

# Etude du biais de différenciation pro-inflammatoire des lymphocytes T CD4+ naïfs dans le modèle de spondyloarthrite du rat transgénique pour le HLA-B27

Bilade Cherqaoui

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# Etude du biais de différenciation pro-inflammatoire des lymphocytes T CD4<sup>+</sup> naïfs dans le modèle de spondyloarthrite du rat transgénique pour le HLA-B27

Elucidating the pro-inflammatory bias of naive CD4+ T lymphocytes in the HLA-B27 transgenic rat model of spondyloarthritis

## Thèse de doctorat de l'université Paris-Saclay

*École doctorale n°577* : Structure et Dynamique des Systèmes Vivants (SDSV) Spécialité de doctorat : Sciences de la vie et de la santé Graduate School : Sciences de la vie et santé Référent : Université de Versailles Saint-Quentin-en-Yvelines

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Structure et dynamique des systèmes vivants (SDSV)

#### Titre : Etude du biais de différenciation pro-inflammatoire des lymphocytes T CD4<sup>+</sup> naïfs dans le modèle de spondyloarthrite du rat transgénique pour le HLA-B27

Mots clés : cellule dendritique ; épigénétique ; HLA-B27 ; lymphocytes T auxiliaires 17 ; spondyloarthrite ; STAT1

Introduction La spondyloarthrite (SpA) est une maladie inflammatoire chronique fréquente. Chez l'homme et dans le modèle du rat transgénique pour le HLA-B27 et la β2-microglobuline humaine (rat-B27, SpA-rat), le développement de la SpA est associé à l'activation de l'axe interleukine (IL)-23/IL-17, impliquant notamment un biais de différenciation des lymphocyte T CD4<sup>+</sup> naïfs en T helper 17 (Th17), favorisé par les cellules dendritiques (DC). Nous avons cherché à comprendre les mécanismes moléculaires conduisant à ce biais de différenciation des lymphocytes T CD4<sup>+</sup> naïfs chez le rat-B27. Résultats Nous avons d'abord démontré que les DC et les lymphocytes T CD4<sup>+</sup> naïfs de rats-B27 contribuaient de façon additive la survenue d'un profil pro-inflammatoire Th17 des lymphocytes T CD4<sup>+</sup>, même avant le début de la SpA-rat. Ceci était lié à un déséquilibre de la balance Th1/Th17 au plan transcriptomique, par ailleurs imprimé au plan épigénomique. STAT1 et STAT3 sont apparus comme des facteurs de transcription clés possiblement responsables de la programmation biaisée des lymphocytes T CD4+ naïfs. Au niveau protéique, un déficit en STAT1 était une caractéristique précoce et persistante des lymphocytes T CD4<sup>+</sup> naïfs du rat-B27, précédant l'apparition de la maladie, alors que STAT3 n'augmentait qu'après l'apparition de la SpA-rat. In vitro, l'expression de l'IL-17A par les lymphocytes T CD4+ naïfs des rats-B27 était inhibée par un activateur transcriptionnel sélectif de STAT1, l'IFNy, et également par l'IL-27 dont les effets étaient liés au rééquilibrage de la balance d'expression entre des gènes dépendants de STAT1 ou de STAT3, en faveur du premier. De plus, l'expression de l'ARNm de Stat1 était diminuée dans les lymphocytes T CD4<sup>+</sup> naïfs des patients atteints de SpA, comparativement à des témoins sains ou atteints de polyarthrite rhumatoïde. La propension des lymphocytes T CD4<sup>+</sup> des patients atteints de SpA à produire un niveau accru d'IL-17A était réduite par l'IL-27 in vitro. In vivo, chez le rat-B27, l'IL-27 réduisait la sévérité de l'arthrite et de la colite, en lien avec la diminution de cellules T effectrices au profil Th17. Conclusion Les lymphocytes T CD4<sup>+</sup> naïfs de rats-B27 présentent un biais intrinsèque favorisant leur différenciation en Th17, imprimé au niveau épigénomique, précédant l'apparition de la maladie et lié à un défaut précoce de la voie STAT1. L'induction de la signalisation STAT1 inhibe le biais de différenciation Th17 in vitro et in vivo. En perspective, nous tâcherons de préciser les mécanismes liant l'expression du HLA-B27 au défaut de STAT1 et de développer l'IL-27 comme une nouvelle cible thérapeutique pour la SpA.

#### Title : Elucidating the pro-inflammatory bias of naive CD4<sup>+</sup> T lymphocytes in the HLA-B27 transgenic rat model of spondyloarthritis

Keywords : epigenetic; dendritic cells; HLA-B27; spondyloarthritis; STAT1; T helper 17 lymphocytes

Introduction Spondyloarthritis (SpA) is a frequent chronic inflammatory disease. Both in human and in HLA-B27/human β2microglobulin transgenic rat model (B27-rat), SpA development is associated with the activation of interleukin (IL)-23/IL-17 axis, including a biased differentiation of naive CD4<sup>+</sup> T cells into T helper-17 (Th17), favored by dendritic cells (DC). We aimed to decipher the transcriptomic and epigenomic mechanisms leading to altered naive CD4<sup>+</sup> T cells differentiation in B27-rat. Results We first demonstrated that both DC and naive CD4<sup>+</sup> T cells from B27-rats contributed additively to a Th17 proinflammatory profile of CD4+ T cells, even before rat-SpA. This was supported by a Th1/Th17 imbalance at the transcriptomic level, that was reflected at the epigenomic level. STAT1 and STAT3 appeared as upstream transcription factors regulating this biased programing of naive CD4<sup>+</sup> T cells. At the protein level, STAT1 deficiency appeared as an early and persistent characteristic of B27-rat naive CD4<sup>+</sup> T cells, even before disease onset, whereas STAT3 protein increased upon rat-SpA development. In vitro, IL-17A expression by B27-rats naive CD4<sup>+</sup> T cells was reversed by a selective STAT1 transcriptional activator, IFNy, or IL-27, which effects were related to STAT1/STAT3-related genes rebalancing. Moreover, Stat1 mRNA expression was decreased in naive CD4<sup>+</sup> T cells from SpA patients as compared to healthy controls and rheumatoid arthritis patients. The propensity of CD4<sup>+</sup> T cells from SpA patients to produce increased level of IL-17A was dampened by IL-27 in vitro. In vivo, in B27-rat, IL-27 reduced arthritis and colitis severity, in relation with a decrease in Th17 effector T cells. Conclusion Naive CD4<sup>+</sup> T cells from B27-rats harbor an intrinsic bias favoring to Th17 differentiation, imprinted at the epigenomic level, preceding disease onset, and related to a precocious STAT1 defect. Induction of STAT1 signaling reversed Th17 differentiation bias in vitro and in vivo. Next perspectives are to decipher the mechanisms linking HLA-B27 to STAT1 defect and to develop IL-27 as a new therapeutic target in SpA.

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## **ABBREVIATIONS**

AINS	anti-inflammatoire non stéroïdien	LILRB2	leukocyte Ig-like receptor 2
APC	cellule présentatrice d'antigène	MAIT	cellule T invariante associée aux muqueuses
ASDAS	ankylosing spondylitis disease activity score	MICI	maladie inflammatoire chronique intestinale
AxSpA	spondyloarthrite à prédominance axiale	NK	lymphocyte natural killer
BASDAI	bath ankylosing spondylitis disease activity index	nrAxSpA	non radiographic axial spondyloarthritis
BASFI	bath ankylosing spondylitis functional index	rat-B27	rat transgénique pour le HLA-B27 et l'hβ2m
BMP	bone morphogenic protein	RE	réticulum endoplasmique
СМН	complexe majeur d'histocomptabilité	RhPso	rhumatisme psoriasique
CpG	cytosine-phosphate-guanine	SA	spondylarthrite ankylosante
CRP	protéine c réactive	SASSS	stoke ankylosing spondylitis spinal score
GM-CSF	granulocyte-macrophage colony-stimulating factor	SE	superenhancer
GWAS	genome wide association study	siRNA	ARN interférent
H3K4Me1	mono-méthylation de la 4° lysine de l'histone H3	snRNA	ARN nucléaire
H3K4Me3	tri-méthylation de la 4° lysine de l'histone H3	SpA	spondyloarthrite
HLA	antigène leucocytaire humain	SpA-rat	spondyloarthrite du rat-B27
(h)β2m	β2 microglobuline (humaine)	STAT	signaling transducer and activator of transcription
IFN	interféron	TCR	récepteur des lymphocytes T
IL	interleukine	TF	facteur transcriptionnel
ILC	innate lymphoid cell	TGFβ	transforming growth factor $\beta$
IRM	imagerie par résonnance magnétique	Th	T helper auxiliaire
JAKi	inhibiteur de janus kinase	τνγα	tumour necrosis factor $\alpha$
JAK	janus kinase	T <sub>REG</sub>	lymphocyte T régulateur
KIR	killer immunoglobulin–like receptor	VS	vitesse de sédimentation

## INTRODUCTION – La spondyloarthrite, maladie hétérogène

La spondyloarthrite (SpA) est une maladie inflammatoire chronique. Elle se caractérise par une grande hétérogénéité clinique et paraclinique (**Figure 1**).

#### Nosologie

La SpA a été identifiée dès les années 1950. Initialement la SpA a été définie comme une **arthrite chronique séronégative**, soit la survenue d'une atteinte articulaire spontanée et persistante inflammatoire sans infection active identifiée ni auto-anticorps circulants dans le sang identifiés. S'est ajoutée à cette notion la présence de signes radiographiques caractéristiques affectant : (i) les articulations sacro-iliaques, avec une possible ankylose (fusion osseuse) ou (ii) les articulations intervertébrales, avec une possible ankylose par ossification des ligaments intervertébraux, pouvant survenir à plusieurs étages (colonne bambou). Enfin, s'est ajoutée à ces notions la présence d'une association caractéristique avec d'autres atteintes inflammatoires extra-articulaires, ainsi que son fort caractère familial et son association à l'allèle B27 de l'antigène leucocytaire humain (HLA) (Brewerton et al., 1974; Moll et al., 1974; Wright, 1978).

#### Epidémiologie

La SpA est une **rhumatisme inflammatoire fréquent**, survenant dans les pays du Nord avec une prévalence de 0.1-1.4% et une incidence de 3-15 patients/100.000 par an (Bohn et al., 2018; Costantino et al., 2013; Dean et al., 2014; Park et al., 2018; Reveille et al., 2012; Wang and Ward, 2018; Wright et al., 2015a).

Cette disparité épidémiologique est en partie expliquée par la variabilité des groupes étudiés, des modalités d'échantillonnage, des classifications utilisées, mais également par des facteurs ethno-géographiques. En effet, il existe un gradient Nord-Sud de répartition de la maladie : les sujets originaires d'Afrique sub-saharienne présentent une prévalence de SpA moindre, alors que les populations Inuits et Eskimos présentent une prévalence de SpA plus élevée. Ce gradient est en partie expliqué par le gradient de prévalence du HLA-B27, dont nous discuterons ci-après (Brown et al., 1997a; Chalmers, 1980; Mijiyawa et al., 2000; Tikly et al., 2014).

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La SpA est une **maladie débutant chez le sujet jeune**, avec une expression des premiers symptômes principalement entre 20 et 30 ans. Dans 5-15% des cas, elle peut débuter avant 16 ans (forme juvénile) ou après 50 ans (forme à début tardif) (Boel et al., 2021; Chen et al., 2012a; Colbert, 2010; Gomez et al., 1997).

La prévalence de la SpA est similaire chez les **hommes et les femmes**. De façon intéressante, le phénotype de la maladie présente des différences selon le genre. En effet, les formes masculines sont plus fréquemment associées à des atteintes rachidiennes et sacro-iliaques radiologiquement visibles (Chimenti et al., 2021; Wright et al., 2020).

## Figure 1. Le SpAzzle



La SpA est une maladie hétérogène qui s'apparente à un puzzle. En effet, chaque patient peut présenter un profil phénotypique différent : présence d'atteintes à prédominance périphérique ou axiale, avec ou sans atteinte radiologique patente, et associée ou non à des atteintes extraarticulaires. Toutes ces anomalies pouvant survenir de façon séquentielle, sur plusieurs années. Son diagnostic est ainsi complexe et long, ce d'autant qu'il n'existe à ce jour pas de biomarqueur spécifique et sensible.

#### Aspects cliniques

La SpA se caractérise par des douleurs et un enraidissement articulaire, débutant et prédominant dans la région lombo-fessière. Ces symptômes fonctionnels sont liés à :

- une inflammation des enthèses (enthésite) zones d'insertion des tendons ou ligaments à l'os ; les enthésites concernent en priorité le rachis, la cage thoracique et le bassin (atteinte axiale), ainsi que très souvent le squelette des membres (atteinte périphérique) ;
- des **arthrites**, qui peuvent être axiales, notamment des articulations sacro-iliaques, et/ou périphériques, notamment au niveau des genoux (souvent asymétriques) ;
- une dactylite, plus rarement, qui correspond à l'inflammation multifocale articulaire et enthésique d'un doigt ou d'un orteil (Navarro-Compán et al., 2021; Sieper and Poddubnyy, 2017).

On classe ainsi les patients selon qu'ils présentent une SpA à **prédominance axiale** (AxSpA), périphérique, ou mixte (Rudwaleit et al., 2009). Cette distinction a des implications en terme d'approche diagnostique, pronostique et thérapeutique; de plus les formes axiales ou périphériques pourraient impliquer des mécanismes physiopathologiques différents (Costantino et al., 2021; Robinson et al., 2021).

Des **atteintes extra-articulaires** sont fréquentes au cours de la SpA, et plus fréquemment observées que dans la population générale. On décrit en effet une association forte avec :

- une uvéite antérieure aiguë 20-25% des patients ;
- un psoriasis cutané 8-20% des patients ;
- une atteinte inflammatoire intestinale, entérocolite, qui peut être (i) infraclinique (c'està-dire asymptomatique et révélée lors d'un examen coloscopique) – estimée à 50% des patients, ou (ii) prendre la forme d'une maladie inflammatoire chronique intestinale (MICI) avérée – 3-10% des patients (Stolwijk et al., 2015; Van Praet et al., 2013, 2014; de Winter et al., 2016).

#### Aspects paracliniques

#### Biologie

Un **syndrome inflammatoire** biologique est observé chez moins d'un patient sur deux, a fortiori en présence d'une MICI associée. Toutefois, l'élévation de la protéine C réactive (CRP) ou l'accélération de la vitesse de sédimentation (VS) ne sont des marqueurs ni sensibles ni spécifiques de la SpA (Rudwaleit et al., 2006).

#### Imagerie

La présence d'**atteintes axiales visibles en imagerie** est un élément central du diagnostic des AxSpA. On peut observer :

- des signes de sacro-iliite sur la radiographie standard ou en tomodensitométrie (déminéralisation, érosion, sclérose/fusion osseuses); l'imagerie par résonnance magnétique (IRM) peut révéler des signes d'inflammation parfois précoces (œdème de la moelle osseuse jouxtant l'articulation) ou un remplacement adipeux de l'os;
- des signes de spondylite des coins vertébraux principalement décelés en IRM ;
- des syndesmophytes, ossification des structures ligamentaires péri-vertébrales, pouvant aller jusqu'à donner l'aspect dit de colonne bambou, décelés en radiographie standard (Braun et al., 2018; Caetano et al., 2021; Maksymowych, 2019; Weber et al., 2010).

On classe ainsi l'AxSpA selon la présence d'une atteinte visible à la radiographie, correspondant à la classique SpA ankylosante (SA) – représentant 30-50% des patients atteints de SpA, ou non radiographique (nrAxSpA) (Sieper and Poddubnyy, 2017). De façon notable, les dommages structuraux peuvent être absents au début de la maladie, et apparaître secondairement au cours de son développement (1–12% en 2 ans et jusqu'à 26–77% après 10 ans). De plus, ils sont peu ou pas réversibles et présentent un risque d'aggravation difficilement prévisible (Costantino et al., 2017; Dougados et al., 2017; Poddubnyy et al., 2011; Wang et al., 2016).

#### Relation avec le HLA-B27

Le **HLA-B27 est le facteur génétique** de prédisposition le plus anciennement décrit et le plus fortement associé à la maladie (Brewerton et al., 1974; Caffrey and James, 1973; Schlosstein et al., 1973). Il est porté par 70-90% des patients atteints de SpA, et d'autant plus fréquemment

au cours de la SA et lorsque la maladie a débuté précocement (Brown et al., 1996, 1997b; Coates et al., 2021; Feldtkeller et al., 2003). Le fait d'être porteur du HLA-B27 multiplie par 40 le risque de développer une SpA (Costantino et al., 2013).

La recherche du HLA-B27 par PCR dans les leucocytes circulants est donc intégrée dans la démarche diagnostique de la SpA, comme un **biomarqueur de la maladie**. Sa sensibilité est bonne, mais sa spécificité est limitée par sa forte prévalence dans la population générale caucasienne, estimée à 5-15% (Khan, 1995; Li et al., 2021; Rudwaleit et al., 2006).

#### Prise en charge de la SpA axiale

#### Diagnostic

Le diagnostic de la SpA repose sur un faisceau d'arguments cliniques, biologiques et d'imagerie. C'est le cumul des différents signes évocateurs, pouvant parfois s'étaler sur plusieurs années, qui étaiera diagnostic. De ce fait, le **délai diagnostique est long**, estimé à 6,7 ans. Ce délai est d'autant plus long chez les femmes, qui présentent une fréquence moindre d'atteintes visibles en imagerie (Jovaní et al., 2017; Sykes et al., 2015; Zhao et al., 2021).

Ce long délai diagnostique de la SpA est sous-tendu par des difficultés nosologiques et lié :

- à l'hétérogénéité phénotypique des patients ;
- au manque de spécificité des symptômes initiaux, pouvant se chroniciser mais sans que n'apparaissent de preuves radiologiques ;
- à l'absence de biomarqueur suffisamment sensible et spécifique (Brown et al., 2020;
   Jones et al., 2019; Robinson et al., 2021).

#### Traitement

Les objectifs de prise en charge de la SpA sont de réduire les symptômes articulaires, éviter les dommages structuraux irréversibles, limiter le handicap et le retentissement socioéconomique liés aux douleurs et limitations articulaires et éviter la survenue d'atteintes extraarticulaires et/ou leur aggravation. Il est également important de limiter le risque cardiovasculaire et d'ostéoporose (déminéralisation osseuse diffuse) secondaire, plus fréquents au cours de la maladie.

La prise en charge repose sur une approche : non médicamenteuse (kinésithérapie, activité physique, mesures hygiéno-diététiques), et médicamenteuse, dont nous précisons ici les principaux points (Cherqaoui et al., 2021; Danve and Deodhar, 2022).

#### Anti-inflammatoires

Les anti-inflammatoires non stéroïdiens (AINS) sont proposés dans un premier temps aux patients ayant une maladie active. Leur usage est associé à des effets indésirables digestifs fréquents et rénaux plus rares.

Les corticostéroïdes utilisés par voie orale sont souvent peu efficaces. Toutefois, l'injection ponctuelle de corticoïdes au sein de grosses articulations inflammatoires permet de réduire l'inflammation locale et soulager le patient.

#### Biothérapies et Inhibiteurs de Janus Kinase (JAKi)

En cas d'échec des AINS, sont proposées des **biothérapies**, anticorps monoclonaux administrés par voie injectable – ou plus récemment des petites molécules, administrées par voie orale. L'objectif est de limiter l'activation d'une voie spécifique de la réponse inflammatoire en ciblant une/des molécule(s) du système immunitaire. Ces traitements comportent un risque infectieux avéré mais modéré, et pour certains, la nécessité d'une vigilance vis-à-vis du risque de cancers.

Deux grandes classes de biothérapies sont indiquées au cours de la SpA : (i) les **anti-Tumour Necrosis Factor**  $\alpha$  (TNF $\alpha$ ) - pour lesquels on bénéficie du plus long recul ; (ii) les **anti-IL-17A et/ou -17F** – d'usage plus récent.

Plus récemment, ont été ajoutés à la pharmacopée de la SpA les **inhibiteurs des Janus kinases** (JAKi) : (i) le tofacitinib, ciblant préférentiellement les JAK1 et JAK3 ; (ii) l'upadacitinib, ciblant préférentiellement la JAK1.

#### Disease Modifying Anti Rheumatic Drugs (DMARD)

Il s'agit de traitement de fond non ciblés, usuellement utilisés au cours de la polyarthrite rhumatoïde, autre rhumatisme inflammatoire chronique. La salazopyrine ou le méthotrexate ont en effet une indication marginale dans les atteintes articulaires périphériques, et restent rarement efficaces chez les patients atteints de SpA.

#### *Réponse et Arrêt des traitements*

L'échec ou l'efficacité insuffisante des biothérapies sont fréquentes, concernant 35-40% des patients. Cela nécessite alors un ajustement de dose ou de classe. Après avoir stabilisé la maladie, on peut tenter de diminuer l'administration d'une biothérapie mais son arrêt est rarement possible, du fait du risque élevé de rechute.

On ne dispose pas de marqueurs permettant de prédire le traitement optimal ou d'en ajuster la dose pour un patient donné. Par ailleurs, le coût sociétal de l'usage au long cours des anticorps monoclonaux reste problématique.

#### Suivi

Pour évaluer le niveau d'activité de la maladie et définir la réponse aux traitements, on dispose de différents outils. Ils sont basés sur des critères composites, associant autoévaluation par le patient (questionnaire ciblant les symptômes principaux de la SpA), évaluation par le clinicien (questionnaire résumant l'examen physique du patient) ± quantification du syndrome inflammatoire biologique (CRP ou VS). L'*Ankylosing Spondylitis Disease Activity Score* (ASDAS), le *Bath Ankylosing Spondylitis Disease Activity Index* (BASDAI), le *Bath ankylosing spondylitis functional index* (BASFI) sont utilisés en pratique malgré leurs limites respectives (Magrey and Ritchlin, 2019; Ogdie et al., 2020). L'évolution des anomalies radiologiques structurales est évaluée par le *Stoke Ankylosing Spondylitis Spinal Score* (SASSS) modifié (van der Heijde et al., 2019; Navarro-Compán et al., 2021). A ces scores s'ajoute l'évaluation de la qualité de vie qui guide également la stratégie de prise en charge et permet d'ajuster les aides non médicamenteuses utiles au patient.

#### Maladies associées à la SpA

Il existe une association non fortuite entre la SpA et la fièvre méditerranéenne familiale, maladie causée par des variants gain de fonction du gène *MEFV* (Akar et al., 2013; Cherqaoui et al., 2018). Certains variants du gène *MEFV* constituent d'ailleurs un facteur de risque indépendant de développer une SpA (Cosan et al., 2010; Li et al., 2019).

#### Concept élargi de SpA

La SpA constitue un **spectre de maladies inflammatoires apparentées**. Ce spectre inclut : la SpA axiale (radiographique/AS ou non radiographique), la SpA périphérique, la SpA

indifférenciée, la SpA à début juvénile, le rhumatisme psoriasique (RhPso), l'arthrite chronique associée aux maladies inflammatoires chroniques intestinales, l'arthrite réactionnelle et les uvéites antérieures aiguës (Baeten et al., 2013; Khan and van der Linden, 1990; Schwartzman et al., 2022). Ces différentes entités partagent de nombreuses similitudes phénotypiques et peuvent coïncider chez un même patient, et présentent une bonne réponse globale aux anti-TNFα. Ces entités partagent d'ailleurs certains mécanismes physiopathologiques, notamment au plan immunitaire et osseux (Bowness, 2015; Linden et al., 2022).

#### **Concept** d'héritabilité

Les maladies du spectre élargie de la SpA sont caractérisées par une **agrégation familiale** fréquente et non fortuite. Cette héritabilité est estimée à 90% pour la SpA elle-même, et repose sur un fond génétique commun non totalement élucidé. Ce fond génétique implique notamment des *loci* n'appartenant pas au système HLA, avec un possible effet cumulatif. Mais le marqueur génétique caractéristique reste l'association forte au HLA-B27, qui expliquerait 25% de l'héritabilité de la SpA (Brown et al., 1997b, 2000; Dernis et al., 2009; Pedersen et al., 2008).

## La SpA, maladie associée au HLA-B27

La forte association entre le HLA-B27 et le spectre élargi des SpA suppose un rôle direct ou indirect du HLA-B27 dans la pathogénie de la SpA. Nous discutons ici du rôle physiologique du HLA-B27 (**Figure 2**) et des hypothèses tentant d'expliquer ses effets pathogènes (**Figure 3**) (Bowness, 2015; Navid et al., 2021).

#### Physiologie du HLA-B27

#### **Structure**

Les HLA, codés sur le bras court du chromosome 6, font partie du complexe majeur d'histocomptabilité (CMH). Les HLA de type B appartiennent au CMH de classe I, au même titre que les type A et C, et sont exprimés à la surface de la majorité des cellules nucléées. L'allèle B27 du HLA est un des allèles B fréquemment observé dans les populations caucasiennes, porté par 5-15% de la population générale (Khan, 1995).

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Structurellement, le HLA-B27 se compose d'une **chaîne lourde**  $\alpha$ , formant un complexe hétérotrimérique en association avec la  $\beta$ 2 microglobuline ( $\beta$ 2m) et un peptide, qui se lie au HLA-B dans le réticulum endoplasmique (RE), grâce au complexe de chargement peptidique (Madden et al., 1992). L'apprêtement dans le RE met en œuvre des protéines chaperonnes, telles que la calnexine et la calréticuline, qui facilitent et contrôlent le repliement des HLA-B. Le grand polymorphisme des chaînes  $\alpha$  permet la présentation d'un grand nombre de peptides différents (Blees et al., 2017). Le peptide est issu de la dégradation des protéines cytoplasmiques par le protéasome, suivi de son transport dans le RE *via* TAP-1 et TAP-2 ; et de son clivage par l'aminopeptidase ERAP1 (Eggensperger and Tampé, 2015; Hammer et al., 2007).

Plus de 100 sous-types de HLA-B27 ont été identifiés à ce jour, différant par la séquence primaire de certains acides aminés mais partageant des caractéristiques structurelles de liaison aux peptides antigéniques (Khan, 2013). Des données indiquent que les variations d'acides aminés modifient les propriétés de fixation peptidique (de Castro, 2009; Fabian et al., 2010; García-Medel et al., 2014; García-Peydró et al., 1999; Guiliano et al., 2017; Hülsmeyer et al., 2004). De plus, **seuls certains sous-types sont associés à la SpA** (B\*27:04, B\*27:05), alors que d'autres ne le sont peu ou pas (B\*27:09, B\*27:06).

#### Fonction

Le **complexe HLA-B27/β2m peptide** est dirigé vers la surface cellulaire en transitant par le Golgi, après vérification de sa bonne conformation. Au même titre que les autres allèles du CMH de classe I, il permet la présentation de peptides intracytoplasmiques d'origine endogène ou exogène (notamment viraux), aux lymphocytes T cytotoxiques CD8<sup>+</sup>, du fait de sa reconnaissance par le récepteur des lymphocytes T (TCR). Le complexe HLA-B27/β2m/peptide joue donc un rôle physiologique majeur dans les réponses immunes antivirales, dites adaptatives car spécifiques de l'antigène (Blum et al., 2013). On peut d'ailleurs noter que le fait d'être porteur du HLA-B27 confère une protection vis-à-vis de certaines infections virales chroniques, telle que l'infection au VIH ou au VHC (Neumann-Haefelin, 2013).

Les lymphocytes natural killers (NK) peuvent également reconnaître le HLA par le biais des *killer immunoglobulin -like receptors* (KIR). En l'occurrence, l'allèle B27 est reconnu par le KIR3DL1, ce qui pourrait dépendre de la nature du peptide qui lui est associé (Malnati et al., 1995; Stewart-Jones et al., 2005).

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Certains travaux suggèrent que le HLA-B27 pourrait également être reconnu par le TCR de lymphocytes T CD4<sup>+</sup> (Boyle et al., 2001). Par ailleurs, le HLA-B joue un rôle essentiel au cours de la sélection du répertoire T au sein du thymus (Klein et al., 2014).



#### Figure 2. Physiologie du HLA-B27

Le schéma de la structure tridimensionnelle du HLA-B27 est repris de la revue de Paul Bowness, 2015. Il comporte la chaîne lourde variable constituée de 3 domaines  $\alpha 1$ ,  $\alpha 2$  et  $\alpha 3$ , et une chaine légère invariante, la  $\beta 2$ microglobuline ( $\beta 2$ m) qui lui est associée par liaison non covalente avec les domaines  $\alpha 2$  et  $\alpha 3$ . Les domaines  $\alpha 1$  et  $\alpha 2$  forment le site de liaison du peptide antigénique (poche), tandis que le domaine  $\alpha 3$ , hydrophobe, constitue le site d'ancrage à la membrane plasmique.

Les antigènes leucocytaires humains (HLA) font partie du complexe majeur d'histocomptabilité (CMH). L'allèle HLA-B27, appartenant au CMH de classe I, est présent chez 5-15% des individus dans les populations Caucasiennes. Il se compose d'une chaîne lourde formant un complexe hétérotrimérique en association avec la β2m et un peptide endogène – lui-même lié au HLA-B dans le réticulum endoplasmique (RE). Ce complexe est lui-même dirigé vers la surface cellulaire. Il permet la présentation de peptides d'origine endogène ou exogène (virus) intracytoplasmiques aux lymphocytes T CD8<sup>+</sup> via le récepteur des lymphocytes T (TCR). Le HLA-B27peut également être reconnu par les lymphocytes natural killers via les killer immunoglobulin-like receptors (KIR).

#### Rôle pathogène du HLA-B27

#### Peptide arthritogène

L'association de la SpA à un allèle du CMH-I soulève l'hypothèse d'un **biais de présentation antigénique** systématique, ou d'un mimétisme moléculaire – c'est à-dire la reconnaissance d'un antigène du Soi comme antigène du non-Soi. Ces deux phénomènes pourraient alors induire une activation lymphocytaire T CD8<sup>+</sup>, notamment au niveau articulaire (Benjamin et al., 1991; Fiorillo et al., 2000; Hermann et al., 1993).

#### Arthrite réactionnelle

Corroborant cette hypothèse, les **arthrite réactionnelles** – entité du spectre des SpA – surviennent 1 à 4 semaines après une infection bactérienne du tractus urogénital (*Chlamydiae trachomatis*) ou intestinal (*Shigella, Salmonella, Yersinia, Campylobacter*) (Zeidler and Hudson, 2021). Tentant de préciser la physiopathologie des arthrites réactionnelles, des données ont mis en évidence :

- une réponse lymphocytaire T CD8<sup>+</sup> dirigée contre des germes connus comme favorisant une arthrite réactionnelle ;
- l'existence de lymphocytes T CD8<sup>+</sup> autoréactifs contre un antigène du soi ;
- une expansion oligoclonale lymphocytaire T (Appel et al., 2004; Fiorillo et al., 2000; Mamedov et al., 2009; May et al., 2002).

L'hypothèse d'un mimétisme moléculaire a également été soulevée (Brooks et al., 1993; Rückert et al., 2006). Toutefois, le caractère pathogène de ces mécanismes n'a pas été démontré, et des données plus récentes ont mis en évidence un profil de peptidome (collection de peptides présentés par un allèle HLA) comparable entre les sous-types HLA-B27 associés ou non au risque de SpA, remettant en question le rôle d'un peptide arthritogène (Schittenhelm et al., 2015).

#### Répertoire du TCR

On observe un **biais du répertoire du TCR** chez les patients atteints de SpA. En effet, une étude effectuée chez des patients porteurs du HLA-B27 a établi l'existence : (i) d'une expansion oligoclonale de lymphocytes T CD4<sup>+</sup> et CD8<sup>+</sup> chez les patients ayant une maladie active ; (ii) une corrélation entre le niveau d'activité de la SpA et l'expression de TCR communs aux lymphocytes T CD4<sup>+</sup> et CD8<sup>+</sup>, suggérant la survenue d'un biais de sélection thymique du TCRαβ

dépendant du HLA-B27 (Zheng et al., 2019). Allant dans le même sens, une étude de plus grande ampleur a mis en évidence : (i) un degré moindre d'expansion clonale des lymphocytes T CD4<sup>+</sup> et CD8<sup>+</sup> chez les patients atteints de SpA que chez les témoins sains ; (ii) une fréquence accrue de lymphocytes T CD8<sup>+</sup> reconnaissant des épitopes restreints, notamment des virus EBV et CMV chez les patients B27+ (Hanson et al., 2020). D'autres travaux ont par ailleurs souligné le lien entre le HLA-B27 et certains clonotypes du TCRβ, et confirmé au sein du liquide synovial de patients atteints de SpA l'expansion clonale de lymphocytes T CD8<sup>+</sup> avec un clonotype spécifique aux patients atteints de SpA (Faham et al., 2017; Komech et al., 2018).

Il n'est toutefois pas établi que ce biais du répertoire du TCR a lieu durant la maturation thymique ni qu'il est à l'origine d'une réponse pro-inflammatoire systémique.

#### ERAP1 et le complexe de chargement peptidique

Les études d'association pangénomique (GWAS) ont mis en évidence chez les patients atteints de SpA une fréquence accrue de certains allèles des gènes *ERAP1* et *ERAP2*, codant pour des aminopeptidases du RE (Cortes et al., 2015; Costantino et al., 2015; Evans et al., 2011; International Genetics of Ankylosing Spondylitis Consortium (IGAS) et al., 2013; Robinson et al., 2015). Comme précédemment introduites, ces enzymes sont essentielles pour l'apprêtement des peptides avant leur association au CMH-I dans le RE (Paladini et al., 2020). Les anomalies de fonction d'ERAP1 semblent être associées à :

- la génération de peptides altérés en termes de taille ou de séquence ;
- un défaut de chargement peptidique et de présentation au HLA-B27 ;
- l'adressage membranaire de chaînes lourdes de HLA-B27 libres, comme cela a été montré pour les monocytes de patients atteints de SpA (Chen et al., 2014; Hammer et al., 2007; Haroon et al., 2012; Kochan et al., 2011; Seregin et al., 2013).

Toutefois, aucune donnée n'a permis d'identifier l'implication des variants d'*ERAP1* connus comme associés à la SpA sur la présentation de peptides arthritogènes ou l'induction d'une réponse pro-inflammatoire systémique.

#### Dimérisation et mauvais repliement du HLA-B27

Le HLA-B27 a la propriété de former de façon accrue des **dimères de chaîne lourde mal repliée**, notamment dans le RE, dans les cellules dendritiques (Allen et al., 1999; Mear et al., 1999; Santos et al., 2008). Ce mauvais repliement du HLA-B27 est en partie conditionné par les

résidus présents au sein de la poche peptidique, ou encore par la formation de ponts disulfures inappropriés (Colbert et al., 2010).

De plus, des lignées cellulaires exprimant le HLA-B27 ont révélé :

- la tendance accrue à l'oligomérisation au sein de vésicules dérivées du RE des soustypes de HLA-B27 associés à la SpA, comparativement à un sous-type non associé ;
- la colocalisation de ces vésicules avec la β2m, sans lien avec le complexe de chargement peptidique ;
- la présence de telles vésicules de façon accrue dans des lignées lymphoblastoïdes B de patients porteurs du HLA-B27 par rapport à celles de sujets témoins (Jah et al., 2020; Jeanty et al., 2014).

Ce mauvais repliement pourrait générer un stress au sein du RE et favoriser une *unfolded protein response* (UPR), réponse physiologique limitant l'accumulation de protéines mal repliées et assurant ainsi l'homéostasie du RE (Navid and Colbert, 2017; Navid et al., 2018). La plupart des données allant dans ce sens ont été obtenues à l'aide d'un modèle de rat transgénique pour le HLA-B27, dont nous discuterons plus loin. Par ailleurs, une équipe a mis en évidence l'expression accrue de GRP78, marqueur d'UPR, au sein des macrophages des articulations périphériques de patients atteints de SpA, qui semblait corrélé avec le taux de TNFα et d'IL-6 circulants, ainsi que le score BASDAI (Dong et al., 2008).

Une autre étude a suggéré que le mauvais repliement du HLA-B27 pourrait induire l'activation de l'**autophagie**. Cela a été montré dans le tissu intestinal de patients atteints de SpA évolutive, où est également observé un excès de mauvais repliement de chaînes lourdes de HLA-B27 ; de plus, au sein des cellules mononuclées de la *lamina propria*, les auteurs ont observé que l'excès d'autophagie était associé à une production accrue d'IL-23 (Ciccia et al., 2014; Goodall et al., 2010).

Toutefois, il existe des données contradictoires quant à l'existence d'une UPR chez les patients atteints de SpA, et aucune donnée n'a permis de confirmer l'hypothèse d'un effet directement pathogène d'un tel défaut de repliement du HLA-B27 (Campbell et al., 2011).

#### Stimulation de récepteurs lymphocytaires

Les dimères de HLA-B27 ou les chaînes lourdes libres, parfois exprimés à la surface des cellules ont la propriété d'interagir avec des *Killer Immunoglobulin-like Receptor* particuliers,

notamment le **KIR3DL2**, exprimés par certains lymphocytes NK et T CD4<sup>+</sup> (Stewart-Jones et al., 2005). Leur rôle *in vivo* a été démontré à l'aide d'un modèle murin transgénique pour le HLA-B27 et pourrait favoriser l'activation d'une signalisation impliquant le TNFα (Kollnberger et al., 2004a). De plus, certains auteurs ont mis en évidence que la liaison du HLA-B27 au KIR3DL2 pourrait conduire à l'activation inappropriée des lymphocytes T et/ou à un profil de type lymphocyte T auxiliaire/helper 17 (Th17) (Bowness et al., 2011; Ridley et al., 2016; Wong-Baeza et al., 2013).

Un autre travail s'est attaché à évaluer les effets des dimères de HLA-B27, ou chaînes lourdes libres, sur les récepteurs *leukocyte Ig-like receptor 2* (LILRB2) – notamment exprimés à la surface des cellules myélo-monocytaires, mettant également en évidence un possible effet sur la signalisation de l'IL-2 (Giles et al., 2012).

Toutefois, la question du mécanisme de formation des dimères de HLA-B27 et leur lien avec le développement de la SpA restent mal compris.

#### Drosophile HLA-B27 et voie BMP/TGFß

La drosophile transgénique pour le HLA-B27 a permis d'évaluer les effets non canoniques du HLA-B27, autrement dit indépendants de la présentation antigénique et même d'une réponse immunitaire adaptative, dont est dépourvue la drosophile (Grandon et al., 2019). Un effet pathogène des sous-types du HLA-B27 associés à la maladie chez l'homme a été mis en évidence et dépendait de la co-expression de la  $\beta$ 2m humaine (h $\beta$ 2m). Le phénotype anormal induit par l'expression du complexe HLA-B27/h $\beta$ 2m dépendait de la voie de signalisation *Bone Morphogenic Protein* (BMP). Cela semblerait impliquer le récepteur Saxophone de la voie BMP/Activine/*Transforming Growth Factor*  $\beta$  (TGF $\beta$ ) – homologues d'ALK1 et ALK2 codés par *ACVRL1* et *ACVR1*. De façon intéressante, la réponse à l'activine et au TGF $\beta$  des lymphocytes T CD4<sup>+</sup> de patients était augmentée, comparativement aux témoins sains. Cette voie est essentielle pour la régulation du métabolisme osseux, mais aussi des réponses inflammatoires et particulièrement de la différenciation des lymphocytes T régulateurs (T<sub>REG</sub>) et Th17.

### Figure 3. Le HLA-B27, pièce du SpAzzle



Il existe une forte association entre SpA et HLA-B27 puisque 70-90% des patients en sont porteurs et que cet allèle multiplie par 40 le risque de développer une SpA. Seuls certains sous-types sont à risque, tels que les HLA-B\*27:04 et -B\*27:05. Les mécanismes de sa pathogénicité restent encore mal compris.

Certaines données suggèrent que la pathogénicité du HLA-B27 pourrait dépendre de :

- son effet sur la sélection d'antigène (Ag) ou un biais du répertoire TCR, qui pourraient favoriser une réponse lymphocytaire CD8<sup>+</sup> (T CD8) anormale au sein des articulations ;
- son effet sur la fonction des cellules dendritiques (DC), dont la fonction principale est les de présenter les peptides liés aux molécules d'HLA aux lymphocytes T
- sa propriété intrinsèque à se dimériser ou à se replier de façon incorrecte, pouvant induire un stress du réticulum endoplasmique (RE) ;
- sa relation avec la voie bone morphogenetic protein (BMP)/transforming growth factor β (TGFβ);
- sa capacité de stimuler directement des récepteurs non canoniques notamment sur les lymphocytes T CD4<sup>+</sup> (T CD4).

