAIT cell TCR triggering is not necessary for wound healing. This result is consistent with the decrease of *Nr4a1*-GFP reporter expression in MAIT cells following excision (Fig. 4D). Still, wound closure was delayed in the MR1<sup>-/-</sup> as compared to the MR1<sup>+/+</sup> parabionts (Fig. 4C), which might be related to a lower number of MAIT cells (Fig. 4B right panel). Since other hematopoietic cells exchange during parabiosis, MR1 presentation may have occurred in the wound of MR1<sup>-/-</sup> animals, or in the MR1<sup>+/+</sup> parabiont before migration. To formally demonstrate that MAIT cell repair function was independent of cognate stimulation, we transferred *in vitro* expanded MAIT cells into excised CD3 $\epsilon$ <sup>-/-</sup> mice. MR1:5-OP-RU tetramer<sup>+</sup>-enriched thymic cells were expanded using 5-OP-RU at day 0, and IL-2 for 10 to 15 days (Fig. S4A and methods). Except for high expression of Ki67 and CD69, which are linked to *in vitro* activation, expanded MAIT cells retained their MAIT17 phenotype (ROR $\gamma$ t<sup>+</sup>Tbet<sup>-</sup>) after expansion, with high CXCR6 expression and more than 50% of the cells being CD103<sup>+</sup>. More than 96% of transferred cells were MAIT cells (Fig. S4B), which is still the case *in vivo* after four days (Fig. S4C). Transferring MAIT cells accelerated wound closure (Fig. S4D). Expanded MAIT cells were then transferred into CD3 $\epsilon$ <sup>-/-</sup> MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals. In this system, only transferred MAIT cells expressed MR1. As above, MAIT cell recruitment into the wound site (Fig. 4E) as well as wound closure (Fig. 4F) were independent of TCR triggering, as confirmed by the absence of *Nr4a1*-GFP

## Results

expression by transferred cells both at transfer and four days later (Fig. S4E). Thus, MAIT cell recruitment and involvement in wound healing do not rely on sustained cognate interactions with MR1 presenting cells.

Fig 4



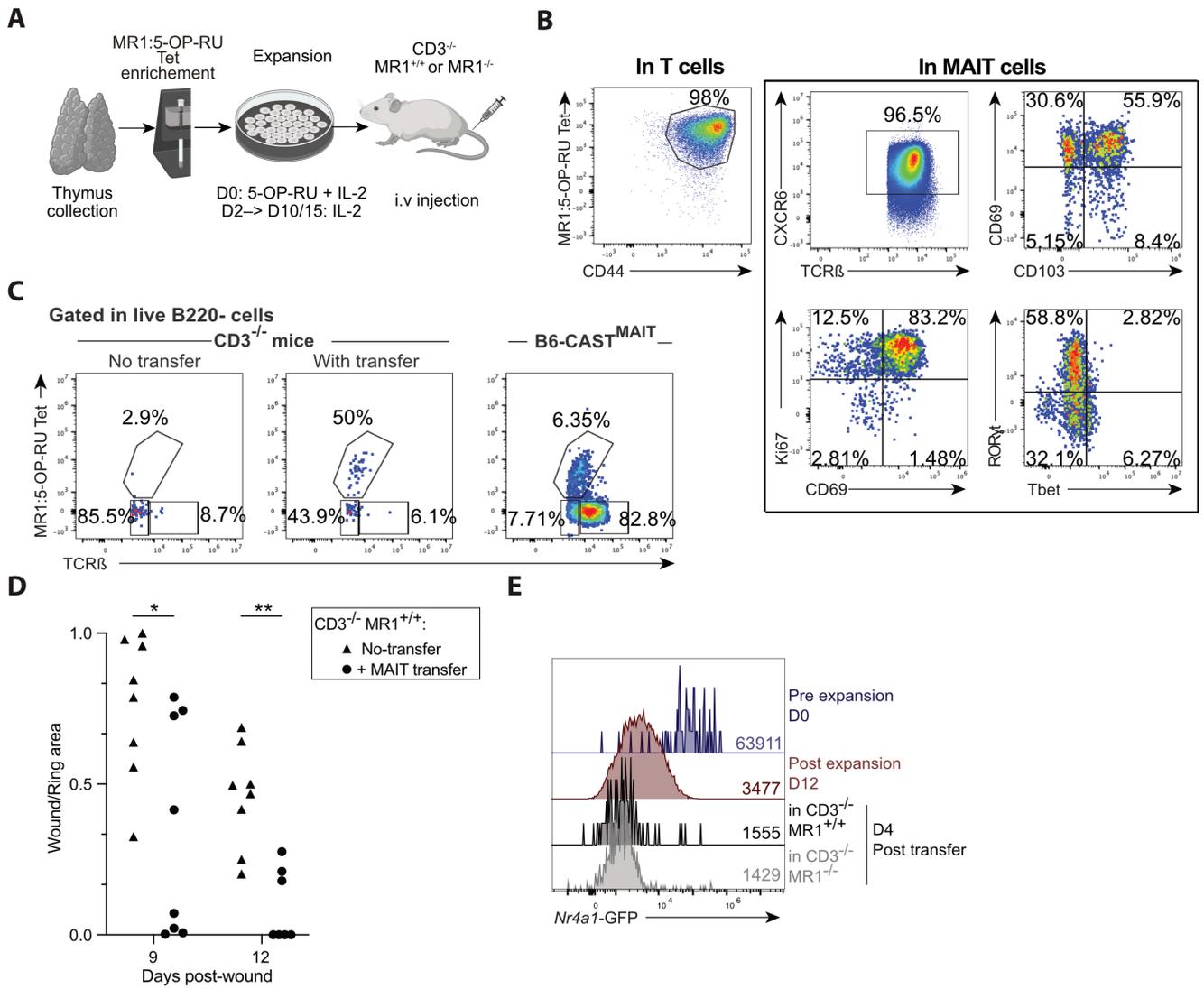
**Fig 4: MAIT cell recruitment and skin healing are independent of MR1.**

- (A)** Grafts were performed on MR1<sup>+/+</sup> animals. MAIT cell staining (left) and numbers (right) in MR1<sup>-/-</sup> and MR1<sup>+/+</sup> grafts before transplant (D0) and longitudinally post-grafting. *Pooled data from 2 independent experiments* (MR1<sup>+/+</sup>: n<sub>D0</sub>=3, n<sub>D6,12</sub>=4; MR1<sup>-/-</sup>: n<sub>D0</sub>=3, n<sub>D6</sub>=4, n<sub>D12</sub>=6). *Unpaired t-test.*
- (B)** MR1<sup>-/-</sup> and MR1<sup>+/+</sup> mice were parabiosed for 5 weeks. MAIT cell staining in the skin of the MR1<sup>-/-</sup> parabiont skin (left) and numbers at wound and control sites (right) for MR1<sup>-/-</sup> and MR1<sup>+/+</sup> parabionts, and MR1<sup>-/-</sup> control mice. *Pooled data from 2 independent experiments* (n<sub>MR1-control</sub>= 3; n<sub>MR1-parabiont</sub>= 9; n<sub>MR1+parabiont</sub>= 9). *Tukey multiple comparison test.*
- (C)** Percent of wound closure in MR1<sup>-/-</sup> and MR1<sup>+/+</sup> parabionts and control MR1<sup>-/-</sup> mice. *Pooled data from 2 independent experiments* (n<sub>MR1<sup>-/-</sup>-alone</sub>= 3; n<sub>MR1<sup>-/-</sup>-paired</sub>= 8; n<sub>MR1<sup>+/+</sup>-paired</sub>= 8). *Mann Whitney and Wilcoxon tests as appropriate.*
- (D)** Mean fluorescence intensity of GFP expression on MAIT cells at wound and control skin sites in *Nr4a1*-GFP animals. *Pooled data from 2 independent experiments* (n=6). *Paired t-test.*
- (E)** MAIT cell numbers at wound and control sites 4 days after transfer into CD3<sup>-/-</sup> MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice. *Pooled data from 2 independent experiments* (n<sub>MR1<sup>+/+</sup></sub>= 4; n<sub>MR1<sup>-/-</sup></sub>=4). *Mann Whitney test.*
- (F)** Longitudinal follow-up of wound surface of transferred CD3<sup>-/-</sup> MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice and non-transferred CD3<sup>-/-</sup> MR1<sup>+/+</sup> control mice. *Pooled data from 2 independent experiments with one blinded* (n<sub>WithTransfer</sub>= 4/4; n<sub>WithoutTransfer</sub>=8).

# Results

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**Fig S4 related to figure 4: MAIT cell phenotype following in vitro expansion and in-vivo transfer.**

- (A)** Experimental cartoon of the thymic MAIT cell expansion protocol (see methods for more details)
- (B)** Frequency and phenotype of MAIT cells after expansion. *Data are representative of 6 independent experiments.*
- (C)** Flow cytometry example of MR1:5-OP-RU Tetramer staining in the skin of CD3<sup>-/-</sup> mice transferred or not with expanded MAIT cells. B6-CAST<sup>MAIT</sup> is presented as control. *Data are representative of 2 independent experiments.*
- (D)** Wound surface (ratio Wound/Ring area) at days 9 and 12 following excision of CD3<sup>-/-</sup> MR1<sup>+/+</sup> mice transferred or not with expanded MAIT cells. *Pooled data from 2 independent experiments (n=7/8). Mann Whitney tests.*
- (E)** Thymic MAIT cells from *Nr4a1*-GFP were expanded. GFP expression on MAIT cells *ex vivo* in the thymus (blue), after *in vitro* expansion (red) and from the skin of transferred CD3<sup>-/-</sup>MR1<sup>+/+</sup> (black) and CD3<sup>-/-</sup>MR1<sup>-/-</sup> (grey). *Data are representative of 2 independent experiments.*

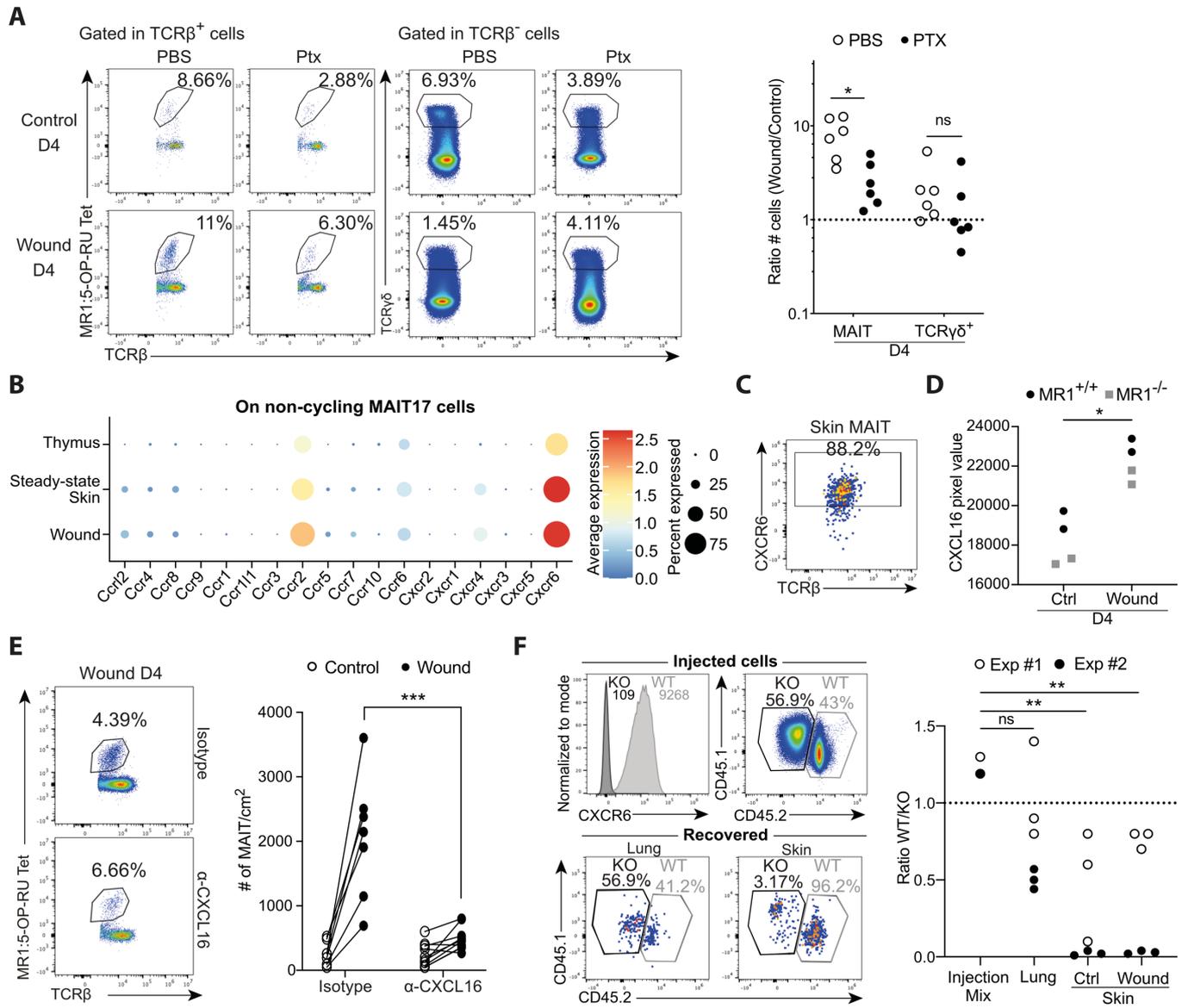
### **CXCR6 is necessary for MAIT cell recruitment to the wound site**

Since MAIT cell migration to the wound was independent of MR1 expression, we assessed the role of chemokines, as proposed for MAIT cell recruitment to the lungs during *F. tularensis* infection (Yu et al., 2020b). We first blocked G protein interactions with chemokine receptors - thereby preventing chemokine signaling - by injecting pertussis toxin (Ptx) before skin excision. Like mainstream T cells (TCR $\beta$ <sup>+</sup> cells excluding MAIT and iNKT cells, Fig. S5A), MAIT cell recruitment to the wound was strongly decreased (Fig. 5A, raw numbers in Fig. S5B). Consistent with their residency profile,  $\gamma\delta$  T cell numbers were not modified, indicating that Ptx injection did not impair cell viability. Analysis of the scRNAseq datasets showed that *Cxcr6*, and to a lesser extent *Ccr2*, were specifically expressed by skin MAIT17 cells (Fig. 5B). At the protein level, CXCR6 was expressed by most skin MAIT cells (Fig. 5C), while CCR2 was expressed by only half of them (Fig. S5C). CXCL16, the ligand for CXCR6, was upregulated in total skin lysate after excision both in MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals (Fig. 5D). By contrast, CCL2, the ligand for CCR2, was highly expressed, independently of the excision (Fig. S5D). We therefore focused on the CXCR6-CXCL16 interaction. *In vivo* blocking of CXCL16 significantly reduced recruitment of MAIT cells into the wound site (Fig. 5E). To formally demonstrate the role of CXCR6 in MAIT cell recruitment into the wound, we deleted *Cxcr6* using CRISPR/Cas9 technology. Thymic MAIT cells were expanded *in vitro* as above (Fig. S4A) before delivery of a ribonucleoprotein complex containing Cas9 and a guide RNA targeting *Cxcr6*. Deletion was highly efficient as shown by the loss of CXCR6 expression (Fig. 5F histogram). A 50/50 mixture of CXCR6<sup>KO</sup> and CXCR6<sup>WT</sup> MAIT cells was injected into CD3 $\epsilon$ <sup>-/-</sup> mice one day after excision. Four days after transfer, most MAIT cells found in the control and wound sites were CXCR6<sup>wt</sup> (Fig. 5F), indicating that CXCR6 is necessary for MAIT cell recruitment into the skin.

# Results

**Fig 5**

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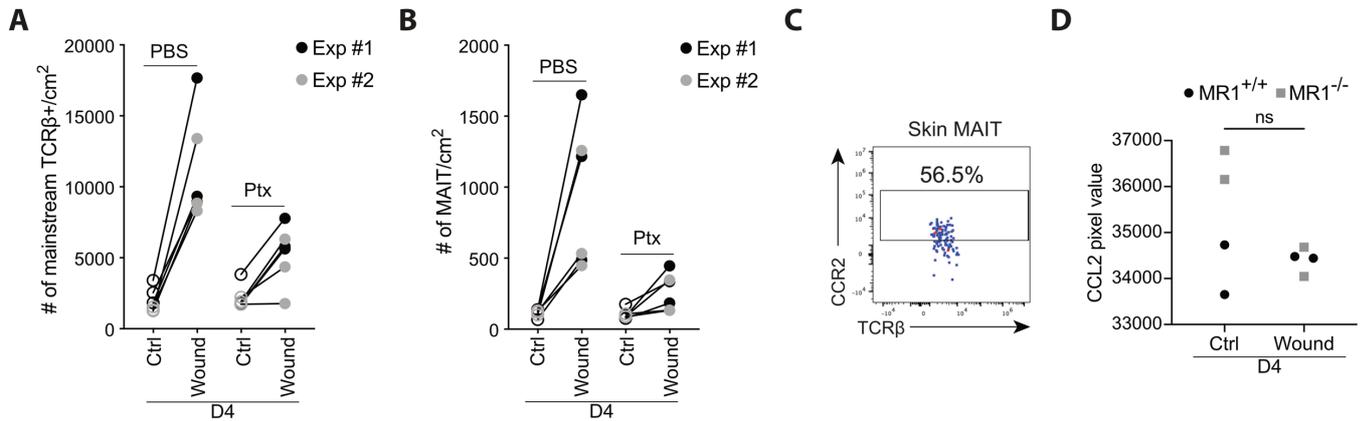


**Fig 5: CXCR6 is necessary for MAIT cell recruitment into the skin.**

- (A)** MAIT and  $\gamma\delta$  T cell staining (left) and numbers (wound/control site) (right) in skin control and wound (D4) sites following Ptx injection. *Pooled data from 2 independent experiments (n=6). Mann Whitney test.*
- (B)** Chemokine receptor expression by non-cycling MAIT17 cells from integrated single-cell datasets as in fig. 1B.
- (C)** CXCR6 expression by MAIT cells. *Data are representative of 10 independent experiments.*
- (D)** CXCL16 protein quantity in total skin lysate from wound (D4) or steady state skin of MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice. *Pooled data from 2 independent experiments (n=4). Mann Whitney test.*
- (E)** MAIT cell staining (left) and numbers (right) in control and wound (D4) skin sites following  $\alpha$ -CXCL16 or isotype control i.p. injection. *Pooled data from 2 independent experiments (n=7/10). Mann Whitney test.*
- (F)** Expanded MAIT cells were deleted for CXCR6 by Crispr/Cas9 modification. Congenic marker and CXCR6 expression on the injected pool (top left) or recovered cells (bottom left). Quantitation of recovered KO cells (ratio of KO/WT) at different sites (right). *Pooled data from 2 independent experiments (n<sub>injection</sub>= 2; n<sub>other</sub>=6). Tukey's multiple comparison test.*

## Supp 5

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**Fig S5 related to figure 5**

- (A)** Mainstream T cell (TCRα<sup>+</sup>MR1:5-OP-RU-Tet-CD1d:aGalCer-Tet<sup>-</sup>) numbers (wound/control site) following G protein signaling blocking *in vivo* via Ptx injection. Pooled data from 2 independent experiments (n=6). Unpaired t-test
- (B)** Raw numbers for figure 5A.
- (C)** Flow cytometry example of CCR2 expression by MAIT cells. Data are representative of 2 independent experiments.
- (D)** Quantitation of CCL2 protein expression in total skin lysates from wound and control skin sites of MR1<sup>+/+</sup> and MR1<sup>-/-</sup> mice. Data are from 2 independent experiments (n=4). Mann Whitney test.

### **MAIT cell-derived Areg promotes wound closure.**

To determine how MAIT cells promote tissue repair, we analyzed cytokines produced *in vivo* at wound sites of MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals (Fig. S6A). In MR1<sup>+/+</sup> animals, we observed an up-regulation of several molecules involved in antibacterial responses (resistin, CXCL10), epithelial proliferation or angiogenesis (PDGF-BB, angiopoietin-1 and -2, FGF-21, WISP-1) and inflammation through recruitment or differentiation of immune cells (CXCL1, CXCL10, IL-1 $\beta$ , IL-17A, (G)M-CSF). These results suggest that the whole repair process was increased at day 4 in MR1<sup>+/+</sup> as compared to MR1<sup>-/-</sup> animals. We assessed the effect of MAIT cells on epidermal proliferation using K14 (keratinocyte marker) and Ki67 immunofluorescence staining (Castela et al., 2017) (Fig. 6A and 6B). The length of the epithelial tongue was increased from day 2 in MR1<sup>+/+</sup> animals (Fig. 6A), demonstrating that MAIT cells are involved early in the repair process. Ki67 staining in the epidermis (but not in the dermis, Fig. S6B) was increased 4 days after excision (Fig. 6B), indicating that MAIT cells stimulated early epithelial proliferation. To assess whether MAIT cells also affected angiogenesis, endothelial cells were stained using a CD31 antibody. The number of vessels was similar between MR1<sup>+/+</sup> and MR1<sup>-/-</sup> wound sections (Fig. S6C). Thus, MAIT cells at early time points mainly favors the proliferation of epithelial cells.

MAIT cell effect could be either direct, through secretion of growth factors, or indirect, by recruiting other immune subsets (as seen in Meierovics et al., 2013). The numbers of mainstream T cells (TCR $\beta$ <sup>+</sup>MR1:5-OP-RU-Tet<sup>-</sup>CD1d: $\alpha$ GalCer-Tet<sup>-</sup>),  $\gamma\delta$  T cells, iNKT cells, Langherans cells as well as Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup> monocytes were similarly modified in MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals throughout wound closure (Fig. S6D). These results suggest that MAIT cells did not impact the recruitment of these populations and that the effect observed on keratinocyte proliferation was direct.

We therefore focused on direct MAIT cell effector functions. scRNAseq analysis identified several pro-repair mediators described as secreted by MAIT cells (Hinks et al., 2019b; Lamichhane et al., 2019b; Leng et al., 2019b) and overexpressed in skin versus thymic samples, including *Furin*, *Tgfb1* and *Hmgb1* (Fig. 6C). We further identified pro-repair mediators specific to cluster 8 (Fig. 2A and Table S2) and upregulated in skin MAIT17 cells (Fig. 6C). Among the most differentially expressed genes in cluster 8 were

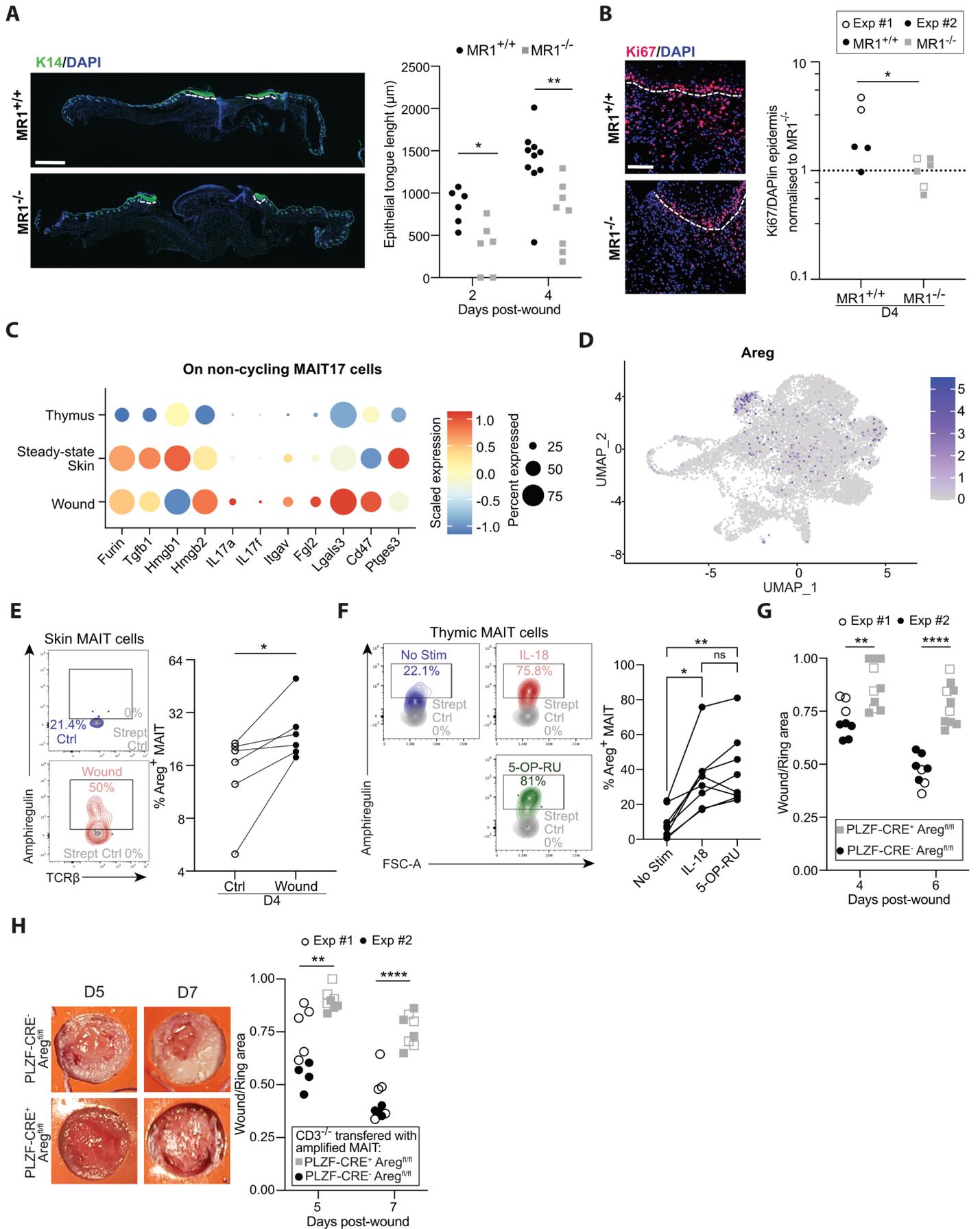
## Results

*Il17a* and *Areg* (Table S2 and Fig. 6C and D), both described to favor tissue repair (reviewed in McGeachy et al., 2019; Zaiss et al., 2015). The role of IL-17 had been already well studied during tissue repair (Konieczny et al., 2022; MacLeod et al., 2013b), and we could not obtain consistent *ex vivo* IL-17 staining on skin MAIT cells (data not shown). Therefore, we decided to focus on *Areg*, as tissue repair signatures associated to *Areg*-producing regulatory T cells were overexpressed by skin MAIT cells (Fig. S2F). *Areg* is an epidermal growth factor like molecule mediating keratinocyte proliferation (Zaiss et al., 2015). *Ex vivo* intracellular staining (no restimulation) confirmed that a higher frequency of skin MAIT cells produced *Areg* at the wound as compared to the control site (Fig. 6E). In humans, MAIT cells expressed *Areg* following TCR stimulation (Lamichhane et al., 2019b) but in our study, wound closure seemed independent of a sustained TCR triggering (Fig. 4), suggesting that *Areg* could be secreted following other, TCR-independent, stimuli. We tested this hypothesis *in vitro* using *ex vivo* thymic MAIT cells as the numbers of skin MAIT cells were too low for *in vitro* testing and the transferred MAIT cells (Fig. S4A) were of thymic origin. IL-18, which is secreted during wound healing (Kämpfer et al., 1999), induced similar levels of *Areg* expression as compared to 5-OP-RU stimulation (Fig. 6F). To determine whether *Areg* secretion was important for skin repair, we compared wound closure in *Areg*<sup>flox/flox</sup> PLZF-Cre<sup>+</sup> and PLZF-Cre<sup>-</sup> animals. The wound surface was increased at day 4 and 6 in PLZF-Cre<sup>+</sup> animals, indicating that *Areg* expression by PLZF-expressing cells was involved in skin wound healing (Fig. 6G). PLZF is expressed by MAIT cells, NKT cells (Savage et al., 2008b), a subset of  $\gamma\delta$  T cells (Kreslavsky et al., 2009) and transiently by 1/3 of embryonic cells during development (Constantinides et al., 2014). To formally investigate the involvement of MAIT cell-derived *Areg* in skin wound healing, we expanded thymic MAIT cells from *Areg*<sup>flox/flox</sup> PLZF-Cre<sup>+</sup> or PLZF-Cre<sup>-</sup> mice as above (Fig. S4A). The resulting >95% pure MAIT cell populations were transferred into excised CD3 $\epsilon$ <sup>-/-</sup> MR1<sup>+/+</sup> animals. Wound closure was significantly delayed when *Areg*-deficient MAIT cells were transferred (Fig. 6H). Thus, *Areg* production by MAIT cells is central to their tissue repair function in skin wound healing.

# Results

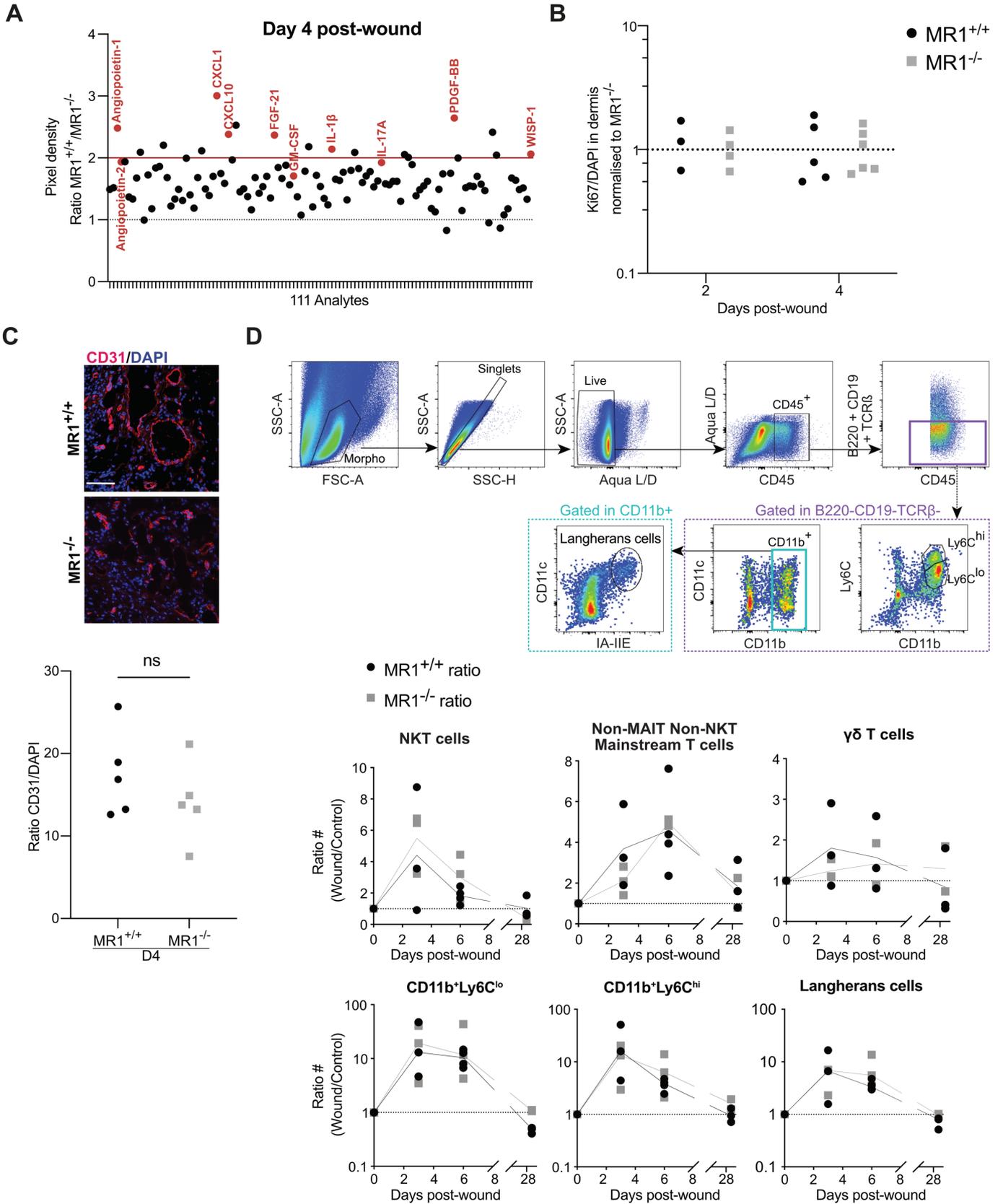
Fig 6

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**Fig 6: MAIT cell-derived Areg exerts a tissue repair function.**

- (A)** Representative immunofluorescence images of wounds from MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals (DAPI in blue, K14 in green) (left). Scale bar represents 100  $\mu$ m. The epidermal tongues are underlined with white dashed lines and their length is quantified (right, D2/D4, 2 tongues per slide). *Pooled data from 1 (D2: n=3) and 2 independent experiments (D4: n=5/4) analyzed blindly. Mann-Whitney test.*
- (B)** Representative immunofluorescence images of wounds from MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals (DAPI in blue, Ki67 in red). The white dashed line separates the epidermal tongue and the underlying dermis (left). Proliferation in the epidermis is quantified by the Ki67/DAPI ratio and normalized to the average expression in MR1<sup>-/-</sup> animals for each experiment (right). *Data are from 2 independent experiments (n=5/6) analyzed blindly. Unpaired t-test.*
- (C)** Dot plot showing RNA expression of repair molecules by non-cycling MAIT17 cells from integrated single-cell datasets as in fig. 1B.
- (D)** Feature plot of Areg expression projected on the UMAP of the integrated datasets.
- (E)** *Ex vivo* Areg staining on skin MAIT cells (Blue: control skin; Red: wound skin; Grey: full staining except the biotinylated anti-Areg antibody). *Pooled data from 2 independent experiments (n=6). Wilcoxon test.*
- (F)** Areg expression by thymic enriched MAIT cells following 36h of in vitro activation by 5-OP-RU or IL-18. *1 experiment (n=8) representative of 2. Dunn's multiple comparison test.*
- (G)** Wound surfaces at days 4 and 6 post excision on PLZF-Cre<sup>-</sup>Areg<sup>fl/fl</sup> (black) and PLZF-Cre<sup>+</sup>Areg<sup>fl/fl</sup> (grey). *Pooled data from 2 independent experiments with one blind (full symbols) (n<sub>CRE-</sub>=8; n<sub>CRE+</sub>=10). Mann Whitney tests.*
- (H)** Representative pictures (left) and wound surfaces (right) post excision (D5 and D7) of CD3 $\epsilon$ <sup>-/-</sup> animals transferred with thymic MAIT cells expanded from PLZF-Cre<sup>-</sup>Areg<sup>fl/fl</sup> (black) and PLZF-Cre<sup>+</sup>Areg<sup>fl/fl</sup> (grey) littermate mice. *Pooled data from 2 independent experiments with one blind (full symbols) (n<sub>CRE-</sub>=9; n<sub>CRE+</sub>=8). Mann Whitney tests.*



**Fig S6 related to figure 6: Investigating the potential impact of MAIT cells in the wounds.**

- (A)** Protein expression in MR1<sup>+/+</sup> as compared to MR1<sup>-/-</sup> wound (D4) lysates (calculated from pixel density: each MR1<sup>+/+</sup> duplicate was divided by the average of MR1<sup>-/-</sup> duplicates in each independent experiment. Geometric mean is represented). *Pooled data from 2 independent experiments (4 MR1<sup>+/+</sup> blots and 3 MR1<sup>-/-</sup> blots).*
- (B)** Quantitation of proliferation in the dermis by immunofluorescence, given by the Ki67/DAPI ratio and normalized to the average expression in MR1<sup>-/-</sup> wounds for each experiment. *Pooled data from 1 (D2, n=3/4) and 2 (n=5/6) independent experiments.*
- (C)** Representative immunofluorescence images of vessels given by CD31 staining (red) in wounds of MR1<sup>+/+</sup> and MR1<sup>-/-</sup> animals four days post-excision (top). Vessel density was quantified by the CD31/DAPI ratio under the two epidermal tongues and in the granulation site in the center, the average of all three measurements for each individual mouse is represented (right). *Pooled data from 2 independent experiments (n=5) analyzed blindly. Mann Whitney test.*
- (D)** Gating strategy used to define Langherans cells, CD11b<sup>+</sup>LY6C<sup>Lo</sup> and CD11b<sup>+</sup>LY6C<sup>hi</sup> CD11b<sup>+</sup> cells (top) and ratio of absolute numbers of NKT,  $\gamma\delta$  T, Langherans, CD11b<sup>+</sup>LY6C<sup>Lo</sup> and CD11b<sup>+</sup>LY6C<sup>hi</sup> cells (wound/control skin site) for each individual mouse. *Data are representative of 2 independent experiments (n=3).*

## Discussion

In this study, we assessed the *in vivo* mechanisms underlying MAIT cell repair functions in an immunocompetent host, using a human-like skin damage model. We showed that MAIT cells express a tissue repair program at steady state in the skin and improve wound closure. The repair function relies on MAIT cell recruitment into the wound, from distant sites including secondary lymphoid organs, through the CXCR6/CXCL16 axis. Surprisingly though, recruitment and wound healing function are independent of concomitant TCR stimulation. MAIT cell presence is associated with increased epithelial proliferation in the epidermis. Lastly, Areg production by MAIT cells is key to their tissue repair function.