#### Rat transgénique HLA-B27

#### Description du modèle

L'expression conjointe du HLA-B27 et de la h $\beta$ 2m induite par transgénèse dans certaines lignées de rats (**rat-B27**), induit spontanément le développement d'une maladie inflammatoire, présentant de nombreuses similitudes anatomocliniques avec la SpA (SpA-rat) (Hammer et al., 1990; Taurog et al., 1993a). En effet, chez le rat-B27, on observe des arthrites périphériques, parfois une atteinte axiale (dépendantes du niveau de surexpression de la h $\beta$ 2m), une MICI (de type rectocolite hémorragique), un psoriasis des phanères (Taurog et al., 1999). Cet effet est :

- spécifique, car les rats transgéniques pour le HLA-B7 restent sains,
- dépendant du nombre de copies exprimées des transgènes HLA-B27 et hβ2m, qui est positivement corrélé avec la fréquence des arthrites,
- dépendant du fond génétique, comme en témoigne l'absence de permissivité des souris et de certaines lignées de rats (Taurog et al., 1993b).

Il est important de noter que le phénotype (intestinal *vs.* articulaire) dépend notamment du fond génétique des lignées utilisées et de facteurs indéterminés (Taurog et al., 1999).

Ces données constituent la preuve de concept du lien – au moins indirect – entre l'expression du complexe HLA-B27/hβ2m, et l'induction d'une réponse inflammatoire dérégulée (Breban et al., 2021a).

#### Effets pathogènes du HLA-B27

Le modèle du rat-B27 a permis d'évaluer la pathogénicité de la molécule de HLA-B27, comme abordé précédemment. Des dimères de chaîne lourde de HLA-B27 ont été observé à la surface des cellules du rat-B27, ainsi qu'une tendance à la formation de formes mal repliées, associées à l'hβ2m et à des protéines chaperonnes dans le RE (Jah et al., 2020; Jeanty et al., 2014; Kollnberger et al., 2004b; Tran et al., 2004).

Le mauvais repliement des molécules de HLA-B27 favorise :

- une UPR au sein des macrophages dérivés de la moelle osseuse ;
- une production accrue d'IL-23 par les macrophages, en réponse à la stimulation par le lipopolysaccharide ;
- le développement de la colite (DeLay et al., 2009; Tran et al., 2006a; Turner et al., 2007).

Toutefois, la maladie articulaire du rat-B27 ne dépend pas de l'UPR, et des données récentes remettent en question le caractère spécifique du phénomène de dimérisation aux seuls soustypes de HLA-B27 associés à la SpA (Lim Kam Sian et al., 2019; Tran et al., 2006b).

#### Immunopathologie de la SpA-rat

L'hypothèse que la SpA-rat dépend d'une **réponse immunitaire dérégulée** a été évaluée grâce au rat-B27. Des études de transfert cellulaire dans ce modèle ont permis de prouver que 2 acteurs cellulaires étaient nécessaires au développement de la maladie : (i) les cellules myéloïdes dérivées de la moelle osseuse exprimant le HLA-B27 avec un niveau de copie élevé ; (ii) les lymphocytes T CD4<sup>+</sup>, dérivés du thymus, qu'ils expriment ou non le HLA-B27 (Breban et al., 1993, 1996).

Autre élément notable, dans certaines lignées de rat-B27, la survenue d'une inflammation testiculaire est un élément précoce qui conditionne le développement de la SpA-rat chez les mâles. Elle est associée à une réponse lymphocytaire T CD4<sup>+</sup> – avec un profil Th17 – et macrophagique, et à la genèse d'auto-anticorps anti-testicules (Taurog et al., 2012).

Par ailleurs, les lymphocytes T CD8<sup>+</sup> ne sont pas indispensables au développement de la SpArat, ce qui invalide au moins partiellement les hypothèses du peptide arthritogène et du mimétisme moléculaire (May et al., 2003; Taurog et al., 2009).

#### Biais de différenciation Th17 au cours de la SpA-rat

Le rat-B27 a également permis de relier l'expression du HLA-B27 au développement d'une réponse immunitaire biaisée, marquée par l'**expansion de lymphocytes Th17** et l'hyperproduction d'IL-17 et de TNF $\alpha$  (DeLay et al., 2009; Glatigny et al., 2012). L'atteinte osseuse axiale et périphérique, ostéogénique et ostéoporotique, est aussi partiellement dépendante de l'IL-17A (van Tok et al., 2019). Ceci est associé à une forte production de TNF $\alpha$ , qui médie une partie de la réponse inflammatoire systémique pathogène (Milia et al., 2011). De plus, les lymphocytes T régulateurs (T<sub>REG</sub>) des rats-B27 ont la particularité de produire plus d'IL-17A et moins d'IL-10 que ceux de rats témoins (Araujo et al., 2014).

Ces données, enrichies par d'autres travaux discutés dans le prochain chapitre, ont mis en avant l'hypothèse d'un rôle clef de l'axe IL-23/IL-17 dans le développement, voire le déclenchement, de la SpA.

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## Figure 4. Le modèle du rat-B27



Le rat transgénique pour le HLA-B27 et la  $\beta$ 2 microglobuline humaine (h $\beta$ 2m) développe spontanément un phénotype anatomoclinique ressemblant à celui de la SpA humaine, avec arthrites, atteinte du squelette axial, psoriasis et colite. Cet effet pathogène est dépendant du nombre de copies de HLA-B27 et d'h $\beta$ 2m, et spécifique du B27, puisque les rats transgéniques pour le HLA-B7 restent sains. Il s'agit donc d'un modèle pertinent pour étudier les mécanismes de développement de la SpA, en lien avec le HLA-B27.

L'immunopathologie de la SpA du rat-B27 implique :

- le rôle nécessaire de cellules myéloïdes HLA-B27<sup>+</sup> et de lymphocytes T CD4<sup>+</sup> ;
- une inflammation systémique et précoce ;
- une répression de la voie de l'interféron (IFN) au sein des cellules dendritiques (DC), associée à une baisse d'expression d'interleukine (IL)-27 et -10 ;
- la différenciation préférentielle de lymphocytes T CD4<sup>+</sup> en T auxiliaires 17 (Th17) proinflammatoires ;
- la présence de T régulateurs (T<sub>REG</sub>) exprimant plus d'IL-17 et moins d'IL-10 que normalement.

## La SpA, maladie médiée par l'axe IL-23/IL-17

La SpA est une maladie inflammatoire chronique qui fait intervenir l'activation inappropriée de cellules du système immunitaire adaptatif et inné, en l'absence d'infection active décelée. Elle se situe ainsi à mi-chemin **entre auto-immunité et auto-inflammation** (Mauro et al., 2021; McGonagle and McDermott, 2006). De nombreuses données corroborent l'hypothèse que la SpA serait médiée par l'activation inappropriée de l'axe IL-23/IL-17 ; nous détaillons ici la fonction et la régulation physiologiques de cet axe (**Figure 6**) et les observations soutenant un effet pathogène de sa dérégulation au cours de la SpA (**Figure 7**) (Gaffen et al., 2014; Lubberts, 2015).

#### Le lymphocyte Th17, acteur central de l'axe IL-23/IL-17

#### Th17 : Généralités

Les lymphocytes T CD4<sup>+</sup> naïfs peuvent se différencier en **lymphocytes T effecteurs** (dits T helper [Th]), se distinguant alors par les cytokines caractéristiques qu'ils produisent. Chaque sous-type de Th assure ainsi des fonctions distinctes, régulant de nombreux aspects de la réponse immunitaire (pro-inflammatoire, mémoire) et de l'homéostasie tissulaire (induction de lésion, d'apoptose, de réparation).

La découverte des lymphocytes Th17 dans les années 1980 a complexifié le paradigme Th1/Th2 (Harrington et al., 2005; Korn et al., 2009; Mosmann et al., 1986; Park et al., 2005). Les Th17 sont notamment localisés au niveau des muqueuses, où ils participent au contrôle physiologique des réponses inflammatoires et **anti-infectieuses** – notamment vis-à-vis de germes extracellulaires tels que les champignons (dont le *candida albicans*) ou le staphylocoque doré (Chen et al., 2011; Cho et al., 2010; Cypowyj et al., 2012; Ivanov et al., 2009; Marks et al., 2009). En complément, les Th1 et les Th2 exercent un rôle clef dans les défenses antivirales et antiparasitaires respectivement. Par ailleurs, les Th17 servent à la veille antitumorale (Bailey et al., 2014).

En outre, les Th17 sont caractérisés par leur implication dans la pathogénie et/ou l'entretien de nombreuses **maladies inflammatoires**, dont la SpA, le psoriasis, le RhPso, les MICI, la polyarthrite rhumatoïde, la sclérose en plaque (Yasuda et al., 2019).

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#### Du lymphocyte T CD4<sup>+</sup> naïf au Th17

#### Stades thymocytaires

Les lymphocytes T sont dérivés des cellules souches hématopoïétiques, différenciées dans la moelle osseuse en progéniteurs lymphoïdes communs, qui migrent ensuite vers le thymus (**Figure 5**). Au cours de leur différenciation, les thymocytes CD4<sup>-</sup> CD8<sup>-</sup>, dits double négatifs, initient le réarrangement du pré-TCR $\alpha\beta$  (ou  $\gamma\delta$ ) puis expriment les molécules CD4 et CD8, devenant alors des thymocytes **double positifs**. Durant leur maturation, les thymocytes sont soumis à une sélection : (i) positive, reposant sur la reconnaissance de faible affinité du TCR par les cellules présentatrices d'antigène (APC) thymiques ; (ii) négative, reposant sur l'apoptose induite des thymocytes trop affins pour les antigènes du Soi (visant à limiter l'immunogénicité dirigée contre des peptides du Soi). La finalisation de la recombinaison du TCR $\alpha\beta$  – spécifique d'un antigène – est associée à l'induction du phénotype **simple positif** CD4<sup>-</sup>CD8<sup>+</sup> ou CD4<sup>+</sup>CD8<sup>-</sup> selon que le TCR $\alpha\beta$  se liera respectivement au CMH-l ou -ll (Klein et al., 2014).

#### *Stade lymphocytaire T CD4*<sup>+</sup> *naïf mature*

Les thymocytes CD4<sup>+</sup> migrent alors vers la périphérie, où ils continuent de maturer du stade de lymphocytes émigrants thymiques récents au stade de lymphocytes T CD4<sup>+</sup> naïfs matures. Ils résident alors dans les organes lymphoïdes secondaires, entre lesquels ils recirculent *via* le sang ou le système lymphatique, sous l'influence de stimulations tissulaires et par effet stochastique (Piconese et al., 2020). Leur maintien dépend de facteurs homéostatiques, tels que l'IL-7 et de la stimulation du CMH (Takada and Jameson, 2009). Deux molécules majeures sont exprimées à la surface des lymphocytes T naïfs CD4<sup>+</sup>, qui leur permet de résider au sein d'un tissu : CCR7 et CD62L (L-sélectine). Le phénotype naïf chez l'homme est caractérisé par l'expression du CD45RA et l'absence du CD45RO (Broek et al., 2018).

#### Différenciation du lymphocyte T CD4<sup>+</sup> naïf

**L'activation du lymphocyte T CD4**<sup>+</sup> **naïf** est principalement induite dans les ganglions périphériques lorsqu'une APC lui présente un antigène – reconnu par le bais du récepteur TCR $\alpha\beta$  spécifique de cet antigène, lui-même complexé au CMH-II (Zhu et al., 2010). L'activation lymphocytaire dépend également d'interactions entre molécules de costimulation. Celle-ci s'accompagne de l'expression de marqueurs d'activation, notamment du CD25, sous-unité  $\alpha$ du récepteur de l'IL-2 – qui conditionne sa capacité de proliférer.



Figure 5. Différenciation thymocytaire

Les lymphocytes T dérivent des cellules souches hématopoïétiques, différenciées dans la moelle en progéniteurs lymphoïdes communs qui migrent vers le thymus. Au cours de leur différenciation thymique, les thymocytes CD4<sup>-</sup> CD8<sup>-</sup>, double négatifs, suivent quatre stades de maturation en initiant le réarrangement du pré-TCR $\alpha\beta$  (ou  $\gamma\delta$ ) puis expriment les molécules CD4 et CD8, devenant alors des thymocytes double positifs. La finalisation de la recombinaison du TCR $\alpha\beta$  – spécifique d'un antigène – est associée à l'induction d'un phénotype simple positif CD4<sup>-</sup>CD8<sup>+</sup> ou CD4<sup>+</sup>CD8<sup>-</sup> selon que le TCR $\alpha\beta$  se lie au CMH-I ou -II respectivement. Les thymocytes CD4<sup>+</sup> migrent alors vers les organes lymphoïdes secondaires, où ils deviennent des lymphocytes T CD4<sup>+</sup> naïfs matures. Ils attendent d'y être stimulés par les cellules présentatrices de l'antigène dont leur TCR est spécifique.

#### **Th17 : cytokines inductrices et produites**

Les **cytokines** produites par l'APC ou présentes dans le milieu durant la phase d'activation, conditionnent la différenciation des lymphocytes T CD4<sup>+</sup> naïfs en : (i) lymphocytes T helper – notamment de type 1, 2 ou 17 ; (ii) lymphocytes  $T_{REG}$ , essentiels pour le contrôle des réponses inflammatoires et le maintien d'une tolérance périphérique ; (iii) lymphocytes folliculaires, régulant la maturation des réponses lymphocytaires B et la mémoire humorale (Muranski and Restifo, 2013; Yin et al., 2021).

#### Cytokines inductrices des Th17

La différenciation des Th17 est induite par la présence dans le milieu d'IL-6, de TGFβ et d'IL-1β, agissant de façon synergique (Figure 6) (Chung et al., 2009; Manel et al., 2008; McGeachy et al., 2007; Veldhoen et al., 2006). Un effet notable de l'action de ces cytokines est d'induire l'expression du récepteur de l'IL-23. L'IL-23 vient alors stabiliser le profil Th17 et peut également favoriser l'induction de Th17 pathogènes (Ahern et al., 2010; Cua et al., 2003; El-Behi et al., 2011). Par ailleurs, l'IL-21 amplifie la différenciation des Th17 (Korn et al., 2007; Zhou et al., 2007).

D'autres facteurs endogènes ou exogènes modulent la différenciation des Th17 et/ou leur profil pathogène, notamment : des produits de voies métaboliques (par exemple, glycolyse ou synthèse d'acides aminés), les propriétés physico-chimiques du milieu (concentration en sel, température), des molécules sécrétées par les cellules épithéliales (protéine SAA), ou sécrétées par les Th17 eux-mêmes au cours de leur différenciation (IL-24) (Chong et al., 2020; DiToro et al., 2020; Karmaus et al., 2019; Lee et al., 2020a; Matthias et al., 2020; Stockinger et al., 2011; Veldhoen et al., 2008; Wagner et al., 2021; Wang et al., 2020).

#### Cytokines produites par les Th17

Une fois différenciés, les lymphocytes Th17 produisent des cytokines ayant des propriétés proinflammatoires, au premier rang desquels les **IL-17A et F**, pouvant agir sous forme homo- ou hétérodimérique. Leur récepteur, l'IL-17R, est exprimé de façon ubiquitaire, notamment au niveau des cellules hématopoïétiques – dont les lymphocytes T, B et neutrophiles – mais aussi des cellules épithéliales, endothéliales ou mésenchymateuses (ostéoblastes, fibroblastes).

L'IL-17 induit la production de cytokines pro-inflammatoires : IL-1 $\beta$ , IL-6, **TNF** $\alpha$  ainsi que des métalloprotéases – impliquées dans les processus de remodelage – ou encore des chimiokines

(CCL20, CXCL1, CXCL8, CXCL10) – attirant notamment les polynucléaires neutrophiles (Korn et al., 2009; Pelletier et al., 2010). Les Th17 sont également caractérisés par leur capacité à produire de l'IL-21, de l'IL-22, du *Granulocyte-macrophage colony-stimulating factor* (GM-CSF) – participant à la granulopoïèse.

Cette signature cytokinique confère aux Th17 un rôle clef dans la protection des muqueuses. A l'inverse, l'expansion ou l'activation dérégulée des Th17 peut induire une réponse inflammatoire exacerbée, et des lésions tissulaires.

#### Contrôle moléculaire de la différenciation en Th17, Th1 et T<sub>REG</sub>

La différenciation des lymphocytes T CD4<sup>+</sup> est un processus dynamique, adapté aux *stimuli* reçus au moment de leur activation (Zhu et al., 2010). Ce processus est finement régulé au plan moléculaire, par le biais de l'activation/répression de **facteurs transcriptionnels (TF)** clefs, établissant un programme transcriptomique spécifique pour chaque sous-type de lymphocyte T différencié. Cela implique entre autres les protéines *Signaling Transducer and Activator of Transcription* (STAT), qui se fixent à l'ADN pour réguler l'expression de nombreux gènes.

#### *Programme Th17*

**RORyt** est un TF clef pour induire la différenciation Th17, notamment nécessaire à la production d'IL-17 et à l'expression de l'IL-23R (Ivanov et al., 2006; Yang et al., 2008). RORyt est induit par l'activation de **STAT3** – elle-même favorisée par les IL-6, -21 et -23 (Capone and Volpe, 2020; Yang et al., 2007; Zhou et al., 2007).

#### Programme Th1

**T-BET** est un TF clef pour induire la différenciation des Th1 (Szabo et al., 2000, 2002). T-BET est induit par l'activation de **STAT1** – elle-même favorisée par l'**IFNγ** (Afkarian et al., 2002). L'activité du TF T-BET conduit à l'expression de gènes cibles, codant notamment pour l'IFNγ, les récepteurs de l'IL-12 ou de chimiokine telle que CXCR3. De façon intéressante, l'activation de STAT4 – elle-même favorisée par l'IL-12 – peut aussi induire un programme transcriptionnel de type Th1.

#### Programme T<sub>REG</sub>

**FOXP3** est un TF clef pour induire la différenciation des T<sub>REG</sub>. En témoignent les patients atteints du syndrome IPEX, dépourvus de T<sub>REG</sub> thymiques du fait de mutations perte de fonction du gène *FOXP3* (Wildin et al., 2001). *FOXP3* est induit par le **TGFβ**. L'activité du TF FOXP3 conduit

à l'expression de gènes cibles, codant notamment pour le TGF $\beta$ , et l'IL-10. De plus, l'expression de FOXP3 est nécessaire au maintien de la fonction immunosuppressive des T<sub>REG</sub> (Fontenot et al., 2003; Kawakami et al., 2021; Williams and Rudensky, 2007).

#### Voie Janus Kinase – STAT

L'activation des protéines STAT est conditionnée par leur phosphorylation, favorisant ensuite leur homo- ou hétéro-dimérisation et leur migration dans le noyau, où ils remplissent leur rôle d'activateurs transcriptionnels en se fixant au promoteur de leurs gènes cibles (**Figure 6**) (Hu et al., 2021).

La phosphorylation des STAT est induite par une cascade de signalisation en aval de l'activation des tyrosine kinases de la famille des JAK. Les JAK, hautement conservées, sont exprimées à la face intra-cellulaire de la membrane plasmique, et associées aux récepteurs de cytokines de type I ou II. Lorsqu'une cytokine extra-cellulaire se lie à son récepteur de surface, les JAK s'autophosphorylent et favorisent l'attraction et la phosphorylation des STAT. Ce mécanisme est impliqué dans la régulation de l'apoptose, de la prolifération, de la migration, du développement et de la différenciation de nombreux sous-types cellulaires, dont les lymphocytes T, B ou NK, macrophages, cellules épithéliales.

La **combinaison des JAK/STAT** dépend du ligand et du récepteur. A titre d'exemple, JAK1 et JAK2 peuvent répondre en aval du récepteur de l'IL-6 ; TYK2 en aval des récepteurs de l'IL-12, de l'IL-23, de l'IL-6 ; JAK1 et JAK2 en aval du récepteur de l'IFN-II ; JAK 1 et TYK2 en aval du récepteur de IFN-I (Salas et al., 2020).

#### Plasticité des Th

Les lymphocytes Th une fois différenciés présentent un certain degré de **plasticité**, permettant le passage d'un phénotype à un autre et favorisant ainsi la dynamique de la réponse, adaptée à l'arrêt du stimulus, au changement de profil cytokinique environnant le Th. Cela est bien décrit en ce qui concerne la possibilité pour des Th17 de basculer vers un profil Th1 ou producteur d'interféron gamma (IFN $\gamma$ ) ou vers un profil T<sub>REG</sub> (Annunziato et al., 2007; Gagliani et al., 2015; Wang et al., 2014).

Les différents programmes transcriptionnels s'inter-régulent, s'opposent et peuvent se chevaucher dans certaines conditions ; c'est notamment le cas pour le programme Th17, inhibé par l'IFNy ou l'IL-27 (*via* STAT1), par l'IL-12 (*via* STAT4), ou par l'IL-2 (*via* STAT5) (Diveu et al.,

2009; Lazarevic et al., 2011; Yang et al., 2011). Un autre exemple de régulation fine entre les profils Th17 et  $T_{REG}$  est la modulation de la signalisation du TGF $\beta$ , dépendant de la dose ou de la voie de signalisation engagée, ainsi que l'effet inhibiteur propre de STAT3 sur l'expression de *Foxp3* (Bettelli et al., 2006; Xu et al., 2021; Zhou et al., 2008).

#### *Immunité de type 3*

En plus des Th17, d'autres cellules immunitaires ont la capacité de produire de l'IL-17, tels que les lymphocytes  $\gamma\delta$ , les lymphocytes NK, les *innate lymphoid cells* (ILC). Cette propriété partagée par différents types lymphocytaires renforce les effets d'une telle réponse, et soustend le concept de **réponse immunitaire de type 3** (Annunziato et al., 2015).


# *Figure 6. Axe IL-23/IL-17 : Lymphocyte T helper 17*

Gauche - L'activation des lymphocytes T CD4<sup>+</sup> est induite lorsque son TCRαβ reconnaît un peptide duquel son TCR est spécifique, présenté par une cellule dendritique, en association au CMH-II. La liaison simultanée des molécules de costimulation induit des signaux de survie et de prolifération du lymphocyte T CD4<sup>+</sup>. Les cytokines alors sécrétées par la cellule dendritique (DC) orientent le profil de différenciation des lymphocytes T CD4<sup>+</sup>.

Droite - Les mécanismes conduisant à la différenciation en T helper 17 (Th17) font notamment intervenir la voie *janus kinase* (JAK) / *signal transducers and activators for transcription* (STAT). La présence dans le milieu d'IL-6, de TGF $\beta$  et d'IL-1 $\beta$  induit l'expression du récepteur de l'IL-23, qui participe – comme l'IL-21 – à stabiliser ou amplifier le programme de différenciation. La liaison des IL-6, -21, -23 à leur récepteur induit l'activation des Janus kinase (JAK) associées, qui à leur tour induisent la phosphorylation et dimérisation de STAT3. Migrant alors dans le noyau, STAT3 agit en coopérant avec d'autres facteurs transcriptionnels (TF) pour induire l'expression d'un TF central des Th17, RORyt. Celui-ci induit alors l'expression de gènes clefs, codant notamment pour les IL-17A, -17F, -1 $\beta$ , -6 ou encore le TNF $\alpha$ . Ceci contribuant à l'initiation d'une réponse inflammatoire, anti-infectieuse ; dans un contexte de déséquilibre, l'expansion ou l'activité excessive des Th17 peut favoriser le développement de maladies inflammatoires, dont la SpA.

#### Modèles de SpA dépendants de l'axe IL-23/IL-17

#### *Rat-B27*

Nous avons précédemment introduit le rôle clef des Th17 au cours de la SpA-rat. Leur expansion anormale est associée au développement de l'atteinte inflammatoire intestinale et probablement articulaire (Glatigny et al., 2012).

Un autre modèle d'intérêt est celui de l'arthrite à adjuvant (à *Mycobacterium tuberculosis*) chez le rat-B27 orchidectomisé. Ce rat développe des arthrites périphériques et axiales après l'injection de l'adjuvant complet de Freund à une dose infra-optimale qui ne suffit pas à induire de pathologie chez un rat non transgénique. Dans ces conditions, il a été montré que l'atteinte articulaire était initiée par le TNF $\alpha$  et l'IL-23 mais que son entretien dépendait d'autres facteurs que ces deux cytokines (van Tok et al., 2017, 2018).

#### **Souris SKG**

La souris SKG est porteuse d'une mutation du gène *Zap70* qui lui confère une haploinsuffisance de ZAP70, protéine impliquée dans la sélection thymique. Cette mutation favorise la survie de lymphocytes T autoréactifs (Rahman and Thomas, 2017). Après injection intrapéritonéale de curdlan (β-1,3 glucane), équivalent d'un challenge bactérien aigu, la souris SKG élevée en conditions sanitaires propres (*specific pathogen-free*) développe un phénotype évocateur de SpA, incluant dactylite, enthésite, arthrites érosive périphériques et atteinte axiale/sacro-iliaque, en association à une uvéite et une iléite. Cette maladie s'accompagne d'un biais de différenciation Th17 et d'une réduction de la population des T<sub>REG</sub>, associée à la production accrue d'IL-23, favorisant la production iléale d'IL-17 (Benham et al., 2014).

## SpA induite par l'IL23 chez la souris

Un autre modèle murin a mis en évidence que l'IL-23 était associée au développement des enthésites, avec une tendance ostéogénique, tant au niveau du squelette périphérique qu'axial, associées à un psoriasis (Sherlock et al., 2012). Ce modèle est induit par injection d'ADN minicirculaire codant pour l'*Il23*, induisant une exposition chronique à l'IL-23. Dans ce modèle, l'inflammation est au moins en partie due à la présence dans les enthèses – site probablement clef pour l'initiation de la maladie chez l'homme – de **lymphocytes T \gamma\delta^+CD4<sup>-</sup>CD8<sup>-</sup>**, capables de produire de l'IL-17 et de l'IL-22 en réponse à l'IL-23. Ces cellules proviennent du thymus et s'activent dans les enthèses, sous l'effet de l'IL-23 systémique (Reinhardt et al., 2016).

#### INTRODUCTION

A l'inverse, une autre équipe a mis en évidence que ce même procédé (ADN mini-circulaire *Il23*) conduisait une ostéoporose systémique, due à une différenciation ostéoclastique préférentielle et principalement médiée par des cellules myéloïdes (Adamopoulos et al., 2011).

## Souris R26 Stat3C<sup>stopfl/fl</sup> CD4Cre

Un souris porteuse d'une mutation gain de fonction de *Stat3* exprimée spécifiquement par les lymphocytes T CD4<sup>+</sup> développe spontanément un tableau évocateur de RhPso, avec psoriasis cutané, arthrites périphériques érosives, enthésites et ostéopénie (Yang et al., 2018). L'étude de cette souris croisée avec un souris déficiente pour l'IL-22 a mis en évidence l'implication d'un biais de différenciation Th17 associé à une activation accrue de la voie du RANKL au sein des cellules stromales, expliquant probablement le phénotype osseux.

## Rôle de l'axe IL-23/IL-17 au cours de la SpA

Les principales données corroborant l'hypothèse d'un rôle clef, voire pathogène, de l'axe IL-23/IL-17 dans le développement et/ou l'entretien de la SpA sont synthétisées en **figure 7**.

#### Aspects génétiques

L'hypothèse du rôle de l'axe IL-17/IL-23 dans la physiopathologie des spondyloarthrites a été renforcée par les données d'association génétique provenant des GWAS (Reveille, 2012). En effet, des **polymorphismes nucléotidiques de l'***IL23R* sont associés à la SpA, et aux maladies apparentées, telles que les MICI, le RhPso, le psoriasis (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2010; Cargill et al., 2007; Duerr et al., 2006).

D'autres gènes impliqués dans la signalisation de l'axe IL-23/IL-17 présentent également des polymorphismes nucléotidiques associés à la SpA, parmi lesquels on peut citer : (i) *CARD9* – capable de moduler la production d'IL-23 ; (ii) *CLEC7A* – impliqué dans la différenciation Th17 ; (iii) *IL12B* – sous-unité commune de l'IL-23 et de l'IL-12, ainsi qu'*IL12RB1* sous-unité commune des récepteurs à l'IL-23 et -12 ; (iv) *IL6R* – codant pour le récepteur de l'IL-6 ; (v) *TYK2* – impliqué dans la signalisation de l'IL-6 et -23 ; (vi) *STAT3* – TF clef pour la différenciation Th17 (Danoy et al., 2010; International Genetics of Ankylosing Spondylitis Consortium (IGAS) et al., 2013; Pointon et al., 2010; Wellcome Trust Case Control Consortium et al., 2007).

#### INTRODUCTION

La preuve du rôle pathogène de ces variants géniques – pris individuellement, ou considérant un effet oligogénique – reste toutefois à faire, ce d'autant qu'ils expliquent une faible part de l'héritabilité de la SpA (Costantino et al., 2018).

## Apport des essais thérapeutiques

L'efficacité du **blocage de l'IL-17A et/ou de l'IL-17F** par injection d'anticorps monoclonaux (secukinumab, ixekizumab, bimekizumab) sur les manifestations articulaires de la SpA corrobore l'hypothèse du rôle de cette cytokine dans l'état inflammatoire des patients (Baeten et al., 2015; Heijde et al., 2020; van der Heijde et al., 2018). Toutefois, bloquer l'IL17 ne présente pas de bénéfice sur les atteintes intestinale ou oculaire des patients (Wang and Maksymowych, 2021).

A l'inverse, le blocage de l'IL-23 par injection d'anticorps monoclonaux (ustekinumab, risankizumab) n'est pas efficace au cours de la SpA axiale, bien qu'efficace sur le psoriasis et sur les enthésites, au cours du RhPso ainsi que sur les MICI (Deodhar et al., 2019; Feagan et al., 2016; Gordon et al., 2018).

Ces résultats divergents de façon inattendue ont souligné que l'IL-23 et -17 pourraient être produites de façon décorrélée dans certaines conditions et présenter des effets dépendants du site inflammatoire (cellules mésenchymateuses osseuses *vs.* entérocytes) et/ou du stade de développement de la SpA (Sieper et al., 2019). Par ailleurs, l'inflammation tissulaire observée au cours de la SpA pourrait être liée à : (i) d'autres cytokines, au premier rang desquels le TNF $\alpha$ , comme le souligne la grande efficacité des anti-TNF $\alpha$  sur toutes les atteintes de la SpA, ainsi que le modèle murin TNF<sup> $\Delta$ ARE</sup> qui surexprime le TNF $\alpha$  et développe une tableau évocateur de SpA avec arthrites, enthésites et colite ; (ii) d'autres types cellulaires, moins dépendants de l'IL-23, comme discuté dans le paragraphe suivant (Baeten and Adamopoulos, 2021; Mandour et al., 2021).

#### Données d'immunophénotypage

## Excès d'IL-17

Les patients atteints de SpA axiale ou d'arthrite réactionnelle ont des **taux d'IL-17 élevés dans le sérum et le liquide synovial** (Chen et al., 2012b; Singh et al., 2007; Wendling et al., 2007). De plus, ces taux semblent diminuer sous anti-TNFα, suggérant leur importance possible dans l'activité inflammatoire de la maladie (Xueyi et al., 2013).

Par ailleurs, la détection d'IL-17 secrétée dans les articulations intervertébrales postérieures semblerait caractéristique des patients atteints de SpA, corroborant l'hypothèse d'un rôle de cette cytokine au niveau axial – atteinte centrale de la SpA (Appel et al., 2011).

Certaines équipes ont abordé la question de la source de l'IL-23, sécrétée théoriquement en amont de la production d'IL-17. De façon intéressante, les macrophages différenciés *in vitro* à partir de cellules myéloïdes de patients atteints de SpA, présentent une capacité de **production accrue d'IL-23**, en réponse à la stimulation par le lipopolysaccharide (Zeng et al., 2011).

Une question corolaire est la détermination de la source principale d'IL-17, pour mieux identifier les acteurs participant à l'activation et/ou la pérennisation de l'axe IL-23/IL-17; ce point est discuté dans les paragraphes suivants (Reinhardt and Prinz, 2018).

## Cellules NKT et lymphocytes Τγδ RORγt<sup>+</sup>

Dans le sang des patients atteints de SpA, les **lymphocytes T NK invariants et les lymphocytes Tyδ RORyt**<sup>+</sup> présentent :

- une capacité accrue de produire de l'IL-17, dépendante de l'expression de RORγt ;
- un net enrichissement quantitatif au sein des articulations inflammatoires, où ces cellules sont une source importante d'IL-17 ;
- un profil transcriptomique différent de celui des cellules de patients atteints de polyarthrite rhumatoïde (Venken et al., 2019).

De plus, les lymphocytes Tγδ RORγt<sup>+</sup> présentent une expression accrue d'IL-23R, dont la stimulation favorise la production d'IL-17 chez les patients atteints de SpA. Et ils semblent pouvoir se localiser électivement au niveau des enthèses vertébrales, où ils expriment un profil de type 3, marqué par l'expression de *RORC*, *IL23R*, *CCR6*, et la production d'IL-17A (Cuthbert et al., 2019; Kenna et al., 2012).

## Cellules T invariantes associées aux muqueuses (MAIT)

Les MAIT, provenant notamment de l'intestin, pourraient être impliquées dans l'activation à distance de l'axe IL-23/IL-17. Chez les patients atteints de SpA, on observe un **excès de MAIT** dans le liquide synovial, où elles présentent :

- un profil activé de type 3 avec expression accrue d'IL-17A et moindre d'IFNγ ;
- une surexpression de l'IL-7R, associée au fait que leur activation dépende plus de l'IL-7
  que de l'IL-23 (Gracey et al., 2016a).

Dans le sang circulant des patients atteints de SpA, une étude souligne l'existence d'un excès de MAIT IL-22<sup>+</sup> ou IL-17A<sup>+</sup>/IFNγ<sup>+</sup> comparativement aux témoins sains (Toussirot et al., 2018). De façon intéressante, les MAIT semblent être la population leucocytaire produisant la plus grande quantité d'IL-17A, et peuvent résider dans les enthèses vertébrales (Rosine and Miceli-Richard, 2021; Rosine et al., 2022).

## Cellules lymphoïdes innées (ILC) de type 3

Les ILC3 représentent une importante source de cytokines pro-inflammatoires. Chez les patients atteints de SpA, on observe que les **ILC de type 3** NKp44+ :

- sont en excès absolu et relatif dans le liquide et le tissu synovial d'articulations inflammatoires par rapport au sang circulant,
- sont en excès dans le sang des patients comparativement à des témoins sains,
- présentent un profil transcriptomique de type 3,

- produisent de l'IL-22, et non de l'IL-17A, après stimulation *in vitro* (Blijdorp et al., 2019). Une autre étude révèle une fréquence accrue des ILC3 au niveau intestinal, sanguin, synovial et de la moelle osseuse chez les patients atteints de SpA, avec une capacité à produire l'IL-22 et l'IL-17 (Ciccia et al., 2015). Leur présence a également été observé dans le liquide synovial d'articulations inflammatoires de patients atteints de RhPso, où ils peuvent produire de l'IL-17A (Leijten et al., 2015). Il a été mis en évidence que la production par les ILC3 d'IL-17A/F dépendait préférentiellement de l'IL-18 et de l'IL-12, notamment produites par les monocytes, et qu'elle était **indépendante de l'IL-23** (Cole et al., 2020). De façon intéressante, une réduction de fréquence des ILC3 circulantes est corrélée à une activité plus sévère de la SpA, témoignant peut-être d'un rôle pathogène de leur afflux au sein des tissus cibles (Leijten et al., 2015; Soare et al., 2018).

## T CD4<sup>+</sup> helper 17

Plusieurs publications ont mis en évidence une **fréquence augmentée de Th17 circulants** chez les patients atteints de SpA, ou de RhPso (Al-Mossawi et al., 2017; Gracey et al., 2016b; Jandus et al., 2008; Shen et al., 2009; Zhang et al., 2012). Dans ces études, les Th17 des patients atteints de SpA présentaient :

- une production accrue d'IL-17 en réponse à une stimulation par le CD3, comparativement à des témoins sains, *a fortiori* lorsqu'on se focalise sur la souspopulation CCR6<sup>+</sup>;
- une capacité supérieure de coexprimer l'IL-17 avec l'IFNγ ou l'IL-22 ;
- un dimorphisme sexuel se caractérisant par leur fréquence plus élevée chez les patients que chez les patientes (Bowness et al., 2011; Gracey et al., 2016b; Jandus et al., 2008; Shen et al., 2009; Zhang et al., 2012).

De façon intéressante, il semble de plus exister une balance entre Th17 et Th1, les second étant moins fréquents chez les patients (Gracey et al., 2016b).

Toutefois, deux autres équipes n'ont pas répliqué ces observation ; de plus, chez des patients ayant une nrAxSpA débutante, la fréquence des Th17 semblerait moindre que chez des témoins sains (Appel et al., 2011; Bautista-Caro et al., 2013).

# Figure 7. Axe IL-23/IL-17, autre pièce du SpAzzle



L'implication de l'axe interleukine (IL)-23/IL-17 au cours de la SpA a été soulignée par de nombreuses données. Les études d'association pangénomique (GWAS) ont révélé une association entre SpA et de nombreux polymorphismes nucléotidiques au voisinage de gènes de l'axe IL-23/IL-17. De plus, l'efficacité clinique des anticorps-monoclonaux anti-IL-17 au cours de la SpA, a confirmé l'importance de cette cytokine dans les réponses inflammatoires des patients. Par ailleurs, les données d'immunophénotypage ont révélé un biais global de différentes sous-populations lymphocytaires (lymphocytes T *natural killers* [NKT], *mucosal associated invariant T* [MAIT], *innate lymphoid cells* [ILC], T helper 17 [Th17]), vers une immunité de type 3, associée à un excès de production ou d'expression d'IL-17 dans des sites clefs de la SpA. Enfin, plusieurs modèles murins présentant un phénotype évocateur de SpA sont associés à l'activation dérégulée de l'axe IL-23/IL-17.

# Rôle de l'environnement au cours de la SpA

Les signaux reçus par le lymphocyte T CD4<sup>+</sup> au cours de son activation déterminent son profil de différenciation. Ces signaux correspondent principalement aux cytokines produites par les APC, qui dépendent du peptide antigénique présenté et du contexte dans lequel elle l'a capté. Ceci suggère que **l'environnement microbien** dans lequel les APC maturent et s'activent conditionne indirectement le profil de réponses adaptatives, dont le devenir des lymphocytes T CD4<sup>+</sup>. De nombreuses données soutiennent l'hypothèse d'un rôle critique des APC (**Figure 3**) et de l'environnement, notamment du microbiote intestinal, au cours de la SpA.

## Cellules présentatrices d'antigènes

## Généralités

Les APC regroupent l'ensemble des cellules possédant la machinerie cellulaire leur permettant de capter des antigènes exogènes/endogènes, de les processer et de les présenter sous forme de peptides dérivés, en association aux molécules du CMH. On décrit principalement : les monocytes/macrophages, les lymphocytes B activés et les cellules dendritiques (DC), qui de part leurs propriétés intrinsèques constituent les APC dites professionnelles..

## Cellules dendritiques

Les DC présentent une hétérogénéité phénotypique. On décrit les :

- DC conventionnelles, dérivées de la moelle osseuse, présentes dans les organes lymphoïdes et non lymphoïdes, migratoires ou résidentes,
  - de type 1, aux caractéristiques tolérogènes et impliquées dans la présentation croisée aux lymphocytes T CD8<sup>+</sup>, la différenciation des T<sub>REG</sub> et Th1,
  - o de type 2, aux caractéristiques pro-inflammatoires ;
- DC plasmacytoïdes, d'origine myéloïde ou lymphoïde, capables de produire de l'IFN de type I en réponse à la reconnaissance d'acides nucléiques et pouvant favoriser la prolifération lymphocytaire ;
- DC dérivées de monocytes, impliquées dans la différenciation lymphocytaire T CD4<sup>+</sup>;
- cellules de Langherans, résidentes de l'épiderme où elles jouent un rôle de veille antiinfectieuse (Yin et al., 2021).

## Rôle spécifique des cDC2 dans la différenciation Th17

Présentes notamment dans les muqueuses intestinale et pulmonaire, les **cDC2 ont un rôle essentiel dans l'induction des Th17**. En effet, les cDC2 ont la capacité de produire d'importantes quantités d'IL-6 et -23, selon les *stimuli* bactériens qu'elles reçoivent, et peuvent également favoriser le passage du TGF $\beta$  – cytokine clef pour la différentiation Th17 – d'une forme latente à une forme active (Bain et al., 2017; Persson et al., 2013; Schlitzer et al., 2013; Segura et al., 2013).

#### *Rat-B27*

Dans le modèle du rat-B27, les expériences de transfert ont démontré que le développement de la SpA-rat dépendait de cellules dérivées de la moelle osseuse exprimant le HLA-B27 à un niveau élevé (Breban et al., 1993).

De plus, il a été mis en évidence plusieurs **altérations fonctionnelles des DC** du rat-B27. Elles présentent notamment un défaut :

- de stimulation des lymphocytes T ;
- d'induction de prolifération lymphocytaire, corrélé à l'intensité de la SpA-rat ;
- de migration (Fert et al., 2008a; Hacquard-Bouder et al., 2004, 2007; Utriainen et al., 2012).

Par ailleurs, la balance entre la proportion des cDC1 tolérogènes et des cDC2 proinflammatoires est inversée en faveur des secondes, au niveau de la rate et des ganglions mésentériques (Dhaenens et al., 2009; Ermoza et al., 2019). De plus, les DC du rat-B27 ont la propriété de favoriser une différenciation lymphocytaire Th17 et des T<sub>REG</sub> produisant plus d'IL-17 et moins d'IL-10 (Araujo et al., 2014; Fert et al., 2014a; Glatigny et al., 2012).

D'un point de vue mécanistique, les effets du HLA-B27 sur l'homéostasie des APC sont mal compris. Cela pourrait impliquer la dimérisation des molécules de HLA-B27, associée à une activation de l'UPR, et/ou un défaut de l'organisation du cytosquelette, et/ou un défaut d'activation de la voie de l'IFN, qui s'associe d'ailleurs à une diminution d'expression de l'*Il27* et de l'*Il10* gènes impliqués respectivement dans la réponse Th1 et régulatrice (Dhaenens et al., 2009; Fert et al., 2014b).

## Modèle de DC déficitaire pour la protéine A20

De façon intéressante, le déficit du gène codant pour l'ubiquitine-ligase A20 dans les DC induit chez la souris une activation dérégulée de cette cellule, accompagnée d'une expansion de lymphocytes T CD4<sup>+</sup> activés (Hammer et al., 2011). Ceci est associé au développement spontané d'une colite, avec des enthésites et des arthrites, corroborant l'hypothèse du rôle clef des DC dans la pathogénie de la SpA et des MICI.

#### **Données humaines**

Dans le sang des patients B27+ atteints de SpA, il existe une fréquence réduite de cDC2 CD1c<sup>+</sup>, en balance avec une fréquence accrue de DC CD14<sup>-</sup>CD16<sup>+</sup>, les secondes favorisant l'expression par les lymphocytes T CD4<sup>+</sup> du CCR6, ainsi que la sécrétion d'IL-1 $\beta$  et d'IL-6 (Wright et al., 2015b). Par ailleurs, les macrophages dérivés de monocytes circulants de patients atteints de SpA présentent – à l'instar des DC du rat-B27 – une expression diminuée de gènes de la voie de l'IFN (Fert et al., 2014b). Les DC dérivées de monocytes de patients présentent – à l'instar des DC du rat-B27 – une expression diminuée de gènes de la voie de l'IFN (Fert et al., 2014b). Les DC dérivées de monocytes de patients présentent – à l'instar des DC du rat-B27 – un défaut de stimulation du lymphocyte T CD4<sup>+</sup> (Talpin et al., 2014a). De plus, la signature transcriptomique des monocytes de patients atteints de SpA est caractérisée par un profil d'activation accrue, et l'expression augmentée de gènes impliqués dans la réponse inflammatoire et la voie du TNF $\alpha$ , avec diminution d'expression de gènes impliqués dans certaines voies métaboliques (Karow et al., 2021).

L'ensemble de ces données suggèrent que le défaut qualitatif et quantitatif de certaines APC au cours de la SpA pourrait favoriser une réponse pro-inflammatoire systémique.

#### Microbiote intestinal et SpA

## Généralités

Le tractus intestinal contient des milliards de germes, des antigènes alimentaires et des métabolites bioactifs ingérés ou produits par les germes en place (Miller and Bäumler, 2021). Ce tractus est séparé du milieu intérieur par une barrière muqueuse, dont la fonction et la perméabilité peuvent varier. Sur le versant interne de cette barrière, le système immunitaire intestinal développe des réponses pro-inflammatoires de défense, ou anti-inflammatoires favorisant la tolérance, pour maintenir l'homéostasie tissulaire (Ansaldo et al., 2021). L'équilibre de cette barrière peut être perturbé par : (i) une dysbiose intestinale – altération qualitative

et/ou quantitative de la composition des microbes commensaux – qui pourrait induire une rupture de la barrière et un biais dans les réponses immunes ; et/ou (ii) une réponse immunitaire intrinsèquement inadaptée, induisant ou entretenant la dysbiose (Brown et al., 2019). L'ensemble peut alors favoriser une inflammation délétère locale, puis systémique, *via* la production de médiateurs pro-inflammatoires circulants qui pourraient stimuler des cellules à distance, ou la migration de cellules pro-inflammatoires vers des sites distants (Britton et al., 2019; Chu et al., 2016; Jiao et al., 2020; Neumann et al., 2019; Tang et al., 2018a).

## Modèles animaux

Chez le rat-B27 ou la souris SKG/curdlan, la maladie murine est **conditionnée par la présence de germes**, comme en témoignent les expérimentations d'élevage en isolateur stérile – abrogeant le développement de la maladie, qui est rétablie lors d'un transfert en milieu non stérile (Rehaume et al., 2014; Taurog et al., 1994).

Dans le modèle du rat-B27, l'intensité de la colite est :

- atténuée par une antibiothérapie précoce, l'usage de probiotiques, l'exclusion du cæcum du reste du tractus intestinal;
- aggravée par la réalisation d'une anse intestinale borgne fermée aux dépends du cæcum, la recolonisation par des bactéries anaérobies (Rath et al., 1996, 1999a, 1999b).

De plus, il existe un lien entre l'expression du HLA-B27, le fond génétique et la dysbiose, contribuant de concert à la modulation du phénotype inflammatoire, *via* une modification des réponses cytokiniques (Gill et al., 2018, 2019). Cette dysbiose est d'ailleurs associée à une modification profonde des métabolites intestinaux, qui peuvent eux-mêmes influencer directement les réponses immunes (Asquith et al., 2017; Brestoff and Artis, 2013).

Par ailleurs, de nombreuses données soulignent l'**influence de l'inflammation intestinale** sur le phénotype des populations myéloïdes ou lymphoïdes, notamment :

- le profil ostéoclastogénique des monocytes CD172a<sup>+</sup>CD43<sup>lo</sup>, influençant l'atteinte axiale;
- le ratio anormal entre DC pro-inflammatoires ou tolérogènes au niveau intestinal, au détriment des secondes ;
- le fait que l'atteinte intestinale dépende de la production d'IL-17 et précède l'atteinte articulaire ;

#### INTRODUCTION

le rôle du microbiote dans la réponse à l'IL-23 de la souris SKG (Ansalone et al., 2017;
 Benham et al., 2014; Ermoza et al., 2019; Glatigny et al., 2012; Utriainen et al., 2012).

Ceci souligne l'importance du microbiote dans le déclenchement d'une réponse inflammatoire exacerbée locale et systémique impliquant :

- des effets pro-inflammatoires d'agents microbiens ou de leurs métabolites dont la quantité et/ou qualité est anormale ;
- une stimulation anormale des APC au niveau intestinal, avec des effets à distance ;
- des caractéristiques intrinsèques de l'hôte qui influent sur le premier et deuxième facteur, comme cela semble être le cas pour le HLA-B27.

## **Données humaines**

La **relation entre anomalies ostéo-articulaires et intestinales** est une caractéristique de la SpA (Gracey et al., 2020). En effet, les patients atteints de SpA se caractérisent par :

- une fréquence élevée d'entérite chronique asymptomatique et parfois d'une authentique MICI ;
- une dysbiose intestinale spécifique, associée à une réduction de la diversité microbienne – comme celle observée au cours des MICI ;
- la surreprésentation de certaines espèces, tel que *Ruminoccocus gnavus*, bactérie mucolytique, dont l'abondance est corrélée positivement à l'activité de la maladie ;
- le développement de manifestations inflammatoires aiguës (arthrite réactionnelle)
  après exposition à certaines bactéries pathogènes digestives ;
- une réduction de la dysbiose après traitement par les anti-TNFα;
- un excès de cellules immunitaires innées de type 3 pouvant circuler de l'intestin vers l'articulation (Breban et al., 2017; Ciccia et al., 2015, 2017; Gill et al., 2015; Rosine et al., 2022; Van Praet et al., 2013).

Il est important de souligner que les sujets sains porteurs du HLA-B27 présentent également une dysbiose, suggérant un effet propre de cet allèle, préliminaire au développement de la maladie (Breban et al., 2017).

Toutefois, restent à déterminer les mécanismes et le fondement biologique de ces corrélations, le lien temporel et les implications propres de ces anomalies intestinales dans le développement, l'activité ou l'évolution de la SpA.

# SpA et épigénétique, interface génome/environnement

L'épigénétique se situe à l'interface entre génétique et environnement (Kanno et al., 2012). Nous décrivons ici les principaux concepts définissant l'épigénétique, les principales modifications de l'épigénome (**Figure 8**), prenant l'exemple de leurs rôles au cours de la différenciation lymphocytaire T CD4<sup>+</sup> et Th17 ; puis on décrira les données suggérant l'implication de phénomènes épigénétiques dans la pathogénie de la SpA.

## Généralités

## **Concepts et définitions**

Toutes les cellules d'un même organisme possèdent le même génome, mais chacune présente la possibilité d'exprimer un profil phénotypique unique, par le biais de l'expression différentielle qualitative et quantitative de chaque gène (Dixon et al., 2015; Xie et al., 2013). L'épigénétique étudie ainsi les *changements d'expression génique héritables lors de la mitose/méiose sans altération de la séquence d'ADN*. Elle concerne les modifications biochimiques (ajout/retrait de groupements chimiques) induites par l'environnement intra- et extra-cellulaire qui interfèrent sur l'expression de l'ADN et de l'ARN, et peuvent conduire à des modifications phénotypiques (Carter and Zhao, 2021; Stricker et al., 2017). Ces changements sont dynamiques, réversibles et possiblement transmissibles lors du développement cellulaire, voire lors de la reproduction (Wadhwa et al., 2009).

## Régulation de l'expression génique

La régulation de l'expression génique fait intervenir de nombreux facteurs externes et internes au génome, au premier rang desquels les éléments cis-régulateurs. Ils correspondent à des régions du génome présentant des sites de fixation pour des TF, et se situent à une distance <1Mb des gènes régulés (Deplancke et al., 2016).

## Activation transcriptionnelle

Les **promoteurs** servent de site de recrutement et point d'ancrage au complexe d'initiation transcriptionnel, le complexe de l'ARN-polymérase II (Sanyal et al., 2012).

Les *enhancers* sont des petites régions d'ADN capables de réguler l'expression de différents gènes, à distance, indépendamment du sens de lecture du promoteur du gène. Des boucles

d'ADN permettent de mettre en contact un enhancers avec de nombreux promoteurs de gènes, et l'enhancer va ainsi moduler l'activité du complexe transcriptionnel sur ces sites. Les *enhancers* servent en effet de point d'ancrage à de nombreux TF, se fixant *via* des séquences spécifiquement reconnues (Shlyueva et al., 2014; Zabidi et al., 2015). Ils se localisent le plus souvent dans des régions où la chromatine est décompactée. La dynamique d'ouverture chromatinienne est souvent initiée par des TF pionniers, qui vont exclure les complexes d'histone (point détaillé ci-dessous), ouvrir la chromatine, et ainsi permettre la fixation secondaire de nombreux TF, établissant ensemble un programme de différenciation cellulaire.

Les *superenhancers* (SE) correspondent à des régions riches en *enhancers* et constituent des modules de cis-régulation en lien avec de nombreux gènes, clefs pour l'établissement d'un programme transcriptionnel de différenciation cellulaire (Whyte et al., 2013).

#### *Répression transcriptionnelle*

Le rôle des *insulators* et les *silencers* dans l'inhibition de l'activité transcriptionnelle – en agissant respectivement sur les *enhancers* ou sur la liaison des TF – est quant à lui plus controversé.



# Figure 8. Principales modifications épigénétiques

L'épigénétique correspond à l'ensemble des changements impactant l'expression de l'ADN et de l'ARN, sans pour autant en modifier leur structure, et pouvant conduire à une modulation du phénotype cellulaire. Ces changements sont dynamiques, réversibles et possiblement transmissibles. On décrit ici les principales modification épigénétiques régulatrices. (1) La méthylation de l'ADN, correspondant à l'apposition d'un groupement méthyle sur certains sites du génome, notamment les îlots de cytosine-phosphate-guanine au niveau des promoteurs des gènes, avec un effet répressif sur sa transcription. (2) Les ARN non codants incluent notamment les micro-ARN, petites molécules d'ARN non codant simple-brin, qui se fixent à certaines séquences complémentaires situées à l'extrémité 3'UTR de l'ARNm et induisent le plus souvent la répression de sa traduction. (3) L'ADN est compacté au sein de la chromatine - dont l'unité élémentaire est le nucléosome, octamère d'histones, elles-mêmes faisant l'objet de modifications post-traductionnelles au niveau des extrémités N-terminales. Ces modifications modulent la force de l'interaction entre l'ADN et le nucléosome, compactant ou décompactant la chromatine. A titre d'exemple, la tri-méthylation de la 27<sup>ème</sup> lysine ou de la 9<sup>ème</sup> lysine de l'histone H3 (H3K27Me3, H3K9Me3) sont des margues répressives, ou bien la mono- ou tri-méthylation de la 4<sup>ème</sup> lysine ou l'acétylation de la 27<sup>ème</sup> lysine de l'histone H3 (H3K4Me1, H3K4Me3, H3K27Ac) sont des marques qui favorisent la transcription.

## Modifications épigénétiques

## Micro-ARN et autres ARN non codants

Les ARN non codants sont des molécules fonctionnelles, ne codant pas pour une protéine, et capables de moduler l'expression des gènes.

## Micro-ARN

Les **micro-ARN** sont des petites molécules d'ARN non codant simple-brin – d'une vingtaine de nucléotides, dont la structure est intégrée au sein d'un complexe protéique dans le cytoplasme (mi-RISC). Les micro-ARN présentent la propriété de se fixer, avec une spécificité variable, à certaines séquences complémentaires situées à l'extrémité 3'UTR de l'ARNm. Ceci conduit principalement à une répression traductionnelle, voire à une dégradation de l'ARNm. Les micro-ARN ont donc un rôle essentiel dans la régulation de l'expression génique, à l'échelle cellulaire, voire tissulaire et systémique – du fait de leur propriété de circulation (Bernstein and Allis, 2005).

## Autres ARN non codants

Les petits ARN interférents (siRNA) sont des ARNs double brin exogènes, se liant spécifiquement à leur ARNm cible et induisant son clivage. Les longs ARN non codants, de plus de 200pb, régulent l'expression post-transcriptionnelle des gènes. Les petits ARN nucléaires (snRNA), de 60-450 nucléotides, participent à l'épissage des ARNm.

## La méthylation de l'ADN

## Définitions et fonctions

Il s'agit de l'apposition d'un groupement méthyle par des méthyltransférases, sur une cytosine, principalement au niveau : d'îlots de cytosine-phosphate-guanine (CpG), souvent situés au niveau des promoteurs des gènes, ou d'éléments répétés et transposons. Des séquences non CpG peuvent aussi être méthylées, notamment dans les cellules souches embryonnaires, disparaissant au cours de la spécification cellulaire (Lister et al., 2009).

Au niveau des promoteurs, **la méthylation de l'ADN influence la différenciation cellulaire**, et permet la régulation de voies clefs pour la fonction cellulaire. La méthylation assure cette fonction en permettant la régulation de l'expression génique – principalement en réprimant la transcription, de l'épissage alternatif (lorsque le groupe méthyle est au niveau du corps du gène), et la modulation de la structure chromatinienne (en partenariat avec des modifications

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d'histones). La méthylation de l'ADN joue également un rôle au cours du développement embryonnaire, de la réplication de l'ADN, et de l'inactivation du chromosome X chez la femme (Maunakea et al., 2010).

## Régulation de la méthylation

La méthylation est effectuée par des ADN méthyltransférases (DNMT1, DNMT2, DNMT3a, DNMT3b et DNMT3L), assurant : (i) le maintien de la méthylation lors de la réplication de l'ADN, (ii) la réparation de l'ADN, (iii) la méthylation *de novo*.

La méthylation est transmise au cours des divisions cellulaires mais reste un phénomène réversible, par déméthylation. La déméthylation peut être active, *via* l'action de déméthylases, ou passive, lors de la division cellulaire (Barwick et al., 2016; Wu and Zhang, 2017).

#### Modifications post-traductionnelles des queues d'histone

## Chromatine : structure et fonctions

La chromatine est la suprastructure correspondant au premier niveau de **compaction de I'ADN** au sein du noyau, qui organise son repliement hiérarchisé. Le nucléosome est l'unité élémentaire de la chromatine, composé de 2 sous-unités identiques, contenant chacune 4 histones (H2A, H2B, H3, H4), s'associant pour former un octamère autour duquel s'enroule l'ADN. H1 permet le rapprochement entre 2 nucléosomes par pontage, augmentant ainsi la fibre de chromatine (Kouzarides, 2007).

La chromatine permet de protéger l'ADN de dégradation exogène et de contrôler l'expression génique, selon son niveau de compaction ou décompaction, états nommés respectivement hétérochromatine ou euchromatine, et qui modulent l'accès de l'ADN à la machinerie transcriptionnelle.

#### Modifications post-traductionnelles des histones

La dynamique structurelle de la chromatine est conditionnée par les **modifications d'histones**. En effet, les extrémités N-terminales des histones font l'objet de modifications posttraductionnelles (marques d'histones). De part leurs propriétés physico-chimiques, ces modifications modulent l'état de compaction chromatinienne directement, en renforçant ou relâchant l'interaction histone-ADN, ou indirectement, en aidant au recrutement des protéines chromatiniennes pouvant elles-mêmes modifier cet état de compaction. Les principales modifications post-traductionnelles d'histone, appelées **marques d'histone**, sont :

- la méthylation, principalement sur les résidus lysines (mono-, di- ou tri-méthylation) ou arginines (mono- ou di-méthylation) des histones H3 et H4,
  - comme la tri- et la mono-méthylation de la 4° lysine de l'histone H3 (marques H3K4Me3 et H3K4Me1), associées à l'activation transcriptionnelle respectivement des promoteurs et des enhancers,
  - o ou comme H3K27Me3, apposée par le groupe Polycomb, et associée à la répression génique,
  - ou encore H3K9Me3, fortement corrélée à la méthylation de l'ADN, et associée à la répression génique ;
- l'acétylation, associée à un relâchement de l'interaction histone-ADN ou au recrutement des TF ou d'autres protéines chromatiniennes comme la marque H3K27Ac, associée à l'activation transcriptionnelle des promoteurs, *enhancers* et SE, les derniers présentant un enrichissement encore plus marqué en cette marque (Figure 9);
- la phosphorylation comme celle de l'histone H3 sur les sérines 10 et 28, associée à un renforcement de l'interaction histone-ADN ;
- l'ubiquitinylation comme H2AK119, associée à la répression de l'expression génique médiée par les protéines Polycomb, ou H2BK120, associée à la réparation de l'ADN (Bannister and Kouzarides, 2011).

Ces modifications, réversibles et dynamiques, sont apposées par des enzymes régissant le *code histone* : (i) *writers* comme les histones acétylases, méthylases, méthyltransférases, phosphatases ou kinases ; (ii) *erasers* comme les histones désacétylases, déméthylases ; (iii) *readers* intervenant dans la lecture du code et modulant ainsi l'activité transcriptionnelle (Haberland et al., 2009).

Certains promoteurs présentent des domaines bivalents, permettant l'activation/répression rapide de la transcription, situés sur des locus géniques critiques pour la différenciation des cellules souches au cours du développement (Mas et al., 2018).

## Etude des marques d'histone

La technique de référence, utilisée dans le cadre de ce travail, est **l'immunoprécipitation de la chromatine suivie d'un séquençage haut débit (ChIP-seq)**. Après une étape de fixation des protéines histones à l'ADN, les cellules sont lysées ; la chromatine ainsi libérée du noyau va être fragmentée puis immunoprécipitée en présence d'anticorps spécifiques dirigés contre une marque d'histone donnée. Le produit d'immunoprécipitation est ensuite lavé, et l'ADN génomique amplifié pour constituer une librairie qui fera l'objet d'un séquençage haut débit, ou d'une analyse ciblée.



## Figure 9. Enhancers, régions cis-régulatrices

L'enrichissement des enhancers, petites régions d'ADN capables de réguler l'expression de différents gènes proches ou distants, **(1)** en marque d'acétylation de la 27<sup>ème</sup> lysine de l'histone H3 favorise **(2)** l'ancrage de facteurs transcriptionnels (TF), qui vont ouvrir la chromatine, et permettre la fixation secondaire de nombreux autres TF, se fixant à l'ADN *via* des séquences spécifiquement reconnues. Ceci va permettre une **(3)** activation transcriptionnelle intense, induisant l'expression de gènes à proximité ou **(4)** à distance. L'établissement d'un tel programme transcriptionnel permet ainsi d'initier la **(5)** différenciation cellulaire, assurant des fonctions adaptées à son environnement.

## *Epigénétique de la différenciation lymphocytaire T CD4*<sup>+</sup>

## **Exemple de la maturation des lymphocytes T CD4<sup>+</sup>**

La différenciation des lymphocytes T CD4<sup>+</sup> est le fruit d'une dynamique épigénétique complexe (Frias et al., 2021; Rothenberg, 2014; Schmidl et al., 2018; Sungnak et al., 2019). L'activation des lymphocytes T CD4<sup>+</sup> naïfs est associée à une modification profonde du profil global de méthylation de l'ADN, notamment sur les sites clefs définissant la signature des Th (Yang et al., 2015). Le profil d'acétylation des histones, la marque activatrice H3K4Me3 ou répressive H3K27Me3, déterminent également l'expression des cytokines signatures des différents types de Th (Avni et al., 2002; Ellmeier and Seiser, 2018; Wei et al., 2009). Le rôle critique des STAT au cours de la différenciation lymphocytaire est également imprimé à l'échelle épigénétique (O'Shea et al., 2011). De façon intéressante, l'épigénétique a permis de mieux comprendre les mécanismes reliant l'activité de voies métaboliques – notamment la glycolyse – au devenir fonctionnel des lymphocytaires sont également imprimées au plan épigénétique (Durek et al., 2016).

#### **Exemple de la différenciation Th17**

La différenciation Th17 est finement régulée au plan épigénétique (Ciofani et al., 2012; Tripathi et al., 2017). Elle fait intervenir des **TF pionniers**, agissant coopérativement (BATF, IRF4) pour favoriser l'ouverture de la chromatine au niveau de sites clefs – et secondairement la fixation de **TF déterminant la signature Th17** (STAT3, RORγt), et en bloquant des TF inhibant le programme Th17 (c-MAF). Il a été bien démontré que les cibles de STAT3 sont déterminantes pour définir une signature Th17 (Durant et al., 2010). De nombreux autres facteurs interviennent au cours de la différenciation Th17, tels qu'ATF7ip modulant la réponse à l'IL-2, Trim33 ATF7ip modulant la réponse au TGFβ, Trim28 modulant l'activité transcriptionnelle de RORγt, le complexe Swi/Snf régulant l'action de RORγt en tant que TF (Jiang et al., 2018; Lee et al., 2020b; Sin et al., 2019; Tanaka et al., 2018).

## Effet environnemental sur l'épigénome

L'épigénétique permet l'adaptation de la cellule à son environnement. A l'échelle d'une cellule, certains *stimuli* modifient directement l'épigénome ; un exemple abordé précédemment est celui de la différenciation des lymphocytes T CD4<sup>+</sup> et des cytokines induisant des modifications

épigénétiques sur les TF clefs qui permettront l'établissement d'un programme transcriptionnel donné (Kanno et al., 2012). De plus, à l'échelle d'un individu, il a bien été montré que l'âge induisait une modification globale de l'épigénome, au même titre que l'ethnie, le genre, et le mode de vie – intégrant des facteurs tel que l'exposition au tabac, à l'alcool, aux ultra-violets, à la pollution, aux médicaments (Bollati and Baccarelli, 2010; van Dongen et al., 2016; Heyn et al., 2012).

#### **Epigénétique et SpA**

#### **Généralités**

Des altérations de l'épigénome pourraient favoriser la survenue de maladies chroniques. Ce concept initialement décrit au cours des cancers, puis des maladies métaboliques a été plus récemment exploré au cours des maladies inflammatoires (Bates, 2020; Chen et al., 2017a).

La forte héritabilité de la SpA n'est que partiellement expliquée par les données génomiques accumulées à ce jour, supposant que d'autres facteurs modulant l'expression génique pourraient être partagés par les patients d'une même famille et notamment, des facteurs épigénétiques (Costantino et al., 2018). Ces dix dernières années, l'épigénome des patients atteints de SpA a été étudié, selon deux angles principaux : (i) évaluer le lien entre modifications épigénétique et développement de la maladie ; (ii) définir des biomarqueurs associés au diagnostic, à l'activité, ou à la réponse thérapeutique de la maladie (Chergaoui et al., 2020).

#### Micro-ARN et SpA

## Micro-ARN comme facteurs pathogènes

Les lymphocytes T CD4<sup>+</sup> et monocytes CD14<sup>+</sup> des patients atteints de SA présentent des modifications significatives quant à l'expression globale des micro-ARN, potentiellement impliqués dans des voies associées à l'inflammation, la perméabilité intestinale ou l'ostéogénèse (Fogel et al., 2019).

Certains travaux soulignent l'implication potentielle de **micro-ARN dans l'activation dérégulée de l'axe IL-23/IL-17**. Les patients atteints de SpA semblent en effet présenter :

 un profil de microARN des Th17 différent des témoins sains, et capable d'influencer la production d'IL-17;

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- un profil de microARN des lymphocytes T différent des témoins sains, et capable d'influencer la production d'IFNγ (Chen et al., 2017b; Lai et al., 2013).

Par ailleurs, l'étude des micro-ARN exprimés dans les fibroblastes de patients atteints de SpA radiographique souligne le rôle possible des mi-R124, 146a ou 21 dans la dérégulation de voies impliquées dans l'ostéogénèse (Tang et al., 2018b).

Trois études ont tenté d'identifier dans des larges cohortes des polymorphismes de certains micro-ARN qui seraient associés aux patients atteints de SpA, comparativement à des témoins sains. Aucune conclusion univoque n'a toutefois pu se dégager (Niu et al., 2015; Xu et al., 2015).

## Micro-ARN comme biomarqueurs

L'utilisation des micro-ARN comme **marqueurs diagnostiques** de SpA reste complexe en terme méthodologique et les nombreuses études publiées à ce jour n'ont pas fait l'objet d'une validation interne ni externe. A titre d'exemple, deux études ont mis en évidence un possible intérêt des miR-146a/miR-155, ou une combinatoire de 7 micro-ARN, comme biomarqueurs de SA (Perez-Sanchez et al., 2018; Qian et al., 2016).

L'utilisation des micro-ARN circulants comme **marqueur d'activité ou de réponse thérapeutique** a aussi été évaluée sans toutefois pouvoir en déduire des résultats définitifs ni reproductibles. On peut citer deux études établissant un lien entre l'expression du : (i) miR-5196 et la réponse aux anti-TNF $\alpha$ ; (ii) miR-132 et la réponse aux AINS (Ciechomska et al., 2018; Guo et al., 2018).

#### Méthylome au cours de la SpA

Deux études pangénomiques ont révélé de nombreux *loci* différentiellement méthylés chez les patients atteints de SA, comparativement à des témoins sains, avec un effet propre possible de l'expression du HLA-B27 sur le méthylome (Coit et al., 2019; Hao et al., 2017). Plus récemment, une étude a mis en évidence que des *loci* différentiellement méthylés chez les patients atteints de SpA, comparativement aux témoins sains, pourraient être liés à la fonction du TCR et à la voie Th17 (Xiao et al., 2022).

## Marques d'histones et SpA

Une étude pionnière a établi une cartographie pangénomique des variants potentiellement causaux de différentes maladies dont la SpA, combinée à l'étude des enhancers dans différentes cellules immunitaires (Farh et al., 2015). De façon intéressante, un enrichissement en polymorphismes nucléotidiques associés à la maladie était observé au niveau des *enhancers* actifs dans les lymphocytes Th0, Th1 et Th17 chez les patients atteints de SpA. Ceci suggère, avec d'autres travaux plus récents, que des variations épigénomiques peuvent être des facteurs étiopathogéniques indépendants de développer une maladie inflammatoire (Ohkura et al., 2020).

De façon intéressante, l'étude du profil de marques d'histone a permis de préciser les mécanismes de régulation de l'expression des gènes ayant des polymorphismes nucléotidiques associés à la susceptibilité à la SpA. Ce type d'étude a concerné : l'enrichissement d'H3K4Me1 au niveau d'un *enhancer* situé à proximité de l'*IL23R* et l'enrichissement d'H3K27Ac/H3K4Me1 au niveau d'un *enhancer* à proximité de *RUNX3* (Roberts et al., 2016; Vecellio et al., 2016).

## **Perspectives thérapeutiques**

L'exposition *in vitro* des lymphocytes T CD4<sup>+</sup> de patients atteints de SpA à un **inhibiteur de I'H3K27Me3 déméthylase** a mis en évidence le rôle clef de cette enzyme sur la différenciation Th17, en modifiant notamment le profil métabolique des cellules exposées (Cribbs et al., 2020).

Un nouveau champ thérapeutique prend en effet l'épigénome comme cible. Les principales avancées ont été effectuées dans le domaine du cancer où certaines drogues sont déjà validées pour un usage chez les patients (Bates, 2020). En effet, des données ont souligné la dépendance de la prolifération dérégulée de certains cancers à la survenue de modifications épigénétiques associées à un effet oncogène. A titre d'exemple, plusieurs inhibiteurs de la méthylation ont ainsi été développés pour traiter les hémopathies malignes (Vidaza®, Décitabine®).

#### **Synthèse**

On peut aujourd'hui certifier que l'épigénome des patients atteints de SpA est altéré. Cet épigénome pourrait être associé à une signature Th17 ou à une dérégulation de l'ostéogenèse. A ce stade, on ne peut affirmer le caractère causal de ces perturbations, ni leurs interactions avec d'autres facteurs environnementaux ou génétiques. De plus, de nombreuses limites méthodologiques gênent l'interprétation des données acquises dans le domaine de l'épigénétique de la SpA. En effet, ces études sont souvent faites sur des populations cellulaires hétérogènes, ou des populations de malades hétérogènes, sans appariement des témoins sur des paramètres connus comme modulateurs de l'épigénome. De plus, pour répondre à la

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question de mécanismes épigénétiques causaux, ou dans l'objectif de définir un biomarqueur diagnostique, il convient de bien choisir le stade de la maladie au moment du prélèvement. Enfin, dans la perspective de développer des traitements ciblant un mécanisme épigénétique dans les maladies inflammatoires – dont la SpA, le préliminaire indispensable reste de mettre en évidence son rôle proprement pathogène ou aggravant.

#### INTRODUCTION

# Problématique et Hypothèses

La SpA est une maladie inflammatoire complexe fortement associée au HLA-B27. Les mécanismes de l'effet pathogène du HLA-B27 restent mal compris. Toutefois, chez l'homme, et chez le rat-B27 – au même titre que d'autres modèles murins de la SpA, le développement de la maladie est associé à une activation inadaptée de l'axe IL-23/IL-17. Entre autres acteurs cellulaires, ceci implique un biais de différenciation des lymphocytes T CD4<sup>+</sup> naïfs en Th17.

Comprendre les **étapes initiales induisant ce biais de différenciation Th17** permettrait d'appréhender les mécanismes étiopathogéniques de la SpA, notamment ceux dus à l'expression du HLA-B27. Les mécanismes à l'origine ce biais pourraient faire intervenir :

- des signaux exogènes perçus au cours de la différenciation lymphocytaire notamment les cytokines sécrétées par la DC ou des molécules induites par la dysbiose notamment intestinale ;
- un effet direct du HLA-B27 sur le programme de différenciation lymphocytaire ;
- une combinaison de ces facteurs, qui pourraient être reliés par des évènements épigénétiques déterminants.

Ce travail est donc basé sur l'**étude des lymphocytes T CD4**<sup>+</sup> **naïfs** dans le modèle du rat-HLA-B27 – choisi pour sa pertinence et l'accessibilité des tissus – ainsi que des lymphocytes T CD4<sup>+</sup> naïfs de patients atteints de SpA. Ce travail s'articule autour de quatre axes d'étude :

- phénotype des lymphocytes T CD4+ naïfs ex vivo et in vitro en condition de stimulation avec ou sans DC (article original 1).
- transcriptomique des lymphocytes T CD4<sup>+</sup> naïfs par approche haut débit ou ciblée (article original 1).
- épigénétique des lymphocytes T CD4<sup>+</sup> naïfs par le biais d'une analyse du profil des marques d'histone sur l'ensemble du génome (article original 1).
- **modulation de la voie STAT1** *in vitro* et *in vivo* (articles originaux 1 et 2 annexe 2).

ARTICLE 1 – Le biais pro-inflammatoire des lymphocytes T CD4<sup>+</sup> naïfs est associé à un déficit intrinsèque de STAT1 au cours de la spondyloarthrite

## Article 1 : Introduction générale

Le rat-B27 est un excellent modèle de SpA, permettant de décrire les effets immunopathologiques induits par l'expression du HLA-B27 (Breban et al., 2021a). Le développement de la SpA-rat dépend d'une part des lymphocytes T CD4<sup>+</sup>, prompts à se différencier en Th17, et de cellules myéloïdes, dont les DC, qui favorisent aussi ce biais de différenciation Th17.

Nous nous sommes donc attachés ici à caractériser le rôle respectif de ces deux sous-types cellulaires dans le développement d'un profil lymphocytaire pro-inflammatoire. Ceci a révélé que les lymphocytes T CD4<sup>+</sup> naïfs présentaient une prédisposition intrinsèque à devenir inflammatoire. Nous avons donc étudié plus précisément les caractéristiques transcriptomiques et épigénomiques lymphocytes T CD4<sup>+</sup> naïfs pour définir les phénomènes survenant en amont, et pouvant être à l'origine de ce biais Th17 pro-inflammatoire.

## Article 1 : Manuscrit

# Intrinsic STAT1 deficiency underlies proinflammatory imprint of naive CD4<sup>+</sup> T cells in spondyloarthritis

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# ABSTRACT

**Introduction**: Spondyloarthritis (SpA) development in human and in HLA-B27/human  $\beta$ 2-microglobulin transgenic rat (B27-rat) are both associated with the activation of interleukin (IL)-23/IL-17 axis, including a biased differentiation of naive CD4<sup>+</sup> T cells into T helper-17 (Th17). We aimed to decipher the transcriptomic and epigenomic mechanisms leading to altered naive CD4<sup>+</sup> T cells differentiation in B27-rat.

**Methods**: The phenotype of B27-rats naïve CD4<sup>+</sup> T cells from mesenteric lymph nodes (mLN), cultivated with conventional type 2 DCs (cDC2) and anti-TCR $\alpha\beta$ , or with anti-CD28 and anti-CD3, was studied by flow cytometry. mLN naive CD4<sup>+</sup> T cells were analyzed by RNA-sequencing

(RNA-seq), and chromatin immunoprecipitation of activating histone marks followed by wholegenome sequencing (ChIP-seq). Age-matched nontransgenic (NTG) littermates were used as controls.

**Results**: cDC2 and naive CD4<sup>+</sup> T cells from B27-rats contributed additively to a Th17 proinflammatory profile of CD4<sup>+</sup> T cells, even before rat-SpA. This was supported by a Th1/Th17 imbalance at the transcriptomic level. This transcriptomic signature was reflected in naive CD4<sup>+</sup> T cells chromatin structure, as activating histone marks were differentially enriched in genes related to Th17 differentiation in B27-rats T cells. Even the predicted superenhancers (SE) specific of B27-rats T cells were related to Th17, whereas NTG rats SE to interferon pathway. STAT1 and STAT3 appeared as upstream transcription factors regulating both transcriptomic and epigenomic signature. At the protein level, STAT1 deficiency appeared as an early and persistent characteristic of B27-rat naive CD4<sup>+</sup> T cells, even before disease onset, whereas STAT3 protein increased upon rat-SpA development. *Stat1* mRNA expression was decreased even in naive CD4<sup>+</sup> T cells from SpA patients as compared to healthy controls and rheumatoid arthritis patients. *In vitro* stimulation of B27-rats naive CD4<sup>+</sup> T cells with either IFNγ or a selective STAT1 transcriptional activator reversed IL-17A expression.

**Conclusion**: Naive CD4<sup>+</sup> T cells from B27-rats harbor an intrinsic bias favoring Th17 differentiation, imprinted at the epigenomic and transcriptomic level, and preceding disease onset. This appeared to be related to a precocious STAT1 defect, observed in naive CD4<sup>+</sup> T cells of SpA patients.

**Keywords:** dendritic cell; epigenetic; HLA-B27; naive CD4 T cells; Spondyloarthritis; STAT1; Th17.

## INTRODUCTION

Spondyloarthritis (SpA) refers to a group of chronic inflammatory disorders of the joint that affects primarily the axial skeleton, and frequently also peripheral joints and extra-articular tissues, including skin (psoriasis), eye (anterior uveitis) and gut (inflammatory bowel disease) <sup>1</sup>. Its association with the human leukocyte antigen (HLA)-B27 was discovered nearly fifty years, yet the mechanism underlying such striking association remains incompletely understood <sup>2</sup>.

Several lines of rats transgenic for HLA-B27 and human β2-microglobulin (hβ2m) (B27rat) develop spontaneously an array of inflammatory manifestations recapitulating the main SpA features (rat-SpA) in a specific way since HLA-B7/hb2m (B7-rat) transgenic rats remain healthy <sup>3</sup>. Thus these B27-rats represent a faithful model to study HLA-B27 role in SpA pathophysiology <sup>4</sup>. Early cell transfer studies evidenced a critical role for CD4<sup>+</sup> T cells in mediating disease manifestations <sup>3,5</sup>. Further characterization of effector CD4<sup>+</sup> T cells showed a selective expansion of proinflammatory CD4<sup>+</sup> T cells exhibiting a T helper-17 (Th17) bias <sup>6</sup>. Such proinflammatory bias was also evidenced in regulatory CD4<sup>+</sup> T cells (Treg) that displayed enhanced interleukin-17 (IL-17) and decreased IL-10 production <sup>7</sup>.

Furthermore, antigen-presenting cells (APCs), including dendritic cells (DCs), from disease-prone B27-rats where shown to support CD4<sup>+</sup> T cell polarization towards Th17 bias, leading to hypothesize that expression of HLA-B27 in APC was a critical trigger of the inflammatory process <sup>6,7</sup>. This theory is consistent with the necessary role played by myeloid-derived cell from SpA-prone B27-rats in inducing rat-SpA <sup>8</sup>. Moreover, several defective functions of DCs were evidenced in B27-rat, including a reverse interferon (IFN) signature shared with macrophages from SpA patients, that could account for skewed T cell differentiation <sup>9,10</sup>.

In the present study, our objective was to decipher the transcriptomic and epigenomic mechanisms leading to altered naive CD4<sup>+</sup> T cells differentiation in B27-rat. We first studied the influence of DCs on naive CD4<sup>+</sup> T cells and then focused on intrinsic predisposition of naive CD4<sup>+</sup> T cells to differentiate into Th17 effector phenotype. This led us to evidence that naive CD4<sup>+</sup> T cells from B27-rat were imprinted to Th17 differentiation and to identify signal transducer and activator of transcription (STAT)1 deficiency as a proximal event underlying such bias.

## METHODS

**Rats.** The SpA-prone B27-rats of the 33-3 line bearing 55 copies of HLA-B\*2705 and 28 copies of h $\beta$ 2m and the healthy B7-rats of the 120-4 line bearing 52 copies of HLA-B\*0702 and 28 copies of h $\beta$ 2m, all on a Fisher (F344) background, were bred and maintained under conventional conditions <sup>6</sup>. Age-matched nontransgenic (NTG) littermates were used as

controls. Across experiments, B27-rats were separated in 3 groups of age, relative to rat-SpA onset: (i)  $\leq$  10 d, breastfed, without any sign of disease (newborn); (ii) between 3 and 4 wk, still breastfed, without any sign of disease (premorbid); (iii) from 2 to 12 mo, with established disease (adult). Study procedures were approved by the Institutional Animal Experimentation Ethical Committee from the Faculty of Health Simone Veil (APAFIS-8910).