Our previous work showed that MAIT cells are tissue-resident in lungs, liver and spleen (Salou et al., 2019b). Here, parabiosis experiments suggest that MAIT cells reside for shorter periods in the skin as compared to the lungs, in contrast to viral-specific TRM (Slütter et al., 2017). Previous results showed no exchange of skin MAIT cells between parabionts within 13 weeks. These contradictory data may result from *S. epidermidis* colonization seven weeks before parabiosis, therefore generating bona fide MAIT TRM in the skin (Constantinides et al., 2019b). Surprisingly however, skin MAIT17 cells in our study overexpressed a residency signature as compared to their thymic counterparts, both at steady state and at the wound site. The presence of photoconverted MAIT cells in the draining LN two days following photoconversion (Fig. S3B) suggests that some skin MAIT cells recirculate, as shown for conventional anti-viral TRM (Fonseca et al., 2020). During wound healing, MAIT cells are recruited into the skin in a CXCR6-CXCL16-dependent manner. This mechanism is likely shared for MAIT cell recruitment to other organs as intranasal instillation of CXCL16 together with 5-OP-RU drives MAIT cell accumulation into the lungs (Yu et al., 2020b). However, the role of CXCR6 is difficult to study *in vivo* as it is necessary for full MAIT cell maturation in the thymus (Koay et al., 2019). *In vitro* expansion allowing CRISPR/Cas9-based genetic modification followed by adoptive transfer solved this issue (Fig. 5F). As human MAIT cells also express CXCR6 (Dusseaux et al., 2011b), recruitment could happen in pathological settings in which CXCL16 is produced such as non-alcoholic fatty liver disease, renal fibrosis or certain cancers (Korbecki et al., 2021).

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Interestingly, egress from secondary lymphoid organs was necessary for MAIT cell accumulation in the wound (Fig. 3H). Although MAIT cells have been mainly described in mucosal tissues, our results suggest that LNs (or any tissue with a S1PR1-dependent egress mechanism) may act as a reservoir (Brinkmann et al., 2010). Accordingly, MAIT cell numbers decreased in the LN draining the wound (Fig. 3G), but this drop was too low to account for the large increase of MAIT cell number at the wound site. The constant albeit low proliferation of skin MAIT cells (Fig. 3A) may contribute to increasing their numbers in the wound, but additional reservoirs likely exist. One hypothesis is that tissue resident MAIT cells (from skin or other organs) return to the circulation, similarly to skin TRM upon reactivation (Fonseca et al., 2020). Alternatively, another pool of non-resident MAIT cells may exist. MAIT cells being negative for CD62L and CCR7, they probably do not circulate from the blood directly to the LN. Instead, they would behave as effector memory T cells, migrating from blood to tissues, exiting tissues through the lymph in a S1PR1-dependent mechanism before going back to blood (Masopust and Soerens, 2019b). In humans, the existence of a circulating pool of MAIT cells is supported by the overlap of the TCR repertoire between MAIT cells from the thoracic duct and the blood (Voillet et al., 2018b). In contrast with our results, FTY720 treatment of mice instilled with *F. tularensis* live vaccine did not hamper MAIT cells accumulation in the lungs (Yu et al., 2020b), suggesting different mechanisms in this model. Additionally, the increase of skin MAIT cell number following *S. epidermidis* association was similar in WT and LN-deficient animals (LTa<sup>-/-</sup>) (Constantinides et al., 2019b) but the impact on skin wound healing was not studied. Altogether, pools of MAIT cells with different circulation profiles may be present in mice. Whether these pools are functionally different and exchange to some extent at steady state or during pathologies remain to be determined.

In our study, MAIT cell recruitment and tissue repair function did not rely on antigen presentation by MR1. This result seems contradictory with the tissue repair program induced by TCR triggering both in humans and mice (Hinks et al., 2019b; Lamichhane et al., 2019b; Leng et al., 2019b). In our study, skin MAIT cells expressed high levels of Nur77 and a strong tissue repair program at steady state. Moreover, MAIT cell were expanded *in vitro* by adding 5-OP-RU once at the beginning of the culture. Thus, our data are consistent with TCR stimulation being necessary for program acquisition, but not for actual tissue repair function. TCR stimulation at steady state is reminiscent of the tonic TCR signaling of pro-repair V $\gamma$ 5V $\delta$ 1 T cells (Chodaczek et al., 2012; Jameson et al., 2002).

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Still, the actual triggering of MAIT cell repair function is probably not dependent on TCR signaling as MAIT cells do not express Nur77 either at the time of transfer or after migration into the wound (Fig. S4E). Similarly, regulatory CD4<sup>+</sup> T cells in the lung following influenza infection secrete Areg even after TCR deletion (Arpaia et al., 2015). Also, NKT cells deleted for their TCR secrete IFN- $\gamma$  following LPS stimulation *in vivo* (Vahl et al., 2013). Licensing mechanisms independent of TCR signaling have been described for commensal specific H2-M3-restricted CD8<sup>+</sup> T cells in the skin. At steady state, these type 17 cells express type 2 repair mediators at the RNA level only. Following tissue injury, alarmins promote secretion of the repair cytokines (Harrison et al., 2019b). A similar translational checkpoint likely exists for MAIT cells, explaining the identical transcriptional program of skin MAIT cells at steady-state and wound sites. This hypothesis is supported by the expression of transcripts encoding ZFP36, ZFP36L1 and ZFP36L2 which regulate the stability of mRNA for cytokines or other immune mediators (Makita et al., 2021). The repair functions may be elicited by cytokines such as IL-18, IL-4, IL-2, IL-7 and IL-21, whose receptors are expressed by skin MAIT cells (scRNAseq, not shown). Accordingly, Areg secretion can be induced following IL-18 stimulation (Fig. 6F). Altogether, these results suggest that skin MAIT cells are in a poised functional state requiring additional signals to exert their tissue repair program.

The pro-repair effect of MAIT cells was mediated at least in part through Areg. In the skin, Areg produced in an autocrine manner promotes keratinocyte proliferation (Cook et al., 1991; Kennedy-Crispin et al., 2012). Accordingly, MAIT cells increased the size of the epidermal tongue as well as keratinocyte proliferation (Fig. 6A/B). Areg is also expressed by lung MAIT17 cells at steady state ((Salou et al., 2019b), unpublished analysis) and following *L. longbeachae* infection (Hinks et al., 2019). This molecule is produced by various cells of the immune system including innate cells as well as regulatory T cells, gingival  $\gamma\delta$ T cells (Krishnan et al., 2018), ILC2 (Monticelli et al., 2011) and tumor-infiltrating CD8<sup>+</sup> T cells (Kwong et al., 2010). The multicellular origin of Areg suggests that multiple cell types may exert the same function. However, in our model, MAIT cell deletion alone delayed wound closure, suggesting non redundant function in the early steps of skin healing. Skin MAIT cells also express ROR $\gamma$ t and probably secrete IL-17, a key effector molecule for the repair function of epidermal  $\gamma\delta$  T cells (Konieczny et al., 2022; MacLeod et al., 2013). The IL-17 secreted by MAIT and  $\gamma\delta$  T cells could act

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similarly, by inducing HIF1 $\alpha$  in epithelial cells and a subsequent shift towards glycolysis to promote their migration (Konieczny et al., 2022). Although a non-redundant role of IL-17 was demonstrated using IL-17R deficient epithelial cells, *Rorc* deletion in  $\gamma\delta$  T cells also affects other effector molecules regulated by ROR $\gamma$ t. Consequently, other effector molecules and other T cell subsets such as MAIT cells are likely important for skin wound healing. Understanding the relative contributions of IL-17 and Areg derived from one or another cell type, including at steady state, would help understanding the fine tuning of epithelial repair.

In summary, our work shows that MAIT cells play a pivotal role in skin wound healing. MAIT cell implication in different types of healing delay such as diabetic wounds would therefore be of interest. Understanding whether MAIT cells have such function in other tissues will assess their full effector potential, to be able to manipulate them towards pro-inflammatory or pro-repair functions.

### Limitations of study

One limitation of our study is the extensive digestion process at 37°C that may modify the transcriptional pattern of skin cells. Therefore, we favored *in vivo* experiments to validate the results obtained using single cell suspensions. Moreover, the transcriptome analysis did not distinguish MAIT cells preexisting in the skin from those recruited to the wound. Whether both subsets perform *in vivo* repair functions is unknown. The MAIT cell transfer into CD3 $\epsilon$ <sup>-/-</sup> mice shows that the recruited ones do elicit the repair process. An additional question which would help improve our understanding of MAIT cell function is their precise location in the skin and their relationship with other cell types.

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### **Author contributions**

Conceptualization: AdP, OL, MS

Formal Analysis: AdP, MA, MM, MS

Funding acquisition: OL

Investigation: AdP, AD, AA, VP, YEM, HB, FL, LP, RG, MS

Methodology: AdP, MA, OL, MS

Project administration: AdP

Resources: DZ, RG, SA

Supervision: OL, MS

Visualization: AdP, MS

Writing – original draft: AdP, MS

Writing – review & editing: AdP, OL, MS

### **Declaration of interest**

The authors declare no competing interests relevant to this work.

## Methods

### Mouse strains

Unless specified otherwise, congenic B6-MAIT<sup>CAST</sup> strain, on an MR1<sup>+</sup> or MR1<sup>-/-</sup> background were used in this study (Cui et al., 2015). Reporter genes under the control of *Rorc* (ROR $\gamma$ t) (Lochner et al., 2008), *Nr4a1* (Nur77) (Zikherman et al., 2012) or *Zbtb16* (PLZF) (Constantinides et al., 2014) promoters were introgressed into the B6-MAIT<sup>CAST</sup> background. CD45.1/1, CD45.1/2, CD45.2/2 animals were generated by crossing CD45.1/1 B6 animals onto CD45.2/2 B6-MAIT<sup>CAST</sup> mice. Photoconvertible Kaede mice were generously provided by R. Golub (Institut Pasteur, Paris) (Tomura et al., 2008). MR1<sup>+/+</sup> and MR1<sup>-/-</sup> CD3e<sup>-/-</sup> mice were generated inhouse by crossing MR1 on a B6 background with CD3<sup>-/-</sup> mice (Malissen et al., 1995). *B6 Areg<sup>flox/flox</sup> (Areg<sup>tm2a(EUCOMM)Hmgu</sup>)* (Minutti et al., 2019) generously provided by D. Zaiss were crossed onto B6-MAIT<sup>Cast</sup> PLZF<sup>GFP<sup>cre</sup></sup> mice (Constantinides et al., 2019b). In all experiments, we accounted for the cage effect on immune cell population (e.g., MAIT cell frequency impacted by microbiota composition) (Constantinides et al., 2019b) by spreading animals and/or litters from the same breeding cages into the different experimental groups. All experiments were conducted in an accredited animal facility by the French Veterinarian Department following ethical guidelines approved by the relevant ethical committee (APAF1S no. 24245-2020021921558370-v1).

### Skin excision

Skin excision was performed as previously described (Dunn et al., 2013). Briefly, after shaving and depilation, a 4 mm full-thickness wound was performed on the back of the mouse using a biopsy punch. Silicone rings were then sutured to prevent epithelial skin contraction. A clean dressing was applied and regularly changed to avoid infection of the wounds. Macroscopic measurements were performed on pictures by calculating wound and ring areas using ImageJ. Wound size was then estimated as a wound to ring area ratio, with 1 being an open wound and 0 a fully closed wound.

## Results

### **Parabiotic surgery**

Aged-matched congenically distinct B6-MAIT<sup>CAST</sup> mice either MR1<sup>+/+</sup> or MR1<sup>-/-</sup> were co-housed for a minimum of 2 weeks before being surgically joined as parabiotic pairs as previously described (Kamran et al., 2013; Salou et al., 2019b). Skin and lungs were collected 5 weeks later.

### **Skin graft**

Graft surgery was performed as described (Cheng et al., 2017). Briefly, either MR1<sup>+/+</sup> or MR1<sup>-/-</sup> B6-MAIT<sup>CAST</sup> donor skin was collected. A 2 cm<sup>2</sup> sample was grafted onto congenically distinct MR1<sup>+/+</sup> B6-MAIT<sup>CAST</sup> mice. Recipients with grafts from identical donors were sacrificed at different time points to follow cellular infiltration kinetics.

### **Kaedes photoconversion**

Mice were anesthetized, shaved on the dorsal side, and exposed to violet light (395nm U.V. light 95 Watts for 60s) (Tomura et al., 2008) to photoconvert skin cells.

### **Tissue processing**

Back skin was shaved, depilated (2 min of hair removal cream, Veet) and full-thickness samples (ie dermis and epidermis) were collected in CO<sub>2</sub> independent medium (Gibco). The following area were sampled: steady state skin, wound site (1 cm<sup>2</sup> encompassing the wound and surrounding rims, meaning that the wound represents 12.6% of the sampled skin, back top left of the mouse) or contralateral control skin (1 cm<sup>2</sup> of skin at the contralateral side as compared to the wound, i.e. back top right). The contralateral site called control is used to normalize the number of MAIT cells present in the wound to account for inter-individual variations (Constantinides et al., 2019b).

Skin single cell suspensions were obtained by putting the samples (flattened, epidermis side up) at 37°C for 45 min in 1 mL of 500 CU Dispase (Corning). The skin sample was then chopped in RPMI 1640 GlutaMAX media supplemented with 1 mM sodium pyruvate, 1 mM non-essential amino acids, 50 µM β-mercaptoethanol, 20 mM HEPES, 100 U/mL penicillin, 100 mg/mL streptomycin, 0.5 mg/mL DNase I (all products

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from Sigma-Aldrich), and 0.25 mg/ml Liberase TL (Roche) and incubated for 1h45 min at 37°C in a 5% CO<sub>2</sub> incubator. After filtering on a 40 µm filter and 2 washing in PBS, BSA 0.5%, 2 mM EDTA, the cell suspension was removed of skin debris using the cell debris removal solution (Miltenyi) following manufacturer's instructions.

Lung single cell suspensions were obtained as described (Salou et al., 2019b). Blood cells were recovered by centrifugation after red blood cell lysis (Biolegend). LNs were scratched onto 40µm filter and cells were washed in PBS 1x, BSA 0.5%, 2mM EDTA before use.

### **Flow cytometry**

Extracellular staining was performed with the relevant titrated antibodies in staining buffer (PBS 0.5% BSA, 2 mM EDTA and anti-FcR 2.4G2 produced in house) for 20 min at 4°C. Staining for transcription factors or cytokines was performed on fixed and permeabilized cells using the appropriate kits (Foxp3 Fixation kit (Thermofisher) and BD Fix/Perm kit, respectively) as per manufacturer instructions, followed by 20 min incubation at 4°C with the relevant titrated antibodies. If needed, tetramer staining was performed before the extracellular staining step, for 30 min at room temperature in staining buffer containing MR1 tetramers loaded with 5-OP-RU or 6-FP with or without CD1d tetramers loaded with PBS-57 (both tetramers from the NIH Tetramer Core Facility; Emory University, GA) and anti-TCRβ. Flow cytometry acquisition was performed on a Cytoflex (Beckman) or Fortessa cytometer (BD). FACS was performed on an Astrios cell sorter (Beckman Coulter). Data were analyzed using FlowJo software.

### ***Ex vivo* amphiregulin production.**

Brefeldin A (Sigma) was injected i.v. (Minutti et al., 2019) 6 hours before sacrifice. Skin was processed as described above, except for addition of Monensin (10 µM, Invitrogen) in every buffer. To prevent extracellular secretion the resulting single cell suspension was maintained for 3h at 37°C with maristamat (10 mM, Sigma), GolgiPlug (1/1000; BD) and GolgiStop (1/1500; BD) (Arpaia et al., 2015) before staining.