Cell culture reagents and monoclonal antibodies (mAb). Cell culture medium was RPMI 1640 with Glutamax I (Life Technologies), supplemented with 10% fetal calf serum, streptomycin/penicillin (100µg/mL), 2% sodium pyruvate, 0.05 mM β-mercaptoethanol and 10 mM HEPES (complete RPMI). Rat recombinant (rr) IL-1β, rrIL-12, mouse (m)rIL-27 and rrIL-6 were from R&D Systems. RrIL-2 was from Immunotools. Antibody neutralizing rIL-4, mouse anti-rat CD3, anti-rat CD28 were from BD. RrGranulocyte macrophage colony stimulating factor (GM-CSF) was from BioLegend. RrIFNy, human anti-human CD14, OX62 (anti-rat CD103) microbeads were from Miltenyi. Fluorochrome-conjugated mouse anti-rat TCRaß, anti-rat CD103 was from BioLegend. Fluorochrome-conjugated mouse anti-rat CD4, anti-rat IFN-y, mouse anti-human CCR7, anti-human CD4, anti-human CD45RA, and anti-human CD45RO, mouse anti-human/mouse STAT1 phosphorylated at tyrosine 701 and STAT3 phosphorylated at tyrosine 705, anti-total STAT1 and STAT3 Abs were from BD. Fluorochrome-conjugated mouse anti-rat CD62L, ICOS, IL-17A and CD25, hamster anti-rat TNF $\alpha$  were from eBioscience. Fluorochrome-conjugated CellTrace Violet (CTV), Live-Dead, mouse anti-rat CD8 and IL-17A enzyme-linked immunosorbent assay (ELISA) kit were from ThermoFisher. Phorbol myristate acetate (PMA), ionomycin, brefeldin A (BFA), protease inhibitor were from Sigma. Fix/Perm buffers for functional or STAT assay were from Tonbo or BD respectively. STAT1 transcriptional activator, 2-(1,8-Naphthyridin-2-ly)phenol (2NP) – known to increase STAT1-dependent genes expression in cell lines – was from Abcam  $^{11,12}$ .

**Isolation of rat T cells.** Single cell suspensions were prepared either from mesenteric lymph nodes (mLN), salivary LN (sLN), or thymus. Subsets of CD4<sup>+</sup> T cells were purified by flow cytometry (ARIA-III®). TCR $\alpha\beta^+$ CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>high</sup> mature naive CD4<sup>+</sup> T cells were isolated from the LN. TCR $\alpha\beta^+$ CD4<sup>+</sup>CD8<sup>-</sup> and TCR $\alpha\beta^+$ CD4<sup>+</sup>CD8<sup>+</sup> thymocytes were isolated from the thymus.

**Isolation of rat DCs.** Conventional type 2 DCs (cDC2) were obtained from spleen digested with collagenase-D (Roche) <sup>13</sup>. Low-density cells collected after centrifugation on a 14.5%

Nycodenz gradient (Nycomed) were magnetically enriched for CD103<sup>+</sup> subset (autoMACS<sup>®</sup> Pro Separator), then sorted by flow cytometry (ARIA-III<sup>®</sup>) as TCR $\alpha\beta$ <sup>-</sup>CD45RA<sup>-</sup>CD4<sup>+</sup>CD103<sup>+</sup>. The cDC2 were matured overnight at 37°C with rrGM-CSF (100ng/mL) before coculture.

**Human T cells.** Axial SpA patients fulfilling the Assessment of SpondyloArthritis international Society classification criteria <sup>14</sup> and age- and sex-matched healthy controls (HC), and rheumatoid arthritis (RA) patients fulfilling the American College of Rheumatology/European League Against Rheumatism classification criteria <sup>15</sup>, were recruited in the department of rheumatology of Ambroise Pare hospital (Boulogne, France). Clinical data are summarized in Supplementary Table 1. Peripheral venous blood PBMC were isolated by FicoII (GE Healthcare) density gradient centrifugation. CD14<sup>+</sup> cells were first removed by positive magnetic selection (AutoMacs<sup>®</sup>). Total CD14<sup>-</sup> PBMC were used for (p)STAT1 and (p)STAT3 assay. CD4<sup>+</sup>CD45RA<sup>high</sup>CD45RO<sup>low</sup>CCR7<sup>+</sup> naive CD4<sup>+</sup> T cells were sorted by flow cytometry (ARIA-III<sup>®</sup>) for quantitative real-time PCR (q-RT-PCR). Written informed consent was obtained from all subjects before study, approved by the local ethical committee.

**Isolated culture of rat naive rat CD4<sup>+</sup> T cells.** Naive CD4<sup>+</sup> T cells were marked with CTV, then cultured ( $10^{5}$ /well) with soluble anti-CD28 ( $1\mu g/mL$ ) in round-bottom wells coated with anti-CD3 ( $2\mu g/mL$ ) during 3 or 6 d, in complete RPMI. In some experiments, rrIL-1 $\beta$  (20ng/mL), rrIFN- $\gamma$  (10ng/mL), and/or STAT1 transcriptional activator (2NP,  $50\mu$ M) were added at the beginning of culture. 2NP dose was optimized in preliminary experiment (Supplementary Fig. 1A,B). Th1 differentiation was induced by adding IL-12 (10ng/mL), anti-IL-4 Ab ( $1\mu g/mL$ ) and IL-2 (5ng/mL) at the beginning of 4d culture.

**Coculture of rat naive CD4<sup>+</sup> T cells and cDC2.** After overnight maturation of cDC2,  $10^5$  naive CD4<sup>+</sup> T cells marked with CTV were incubated with  $10^4$  cDC2, in the presence of soluble anti-TCR $\alpha\beta$  (1 $\mu$ g/mL), during 3 or 6 d, in complete RPMI.

**Rat CD4<sup>+</sup> T cells functional assay.** After culture, T cells were stimulated with PMA (10 ng/mL), ionomycin (1  $\mu$ g/mL) for 4h, in the presence of BFA (2  $\mu$ g/mL). Live-Dead and CTV staining identified live cells that had divided. The expression of cell surface activation markers (CD25, ICOS) or intra-cellular cytokines (IL-17A, TNF $\alpha$ , IFN $\gamma$ ) was evaluated by flow cytometry (Fortessa®). In some experiments, IL-17A production in the culture supernatants was evaluated by ELISA.

**Determination of total STAT1 and 3 intra-cellular content by flow cytometry.** Single-cell suspensions of rat mLN, or CD14<sup>-</sup> human PBMC, were marked to identify CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>+</sup> naive CD4<sup>+</sup> T cells. After incubation with Fix then Perm buffers, cells were stained with anti-STAT1 and anti-STAT3 Abs. The mean fluorescent intensity (MFI) was quantified by flow cytometry (Fortessa®).

**Analysis of STAT1 and 3 phosphorylation by flow cytometry.** Single-cell suspensions of rat mLN were stained to identify CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>+</sup> naive CD4<sup>+</sup> T cells. Then they were stimulated with mrIL-27 (5 ng/mL) or rrIL-6 (10 ng/mL) for 10 minutes at 37°C. After incubation with Fix I then Perm III buffers, cells were stained with anti-pSTAT1 and anti-pSTAT3 Abs. The MFI was quantified by flow cytometry (Fortessa®).

**RNA sequencing (RNA-seq).** Total RNA was purified from mLN naive CD4<sup>+</sup>T cells sorted from 3 mo- (RNeasy micro kit, QIAGEN) or 4 wk-old rats (Monarch<sup>®</sup> Total RNA Miniprep Kit, NEB). Samples with RNA integrity number >7 were sequenced. Libraries were prepared using NEBNext<sup>®</sup> Low Input RNA Library Prep Kit and sequenced on an Illumina HiSeq 2000, paired end 75 bases. Sequencing was performed at the genomic platforms of Cochin Institute – Sorbonne University, or Faculty of Health Simone Veil–Versailles-Saint-Quentin/Paris-Saclay University. Transcripts were aligned to the rat genome Rnr\_6.0, counts normalized, and differential analysis performed using STAR version 2.7.1 and DESeq2, with R (3.6.1). Pathways analysis was performed using QIAGEN Ingenuity Pathway Assist (IPA) <sup>16,17</sup>. Volcano plots were designed with EnhancedVolcano.

**q-RT-PCR.** Naive rat or human CD4<sup>+</sup> T cell and rat thymocytes pellets were collected in TRIzol RNA Isolation Reagents (ThermoFisher), then RNA was extracted using chloroform/ethanol (rat) or Monarch<sup>®</sup> Total RNA Miniprep Kit (human). cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher), followed by q-RT-PCR (CFX384 Touch Real-Time PCR Detection System) using SsoAdvanced<sup>™</sup> Universal SYBR® Green Supermix (BioRad). Duplicates were run for each sample in a 384-well plate; *Gapdh/GADPH* and *Actin/ACTIN* were used as endogenous reference genes. For each sample/target gene, the mRNA abundance was normalized to the reference genes <sup>18</sup>. Primers sequences are provided in Supplementary Table 2.

#### ARTICLE ORIGINAL (1)

Chromatin immunoprecipitation followed by whole-genome sequencing (ChIP-seq). mLN naive CD4<sup>+</sup> T cells (2-7 x 10<sup>5</sup> cells) were sorted from or 4 wk- or 3 mo-old rats, then fixed using 32% paraformaldehyde (Electron Microscopy Sciences) for 8 min. After cell and nuclei lysis, chromatin was fragmented on Bioruptor-PICO (Diagenode), immunoprecipitated overnight at 4°C using the following rabbit polyclonal Abs: IgG (Diagenode, 1µg), m/r anti-H3K4Me3 (Diagenode, 1µg), m/r anti-H3K27Ac Abs (Active Motif, 5µg), and tagmented using the ChIP-mentation Kit for Histones (Diagenode). Libraries were prepared according to the ChIP-mentation Kit guidelines and sequenced using the NextSeg 500/550 (Illumina). Sequencing was performed at the genomic platforms of Integrative Institute of Cellular Biology - Paris-Saclay University, or Faculty of Health Simone Veil-Versailles-Saint-Quentin/Paris-Saclay University. Reads were trimmed with fastp and aligned to the rat genome Rnr\_6.0 with Bowtie2 using the --sensitive parameter <sup>19,20</sup>. Uniquely mapped non-redundant reads were used for peak calling with MACS2, compared to IgG signal <sup>21</sup>. Peaks were visualized using IGV <sup>22</sup>. Distribution across the different genomic features (promoter, exon, 5' or 3' UTR, intronic or intergenic) was performed using ChIPseeker<sup>23</sup>. Differential peaks enrichment analysis was performed using DiffBind, after overlapping/merging replicates peak sets and reads counting <sup>24</sup>. Superenhancers (SE) were retrieved using ROSE, after enhancers ranking depending on H3K27Ac peak enrichment <sup>25</sup>. Transcription factors (TF) binding motif scanning was performed using Homer, identifying regulatory elements enriched in dataset relative to a database of motif fixation sites <sup>26</sup>. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis was performed using g:Profiler.

**Statistical analyses.** Statistical analysis was performed using GraphPad Prism 9. Statistical significance was determined by paired or unpaired Student's *t* test, 1-way or 2-way Anova followed by Tukey's multiple comparisons test, simple linear regression. *P* values were corrected with Benjamini-Hochberg process for RNA-seq and ChIP-seq. *P* values <0.05 were considered significant.

## RESULTS

# DCs and naive CD4<sup>+</sup> T cells from B27-rat jointly contribute to the proinflammatory phenotype of CD4<sup>+</sup> T cells

We had previously shown that B27-rats DCs cocultured with CD4<sup>+</sup> T cells from NTG rats could induce Th17 bias, possibly accounting for the expansion of Th17 cells that occurs along with rat-SpA development <sup>6</sup>. To examine the respective influence of CD4<sup>+</sup> T cells and DCs in such proinflammatory pattern we performed cross-priming of naive mLN naive CD4<sup>+</sup> T cells with cDC2 from B27-rats and NTG littermates, in the presence of soluble anti-TCR $\alpha\beta$ . The survival and proliferation rates of CD4<sup>+</sup> T cells were comparable in all conditions (data not shown). Even before disease onset, cDC2 and naive CD4<sup>+</sup> T cells from B27-rats contributed additively to the proinflammatory activation profile of CD4<sup>+</sup> T cells, characterized by heightened expression of CD25 and ICOS cell surface markers and Th17-like phenotype characterized by increased expression of TNF $\alpha$  and IL-17A and increased production of IL-17A in supernatant (Fig. 1A, Supplementary Fig. 2A). Noteworthy, numbers of naive and effector CD4<sup>+</sup> T cells were comparable in the mLN of premorbid B27-rats and NTG littermates, showing that they had not undergone *in vivo* pre-activation (Supplementary Fig. 2B). Similar proinflammatory activation profile was observed with naive CD4<sup>+</sup> T cells from B27-rats with established disease (Fig. 1B, Supplementary Fig. 2C).

## Naive CD4<sup>+</sup> T cells from B27-rat are intrinsically prone to Th17 differentiation

We next examined how B27-rats naive CD4<sup>+</sup> T cells responded to *in vitro* activation, in the absence of cDC2. After 3 d of stimulation with anti-CD3 and anti-CD28, naive CD4<sup>+</sup> T cells from premorbid B27-rats displayed a slight increased survival and a comparable proliferation rate, as compared to NTG littermates T cells (Supplementary Fig. 3A). Consistent with the foregoing results they expressed increased activation markers (CD25 and ICOS) and Th17-like phenotype (Fig. 2A, Supplementary Fig. 3B). In naive CD4<sup>+</sup> T cells from B27-rats with established rat-SpA, similar results were observed (Fig. 2B, Supplementary Fig. 3C,D). In contrast, naive CD4<sup>+</sup> T cells from healthy B7-rats behaved like NTG rats T cells (Supplementary Fig. 3E).

To investigate what intrinsic determinants of naive CD4<sup>+</sup> T cells from B27-rats may drive their propensity to develop proinflammatory phenotype upon stimulation, we compared the transcriptome of *ex vivo* mLN naive CD4<sup>+</sup> T cells between premorbid B27-rats and NTG littermates (Supplementary Table 3). The 20 most significantly enriched pathways of differentially expressed genes were mostly related to T cell differentiation and function (Fig. 2C, Supplementary Table 4). The Th17 activation pathway appeared the most activated in B27rats, conversely IFN and Th1 pathways were the most inhibited, according to IPA activation zscore (Fig. 2C-E). In naive CD4<sup>+</sup> T cells from B27-rats with established SpA, we observed a similar profile of enriched pathways as compared to NTG littermates, supporting the potential role of Th17/Th1 imbalance in rat-SpA development (Fig. 2E, Supplementary Figure 4A,B, Supplementary Table 5). Mapping the top differentially expressed genes related to Th17 and IFN/Th1 pathways, we confirmed such clear-cut signature of premorbid B27-naive CD4<sup>+</sup> T cells transcriptome, which reinforced in B27-rats with established rat-SpA (Fig. 2E). IPA allowed to predict upstream TF that might account for the transcriptomic signature in B27-rat naive CD4<sup>+</sup> T cells before and after rat-SpA onset (Fig. 2F, Supplementary Table 6). Among them, STAT1 – predicted to be less active in B27-rats T cells – and STAT3 – predicted to be more active – appeared of special interest since they are critical TF for Th1 and Th17 differentiation respectively and may cross-regulate each other <sup>27,28</sup>.

## Naive CD4<sup>+</sup> T cells imprint is determined at the epigenome level

We next asked whether the transcriptomic signature of B27-rat naive CD4<sup>+</sup> T cells was reflected in their chromatin structures. We performed ChIP-seq to profile the whole-genome enrichment of 2 activating histone marks in *ex vivo* sorted naive CD4<sup>+</sup> T cells from B27-rats and NTG littermates: H3K4Me3 (promoters), H3K27Ac (enhancers). Histone marks distribution across the different genomic features was comparable between B27-rats and NTG littermates (Supplementary Fig. 5A). The foregoing RNA-seq data evidenced that mRNA expression of histone modifiers was comparable between B27-rats and NTG littermates Table 7).

H3K4Me3 and H3K27Ac were differentially enriched in naive CD4<sup>+</sup> T cells from B27-rats with established disease, as compared to NTG littermates, in 32 and 126 sites respectively (Supplementary Fig. 5B, Supplementary Table 8). We pooled both of those activating histone marks to increase KEGG analysis power. Promoters at these locus and enhancers at proximity were related to osteoclast and Th17 differentiation (Fig. 3A, Supplementary Table 9). Among the genes related to Th17 differentiation, *ll1r1* (Fig. 3B), *Socs3*, *Ahr* and Trem2, presented an increased enrichment for one or both of those histone marks in B27-rats T cells and were significantly up-regulated at the transcriptomic level (Fig. 2D). This unsupervised genome-wide

approach also detected in B27-rats T cells a decreased enrichment for H3K4Me3 histone mark in genes related to IFN pathway – such as *Usp18* (Fig. 3C), *Olah* and *Oasl* that were significantly down-regulated at the transcriptomic level (Fig. 2D). Even before disease onset, B27-rats naive CD4<sup>+</sup> T cells harbored a biased epigenomic signature including increased enrichment for H3K4Me3 histone mark at *Il1r1* promoter (Supplementary Fig. 5C). Otherwise, at the epigenomic level, naive CD4<sup>+</sup> T cells of healthy B7-rats, behaved like NTG rather than B27-rats T cells (Supplementary Fig. 5D).

Cis-regulatory sequences, called enhancers, are retrieved using H3K27Ac enrichment profile. Some of these enhancers favors the fixation of numerous TF leading to high transcriptional activity of related promoters; these SE play a key role in determining the cell fate, especially during naive CD4<sup>+</sup> T cells differentiation <sup>29</sup>. Indeed, differential enhancers/SE activity could account for skewed T cell differentiation during SpA and rat-SpA<sup>30</sup>. We retrieved the SE and defined the genes located at proximity (Supplementary Fig. 5E). RNA-seq data confirmed the increased transcription level of genes near SE, as compared to those near conventional enhancers (Supplementary Fig. 5F). Thus, we identified SE-related genes specific of NTG or B27-rats naive CD4<sup>+</sup> T cells (Supplementary Fig. 5E, Supplementary Table 10). B27rats SE-related genes were involved in the regulation of immune system process, leukocyte differentiation and T cell activation GO pathways (Supplementary Table 11) and several of them were related to Th17 and NFkB pathways (Fig. 3C). In contrast, several NTG rats SE-related genes were related to IFN pathway (Fig. 3C). Next, we defined the *de novo* TF binding motifs that could preferentially bind on these SE (Supplementary Table 12). Among them, TF critical for IFN pathway were predicted to bind on NTG SE, whereas TF critical for Th17/NFkB pathways were predicted to bind on B27-SE (Fig. 3D). ZNF341, a regulator of both STAT1 and STAT3 transcription activity, was predicted to bind to both NTG and B27 SE on distinct binding sites <sup>31</sup>. Using IPA, we defined the upstream TF predicted to control the expression of SE-related genes (Fig. 3E). Among them, STAT1 and STAT3 appeared as upstream TF regulating NTG and B27-rats SE-related genes, respectively. Even before disease onset, naive CD4<sup>+</sup> T cells harbored specific SE in B27-rats, related to genes critical for T cell activation and Th17 differentiation, such as Ccr7, Il2ra, Tnfrsf14, Ddit3 and Stat3 (Supplementary Table 13). The TF predicted to preferentially bind on B27-SE (Supplementary Table 14), including IRF4, SMAD3 and BATF, were related to Th17 differentiation and inflammatory bowel disease (Supplementary Fig. 5H, Supplementary Table 15).
Altogether, these data confirmed that naive CD4<sup>+</sup> T cells from B27-rat are imprinted at the epigenomic level, even before disease onset, predisposing them to differentiate preferentially into Th17 cells. This could involve an imbalance of STAT1 and STAT3 transcriptional effects, in favor of the latest.

#### STAT1 deficiency is an early and persistent hallmark of B27-rat naive CD4<sup>+</sup> T cells

To further examine STAT1/STAT3 balance in B27-rat naive CD4<sup>+</sup> T cells, we assessed protein expression by flow cytometry. Before disease onset, total STAT1 expression was decreased in B27-rat mLN naive CD4<sup>+</sup> T cells, as compared to NTG littermates, whereas STAT3 expression was comparable between both conditions (Fig. 4A). After disease onset, decreased STAT1 persisted, while STAT3 became higher in B27- than in NTG rats naive CD4<sup>+</sup> T cells (Fig. 4B). Otherwise, expression levels of STAT5 and STAT6, critical for T cells differentiation, were comparable in naive CD4<sup>+</sup> T cells of B27- and NTG rats (Supplementary Fig. 6A). As STATs are activated through phosphorylation, we examined the expression levels of pSTAT1 in naive CD4<sup>+</sup> T cells after exposure to IL-27, known to induce its phosphorylation. Before and after disease onset, pSTAT1 was decreased in naive CD4<sup>+</sup> T cells from B27-rats mLN, as compared to NTG condition (Fig. 4C,D), whereas pSTAT3 appeared comparable between both conditions (Supplementary Fig. 6B,C). Thus, STAT1 protein deficiency appeared as an early and persistent characteristic of B27-rat naive CD4<sup>+</sup> T cells, even before disease onset, whereas STAT3 protein increased upon rat-SpA development.

In support of the foregoing interpretation, STAT1 deficiency was also present in thymic precursors of naive CD4<sup>+</sup> T cells from premorbid B27-rats. Hence, *Stat1* mRNA expression was decreased in CD4<sup>+</sup> single-positive (CD4SP) and/or CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) thymocytes of newborn (Fig. 4E) and premorbid (Fig. 4F) B27-rats, whereas *Stat3* mRNA expression did not differ as compared to thymocytes from NTG rats (Supplementary Fig. 7A). We further evaluated the expression of a selection of genes regulated by STAT1 or STAT3 in thymocytes. In premorbid B27-rats, several genes whose expression is induced by STAT1, including *Usp18*, *lfit2* and *Tbx21*, were decreased whereas a majority of STAT3-induced genes (*Rorc, Batf, Socs3*) did not differ from NTG rats levels (Supplementary Fig. 7B). Interestingly however, among the latter genes, *ll1r1* expression appeared already increased in DP thymocytes from premorbid and even in thymocytes from some newborn B27-rats (Supplementary Fig. 7C). Otherwise, the mRNA expression of *Stat2, Stat4, Stat5, Stat6* and *Foxp3* in CD4SP

B27- and NTG rats (data not shown). Noteworthy, regarding *Stat1* mRNA expression, CD4SP from healthy B7-rats behaved like NTG rats (Supplementary Fig. 7D).

Altogether, these data indicate that the proinflammatory activation profile of naive CD4<sup>+</sup> T cells from B27-rats might be consecutive to defective *Stat1* expression, already present in newborn CD4SP.

# STAT1 deficiency is characteristic of naive CD4<sup>+</sup> T cells from axial SpA patients

We next evaluated STAT1 expression in circulating naive CD4<sup>+</sup> T cells from HLA-B27<sup>+</sup> axial SpA patients, in comparison with HC and RA patients. Total STAT1 and STAT3 proteins were comparably expressed in naive CD4<sup>+</sup> T cells between all 3 groups (Supplementary Fig. 8A). In contrast, *STAT1* mRNA expression level was decreased in naive CD4<sup>+</sup> T cells from axial SpA patients, as compared to both HC and RA patients, whereas *STAT3* appeared increased in RA patients as compared to both other groups that were comparable to each other (Fig. 4G, Supplementary Fig. 8B). Of interest, *STAT1/STAT3* ratio was specifically decrease in naive CD4<sup>+</sup> T cells from axial SpA patients, as compared to HC, suggesting that imbalance in expression of these TF might be a critical event during SpA (Fig. 4H).

# STAT1 deficiency associated with *ll1r1* upregulation, that favors a Th17 differentiation bias in B27-rat naive CD4<sup>+</sup> T cells

*Stat1* expression was decreased in LN naive CD4<sup>+</sup> T cells from premorbid B27-rats, as compared to NTG rats T cells, without distinction between mLN draining gut, i.e. a rat-SpA target tissue, and sLN draining irrelevant tissue (Fig. 5A), suggesting that this signature might be ubiquitous and favor pro-inflammatory bias in a tissue-dependent way. A major cytokine secreted by inflammatory tissue is IL-1β<sup>32,33</sup>. Moreover, the upregulation of IL-1β receptor, *Il1r1*, appeared as an early event favored by a strong epigenomic imprint in mLN B27-rats naive CD4<sup>+</sup> T cells (Fig. 2D,E,3B, Supplementary Fig. 4A,5C), that might begin even in newborn thymocytes (Supplementary Fig. 7C). Thus, we examined the putative link between *Stat1* deficiency, IL-1 pathway and Th17 bias. We first confirmed that *Il1r1* was also increased in sLN as well as in mLN B27-rats naive CD4<sup>+</sup> T cells (Fig. 5B) and observed that *Stat1* negatively correlated with *Il1r1* expression, suggesting that they may cross-regulate each other (Fig. 5B,C). Hence, the presence of rrIL-1β during activation of mLN naive CD4<sup>+</sup> T cells from premorbid B27-rats, but not NTG littermates, resulted in heightened production of IL-17A (Fig. 5D,E). Thus, the

hypersensitivity of naive CD4<sup>+</sup> T cells of B27-rats to IL-1 $\beta$  reinforces Th17-biased differentiation in mLN, possibly related to *ll1r1* upregulation and *Stat1* deficiency.

# STAT1 signaling in B27-rat naive CD4<sup>+</sup> T cells inhibits IL-17A expression

We next examined the functional consequences of STAT1 deficiency upon naive CD4<sup>+</sup> T cells differentiation. In the presence of IL-12 and anti-IL-4, T-bet, TNF $\alpha$  and IFN $\gamma$  were induced similarly in B27-rats and NTG littermates, without IL-17A, showing efficient Th1 differentiation of B27-rat naive CD4<sup>+</sup> T cells despite decreased basal level of STAT1 (Figure 6A). Interestingly, the addition of IFN $\gamma$  – which signals through STAT1 – led to a decrease of IL-17A expression (Fig. 6B). Addition of 2NP, a selective STAT1 transcriptional activator, during naive CD4<sup>+</sup> T cells activation specifically reversed the increased IL-17A expression in premorbid and adult B27-rats T cells (Fig. 6C), even in the presence of IL-1 $\beta$ , that otherwise increased Th17 differentiation as described above (Fig. 6D). We thus confirmed that activating STAT1 signaling could reverse the Th17 bias imprinted in B27-rat naive CD4<sup>+</sup> T cells.

# DISCUSSION

Rat-SpA that develops spontaneously in the 33-3 line of B27-rats appears dependent on CD4<sup>+</sup> T cells and is associated with uncontrolled expansion of pro-inflammatory Th17 cells producing TNFα and IL-17<sup>5,6</sup>. However, a primary role of B27-rat myeloid-derived cells was previously established, leading to propose that expression of HLA-B27 in APC could be a key mechanism underlying rat-SpA <sup>8</sup>. Consistently, DCs from B27-rat exhibit altered functions that correlate with rat-SpA susceptibility and are shared with SpA patients <sup>3,9,34</sup>. Interestingly, some of those alterations, including reverse-IFN signature and decreased IL-27 and IL-10 expression could contribute to Th17 expansion <sup>3,9,18</sup>.

Indeed, cDC2 from premorbid B27-rats induced heightened activation and expression of TNF $\alpha$  and IL-17 in cocultured naive CD4<sup>+</sup> T cells, whether from B27- or NTG rat, as shown in the present study. However, naive B27-rat CD4<sup>+</sup> T cells were also found prone by themselves to develop such pro-inflammatory profile. This led us to examine *ex vivo* the basal transcriptome imprint of B27-rat naive CD4<sup>+</sup> T cell and to uncover two opposite major signatures: defective IFN and heightened Th17 patterns, notably involving key TF of both pathways, including decreased expression of *Stat1* and *Tbx21* on the one hand and increased

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expression of *Stat3*, *Ahr* and *Batf* on the other. An imbalance between both pathways was present in premorbid rats and deepened in B27-rats with established disease, reinforcing its relevance to rat-SpA disease process. This observation appears particularly compelling, since TF from both pathways are known to cross-regulate each other <sup>27,35,36</sup>. Moreover, several of those features were similar to the transcriptomic signature previously described in B27-rat cDC2, i.e. reverse-IFN signature (including decreased *Stat1*) and increased *Socs3*, a negative regulator of IFN <sup>18</sup>. As for cDC2, it was specific for B27-rat since naive CD4<sup>+</sup> T cells from B7-rat behaved alike NTG rat cells.

Epigenomic study of histone marks associated with active promoters and enhancers including SE which are regions critical to determine CD4<sup>+</sup> T cell differentiation - consistently revealed that naive CD4<sup>+</sup> T cells from B27-rats were skewed at the chromatin level to differentiate into Th17 cells and down-regulate several genes of the IFN pathway. Algorithms used to determine which TF might bind to SE, predicted that IRF4 and STAT3, two TF involved in Th17 differentiation, were expected to bind to B27-rat-specific SE, whereas conversely greater binding of STAT1 to NTG rat-specific SE was likely. This highlighted a possible imbalance between those TF as a triggering event in skewed differentiation of B27-rat CD4<sup>+</sup> T cells.

We next examined the earliest stage of CD4<sup>+</sup> T cell development in B27-rat and found that several of the IFN pathway-related genes -i.e. *Stat1*, *Ifit2*, *Usp18*- were already down-regulated in the developing thymocytes from premorbid and even newborn B27-rats, in particular in the CD4<sup>+</sup> SP thymocytes that localize to the thymic medulla after positive selection, before being seeded to the periphery <sup>37</sup>. In contrast, this was not the case for any of the Th17 pathway-related genes, to the exception of *Il1r1*. Thus, it can be inferred that the primary alteration in naive CD4<sup>+</sup> T cells from B27-rat most likely resides in the reverse-IFN signature and particularly in decreased *Stat1* expression. This could be due to an altered response to type-I IFN that is produced constitutively in the thymic medulla <sup>38</sup> and could favor a secondary heightened Th17 response which appears as a hallmark of rat-SpA <sup>6</sup>. This interpretation is consistent with the decrease of STAT1 protein and phosphorylation, already present in naive CD4<sup>+</sup> T cells from premorbid B27-rat, whereas increased STAT3 was only detected in B27-rats with established disease. It is also supported by the negative regulation of IL-17 production induced *in vitro* by addition of IFNγ or a of a transcriptional activator of STAT1 during stimulation of B27-rat naive CD4<sup>+</sup> T cells. Noteworthy, inhibition of Th17 expansion was also

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achieved by adding IL-27 to B27-rat CD4<sup>+</sup> T cell in vitro, allowing to restore the balance between STAT1 and STAT3 activation, and *in vivo* allowing to prevent rat-SpA development (Jouhault Q et al, *submitted manuscript*).

Regarding the up-regulation of *ll1r1* that was already detected in B27-rat thymocytes, it was possibly secondary to *Stat1* down-regulation given the negatively correlated expression of both genes and the mutual antagonism between IFN and IL-1 signaling pathways <sup>39</sup>. Increased expression of this cytokine receptor gene could contribute to enhance the differentiation of pro-inflammatory Th17 cells in sites where IL-1 is produced, notably in the gut upon exposure to microbiota, which is a required trigger of rat-SpA in the 33-3 line <sup>40–42</sup>.

Like B27-rat, SpA patients display a Th17 bias that appears critically important, as confirmed by the efficacy of anti-IL-17 mAb treatments in those patients <sup>43,44</sup>. Thus, our observation of a similarly decreased *STAT1* expression and *STAT1/STAT3* imbalance in naive CD4<sup>+</sup> T cells from HLA-B27<sup>+</sup> SpA patients supports the relevance of this observation to human SpA.

Besides naive CD4<sup>+</sup> T cells, it is striking that other types of immune cells from B27-rat, i.e. cDC2 and cDC1 (<sup>18</sup> and Fert I, Araujo LM, Breban M, unpublished observation) as well as monocytes-derived macrophages from SpA patients <sup>18,45</sup> share a similar reverse-IFN signature, including down-regulation of STAT1, the major TF of the pathway. What molecular event is responsible for the signature depicted herein in naive CD4<sup>+</sup> T cells from B27-rat, remains to be determined. However, it can be speculated that it happens as a direct cellular consequence of HLA-B27 expression. Some of the peculiar behaviors of HLA-B27, including its tendency to aggregate in endoplasmic reticulum-derived vesicles, to induce an unfolded protein response, or to interfere with TGF $\beta$  receptor family signaling could theoretically account for it <sup>2,46,47</sup>. In turn, STAT1 deficiency could favor Th17 differentiation either directly by altering the cellular response to stimulation <sup>35</sup> and/or indirectly by affecting the control of gut microbiota, which is consistent with the gut dysbiosis that is present both in B27-rat and SpA patients <sup>48,49</sup>.

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# Article 1 : Figures



Figure 1. DCs and naive CD4<sup>+</sup> T cells from B27-rat contribute additively to the proinflammatory fate of CD4<sup>+</sup> T cells

**Figure 1** - Cocultures of splenic cDC2 with mLN naive CD4<sup>+</sup> T cells were performed in the presence of soluble anti-TCR $\alpha\beta$  mAb. Cells were harvested from (**A**) 3-4 wk-old premorbid B27-rats (n = 6), or from (**B**) 3-9 mo-old B27-rats with established rat-SpA (n = 6), and age-matched NTG littermates. (**A**) After 3 d, CD4<sup>+</sup> T cells were analyzed by flow cytometry for cell-surface expression of CD25 and ICOS, and intra-cellular expression of TNF $\alpha$  and IL-17A. Data are gated on live CD4<sup>+</sup> T cells that had divided (according to CTV staining). (**B**) After 6 d, IL-17A concentration was assessed by ELISA in supernatants. Histograms correspond to the mean ± SEM of 6 independent cocultures. Each dot represents an independent coculture. p-values were calculated by 2-way Anova; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.



#### Figure 2. Naive CD4+ T cells from B27-rat are intrinsically prone to Th17 differentiation

**Figure 2** - mLN naive CD4<sup>+</sup> T cells were isolated from (**A**) 3-4 wk-old premorbid B27-rats (n = 10), or from (**B**) 3-6 mo old B27-rats with established rat-SpA (n = 4) and age-matched NTG littermates, then cultured in anti-CD3 coated wells, in the presence of soluble anti-CD28 mAb. (**A**) After 3 d, CD4<sup>+</sup> T cells were analyzed by flow cytometry for cell surface expression of CD25 and ICOS, and intra-cellular expression of TNF $\alpha$  and IL-17A. Data are gated on live CD4<sup>+</sup> T cells that had divided. (**B**) After 6 d, IL-17A was assessed by ELISA in supernatants. (**A**,**B**) Histograms correspond

to the mean  $\pm$  SEM. Each dot represents an independent rat. p-values were calculated by unpaired t-test; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. (C-F) RNA-seq was performed on mLN naive CD4<sup>+</sup> T cells isolated from (C-F) 3 wk-old premorbid B27-rats (n = 7) and age-matched NTG littermates (n = 5), or from (E,F) 3-mo old B27-rats with established rat-SpA and age-matched NTG littermates (n = 7 per group). (C) The graph shows the top 20 IPA terms, enriched in genes differentially expressed in B27-rats, as compared to NTG littermates naive CD4<sup>+</sup> T cells. The zscore refers to the activation (red), inhibition (blue) or undefined (white) state of the pathway in B27-rats relative to NTG littermates. Adjusted-p value is expressed in -log. (D) Volcano plot displaying log<sub>2</sub> fold-change versus -log<sub>10</sub> adjusted p value for each gene expressed in naive CD4<sup>+</sup> T cells from B27-rats, as compared to NTG littermates T cells. Differentially expressed genes involved in IFN/Th1 and Th17 pathways are labeled in boxes. (E) Expression heatmap showing the log<sub>2</sub> fold-change expression of genes related to IFN/Th1 and Th17 pathways, in naive CD4<sup>+</sup> T cells, as compared to NTG littermates T cells, before (3 wk-old, premorbid) of after (3 mo-old, adult) rat-SpA onset. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001. (F) The table shows the upstream TF predicted to explain the differential transcriptomic signature in naive CD4<sup>+</sup> T cells from B27-rats, as compared to NTG littermates T cells. log(adj p value) refers to the comparison of the dataset to IPA database for each TF, calculated by Fisher's exact test. The z-score refers to the activated (red), inhibited (blue) or undefined (white) state of the corresponding pathway in B27-rats relative to NTG littermates. Log2FC(RNA) refers to the relative gene expression in naive CD4<sup>+</sup> T cells from B27-rats, as compared to NTG littermates T cells, from RNA-seg dataset (blue if <0, red if >0, bold if significant).





**Figure 3** - ChIP-seq was performed with Abs specific for H3K4Me3 and H3K27Ac activating histone marks on mLN naive CD4<sup>+</sup> T cells from 3 mo-old B27-rats with established rat-SpA (n = 3). (**A**) The left graph shows the 2 KEGG pathways enriched for genes harboring a differential enrichment of H3K4Me3 and/or H3K27Ac histone marks, in B27-rats compared to NTG littermates naive CD4<sup>+</sup> T cells (adjusted-p value expressed in -log<sub>10</sub>). The right graph shows genes common to both KEGG pathways and Log2FC(RNA) refers to the relative gene expression in naive

CD4<sup>+</sup> T cells from B27-rats, as compared to NTG littermates T cells from RNA-seq dataset. \*\*\*\* adjusted p<0.0001. (**B**) Integrated genome viewer snapshots of genes involved in Th17 (*ll1r1*), and IFN pathways (*Usp18*). Differentially enriched peaks are highlighted in yellow. Graphed dataset from 1 pair of B27/NTG rats, representative of 3. (**C**) Vein diagram showing the genes at proximity of the SE of B27-rats naive CD4<sup>+</sup> T cells and those of NTG littermates (ROSE). Examples of SE-related genes specific of NTG and B27-rats, involved in IFN (blue) or Th17 (red) pathways. (**D**) Examples of *de novo* binding motifs of TF predicted to fix on NTG and B27-SE. (**E**) Upstream TF predicted to modulate the expression of NTG and B27-rat-specific SE genes. -log (adj p value) refers to the comparison of the genes dataset to IPA database for each TF, calculated by Fisher's exact test. Log2FC(RNA) refers to the relative gene expression in naive CD4<sup>+</sup> T cells from B27-rats before (premorbid; 3 wk-old) of after (adult; 3 mo-old) rat-SpA onset, as compared to NTG littermates T cells, from RNA-seq dataset (blue if <0, red if >0, bold if significant). TF are in bold if the direction of Log2FC(RNA) remained consistent over time.

#### Figure 4. STAT1 deficiency is an early and persistent hallmark of B27-rat naive CD4+ T cells



**Figure 4** - (**A-D**) Single cell suspension was obtained from mLN of (**A,C**) 3 wk- or (**B,D**) 3-12 mo-old B27-rats and NTG littermates. (**A,B**) Total STAT1 and STAT3 protein MFI was assessed in naive CD4<sup>+</sup> T cells by flow cytometry. Histograms correspond to the mean ± SEM of 8 to 18 rats per group. Each dot represents an independent rat. (**C,D**) Phosphorylated STAT1 MFI was assessed by flow cytometry in naive CD4<sup>+</sup> T cells, after IL-27 stimulation for 10 minutes. Histograms correspond to the mean ± SEM of 7 to 12 rats per group. Each dot represents an independent rat. (**E,F**) *Stat1* gene mRNA expression was assessed by q-RT-PCR in CD4<sup>+</sup> single-positive (CD4SP) and CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) thymocytes, sorted from the thymus of (**E**) 10 d- or (**F**) 3 wk-old B27-rats and NTG littermates. Data are the mean ± SEM of 5 to 8 rats per group. (**G,H**) Naive CD4<sup>+</sup> T cells were sorted from the PBMC of SpA

patients (n = 28), RA patients (n = 8) and HC (n = 16) and mRNA expression was assessed by q-RT-PCR. (G) *STAT1* gene mRNA expression. Data are the mean  $\pm$  SEM. p-values were calculated by (A-D) paired t-test or (E,F) unpaired t-test or (G,H) 1-way Anova; \* p<0.05, \*\* p<0.01, \*\*\*\* p<0.0001.



**Figure 5.** STAT1 deficiency associates with *ll1r1* upregulation that favors a Th17 differentiation bias in B27-rat naive CD4<sup>+</sup> T cells

**Figure 5** - Naive CD4<sup>+</sup> T cells were sorted from (**A-C**) sLN or mLN, or (**D-E**) mLN, from 3-4 wk-old B27-rats and NTG age-matched littermates (n = 6-8). (**A-C**) *Stat1* or *ll1r1* mRNA expression was assessed by q-RT-PCR; (**C**) correlation between both genes was evaluated separately in B27-rats (right panel) and NTG littermates (left panel). Data are shown as the mean  $\pm$  SEM. Each dot represents an independent rat. (**D,E**) Naive CD4<sup>+</sup> T cells were cultured in anti-CD3 coated-wells, in the presence of soluble anti-CD28 mAb, with or without IL-1 $\beta$ , for 3 d. (**D**) CD4<sup>+</sup> T cells were analyzed by flow cytometry for cell-surface expression of CD25 and ICOS, and intra-cellular expression of TNF $\alpha$  and

IL-17A.Data gated on live CD4<sup>+</sup> T cells are shown as the mean  $\pm$  SEM of 10 rats per group. (**E**) IL-17A concentration was assessed by ELISA in supernatants. p-values were calculated by (**A**,**B**) unpaired t-test or (**C**) linear regression or (**D**,**E**) 2-way Anova; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.001.



Figure 6. STAT1 signaling in B27-rats naive CD4<sup>+</sup> T cells inhibits IL-17A expression

**Figure 6** - mLN naive CD4<sup>+</sup> T cells were sorted from (**A**-**D**) 3-4 wk-old or (**C**) 2-5 mo-old B27-rats and (**A**,**C**,**D**) NTG age-matched littermates, cultured in anti-CD3 coated-wells in the presence of soluble anti-CD28 mAb and of (**A**) rrIL-12, rrIL-2 and rat neutralizing anti-IL-4, or (**B**) rrIFN- $\gamma$ , or (**C**,**D**) STAT1 transcriptional activator (2NP; 50µM), and (**D**) IL-1 $\beta$ . After (**A**) 4 d or (**B**-**D**) 3 d, intra-cellular expression of T-bet, IFN $\gamma$ , TNF $\alpha$  and IL-17A was assessed by flow cytometry in gated live CD4<sup>+</sup> T cells. Data are shown as the mean ± SEM of 4 to 6 independent experiments. p-values were determined by (**A**,**B**) unpaired t-test or (**C**,**D**) 2-way Anova; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

	Healthy Control	SpA	RA
Ν	32	39	8
B27 positive (%)	0	37 (95)	1 # (14)
Gender - F/M	11/18	16/23	7/1
Age at sampling (mean ± SD)	54.3 ± 13.3	55.6 ± 12.3	66.6 ± 8.9
BMI (mean ± SD)	$26.2 \pm 4.9$	25.6 ± 4.0	25.1 ± 5.6
Age of disease onset (mean ± SD)	-	30.1 ± 10.7	54.5 ± 15.8
Duration of disease (mean ± SD)	-	25.4 ± 9.7	12.1 ± 12.6
Axial involvement (%)	-	39 (100)	-
Radiographic/MRI sacroiliitis (%)	-	20 (50)	-
SpA: Oligoarthritis (%)	-	13 (33)	-
Enthesitis (%)	-	30 (88)	-
Psoriasis (%)	-	8 (21)	-
Uveitis (%)	-	8 (21)	-
Inflammatory bowel disease (%)	-	5 (13)	0
NSAIDs (%)	-	23 (20)	-
Biologics (%)	-	23 (59)	7 (88)
Anti-TNF (%)	-	20 (51)	3 (38)
Anti-IL17 (%)	-	2 (5)	-
Other * (%)	-	1 (3)	4 (50)
MTX or SSZ (%)	-	2 (5)	4 (50)
CTS (%)	-	0	5 (63)
BASDAI (mean ± SD)	-	2.8 ± 2.2	-
BASFI (mean ± SD)	-	2.2 ± 2.4	-
DAS28-ESR (mean ± SD)	-	-	3.7 ± 1.2

# Article 1 : Données complémentaires

**Supplementary Table 1.** Healthy controls (HC), axial SpA and RA patients were recruited for mRNA expression quantification by q-RT-PCR and/or total STAT1 and STAT3 proteins expression quantification by flow cytometry. F: female, M: male, BMI: body mass index, MRI: magnetic resonance imaging, NSAID: non-steroid anti-inflammatory drug, MTX: methotrexate, SSZ: sulfasalazine, CTS: corticosteroid, BASDAI: Bath ankylosing spondylitis disease activity Index, BASFI: Bath ankylosing spondylitis functional index, DAS28-ESR: Disease activity score 28–erythrocyte sedimentation rate. \* 1 SpA patient was treated with upadacitinib; 1 RA patient was treated with abatacept, 2 with tocilizumab, 1 with sarilumab; # 7/8 RA patients were typed for HLA-B27.

Ingenuity Canonical Pathways	-d)6ol-	/alue) 👻 p-value '	-log(B-H p-value)	<ul> <li>B-H p-value v1</li> </ul>	Ratio	z-score *	Molecules
IL-9 Signaling	96'6	0 1,05E-10	7,400	3,98E-08	25,7%	0,707	BCL3, IRS1, JAK3, PIK3CD, PIK3R1, PIK3R5, REL, SOCS3, STAT3
Natural Killer Cell Signaling	9,25	0 5,62E-10	6,970	1,07E-07	8,9%	1,604	B2M,CD247,FASLG,HLA-A,HLA-E,HSPA5,JAK3,KLRD1,MAP3K5,PIK3CD,PIK3R1,PIK3R5,PXN,REL,RRAS
T Cell Receptor Signaling	7,54	0 2,88E-08	5,560	2,75E-06	7,3%	-1,069	B2M,CD247,H2-T24,HLA-A,HLA-E,ITK,MALT1,PIK3CD,PIK3R1,PIK3R5,PPP3CA,REL,RRAS,TCF3
Th1 Pathway	7,63	0 2,34E-08	5,560	2,75E-06	10,4%	-1,414	CD247,HLA-A,JAK3,KLRD1,PIK3CD,PIK3R1,PIK3R5,RUNX3,SOCS3,STAT3,TBX21
T Cell Exhaustion Signaling Pathway	7,23	5,89E-08	5,350	4,47E-06	8,4%	1,414	BATF,CD247,HLA-A,HLA-E,JAK3,LAG3,PIK3CD,PIK3R1,PIK3R5,RRAS,STAT3,TBX21
temic Lupus Erythematosus In T Cell Signaling Pathway	6,20	0 6,31E-07	4,490	3,24E-05	6,1%	-1,732	B2M,CD247,EZR,FASLG,H2-T24,HLA-A,HLA-E,PIK3CD,PIK3R1,PIK3R5,PPP3CA,RRAS,STAT3
Th2 Pathway	6,12	0 7,59E-07	4,490	3,24E-05	8,4%	1,000	CCR4, CD247, HLA-A, JAK3, PIK3CD, PIK3R1, PIK3R5, RUNX3, SOCS3, TBX21
Role of JAK1 and JAK3 in yc Cytokine Signaling	6,17	0 6,76E-07	4,490	3,24E-05	11,9%	NaN	IRS1, JAK3, PIK3CD, PIK3R1, PIK3R5, RRAS, SOCS3, STAT3
iCOS-iCOSL Signaling in T Helper Cells	5,71	0 1,95E-06	4,240	5,75E-05	8,7%	-0,816	CD247,HLA-A,ITK,PIK3CD,PIK3R1,PIK3R5,PLEKHA1,PPP3CA,REL
IG F-1 Signaling	5,78	0 1,66E-06	4,240	5,75E-05	8,9%	1,414	IG FBP4, IRS1, PIK3CD, PIK3R1, PIK3R5, PXN, RRAS, SOCS3, STAT3
Mouse Embryonic Stem Cell Pluripotency	5,74	0 1,82E-06	4,240	5,75E-05	8,8%	2,333	ID2,IL6ST,JAK3,PIK3CD,PIK3R1,PIK3R5,RRAS,STAT3,TCF3
JAK/Stat Signaling	5,53	0 2,95E-06	4,130	7,41E-05	9,9%	1,134	JAK3, PIK3CD, PIK3R1, PIK3R5, REL, RRAS, SOCS3, STAT3
B Cell Receptor Signaling	5,48	3,31E-06	4,120	7,59E-05	6,4%	0,333	CD22,EGR1,MALT1,MAP3K5,PIK3CD,PIK3R1,PIK3R5,PPP3CA,REL,RRAS,TCF3
Acute Phase Response Signaling	5,48	0 3,31E-06	4,120	7,59E-05	6,4%	1,667	IL1R1,IL6ST,MAP3K5,NR3C1,PIK3CD,PIK3R1,REL,RRAS,SOCS3,STAT3,TCF3
CD28 Signaling in T Helper Cells	5,40	0 3,98E-06	4,100	7,94E-05	8,0%	-0,816	CD247,HLA-A,ITK,MALT1,PIK3CD,PIK3R1,PIK3R5,PPP3CA,REL
Prolactin Signaling	5,41	0 3,89E-06	4,100	7,94E-05	9,5%	1,134	IRS1, NR3C1, PIK3CD, PIK3R1, PIK3R5, RRAS, SOCS3, STAT3
NGF Signaling	5,31	0 4,90E-06	4,030	9,33E-05	7,8%	1,414	MAP3K5, PIK3CD, PIK3R1, PIK3R5, REL, RRAS, SMPD1, SMPD2, TRIO
IL-23 Signaling Pathway	5,04	0 9,12E-06	3,850	1,41E-04	13,3%	0,447	PIK3CD, PIK3R1, PIK3R5, REL, SOCS3, STAT3
IL-6 Signaling	5,01	0 9,77E-06	3,840	1,45E-04	7,2%	1,414	IL1R1,IL6ST,PIK3CD,PIK3R1,PIK3R5,REL,RRAS,SOCS3,STAT3
Th17 Activation Pathway	4,98	1,05E-05	3,830	1,48E-04	10,1%	2,236	BATF,CD247,IL1R1,JAK3,REL,SOCS3,STAT3
Virus Entry via Endocytic Pathways	4,94	0 1,15E-05	3,810	1,55E-04	8,3%	NaN	B2M,HLA-A,HLA-E,PIK3CD,PIK3R1,PIK3R5,RRAS,TFRC
temic Lupus Erythematosus In B Cell Signaling Pathway	4,71	0 1,95E-05	3,660	2,19E-04	4,8%	-0,302	CD22,FASLG,IFIT2,IL6ST,MALT1,PIK3CD,PIK3R1,PIK3R5,PPP3CA,REL, RRAS,STAT3
Type I Diabetes Mellitus Signaling	4,72	0 1,91E-05	3,660	2,19E-04	7,7%	0,447	CD247,FASLG,HLA-A,HLA-E,IL1R1,MAP3K5,REL,SOCS3
Tumor Microenvironment Pathway	4,73	0 1,86E-05	3,660	2,19E-04	5,9%	1,000	FASLG, HLA-A, HLA-E, MMP9, PIK3CD, PIK3R1, PIK3R5, REL, RRAS, STAT3
IL-15 Signaling	4,74	1,82E-05	3,660	2,19E-04	9,3%	1,633	JAK3,PIK3CD,PIK3R1,PIK3R5,REL,RRAS,STAT3
MSP-RON Signaling In Macrophages Pathway	4,72	0 1,91E-05	3,660	2,19E-04	7,7%	1,890	PIK3CD, PIK3R1, PIK3R5, REL, RRAS, SBNO2, SOCS3, STAT3
toxic T Lymphocyte-mediated Apoptosis of Target Cells	4,76	0 1,74E-05	3,660	2,19E-04	16,7%	NaN	B2M,CD247,FASLG,HLA-A,HLA-E
SPINK1 General Cancer Pathway	4,57	0 2,69E-05	3,550	2,82E-04	11,1%	1,633	JAK3,PIK3CD,PIK3R1,PIK3R5,RRAS,STAT3
Allograft Rejection Signaling	4,57	0 2,69E-05	3,550	2,82E-04	11,1%	NaN	B2M,CD247,FASLG,H2-T24,HLA-A,HLA-E
	Dethinio			-	2		

# ARTICLE ORIGINAL (1)

Upstream TF	Expr Log Ratio	Common adult-premorbid	Activation z-score	B-H corrected p-value	Target Molecules in Dataset
STAT3	0,462	yes	2,393	1,90E-04	AHR, BATF, CHI3L1, FAS, GADD45G, HK2, ICAM1, IGF1R, MMP9, RORA, SBNO2, SOCS3, STAT3
BRCA1	-0,137	yes	-2,216	5,91E-03	IFIT2,IGF1R,IRF7,MX1,TAP1
EGR3	-0,217	yes		1,91E-02	FASLG, SOCS3
MTA1	-0,096	yes		1,91E-02	MMP9,STAT3
STAT1	-0,106	yes	-0,2	2,33E-02	EIF2AK2,ICAM1,IGF1R,PSMB8,PSMB9,TAP1
ETS1	0,093	yes		3,92E-02	CD79A,FASLG,MMP9,RUNX2
RELA	0,138		0,647	9,69E-04	BIRC3, CHI3L1, FASLG, ICAM1, IGF1R, MMP9, NFKBIA, SELP, VCAM1
POU2F1	0,105			1,79E-03	CD79A,CD79B,HLA-DRA,VCAM1
STAT2	-0,192			9,58E-03	MX1,OAS1,PSMB8
NFATC1	0,186			1,59E-02	ACP5,CYBB,FAS,FASLG
STAT6	0,115			1,59E-02	ACP5, FCER2, FOXP3, VCAM1
CIITA	-0,621			1,91E-02	HLA-A,HLA-DQB1,HLA-DRA
KLF2	0,404			1,91E-02	CCR3,CXCR3,SELL
POU2AF1	-0,162			1,91E-02	CD79A,CD79B
IRF1	-0,051			2,51E-02	FASLG, IFI44, ISG15, PSMB9, VCAM1
FOXC2	na			2,52E-02	ITGB5,MMP9,RUNX2
SIRT2	-0,106			2,52E-02	FDFT1,IDI1,SQLE
PML	0,04			2,78E-02	PSMB8,PSMB9,TAP1
JUN	0,504		-1,154	3,27E-02	ACP5, ANXA1, ANXA2, FAS, FASLG, IGF1R, MMP9, RUNX2, SPHK1
EOMES	-0,773			3,31E-02	FASLG,IL2RB
FOXO3	0,194		-1,067	3,31E-02	FASLG, FOXP3, ICAM1, MMP9, PRNP
IRF7	-0,396			3,31E-02	CTLA4, FOXP3, ISG15
IRF8	-0,168			3,31E-02	CD80,ICAM1,ID3,ISG15
SP110	-0,247		1,89	3,42E-02	ATF6,BCL3,CD74,CLU,IL1R1,MX1,OAS1
MAF	0,67			3,66E-02	CXCR5,FDFT1,IDI1
TBX21	-0,811			3,66E-02	CXCR3,ICOS,IL2RB
NFKBIA	0,355			3,92E-02	FASLG,ICAM1,MMP9,VCAM1
IRF2	0,062			4,05E-02	ISG15,VCAM1
MYC	0,021		0,53	4,06E-02	ANXA4,ANXA6,ASNS,BCAT1,CLU,CPD,E2F2,FASLG,GM2A,HK2,HLA-E,LXN,OAS1,PSMB8,Scd2,SPARC
FOXP3	1,589			4,84E-02	FASLG,FOXP3,MMP9
BRCA1	-0,021	yes	-2,213	6,42E-03	DDIT3,IFIT2,MX1,NR3C1,TAP1
STAT3	0,223	yes	1,095	7,28E-03	BATF, EGR1, MMP9, SBNO2, SOCS3, STAT3
STAT1	-0,02	yes	-1,922	1,04E-02	EIF2AK2,FURIN,TAP1,TBX21
EGR3	0,05	yes		2,22E-02	FASLG,SOCS3
MTA1	0,032	yes		2,22E-02	MMP9,STAT3
ETS1	0,022	yes		2,62E-02	EGR1,FASLG,MMP9,RUNX3
CREB3	-0,001			1,51E-02	DDIT3,HSPA5,MMP9
EGR2	0,204		-0,447	1,51E-02	EGR1,FASLG,HSPA5,ID2,LAG3,SOCS3,TFRC
CREBZF	-0,018			2,22E-02	DDIT3,HSPA5
E2F1	na		-1,131	2,37E-02	ATAD2,CDC25B,E2F2,EIF2AK2,MCM5,PCNA,TCF3
XBP1	-0,004			2,62E-02	DDIT3,DNAJB9,HSPA5

**Supplementary Table 6.** RNAseq premorbid (lower panel) & adult (upper panel) B27 vs NTG - Upstream TF; Ingenuity - BH correction, Expr Log Ratio corresponding to log2FC(RNA B27vsNTG)

	Premor	bid rats	Adul	t rats		
gene	adjusted p value	log2 Fold Change	adjusted p value	log2 Fold Change	ensembl	description
Ash1I	7E-01	0,0	5E-01	0,2	ENSRNOG0000020386	ASH1 like histone lysine methyltransferase(Ash1I)
Ash2l	1E+00	0,0	7E-01	-0,1	ENSRNOG0000014875	ASH2 like histone lysine methyltransferase complex subunit(Ash2I)
Bmi1	7E-01	0,0	9E-01	-0,1	ENSRNOG0000016585	BMI1 proto-oncogene, polycomb ring finger(Bmi1)
Ehmt1	1E+00	0,0	9E-01	0,1	ENSRNOG0000007242	euchromatic histone lysine methyltransferase 1(Ehmt1)
Ehmt2	8E-01	0,0	9E-01	0,1	ENSRNOG0000030630	euchromatic histone lysine methyltransferase 2(Ehmt2)
Ezh2	1E+00	0,0	8E-01	0,2	ENSRNOG0000006048	enhancer of zeste 2 polycomb repressive complex 2 subunit(Ezh2)
Hdac1	5E-01	0,1	9E-01	0,0	ENSRNOG0000009568	histone deacetylase 1(Hdac1)
Hdac10	1E+00	0,0	6E-01	-0,2	ENSRNOG0000031915	histone deacetylase 10(Hdac10)
Hdac1I	na	0,0	1E+00	0,0	ENSRNOG0000013695	histone deacetylase 1-like(Hdac1I)
Hdac2	8E-01	0,0	9E-01	-0,1	ENSRNOG0000000604	histone deacetylase 2(Hdac2)
Hdac3	1E+00	0,0	9E-01	0,0	ENSRNOG0000019618	histone deacetylase 3(Hdac3)
Hdac4	3E-01	0,1	4E-01	0,4	ENSRNOG0000020372	histone deacetylase 4(Hdac4)
Hdac5	7E-01	0,0	1E+00	0,0	ENSRNOG0000020905	histone deacetylase 5(Hdac5)
Hdac6	na	na	1E-01	0,4	ENSRNOG0000048738	histone deacetylase 6(Hdac6)
Hdac6.2	na	na	na 7E.04	na	ENSRNOG000004/281	histone deacetylase 6(LOC108348065)
	5E-01	0,1	7E-01	0,2	ENSRINOG00000055597	histone deacetylase 7(Hdac7)
	1E:00	na	1E+00	0,0	ENSRINOG0000001122	histone deacetylase 8(Hdac8)
Kdm1a	0E 01	0,0	9E-01	0,1	ENSRINOG00000014445	heterochromatin protein 1, binding protein 3(Hp1bp3)
Kdm1b	9E-01	0,0	0E-01	-0,1	ENSRINOG00000022372	lysine demethylase TA(Kdm1h)
Kdm2a	1E+00	-0,1	9E-01	-0,1	ENSRINOG00000010319	lysine demethylase 1B(Kdm1b)
Kdm2b	1E+00	0,0	1E±00	0,0	ENSRNOG0000019143	lysine demethylase 2B(Kdm2b)
Kdm3a	7E-01	0,0	9E-01	0,0	ENSRNOG0000023702	lysine demethylase 30(Kdm3a)
Kdm3b	9E-01	0,0	9E-01	0,0	ENSRNOG0000050200	lysine demethylase 3B(Kdm3b)
Kdm4a	7E-01	0.0	8E-01	0.3	ENSRNOG0000019956	lysine demethylase 4A(Kdm4a)
Kdm4b	1E+00	0.0	9E-01	0,0	ENSRNOG0000049221	lysine demethylase 4B(Kdm4b)
Kdm4c	4E-01	-0,1	9E-01	-0,1	ENSRNOG0000006644	lysine demethylase 4C(Kdm4c)
Kdm5a	3E-01	-0,1	1E+00	0,0	ENSRNOG0000010591	lysine demethylase 5A(Kdm5a)
Kdm5b	9E-01	0,0	9E-01	-0,1	ENSRNOG0000060544	lysine demethylase 5B(Kdm5b)
Kdm5c	na	na	7E-01	-0,2	ENSRNOG0000057706	-
Kdm6a	na	na	3E-01	-0,2	ENSRNOG0000052721	lysine (K)-specific demethylase 6A(NEWGENE_1565481)
Kdm6b	6E-01	-0,1	1E+00	0,0	ENSRNOG0000037613	lysine demethylase 6B(Kdm6b)
Kdm7a	4E-01	-0,1	5E-01	-0,3	ENSRNOG0000052445	-
Kdm8	9E-01	0,0	1E+00	0,0	ENSRNOG0000015180	lysine demethylase 8(Kdm8)
L3mbtl2	7E-01	0,0	9E-01	0,0	ENSRNOG0000024743	L3MBTL2 polycomb repressive complex 1 subunit(L3mbtl2)
L3mbtl3	6E-01	0,0	1E-01	0,2	ENSRNOG0000011720	l(3)mbt-like 3 (Drosophila)(L3mbtl3)
Obi1	1E+00	0,0	8E-01	0,1	ENSRNOG0000009690	ring finger protein 219(Rnf219)
Pcgf2	na	0,0	na	na	ENSRNOG0000012705	polycomb group ring finger 2(Pcgf2)
Phc1	9E-01	0,0	1E+00	0,0	ENSRNOG0000015191	polyhomeotic homolog 1(Phc1)
Phc2	9E-01	0,0	1E+00	0,0	ENSRNOG0000006004	polyhomeotic homolog 2(Phc2)
Phc3	1E+00	0,0	6E-01	0,1	ENSRNOG0000009491	polyhomeotic homolog 3(Phc3)
Prc1	1E-01	0,0	3E-01	0,5	ENSRNOG0000013057	protein regulator of cytokinesis 1(Prc1)
Rbbp5	3E-01	-0,1	9E-01	0,1	ENSRNOG0000021289	RB binding protein 5, histone lysine methyltransferase complex subunit(Rbbp5)
Rest	6E-02	-0,1	3E-01	0,3	ENSRNOG0000002074	RE1-silencing transcription factor(Rest)
Ring1	7E-01	0,0	9E-01	0,2	ENSRNOG0000000467	ring finger protein 1(Ring1)
Rybp	8E-01	0,0	1E+00	0,0	ENSRNOG0000005353	-
Suv39h111	na	na	1E+00	0,0	ENSRNOG0000039576	suppressor of variegation 3-9 homolog 1 (Drosophila)(Suv39h1)
Suv39h2	9E-01	0,0	9E-01	0,1	ENSKNUG00000015585	suppressor or variegation 3-9 homolog 2(Suv39h2)
Suz12	7E-01	0.0	7E-01	-0.2	ENSRNOG0000058663	-

**Supplementary Table 7.** RNAseq premorbid & adult B27 vs NTG - Histone modifiers genes; BH correction / red if log2FC>0, green if log2FC<0, yellow if adj-p value<5E-2