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### **Single cell RNA sequencing**

Single cell suspensions of 18 wounds (1 cm<sup>2</sup>) or steady state back skin (same location as the wound) were pooled together and live TCR $\beta$ <sup>+</sup>MR1:5-OP-RU<sup>+</sup> were isolated by Aria cell sorter (BD) in 10% FCS CO<sub>2</sub>-independent medium. 7,000 cells for each sample were loaded onto a chromium 3' chip following the Single Cell 3' Kit V3(10X Genomics). Generation and acquisition of the sequencing reads were performed according to the manufacturer recommendations (10X Genomics) by the ICGex NGS platform of the Institut Curie.

### **Single cell RNA-seq preprocessing**

The reads were aligned and feature-barcode matrices were generated using the Cell ranger pipeline version 3.1.0. The reference genome used is the mm10-3.0.0.

### **Single cell RNA analysis**

All analyses were performed using R version 4.1.0 and the following packages: Seurat\_4.1.0, clustree\_0.4.4, ggplot2\_3.3.5 and dplyr\_1.0.8. Based on the distribution of the numbers of genes and molecules detected per cell, the following filters were applied to remove outliers: nFeature\_RNA > 1,200 & nCount\_RNA > 3,500 & nCount\_RNA < 25,000 for the control site, and nFeature\_RNA > 1,200 & nCount\_RNA > 3,000 & nCount\_RNA < 33,000 for the wound site, respectively. Cells containing more than 15% of mitochondrial genes were considered as dying cells and filtered out. Following preliminary analyses, some contaminating cells representing less than 0.15% of the total cells were removed, based on the expression of either F4/80, CD11b, CD11c, CD64, CD20 or CD206. The thymic dataset (Legoux et al., 2019c) was filtered based on the following arguments: nFeature\_RNA > 800 & nCount\_RNA < 22,000 and less than 10% of mitochondrial genes. The cells with C1qc expression (more than 0.01%) were also removed. In summary, we obtained 3 datasets with comparable cell numbers (3,937 for skin distal, 4,368 for skin wound and 3,428 for thymus) and median number of features (2,458 for skin distal, 2,439.5 for skin wound and 1,812 for thymus).

All three datasets were integrated together using the corresponding Seurat vignette (Stuart et al., 2019). The variable features number was set to 2,000 for the skin

## Results

datasets and encompassed Tbx21 and Mki67. Given that most MAIT cells in the skin are MAIT17 cells, neither Rorc nor Zbtb16 were present in the VariableFeatures lists. This number was raised to 2500 for the thymus, to encompass Rorc and Zbtb16. Following normalization of each dataset and linear transformation (ScaleData), the anchors were identified using the default parameters, except the number of Integration Features which was raised to 4000 to encompass Zbtb16 and Rorc. The data were then integrated. A principal component analysis was run, and the number of principal components to use for downstream clustering (n=25) was determined as proposed in the Seurat Vignette ([https://satijalab.org/seurat/articles/pbmc3k\\_tutorial.html](https://satijalab.org/seurat/articles/pbmc3k_tutorial.html)). Graph-based clustering (Louvain) was performed using the default parameters, and a UMAP (dims=25) was constructed with a resolution of 0.4 based on the stability observed with the package clustree. The differentially expressed genes were determined using the FindAllMarkers() function (using a logistic regression, test.use = "LR", and testing the effect of the dataset, latent.vars = "orig.ident" to correct for the batch effect). The same analysis was performed to determine the genes specific for the skin datasets, after sub-setting the non-cycling MAIT17 cells (FindMarkers()). The signatures used throughout the analysis were gene lists from analyses in (Arpaia et al., 2015; Baranek et al., 2020; Burzyn et al., 2013; Legoux et al., 2019d; Linehan et al., 2018b; Milner et al., 2017; Salou et al., 2019b; Yanai et al., 2016) and are presented in table S1.

### **Migration inhibition protocols (FTY20, Pertussis toxin and $\alpha$ -CXCL16 treatments)**

FTY720 (Sigma) 0.5 mg/kg or PBS alone was injected daily from the day prior to skin excision, until organ collection (Brinkmann et al., 2002). Pertussis toxin (Burns, 1988) treatment (1  $\mu$ g in 100  $\mu$ l i.p., Gibco) was performed one day prior to skin excision and daily until organ collection. *In vivo* CXCL16 blocking was done by injecting i.p 100  $\mu$ g of anti-CXCL16 antibody or 100  $\mu$ g of IgG isotype (R&D Systems) one day prior to skin excision and daily until organ collection.

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### **MAIT expansion and adoptive transfer**

Thymic single-cell suspensions were obtained by mechanical dissociation through a 40 µm cell strainer. Cells were first incubated with MR1:6FP tetramer to avoid unspecific staining, stained using MR1:5-OP-RU-PE tetramer. Enrichment by positive selection on LS columns (Miltenyi) was performed after staining the cells with anti-PE microbeads (Miltenyi).

$2 \times 10^6$  cells/mL were plated in complete RPMI 1640 media (10% FCS, 100U Penicillin/Streptomycin, 10 mM HEPES, 1 mM Sodium Pyruvate, 1X Non-essential Amino Acid, 50 µM β-mercaptoethanol) and stimulated with 150 nM 5-OP-RU and 10 ng/mL of rmlL-2 (Peprotech). MAIT cells were expanded for 10 to 15 days by addition of rmlL-2 (every 2 days) before adoptive transfer or genetic manipulation.

### **CXCR6 CRISPR-Cas9 knock-out**

To create a CXCR6-specific RNP complex, oligos crRNA\_CXCR6\_AA (100 pmole) and tracrRNA (100 pmole) were first annealed using a slow ramp reaching 23°C and incubated at room temperature 10 min with 10 µg S.p Hifi Cas9 Nuclease V3. 2.106 (all reagents from IDT). Expanded MAIT cells were transfected according to the manufacturer instruction (Lonza). Briefly,  $2 \times 10^6$  cells were resuspended in nucleofection solution (Lonza) with 3 µl RNP complex, transferred to nucleofection cuvette strips, electroporated using the DN110 program (4D-Nucleofector Core Unit: Lonza, AAF-1002B) and incubated in complete RPMI 1640 media at 32 °C for 24 hours to force non homologous repair recombination. Transfected cells were further cultured for 2-3 days before transfer. Knock-out efficiency of CXCR6 was evaluated for each experiment on the day of injection by flow-cytometry.

### **Proteome array**

Skin samples (1 cm<sup>2</sup>) were chopped in PBS containing 1% Triton and protease inhibitors, flash-frozen, and then thawed before scratching on a 40 µm filter and centrifugation at 14,000 g for 5 min. 200 µg (as determined by BCA assay from ThermoFisher), of each sample was then added to each blot following manufacturer's instruction (R&D). Blots were imaged using the ChemiDoc imaging system (BIORAD). The

## Results

resulting pixel densities of the protein immune-blot dots were quantified with image J (NIH) following manufacturer's instruction (R&D). Two measurements from each sample were obtained and the pair of duplicate spots was averaged, each MR1<sup>+/+</sup> average values was then divided by the average of MR1<sup>-/-</sup> duplicates.

### **Immunostaining**

Wounds were embedded in OCT (Tissue-Tek) and stored at -80°. Five µm OCT sections were cut using a Cryostat (LEICA). For each skin sample, Hematoxylin and eosin staining was used to determine the middle of the wound, given by the distance between first visible hair follicles on each side of the open wound. All immunostainings were done no more than 5 sections away (25 µm) from that center. Sections were air-dried for 10 min and fixed with 4% paraformaldehyde (EMS) for 5 min. Sections were washed 3 times in PBS 0.05% Tween and then blocked for one hour in permeabilization and blocking (PB) buffer (0.5% skim milk powder, 0.25% gelatine from cold water fish skin, 0.5% Triton X-100, 20 mM HEPES, 0.9% NaCl, pH 7.2 (all reagents from Sigma-Aldrich)). Primary antibodies were diluted in PB buffer and incubated overnight at 4°C, washed 3 times in PBS tween (0.05%). Slides were then stained with secondary antibodies for 1h at room temperature, washed 3 times in PBS tween (0.05%), stained with DAPI (Sigma), washed twice in PBS tween (0.05%), mounted with fluoromount (DAKO) and imaged using an EVOS-M500 microscope (ThermoFisher). Primary antibodies were used as follows: rabbit anti-K14 (1:1000; Biolegend), rabbit anti-mouse Ki67 (1:500; Cell signalling 9129S), rat anti-mouse CD31 (1:100; BD Biosciences) or isotypes. Alexa Fluor-conjugated antibodies (ThermoFisher Scientific) were used at 1:1000 as secondary antibodies.

### **Thymic MAIT cell *in vitro* activation**

Thymic single-cell suspensions were obtained by mechanical dissociation through a 40 µm cell strainer. Enrichment in mature cells (CD24<sup>-</sup>) was achieved by negative selection using LS columns (Miltenyi) after staining the cells with anti-CD24-FITC antibody (Invitrogen) and anti-FITC microbeads (Miltenyi). 1 x 10<sup>6</sup> cells/mL were plated for 36h in complete RPMI 1640 media (10% FCS, 100 U Penicillin/Streptomycin, 10 mM HEPES, 1 mM Sodium Pyruvate, 1X Non-essential Amino Acid, 50 µM β-mercaptoethanol)

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alone or with addition of 5-OP-RU (1.5  $\mu$ M, 5-OP-RU synthesised in house) or of rmlL-18 (10 ng/mL, R&D).

### **Statistical method**

For each experiment, number of independent experiments, replicates and the statistical tests used are indicated in the figure legends. The following statistical test were used and calculated by GraphPad Prism v8 (GraphPad): Mann-Whitney, Wilcoxon, unpaired t-test, paired t test, sídák's multiple comparison test, Tukey's multiple comparison test (with p values: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001 and \*\*\*\*p<0.0001 and "ns" if the comparison was non-significant).

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Marion Salou (marion.salou@curie.fr).

## Results

### Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Antibodies for Cytometry		
Amphiregulin (Biot)	R&D system	Cat#BAF989
Aqua L/D	ThermoFisher	Cat#L34957
B220 (AF700)	eBioscience	Cat#56-0452-82
CCR2 (AF700)	R&D system	Cat#FAB5538N
CD103 (BV786)	BD	Cat#564322
CD103 (PerCP-eF710)	Invitrogen	Cat#46-1031-82
CD11b (APC)	eBiosciences	Cat#17-0112-83
CD11c (PETR)	LifeTechnologies	Cat#MCD11C17
CD19 (AF700)	Biolegend	Cat#115528
CD19 (FITC)	eBioscience	Cat#11-0191-85
CD44 (BV605)	Biolegend	Cat#103047
CD45.1 (PE-Cy7)	Biolegend	Cat#110730
CD45.2 (AF700)	Biolegend	Cat#109822
CD24 (FITC)	Invitrogen	Cat# 11-0242-82
CD69 (PC7 & PE-Dazzle 594)	Biolegend	Cat#104512
CXCR6 (PETR)	Biolegend	Cat#151117
Ki67 (PE-Cy7)	Biolegend	Cat#652426
Ly6C (BV785)	Biolegend	Cat#128041
RORgt (BV786)	BD	Cat#564723
Tbet (APC)	Invitrogen	Cat#17-5825-82
TCRb (APC-Cy7)	Biolegend	Cat#109220
TCRgd (BV605)	Biolegend	Cat#118129
Tet CD1d (BV421)	NIH tetramer core facility	
Tet MR1 (APC)	NIH tetramer core facility	
Tet MR1 (PE)	NIH tetramer core facility	
Antibodies for Immunohistochemistry and Immunofluorescence		
DAPI	Sigma	Cat#MBD0015
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Fisher	Cat#A-21206
Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647	Fisher	Cat#A32933
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Cyanine3	Fisher	Cat#A10522
Keratin 14 Polyclonal Chicken Antibody, Purified Antibody	Biolegend	Cat#905301
Ki-67 (D3B5) Rabbit mAb	Ozyme	Cat#9129S
Purified Chicken IgY Isotype Ctrl Antibody	BIOLEGEND	Cat#402101
Purified Rat Anti-Mouse CD31	BD	Cat#550274
Purified Rat IgG2a κ Isotype Control	BD	Cat#559073
Rabbit (DA1E) mAb IgG XP® Isotype Control	Ozyme	Cat#3900S
Bacterial and virus strains		
Biological samples		
Chemicals, peptides, and recombinant proteins		

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24G2	Institut Curie, produced in house	N/A
Alt-R S.p. HiFi Cas9 Nuclease V3	IDT	Cat#1081061
Anti-mCXCL16	R&D Systems	Cat#MAB503
Anti-PE microbeads	Miltenyi	Cat#130-048-801
Anti-FITC microbeads	Miltenyi	Cat# 130-048-701
B-mercaptoethanol	Sigma	Cat#M3148
BCA Assay	ThermoFisher	Cat#23227
Bovine Serum Albumin	SIGMA	Cat#A7906
Brefeldin A	SIGMA	Cat#B6542
CO2 independent medium	Gibco	Cat#18045088
DAPI	Sigma	Cat#MBD0015
Debris removal solution	Miltenyi	Cat#130-109-398
Dispase	Corning	Cat#354235
DNase 1	Roche	Cat#5401020001
EDTA	Gibco	Cat#15575-038
Fetal Calf Serum	Eurobio	Cat#CVFSVF00-01
Fluorescent Mounting Medium	Dako	Cat#53023
FTY-720	Sigma	Cat#SML0700-25mg
gelatine from cold water fish skin	Sigma	Cat#67765
GolgiPlug	BD	Cat#555029
GolgiStop	BD	Cat#554724
HEPES	Gibco	Cat#15630-056
Liberase TL	Sigma	Cat#10104159001
Live and Dead AQUA	Thermo Fischer Scientific	Cat#L34957
LS columns	Miltenyi	Cat#130-042-401
Maristamat	Sigma	Cat#M2699
Monensin	Invitrogen	Cat#00-4505-51
Non-essential amino acid	ThermoFisher	Cat#11140050
OCT	Tissue-Tek	Cat#16-004004
Paraformaldehyde	EMS	Cat#15710
PBS	Eurobio scientific	Cat#CS1PB501-01
Penicillin/Streptomycin	Gibco	Cat#15140-122
Pertussis Toxin	Gibco	Cat#PHZ1174
Protease inhibitors	Roche	Cat#11697498001
Rat IgG2A Isotype Control	R&D Systems	Cat#MAB006
RBC lysis buffer	Biologend	Cat#420302
RPMI 1640 GlutaMAX	Gibco	Cat#61870036
skim milk powder	Régilait	Cat#B0110287
Sodium Chloride	VWR Chemicals	Cat#27810-295
Sodium Pyruvate (NaPyr)	ThermoFisher	Cat#11360070
Triton X-100	Sigma	Cat#11332481001
Tween	Sigma	Cat#P9416
5-OP-RU	Curie Institute	(Soudais et al., 2015)
<b>Critical commercial assays</b>		
Cytofix/Cytoperm Solution Kit	BD	Cat#554714
FoxP3 Transcription factor Permeabilization buffer	Thermofischer	Cat#00-5523-00
mrmIL-2	Peprtech	Cat#212-12
rmlL-18	R&D	Cat# 9139-IL
Proteome array	R&D	Cat#ARY028
Solution P3 Primary Cell 4DNucleofector X kit S	Lonza	Cat#VAXP-3032
Deposited data		

## Results

Skin single cell datasets	This paper	GSE207348 Deposit pending
Thymic single cell dataset	Legoux et al., 2019	E-MTAB-7704
Experimental models: Cell lines		
Experimental models: Organisms/strains		
Areg <sup>flox/flox</sup>	Bred in Institut Curie - provided by D. Zaiss	(Minutti et al., 2019)
PLZF-GFPcre	Institut Curie	(Constantinides et al., 2014)
CD3 <sup>-/-</sup> MR1 <sup>+/+</sup> and MR1 <sup>-/-</sup>	Institut Curie	(Malissen et al., 1995).
CD45.1/1 and CD45.1/2 B6-MAIT <sup>CAST</sup>	Institut Curie	N/A
CD45.2/2 B6-MAIT <sup>CAST</sup> MR1 <sup>+/+</sup> or MR1 <sup>-/-</sup>	Institut Curie	(Cui et al., 2015; Treiner et al., 2003)
Kaede B6	Rachel Golub, Institut Pasteur	(Tomura et al., 2008)
Nur77-GFP B6-MAIT <sup>CAST</sup>	Institut Curie	(Zikherman et al., 2012)
RORgt-GFP B6-MAIT <sup>CAST</sup>	Institut Curie	(Lochner et al., 2008)
Oligonucleotides		
Alt-R CRISPR-Cas9 crRNA : Mm_CXCR6.1_AA : /AltR1/rCrU rGrUrA rCrGrA rUrGrG rGrCrA rCrUrA rCrGrA rGrUrU rUrUrA rGrArG rCrUrA rUrGrC rU/AltR2/	IDT	N/A
Alt-R® CRISPR-Cas9 tracrRNA	IDT	Cat#1072534
Recombinant DNA		
Software and algorithms		
Astrios software (Summit)	BECKMAN COULTER (Summit v62)	<a href="https://www.beckman.fr/flow-cytometry/cell-sorters/moflo-astrios-eq">https://www.beckman.fr/flow-cytometry/cell-sorters/moflo-astrios-eq</a>
Cytoflex software (CytExpert)	BECKMAN COULTER (V2.4)	<a href="https://www.beckman.fr/flow-cytometry/research-flow-cytometers/cytoflex/software">https://www.beckman.fr/flow-cytometry/research-flow-cytometers/cytoflex/software</a>
FlowJo	BD (V10.8.0)	<a href="https://www.flowjo.com/">https://www.flowjo.com/</a>
Fortessa software (BD FACSDiva software)	BD (V6)	<a href="https://www.bdbiosciences.com/en-eu/products/software/instrument-software/bd-facsdiva-software">https://www.bdbiosciences.com/en-eu/products/software/instrument-software/bd-facsdiva-software</a>
Image J Software	Schneider et al., 2012	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
Prism	GraphPad (V8)	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
Other		
Astrio	BECKMAN COULTER	Cat#B25982