# ARTICLE ORIGINAL (1)

(0)	Pank	Motif	<b>D</b> -value	log P-pyaluo	% of Targete	% of Background	STD/Rg STD)	Rost Match/Details	NTG or P27 epocific
	Rdlik		4E 74	1 642E - 02	% OF Targets	% OF Background	31D(Bg 31D)	ZNEE40/ZW/JE/2002 ZNEE40 CED Child San(CSEE0244)/Jamas(0.656)	NTG OF BZ7 Specific
Ĕ	-		45.64	-1.043E+02	0.00%	0.00%	170.10p (0.00p)	NEICHA0164 0/ January 0 642)	yes
z	2	ACGICGTAAGICAGICCGTACIGAATCGCGTAACGTACGTACGTACGTACGTACGTAAGICATCG	12-01	-1.418E+02	0.92%	0.01%	165.60p (128.40p)	NPIC/MAD 161.2/Jaspai (0.613)	yes
	3		12-00	-1.401E+02	1.06%	0.02%	161.20p (109.80p)	HOACT2/MA0906.1/Jaspar(0.653)	yes
	4	ACTGAGTCCGTAACTGCGTACGTACGTAAGTCACGTCGTAAGTCCGTA	1E-54	-1.266E+02	1.04%	0.02%	145.7bp (165.7bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.638)	yes
	5	AGTCACGTACTGAGTCACGTACGTACGTAGTCCGTAAGTCAGTC	1E-54	-1.246E+02	0.87%	0.01%	117.8bp (169.0bp)	ZNF165(Zf)/WHIM12-ZNF165-ChIP-Seq(GSE65937)/Homer(0.674)	yes
	6	CGTAAGCTACGTCGTACTGACGTAACGTACGTCTGAAGTCGTCAACTG	1E-53	-1.229E+02	1.09%	0.03%	128.1bp (150.7bp)	PROP1/MA0715.1/Jaspar(0.725)	yes
	7	AGCTACTGACTGATCGACGTGTACCGTAACGTAGTCAGTC	1E-52	-1.206E+02	1.52%	0.09%	150.8bp (156.9bp)	PRDM10(Zf)/HEK293-PRDM10.eGFP-ChIP-Seq(Encode)/Homer(0.761)	yes
	8	ACTGCATGGATCACTGTCGAATCGACGTAGTCACGTACTG	1E-51	-1.196E+02	4.64%	1.23%	142.7bp (152.8bp)	PB0151.1_Myf6_2/Jaspar(0.744)	yes
	9	ATCGTCAGGTCACGTAACGTGTCATAGCTGCAACGTCATGCTGACGTA	1E-47	-1.092E+02	3.41%	0.74%	162.6bp (136.9bp)	PB0141.1_Isgf3g_2/Jaspar(0.626)	yes
	10	ACTGCTAGGTACACGTACGTACTGGTACCGTAACTGCGTACGTA	1E-46	-1.074E+02	1.23%	0.06%	157.3bp (131.1bp)	PB0145.1_Mafb_2/Jaspar(0.772)	yes
	11	AGTCACGTACTGACGTAGTCAGTCCGTAACTGAGTCAGTC	1E-46	-1.068E+02	0.56%	0.00%	145.0bp (52.6bp)	POL011.1_XCPE1/Jaspar(0.642)	yes
	12	TCAGCGTATGACAGCTACGTTAGCAGTCATCGACTGTCGACGTACATG	1E-46	-1.068E+02	0.56%	0.00%	145.5bp (0.4bp)	ETV4(ETS)/HepG2-ETV4-ChIP-Seq(ENCODE)/Homer(0.768)	yes
	13	AGTCACTGACTGACTGACTGACGTACTGACGTAGTCAGTC	1E-43	-1.013E+02	0.53%	0.00%	127.2bp (2.2bp)	NFkB-p50.p52(RHD)/Monocyte-p50-ChIP-Chip(Schreiber et al.)/Homer(0.637)	ves
	14	AGTCACGTACTGACTGACTGAGTCCGTAATCGACGTACGT	1E-41	-9.582E+01	0.51%	0.00%	188 3hp (42 8hp)	ZNE416(Zf)/HEK293-ZNE416 GEP-ChIP-Seg(GSE58341)/Homer(0 737)	VAS
	15	AGTCAGTCAGTCACGTACGTCGTAACTGCGTACGTAAGTCAGTC	1E-41	-9.582E+01	0.51%	0.00%	147 8hp (114 2hp)	PB0194 1 Zbtb12 2/Jaspar(0 736)	no
	16	ACGTACGTACTGACGTCGTACGTACGTAACTGAGTCCGTACGTA	1E-41	-9 582E+01	0.51%	0.00%	185 3bp (0.0bp)	EOXN3/MA1489 1/ Jacpar(0.680)	Ves
	47		45 30	0.0015.01	4.50%	0.00%	150.00p (0.00p)	ZDTD26/444570 4/ Januar(0.640)	900
	17		1E-39	-9.091E+01	1.52%	0.16%	152.90p (156.10p)	ZB1B26/WA1579.1/Jaspar(0.646)	yes
	18	ACTGCGTAACGTAGTCACTGCGTACGTACGATAGTCACGT	1E-39	-9.075E+01	0.60%	0.01%	170.20p (93.00p)	IRF6/MA1509.1/Jaspar(0.751)	yes
	19	AGTCACGTACTGACTGCGTAAGTCACGTAGTCACTGGTCA	1E-37	-8.722E+01	1.01%	0.05%	152.9bp (150.3bp)	VDR/MA0693.2/Jaspar(0.622)	no
	20	ACGTCGTAAGTCCGTAACGTACTGAGTCAGTCACTGCGTA	1E-37	-8.623E+01	0.58%	0.01%	155.5bp (146.5bp)	TEAD3/MA0808.1/Jaspar(0.652)	yes
	21	AGTCACTGACTGCGTAACGTCGTAATCGACTGAGTCACGT	1E-37	-8.540E+01	0.68%	0.01%	140.4bp (180.0bp)	NFY(CCAAT)/Promoter/Homer(0.625)	yes
	22	AGTCAGTCACTGCGTACTAGAGTCACGTAGTCAGTCAGTC	1E-36	-8.323E+01	2.85%	0.68%	165.3bp (150.5bp)	POL013.1_MED-1/Jaspar(0.649)	yes
	23	ACGTACTGAGTCCGTAACTGACTGCGTACGTAACTGCGTAACGTAGTC	1E-35	-8.117E+01	0.68%	0.02%	162.6bp (173.0bp)	ETV4/MA0764.2/Jaspar(0.721)	yes
	24	CGTAACTGAGTCCGTAAGTCACTGCGTAACTGGTACACGT	1E-34	-8.025E+01	1.09%	0.08%	117.7bp (139.1bp)	ZNF519(Zf)/HEK293-ZNF519.GFP-ChIP-Seq(GSE58341)/Homer(0.712)	yes
	25	AGTCACTGACTGACGTACTGACTGACGTACGTACGTACGT	1E-34	-7.963E+01	0.43%	0.00%	215.0bp (0.0bp)	RUNX(Runt)/HPC7-Runx1-ChIP-Seq(GSE22178)/Homer(0.677)	yes
	26	ACTGAGTCCGTACGTAAGTCCGTAACTGAGTCCGTAACGTAGTCAGTC	1E-33	-7.731E+01	0.53%	0.01%	57.0bp (132.4bp)	ZNF341/MA1655.1/Jaspar(0.664)	no
	27	ACGTCGTACGTACGTAACTGCGTAACTGGTACACGTACGT	1E-32	-7.522E+01	0.56%	0.01%	133.5bp (93.8bp)	PH0151.1_Pou6f1_1/Jaspar(0.677)	yes
	28	AGTCACTGAGTCAGTCACTGACGTCGTACGTAACTGACTG	1E-32	-7.434E+01	0.41%	0.00%	158.3bp (0.0bp)	PB0086.1 Tcfap2b 1/Jaspar(0.648)	ves
	29	ACTGACGTACGTCGTACTAGACGTCTGAAGTCACTGACTG	1E-32	-7 408E+01	0.75%	0.03%	144 0hp (162 7hp)	NR1D2/MA1532 1/Jaspar(0.598)	Ves
	20		45 30	7.000E+01	4.94%	4.00%	130.4bp (147.2bp)	OVOL 2/MA1545 4 (January 0, 205)	yas
	30		12-30	-7.009E+01	4.01%	1.69%	130.40p (147.30p)	OVOE2/MA 1545. 1/Jaspai (0.705)	yes
	31		1E-30	-0.911E+01	0.39%	0.00%	10.000 (U.UDD)	PD0090.1_2(p10/_1/Jaspar(0.659)	yes
	32		1E-29	-6./41E+01	0.77%	0.04%	120.70p (164.2bp)	INSM1/MA0155.1/Jaspar(0.715)	yes
	33	AGTCCGTAAGTCACTGCGTAACTGCGTACGTAAGTCACTG	1E-29	-6.724E+01	0.43%	0.01%	145.3bp (51.5bp)	PRDM1/MA0508.3/Jaspar(0.648)	yes
	34	ACGIAGICACGTACGTACGTCGTAACGTACGTACGTACTGACTGACGT	1E-29	-6.724E+01	0.43%	0.01%	119.7bp (154.0bp)	MF0008.1_MADS_class/Jaspar(0.694)	yes
	35	ACTGACTGCGTAACTGACGTACTGACGTCGTAAGTCACGT	1E-28	-6.503E+01	1.04%	0.10%	144.8bp (149.0bp)	MSANTD3/MA1523.1/Jaspar(0.688)	yes
	36	ACTGACTGACTGACGTCGTAACGTAGTCACTGAGTCACGT	1E-27	-6.265E+01	0.41%	0.01%	166.6bp (58.0bp)	PH0162.1_Six2/Jaspar(0.735)	yes
	37	CGTAACTGACGTAGTCACTGACTGCGTAACGTCGTAAGTC	1E-27	-6.237E+01	0.51%	0.01%	193.8bp (133.1bp)	PB0160.1_Rfxdc2_2/Jaspar(0.617)	no
	38	AGTCAGCTACGTACGTACTGACGTACGTCGTAACGTACGT	1E-26	-6.147E+01	1.57%	0.28%	147.5bp (153.0bp)	PB0119.1_Foxa2_2/Jaspar(0.901)	yes
	39	ACTGACGTAGTCACTGACTGACGTACTGCGTAACGT	1E-26	-6.003E+01	0.43%	0.01%	168.2bp (65.3bp)	SREBF1/MA0595.1/Jaspar(0.817)	yes
	40	AGTCCGTAACGTAGTCACTGCGTACGTAACTGACGTCGTA	1E-25	-5.811E+01	0.39%	0.01%	143.0bp (139.1bp)	TCF7/MA0769.2/Jaspar(0.652)	yes
	41	ACGTAGTCAGTCAGTCACGTAGTCACTGACGTAGTCACGT	1E-24	-5.709E+01	0.70%	0.04%	176.1bp (96.0bp)	PB0200.1_Zfp187_2/Jaspar(0.631)	yes
	42	AGTCACTGATGCAGCTCGTACGTAATCGCTGA	1E-24	-5.666E+01	4 1 1%	1.67%	146 6bp (155 6bp)	PB0112.1 E2E2 2/Jaspar(0.637)	VAS
	43	CGATACGTCAGTATGCTCGAAGTCCTGACGTA	1E-23	-5.436E+01	17 37%	11.96%	145 4bp (145 1bp)	CEBPD/MA0836 2/ Jaspar(0.799)	Ves
	44	CGTAACTGACGTCGTAATCGAGTCACTGAGCT	15.22	-5.292E+01	2.20%	0.62%	162.2bp (168.4bp)	BB0154 1 Oct 2/Jacpar(0.734)	)co
	45		45.04	-3.302E+01	2.20%	0.0276	133.30p (130.40p)	TDV54440007 4/ Januar(0.659)	110
	40		45.04	4.014E+01	2.20%	4.000/6	113.30p (81.30p)	https://www.ood/1.i/Saspar(0.000)	yes
	46	GTCACGTAAGTCACTGAGTCACGTACGTACTG	1E-21	-4.911E+01	3.29%	1.28%	154.70p (159.30p)	Nr2e3/MAU164.1/Jaspar(U.721)	yes
	47	ACGTATGCCGTAACTGACGTATGCGTCACTAG	1E-18	-4.275E+01	20.19%	15.03%	151.1bp (146.8bp)	MatA(bZIP)/Islet-MatA-ChIP-Seq(GSE30298)/Homer(0.755)	yes
	48	ACTGAGTCAGTCACGTACGTACGTACGTACGTACGTACGT	1E-17	-3.915E+01	0.24%	0.00%	95.2bp (0.0bp)	Sox3/MA0514.1/Jaspar(0.677)	yes
	49	GTCACGTACGATATCGCTGACGATCGTACGTACTGATGACACGTCTGA	1E-16	-3.693E+01	1.04%	0.21%	120.5bp (151.0bp)	HNF1A/MA0046.2/Jaspar(0.686)	yes
	50	ACTGATCGCGTAACGTACGTACTGAGTCACTG	1E-16	-3.685E+01	4.52%	2.34%	153.4bp (149.2bp)	Ddit3::Cebpa/MA0019.1/Jaspar(0.692)	yes
	51	CGTACGTAAGTCCGTAACTGAGTCACTGAGTC	1E-13	-3.137E+01	1.40%	0.43%	153.7bp (160.3bp)	ZNF341/MA1655.1/Jaspar(0.780)	yes
	52	CGTAACTGCGTAACTGACTGCGTAACGTAGTC	1E-13	-3.059E+01	5.56%	3.29%	140.0bp (143.7bp)	DPRX/MA1480.1/Jaspar(0.654)	yes
	53	CGTAAGTCCGTAACTGACGTCGTAACGTCGTA	1E-12	-2.962E+01	2.83%	1.33%	122.4bp (148.8bp)	ZBTB32/MA1580.1/Jaspar(0.758)	yes
	54	CGTACGTAACGTACTGACGTAGTCACTGACTG	1E-11	-2.578E+01	0.94%	0.25%	109.7bp (158.9bp)	YY2/MA0748.2/Jaspar(0.725)	yes
	55	ACGTAGTCCGTAACTGACGTACTGACGTACTGACGTAGTCCGTAACTG	1E-08	-2.060E+01	0.17%	0.01%	8.9bp (47.4bp)	Reverb(NR).DR2/RAW-Reverba.biotin-ChIP-Seg(GSE45914)/Homer(0.704)	ves
	Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	NTG or B27 specific
27	Rank 1	Motif AGTCCGTAACGTCGTACGTACGTAACTGAGTCACCTGGTCAACGTACGT	P-value 1,00E-21	log P-pvalue -5.001e+01	% of Targets 0.32%	% of Background 0.00%	STD(Bg STD) 186.6bp (131.7bp)	Best Match/Details CDX4(Homeobox)/Myc-ChIP-Seq(GSE48254)/Homer(0.655)	NTG or B27 specific yes
B27	Rank 1 2	Motif AGTCCGTAACGTCGTACGTACGTACGTACGTACGTCACCGGTCAACGTACGT	P-value 1,00E-21 1,00E-20	log P-pvalue -5.001e+01 -4.689e+01	% of Targets 0.32% 1.08%	% of Background 0.00% 0.16%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp)	Best Match/Details CDX4(Homeobox)/Myt-ChP-Seq(GSE48254)/Homer(0.655) PB0032.1_IRC900814_1/Jaspar(0.759)	NTG or B27 specific yes yes
B27	Rank 1 2 3	Motif AGTCCGTAACGTGGTAGGTACGTAGTGAGTCACTGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01	% of Targets 0.32% 1.08% 0.29%	% of Background 0.00% 0.16% 0.00%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp)	Best Match/Details CDX4(Homeobox)/Myc-ChP-Seq(GSE48254)/Homer(0.655) P80032_1.1(RC000E1_1.01aspart(0.759) 281181/2(2)/HcR233-ZB112_GFP-ChIP-Seq(GSE5841/Homer(0.603)	NTG or B27 specific yes yes no
B27	Rank 1 2 3 4	Motif AGTCCGTAACGTCGTACGTACGTACCTGAGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01	% of Targets 0.32% 1.08% 0.29% 8.47%	% of Background 0.00% 0.16% 0.00% 4.94%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp)	Best Match/Details CDX4(Homeobox)/Myc-ChP-Seq(GSE48254)/Homer(0.655) PB0032-1, IRC000614, /104spar(0.759) 28TB12(21)/HEK293-28TB12.GFP-ChIP-Seq(GSE58341)/Homer(0.603) SN4/2/MA0745.2/Jaspar(0.603)	NTG or B27 specific yes yes no yes
B27	Rank 1 2 3 4 5	Motif AGTCCGTAACGTCGTACGTACGTAACGTCAGTCACGTGACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp)	Best Match/Details           CDX4(Homecbou/Mybc:ChP-Seq(GSSE48254)Homer(0.665)           PB0032:1_IRC00614, 1./Jaspar(0.759)           ZBTB12(ZI)HEK239-ZBTB12(ZI)H-Seq(GSE68341)Homer(0.603)           SN40ZMA0745.2/Jaspar(0.683)           ETV1MA0712 Julyaspar(0.683)	NTG or B27 specific yes yes no yes yes
B27	Rank 1 2 3 4 5 6	Motif AGTCCGTAACGTCGTACGTACGTACGTACGTCAGTCAGTC	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.158e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 183.2bp (163.3bp)	Best Match/Details           CDX4(Homedbox)/Myc-ChIP-Seq(GSE48254)/Homer(0.655)           PB0302-1_IRC000614_1/L3aspar(0.759)           Z8TB12(27)/HE/263-28TB12-GP-OhP-Seq(GSE384)/Homer(0.603)           SN42/MA0745 2/Jaspar(0.683)           ETV1/MA0761 2/Jaspar(0.853)           ETV1/MA0761 2/Jaspar(0.851)	NTG or B27 specific yes yes no yes yes yes
B27	Rank 1 2 3 4 5 6 7	Notif AGTCCGTAACGTCGTACGTAACGTAGTCAGTCAGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.158e+01 -4.061e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 183.2bp (163.3bp) 142.1bp (144.4bb)	Best Match/Details           CDX4(Homeckou/Myv-ChP-Seq(GSE48254)-Homer(0.655)           PB0932.1.18Cc00841.1(Jaspar0.759)           ZBTB12(ZI)HEX29.2BTB12.CFP-ChIP-Seq(GSE58341)-Homer(0.603)           SN4/2/MA0745.2Jaspar(0.683)           ET1V1AM0715.Jaspar(0.852)           ET1V1AM0715.Jaspar(0.852)           VBR/M0823.2Laspar(0.759)	NTG or B27 specific yes yes no yes yes yes no
B27	Rank 1 2 3 4 5 6 7 8	Motif AGTCCGTAACGTCGTACGTACGTACGTACGTACGTCAGGTACGGTACGTAC	P-value 1,00E-21 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.158e+01 -4.061e+01 -4.054e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 183.2bp (163.3bp) 142.1bp (144.4bp) 138.2bp (140.7bn)	Best Match/Details           CDX4(Homecbox)/Myc-Ch4P-Seq(GSE48254)/Homer(0.655)           PB0321_IRC300614_1/L34spar(0.759)           ZBTB12(2)/HC282-SZB1212_GSE70-Ch4P-Seq(GSE58341)/Homer(0.603)           SN402/MA0745_2/L34spar(0.832)           ETV1/MA0761_2/L34spar(0.832)           Nanog(Homecbox)/mES-Nanog-Ch4P-Seq(GSE11724)/Homer(0.749)           VDR/MA0683_2/L34spar(0.759)           EDX1/MA4761_L144871_L069x01           EDX1/MA4761_L14471_L069x01	NTG or B27 specific yes no yes yes yes no ves
B27	Rank 1 2 3 4 5 6 7 8 0	Motif AGTCCGTAACGTACGTACGTACGTACGTACGTACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-17	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.158e+01 -4.054e+01 -3.479ea04	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 183.2bp (145.3bp) 142.1bp (144.4bp) 138.2bp (140.7bp) 141.7bp (141.7bc)	Best Match/Defails           CDX4(Homeckov)Mybc-ChP-Seq(GSE548254)/Homer(0.665)           PB0032_11,RC500814_1.01aspar0.759)           ZBTB12(ZI)/HEK289-ZBTB12.GFP-ChIP-Seq(GSE58341)/Homer(0.603)           SNM2/MA0745_2.Jsapar(0.683)           ETV1MA0715_2.Jsapar(0.682)           Nanog(Homeobou)/mES-Nanog-CAIP-Seq(GSE1724)/Homer(0.749)           VDR:MA0632.Jsapar(0.759)           FXVE:MA1487_11/aspar(0.869)           FXVE:MA1487_1143870(0.869)           SNA2-Skug27MMA0FP1 1/Temeraf0.663	NTG or B27 specific yes yes yes yes yes no yes yes
B27	Rank 1 2 3 4 5 6 7 8 9 10	Motif AGTCGTAACGTCGTACGTACGTAACGTAAGTCAACGTAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14	log P-pvalue -5.001e+01 -4.689e+01 -4.499e+01 -4.213e+01 -4.158e+01 -4.061e+01 -4.054e+01 -3.479e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 33.20%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (136.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 132.2bp (145.6bp) 142.1bp (144.4bp) 138.2bp (140.7bp) 141.7bp (141.7bp) 141.7bp (144.4bc)	Best Match/Details           CDX4(Homecbou/Myrc;ChP-Seq(GSE48254)Homer(0.655)           PB0032.1.1RC000814_1/Jaspar(0.789)           ZBTB12(ZI)HEK282-ZBTB12_GFP-ChP-Seq(GSE58341)Homer(0.603)           SNU2/MA0745_2/Jaspar(0.683)           ETV14MA0712_Jaspar(0.832)           Nanog(Homecbou)/mES-Nanog-CNIP-Seq(GSE11724)Homer(0.749)           VDR/MA0683_2/Jaspar(0.789)           FOX:MA0487_1/Jaspar(0.689)           POX:MA147_1/Jaspar(0.689)           POX:MA147_1/Jaspar(0.689)           PD0791_17764_2           PD0791_17764_2           PD0791_17764_2	NTG or B27 specific yes yes no yes yes no yes yes yes
B27	Rank 1 2 3 4 5 6 7 8 9 10 44	Notif AGTCGTAACGTCGTACGTACGTAGCTAGCTGAGCACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 4,00E-45	log P-pvalue -5.001e+01 -4.689e+01 -4.213e+01 -4.158e+01 -4.061e+01 -4.054e+01 -3.479e+01 -3.298e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 27.66%	STD(8g STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (136.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 133.2bp (143.4bp) 138.2bp (144.4bp) 138.2bp (144.7bp) 141.7bp (141.7bp) 141.9bp (145.6bp) 141.6bp (145.6bp) 142.6bp (145.6bp) 142.6bp (145.6bp) 142.6bp (145.6bp) 143.2bp (145.6bp) 144.6bp (145.6bp) 144.6bp (145.6bp) 144.6bp (145.6bp) 144.6bp (145.6bp) 144.6bp (145.6bp) 145.6bp (145.6bp) 145.6bp (145.6bp) 145.6bp (145.6bp) 146.6bp (145.6bp) 146.6bp (145.6bp) 147.6bp (145.6bp)	Best Match/Details           CDX4(Homedbox)/Myr-CHP-Seq(GSE48254)/Homer(0.655)           P60302.1, IRC500614, 1/Jaspar(0.759)           ZBTB12(Z)/HE282-SZBT12C/FO-IP-Seq(GSE3834)/Homer(0.603)           SN4I2/MA0745.2/Jaspar(0.683)           ETV1/MA0761.2/Jaspar(0.852)           Nanog(Homeobx)/mES-Nanog-CHP-Seq(GSE11724)/Homer(0.749)           VDR/MA0683.2/Laspar(0.759)           FOXE1/MA1487.1/Jaspar(0.869)           Noc-S_(vir.2.)/AM40503.1/Jaspar(0.686)           P80031.1_Z[P691_2/Jaspar(0.855)           B00051.1_Bend 1/ formed 1/2 more 1/2	NTG or B27 specific yes yes yes yes yes yes yes yes yes
B27	Rank 1 2 3 4 5 6 7 8 9 10 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Motif AGTCCGTAACGTACGTACGTAACGTAAGTCAACGTACGTA	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-14	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.213e+01 -4.213e+01 -4.054e+01 -4.054e+01 -3.270e+01 -3.270e+01 -3.270e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 33.39% 1.47%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.042%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.3bp) 142.1bp (144.4bp) 142.2bp (143.7bp) 141.7bp (141.7bp) 141.9bp (145.4bp) 141.9bp (145.4bp) 141.9bp (145.4bp) 145.9bp (145.7bp) 145.9bp (145.7bp) 145.7bp (145.7bp) 1	Best Match/Details           CDX4(Homeckow)Myby:ChP-Seq(GSS148254)Htxmer(0.655)           PB00321_13RC00614_11/Jaspar(0.759)           ZBTB12(ZI)HEX29-ZBTB12_GFP-ChP-Seq(GSE548241)Homer(0.603)           SN402/MA0745_2Jaspar(0.853)           ETV1MA071_Jaspar(0.852)           ETV1MA071_Jaspar(0.853)           Nanog(Homecbox)/mES-Nanog-C3IP-Seq(GSE11724)/Homer(0.749)           VDR/MA0693_2Jaspar(0.759)           FOX:E1MA1487_1Jaspar(0.669)           Nex-26_Vmr_2/MA0693_1Jaspar(0.669)           P02023_1_2Pepi1_2Jaspar(0.653)           P02025_1_Pepi1_Jaspar(0.733)           P02026_1_Depi1_Maspar(0.733)	NTG or B27 specific yes no yes yes yes no yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12	Notif AGTCCGTAACGTCGTACGTAACGTAGTCAGTCAGCTGGCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-13	log P-pvalue -5.001e+01 -4.6899e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.054e+01 -4.054e+01 -3.298e+01 -3.298e+01 -3.270e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 33.39% 1.47% 0.21%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.00% 0.02%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 125.8bp (36.4bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 135.2bp (144.5bp) 142.1bp (144.4bp) 141.7bp (141.7bp) 141.7bp (144.7bp) 141.5bp (144.5bp) 150.1bp (145.5bp) 150.1bp (185.8bp) 150.1bp (185.8bp) 15	Best Match/Details           CDX4(Homecbox)/Myc-CHP-Seq(CSE48254)/Homer(0.655)           PB0032.1_IRC300614_1/L3aspar(0.759)           ZBTB12(2)/HC243>ZBT12.6/SPC-OHP-Seq(CSE58341)/Homer(0.603)           SMA02/MA0745_2.1aspar(0.693)           ETV1/MA0761_2/Laspar(0.832)           Nanog(Homecbox)/mES-Nanog-CMP-Seq(CSE11724)/Homer(0.749)           VDR/MA0683_2/Laspar(0.759)           FOXE1/MA4471_L1dspar(0.669)           No2-5(var.2)/MA0503_1/Laspar(0.669)           P00025_1_PB01L14spar(0.733)           S0X10/MA0424_2L3spar(0.55)           P00025_1_PB01L14spar(0.733)           S0X10/MA04242_L3spar(0.559)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13	Notif AGTCCGTAACGTACGTACGTAACGTAGTCAGTCAGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-13	log P-pvalue -5.001e+01 -4.689e+01 -4.502e+01 -4.499e+01 -4.213e+01 -4.051e+01 -4.054e+01 -3.270e+01 -3.270e+01 -3.064e+01 -3.034e+01 -3.034e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 1.47% 0.21% 1.21%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.00% 0.32%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 133.2bp (145.6bp) 133.2bp (145.7bp) 141.7bp (141.7bp) 141.7bp (141.7bp) 141.5bp (144.5bp) 150.1bp (88.9bp) 150.1bp (88.9bp)	Best Match/Details           CDX4(Homeckou/Myv-ChP-Seq(GSE548254)H-kmer(0.655)           PB0032.1;R5C00814,1(Jaspard)C750)           ZBTB12(ZI)H-KE33-ZBTB12.GFP-ChIP-Seq(GSE548341)H-kmer(0.603)           SN402/MA0745.2/Jaspar(0.683)           ETV1(MA0715.2/Jaspar(0.683)           ETV1(MA0715.2/Jaspar(0.832)           Nanog(Homeobou/mES-Nanog-CAIP-Seq(GSE1724)H-bmer(0.749)           VDR:MA053.2/Jaspar(0.759)           FOXE (MA1487.1/Jaspar(0.686)           P00203.1_ZP691_2/Jaspar(0.635)           P00202.1_Pelg1_1_Jaspar(0.635)           P00203.1_Depl1_2_Jaspar(0.586)           NOX1CMA042.2_Jaspar(0.73)           S0X1CMA0442.2_Jaspar(0.73)           S0X1CMA042.2_Jaspar(0.736)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14	Motif AGTCCGTAACGTACGTACGTAACGTAAGTCAAGGTACGTAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-13	log P-pvalue -5.001e+01 -4.689e+01 -4.692e+01 -4.499e+01 -4.158e+01 -4.051e+01 -4.054e+01 -3.079e+01 -3.298e+01 -3.034e+01 -3.034e+01 -3.034e+01 -3.012e+01 -3.0	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 12.61% 3.97% 1.16% 3.39% 1.47% 0.21% 0.21% 0.21% 0.21%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.00% 0.32% 15.78%	STD(Bg STD) 186.6bp (131.7bp) 157.6bp (136.4bp) 125.8bp (36.4bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 133.2bp (143.7bp) 142.1bp (144.4bp) 143.3bp (144.4bp) 144.5bp (144.5bp) 144.5bp (144.5bp) 150.1bp (145.4bp) 150.4bp (142.1bp) 140.1bp (145.2bp) 140.1bp (145.2bp) 1	Best Match/Details           CDX4(Homechow)Mys: CHP-Seq(GSE48254)Homer(0.655)           PB0032.1.1RC000814_1/Jaspar(0.769)           ZBTB12(ZI)HEK283-ZBTB12 (ZFP-ChIP-Seq(GSE58341)Homer(0.603)           SN402/MA0745_2/Jaspar(0.689)           ET1V1MA0712 (Jaspar(0.682)           VDRMA0683_2/Jaspar(0.769)           VDRMA0683_2/Jaspar(0.769)           FDV1MA0745_1/Jaspar(0.689)           PO2EY_IAHAT1_JJaspar(0.689)           PD6025_1_ZPB61_2/Jaspar(0.685)           PB0035_1_ZPB61_2/Jaspar(0.713)           SOX10MA0422_2Jaspar(0.780)           RV41/MA155_1_Jaspar(0.730)           ZDF54/JIAHA555_1Jaspar(0.730)	NTG or 827 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15	Notif AGTCCGTAACGTCGTACGTACGTACGTCACGTCACGTCA	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-14 1,00E-14 1,00E-13 1,00E-13 1,00E-12 1,00E-12	log P-pvalue 5.001e+01 -4.689e+01 -4.689e+01 -4.499e+01 -4.213e+01 -4.213e+01 -4.213e+01 -4.054e+01 -3.270e+01 -3.270e+01 -3.034e+01 -3.03	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.3.9% 1.47% 0.21% 1.21% 1.21% 20.30% 20.0%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.00% 0.32% 15.78% 0.77% 0.77%	STD(8g STD) 186.6bp (131.7bp) 187.6bp (136.4bp) 157.6bp (136.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 132.2bp (143.7bp) 142.1bp (144.4bp) 143.2bp (144.7bp) 144.5bp (144.7bp) 144.5bp (144.7bp) 150.1bp (48.3bp) 150.4bp (145.2bp) 140.2bp (145.2bp) 1	Best Match/Details           CDX4(Homeckou/Myv-Ch4P-Seq(GSE48254)Homer(0.655)           PB0932.1.18Cc0041.1/laspar(0.759)           28TB12(2I)HEX283-28TB12.GFP-Ch1P-Seq(GSE58341)Homer(0.603)           SN402/MA0745.2/laspar(0.853)           ET1V1MA0715.2/laspar(0.853)           ET1V1MA0715.2/laspar(0.853)           Nanog(Homeoboy/InES-Nanog-Ch4P-Seq(GSE11724)Homer(0.749)           VDR1AM0823.2/laspar(0.759)           FOVEX.IMA1497.1(laspar(0.868))           FOVEX.IMA1497.1(laspar(0.686))           PB0020.1.2/p691_2.2/laspar(0.635)           PB0020.2.1.2/p691_2.2/laspar(0.635)           PB0020.2.1.2/p691_2.2/laspar(0.733)           SOX10MA042.2.2/laspar(0.736)           P26-34.4/IMA1112.2/laspar(0.736)           P2634.1/IA55.1/laspar(0.736)           PB0050.1.0er1_1.1/laspar(0.776)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16	Motif AGTCCGTAACGTACGTACGTAACGTAAGTCAACGTACGTA	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-12 1,00E-11	log P-pvalue 5.001e-01 4.689e+01 4.689e+01 4.499e+01 4.499e+01 4.213e+01 4.056e+01 3.3479e+01 3.3064e+01 3.3064e+01 3.304e+01 2.2860e+01 -2.262e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 12.61% 3.39% 1.16% 3.39% 1.47% 0.21% 1.21% 0.21% 1.21% 0.20%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.00% 0.32% 15.78% 0.01%	STD(8g STD)           186.6bp (131.7bp)           157.6bp (136.4bp)           152.8bp (36.4bp)           152.8bp (36.4bp)           152.8bp (145.8bp)           153.2bp (145.8bp)           153.2bp (145.8bp)           153.2bp (145.8bp)           142.8bp (144.5bp)           141.3bp (144.7bp)           141.9bp (144.5bp)           150.1bp (88.6bp)           150.4bp (142.1bp)           162.2bp (142.5bp)           162.2bp (143.5bp)	Best Match/Details           CDX4(Homecbox)/Mys-ChP-Seq(GSE48254)Hsmer(0.655)           PB0032_1_IRC00614_1/Jaspar(0.759)           ZBTB12(ZI)HEK232-ZBTB12_GFP-ChP-Seq(GSE58341)Homer(0.603)           SN402/MA0745_2/Jaspar(0.853)           ETV14MA071_2/aspar(0.852)           Narog(Homecbox)/mES-Narog-ChIP-Seq(GSE11724)Homer(0.749)           VDRAM0613_JJaspar(0.832)           Narog(Homecbox)/mES-Narog-ChIP-Seq(GSE11724)Homer(0.749)           VDRAM0613_JJaspar(0.689)           P00205_1_PE091_2Jaspar(0.669)           P00205_1_PE091_2Jaspar(0.655)           P00205_1_PE091_Jaspar(0.659)           NR44/MA112_JJaspar(0.739)           SOX10MA042_JJaspar(0.739)           ZNF341/MA1655_JJaspar(0.739)           ZNF341/MA1655_JJaspar(0.739)           ZNF341/MA1655_JJaspar(0.739)           P00505_1_OT_1_Jaspar(0.772)           NFk8-p65(RHD)/GM12787-p65-CNF-Seq(GSE19455)/Homer(0.663)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17	Notif AGTCGTAACGTGTACGTAGGTAACGTAGTCAGTGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-12 1,00E-11 1,00E-11 1,00E-11	log P-pvalue -5.001e+01 -4.839e+01 -4.502e+01 -4.502e+01 -4.512e+01 -4.158e+01 -4.061e+01 -3.270e+01 -3.270e+01 -3.238e+01 -3.034e+01 -3.034e+01 -3.034e+01 -3.034e+01 -2.860e+01 -2.762e+01 -2.7	% of Targets 0.32% 1.08% 0.29% 8.47% 0.39% 12.57% 0.39% 2.2.61% 3.3.97% 1.16% 3.3.9% 1.47% 0.21% 2.00% 0.21% 1.21% 2.00% 0.29%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 18.00% 0.26% 27.66% 0.42% 0.02% 15.78% 0.01% 15.76%	STD(Bg STD) 186.6bp (131.7bp) 187.6bp (136.4bp) 182.8bp (136.4bp) 142.6bp (145.6bp) 132.2bp (145.6bp) 133.2bp (143.0bp) 133.2bp (140.7bp) 141.7bp (144.7bp) 141.7bp (144.7bp) 141.7bp (144.7bp) 150.1bp (183.0bp) 150.4bp (142.5bp) 140.2bp (142.5bp) 144.6bp (145.2bp) 144.6bp (145.2bp)	Best Match/Details           CDX4(Homeotox)Mys-ChP-Seq(GSE48254)Homer(0.665)           PB0032.1,1R5:00014.1./14aspar(0.759)           28TB12(ZI)HEK299-ZBTB12.GFP-ChIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2.itspar(0.683)           ETV1MA0715.2.itspar(0.682)           Nanog(Homeotox)MFS-Nanog-CAIP-Seq(GSE1724)Homer(0.749)           VDR:MA0633.2.itspar(0.759)           VDR:MA0633.2.itspar(0.683)           P60203.1, ZFp601.2.itspar(0.685)           P80203.1, ZFp601.2.itspar(0.768)           OX10MA042.2.itspar(0.685)           P80203.1, ZFp601.2.itspar(0.738)           P80305.1, Open1.1.itspar(0.738)           P80305.1, ZFP3.5.itspar(0.738)           P80305.1, ZFP3.5.itspar(0.765)           P80305.1, ZFP3.5.itspar(0.765)	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro ro ro ro ro ro
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18	Notif AGTCCGTAACGTACGTACGTAACGTAGTCAGTCAGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-17 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-11 1,00	log P-pvalue -5.001e-01 -4.502e+01 -4.502e+01 -4.498e+01 -4.498e+01 -4.054e+01 -3.054e+01 -3.278e+01 -3.278e+01 -3.034e+01 -3.034e+01 -3.034e+01 -2.808e+01 -2.868e+01 -2.858e+01 -2.858e+01	% of Targets 0.32% 1.08% 0.29% 12.57% 0.39% 12.57% 3.09% 1.16% 33.39% 1.47% 0.21% 1.47% 0.21% 1.20% 0.20% 2.00% 0.29% 0.39%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.42% 0.00% 0.32% 15.78% 0.01% 0.01%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.6bp)           183.2bp (145.6bp)           183.2bp (145.6bp)           183.2bp (145.7bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           150.1bp (144.7bp)           150.1bp (144.7bp)           150.1bp (145.4bp)           160.4bp (142.1bp)           140.1bp (145.2bp)           162.2bp (146.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           163.2bp (145.2bp)           164.2bp (145.2bp)           165.2bp (145.7bp)           165.2bp (145.7bp)           165.2bp (145.7bp)           165.2bp (145.7bp)           165.2bp (145.7bp)           165.2bp (145.7bp)	Best Match/Details           CDX4(Homeckou/Myv-ChP-Seq(GSE548254)Hsmer(0.655)           PB00321_1BC00614_11/Jaspar(0.579)           ZBTB12(Z))HEX23-ZBTB12_GFP-ChP-Seq(GSE54824)(Hsmer(0.603)           SN402/MA0745_2Jaspar(0.653)           ETV1MA071_2Jaspar(0.632)           ETV1MA071_2Jaspar(0.632)           Nanog(Homeckou/MES-Nanog-CHP-Seq(GSE11724)(Homer(0.749)           VDR/MA0633_2Jaspar(0.759)           FOXE:MAA1487_1Jaspar(0.669)           Nex2-6(wr:2)MA0633_1Jaspar(0.669)           Nex0-6(wr:2)Ma0633_2Jaspar(0.653)           PB0003_1_PB01_2Jaspar(0.635)           PB0003_1_PB01_2Jaspar(0.739)           SOX100MA0422_JJaspar(0.739)           PB0051_DC1_TASpar(0.758)           PB05051_DC1_TASpar(0.758)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TASpar(0.761)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)           PB05051_DC1_TJaspar(0.763)	NTG or B27 specific yes yes no yes yes yes yes yes yes yes yes yes no no no no no yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19	Notif AGTCGTAACGTGACGTACGTAACGTAGTCACTGGTCAACGTACGT	P-value 1,00E-21 1,00E-19 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-17 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10	log P-pvalue -5.001e+01 -4.858e+01 -4.499e+01 -4.499e+01 -4.499e+01 -4.051e+01 -4.051e+01 -4.056e+01 -3.298e+01 -3.298e+01 -3.054e+01 -2.850e+01 -2.551e+01 -2.551e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.30% 1.16% 3.33% 0.21% 0.21%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 18.0% 0.26% 27.66% 0.42% 0.02% 0.42% 0.00% 15.78% 0.01%	STD(8g STD) 186.6bp (131.7bp) 175.6bp (136.4bp) 128.8bp (36.4bp) 135.2bp (146.8bp) 135.2bp (146.8bp) 135.2bp (146.8bp) 142.1bp (144.7bp) 141.7bp (141.7bp) 141.7bp (141.7bp) 141.5bp (144.5bp) 150.4bp (142.7bp) 150.4bp (142.7bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 150.2bp (142.7bp) 150.2bp (145.7bp) 150.2bp (175.0bp) 150.2bp (150.7bp) 150.2bp (150.7bp) 1	Best Match/Details           CDX4(Homechou/Myrc:CHP-Seq(GSE48254)Homer(0.655)           PB0032.1_IRC0006H1_1/Jappar(0.765)           ZBTB1(2/2)HEX282-ZBTB12 (ZFP-ChP-Seq(GSE58341)Homer(0.603)           SMU2/MA0745_2/Jappar(0.639)           ETV1MA0761_2/Jappar(0.632)           Nanog(Homechou/ImES-Nanog-ChIP-Seq(GSE1724)Homer(0.749)           VDR/MA0683_2/Jappar(0.759)           FCV1MA0761_2/Jappar(0.630)           PB0032_1_ZPp01_2/Jappar(0.635)           PB0032_1_ZPp01_2/Jappar(0.635)           PB0032_1_ZPp01_2/Jappar(0.733)           SOX10MA0422_2Jappar(0.739)           ZNF341/MA1655_1/Jappar(0.736)           PB0035_1_Phot_1_Z/Jappar(0.736)           ZNF341/MA1655_1/Jappar(0.766)           PB0035_1_Det_1_1/Jappar(0.766)           PB0035_1_Det_1_1/Jappar(0.767)           PB0035_1_Det_1_1/Jappar(0.766)           PB0035_1_Det_1_1/Jappar(0.767)           PB0035_1_Det_1_1/Jappar(0.767)           PB0035_1_Det_1_M1_M1655_1/Jappar(0.766)           PB0035_1_Det_1_M1_M1655_1/Jappar(0.772)           PB035_1_JAppar(0.774)           PB035_1_PD0_M174_FOXAL-PLP-Seq(GSE20831)Homer(0.663)           PB035_1_M16_1_Jappar(0.776)           PB035_1_M16_1_Jappar(0.776)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20	Notif AGTCCGTAACGTACGTACGTAACGTAAGTCAACGTACGTA	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-12 1,00E-12 1,00E-12 1,00E-13 1,00E-13 1,00E-13 1,00E-14 1,00E-14 1,00E-15 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-1	log P-pvalue -5.001e+01 -4.658e+01 -4.502e+01 -4.502e+01 -4.502e+01 -4.213e+01 -4.054e+01 -3.054e+01 -3.054e+01 -3.054e+01 -3.054e+01 -2.816e+01 -2.816e+01 -2.857e+01 -2.475e+01 -2.475e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.39% 1.16% 0.39% 0.21% 1.21% 0.21% 1.21% 0.21% 1.33% 0.29% 0.29% 0.29% 0.29% 0.21%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.00% 0.32% 0.07% 0.01% 15.76% 0.03% 0.00% 0.00% 0.00%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.6bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           182.8bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           150.1bp (145.8bp)           150.1bp (145.8bp)           150.1bp (145.8bp)           160.4bp (145.2bp)           162.2bp (145.8bp)           162.2bp (145.2bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           153.2bp (146.2bp)           154.2bp (168.0bp)           152.2bp (175.0bp)           128.2bp (14.6bp)           133.4bp (146.4cp)	Best Match/Details           CDX4(Homeckou/Myv-ChP-Seq(GSE348254)Homer(0.655)           PB00321.18C00814.1(Jaspar(0.759)           ZBTB12(Z))HEX293-ZBTB12 (ZFP-ChIP-Seq(GSE548341)Homer(0.603)           SN402/MA0745.2/Jaspar(0.683)           ET1V1MA0715.2/Jaspar(0.683)           ET1V1MA0715.2/Jaspar(0.683)           PR00261.18C00812.2/Jaspar(0.769)           VDR1MA053.2/Jaspar(0.769)           FOXE (MA1487.1/Jaspar(0.669)           Nexc45(vm:2)MA0503.1/Jaspar(0.685)           PB0032.1_PB01_2.2/Jaspar(0.635)           PB0032.1_PB01_2.2/Jaspar(0.635)           PB0032.1_PB01_2.2/Jaspar(0.738)           SOX10(MA0422.2/Jaspar(0.738)           PB0052.1_DP1_1/Jaspar(0.758)           PB0052.1_DP1_1/Jaspar(0.768)           PB0052.1_DP1_1/Jaspar(0.758)           PB0052.1_DP1_1/Jaspar(0.758)           PB0052.1_DP1_1/Jaspar(0.758)           PB0052.1_DP1_1/Jaspar(0.758)           PB0052.1_DP1_1/Jaspar(0.758)           PB1050.1_RAC2_2.2/Jaspar(0.757)           PKB26.1[PD1/CM12787-P65-CHP-Seq(GSE19485)/homer(0.663)           PB0056.1_Jef1_Jaspar(0.703)           FOXA1(Forh/esd)(M2787-P65-CHP-Seq(GSE20453)/homer(0.760)           PB0056.1_Det2_2.2/Jaspar(0.767)           PKB26.2[RAD42_2.2/Jaspar(0.763)           FOXA1(Forh/esd)(M285.1/Jaspar(0.703)	NTG or B27 specific yes yes no yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           20           21	Metil AGTCGTAACGTAGTAGCTAACTGAGTCACTGAGCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-18 1,00E-17 1,00E-15 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-14 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-10 1,00E-10	log P-pvalue -5.001e+01 -4.858e+01 -4.4592e+01 -4.4592e+01 -4.459e+01 -4.051e+01 -4.051e+01 -3.278e+01 -3.278e+01 -3.056e+01 -3.056e+01 -2.858e+01 -2.557e+01 -2.557e+01 -2.425e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.39% 1.16% 0.21% 0.21% 0.21% 0.21% 0.29% 0.29% 0.39% 0.39% 0.39% 0.32%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 0.26% 27.66% 0.42% 0.42% 0.00% 0.32% 15.78% 0.77% 0.01% 15.78% 0.01% 15.78% 0.01% 0.00% 0	STD(8g STD) 186.6bp (131.7bp) 175.6bp (136.4bp) 125.8bp (36.4bp) 125.8bp (36.4bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 135.2bp (143.7bp) 141.7bp (141.7bp) 141.3bp (145.7bp) 141.5bp (144.5bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 144.5bp (145.2bp) 144.5bp (145.2bp) 144.5bp (145.2bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 125.2bp (145.3bp) 126.2bp (145.3bp) 12	Best Match/Details           CDX4(Homecbou/Myrc,ChP-Seq(GSE48254)Htxmer(0.655)           PB0032.1 JRC000814, 1/Jaspar(0.789)           ZSTB12(ZI)HEK282-ZBTB12 (ZI-P-ChP-Seq(GSE58341)Htxmer(0.603)           SN402/MA0745.2/Jaspar(0.803)           ETV14MA0712 /Jaspar(0.832)           Nanog(Homecbou/JmES-Nanog-CNIP-Seq(GSE11724)Htxmer(0.749)           VDR/MA0683.2/Jaspar(0.789)           FDV14MA0745.2/Jaspar(0.869)           PAC25447.1/Jaspar(0.669)           Nex2-6/vm2.2/MA0603.1/Jaspar(0.689)           PB0052.1, ZPie01 / 2/Jaspar(0.685)           PB0052.1, ZPie01 / 2/Jaspar(0.739)           SOX10MA0422 /Jaspar(0.739)           ZNF34/IMA152.1/Jaspar(0.739)           PB0055.1, ZPie01 / 1/Jaspar(0.739)           PB0055.1, Pie1, 1/Jaspar(0.779)           NFk8-p65(RHD)(GM1272-rp6-CHP-Seq(GSE1486))Htxmer(0.663)           PB0150.1, Rhxdc, 2/Jaspar(0.770)           PF00450.1, Tel, 1/Jaspar(0.703)           POXAI/MA171.1/Jaspar(0.703)           FOXAI(Forkhead/MCF7-FOXA1-CHP-Seq(GSE28831)Htxmer(0.780)           H4ME0X1/MA271.1/Jaspar(0.661)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22	Notif AGTCCGTAACGTACGTACGTACGTAACGTAGCTCAGCACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-17 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-10 1,00E-10	Iog P-pvalue -5.001e+01 -4.638e+01 -4.502e+01 -4.502e+01 -4.4502e+01 -4.213e+01 -4.158e+01 -3.479e+01 -3.048e+01 -3.034e+01 -3.034e+01 -3.034e+01 -3.034e+01 -2.875e+01 -2.875e+01 -2.475e+01 -2.475e+01 -2.425e+01 -2.456e+01 -2.456e+01 -2.456e+01 -2.456e+01 -2.456e+01 -2.	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.37% 0.21% 1.16% 1.47% 0.21% 0.21% 0.29%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.00% 0.32% 15.76% 0.03% 0.03% 0.00% 3.94% 0.02% 3.98%	STD(8g STD) 186.6bp (131.7bp) 187.6bp (136.4bp) 182.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 138.2bp (144.7bp) 142.1bp (144.7bp) 141.9bp (145.4bp) 140.2bp (145.4bp) 150.1bp (38.9bp) 150.4bp (142.5bp) 162.2bp (180.7bp) 162.2bp (180.7bp) 152.2bp (175.0bp) 152.2bp (146.4bp) 153.4bp (146.4bp) 153.4bp (146.4bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 154.6bp (147.2bp) 155.2bp (145.6bp) 155.2bp (145.6bp) 15	Best Match/Details           CDX4(Homedoox)Myo-ChP-Seq(GSE548254)Homer(0.655)           PB0032.11/8C00814.1(Jaspar(0.759)           ZBTB12(ZI)HEX23-ZBTB12.CFP-ChIP-Seq(GSE548341)Homer(0.603)           SNAU2/MA0745.2/Jaspar(0.683)           ET1V1MA0715.2/Jaspar(0.683)           ET1V1MA0715.2/Jaspar(0.683)           PR0025.11/8C00912.2/aspar(0.759)           VDR1MA053.2/Jaspar(0.686)           P00025.1_PE012.2/aspar(0.686)           P00025.1_PE012.2/aspar(0.655)           P00025.1_PE012.2/aspar(0.655)           P00025.1_PE012.2/aspar(0.655)           P00025.1_PE012.2/aspar(0.730)           2XF3411A11655.1/Jaspar(0.730)           P00055.1_DE01.2/aspar(0.730)           2XF3411A11655.1/adap(0.772)           PKB456[CD1041122787-65C-PLP-Seq(CSE19485)Homer(0.663)           P00050.1_De11.1/aspar(0.767)           P01050.1_Acdt.2_Jaspar(0.757)           PXB456[CD104112787-65C-PLP-Seq(CSE19485)Homer(0.663)           P01050.1_Acdt.2_Jaspar(0.757)           PXB456[CD10412787-65C-PLP-Seq(CSE19485)Homer(0.760)           P01050.1_Acdt.2_Jaspar(0.757)           FXB456[CD10412787-65C-PLP-Seq(CSE19485]Homer(0.760)           P01050.1_Acdt.2_Jaspar(0.757)           FXB456[CPC104122.243870-FC03)           FXB456[CPC1041242.243870-FC03)           P01050.1_Acdt.2_Jaspar(0.750) <tr< td=""><td>NTG or B27 specific yes yes no yes yes yes yes yes yes yes yes yes yes</td></tr<>	NTG or B27 specific yes yes no yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23	Motif AGTCCGTAACGTACGTACGTACGTAACGTAGGTCAACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-15 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-13 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-00 1,00	log P-pvalue -5.001e-01 -4.889e+01 -4.802e+01 -4.4502e+01 -4.4213e+01 -4.158e+01 -4.213e+01 -4.213e+01 -3.270e+01 -3.270e+01 -3.2762e+01 -2.265e+01 -2.557e+01 -2.475e+01 -2.475e+01 -2.245e+01 -2.255e+01 -	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.39% 1.16% 3.33% 0.21% 1.21% 0.21% 0.29% 0.29% 0.29% 0.29% 0.29% 0.39% 0.21% 6.21% 0.39% 0.32% 0.32% 0.32% 0.32% 0.32% 0.32% 0.32% 0.32% 0.29% 0.29% 0.29% 0.20% 0.32% 0.20% 0.20% 0.32% 0.20% 0.20% 0.32% 0.20% 0.20% 0.30% 0.20% 0.20% 0.32% 0.20% 0.20% 0.30% 0.2	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 27.66% 0.42% 0.42% 0.00% 0.32% 15.78% 0.77% 0.01% 15.76% 0.01% 0.03% 0.00% 3.94% 0.00% 3.94% 1.70%	STD(8g STD) 186.6bp (131.7bp) 175.76bp (136.4bp) 125.8bp (36.4bp) 125.8bp (36.4bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 135.2bp (143.7bp) 141.2bp (144.7bp) 141.3bp (144.5bp) 141.3bp (144.5bp) 141.5bp (144.5bp) 142.5bp (144.5bp) 142.2bp (145.7bp) 142.2bp (145.7bp) 133.4bp (145.7bp) 133.4bp (145.7bp) 133.4bp (146.3bp) 144.2bp (145.7bp) 133.4bp (146.3bp) 144.2bp (143.5bp) 144.2bp (143.5bp) 1	Best Match/Details           CDX4(Homecbox)Myp-ChP-Seq(GSE48254)Hsmer(0.655)           PB0322_1_IRC00614_1/Jaspar(0.759)           ZBTB12(ZI)HEC23-ZBTB12_GFP-ChP-Seq(GSE58341)Homer(0.603)           SN402/MAP45_2_Jaspar(0.853)           ETV14MA071_Jaspar(0.852)           Narog(Homecbox)/mE5_Nanog-ChIP-Seq(GSE11724)Homer(0.749)           VDR7MA0613_JJaspar(0.832)           Narog(Homecbox)/mE5_Nanog-ChIP-Seq(GSE11724)Homer(0.749)           VDR7MA0613_JJaspar(0.669)           P00205_1_PB01_Jaspar(0.669)           P00205_1_PB01_Jaspar(0.669)           P00205_1_PB01_Jaspar(0.655)           P00205_1_PB01_Jaspar(0.739)           SOX10MA0422_Jaspar(0.739)           P00505_1_OT_1_Jaspar(0.772)           Nrk4_MMA112_JJaspar(0.773)           P00505_1_OT_1_Jaspar(0.776)           P00505_1_OT_1_Jaspar(0.776)           P00505_1_OT_1_Jaspar(0.776)           P00505_1_OT_1_Jaspar(0.776)           P00505_1_OT_1_Jaspar(0.770)           P00505_1_R-1_G_1_Jaspar(0.770)           P00505_1_OT_1_Jaspar(0.770)           P00505_1_OT_1_Jaspar(0.766)           P00505_1_OT_1_Jaspar(0.703)           P00405_1/MA0405_J/Jaspar(0.803)           P00405_1/MA0405_J/Jaspar(0.803)           P00405_1/Jaspar(0.801)           P00405_1/MA111_1_Jaspar(0.801)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24	Notif AGTCGTAACGTGACGTAGCTAACGTAACGTAACGTAACG	P-value 1,00E-21 1,00E-20 1,00E-19 1,00E-19 1,00E-19 1,00E-18 1,00E-17 1,00E-17 1,00E-17 1,00E-17 1,00E-14 1,00E-13 1,00E-13 1,00E-13 1,00E-13 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-09 1,00E-0 1	log P-pvalue           5:001e+01           4:502e+01           4:502e+01           4:502e+01           4:213e+01           4:213e+01           4:326e+01           4:051e+01           3:208e+01           4:326e+01           3:208e+01           3:208e+01           3:208e+01           2:268e+01           2:268e+01           2:2516e+01           2:2516e+01           2:2516e+01           2:2516e+01           2:258e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.39% 1.16% 3.39% 1.47% 0.21% 1.41% 20.30% 2.00% 0.21% 1.93% 0.39% 0.39% 0.21% 1.03% 1.83% 1.02% 1.43%1.43% 1.43% 1.43% 1.43% 1.43% 1.43% 1.43%1.43% 1.43% 1.43% 1.43% 1.43%1.43% 1.43% 1.43% 1.43% 1.43% 1.43%1.43% 1.43% 1.43% 1.43%1.43% 1.43% 1.43%1.43% 1.43% 1.43%1.43%1.43%1.43% 1.43%1.43%1.43%1.43%1.