## Results

Biopsy punch (4Ø)	Stiefel	Cat#600210
ChemiDoc Imaging System	BIORAD	Cat#17001402
Chromium 3' Chip	10X Genomics	
Cryostat	LEICA	Cat#CM1950
Cytoflex LX	BECKMAN COULTER	Cat#C00445
Fortessa LSR	BD	Cat#23-11617-01
Hair removal cream	Veet	Cat#EA_3108955
Microscope pour immunostaining	ThermoFischer	Cat#EVOS_M500
Silicone sheet (5mm thick)	Grace Bio-Labs	Cat#GBL664581-5EA

**Table S1: Signatures used for the scRNAseq analysis**

<b>Signature</b>	<b>MAIT0</b>	<b>MAIT1</b>	<b>Cycling_G2M</b>	<b>Cycling_S</b>	<b>MAIT17</b>	<b>ISG</b>
<i>Reference</i>	<i>Legoux et al., 2019a</i>	<i>Salou et al., 2019a</i>	<i>Legoux et al., 2019a</i>	<i>Legoux et al., 2019a</i>	<i>Salou et al., 2019a</i>	<i>Baranek et al., 2020</i>
	Ifi2712a	Klrc1	Tuba1b	Actb	Rorc	Ifit3
	Ms4a4b	Klrd1	Stmn1	Ptma	Itgae	Isg15
	Izumo1r	Samd3	Tubb5	Slc25a5	Il23r	Stat1
	Ms4a6b	Plac8	Ptma	Ppia	Serpib1a	Irf7
	Lef1	Il12rb2	Pclaf	Hmgn2	Mmp25	Bst2
	Slamf6	Ifng	H2afz	Lgals1	1700040D17Rik	
	Igfbp4	Klrb1c	Hmgb2	Cfl1	Pxdc1	
	Satb1	Serpina3g	Hmgn2	Gapdh	Il1r1	
	Cd28	Fasl	Hmgb1	Ran	Lingo4	
	Ccr7	Klrk1	Cks1b	H2afz	Ccr8	
	Plac8	Tbx21	Top2a	Ldha		
	Gimap4	Slamf7	Dut	Eif5a		
	Tesc	Zfp683	Hnrnpab	Hmgb1		
	Ass1	Art2b	Lmnb1	Npm1		
	Inpp4b		Dek	Actg1		
	Cd200r1		Ran	Ybx1		
	Il6ra		Ppia	Hmgb1		
	Tspan32		Smsc2	Ranbp1		
	Drosha		Hist1h2ap	Set		
	Actn1		Ranbp1	Mif		

<b>Signature Part 1</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue residency</b>	<b>Circulating</b>
<i>Reference</i>	<i>Linehan et al., 2018</i>	<i>Burzyn et al., 2013</i>	<i>Arpaia et al., 2015</i>	<i>Yanai et al., 2016</i>	<i>Milner et al., 2017</i>	<i>Milner et al., 2017</i>
	Flg	Pcyt1a	Il18r1	Adrb2	2900026A02Rik	1700025G04Rik
	Flg2	Pcsk1	Il17re	Aimp1	AA467197	A430078G23Rik
	Furin	Ccr1	Itgb1	Aqp3	Abcb1b	Acss1
	Areg	Areg	Ramp3	Arhgap1	Adam19	Aff3
	Bmp2	Frmd5	Tnfsf11	Arnt	Atf3	Apobec2
	Bmp4	Havcr2	Cd40lg	B4galt1	B4galnt4	Arhgap26
	Bmp7	Slc15a3	Plcb4	Bcl11b	Bag3	Arhgef18

Results

<b>Signature Part 2</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue residency</b>	<b>Circulating</b>
Ngf	Ccr3	B3gnt5	Bub3	Bhlhe40	Armc7
Tgfa	Il10rb	Cep290	Cap2	Btg2	As3mt
Tgfb1	Lyn	Tmem176b	Casp1	Btg3	Aven
Tgfb2	Npnt	Plxnc1	Cd151	Ccl4	B3gnt5
Tgfb3	Ctsh	Tmem176a	Cd9	Cd244	Bin2
Pdgfa	Arnt2	Lif	Cdkn1b	Cd69	Car5b
Pdgfb	Il23r	Nrp1	Cebpg	Cdh1	Cdc14b
Csf2	Tubb6	Itga7	Csf2	Chd7	Cmah
Fgf1	Neb	Colq	Ctnnb1	Chn2	D1Ert622e
Fgf7	Snx9	Sema4f	Epb41	Cish	Dapl1
Fgf10	Sik1		Fam129b	Coq10b	Dennd2d
Fgf22	Rgs2		Fgfr1op2	Cpd	Dgka
Igf1	Dgat2		Fhl2	Crem	Dock5
Igf2	Fam46a		Flna	Ctla4	Dusp7
Vegfa	Trf		Hmox2	Dennd4a	Ehd3
Ctgf	Kcna4		Icam1	Dgat1	Elovl7
Mmp3	Arl5a		Icos	Dnaja1	Eomes
Mmp10	Tnfrsf10b		Il22	Dnaja4	F2rl2
Mmp13	Padi2		Il33	Dnajib1	Fam49a
Mmp15	Gpr55		Ilk	Dnajib4	Fam53b
Mmp25	Sdc4		Itga3	Dnajib6	Fgf13
Mmp28	Cd200r1		Itga9	Dusp1	Flna
Defb1	Pparg		Itgb1	Dusp4	Gab3
Defb6	Camk2n1		Jun	Dusp6	Gramd4
Epgn	Ehd4		Junb	Egr1	Gzmm
Hbegf	Tmbim1		Klf10	Ehd1	Hao
Egf	Ifrd1		Klf4	El12	Icam2
Mmp2	Serp1nb6a		Klk8	Fasl	Il17ra
Tnf	Plxnd1		Lgals1	Fbxo30	Il18rap
Il1a	Rrad		Map3k1	Fgl2	Kbtbd11
Il1b	Rasgef1b		Map3k5	Fmn13	Kcnj8
Csf1	Plin2		Mfge8	Fos	Klf2
Ccl2	Msr3		Mgat5	Fosl2	Klhdc1
Ccl3	Per1		Mif	Gabarapl1	Klr1b1c
Cxcl1	Sgms1		Myc	Gadd45b	Klrg1
Cxcl2	Dusp14		Myd88	Gem	Lair1
Cxcl10	Spty2d1		Ncoa3	Glrx	Lfng
Cxcl15	Myo1d		Nf1	Gpr171	Limd1
Il6	Coq10b		Nfe2l2	Gzmb	Lpin1
Pgf	Il9r		Nfic	H3f3b	Ly9
Angpt1	Evi5		Nr4a1	Havcr2	Mboat1

Results

Signature Part 3	Tissue repair	Tissue repair	Tissue repair	Tissue residency	Circulating
	Angpt2	Cables1		Panx1	Hip1
Angpt4	Cd80		Pkd1	Hpgds	Nck2
Cyr61	Ehd1		Prkca	Hspa5	Nod1
Inhba	Nr1d1		Prkce	Icos	Nsg2
Inhbb	Alcam		Prkch	Ifng	Pde2a
Btc	Epcam		Ptk2	Ifrd1	Prss12
Ereg	Arl5b		Ptk2b	Il21r	Prune1
Nrg1	Ets2		Rac1	Il4ra	Pycard
Nrg2	Acot11		Rac2	Inpp4b	Qprt
Nrg4	Neb1		Raf1	Irf4	Rap1gap2
Nrg3	Klf4		Ramp1	Isg20	Rasa3
Lep	Klf8		Ripk3	Isy1	Rasgrp2
Vegfb	Slc25a19		Sdc1	Itga1	Rgs19
Vegfc	Cnm2		Sdc4	Jun	S1pr1
Vegfd	L1cam		Siah2	Junb	S1pr4
Pdgfc	Gprc5a		Smad4	Kdm6b	Samd3
Hmgb1	Syt13		Smad7	Litaf	Sell
Ptges2	Tagln2		Sod1	Lmnb1	Sfxn3
Cxcl12	Axl		Stat3	Ly6g5b	Sh2d1a
Shh	Zdhhc23		Tcf3	Mxd1	Sh3bp5
Dhh	Ky		Tgfb1	Mxi1	Sidt1
Ihh	Ttbk2		Thra	Neurl3	Sike1
Disp1	Fam129b		Thy1	Nfil3	Slamf6
Dll1	Trim46		Tnf	Nfkbie	Smpdl3b
Dll3	Gla		Tnfsf14	Nr4a1	Spn
Dll4	Gm2a		Trp53	Nr4a2	St3gal1
Jag1	Cd74		Vav1	Nr4a3	Stard10
Jag2	Clic4		Vav3	Ntan1	Tcf7
Wnt1	Slc16a3		Vegfa	Odc1	Tlr1
Wnt2	Dusp3		Vim	P2ry10	Tmem71
Wnt2b	Pdgfb		Slc6a6	P4hb	Tnfrsf26
Wnt3	Itgav		Itgb2	Pdcd1	Traf3ip3
Wnt3a	Tdpoz4		Hif1a	Per1	Ubxn2b
Wnt4	Rnf125		Clic4	Plk3	Usp33
Wnt5a	Lgmn		Akt1	Plscr1	Ypel1
Wnt5b	Crem		Anxa1	Pmepa1	Zeb2
Wnt6	Tnfrsf9		Dst	Ppp1r15a	Zfp595
Wnt7a	Rassf6		Egr1	Prdx6	Zfp760
Wnt7b	Bmpr1a		Kit	Ptp4a1	Nfatc3
Wnt8a	N4bp1		Plaur	Qpct	
Wnt8b	Rab11fip1		Pten	Rbpj	

Results

<b>Signature Part 4</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue repair</b>	<b>Tissue residency</b>	<b>Circulating</b>
	Wnt9a Wnt10a Wnt10b Wnt11 Wnt16 Chat Thbs1 Hif1a Hgf Wnt9b	Zc3h12c Hpse Dot1l Zfp36 Icam1 Rab4a Plxdc1 Rorc Asns Irak3 Kdm6b Ero1l Fos Cpm Got1		Il4 Cd44 Ppard Ncoa6	Rgs1 Rgs16 Rhub Rnf149 Rrad Sik1 Skil Slc16a6 Slc7a5 Spty2d1 Ssbp2 Stk17b Tgif1 Tigit Tiparp Tjp1 Tnf Tnfaip3 Tnfrsf1b Tnfrsf9 Tra2a Trib1 Trp53inp2 Ube2s Vps37b Wsb1 Xcl1 Zfand5 Zfp36l1 Hsp90aa1 Rgs10	

**Table S2: Differentially expressed genes determined using the FindAllMarkers() function (test.use = LR, Latent.vars = orig.ident)**

\*For space issues only the 20 most differentially expressed genes for each clusters are represented here

Gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Vps37b	0,34	0,91	0,63	6,52E-276	0
Ifrd1	0,29	0,86	0,57	3,95E-154	0
Gadd45b	0,29	0,86	0,57	1,05E-138	0
Pim1	0,29	0,92	0,67	9,30E-229	0
Gm20186	0,29	0,59	0,38	4,14E-73	0
Ramp3	0,28	0,89	0,56	8,69E-190	0
Nfkbia	0,28	0,95	0,77	6,81E-183	0
Rora	0,27	0,94	0,73	5,37E-206	0
Nfkb1	0,26	0,88	0,63	3,55E-131	0
Neurl3	0,26	0,79	0,46	2,52E-108	0
Arl5c	0,26	0,77	0,49	1,18E-115	0
Dennd4a	0,25	0,80	0,47	5,65E-143	0
Kdm6b	0,25	0,87	0,57	1,96E-170	0
Il2ra	0,25	0,69	0,40	1,23E-93	0
Ifngr1	0,25	0,92	0,68	4,04E-171	0
Nr4a1	0,24	0,84	0,53	2,12E-103	0
Dusp5	0,24	0,84	0,52	6,72E-130	0
Sytl3	0,24	0,77	0,48	1,39E-115	0
Hspa1b	1,00	0,88	0,24	0	1
Hspa1a	1,00	0,82	0,23	0	1
Jun	0,71	0,94	0,61	0	1
Fos	0,65	0,97	0,70	0	1
Zfp36	0,64	0,90	0,60	6,85E-299	1
Dusp1	0,60	0,91	0,67	0	1
Dnajb1	0,54	0,75	0,45	4,08E-199	1
Klf6	0,51	0,95	0,65	5,48E-303	1
Fosb	0,49	0,92	0,56	1,30E-219	1
Klf2	0,45	0,62	0,30	5,25E-203	1
Ubc	0,44	0,99	0,91	4,35E-211	1
Btg2	0,41	0,96	0,84	2,26E-178	1
Hsp90aa1	0,41	0,97	0,88	1,50E-130	1
Hsph1	0,38	0,67	0,31	3,98E-151	1
Phlda1	0,36	0,51	0,27	7,10E-76	1
Cd69	0,36	0,72	0,48	6,96E-73	1
Junb	0,31	0,99	0,92	1,20E-62	1
Egr1	0,31	0,38	0,12	5,76E-122	1

Results

gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Txnip	0,30	0,76	0,39	6,47E-134	1
Ier2	0,29	0,86	0,74	3,34E-44	1
Gzmb	0,37	0,30	0,10	2,22E-61	2
Gzmc	0,20	0,19	0,05	1,98E-30	2
Hsp90b1	0,17	0,94	0,83	2,04E-47	2
Hspa5	0,16	0,94	0,87	6,20E-34	2
Nme1	0,15	0,95	0,73	4,95E-108	2
Mif	0,15	0,98	0,84	3,76E-100	2
Azin1	0,14	0,64	0,44	2,45E-32	2
Ppp1r14b	0,14	0,92	0,74	2,15E-74	2
Lgals1	0,14	1,00	0,95	1,01E-72	2
Manf	0,14	0,90	0,72	2,72E-52	2
Eif5a	0,13	1,00	0,95	4,72E-106	2
Calr	0,13	0,95	0,87	1,90E-44	2
Lgals3	0,13	1,00	0,93	2,33E-66	2
Npm1	0,13	1,00	0,98	5,00E-100	2
Pdia6	0,13	0,78	0,55	1,59E-45	2
Slc25a5	0,12	0,99	0,92	2,13E-93	2
Actg1	0,12	1,00	1,00	8,34E-77	2
Cfl1	0,12	1,00	0,99	3,18E-113	2
Atp5g1	0,12	0,94	0,76	1,81E-71	2
Prdx1	0,12	0,96	0,79	1,49E-72	2
AY036118	0,34	0,82	0,63	4,65E-46	3
Gm42418	0,30	0,97	0,88	8,81E-42	3
Krt83	0,28	0,57	0,27	9,99E-109	3
Rgs1	0,27	0,74	0,61	1,43E-30	3
Cd72	0,24	0,61	0,31	2,86E-102	3
Rorc	0,23	0,99	0,87	9,61E-110	3
Rnf208	0,21	0,47	0,11	3,27E-181	3
Itgae	0,19	0,86	0,60	2,37E-108	3
Capg	0,18	0,99	0,92	2,45E-159	3
Ccr6	0,18	0,69	0,40	1,31E-69	3
Maf	0,17	0,97	0,81	5,51E-58	3
Ly6g5b	0,17	0,72	0,40	4,38E-106	3
Gng2	0,16	0,80	0,56	1,84E-55	3
Znrf1	0,16	0,92	0,73	8,48E-81	3
Sdc4	0,15	0,43	0,21	7,02E-25	3
Crmp1	0,15	0,51	0,25	7,99E-70	3
Lars2	0,15	0,57	0,37	2,17E-18	3
Ctsw	0,15	0,74	0,46	2,87E-122	3
Il17a	0,15	0,21	0,13	0,02036929	3
B930036N10Rik	0,15	0,72	0,55	5,29E-23	3

Results

gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Cd7	0,41	0,74	0,50	1,76E-18	4
Sptssa	0,31	0,89	0,84	8,92E-29	4
Ypel3	0,31	0,69	0,55	3,66E-25	4
Rpl35	0,29	1,00	1,00	7,33E-07	4
Rps17	0,27	1,00	1,00	5,98E-07	4
Actb	0,26	1,00	1,00	1,14E-58	4
Sema4b	0,25	0,43	0,32	6,57E-53	4
Ramp1	0,25	0,90	0,85	1,60E-16	4
Tcrg-C1	0,25	0,67	0,59	0,01970408	4
Rps19	0,25	1,00	1,00	2,98E-22	4
Rpl18a	0,25	1,00	1,00	1,98E-18	4
Rps18	0,23	1,00	1,00	1,53E-06	4
Eef1g	0,22	0,95	0,94	2,31E-09	4
Rps28	0,22	1,00	1,00	8,17E-12	4
Il17re	0,22	0,44	0,37	6,02E-12	4
Slc39a1	0,21	0,33	0,23	3,83E-23	4
Camk2d	0,20	0,47	0,43	6,57E-10	4
Gm2a	0,20	0,66	0,72	4,50E-29	4
Selenop	0,20	0,75	0,75	1,40E-12	4
Rpl36al	0,19	0,90	0,92	1,77E-06	4
Ramp3	0,51	0,98	0,65	1,70E-269	5
Eprs	0,50	0,96	0,67	1,47E-193	5
Odc1	0,49	0,93	0,71	5,56E-120	5
Crem	0,49	0,93	0,49	1,22E-211	5
Neurl3	0,43	0,95	0,54	1,39E-153	5
Dgat1	0,42	0,95	0,51	8,67E-158	5
Vps37b	0,39	0,98	0,70	6,84E-171	5
Fth1	0,38	1,00	1,00	2,97E-137	5
Irs2	0,38	0,80	0,39	9,63E-110	5
Isy1	0,38	0,93	0,58	2,28E-161	5
Usmg5	0,37	1,00	0,90	2,25E-208	5
Paxbp1	0,36	0,91	0,55	7,57E-131	5
Bpnt1	0,36	0,82	0,33	5,41E-163	5
Cd164	0,36	0,95	0,70	1,07E-148	5
Nfkb1	0,35	0,98	0,69	1,48E-117	5
Emb	0,33	1,00	0,93	5,23E-175	5
Cenpa	0,33	0,83	0,47	2,76E-83	5
Il2rb	0,33	0,96	0,62	4,23E-144	5
Rassf3	0,33	0,82	0,41	1,62E-101	5
Cwc25	0,32	0,82	0,45	2,83E-68	5
Stmn1	0,85	0,92	0,11	0	6
Hist1h2ap	0,83	0,45	0,06	7,13E-176	6

Results

gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Tuba1b	0,75	0,94	0,40	0	6
Pclaf	0,73	0,67	0,02	0	6
Hmgn2	0,63	0,94	0,35	0	6
Dut	0,55	0,91	0,23	0	6
Ran	0,55	0,99	0,77	0	6
Tubb5	0,52	0,97	0,74	5,07E-203	6
Top2a	0,52	0,55	0,08	2,18E-265	6
Hmgb2	0,50	0,99	0,86	0	6
Cks1b	0,50	0,77	0,07	0	6
Ranbp1	0,48	0,97	0,59	0	6
Hist1h2ae	0,44	0,43	0,04	5,38E-200	6
Ptma	0,43	1,00	1,00	0	6
Phgdh	0,42	0,87	0,27	5,35E-220	6
Selenoh	0,41	0,91	0,36	7,74E-256	6
H2afz	0,40	1,00	0,90	2.5398e-314	6
Slc25a5	0,40	1,00	0,92	1,00E-251	6
Rrm2	0,40	0,46	0,02	1,19E-245	6
Birc5	0,40	0,45	0,01	1,81E-269	6
Ifi2712a	0,83	0,85	0,27	7,67E-162	7
Ms4a4b	0,69	0,71	0,09	2,58E-151	7
Izumolr	0,67	0,57	0,02	1,46E-165	7
Lef1	0,63	0,72	0,13	2,33E-149	7
Igfbp4	0,56	0,41	0,03	2,94E-94	7
Itm2a	0,55	0,32	0,02	1,53E-44	7
Plac8	0,51	0,34	0,02	1,87E-74	7
Ms4a6b	0,51	0,70	0,19	3,17E-114	7
Id3	0,49	0,48	0,11	1,73E-41	7
Gimap4	0,48	0,60	0,09	1,35E-114	7
Ccr9	0,47	0,37	0,02	6,67E-104	7
Limd2	0,40	0,95	0,84	4,17E-52	7
Bcl2	0,38	0,30	0,14	1,87E-24	7
Rgs10	0,38	0,84	0,60	5,43E-09	7
Cd2	0,38	0,78	0,59	1,19E-101	7
Slamf6	0,38	0,49	0,03	6,83E-110	7
Cd247	0,37	0,69	0,45	1,20E-54	7
Tspan32	0,36	0,58	0,22	3,96E-82	7
Cd8b1	0,35	0,17	0,09	3,96E-17	7
Cd28	0,35	0,80	0,42	2,23E-105	7
Hopx	0,64	0,65	0,19	1,30E-121	8
Il17a	0,59	0,41	0,12	4,59E-32	8
Zeb2	0,56	0,65	0,15	2,43E-153	8
Traf1	0,53	0,93	0,47	7,27E-174	8

## Results

gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Areg	0,53	0,15	0,03	2,83E-24	8
Crip1	0,49	0,99	0,91	4,28E-92	8
Fth1	0,42	1,00	1,00	3,40E-92	8
Ostf1	0,42	0,96	0,74	5,26E-228	8
Gm42031	0,39	0,46	0,21	5,54E-39	8
Samsn1	0,38	0,94	0,56	1,09E-96	8
Rbpj	0,37	0,80	0,40	8,66E-88	8
Bhlhe40	0,36	0,79	0,39	3,63E-73	8
Hlf	0,35	0,65	0,30	2,35E-54	8
Hif1a	0,34	0,84	0,50	2,44E-90	8
Stx11	0,34	0,72	0,30	2,02E-89	8
Uhrf2	0,33	0,82	0,54	1,16E-50	8
Ubald2	0,33	0,96	0,66	6,75E-79	8
Itgav	0,33	0,56	0,23	1,14E-56	8
Sgip1	0,32	0,21	0,07	1,24E-21	8
Rnf157	0,31	0,66	0,24	2,73E-69	8
Ccl5	1,15	0,39	0,01	1,41E-143	9
Nkg7	0,82	0,89	0,29	2,51E-251	9
Ms4a4b	0,78	0,89	0,10	1,10E-175	9
Ly6c2	0,78	0,39	0,01	2,61E-123	9
AW112010	0,70	0,94	0,61	4,20E-159	9
Gimap4	0,69	0,78	0,10	7,05E-167	9
Klrd1	0,61	0,46	0,01	3,92E-167	9
Xcl1	0,58	0,42	0,00	1,42E-143	9
Gimap3	0,58	0,76	0,16	8,04E-160	9
Klra9	0,55	0,23	0,00	2,76E-85	9
Ifi2712a	0,55	0,72	0,29	7,59E-27	9
Bcl2	0,54	0,59	0,14	7,02E-72	9
Ctsw	0,54	0,92	0,47	2,02E-120	9
Ms4a6b	0,54	0,73	0,21	1,59E-82	9
Dusp2	0,53	0,77	0,39	4,68E-102	9
Gimap6	0,52	0,71	0,28	9,10E-100	9
Klrl1	0,46	0,53	0,34	4,76E-109	9
Ctsd	0,43	0,82	0,60	5,40E-86	9
Cxcr3	0,41	0,47	0,03	2,90E-139	9
Gimap1	0,38	0,68	0,35	1,36E-56	9
Isg15	0,69	0,78	0,21	6,18E-133	10
Cxcl10	0,67	0,29	0,12	2,06E-18	10
Ifit1	0,58	0,61	0,04	9,41E-134	10
Bst2	0,55	0,85	0,40	1,53E-128	10
Ifi203	0,51	0,88	0,45	3,43E-127	10
Slfn5	0,46	0,61	0,15	1,87E-102	10

Results

gene	Avg_log10C	pct.1	pct.2	p_val_adj	cluster
Stat1	0,46	0,88	0,34	2,03E-113	10
Rtp4	0,43	0,74	0,11	8,52E-130	10
Zbp1	0,42	0,78	0,20	2,20E-121	10
Ifi206	0,41	0,66	0,18	3,50E-90	10
Ly6a	0,41	0,65	0,29	1,29E-37	10
Mndal	0,39	0,88	0,50	6,22E-89	10
Isg20	0,39	0,57	0,11	7,73E-95	10
Ifi209	0,39	0,70	0,21	1,40E-85	10
Trim30a	0,37	0,66	0,22	7,89E-80	10
AW112010	0,37	0,89	0,61	6,15E-47	10
Ifit3	0,37	0,34	0,01	3,34E-69	10
Rnf213	0,35	0,60	0,14	6,00E-91	10
Rsad2	0,35	0,33	0,01	6,89E-78	10
Ifi47	0,34	0,67	0,24	5,61E-64	10
Gm42418	0,86	1,00	0,89	6,53E-39	11
Hspa1b	0,78	0,64	0,31	2,96E-06	11
Hspa1a	0,77	0,67	0,29	5,15E-08	11
Atf3	0,64	0,81	0,48	9,38E-09	11
Lars2	0,64	0,81	0,39	1,65E-31	11
Dnajb1	0,59	0,67	0,48	1,51E-07	11
Tnfaip3	0,58	0,97	0,69	1,71E-33	11
Ier2	0,57	0,96	0,75	1,54E-21	11
Jun	0,56	0,87	0,65	1,56E-07	11
Nr4a1	0,56	0,96	0,63	3,06E-20	11
Hsph1	0,56	0,51	0,35	0,0001231	11
Klf6	0,56	0,83	0,68	6,09E-09	11
Btg2	0,55	1,00	0,85	4,01E-20	11
Fosb	0,53	0,88	0,60	9,89E-12	11
Cd69	0,53	0,80	0,50	2,79E-10	11
Zfp36	0,53	0,87	0,63	1,15E-05	11
Junb	0,53	0,99	0,92	2,09E-20	11
Ifrd1	0,52	0,88	0,66	5,58E-08	11
Hsp90aa1	0,52	0,96	0,89	0,00341497	11
Gadd45b	0,51	0,94	0,67	2,18E-05	11
Itm2a	1,34	0,93	0,03	4,66E-72	12
Lef1	1,32	1,00	0,15	6,62E-98	12
Nrgn	1,19	0,77	0,03	7,91E-69	12
Gm43352	1,01	0,81	0,02	1,13E-75	12
Bcl2	0,96	0,90	0,15	1,79E-51	12
Slc29a1	0,96	0,68	0,16	1,57E-51	12
Tox	0,90	0,97	0,36	9,28E-73	12
Id3	0,89	0,71	0,13	4,35E-25	12

## Results

<b>gene</b>	<b>Avg_log10C</b>	<b>pct.1</b>	<b>pct.2</b>	<b>p_val_adj</b>	<b>cluster</b>
Cd28	0,86	0,97	0,44	3,23E-68	12
Slamf6	0,84	0,65	0,05	2,54E-40	12
Cldn10	0,80	0,63	0,01	3,28E-50	12
Tubb5	0,79	0,97	0,76	1,77E-41	12
Cd27	0,78	0,93	0,41	4,75E-52	12
Izumo1r	0,78	0,71	0,05	4,03E-23	12
Hivep3	0,74	0,63	0,04	2,05E-53	12
Rgs10	0,69	0,93	0,61	5,84E-25	12
Sox4	0,68	0,71	0,11	1,39E-33	12
Cd2	0,67	0,85	0,60	1,11E-35	12
Ubac2	0,67	0,62	0,33	1,03E-25	12
Myb	0,65	0,60	0,06	1,55E-43	12

**Table S3: Differentially expressed genes determined using the FindMarkers() function by orig.ident (test.use = LR)**

List of genes differentially expressed for the following comparisons, with an avg\_log10FC>0.25 and p\_val\_adj<0.05

<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
40787	3110043O21Rik	40787
4930523C07Rik	4930523C07Rik	4930523C07Rik
4932438A13Rik	4932438A13Rik	4932438A13Rik
AC160336.1	AC160336.1	AC160336.1
Adam8	Aebp2	Adgre5
Adgre5	Aff1	Aebp2
Aebp2	Aff4	Aff1
Aff4	Ago2	Aff4
Ago2	Ahnak	Ago2
Ahnak	Akap13	Ahnak
Akap13	Ankrd11	Akap13
Ankrd11	Arap2	Ankrd11
Anxa2	Arf4	Anxa2
Arap2	Arl5b	Arap2
Arid4b	Arl5c	Arf4
Arl5b	Arpc3	Arl5b
Arl5c	Atf3	Arl5c
Arpc3	Atp2b1	Arpc3
Atf3	Avpi1	Atf3
Atf4	AY036118	Atf4
Atf6	Bcl2a1b	Atf6
Atp2b1	Bcl2a1d	Avpi1
Avpi1	Bhlhe40	AY036118
AY036118	Birc3	Bcl2a1b
Bcl2a1b	Bpnt1	Bcl2a1d
Bhlhe40	Brd1	Bhlhe40
Birc3	Btg1	Birc3
Bpnt1	Btg2	Bpnt1
Brd1	Ccn11	Brd1
Btg1	Ccr2	Btg1
Btg2	Cd164	Btg2
Calr	Cd40lg	Calr
Ccdc88c	Cd44	Ccdc88c
Ccn11	Cd69	Ccn11
Cd164	Cd83	Ccr2

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Cd40lg	Cdk11b	Cd164
Cd44	Cdkn1a	Cd40lg
Cd69	Cebpb	Cd44
Cd83	Cenpa	Cd69
Cdkn1a	Chd2	Cd83
Cdkn1b	Chd7	Cdk11b
Cebpb	Cish	Cdkn1a
Cenpa	Clcn3	Cebpb
Cep170	Clk1	Cenpa
Chd2	Coq10b	Chd2
Chd4	Crem	Chd4
Chd7	Crip1	Chd7
Cish	Csrnp1	Cish
Clcn3	Ctla2a	Clcn3
Clk1	Cwc25	Clk1
Coq10b	Cxcl10	Coq10b
Crem	Cxcl2	Crip1
Crip1	Cxcr4	Csde1
Csde1	Cxcr6	Csrnp1
Csrnp1	Cytip	Ctla2a
Ctla2a	D16Ert472e	Cwc25
Cwc25	D8Ert4738e	Cxcl10
Cxcr4	Ddx3x	Cxcr4
Cxcr6	Ddx5	Cxcr6
Cytip	Dennd4a	Cytip
D16Ert472e	Dennd5a	D16Ert472e
Ddx3x	Dgat1	D8Ert4738e
Ddx5	Diaph1	Ddx3x
Dennd4a	Dnaja1	Ddx5
Dgat1	Dnajib1	Dennd4a
Diaph1	Dnajib6	Dennd5a
Dnaja1	Dot11	Dgat1
Dnajib1	Dusp1	Diaph1
Dnajib6	Dusp10	Dnaja1
Dot11	Dusp5	Dnajib1
Dusp1	Ell2	Dnajib6
Dusp5	Emb	Dot11
Elmsan1	Emd	Dusp1
Emb	Eprs	Dusp5
Emp1	Ets1	Ell2
Eprs	Ezr	Emb

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Ets1	Fam107b	Emp1
Ezr	Fam110a	Eprs
Fam110a	Fosb	Ezr
Fnbp1	Fosl2	Fam107b
Fosb	Fth1	Fam110a
Fosl2	Furin	Fnbp1
Fth1	Gadd45b	Fosb
Furin	Gch1	Fosl2
Gadd45b	Gem	Fth1
Gch1	Gls	Furin
Gem	Gm20186	Gadd45b
Gls	Gm26669	Gch1
Gm20186	Gm42031	Gem
Gm26532	Gm42418	Gls
Gm26669	Gm45716	Gm20186
Gm42418	Gm47283	Gm26669
Gm47283	Gna13	Gm42031
Gna13	Got1	Gm42418
Got1	Gpbp1	Gm47283
Gpbp1	Gpr132	Gna13
Gpr132	Gpr183	Got1
Grap2	Gramd3	Gpbp1
H2-K1	Grap2	Gpr132
H2-Q7	Gzmb	Gpr183
Hif1a	H2-D1	Grap2
Hilpda	H2-K1	Gzmb
Hk2	H2-Q6	H2-D1
Hlf	H2-Q7	H2-K1
Hnrnpa0	Hif1a	H2-Q6
Hopx	Hilpda	H2-Q7
Hsp90aa1	Hivep2	Hif1a
Hsp90b1	Hk2	Hilpda
Hspa1a	Hmgb2	Hk2
Hspa1b	Hnrnpa0	Hlf
Hspa5	Hopx	Hmgb2
Hsph1	Hsp90aa1	Hnrnpa0
Icam1	Hsp90b1	Hopx
Ier5	Hspa1a	Hsp90aa1
Ier5l	Hspa1b	Hsp90b1
Ifngr1	Hspa5	Hspa1a
Ifrd1	Hsph1	Hspa1b

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Ikzf3	Icam1	Hspa5
Il17a	Icos	Hsph1
Il2rb	Ier5	Ier2
Il2rg	Ier5l	Ier5
Il4ra	Ifnar1	Ier5l
Iqgap1	Ifngr1	Ifnar1
Irak2	Ifrd1	Ifngr1
Irf2bp2	Ikzf3	Ifrd1
Irs2	Il17a	Ikzf3
Isy1	Il2ra	Il17a
Itk	Il2rb	Il2ra
Itpkb	Il2rg	Il2rb
Jmjd1c	Il4ra	Il2rg
Jun	Irak2	Il4ra
Junb	Irf2bp2	Iqgap1
Jund	Irs2	Irak2
Kdm2b	Isy1	Irf2bp2
Kdm6b	Itk	Irs2
Klf4	Itpkb	Isy1
Klf6	Jmjd1c	Itk
Klrk1	Jmy	Itpkb
Kpna1	Jun	Jmjd1c
Kras	Junb	Jmy
Lars2	Jund	Jun
Lgals1	Kbtbd11	Junb
Lmna	Kctd12	Jund
Lrrfip1	Kdm6b	Kctd12
Maf	Klf4	Kdm2b
Map3k14	Kpna1	Kdm6b
Map3k8	Kras	Klf4
Map4k4	Lars2	Klf6
Mapk6	Lmna	Klrk1
March7	Macf1	Kpna1
Mast4	Maf	Kras
Mbd2	Map3k14	Lars2
Mcl1	Map3k8	Lmna
Msn	Map4k4	Lrrfip1
mt-Co2	March7	Macf1
mt-Nd2	Mast4	Maf
mt-Nd4l	Mbd2	Map3k14
mt-Nd5	Mcl1	Map3k8

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Myo1e	Mier1	Map4k4
Ncor1	mt-Co1	Mapk6
Nebl	mt-Nd2	Mast4
Neurl3	mt-Nd4l	Mbd2
Nfil3	mt-Nd5	Mcl1
Nfkb1	Mxd1	mt-Co2
Nfkbia	Myo1e	mt-Nd2
Nfkbie	Nabp1	mt-Nd4l
Nfkbiz	Neat1	mt-Nd5
Nkg7	Neurl3	Myo1e
Nktr	Nfil3	Ncor1
Nr1d1	Nfkb1	Neat1
Nr3c1	Nfkbia	Neurl3
Nr4a1	Nfkbid	Nfil3
Nr4a3	Nfkbie	Nfkb1
Nrip1	Nfkbiz	Nfkbia
Nup98	Nktr	Nfkbid
Odc1	Nr3c1	Nfkbie
Orai1	Nr4a1	Nfkbiz
P2ry10	Nr4a3	Nkg7
Paxbp1	Nrip1	Nktr
Pcgf5	Nup98	Nr3c1
Pde4b	Odc1	Nr4a1
Per1	Orai1	Nr4a3
Pfkp	Osbp19	Nrip1
Pi4k2a	P2ry10	Nup98
Pim1	Paxbp1	Odc1
Pim3	Pcgf5	Orai1
Plec	Pde4b	Osbp19
Plp2	Per1	P2ry10
Pnrc1	Pfkp	Pcgf5
Ppfia1	Phlda1	Pde4b
Ppp1r15a	Pim1	Per1
Ppp2ca	Plcx2	Pfkp
Prkd3	Plin2	Phlda1
Prr7	Pnrc1	Pim1
Prrc2c	Ppfia1	Pim3
Ptp4a1	Ppp1cc	Plin2
Ptp4a2	Ppp1r15a	Pnrc1
Ptprc	Ppp2ca	Ppfia1
Purb	Prkd3	Ppp1cc