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.42% 0.32% 15.78% 0.03% 0.07% 3.94% 0.02% 3.94% 1.70% 9.85%	STD(8g STD) 186.8bp (131.7bp) 187.8bp (136.4bp) 128.8bp (36.4bp) 142.8bp (36.4bp) 142.8bp (145.8bp) 135.2bp (145.8bp) 133.2bp (144.8bp) 133.2bp (144.4bp) 133.2bp (144.7bp) 141.9bp (145.4bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 150.4bp (145.2bp) 152.8bp (150.0bp) 152.8bp (150.0bp) 153.3bp (146.8bp) 133.4bp (146.4bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 134.4bp (147.2bp) 134.4bp (147.2bp) 134.4bp (147.2bp) 134.4bp (147.2bp) 134.4bp (147.2bp) 134.4bp (147.2bp) 134.4bp (146.7bp) 134.4bp (146.7bp) 134.4bp (146.7bp) 134.4bp (146.7bp) 134.4bp (146.7bp) 134.4bp (146.7bp) 134.2bp (146.8bp) 134.2bp (146.8bp) 135.2bp (146.8bp) 135.2bp (146.8bp) 136.2bp (146.8bp) 137.2bp (146.8bp) 13	Best Match/Details           CDX4(Homeobox)Mybc-Ch4P-Seq(GSE848254)Homer(0.655)           PB0932.1.18Cc00841.1(Jaspar(0.759)           28TB12(21)HEX29.28TB12.CFP-Ch1P-Seq(GSE58341)Homer(0.603)           SN402/MA0745.2Jaspar(0.683)           ET1V1AM0715.Jaspar(0.683)           ET1V1AM0715.Jaspar(0.683)           Nanog(Homeobox)/mES-Nanog-Ch4P-Seq(GSE11724)Home(0.749)           VDR:MA0683.Jaspar(0.763)           P60025.1.72691.Jaspar(0.680)           Ne02.5(nz.2)/MA0603.Jaspar(0.763)           P60025.1.72691.Jaspar(0.685)           NR02.5(nz.2)/MA0603.Jaspar(0.733)           SOX10/MA042.Jaspar(0.685)           P80025.1.72691.Jaspar(0.738)           P80025.1.72691.Jaspar(0.738)           P80025.1.72691.Jaspar(0.738)           P80025.1.041.112.2Jaspar(0.738)           P80025.1.041.112.2Jaspar(0.738)           P80025.1.041.112.2Jaspar(0.738)           P80055.1.041.112.2Jaspar(0.738)           P80055.1.041.112.2Jaspar(0.738)           P80055.1.041.2.2Jaspar(0.738)           P80055.1.041.2.2Jaspar(0.738)           P80055.1.041.2.2Jaspar(0.738)           P80150.1.7M44.2.2Jaspar(0.756)           P80150.1.7M44.2.2Jaspar(0.757)           P0150.1.7M44.2.2.3Jaspar(0.758)           P0150.1.7M44.2.2.3Jaspar(0.758)           P02051.1.7M54.2.2.3Jaspar(0.758)	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro ro ro ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25	Notif AGTCCGTAACGTACGTACGTAACGTAAGTCAAGTCACGTCAGCACGTACGT	P-value 1,00E-21 1,00E-20 1,00E-10 1,00E-10 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00	log P-pvalue -5.001e+01 -4.889e+01 -4.899e+01 -4.902e+01 -4.902e+01 -4.928e+01 -4.128e+01 -4.128e+01 -3.298e+01 -3.298e+01 -3.298e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.475e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.587e+01 -2.475e+01 -2.4	% of Targets 0.32% 1.08% 0.29% 0.29% 0.29% 1.47% 0.39% 2.261% 0.39% 1.47% 0.21% 0.29% 1.21% 0.29% 0.29% 0.29% 0.29% 0.29% 0.30% 0.30% 00%0	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.42% 0.42% 0.00% 0.32% 15.78% 0.01% 0.01% 0.03% 0.01% 0.03% 0.03% 0.00%	STD(8g STD)           186.8bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           141.3bp (144.7bp)           141.9bp (144.7bp)           144.2bp (144.7bp)           150.1bp (145.2bp)           150.1bp (145.2bp)           162.2bp (145.8bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           163.2bp (145.2bp)           164.2bp (145.2bp)           162.2bp (145.8bp)           124.2bp (145.2bp)           124.2bp (146.2bp)           124.2bp (146.2bp)           124.2bp (146.2bp)           124.2bp (146.2bp)           126.2bp (160.0bp)           127.0bp (146.2bp)           128.0bp (0.0bp)	Best Match/Details           CDX4(Homeckou/Myv-ChP-Seq(GSE48254)Homer(0.655)           PB0032.1.JRC00814, 1/Jaspar(0.759)           ZBTB12(Z))HEX29-ZBTB12 (ZFP-ChP-Seq(GSE548241)Homer(0.603)           SN402/MA0745.2.Jaspar(0.853)           ETV1MA071 J/Jaspar(0.832)           ETV1MA071 J/Jaspar(0.832)           Nanog(Homeobou/mES-Nanog-CHP-Seq(GSE11724)Homer(0.749)           VDR/MA0803 J/Jaspar(0.759)           FOX:FLMA1487.1/Jaspar(0.669)           Nex2-S(wr.2)MA0503 J/Jaspar(0.689)           P00021.1_Ppd1_JJaspar(0.669)           Nex0-S(wr.2)MA0503 J/Jaspar(0.739)           P00021.1_Ppd1_JJaspar(0.739)           SOX10MA0442 J/Jaspar(0.739)           P00021.1_Ppd1_JJaspar(0.739)           P00021.1_Ppd1_JJaspar(0.739)           P00021.1_Ppd1_JJaspar(0.739)           P00051.0_LOL_JASpar(0.739)           P00051.0_LOL_JASpar(0.739)           P00051.1_DLOL_JJJASpar(0.730)           P00051.1_DLOL_JJASpar(0.730)           P00051.1_LOL_JASpar(0.730)           P00051.1_DLOL_JASpar(0.730)           P00051.1_PDL0L_JASpar(0.730)           P00051.1_RDL0L_JASpar(0.730)           P00051.1_LOL_JASPASS.1_JASPAR(0.830)           P00051.1_LOL_JASPASS.1_JASPAR(0.831)Homer(0.780)           FDXA1(Forthead)MCFT-FDXA1-CHP-Seq(GSE3831)Homer(0.780)           FDXA1(Forthead)M	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26	Notif AGTCOGTAACGTGATAGGTAACGTAAGTCAGTCATGGTCAACGTACGT	P-value 1,00E-211 1,00E-201 1,00E-10 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,	log P-pvalue           -5.001++01           -4.689++01           -4.689++01           -4.213++01           -4.213++01           -4.061++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.064++01           -3.064++01           -3.064++01           -3.064++01           -3.064++01           -2.850++01           -2.850++01           -2.857++01           -2.455++01           -2.425++01           -2.425++01           -2.256++01           -2.256++01           -2.258++01           -2.258++01           -2.258++01           -2.425++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01	% of Targets 0.32% 0.29% 0.29% 8.47% 12.57% 13.97% 1.16% 33.39% 1.47% 0.39% 0.29% 1.47% 0.29% 1.21% 20.00% 0.29% 19.93% 0.29% 19.93% 0.21% 6.21% 0.32% 19.93% 0.21% 19.93% 0.21% 19.93% 19.93% 19.93% 19.93% 19.93% 19.93% 19.95%	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.00% 0.32% 15.78% 0.07% 0.02% 39.94% 1.70% 9.85% 0.00%	STD(8g STD) 186.8bp (131.7bp) 177.8bp (136.4bp) 128.9bp (36.4bp) 128.2bp (36.4bp) 128.2bp (145.8bp) 135.2bp (145.8bp) 132.2bp (144.7bp) 142.1bp (144.7bp) 143.2bp (144.7bp) 141.3bp (145.4bp) 140.1bp (145.2bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 150.4bp (145.2bp) 152.2bp (150.3bp) 152.2bp (150.3bp) 142.2bp (145.2bp) 132.4bp (145.2bp) 144.8bp (145.2bp) 144.2bp (146.2bp) 142.7bp (146.2bp) 138.0bp (0.0bp) 130.0bp (0.0bp	Best Match/Details           CDX4(Homeotox)/Myo-ChP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C0041.1/Lispaper(0.579)           28TB12(21)HEX29.28TB12.GFP-ChIP-Seq(GSE58341)Homer(0.603)           SNAU2/MA0745.2/Lispar(0.653)           ETV1MA0715.2/shaper(0.683)           ETV1MA0715.2/shaper(0.683)           Nanog(Homeotox)/mES-Nanog-ChP-Seq(GSE1724)/Homer(0.749)           VDR1A0693.2/Lispar(0.759)           FOXE I/MA1497.1/Lispar(0.683)           P60025.1.7p8(12/lispar(0.769)           Nex2-S(ver.2)/MA0503.1/Lispar(0.686)           P60025.1.7p8(12/lispar(0.733)           SOX10/MA042.2.2/lispar(0.736)           P80025.1.7p8(11/lispar(0.736)           P80055.1.0p4(1.0/138)           P80055.1.0p4(1.0/138)           P80055.1.0p4(1.0/138)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.1p4(1.0/238)           P80055.1.1p4(1.0/238)           P80055.1.0p4(1.0/238)           P80055.1.1p4(2.2)Lispar(0.765)           P80055.1.1p4(2.2)Lispar(0.6	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27	Notif AGTCGTAACGTGACGTACGTACGTAACGTAGGTCACGTCAGCACGTACGT	P-value 1.00E-21 1.00E-20 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-10 1.00E-1	log P-pvalue           -5.001e+01           -4.680e+01           -4.680e+01           -4.680e+01           -4.680e+01           -4.213e+01           -4.213e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -2.611e+01           -2.615e+01           -2.475e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.256e+01           -2.162e+01	% of Targets 0.32% 1.08% 0.29% 0.29% 1.35% 0.39% 2.261% 3.97% 1.16% 3.39% 1.47% 0.21% 0.29% 1.21% 0.39% 0.29% 0.29% 0.29% 0.29% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.29% 0.21% 0.29% 0.20	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.02% 0.02% 15.78% 0.00% 0.03% 0.03% 0.00% 0.02% 3.98% 1.70% 9.65% 0.00% 0.00% 0.00%	STD(8g STD)           186.8bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.8bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           184.2bp (144.7bp)           184.2bp (144.7bp)           184.2bp (144.7bp)           185.2bp (144.7bp)           180.2bp (144.2bp)           180.2bp (145.2bp)           180.2bp (145.2bp)           182.2bp (145.2bp)           183.2bp (146.2bp)           184.2bp (145.2bp)           182.2bp (140.7bp)           184.2bp (145.2bp)           182.2bp (140.7bp)           184.2bp (145.2bp)           182.2bp (140.7bp)           184.2bp (143.2bp)           184.2bp (143.2bp)           184.2bp (143.2bp)           184.2bp (143.2bp)           184.2bp (145.2bp)           184.2bp (145.2bp)           184.2bp (145.2bp)           184.2bp (145.2bp)           184.2bp (146.2bp)           184.2bp (146.2bp)           184.2bp (146.2bp)           184.2bp (146.2bp)           184.2bp (146.2bp)	Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE48254)Homer(0.655)           PB00321_11RC006H1_11/aspar(0.579)           ZBTB12(Z)/HEK23>-ZBTB12_GFP-ChP-Seq(GSE54341)Homer(0.603)           SN402/MA0745_21/aspar(0.633)           ETV1MA071_21/aspar(0.632)           ETV1MA071_21/aspar(0.632)           Nanog(Homeokou/MES-Nanog-CHP-Seq(GSE11724)(Homer(0.749)           VDR/MA0603_21/aspar(0.759)           FOXE:MA1487_11/aspar(0.669)           Nex-64(wr.2)MA0503_11/aspar(0.669)           Nex0-64(wr.2)MA0503_11/aspar(0.659)           P00003_1_PB01_22/aspar(0.635)           P00003_1_PB01_22/aspar(0.539)           P00003_1_PB01_1/aspar(0.736)           P00003_1_PD01_11/aspar(0.769)           P00003_1_PD1_1/aspar(0.760)           P0003_1_PD1_1/aspar(0.760)           P0003_1_PD1_1/aspar(0.760)           P0003_1_PD1_1/aspar(0.760)           P0003_1_PD1_1/aspar(0.760)           P0005_1_D1_OT1_1/aspar(0.760)           P0005_1_PD1_OT1_1/aspar(0.760)           P0005_1_PD1_OMES5_1/aspar(0.761)           P0005_1_PD1_OMES5_1/aspar(0.762)           P0005_1_PD1_OMES5_1/aspar(0.853)           P0005_1_PD1_OMES5_1/aspar(0.851)Homer(0.780)           P0005_1_PD1_OMES5_1/aspar(0.851)           P0005_1_PD1_OMES5_1/aspar(0.851)           P0005_1_PD1_OMES5_1/aspar(0.850	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro ro ro ro ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27           70	Notif AGTCGTAACGTGACGTAGCTAACGTAGTCACGTGACGTACGT	P-value 1,00E-21 1,00E-10 1,00E-10 1,00E-10 1,00E-11 1,00E-10 1,00	log P-pvalue           5.001e+01           -4.689e+01           -4.689e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.061e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.064e+01           -3.064e+01           -3.306e+01           -2.857e+01           -2.857e+01           -2.857e+01           -2.857e+01           -2.425e+01           -2.425e+01           -2.425e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.162e+01           -	% of Targets 0.32% 0.22% 0.29% 8.47% 12.57% 3.39% 1.47% 0.39% 1.47% 0.21% 0.21% 0.21% 0.21% 0.29% 1.43% 0.21% 0.21% 0.33% 1.43% 1.43% 0.21% 0.32% 45.07% 3.18% 1.45% 0.16% 0.16% 0.16% 0.16%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.26% 0.42% 0.26% 0.42% 0.07% 0.01% 0.77% 0.01% 0.03% 0.03% 0.00% 0.02% 39.98% 1.70% 0.00	STD(8g STD)           186.8bp (131.7bp)           176.7bp (136.4bp)           157.8bp (36.4bp)           128.8bp (36.4bp)           128.8bp (36.4bp)           132.8bp (145.8bp)           133.2bp (145.8bp)           133.2bp (145.8bp)           134.2bp (143.7bp)           134.2bp (144.7bp)           141.3bp (144.7bp)           141.3bp (145.2bp)           140.1bp (145.2bp)           140.1bp (145.2bp)           140.2bp (142.5bp)           140.2bp (142.5bp)           142.8bp (143.2bp)           142.8bp (144.7bp)           142.8bp (143.2bp)           142.8bp (143.2bp)           142.8bp (143.2bp)           142.8bp (143.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           138.0bp (0.0bp)           138.0bp (0.0bp)           130.4bp (20.7bp)	Best Match/Details           CDX4(Homeckov)Mys-CRIP-Seq(GSE48254)Homer(0.655)           PB0932.1.18Cc00814.1./laspar(0.759)           228TB12(ZI)HEK289-ZBTB12.GFP-CRIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2./laspar(0.853)           ETV1MA0715.2./laspar(0.852)           ETV1MA0715.2./laspar(0.852)           Nanog(Homeobo)(mES-Nanog-CMP-Seq(GSE1724)Homer(0.749)           VDR:MA0632.2./laspar(0.759)           FOXE:INA1487.1(laspar(0.856)           P0005.1.7peij1_1.42par(0.355)           P0005.1.7peij1_1.42par(0.355)           P0005.1.7peij1_1.42par(0.73)           SDX10MA0422.2.laspar(0.736)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij1_1.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)           P0005.1.7peij2_2.02psir(0.756)	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes ro ro ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           27           28           27           28           20	Notif AGTCCGTAACGTACGTACGTACGTACGTACGTACGTCACGTACGT	P-value 1,00E-20 20 1,00E-10 20 1,00E-10 20 1,00E-19 1 1,00E-18 1 1,00E-18 1 1,00E-18 1 1,00E-17 1 1,00E-17 1 1,00E-17 1 1,00E-17 1 1,00E-17 1 1,00E-17 1 1,00E-10 1,	log P-pvalue           -5.0019-01           -5.0019-01           -4.6898-01           -4.6898-01           -4.6898-01           -4.6898-01           -4.6898-01           -4.6898-01           -4.6918-01           -4.2138-01           -4.0518-01           -4.0518-01           -3.0788-01           -3.2988-01           -3.2988-01           -2.6587-01           -2.6587-01           -2.6587-01           -2.2587-01           -2.2587-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.2588-01           -2.1628-01           -2.1628-01           -2.1288-01           -2.1288-01           -2.1288-01           -2.1288-01           -2.1288-01           -2.1288-01           -2.1288-01           -2.1288-01	% of Targets 0.32% 1.09% 0.29% 0.29% 0.29% 12.57% 0.39% 22.61% 1.21% 2.39% 0.21% 0.21% 0.21% 0.21% 0.29% 0.29% 0.29% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.29% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.29% 0.21% 0.29% 0.29% 0.29% 0.21% 0.29% 0.29% 0.21% 0.29% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.29% 0.21% 0.2	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.42% 0.02% 0.32% 0.75% 0.03% 0.03% 0.03% 0.03% 0.03% 0.00% 0.02% 0.00%	STD(8g STD)           186.6bp (131.7bp)           127.6bp (136.4bp)           127.8bp (36.4bp)           128.2bp (36.4bp)           132.2bp (145.8bp)           133.2bp (145.8bp)           133.2bp (145.8bp)           133.2bp (145.8bp)           135.2bp (144.7bp)           141.7bp (141.7bp)           141.9bp (145.4bp)           150.1bp (145.8bp)           150.1bp (145.2bp)           160.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (180.8bp)           163.2bp (142.5bp)           164.2bp (145.2bp)           162.2bp (175.0bp)           124.2bp (146.2bp)           126.2bp (120.7bp)           126.2bp (120.7bp)           126.2bp (120.7bp)	Best Match/Details           CDX4(Homeckou/Myc-ChP-Seq(GSE348254)Homer(0.655)           PB00321_IEG20614_1/Lidspar(0.579)           ZBTB12(Z)/HEK239-ZBTB12_GPP-ChIP-Seq(GSE548341)Homer(0.603)           SN402/MA0745_2/Lispar(0.683)           ET1V1MA0745_2/Lispar(0.683)           ET1V1MA0715_2/Lispar(0.683)           Nanog(Homeobou/mES-Nanog-CAIP-Seq(GSE11724)Homer(0.749)           VDR:MA0603_2/Lispar(0.759)           FOXE (MA1487_1/Lispar(0.669)           Nex2-6(vm.2)MA0503_1/Lispar(0.769)           P00021_TPB01_2/Lispar(0.655)           P00022_1_PB01_2/Lispar(0.655)           P00023_1_DP01_2/Lispar(0.659)           NR4A1/MA1112_2/Lispar(0.738)           SOX10(MA04422_2/Lispar(0.768))           P00025_1_PB01_1_Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.758)           P00035_1_DP01_C2_Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00035_1_IPA0_2_2/Lispar(0.768)           P00045_1_IPA0_2_2_Lispar(0.863)           P00405_1_IPA0_2_2_Lispar(0.863)           P0140_1_IPA0_2_2_Lispar	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           9           20           21           22           23           24           25           26           27           28           29           21           22           23           24           25           26           27           28           29	Motif AGTCGTAACGTGGTACGTAGGTAACGTAGGTCACGTGGTCACGGTAGGT CAGTCAGTAGTCGCATACGTGAGTCAGTGGTCACGTAGGTAG	P-value 1,00E-210 1,00E-90 1,00E-90 1,00E-90 1,00E-91 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-10 1,00E-00 1,0	log P-pvalue           5.001e+01           -4.083e+01           -4.838e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.061e+01           -3.078e+01           -3.470e+01           -3.047e+01           -3.047e+01           -3.056e+01           -2.762e+01           -2.762e+01           -2.516e+01           -2.255e+01           -2.255e+01           -2.102e+01           -2.102e+01           -2.255e+01           -2.2102e+01           -2.102e+01           -2.255e+01           -2.102e+01	% of Targets 0.32% 0.22% 0.29% 8.47% 12.57% 0.39% 22.61% 33.39% 1.47% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.33% 0.39% 0.21% 0.22% 0.22% 0.22% 0.22% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.22% 0.2%% 0.2%% 0.2%% 0.2%% 0.2%% 0.2%% 0.2%% 0.2%% 0.2%%0.2%% 0.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.26% 0.42% 0.02% 0.02% 0.77% 0.01% 15.78% 0.01% 0.03% 0.03% 0.03% 0.03% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.01% 0.00	STD(8g STD) 186.6bp (131.7bp) 175.6bp (136.4bp) 125.8bp (36.4bp) 125.8bp (36.4bp) 135.2bp (145.6bp) 135.2bp (145.6bp) 135.2bp (143.7bp) 141.7bp (144.7bp) 141.7bp (144.7bp) 141.7bp (145.7bp) 144.5bp (144.5bp) 140.2bp (142.7bp) 140.2bp (142.7bp) 140.2bp (142.7bp) 140.2bp (142.7bp) 140.2bp (142.7bp) 140.2bp (142.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 133.4bp (146.7bp) 135.7bp (146.7bp) 135.7bp (146.7bp) 135.7bp (146.7bp) 135.7bp (146.7bp) 135.7bp (146.7bp) 135.7bp (136.7bp) 135.7bp (136.7bp) 135.7bp (130.7bp) 135.7bp (130.7bp) 13	Best Match/Details           CDX4(Homeckov)Myv-CNP-Seq(GSE48254)Homer(0.655)           PB0032.1,18C20041.1/Llaspar(0.759)           28TB12(ZI)HEK293-28TB12.GFP-CNIP-Seq(GSE58341)Homer(0.603)           SNMu2/MA0745.2/Laspar(0.633)           ETV1MA0715.2/Laspar(0.632)           Nanog(Homeobo)(mES-Nanog-CAIP-Seq(GSE1724)Homer(0.749)           VDR:MA0633.3/Laspar(0.635)           PE028.1, 127, 127, 127, 129, 120, 120, 120, 120, 120, 120, 120, 120	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27           28           29           30	Notif AGTCOGTAACGTACGTACGTAACGTAAGTCACGTCAGCACGTACGT	P-value 1.00E-20 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-10 1.00E-00 1.00	log P-pvalue           -5.001e+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.489e+01           -4.213e+01           -4.213e+01           -4.051e+01           -4.051e+01           -3.298e+01           -3.298e+01           -3.298e+01           -3.064e+01           -3.064e+01           -3.064e+01           -3.064e+01           -2.686e+01           -2.667e+01           -2.675e+01           -2.475e+01           -2.2556e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.258e+01           -2.162e+01           -2.162e+01           -2.128e+01           -2.128e+01 <td< td=""><td>% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 2.261% 1.21% 2.00% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.39% 0.21% 0.39% 0.21% 0.35% 0.16% 0.16% 0.16% 0.16% 0.18% 0.24%</td><td>% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.00% 0.32% 15.78% 0.03% 0.00% 0.03% 1.70% 0.00</td><td>STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           157.6bp (136.4bp)           128.8bp (36.4bp)           142.6bp (145.6bp)           135.2bp (145.7bp)           135.2bp (145.7bp)           138.2bp (140.7bp)           138.2bp (140.7bp)           141.7bp (145.4bp)           141.9bp (145.4bp)           150.1bp (38.0bp)           150.1bp (180.7bp)           162.2bp (145.2bp)           150.2bp (145.2bp)           150.2bp (145.2bp)           152.2bp (145.2bp)           152.2bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           153.8bp (140.0bp)           153.8bp (140.0bp)           153.8bp (140.0bp)           155.3bp (153.1bp)           155.3bp (153.1bp)           155.3bp (153.1bp)           156.7bp (132.1bp)</td><td>Best Match/Details           CDX4(Homedool/Myc-ChP-Seq(GSE548254)Homer(0.655)           PB0032 1, IfScor081, 1.(Jaspar(0.759)           ZBTB12(ZI)+EK239-ZBTB12, CFP-ChIP-Seq(GSE58341)Homer(0.603)           SNAU2/MA0745 2.Jaspar(0.633)           ET1V1MA0715 2.Jaspar(0.633)           F1V1MA0715 2.Jaspar(0.632)           PB0022 1, IFSCOR12 Jaspar(0.632)           PR0025 1, IFSCOR12 Jaspar(0.632)           PR0025 1, IPSCOR12 Jaspar(0.689)           Noc-6(wr 2)/MA0603 1.Jaspar(0.689)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.739)           ZNF341MA1655 1.Jaspar(0.739)           ZNF341MA1655 1.Jaspar(0.730)           PB0050 1, Dart 1, Jaspar(0.730)           ZNF341MA1655 1.Jaspar(0.730)           PB1050 1, MA0627 2.Jaspar(0.730)           PB1050 1, MA0627 2.Jaspar(0.750)           PB1050 1, MA165 2.Jaspar(0.650)           PB1050 1, MA0637 1.Jaspar(0.650)           FCXA1(Forkhaud)MCF7-FCXA1-CHP-Seq(GSE584341)Homer(0.780)           FCXA24(Forkhaud)MCF7-FCXA1-CHP-Seq(GSE58341)Homer(0.590)</td><td>NTG or B27 specific yes yes yes yes yes yes yes yes yes yes</td></td<>	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 2.261% 1.21% 2.00% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.39% 0.21% 0.39% 0.21% 0.35% 0.16% 0.16% 0.16% 0.16% 0.18% 0.24%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.00% 0.32% 15.78% 0.03% 0.00% 0.03% 1.70% 0.00	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           157.6bp (136.4bp)           128.8bp (36.4bp)           142.6bp (145.6bp)           135.2bp (145.7bp)           135.2bp (145.7bp)           138.2bp (140.7bp)           138.2bp (140.7bp)           141.7bp (145.4bp)           141.9bp (145.4bp)           150.1bp (38.0bp)           150.1bp (180.7bp)           162.2bp (145.2bp)           150.2bp (145.2bp)           150.2bp (145.2bp)           152.2bp (145.2bp)           152.2bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           153.8bp (140.0bp)           153.8bp (140.0bp)           153.8bp (140.0bp)           155.3bp (153.1bp)           155.3bp (153.1bp)           155.3bp (153.1bp)           156.7bp (132.1bp)	Best Match/Details           CDX4(Homedool/Myc-ChP-Seq(GSE548254)Homer(0.655)           PB0032 1, IfScor081, 1.(Jaspar(0.759)           ZBTB12(ZI)+EK239-ZBTB12, CFP-ChIP-Seq(GSE58341)Homer(0.603)           SNAU2/MA0745 2.Jaspar(0.633)           ET1V1MA0715 2.Jaspar(0.633)           F1V1MA0715 2.Jaspar(0.632)           PB0022 1, IFSCOR12 Jaspar(0.632)           PR0025 1, IFSCOR12 Jaspar(0.632)           PR0025 1, IPSCOR12 Jaspar(0.689)           Noc-6(wr 2)/MA0603 1.Jaspar(0.689)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.635)           PB0025 1, PB01 2, Jaspar(0.739)           ZNF341MA1655 1.Jaspar(0.739)           ZNF341MA1655 1.Jaspar(0.730)           PB0050 1, Dart 1, Jaspar(0.730)           ZNF341MA1655 1.Jaspar(0.730)           PB1050 1, MA0627 2.Jaspar(0.730)           PB1050 1, MA0627 2.Jaspar(0.750)           PB1050 1, MA165 2.Jaspar(0.650)           PB1050 1, MA0637 1.Jaspar(0.650)           FCXA1(Forkhaud)MCF7-FCXA1-CHP-Seq(GSE584341)Homer(0.780)           FCXA24(Forkhaud)MCF7-FCXA1-CHP-Seq(GSE58341)Homer(0.590)	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           27           28           29           30           31	Metir AGTCGTAACGTAGTAGCTAACTGAGTCACTGGTCAACGTAGCT CAGTCACTGATCGTACGTAGCTGAGCTCAGCTAGCTAGCT	P-value 1,00E-210 1,00E-90 1,00E-10 1,00E-10 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-10 1,00E-00 1,0	log P-pvalue           5.001e+01           -4.083e+01           -4.838e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -3.238e+01           -3.479e+01           -3.3479e+01           -3.064e+01           -3.064e+01           -3.064e+01           -3.064e+01           -2.367e+01           -2.857e+01           -2.857e+01           -2.255e+01           -2.255e+01           -2.2425e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.255e+01           -2.162e+01           -2.162e+01           -2.128e+01           -2.128e+01           -2.128e+01           -2.128e+01           -2.128e+01           -2.038e+01           -2.038e+01           -2.038e+01           -2.038e+01	% of Targets 0.32% 1.08% 0.29% 0.29% 0.39% 2.61% 0.39% 1.16% 3.39% 1.47% 0.21% 0.21% 0.29% 0.29% 0.29% 0.29% 0.29% 0.29% 0.39% 0.29% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.29%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.00% 0.32% 15.78% 0.01% 0.03% 0.03% 0.00%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           157.6bp (136.4bp)           125.8bp (36.4bp)           135.2bp (145.6bp)           135.2bp (145.8bp)           135.2bp (145.8bp)           135.2bp (145.8bp)           135.2bp (145.3bp)           142.8bp (144.5bp)           141.3bp (144.5bp)           150.1bp (144.2bp)           150.1bp (145.2bp)           162.2bp (145.8bp)           163.4bp (143.2bp)           163.2bp (155.3bp (134.8bp)           128.2bp (140.0bp)           128.2bp (140.0bp)           138.4bp (146.3bp)           142.8bp (140.0bp)           138.0bp (0.0bp)           138.0bp (0.0bp)           138.0bp (131.8bp)           136.7bp (132.0bp)           136.7bp (132.1bp)           135.8bp (134.8bp)           136.7bp (132.2bp)           135.8bp (134.8bp)           136.7bp (132.3bp)           135.8bp (134.8bp)	Best Match/Details           CDX4(Homeckov)Myc-CNP-Seq(GSE48254)Homer(0.655)           PB0032.1,R5:00014.1./laspar(0.759)           ZBTB12(ZI)HEK289-ZBTB12.GFP-CNP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2.ispar(0.683)           ETV1MA0715.2.ispar(0.682)           Nanog(Homeokov)MrS-Nanog-CAP-Seq(GSE1724)Homer(0.769)           VDR:MA0633.3.ispar(0.759)           P60203.1, ZFp691_2/Jaspar(0.683)           P60203.1, ZFp691_2/Jaspar(0.685)           P80203.1, ZFp691_2/Jaspar(0.685)           P80203.1, ZFp691_2/Jaspar(0.685)           P80203.1, ZFp691_2/Jaspar(0.763)           P80203.1, ZFp691_2/Jaspar(0.763)           P80203.1, ZFp691_2/Jaspar(0.763)           P80205.1, Pag1_1./Laspar(0.763)           P80205.1, Pag1_1./Laspar(0.763)           P80205.1, Pag1_1.7/Laspar(0.763)           P80505.1, Oxn1_1.M3spar(0.763)           P80505.1, Oxn1_1.	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           277           28           29           30           31           32	Notif AGTCOGTAACGTACGTAGGTAACGTAACGTAGGTCAGCGTAGCGTAACGTAGCGTAACGTAGCGTAGCGTAACGTAGGTCAGCAGGTAGCGTAACGTAGGTCAGCAGGTAGCGTACGTA	P-value 1,00E-212 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-11 1,00E-10 1,00E-00 1,0	log P-pvalue           -5.001e+01           -4.689e+01           -4.689e+01           -4.489e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -3.058e+01           -4.058e+01           -3.078e+01           -3.298e+01           -3.068e+01           -3.068e+01           -3.068e+01           -3.068e+01           -2.868e+01           -2.868e+01           -2.868e+01           -2.868e+01           -2.868e+01           -2.878e+01           -2.188e+01           -2.188e+01           -2.188e+01           -2.188e+01           -2.188e+01           -2.188e+01           -2.088e+01           -1.051e+01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 3.97% 1.16% 3.39% 1.47% 0.29% 1.16% 3.39% 0.21% 0.21% 0.20% 0.29% 1.21% 0.20% 0.29% 0.29% 0.29% 0.29% 0.21% 0.29% 0.21% 0.39% 0.21% 0.39% 0.21% 0.24% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.24%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.02% 0.32% 15.76% 0.03% 0.00% 0.03% 15.76% 0.00% 0.0	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           128.8bp (36.4bp)           142.6bp (145.6bp)           135.2bp (144.6bp)           135.2bp (144.6bp)           138.2bp (144.7bp)           142.6bp (145.4bp)           143.2bp (144.7bp)           143.2bp (144.7bp)           144.5bp (144.2bp)           140.2bp (142.5bp)           140.2bp (142.5bp)           150.4bp (142.5bp)           152.2bp (146.4bp)           133.4bp (146.4bp)           126.2bp (175.0bp)           128.2bp (146.4bp)           128.2bp (146.4bp)           128.2bp (146.2bp)           128.2bp (146.2bp)           128.2bp (146.2bp)           138.0bp (0.0bp)           210.4bp (35.6bp) (132.0bp)           135.0bp (120.7bp)           135.0bp (134.1bp)           135.0bp (134.1bp)           135.0bp (134.1bp)           135.0bp (134.1bp)           135.0bp (134.1bp)           135.0bp (134.1bp)           136.7bp (135.1bp)           136.7bp (135.1bp)           137.7bp (135.1bp)           136.7bp (135.1bp)           137.7bp (135.1bp)	Best Match/Details           CDX4(Homeobox)Myc-CNP-Seq(GSE848254)Homer(0.655)           PB0932.1.18C00814.1.(Isspar(0.759)           ZBTB12(21)HEX29.2BTB12.CFP-ChIP-Seq(GSE58341)Homer(0.603)           SNAU2MA0745.2.Jaspar(0.853)           ET1V1AA071.2.Jaspar(0.853)           FTV1MA074.2.Jaspar(0.853)           PB0025.1.18C0014.1.(Isspar(0.850)           Nanog(Homeobox)(mES-Nanog-CAIP-Seq(GSE11724)(Home(0.769)           VDR:MA0633.2.Jaspar(0.759)           FOXE.VMA1487.1.Jaspar(0.868)           PB0025.1.PE01_2.Jaspar(0.655)           PB0025.1.Pe01_2.Jaspar(0.655)           PB0025.1.Pe01_2.Jaspar(0.655)           PB0025.1.Pe01_2.Jaspar(0.73)           SOX10/MA442.2.Jaspar(0.73)           SOX10/MA442.2.Jaspar(0.73)           PB0025.1.Pe01_2.Jaspar(0.73)           PB0025.1.Pe01_2.Maspar(0.73)           ZXF341MA1655.1.Jaspar(0.73)           PB0035.1.fml, 1.Jaspar(0.73)           PB0160.1.FMA2.2.Jaspar(0.73)           PB0160.1.FMA4112.2.Jaspar(0.767)           PB0160.1.FMA2.2.Jaspar(0.767)           PB0160.1.FMA2.2.Jaspar(0.876)           TEAD2/MA171.1.Jaspar(0.860)           TEAD2/MA171.1.Jaspar(0.861)           TEAD2/MA171.1.Jaspar(0.861)           TEAD2/MA172.1.Jaspar(0.866)           TEAD2/MA172.1.Jaspar(0.862)           CATM	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           20           21           22           23           24           25           26           27           28           30           31           32           33	Metil AGTCGTAACGTACGTACGTAACGTAAGTCAACGTACGTAACGTAACGTAAGTCAGTC	P-value 1.000E-210 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-10 1.000E-000E-000E-000E-000E-000E-000E-000	log P-pvalue           -5.001e+01           -4.680e+01           -4.680e+01           -4.680e+01           -4.690e+01           -4.050e+01           -4.213e+01           -4.056e+01           -4.056e+01           -3.056e+01           -3.208e+01           -3.208e+01           -3.208e+01           -3.034e+01           -3.034e+01           -3.034e+01           -2.616e+01           -2.656e+01           -2.567e+01           -2.567e+01           -2.567e+01           -2.567e+01           -2.567e+01           -2.256e+01           -2.256e+01           -2.256e+01           -2.256e+01           -2.256e+01           -2.256e+01           -2.162e+01           -2.162e+01           -2.162e+01           -2.162e+01           -2.162e+01           -2.128e+01           -2.093e+01           -2.093e+01           -2.093e+01           -2.093e+01           -2.093e+01           -2.093e+01           -2.093e+01	% of Targets 0.32% 1.08% 0.29% 0.29% 0.29% 12.57% 0.39% 22.61% 0.39% 1.16% 3.39% 1.47% 0.21% 0.29% 1.21% 20.00% 0.29% 0.29% 0.39% 0.39% 0.39% 0.32% 0.39% 0.32% 0.39% 0.31% 0.32% 0.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.01% 0.32% 15.78% 0.01% 0.03% 0.03% 0.03% 0.00% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.01% 0.00% 0.01% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.00% 0.01% 0.00%	STD(8g STD)           186.8bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           184.2bp (144.7bp)           144.3bp (144.5bp)           150.1bp (88.3bp)           150.1bp (145.2bp)           150.4bp (142.1bp)           162.2bp (145.8bp)           162.2bp (145.8bp)           162.2bp (145.8bp)           162.2bp (145.8bp)           162.2bp (145.8bp)           162.2bp (145.8bp)           164.4bp (147.2bp)           164.2bp (143.5bp)           144.4bp (147.2bp)           142.8bp (146.8bp)           142.8bp (140.0bp)           142.8bp (140.0bp)           142.7bp (146.2bp)           153.0bp (134.1bp)           153.0bp (134.1bp)           153.7bp (132.1bp)           155.3bp (132.1bp)           155.3bp (132.1bp)           155.3bp (153.0bp)           155.3bp (151.7bp)           155.3bp (151.7bp)           155.3bp (151.7bp)           155.3bp (151.7bp)	Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE348254)Homer(0.655)           PB00321_1JRC00814_1/Jappar(0.759)           ZBTB12(ZI)HEX23-ZBTB12_GFP-ChP-Seq(GSE548241)Homer(0.603)           SN402/MA0745_2Jappar(0.683)           ETV1MA071_2Jappar(0.682)           PL00221_1JRC00817_2Jappar(0.683)           Nanog(Homeobou/mES-Nanog-CHP-Seq(GSE1172A)/Homer(0.749)           VDR/MA0683_2Jappar(0.759)           POX25_1DP01_2Jappar(0.689)           POX021_1Papp1_1/Jappar(0.689)           P00021_1Papp1_1Jappar(0.73)           SOX10MA0442_2Jappar(0.73)           SOX10MA0442_2Jappar(0.73)           P00021_1Papp1_1/Jappar(0.730)           P00021_1Papp1_1/Jappar(0.730)           P00021_1Papp1_1/Jappar(0.730)           P00031_1Papp1_2/Jappar(0.730)           P00031_1Papp1_1/Jappar(0.730)           P00031_1Papp1_1/Jappar(0.730)           P00031_1Papp1_2/Jappar(0.730)           P00031_1Papp1_2/Jappar(0.730)           P00031_1Papp1_2/Jappar(0.730)           P00031_1Papp1_2/Jappar(0.730)           P0014_11AAAAAA           P0015_11AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           31           32           33           34	Notif AGTCOGTAACGTAGCTAGCTAACGTAGCTAGCTGAGCAGCTAGCT	P-value 1,00E-212 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-10 1,00E-00 1,0	log P-pvalue           -5.001++01           -4.689++01           -4.689++01           -4.689++01           -4.213++01           -4.213++01           -4.061++01           -4.061++01           -4.061++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.002++01           -3.002++01           -2.808++01           -2.816++01           -2.816++01           -2.826++01           -2.256++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.182++01           -2.182++01           -2.089++01           -1.851++01           -1.851++01           -1.830++01           -1.830++01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.3.9% 1.47% 0.21% 2.00% 0.21% 1.21% 2.00% 0.21% 0.21% 6.21% 0.32% 0.41% 0.32% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.24% 0.34% 0.24%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.32% 15.78% 0.07% 0.01% 0.03% 0.00% 0.01% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00	STD(8g STD) 186.6bp (131.7bp) 187.6bp (136.4bp) 182.8bp (36.4bp) 142.6bp (145.6bp) 135.2bp (145.6bp) 135.2bp (144.6bp) 133.2bp (144.7bp) 142.1bp (144.7bp) 143.2bp (144.7bp) 143.2bp (144.7bp) 141.9bp (145.4bp) 140.2bp (145.4bp) 150.1bp (38.9bp) 150.4bp (142.5bp) 140.2bp (142.5bp) 142.2bp (145.2bp) 126.2bp (145.2bp) 126.2bp (145.2bp) 126.2bp (146.4bp) 126.2bp (146.4bp) 126.2bp (146.4bp) 126.2bp (146.4bp) 133.4bp (146.4bp) 132.8bp (10.0bp) 126.2bp (146.2bp) 138.0bp (10.0bp) 125.3bp (130.7bp) 125.3bp (131.7bp) 125.3bp (134.1bp) 125.3bp (134.1bp) 125.	Best Match/Details           CDX4(Homeobox)Myc-CNP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C00814.1.(Isspar(0.759)           28TB12(21)HEX29.28TB12.CFP-CNIP-Seq(GSE58341)Homer(0.603)           SN402/MA0745.2.Jaspar(0.853)           ET1V1MA074.2.Jaspar(0.853)           FV1VMA074.2.Jaspar(0.853)           Nanog(Homeobox)/mES-Nanog-CAIP-Seq(GSE11724)Homer(0.749)           VDR:MA0832.Jaspar(0.759)           FE0XE.1.Zp663.1.Jaspar(0.868)           Nex2-5(wr.2)MA0503.2.Jaspar(0.739)           PB0020.1.Zp861.2.Jaspar(0.855)           PB0020.1.Zp861.2.Jaspar(0.855)           PB0020.1.Zp861.2.Jaspar(0.739)           Nex2-5(wr.2)MA0503.1.Jaspar(0.768)           PB0020.1.Zp861.2.Jaspar(0.739)           PB0020.1.Zp861.2.Jaspar(0.739)           PB0020.1.Zp861.2.Jaspar(0.739)           PB0020.1.Zp861.1.Jaspar(0.739)           PB0020.1.Zp861.1.Jaspar(0.739)           PB0050.1.Hol.Laspar(0.739)           PB0160.1.FM4.2.Jaspar(0.739)           PD0160.1.FM4.2.Jaspar(0.730)           PD0160.1.FM4.2.Jaspar(0.730)           PD0160.1.FM4.2.Jaspar(0.760)           PD0160.1.FM4.2.Jaspar(0.760)           PD17.1.TM4.274.1.Jaspar(0.760)           PD17.1.TM4724.1.Jaspar(0.660)           TFAP28/2wr.3)MA0815.1.Jaspar(0.660)           TFAP28/2wr.3)MA0815.1.Jaspar(0.660) <td>NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro ro ro ro ro ro yes yes yes yes yes yes yes yes yes yes</td>	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro ro ro ro ro ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           201           22           23           24           25           26           27           28           29           30           32           33           34           35	Metil AGTCGTAACGTAGTAGCTAACTGAGTCACTGGTCAGCGTAACGTAGCTAGC	P-value 1.000E-210 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-10 1.000E-000E-000E-000E-000E-000E-000E-000	log P-pvalue           -5.001e+01           -4.680e+01           -4.680e+01           -4.680e+01           -4.680e+01           -4.213e+01           -4.213e+01           -3.054e+01           -3.054e+01           -3.228e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -3.054e+01           -2.850e+01           -2.850e+01           -2.611e+01           -2.615e+01           -2.255e+01           -2.162e+01	% of Targets 0.32% 1.08% 0.29% 1.08% 0.29% 12.57% 0.39% 22.61% 3.39% 1.47% 0.29% 1.41% 20.00% 0.29% 1.