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Pxdc1	Prr7	Ppp1r15a
Rab11fip1	Prrc2c	Ppp2ca
Rab8b	Ptp4a2	Prr7
Ramp3	Ptpn22	Prrc2c
Ranbp2	Ptprc	Ptp4a1
Rassf3	Purb	Ptp4a2
Rbpj	Rab21	Ptpn22
Rel	Rab8b	Ptprc
Rgcc	Ramp3	Purb
Rgs2	Ranbp2	Rab21
Rictor	Rassf3	Rab8b
Riok1	Rbm33	Ramp3
Rock1	Rbpj	Ranbp2
Rora	Rel	Rassf3
Rrad	Rgcc	Rbm33
S100a4	Rgs2	Rbpj
S100a6	Riok1	Rel
Samsn1	Rnf157	Rgcc
Satb1	Rock1	Rgs2
Sdc4	Rora	Rictor
Sf3b1	Rps21	Riok1
Sik1	Rrad	Rnf157
Slc38a1	S100a4	Rock1
Slc38a2	S100a6	Rora
Slc7a1	Samsn1	Rrad
Slk	Satb1	S100a4
Smap2	Sdhaf1	S100a6
Snx18	Sf3b1	Samsn1
Spag9	Sfpq	Satb1
Spty2d1	Sik1	Sdc4
Srgn	Sipa111	Slc38a1
Stk17b	Skil	Slc38a2
Stk4	Slc15a3	Slk
Syt13	Slc38a1	Smap2
Tax1bp1	Slc38a2	Snx18
Tgif1	Slk	Spag9
Tgoln1	Smap2	Spty2d1
Tiparp	Spag9	Srgn
Tmem64	Spty2d1	Stat4
Tnf	Srgn	Stk17b
Tnfaip3	Stat4	Stk4

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<b>Skin steady state vs Thymus</b>	<b>Skin wound vs Thymus</b>	<b>Skin (steady state and wound) vs Thymus</b>
Tnfaip8	Stk17b	Tgif1
Tnip1	Stk4	Tgoln1
Tpt1	Tgif1	Tnfaip3
Tra2a	Tgoln1	Tnfaip8
Tra2b	Tiparp	Tnfrsf1b
Traf1	Tmem64	Tnip1
Tsc22d3	Tnfaip3	Tob2
Txnip	Tnfaip8	Tpt1
Ubald2	Tnfrsf1b	Tra2a
Ubc	Tnip1	Traf1
Ube2s	Tob2	Traf4
Ubl3	Tpt1	Tsc22d3
Ugcg	Tra2a	Ubald2
Uhrf2	Traf1	Ubc
Vgl14	Traf4	Ube2s
Vim	Tsc22d3	Ubl3
Vps37b	Ubald2	Ugcg
Wnk1	Ubc	Uhrf2
Ythdc1	Ubl3	Vgl14
Zc3h12a	Ugcg	Vim
Zc3hav1	Usmg5	Vps37b
Zeb1	Vgl14	Wnk1
Zfp36	Vps37b	Ythdc1
Zfp3611	Ythdc1	Zc3h12a
Zfp3612	Zc3h12a	Zc3hav1
Zfp91	Zc3hav1	Zcchc11
	Zcchc11	Zeb1
	Zdhhc18	Zeb2
	Zeb1	Zfp36
	Zeb2	Zfp3611
	Zfp36	Zfp3612
	Zfp3611	Zfp91
	Zfp3612	

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## Discussion and perspectives

## A. Afferent signals and repair function of MAIT cells

A major area of research within this work was the study of the afferent signals required for MAIT cell dynamics and pro-repair function. Given the published *in vitro* and *in vivo* literature that showed the induction of a pro-repair effector function of MAIT cells following TCR stimulation (Constantinides et al., 2019a; Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a), we studied its impact in our model. We also aimed at understanding the repair function MAIT cells played within the skin in the early stages of wound closure.

### i. Afferent signals

#### **TCR necessity?**

In our setting, MR1 expression was unnecessary for the recruitment of MAIT cells within the wound and their pro-repair function following *in vivo* transfer, indicating a TCR-independent function. During our expansion experiments, we stimulated MAIT cells through their TCR (via the addition of 5-OP-RU) to expand our cells. 5-OP-RU was added only on the first day of culture. Moreover, TCR stimulation of MAIT cells *in vitro* and *Staphylococcus epidermis* association *in vivo* demonstrated a skewing of MAIT cells towards a pro-repair phenotype (Constantinides et al., 2019a; Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a). It is therefore possible that exposure to 5-OP-RU (i.e., in our expansion; released by the bacteria that rapidly colonize the wound) could induce the tissue repair effector function of MAIT cells. We have saved some cells from our *in vitro* cell expansions for transcriptomic analysis. This will allow us to evaluate whether the expansion of MAIT cells has “skewed” their phenotype. From our experiments, we can only conclude that sustained TCR signaling is not required for MAIT cell pro-repair function.

#### **Alarmins?**

One puzzling result in our study was the near-identical transcriptomic profile between MAIT cells from the wound site as compared to the control site. As discussed in the “Limitations of study” paragraph, prolonged incubation at 37°C (necessary for the skin digestion) could modify the transcriptional profile of MAIT cells. To address this, we are

currently performing single cell analysis on MAIT cells that underwent digestion and cells that have not. However, it appears highly unlikely that digestion alone would be responsible for the loss of differences between MAIT cells at steady state and in the wound (Crawl et al., 2022).

Supposing that digestion is not a confounding factor, this transcriptomic similarity would indicate that MAIT cells within the skin are already transcriptionally poised towards a repair function. The transcriptional pro-repair profile in non-inflamed skin has been shown for other repair cells: H2-M3 restricted T cells (Harrison et al., 2019a). Despite transcriptional expression of pro-repair genes (e.g., IL-5, IL-13) in H2-M3 T-cells, there is no protein expression at steady-state, indicating the presence of a translational checkpoint (Harrison et al., 2019a). Protein expression of H2-M3 T-cells occurs following alarmin (e.g., IL-1, IL-18, IL-25 and IL-33) mediated stimulation (Harrison et al., 2019a). The significant increase in the Areg protein expression by MAIT cells from the wound as compared to the control skin despite no significant differences at the transcriptomic level (data not shown) supports the existence of this checkpoint.

### ***In silico* Single cell transcriptomic analysis**

To determine which afferent signals other than the MAIT cell cognate ligand would elicit the repair function of MAIT cells, we looked in our single cell data for receptors present at the surface of MAIT cells in the skin.

Several candidates are provided by the literature including type I IFNs that are produced by plasmacytoid DCs during skin damage in response to commensal bacteria and promote wound closure by inducing the expression of growth factors by macrophages and fibroblast (Di Domizio et al., 2020). IL-18 is also a promising candidate as it leads to production of pro-repair mediators by H2-M3 cells and its receptor is highly expressed by skin MAIT cells as seen in our single-cell data (Harrison et al., 2019a). Given the pro-repair function that it elicits in Tregs, IL-33 would appear as a promising candidate. However, from our single-cell analysis its receptor is not expressed by skin MAIT cells.

Following the identification of potential candidates, we would test *in vitro* and *in vivo* their effect on MAIT cell repair function as detailed hereafter.

### **In vitro**

It is important to assess whether TCR-stimulation (via 5-OP-RU addition) or non-cognate signals (e.g., type I IFNs, IL-18) lead to the production of tissue repair mediators (e.g., Areg) by MAIT cells. *In vitro*, we could sort MAIT cells collected *ex vivo* from the skin before exposing them to different stimulations (e.g., 5-OP-RU, IL-18) and assessing their production of effector mediators (e.g., Areg) in the supernatant (e.g., via Elisa) or by flow-cytometry (intracellularly).

### **In vivo**

To evaluate whether MAIT cells rely on TCR-mediated signals to mediate their functions *in vivo*, two points must be assessed: 1) Necessity of TCR-triggering for long-term MAIT cell retention within the skin. 2) Requirement of TCR-signaling for the repair function of MAIT cells in the absence of pre-amplification. Both questions can be answered using the **MR1 conditional knockout mouse (*Mr1<sup>f/f</sup>)*** and **mono-colonized germ-free mice** that have been recently generated in the host lab. We will colonize breeding GF mice with an antibiotic-sensitive 5-OP-RU producing bacteria. After weaning of the progeny, we will verify the presence of MAIT cells in peripheral organs including the skin. Once the bacterial strain is removed by antibiotic treatment, we will evaluate whether MAIT cells remain within the skin and for how long. If they do, we will perform skin excision to assess if MAIT cells still promote wound healing. In this mono-colonized germ-free setting, we will still need to check for a skewing of the resulting MAIT cells (e.g., Th1 versus Th17) by performing a transcriptomic analysis to compare MAIT cells from colonized, short-term bacterial-depleted (with antibiotics), long-term bacterial-depleted and the usual SPF mice.

Lastly, to assess if other signals (e.g., IL-18) are needed for MAIT cell pro-repair function in our model, we will use knock-out mice, if they are available and not detrimental to the development of MAIT cells (e.g., impair thymic development of MAIT cells in IL-18<sup>-/-</sup> mice). We could also block afferent signals using neutralizing antibodies (e.g., anti-IL-18). Our readouts would be wound closure and MAIT cell secretory capabilities.

## Discussion and perspectives

Understanding afferent signals that would induce the repair function of MAIT cells is a key step in unravelling MAIT cell biology. Moreover, given that both human and murine MAIT cells upregulate tissue-repair mediators following TCR-mediated stimulation, this opens up therapeutical perspectives for MAIT cells (Hinks et al., 2019a; Lamichhane et al., 2019a; Leng et al., 2019a).

Accordingly, to assess if TCR-stimulation improves repair, I have attempted to apply 5-OP-RU topically post-wounding. 5-OP-RU supplementation led to the formation of a thick scab despite the presence of a dressing as compared to untreated wounds (data not shown), thereby preventing macroscopic measurements of wound closure. Scabs can contribute to wound healing by serving as a surface for cell migration and movement of the wound margins (Phillips, 2000). To address if 5-OP-RU topical application is a viable therapeutical option, this experiment would need to be repeated and wound closure measurements performed via histology.

Therefore, understanding the afferent signals and impact they have on MAIT cell biology would help not only further decipher MAIT cell biology, but could also provide different therapeutic perspectives (e.g., repair, anti-inflammatory, etc.). In a therapeutic perspective, it would be critical to evaluate the impact of MAIT cell cognate/non-cognate stimulation on wound repair and its quality longitudinally.

### ii. Repair quality and wound kinetics.

#### **A function for MAIT cells beyond the early stages of repair?**

The prepositioning of MAIT cells at steady state in the skin and the macroscopic observation of a significant acceleration of the early stages of wound closure in their presence prompted us to study MAIT cells and the skin in the first days post-wounding.

In this study, we performed longitudinal assessments of other immune cells (e.g., monocytes, mainstream T cells) on days 3, 6, and the very late day 29. We did not observe changes in any hematopoietic subset frequency and phenotype. Still, we cannot rule out the possibility that, in addition to their early tissue-repair function, MAIT cells may act directly or modulate other immune cells during the later stages of repair. Tregs, for instance, play a role in both the early stages (e.g., inhibition of neutrophil recruitment)

## Discussion and perspectives

and the late stages (e.g., matrix remodeling) of wound healing (Dobaczewski et al., 2010; Li et al., 2018) (Fig 18). Therefore, it would be critical to evaluate the impact of MAIT cells throughout wound repair.

To assess this potential late effect, we will perform another time course analysis between days 6 and 29, analyzing the impact of MAIT cell presence on other cell types.

### **Repair quality?**

It would seem logical that the acceleration of wound healing by MAIT cells would lead to a better quality of repair as “prolonged inflammation impairs wound healing and increases scarring” (Qian et al., 2016), but this remains to be addressed. Assessment of repair quality could be done by evaluating collagen deposition and the skin tensile strength.

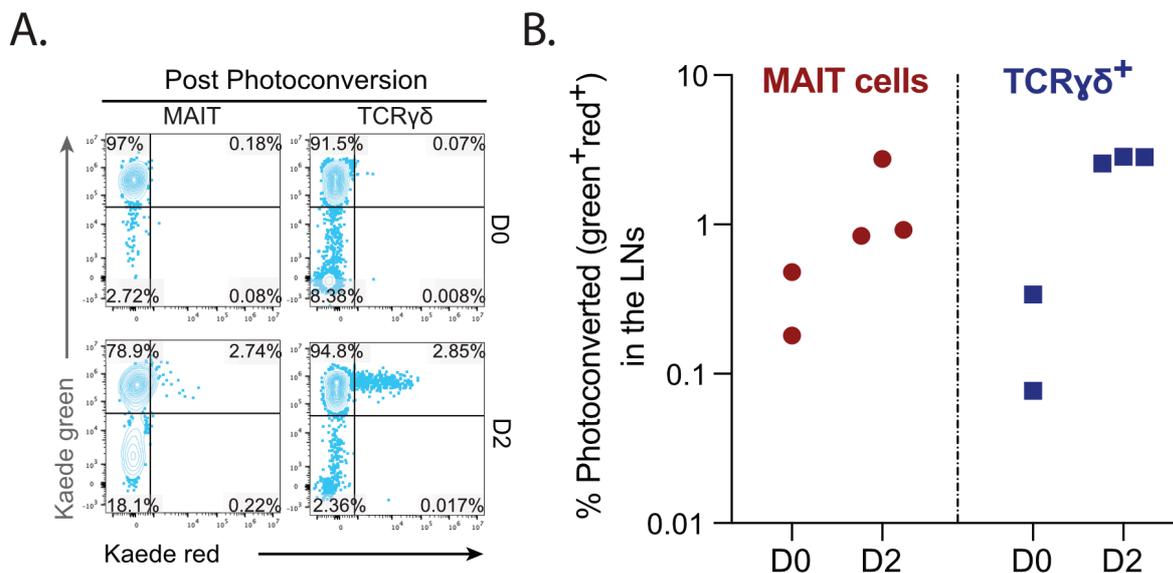
Some physical discomfort may result from the previously mentioned keloid and hypertrophic scars such as: itchiness, tightness, functional impairment, and pain (El Kinani and Duteille, 2020). Given the societal (i.e., financial) and personal burdens of abnormal scarring, a reduced or better quality-scarring induced by MAIT cells would confer an additional solid incentive for the therapeutical use of MAIT cells in wound healing.

## **B. The Lymph-Node puzzle**

In our skin excision setting, following injury, MAIT cell numbers significantly decrease within the skin-draining lymph nodes (LNs). Moreover, blocking MAIT cell LN-egress via FTY-720 results in a loss of MAIT cell accumulation within the wound site. Loss of accumulation indicates transit in the LNs before MAIT cell recruitment to the wound. Because this LN transit occurs during wound healing, we could analyze the dynamics and potential function of MAIT cells within LNs at steady-state and after wounding.

### At steady state

Type 17 V $\gamma$ 4 and V $\gamma$ 6  $\gamma\delta$  T cells effectively migrate from the skin to the LNs and from the draining LNs back to the skin (McKenzie et al., 2017; Van Rhijn et al., 2007). Mainstream TRM cells, are also found in the LNs (Beura et al., 2018). Transcriptomic analysis revealed that some LN TRM cells share an expression profile with their skin counterpart, implying that some LN TRM cells could be the offsprings of skin TRMs. Moreover, following peptide challenge, the Kaedes photoconvertible mice demonstrated migration of skin TRMs to the LNs (Beura et al., 2018).



**Fig 21: (A) Flow cytometry example and (B) frequency of photoconverted MAIT and  $\gamma\delta$  T cells in the inguinal and brachial lymph nodes.** Data is of one experiment with: 2 mice analyzed at day 0 and 3 mice at day 2. This preliminary experiment requires repetition to validate its reproducibility. \*

\* This preliminary experiment was repeated and is now included in the revised version of the paper that was added to this manuscript.

Using Kaedes mice, we observed a decrease in the proportions of photo labeled MAIT cells in the skin. In a preliminary experiment, we collected the skin draining LNs and looked for skin photo-converted MAIT cells in the draining LNs. Validating our experimental setting, two days after photoconversion we found an increase in both the numbers (not shown) and frequency of photoconverted T cells in the LNs as compared to day 0 (collected right after the 60sec photoconversion), as previously described in the literature (Van Rhijn et al., 2007) (Fig 21). Surprisingly, some photo-converted MAIT cells also appeared within the LNs, indicating a skin to LN migration of MAIT cells (Fig 21). To conclude on this additional dynamic would require a repetition of the Kaedes experiment.

## Discussion and perspectives

As an alternate and complementary model, we will use the “FITC-painting” technique on our B6-CAST<sup>MAIT</sup> mouse, allowing us to track skin MAIT cells (Tadayon et al., 2021).

Nonetheless, this preliminary result of a skin to LNs migration could be indicative of a function for MAIT cells within LNs and/or a required transit to acquire specific effector characteristics.

### **Afferent or efferent functions of the LNs.**

In both the photo-conversion and “FITC-painting” models, the skin is slightly inflamed (Friess et al., 2022; Ober-Blöbaum et al., 2017). Therefore, to unravel the MAIT cell biology at steady state in LNs, a transcriptomic analysis at the single cell level could be useful. Transcriptomic analysis would allow to determine the existence of a differential or common programs between MAIT cells from the LNs and skin. Accordingly, we performed such single-cell transcriptomic analysis (scRNA-seq) on sorted MAIT cells from the inguinal and brachial skin-draining LN. To evaluate if the program acquired by MAIT cells would be LN specific, we also sorted MAIT cells from other draining LNs (mediastinal, mesenteric). Analysis of this scRNA-seq data is ongoing.

These transcriptomic datasets could also provide clues on the existence of effector functions by LN MAIT cells. Related preset cells, NKT cells and  $\gamma\delta$  T cells are capable of modulating other immune cells in the LNs (Gaya et al., 2018; Nakamizo et al., 2015). Upon viral infection, NKT cells in the LNs produce IL-4 at the follicular border, which promotes B cell anti-viral functions (e.g., IgG1 class-switch; germinal center formation; metabolic changes) (Gaya et al., 2018). Within the LNs, V $\gamma$ 4  $\gamma\delta$  T cells, located in the interfollicular T-cell zone, stimulate dendritic cells to produce IL-12 (probably via the secretion of TNF- $\alpha$ ) which drives CD8<sup>+</sup> T-cell IFN- $\gamma$  production (Nakamizo et al., 2015). MAIT cells are also found capable of modulating other immune cells (e.g., by promoting inflammatory monocyte differentiation into dendritic cells in the lung (Meierovics and Cowley, 2016)) and could do so within the LNs. On top of the transcriptomic data, immunohistochemical analysis would provide key clues on the possible functions of MAIT cells within the lymph nodes (e.g., if located near the interfollicular T-cell zone = possible role on T cells).

Interestingly, in parabiotic mice within the mediastinal LNs, MAIT cells are mainly from the parabiont analyzed and not partner-derived and even more so during lung

inflammation (influenza-mediated), indicating a true tissue residency for medLN MAIT cells (**Salou et al., 2022 – EMBO conference**). This result also pertains for the V $\gamma$ 4  $\gamma\delta$ -T cells subtype that we found photoconverted in the popliteal LNs whilst in parabiosis mice  $\frac{3}{4}$  of the  $\gamma\delta$ -T cells do not recirculate (Nakamizo et al., 2015; Zhang et al., 2016). We would need to confirm the absence of MAIT cell exchange between parabionts in the skin draining lymph-node. While  $\frac{3}{4}$  of the V $\gamma$ 4  $\gamma\delta$ -T cells do not recirculate  $\frac{1}{4}$  does, a heterogeneity is therefore possible within the LNs (e.g., recirculating *versus* residential) that may be associated with different functions. Our scRNA-seq data and parabiosis experiments will help to assess whether such a heterogeneity exist for MAIT cells in the skin draining LNs.

Overall, the dynamics in the LNs we demonstrated in the result section as well as the one from our preliminary experiment opens many fascinating questions on MAIT cells biology: How often do MAIT cells exchange between the skin and LNs? Do MAIT cells patrol the skin? What role(s) do MAIT cells play within this secondary lymphoid organ? Understanding these pending questions could not only provide new insight on MAIT cell function within the skin and skin draining LNs but help in the general understanding of their effector role/potential.

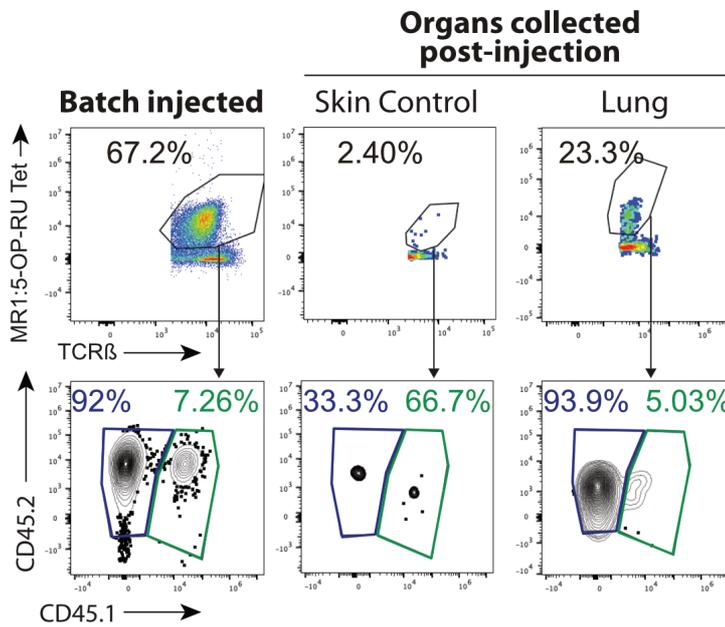
### C. Skin MAIT cells: Tissue imprinting of MAIT cells?

When we merged our skin scRNA-seq dataset and a thymic bulk RNA-seq dataset of sorted MAIT cells, some skin-specific cluster emerged. The transcriptomic existence of skin-specific clusters suggests that MAIT cells could be modified when reaching the skin. Moreover, merging MAIT cell peripheral datasets (e.g., Lung-liver) revealed some differentially expressed genes, indicating not only extra-thymic programming (i.e., thymus *versus* periphery) but also an inter-organ specificity (i.e., lung *versus* liver) (Salou et al., 2019a) (Fig 9).

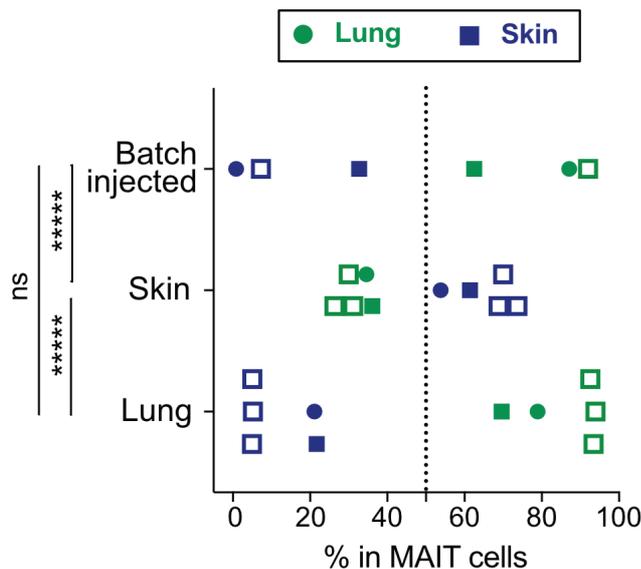
The existence of tissue-specific programs has been previously described for other cell types (e.g., macrophages, ILCs, TRMs, and Tregs). While Tregs retain common Treg characteristics in humans and mice, they also appear to adapt to their microenvironment by gradually acquiring tissue-specific programming (e.g., upregulation of *Itgae* in the skin as compared to the colon) (Miragaia et al., 2019; Povolero et al., 2018). For macrophages,

although ontogeny itself is important, differential histone modifications and RNAseq data indicate that additional local microenvironmental cues (e.g., the microbiota) lead to distinct organ-specific imprinting of the cells (Lavin et al., 2014). Additionally, transferring peritoneal macrophages to the alveolar cavity of mice results in a reprogramming of macrophages (e.g., downregulation of *Gata6* upregulation of *Pparg*). This dependency on environmental cues provided by the microbiota is also necessary for the epigenetic profile of tissue-ILCs, as demonstrated by comparing profiles of germ-free- or antibiotic-treated- and SPF- mice (Gury-BenAri et al., 2016).

A.



B.



**Fig 22: Skin MAIT cells return preferentially to the skin.** (A) Representative flow-cytometry plots and (B) quantification of the frequency of expanded lung (green) or skin (blue) MAIT cells in the: injected cell batch, skin (Control or Wound) or lung of CD3<sup>-/-</sup> MR1<sup>+/+</sup> mice 48h after transfer. Data is of 2 experiments (full or empty forms) with 3 batch injected (full square, empty square and full circle) into 5 mice (symbols corresponding to the batch injected). Statistical test: Tukey's multiple comparisons (with: \*\*\*\* p-value < 0.0001 and ns = +0.05)

This preliminary experiment requires repetition to validate its reproducibility (some staining issues in the second experiment, full forms).

## Discussion and perspectives

Transcriptomic analysis of TRMs from the peripheral organs (e.g., skin, liver) reveals on top of a common core signature (e.g., upregulated *Cxcr6*, *Cd69*; downregulated *S1pr1*, *Ccr7*) an inter-organ specificity (e.g., upregulated *Itgae* in the skin *versus* the liver) (Christo et al., 2021b). Like macrophages, the transfer of liver TRMs into the skin leads to the acquisition of skin properties (e.g., *Itgae*, *Cd244* expression) (Christo et al., 2021b).

To assess the existence of a tissue-specific program, I performed a preliminary experiment –Fig 22 – injecting MAIT cells into CD3<sup>-/-</sup> recipients from the skin and the lung following the protocol for thymic MAIT cell expansion (described in methodology section of the draft).

Because of issues regarding the expansion of the skin MAIT cells (very little MAIT cells in the skin to begin), I injected up to  $\approx 10$  times more lung than skin expanded MAIT cells (Fig 22 A, B). Strikingly, more than 2/3 of the cells recovered in the skin were of skin origin (Fig 22 A, B). This enrichment indicates a preferential targeting and/or retention of injected skin cells in the skin. Surprisingly, this tissue imprinting appears very potent as it remains despite about 10 days of *ex vivo* amplification.

Importantly, TGF $\beta$  signaling is important for differentiation and retention of TRMs within the epithelial tissues (e.g., up-regulation of *Itgae* in the gut) (Zhang and Bevan, 2013). Therefore, it was hypothesized that the differences observed between the skin and the liver TRMs could be in part driven by TGF $\beta$  signaling (Christo et al., 2021b). When preventing TGF $\beta$  signaling (TGF $\beta$ RII-deficient), skin TRMs showed a strong altered gene expression (>2000 DEGs) as compared to wild-type whereas liver TRMs showed a conserved gene expression ( $\approx 200$ DEG) (Christo et al., 2021b), highlighting the impact of TGF $\beta$  which may hardwire the transcriptional identity of skin TRM cells. In our single-cell transcriptomic data, we observed a specific up-regulation of genes associated with TGF $\beta$ - signaling (e.g., *Tgifi*) by skin MAIT cells as compared to their thymic counterpart. Altogether this suggests a potential skin specific program for MAIT cells that could be reminiscent of the one described for mainstream TRM T cells.

Moreover, the transcriptomic difference between TRM cells from the skin and liver is associated with functional differences (Christo et al., 2021b). Briefly, livers of antigen-stimulated mice (necessary for TRMs development) were transplanted into a naïve mouse that was then challenged with the TRM antigen to reactivate the donor-derived liver TRM cells (Christo et al., 2021b). Following antigen-challenge, a

## Discussion and perspectives

significant number of donor-derived ex-liver TRM cells were found within the recipients' peripheral organs (spleen, liver, skin) (Christo et al., 2021b), demonstrating that following restimulation liver TRMs can leave the liver to repopulate the periphery, a mechanism called: transdifferentiation (Christo et al., 2021b). Meanwhile, skin TRM cells injected into antigen-challenged mice failed to repopulate the liver and spleen indicating little to no transdifferentiation (Christo et al., 2021b). The loss of the capacity to repopulate the periphery in the event of a secondary challenge was associated to the CD103 (*Itgae*) status of the cells, itself linked to TGF $\beta$ - signaling (Christo et al., 2021b).

In our setting we also observe protein expression of CD103 (*Itgae*) by skin MAIT cells. A next experimental step would be to assess the transdifferentiating potential of MAIT cells by looking at other organs (e.g., spleen, liver, lung) following inflammatory challenge.

The concept of imprinting, which would provide additional characteristics to MAIT cells in addition to their subset identity (i.e., MAIT17/MAIT1), is novel, and as such, it opens new avenues for research into their biology. How strong is this imprinting? Is it retained following *in vivo* stimulation? Does it confer additional or different effector functions to MAIT cells? Furthermore, evaluating the different transdifferentiation potentials of these cells based on their origin could aid in further understanding their plasticity and effector potential. Can MAIT cells from any organs transdifferentiate? When does transdifferentiation occur (e.g., in case of inflammation, infection)?

# Conclusion

In this work, we show that in an unmanipulated setting (i.e., no *Staphylococcus epidermidis* association) and normal host (i.e., no  $\gamma\delta$  T cells deletion), MAIT cells accelerate skin wound healing. Analyzing the steady-state skin, we observed that skin MAIT cells are type 17 and already express a tissue repair transcriptomic profile as seen by scRNA-seq analysis. We evidenced that the presence of MAIT cells improves skin re-epithelization (i.e., increasing epithelial tongue length and epidermal cell proliferation). Moreover, we showed that MAIT cells production of the epidermal-growth factor Areg is involved in their wound-healing function.

Although MAIT cells exhibit tissue resident characteristics in the skin, they appear to have a shorter residence in the skin than in the lung. We showed that MAIT cells are recruited and transit through the lymph nodes in the context of skin inflammation. Studying MR1-mediated cognate interaction as a possible afferent signaling source of MAIT cells, we found that MR1 expression is unnecessary for MAIT cell recruitment into the skin and their tissue repair function. Independence on MR1 expression shows that sustained TCR stimulation is not required for MAIT cell function and skin dynamics. Instead, we discovered that the CXCR6-CXL16 signaling axis is critical for MAIT cell influx into the skin.

As discussed previously, additional explorations are required to fully elucidate the properties of skin MAIT cells. However, by providing *de novo* mechanisms, these findings contribute to a better understanding of MAIT cell biology and shed light on their dynamics and effector functions in the skin. Given the prevalence of MAIT cells in humans, their previously established *in vitro* healing properties, and these new data on their *in vivo* pro-repair function in a human-like model, their use/modulation in wound treatment-therapies would be worth exploring.

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## RÉSUMÉ

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Les dommages tissulaires d'origine mécanique, chimique ou infectieuse affectent toutes les barrières épithéliales, y compris la peau. La résolution rapide de ces dommages est essentielle pour que ces barrières recouvrent leur intégrité et fonction, elle implique l'action conjointe de cellules immunitaires infiltrant ces tissus. L'intérêt entourant l'étude des cellules MAIT (cellules T immunitaires associées aux muqueuses) dans la réparation provient de leur fréquence élevée dans la peau (jusqu'à 10% des cellules T) et de leur capacité à sécréter rapidement diverses molécules effectrices. Récemment, il a été démontré à la fois in vitro et in vivo que les cellules MAIT sont impliquées dans la cicatrisation des plaies. Cependant, les mécanismes qui sous-tendent cette fonction pro-réparatrice demeurent inconnus. En utilisant la souche congénique B6-MAITCAST (10 fois plus de cellules MAIT que les souris C57BL/6) et un modèle d'excision mimant le processus de réparation humaine (Dunn et al., 2013), nous avons cherché à élucider ces mécanismes. Grâce à ces outils, nous montrons que les cellules MAIT accélèrent la fermeture des plaies. Ayant observé l'augmentation du nombre et de la fréquence des cellules MAIT au sein des plaies sans augmentation de leur prolifération, nous avons émis l'hypothèse qu'elles étaient recrutées. À l'aide de greffe de peau, nous avons mis en évidence que, contrairement aux cellules  $\gamma\delta$ -T résidentes, les cellules MAIT sont recrutées au site inflammatoire. La souris Kaedes (où la lumière ultraviolette induit un changement de fluorescence) et le traitement par FTY720 démontrent que les cellules recrutées transitent par les ganglions lymphatiques et ne migrent pas depuis d'autres endroits de la peau. Nous avons ensuite cherché à savoir si la signalisation du TCR, via MR1, la molécule de présentation de l'antigène des cellules MAIT, était nécessaire pour leur recrutement et fonction réparatrice. Pour étudier la dépendance des cellules MAIT à la signalisation TCR, nous avons réalisé une parabiose d'animaux MR1+ et MR1- et une greffe de peau MR1- sur des animaux MR1+, ceci a montré que leur recrutement était MR1 indépendant. Les expériences de blocage de CXCL16 et de suppression génétique de CXCR6 sur les cellules MAIT par CRISPR-Cas9 montrent que le recrutement est dépendant de la signalisation CXCL16-CXCR6. Nos expériences de transfert adoptif démontrent également que la fermeture de la plaie est indépendante de la signalisation du TCR.

Concernant leur fonction effectrice, notre analyse scRNAseq suggère que le programme de réparation des cellules MAIT acquis dans la peau à l'état basal. De plus, nous observons qu'en présence des cellules MAIT il y a une augmentation significative de la longueur des langues épithéliales et de la prolifération au sein de ces langues. Ainsi, nous avons présumé que les cellules MAIT pouvaient agir directement sur la croissance des kératinocytes. Cette théorie est appuyée par le scRNAseq montrant une augmentation de l'expression de signatures associées à des cellules pro-réparatrices produisant de l'amphireguline (Areg) par les cellules MAIT cutanées. Conjointement, nous observons la nécessité de l'Areg produite par les cellules PLZF dans notre modèle et l'augmentation de sa production par les cellules MAIT. Des expériences de transfert de cellules MAIT déficientes en Areg démontrent que l'Areg produite par les cellules MAIT est déterminante pour la réparation des plaies.

Dans l'ensemble, notre étude décrit la dynamique des cellules MAIT dans la peau, les signaux sur lesquels elles s'appuient pour leur recrutement et la manière dont elles promeuvent la réparation des tissus. Elle fournit également des preuves d'un mécanisme médié par l'Areg sous-tendant cette fonction. Ces connaissances sur la biologie des cellules MAIT fournissent de nouveaux axes thérapeutiques qui pourraient atténuer la douleur, l'inconfort et les complications causés par les plaies non ou mal cicatrisées.

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## MOTS CLÉS