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.25% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.22% 0.22% 0.39% 0.22% 0.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.42% 0.02% 0.02% 0.03% 0.03% 0.03% 0.00% 0.03% 0.00%	STD(8g STD)           186.8bp (131.7cp)           187.6bp (136.4bp)           187.6bp (145.8bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           184.2bp (144.7bp)           184.2bp (144.7bp)           184.2bp (144.7bp)           185.4bp (144.7bp)           180.2bp (144.7bp)           180.2bp (144.7bp)           180.2bp (145.7bp)           182.2bp (146.7bp)           182.2bp (146.7bp)           182.2bp (146.7bp)           182.2bp (146.7bp)           182.2bp (146.7bp)           182.2bp (146.7bp)           183.2bp (146.7bp)           183.2bp (146.7bp)           183.2bp (146.7bp)           183.2bp (148.7bp)           183.2bp (148.7bp)           185.7bp (123.2bp)           185.7bp (123.2bp)           185.7bp (123.2bp)           185.7bp (123.2bp)           185.7bp (123.2bp)           185.7bp (123.2bp)           185.7bp (124.2bp)           185.7bp (125.7bp)           185.7bp (125.7bp)           185.7bp (125.7bp)	Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE48254)Homer(0.655)           PB00321_1BC00614_11/Japar(0.650)           ZBTB12(Z)/HEK23>ZBTB12 (ZPP-ChP-Seq(GSE58341)Homer(0.603)           SN402/MA0745_2Japar(0.633)           ETV1MA071_2Japar(0.632)           PE1021_D12/shapar(0.759)           VDR/MA0603_2Japar(0.759)           POS02_1_D12/shapar(0.649)           VDR/MA0603_2Japar(0.759)           PO025_1_PB01_2Japar(0.659)           PO025_1_PB01_2Japar(0.659)           P00205_1_PB01_2Japar(0.659)           P00205_1_PB01_2Japar(0.730)           P00205_1_PB01_2Japar(0.730)           P00205_1_PB01_2Japar(0.730)           P00205_1_PB01_2Japar(0.730)           P00205_1_PB01_1Japar(0.730)           P00205_1_PB01_2Japar(0.730)           P00205_1_PD1_MI12277-p65-CHP-Seq(GSE19485)Homer(0.630)           P00205_1_PD1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_1_PD1_PS01_2SE3431(PB01)           P00205_PD1_PS01_PS01_2SE3431(PB01)           P	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           4           15           16           17           18           19           20           21           22           24           25           26           27           28           29           30           31           32           33           34           35	Notif AGTCGTAACGTGTACGTAGGTAACGTAGGTCAGGTGACGTAGGTAACGTAGGTCAGGTCAGGTAGGT	P-value 1.000=210 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=000 1.000=000 1.000=000 1.000=0000000000	log P-pvalue           5.001++01           -4.689++01           -4.689++01           -4.213++01           -4.213++01           -4.208++01           -4.061++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -2.668++01           -2.880++01           -2.878++01           -2.878++01           -2.8578++01           -2.4558++01           -2.4558++01           -2.4558++01           -2.4258++01           -2.2568++01           -2.2568++01           -2.2568++01           -2.2568++01           -2.2568++01           -2.2568++01           -2.2568++01           -2.2580+01           -2.158++01           -2.1628++01           -2.1628++01           -2.1628++01           -2.1628++01           -2.0838++01           -1.8308++01           -1.8308++01           -1.8308++01           -1.8308++01           -1.8308++01           -1.8308++01	% of Targets 0.32% 0.22% 0.29% 8.47% 0.29% 8.47% 12.57% 3.39% 1.47% 0.39% 0.29% 1.47% 0.21% 2.00% 0.29% 19.93% 0.29% 19.93% 0.29% 19.93% 0.29% 19.93% 0.21% 6.21% 0.18% 0.18% 0.18% 0.18% 0.18% 0.18% 0.18% 0.24% 5.7%	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.26% 0.42% 0.42% 0.02% 0.32% 15.76% 0.03% 0.07% 0.01% 0.02% 3.94% 1.70% 0.00%	STD(8g STD) 186.8bp (131.7bp) 187.8bp (136.4bp) 182.8bp (36.4bp) 142.8bp (36.4bp) 142.8bp (145.8bp) 135.2bp (145.8bp) 133.2bp (144.8bp) 142.1bp (144.4bp) 133.2bp (144.7bp) 143.9bp (144.7bp) 143.9bp (145.4bp) 140.1bp (145.2bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 142.8bp (145.2bp) 142.8bp (145.2bp) 133.4bp (146.2bp) 133.4bp (146.2bp) 142.7bp (145.2bp) 142.7bp (145.7bp) 142.7bp (145.7bp) 14	Best Match/Details           CDX4(Homeckou/Myc-ChP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C0041.1/Lispapr(0.579)           28TB12(21)HEX23-28TB12.CFP-ChIP-Seq(GSE58341)Homer(0.603)           SN402/MA0745.2/Lispapr(0.653)           ET1V1MA0715.2/Lispapr(0.653)           FV1VMA0715.2/Lispapr(0.659)           Nanog(Homeokou/InES-Nanog-ChIP-Seq(GSE1724)Homer(0.749)           VDR1And0823.2/Lispapr(0.759)           FOXE: INA1497.1/Lispapr(0.650)           Nanog(Homeokou/InES-Nanog-ChIP-Seq(GSE11724)Homer(0.749)           VDR1And0823.2/Lispapr(0.759)           FOXE: INA1497.1/Lispapr(0.650)           P60005.1.7peig11/Lispapr(0.650)           D20035.1.7peig11/Lispapr(0.730)           P80050.1.0er1_1.1/Lispapr(0.730)           P80050.1.0er1_1.1/Lispapr(0.730)           P80150.1.Thefs.1/Lispapr(0.761)           P80150.1.Thefs.2.2/Lispapr(0.766)           P80150.1.Thefs.2.2/Lispapr(0.765)           P80150.1.Thefs.2.2/Lispapr(0.766)           P80150.1.Thefs.2.2/Lispapr(0.766)           P80150.1.Thefs.1/Lispapr(0.803)           TEA22/MAT121.1/Lispapr(0.801)           ZBTB18(Z)HeK202-ZBTB18.GFP-ChP-Seq(GSE34341)Homer(0.560)           P80150.1.Thefs.2.2/Lispapr(0.803)           TEA224/Lispapr(0.801)           ZBTB18(Z)HeK202-ZBTB18.GFP-ChP-Seq(GSE34341)Homer(0.500)           P0	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           9           20           21           22           23           24           25           26           27           30           31           32           33           34           35           36           37	Notif AGTCCGTAACGTGACGTACGTAACGTAGGTCAGTGAGCACGTACGT	P-value 1.000E-210 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-10 1.000E-000E-000E-000E-000E-000E-000E-000	log P-pvalue           -5.001++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.689++01           -4.051++01           -4.051++01           -3.054++01           -3.298++01           -2.658++01           -2.658++01           -2.658++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.258++01           -2.2128++01           -2.128++01           -2.128++01           -2.128++01           -2.128++01           -2.128++01           -2.138++01           -1.839++01           -1.839++01           -1.839++01           -1.839++01           -1.839++01 <td< td=""><td>% of Targets 0.32% 1.05% 0.29% 0.29% 0.29% 12.57% 0.39% 22.61% 0.39% 22.61% 0.39% 0.21% 0.21% 0.21% 0.21% 0.21% 0.39% 0.24% 0.39% 0.24% 0.39% 0.24% 0.39% 0.24% 0.34% 0.24% 0.34% 0.</td><td>% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.32% 0.75% 0.03% 0.03% 0.03% 0.03% 0.03% 0.03% 0.00% 0.03% 0.00% 0.00% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.03% 0.01% 0.00% 0.03% 0.01% 0.00%</td><td>STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.4bp)           183.2bp (144.5bp)           183.2bp (144.7bp)           141.7bp (147.7bp)           145.2bp (144.5bp)           150.1bp (145.2bp)           160.2bp (144.2bp)           160.2bp (144.2bp)           162.2bp (180.7bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           163.2bp (146.2bp)           164.2bp (146.2bp)           165.3bp (120.1bp)           165.3bp (120.1bp)           155.3bp (151.2bp)           155.3bp (151.2bp)           157.9bp (161.5bp)           153.2bp (116.5bp)           153.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)</td><td>Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE34825(H+kmer(0.655))           PB00321_IEG20814_11(aspar(0.579))           ZBTB12(Z))+EK239-ZBTB12_GPP-ChIP-Seq(GSE548341)Homer(0.603)           SN402/MA0745_21/aspar(0.683)           ET1V1MA0745_21/aspar(0.683)           ET1V1MA071_21/aspar(0.683)           Nanog(Homeobou/mES-Nanog-CAIP-Seq(GSE11724)Homer(0.749)           VDR:MA0633_21/aspar(0.759)           FOXE (MA1487_11/aspar(0.669)           Nex-6_Ver_21/MA053_21/aspar(0.759)           FOXE (MA1487_11/aspar(0.655)           PB0032_1_PBig1_1_Ulaspar(0.655)           PB0032_1_PBig1_1_Ulaspar(0.738)           SOX10 (MA0422_21/aspar(0.738))           PB0032_1_IPBig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_DFI_Ulaspar(0.730)           FOXA1(Forthward)/MC7+FOXA1-CHP-Seq(GSE20431)Homer(0.760)           PB0160_1_DFA0CA1_22_Jaspar(0.676)           PB0160_1_DFA0CA1_22_Jaspar(0.676)           ZBTB16(2)/HEXE2_23-ZBTB16(SE20431)Homer(0.780)           FTA22AWA1121_11/aspar(0.680)           PD1603_DFIACE_2_Jaspar(0.676)           VENTXMA0724_13aspar(0.650)           VENTXMA0724_13aspar(0.560)           VENTXMA0724_13aspar(0.561)      <tr< td=""><td>NTG or B27 specific yes yes yes yes yes yes yes yes yes yes</td></tr<></td></td<>	% of Targets 0.32% 1.05% 0.29% 0.29% 0.29% 12.57% 0.39% 22.61% 0.39% 22.61% 0.39% 0.21% 0.21% 0.21% 0.21% 0.21% 0.39% 0.24% 0.39% 0.24% 0.39% 0.24% 0.39% 0.24% 0.34% 0.24% 0.34% 0.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.02% 0.32% 0.75% 0.03% 0.03% 0.03% 0.03% 0.03% 0.03% 0.00% 0.03% 0.00% 0.00% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.03% 0.01% 0.00% 0.03% 0.01% 0.00%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.4bp)           183.2bp (144.5bp)           183.2bp (144.7bp)           141.7bp (147.7bp)           145.2bp (144.5bp)           150.1bp (145.2bp)           160.2bp (144.2bp)           160.2bp (144.2bp)           162.2bp (180.7bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           162.2bp (146.2bp)           163.2bp (146.2bp)           164.2bp (146.2bp)           165.3bp (120.1bp)           165.3bp (120.1bp)           155.3bp (151.2bp)           155.3bp (151.2bp)           157.9bp (161.5bp)           153.2bp (116.5bp)           153.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)           154.2bp (116.5bp)	Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE34825(H+kmer(0.655))           PB00321_IEG20814_11(aspar(0.579))           ZBTB12(Z))+EK239-ZBTB12_GPP-ChIP-Seq(GSE548341)Homer(0.603)           SN402/MA0745_21/aspar(0.683)           ET1V1MA0745_21/aspar(0.683)           ET1V1MA071_21/aspar(0.683)           Nanog(Homeobou/mES-Nanog-CAIP-Seq(GSE11724)Homer(0.749)           VDR:MA0633_21/aspar(0.759)           FOXE (MA1487_11/aspar(0.669)           Nex-6_Ver_21/MA053_21/aspar(0.759)           FOXE (MA1487_11/aspar(0.655)           PB0032_1_PBig1_1_Ulaspar(0.655)           PB0032_1_PBig1_1_Ulaspar(0.738)           SOX10 (MA0422_21/aspar(0.738))           PB0032_1_IPBig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_1_Ulaspar(0.738)           PB0032_1_DFig1_DFI_Ulaspar(0.730)           FOXA1(Forthward)/MC7+FOXA1-CHP-Seq(GSE20431)Homer(0.760)           PB0160_1_DFA0CA1_22_Jaspar(0.676)           PB0160_1_DFA0CA1_22_Jaspar(0.676)           ZBTB16(2)/HEXE2_23-ZBTB16(SE20431)Homer(0.780)           FTA22AWA1121_11/aspar(0.680)           PD1603_DFIACE_2_Jaspar(0.676)           VENTXMA0724_13aspar(0.650)           VENTXMA0724_13aspar(0.560)           VENTXMA0724_13aspar(0.561) <tr< td=""><td>NTG or B27 specific yes yes yes yes yes yes yes yes yes yes</td></tr<>	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27           28           30           31           32           33           34           35           36           37           38	Notif AGTCGTAACGTGGTACGTAGGTAACGTAGGTCAGGTGACGTAGGTAG	P-value 1.000=210 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=000 1.000=0000000000	log P-pvalue           5.001++01           -4.689++01           -4.689++01           -4.213++01           -4.213++01           -4.213++01           -3.470++01           -3.470++01           -3.470++01           -3.470++01           -3.470++01           -3.470++01           -3.306++01           -3.306++01           -2.850++01           -2.850++01           -2.850++01           -2.850++01           -2.850++01           -2.850++01           -2.850++01           -2.850++01           -2.4250++01           -2.4250++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.183++01           -1.839++01           -1.830++01           -1.830++01           -1.830++01 <td< td=""><td>% of Targets 0.32% 0.22% 0.29% 8.47% 0.29% 8.47% 12.57% 3.39% 1.47% 0.39% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.63% 0.29% 0.39% 0.21% 0.32% 0.45% 0.18% 0.18% 0.18% 0.18% 0.18% 0.18% 0.24</td><td>% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 1.2.11% 1.80% 0.26% 0.26% 0.42% 0.27.66% 0.42% 0.00% 0.32% 15.77% 0.01% 0.02% 3.94% 0.02% 3.94% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.02% 3.94% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00%</td><td>STD(8g STD)           186.8bp (131.7bp)           177.6bp (136.4bp)           177.6bp (136.4bp)           128.8bp (36.4bp)           128.8bp (36.4bp)           128.8bp (145.8bp)           135.2bp (145.8bp)           135.2bp (145.8bp)           138.2bp (146.7bp)           141.8bp (146.4bp)           141.8bp (145.4bp)           140.2bp (145.4bp)           140.2bp (145.2bp)           140.1bp (145.2bp)           140.2bp (142.5bp)           142.8bp (146.2bp)           142.8bp (146.2bp)           133.4bp (146.3bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           135.4bp (146.3bp)           134.2bp (146.3bp)           135.4bp (146.2bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (146.2bp)</td><td>Best Match/Details           CDX4(Homeckou/Myc-ChP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C00814.1./laspar(0.655)           ZBTB12(ZI)HEX23&gt;ZBTB12.GFP-ChIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2./laspar(0.851)           ETV1MA0715.2/laspar(0.852)           ETV1MA0715.2/laspar(0.852)           Nanog(Homeobo)(mES-Nanog-CAPI-Seq(GSE1724)Homer(0.749)           VDR:MA0632.2/laspar(0.759)           FOXE:INA1487.1/laspar(0.852)           P60052.1., PLog1_1.2/laspar(0.656)           Nex2.5/ver.2/MA0503.1/laspar(0.555)           P60052.1., PLog1_1.2/laspar(0.355)           P60052.1., PLog1_1.2/laspar(0.356)           DX01X01MA042.2.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           P80050.1.Ort.1.10529(GSE18451)Homer(0.663)           P80050.1.Ort.1.10529(GSE18451)Homer(0.663)           P80050.1.Ort.1.01F-Seq(GSE28831)Homer(0.730)           PD4060.1.Ref. 2/laspar(0.730)           PD4060.1.Ref.2.2.2.alspar(0.676)           PB0050.1.Gort.1.1/laspar(0.661)           ZXBTB18(ZI)HEK292-ZB1816.GFP-ChIP-Seq(GSE28831)Homer(0.730)           PD4061.1.Tef.1.laspar(0.660)           TEA22/MA1121.1.1/laspar(0.660)           TEA22/MA1121.1.1/laspar(0.6</td><td>NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes</td></td<>	% of Targets 0.32% 0.22% 0.29% 8.47% 0.29% 8.47% 12.57% 3.39% 1.47% 0.39% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.63% 0.29% 0.39% 0.21% 0.32% 0.45% 0.18% 0.18% 0.18% 0.18% 0.18% 0.18% 0.24	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 1.2.11% 1.80% 0.26% 0.26% 0.42% 0.27.66% 0.42% 0.00% 0.32% 15.77% 0.01% 0.02% 3.94% 0.02% 3.94% 0.00% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.02% 3.94% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00% 0.01% 0.02% 0.00%	STD(8g STD)           186.8bp (131.7bp)           177.6bp (136.4bp)           177.6bp (136.4bp)           128.8bp (36.4bp)           128.8bp (36.4bp)           128.8bp (145.8bp)           135.2bp (145.8bp)           135.2bp (145.8bp)           138.2bp (146.7bp)           141.8bp (146.4bp)           141.8bp (145.4bp)           140.2bp (145.4bp)           140.2bp (145.2bp)           140.1bp (145.2bp)           140.2bp (142.5bp)           142.8bp (146.2bp)           142.8bp (146.2bp)           133.4bp (146.3bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           134.4bp (146.2bp)           135.4bp (146.3bp)           134.2bp (146.3bp)           135.4bp (146.2bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (135.8bp)           135.4bp (146.2bp)	Best Match/Details           CDX4(Homeckou/Myc-ChP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C00814.1./laspar(0.655)           ZBTB12(ZI)HEX23>ZBTB12.GFP-ChIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2./laspar(0.851)           ETV1MA0715.2/laspar(0.852)           ETV1MA0715.2/laspar(0.852)           Nanog(Homeobo)(mES-Nanog-CAPI-Seq(GSE1724)Homer(0.749)           VDR:MA0632.2/laspar(0.759)           FOXE:INA1487.1/laspar(0.852)           P60052.1., PLog1_1.2/laspar(0.656)           Nex2.5/ver.2/MA0503.1/laspar(0.555)           P60052.1., PLog1_1.2/laspar(0.355)           P60052.1., PLog1_1.2/laspar(0.356)           DX01X01MA042.2.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           ZXP341/MA152.2/laspar(0.739)           P80050.1.Ort.1.10529(GSE18451)Homer(0.663)           P80050.1.Ort.1.10529(GSE18451)Homer(0.663)           P80050.1.Ort.1.01F-Seq(GSE28831)Homer(0.730)           PD4060.1.Ref. 2/laspar(0.730)           PD4060.1.Ref.2.2.2.alspar(0.676)           PB0050.1.Gort.1.1/laspar(0.661)           ZXBTB18(ZI)HEK292-ZB1816.GFP-ChIP-Seq(GSE28831)Homer(0.730)           PD4061.1.Tef.1.laspar(0.660)           TEA22/MA1121.1.1/laspar(0.660)           TEA22/MA1121.1.1/laspar(0.6	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           11           12           13           14           15           16           17           18           20           21           22           23           24           25           26           27           28           29           30           31           32           33           34           35           37           38	Notif AGTCOGTAACGTOGTACGTACGTAACGTAGGTCAGGTGAGGTCAGGTAACGTAGGT CAGTCAGTGATGCGTAGGTGAGTCAGGTCAGGTCAGGTAGGT	P-value 1.000E-210 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-10 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-11 1.000E-10 1.000E-000E-000E-000E-000E-000E-000E-000	log P-pvalue           -5.001+-01           -5.001+-01           -4.689+-01           -4.689+-01           -4.489+-01           -4.489+-01           -4.489+-01           -4.439+-01           -4.213+-01           -3.078+-01           -3.298+-01           -3.298+-01           -3.298+-01           -3.064+-01           -3.064+-01           -3.064+-01           -3.064+-01           -2.868+-01           -2.868+-01           -2.878+-01           -2.878+-01           -2.475+-01           -2.475+-01           -2.258+-01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01           -2.162++01	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 22.61% 1.21% 20.0% 0.21% 6.21% 0.21% 6.21% 0.39% 0.21% 6.21% 0.39% 0.21% 6.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.25% 0.39% 0.21% 0.39% 0.25% 0.39% 0.21% 0.39% 0.21% 0.39% 0.25% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.16% 0.30% 0.16% 0.30% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.34% 0.16% 0.32% 0.16% 0.32% 0.16% 0.32% 0.21% 0.35% 0.21% 0.35% 0.21% 0.35% 0.25% 0.35% 0.25% 0.35% 0.25% 0.35% 0.25% 0.35% 0.35% 0.25% 0.35% 0.35% 0.25% 0.35% 0.	% of Background           0.00%           0.16%           0.00%           4.94%           8.33%           0.01%           17.11%           1.80%           0.26%           0.42%           0.03%           0.32%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.00%           0.00%           0.01%           0.00%           0.01%           0.03%           1.70%           9.65%           0.03%           5.12%           2.1.33%           0.11%           0.02%           3.37%           1.00%           1.00%	STD(8g STD)           186.6bp (131.7bp)           128.7bp (136.4bp)           127.8bp (36.4bp)           128.2bp (36.4bp)           128.2bp (145.8bp)           135.2bp (145.8bp)           135.2bp (145.8bp)           138.2bp (145.8bp)           138.2bp (145.8bp)           138.2bp (145.8bp)           150.1bp (145.8bp)           150.1bp (145.8bp)           150.1bp (145.2bp)           150.1bp (145.2bp)           150.2bp (145.2bp)           152.2bp (15.7bp)           128.2bp (145.2bp)           133.4bp (146.2bp)           133.4bp (146.2bp)           134.2bp (145.2bp)           144.2bp (145.2bp)           152.2bp (175.0bp)           124.2bp (145.2bp)           134.4bp (145.2bp)           134.4bp (145.2bp)           144.2bp (145.2bp)           142.7bp (20.1bp)           142.7bp (20.1bp)           143.4bp (145.2bp)           135.0bp (134.1bp)           136.0bp (134.1bp)           136.7bp (132.1bp)           136.7bp (132.1bp)           137.0bp (151.1bp)           138.2bp (145.2bp)           134.2bp (145.2bp)           124.2bp (145.2bp)	Best Match/Details           CDX4(Homedool/Myc-ChP-Seq(GSE48254)Homer(0.655)           PB0032 1, IfScor081, 1.(Jaspar(0.655)           2BTB12(21)HEX23-2BTB12, CFP-ChP-Seq(GSE58341)Homer(0.603)           SNAU2MA0745 2.Jaspar(0.633)           ETV1(MA0715 2.Jaspar(0.632)           DEVIDED           DEVIDED           PD022 1, IFSCOR1 2.Jaspar(0.632)           Nanog(Homedool/mES-Nanog-CAP-Seq(GSE1724)Homer(0.749)           VDR/MA053 2.Jaspar(0.632)           PD0203 1, ZP01 2.Jaspar(0.689)           Nexc-6(ver.2)MA0603 7.Jaspar(0.689)           PD0203 1, ZP01 2.Jaspar(0.635)           PD0203 1, ZP01 2.Jaspar(0.635)           PD0203 1, ZP01 2.Jaspar(0.739)           SOX10(MA0422 2.Jaspar(0.739)           SOX10(MA0422 2.Jaspar(0.739)           PB0203 1, ZP01 2.Jaspar(0.659)           PB0203 1, ZP01 2.Jaspar(0.739)           PB050 1. D01 2.Jaspar(0.730)           SOX10(MA0422 2.Jaspar(0.730)           SOX10(MA0422 2.Jaspar(0.730)           PB0150 1. TAsbar(0.730)           PD0150 1. TAsbar(0.750)           PD0150 1. TAsbar(0.750)           PD150 1. TAsbar(0.550)           ZETB18(Z)/HEX25-ZETB18 (SCE28831)Homer(0.760)           PD1502 1. TAsbar(0.550)           ZETB18(Z)/HEX25-ZETB18 (SCE28831)Homer(0.550)           VEHT/MA0724 1.Jas	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           28           30           31           22           230           31           24           25           28           30           31           32           33           34           35           36           37           38           39           40	Motif AGTCGTAACGTGGTACGTAGCGTAACGTGAGTCACGTGGTCAGCGTAGGTAG	P-value 1.00E-20 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-10 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-01 1.00E-00 1.00E-0	log P-pvalue           5.001e+01           -4.689e+01           -4.689e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.081e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.470e+01           -3.306e+01           -3.306e+01           -2.850e+01           -2.857e+01           -2.857e+01           -2.857e+01           -2.857e+01           -2.856e+01           -2.856e+01           -2.856e+01           -2.158e+01           -3.158e+01           -1.551e+01           -1.838e+01           -1.776e+01           -1.777e+01           -1.372e+01           -1.330a+01	% of Targets 0.22% 0.22% 0.29% 8.47% 12.57% 3.39% 12.57% 3.39% 1.47% 0.29% 2.2.61% 3.39% 1.47% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.32% 0.16% 0.18% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24%	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 18.01% 0.26% 0.26% 0.42% 0.26% 0.42% 0.01% 0.27% 0.01% 0.77% 0.01% 0.02% 0.00% 0.02% 0.00% 0.00% 0.02% 0.00%	STD(8g STD)           186.8bp (131.7bp)           176.7bp (136.4bp)           177.8bp (136.4bp)           128.8bp (36.4bp)           128.8bp (36.4bp)           128.2bp (146.5bp)           135.2bp (145.8bp)           135.2bp (146.7bp)           138.2bp (140.7bp)           141.7bp (141.7bp)           143.2bp (144.3bp)           143.2bp (144.5bp)           150.1bp (86.8bp)           150.1bp (86.8bp)           140.1bp (145.2bp)           140.2bp (142.5bp)           140.2bp (142.5bp)           142.2bp (143.2bp)           144.2bp (143.2bp)           144.2bp (143.2bp)           144.2bp (143.2bp)           144.2bp (143.2bp)           144.2bp (143.2bp)           142.7bp (146.2bp)           135.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (132.1bp)           135.2bp (132.1bp)           135.2bp (132.1bp)           135.2bp (144.5bp)           135.2bp (132.1bp)           135.2bp (134.1bp)           136.2bp (134.1bp)           136.2bp (134.1bp)           136.2bp (134.1bp)           136.2bp (144.4bp)           132.2bp (144.4bp)	Best Match/Details           CDX4(Homeotox)/Myv-CNP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C00014.1./Idagard(0.757)           ZBTB12(ZI)/HEX29-ZBTB12.GFP-CNIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2./lagar(0.635)           ETV1MA0715.2./lagar(0.635)           DEV104.0715.2./lagar(0.635)           Nanog(Homeotox)/IMS2.2./lagar(0.635)           PO0203.1.7.60033.1/lagar(0.636)           PO0205.1.7.601           PO0205.1.7.601           DVR.MA06033.1/lagar(0.635)           P00205.1.7.601           DVR.MA0603.1/lagar(0.635)           P00205.1.7.601           DVR.MA0605.1.4.801           DVR.MA0605.1.4.801           DVR.MA0605.1.4.801           DVR.MA0605.1.9.601           DVR.MA0605.1.9.601           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.0149           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0615.1.4.801           DVR.MA0605.1.4.801           DVR.MA061	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27           30           21           22           23           34           35           36           37           38           39           40	Notif AGTCOGTAACGTACGTAGGTAACGTAAGTCAGTCAGGTCAG	P-value 1.0002-12 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-10 1.0002-0002-0002-0002-0002-0002-0002-000	log P-pvalue           5:001++01           -4:659++01           -4:659++01           -4:659++01           -4:659++01           -4:213++01           -4:213++01           -4:213++01           -3:268++01           -4:051++01           -3:278++01           -3:268++01           -3:064++01           -3:064++01           -3:064++01           -3:064++01           -2:662++01           -2:678++01           -2:678++01           -2:518++01           -2:518++01           -2:518++01           -2:258++01           -2:258++01           -2:162++01           -2:258++01           -2:182++01           -2:288++01           -2:182++01           -2:288++01           -1:258++01           -2:288++01           -1:389++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -1:388++01           -	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 1.47% 0.21% 1.21% 20.00% 2.00% 0.29% 1.21% 1.21% 2.00% 0.29% 0.39% 0.21% 6.21% 0.32% 45.07% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.24% 5.7% 0.55% 0.24% 5.10% 5.	% of Background 0.00% 0.16% 0.10% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.02% 0.32% 0.00% 0.32% 0.00% 0.03% 0.00% 0.03% 0.00% 0.03% 0.00%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           142.6bp (145.6bp)           135.2bp (145.7bp)           142.6bp (145.6bp)           138.2bp (144.7bp)           143.2bp (145.7bp)           143.2bp (144.7bp)           144.7bp (145.7bp)           140.2bp (145.7bp)           140.2bp (145.7bp)           140.2bp (145.7bp)           140.2bp (145.7bp)           142.2bp (146.7bp)           142.2bp (146.7bp)           142.2bp (146.7bp)           142.2bp (146.7bp)           142.2bp (146.7bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           142.7bp (146.2bp)           138.0bp (10.0bp)           138.0bp (142.7bp)           138.0bp (142.7bp)           138.0bp (142.7bp)           138.0bp (142.7bp)           142.7bp (146.4bp)           138.0bp (142.7bp)           142.7bp (146.4bp)           138.0bp (147.7bp)           138.0bp (147.7bp)           142.7bp (146.4bp)	Best Match/Details           CDX4(Homedool/Myc-ChP-Seq(GSE48254)Homer(0.655)           PB0932 1, IRScotle 1, Utsapar(0.759)           ZBTB12(ZI)+EK293-ZBTB12, CFP-ChIP-Seq(GSE58341)Homer(0.603)           SNAU2MA0745 2, Jaspar(0.853)           ETV1MA0715 2, Jaspar(0.851)           DETV1MA0715 2, Jaspar(0.852)           Nanog(Homedool/mES-Nanog-CAIP-Seq(CSE11724)/Homer(0.749)           VDR:MA0632 2, Jaspar(0.759)           FOXE VMA1487 1/Jaspar(0.868)           PB0020 1, ZP601 2, Jaspar(0.655)           PB0020 1, ZP601 2, Jaspar(0.739)           ZNF341MA1655 1, Jaspar(0.739)           ZNF341MA1655 1, Jaspar(0.739)           PB0030 1, Dent 1, Jaspar(0.739)           PB0160 1, TArba 2, Zlaspar(0.739)           PB0160 1, TArba 2, Zlaspar(0.739)           PB0160 1, TArba 2, Zlaspar(0.767)           PB0160 1, TArba 2, Zlaspar(0.676)           PB0170 1, TArba 2, Zlaspar(0.676)           PB0170 1, TArba 2, Zlaspar(0.656)           TFAP28(war.3)MA0813 1, Jlaspar(0.586)           TFAP28(war.3)MA0813 1, Jlaspar(0.566) <td>NTG or B27 specific yes yes yes yes yes yes yes yes yes yes</td>	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
827	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           20           30           31           32           33           34           35           36           39           30           31           32           33           34           35           36           37           38           390           41	Metir AGTCGTAACGTAGTAGCTAACGTAGTCAGTCAGCTAGCGTAACGTAGCTAGC	P-value 1.00E-20 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-10 1.00E-0	log P-pvalue           5.001e+01           -5.001e+01           -4.683e+01           -4.683e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -4.213e+01           -3.238e+01           -3.3479e+01           -3.3479e+01           -3.064e+01           -3.064e+01           -2.856e+01           -2.762e+01           -2.762e+01           -2.516e+01           -2.256e+01           -2.138e+01           -2.128e+01           -1.830e+01           -1.830e+01           -1.330e+01           -1.333e+01 <td< td=""><td>% of Targets 0.22% 0.22% 0.29% 8.47% 12.57% 0.39% 22.61% 33.39% 1.47% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.23% 1.47% 0.29% 0.29% 0.29% 0.29% 0.29% 0.21% 0.25% 0.21% 0.22% 0.</td><td>% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.26% 0.42% 0.02% 0.32% 0.77% 0.01% 15.78% 0.00% 0.03% 0.03% 0.03% 0.03% 0.00% 0.03% 0.00% 0.03% 0.00</td><td>STD(8g STD)           186.8bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.8bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           184.2bp (144.8bp)           144.3bp (144.8bp)           144.3bp (144.8bp)           150.1bp (84.8bp)           150.4bp (142.7bp)           160.2bp (145.8bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           162.2bp (143.5bp)           163.3bp (144.7bp)           164.2bp (143.5bp)           165.3bp (123.0bp)           164.2bp (145.7bp)           155.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (145.7bp)           165.3bp (145.7bp)           164.3bp) (144.7bp)</td><td>Best Match/Details           CDX4(Homeckov)Mys-CNP-Seq(GSE48254)Homer(0.655)           PB0032.1, IRSC0014.1./Idsaprd.0759)           28TB12(ZI)HEK293-28TB12.GFP-CNP-Seq(GSE58341)Homer(0.603)           SNMu2/MA0745.2.Jaspar(0.632)           ETV1MA0715.2.Jaspar(0.632)           Nanog(Homeckov)MrS-Nanog-CMP-Seq(GSE11724)Homer(0.749)           VDR:MA0633.3.Jaspar(0.759)           P6023.1, JE2604.2.Jaspar(0.652)           P80203.1, ZEp601.2.Jaspar(0.655)           P80203.1, ZEp601.2.Jaspar(0.656)           P80203.1, ZEp601.2.Jaspar(0.655)           P80203.1, ZEp601.2.Jaspar(0.656)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Ma</td><td>NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes</td></td<>	% of Targets 0.22% 0.22% 0.29% 8.47% 12.57% 0.39% 22.61% 33.39% 1.47% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.23% 1.47% 0.29% 0.29% 0.29% 0.29% 0.29% 0.21% 0.25% 0.21% 0.22% 0.	% of Background 0.00% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.26% 0.42% 0.02% 0.32% 0.77% 0.01% 15.78% 0.00% 0.03% 0.03% 0.03% 0.03% 0.00% 0.03% 0.00% 0.03% 0.00	STD(8g STD)           186.8bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.8bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           184.2bp (144.8bp)           144.3bp (144.8bp)           144.3bp (144.8bp)           150.1bp (84.8bp)           150.4bp (142.7bp)           160.2bp (145.8bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           162.2bp (168.0bp)           162.2bp (143.5bp)           163.3bp (144.7bp)           164.2bp (143.5bp)           165.3bp (123.0bp)           164.2bp (145.7bp)           155.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (123.0bp)           165.3bp (145.7bp)           165.3bp (145.7bp)           164.3bp) (144.7bp)	Best Match/Details           CDX4(Homeckov)Mys-CNP-Seq(GSE48254)Homer(0.655)           PB0032.1, IRSC0014.1./Idsaprd.0759)           28TB12(ZI)HEK293-28TB12.GFP-CNP-Seq(GSE58341)Homer(0.603)           SNMu2/MA0745.2.Jaspar(0.632)           ETV1MA0715.2.Jaspar(0.632)           Nanog(Homeckov)MrS-Nanog-CMP-Seq(GSE11724)Homer(0.749)           VDR:MA0633.3.Jaspar(0.759)           P6023.1, JE2604.2.Jaspar(0.652)           P80203.1, ZEp601.2.Jaspar(0.655)           P80203.1, ZEp601.2.Jaspar(0.656)           P80203.1, ZEp601.2.Jaspar(0.655)           P80203.1, ZEp601.2.Jaspar(0.656)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P80203.1, ZEp601.2.Jaspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.738)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Maspar(0.758)           P8050.1, Cert.1.Ma	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           27           28           29           30           31           32           34           35           36           37           39           40           41           42	Notif AGTCOGTAACGTACGTAGGTAACGTAAGTCAGTCAGGTCAG	P-value 1.000=210 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=10 1.000=000 1.000=0000000000	log P-pvalue           5:001++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -4:059++01           -3:208++01           -3:208++01           -3:208++01           -3:002++01           -3:002++01           -3:002++01           -2:058++01           -2:058++01           -2:058++01           -2:058++01           -2:058++01           -2:058++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:102++01           -2:039++01           -1:050++01           -1:050++01           -1:050++01           -1:050++01           -1:050++01           -1:050++01           -	% of Targets 0.32% 1.08% 0.29% 8.47% 12.57% 0.39% 22.61% 3.97% 1.16% 3.39% 1.47% 0.21% 1.21% 20.00% 0.21% 1.21% 20.00% 0.29% 1.47% 0.21% 6.21% 0.32% 0.41% 0.32% 0.41% 0.32% 0.41% 0.48% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.16% 0.18% 0.24% 0.55% 0.24% 0.55% 0.24% 0.55% 0.24% 0.55% 0.24% 0.55% 0.24% 0.51% 0.24% 0.51% 0.24% 0.55% 0.24% 0.55% 0.24% 0.24% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.24% 0.24% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.25% 0.24% 0.25% 0.24% 0.25% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 17.11% 1.80% 0.26% 0.42% 0.42% 0.42% 0.00% 0.32% 15.76% 0.00% 0.07% 0.01% 0.00	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (146.5bp)           183.2bp (144.5bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           141.9bp (145.4bp)           141.9bp (145.4bp)           140.2bp (144.2bp)           140.1bp (145.2bp)           150.1bp (38.9bp)           150.4bp (142.5bp)           162.2bp (175.0bp)           133.4bp (146.4bp)           133.4bp (146.4bp)           134.2bp (146.2bp)           134.2bp (146.2bp)           134.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (146.2bp)           135.2bp (132.1bp)           135.2bp (131.1bp)           135.2bp (145.2bp)           135.2bp (145.2bp)           142.2bp (146.2bp)           142.2bp (146.2bp)           142.2bp (146.2bp)           142.2bp (146.2bp)           135.2bp (147.1bp)           135.4bp (146.2bp)	Best Match/Details           CDX4(Homeobox)Myc-CNP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C00814.1.(Isspar(0.655)           ZBTB12(21)HEX29.2BTB12.CFP-CNIP-Seq(GSE58341)Homer(0.603)           SNAU2MA0745.2.Jaspar(0.635)           ETV1MA0745.2.Jaspar(0.852)           Nanog(Homeobox)(mES-Nanog-CAIP-Seq(GSE1724)Homer(0.749)           VDR:MA0823.2Jaspar(0.759)           PE0203.1.2Cp(0.759)           PE0203.1.2p6(1.2)Jaspar(0.669)           No.2-5(w.2)MA0503.2Jaspar(0.739)           PB0203.1.2p6(1.2)Jaspar(0.655)           PB0203.1.2p6(1.2)Jaspar(0.655)           PB0203.1.2p6(1.2)Jaspar(0.739)           SOX10(MAA42.2)Jaspar(0.739)           SOX10(MA442.2)Jaspar(0.739)           PB0203.1.2p6(1.2)Jaspar(0.739)           PB0203.1.2p6(1.2)Jaspar(0.739)           PB0203.1.7b6.1/Jaspar(0.739)           PB0203.1.7b7.1/Jaspar(0.739)           PB0305.1.7b7.1/Jaspar(0.739)           PB0160.1.7b7.2p6(GSE18431)Homer(0.763)           PB0160.1.7b7.2p6(GSE28431)Homer(0.763)           PD0160.1.7b7.2p6(GSE28431)Homer(0.763)           PD0161.7b7.2p6(GSE28431)Homer(0.760)           PD141.12.2Jaspar(0.703)           PD141.12.2Jaspar(0.703)           PD141.1.7b7.2p6(GSE28431)Homer(0.566)           TF2P28(xr)MA0124.1Jaspar(0.566)           TF2P28(xr)MA0124.1Jaspar(0.5	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           9           20           21           22           23           24           25           26           20           212           22           23           30           312           32           33           34           35           36           377           38           39           30           312           333           36           377           38           39           30           31           32           337      38	Metir AGTCGTAACGTACGTACGTACGTAACTGAGTCACTGGTCACGTACGT	P-value (0.00-21 0.00-20 1.00-19 1.	log P-pvalue           -5.001+01           -5.001+01           -4.689+01           -4.689+01           -4.689+01           -4.689+01           -4.689+01           -4.213+01           -4.213+01           -4.051+01           -4.051+01           -4.054+01           -3.078+01           -3.298+01           -3.298+01           -3.064+01           -2.860+01           -2.860+01           -2.860+01           -2.611+01           -2.6128+01           -2.4758+01           -2.2558+01           -2.2558+01           -2.2568+01           -2.2568+01           -2.2588+01           -2.2588+01           -2.2588+01           -2.2588+01           -2.2588+01           -2.2588+01           -2.128+01           -2.128+01           -2.128+01           -2.128+01           -2.128+01           -3.838+01           -1.838+01           -1.767+01           -1.778+01           -1.338+01           -1.338+01      <	% of Targets 0.32% 1.08% 0.29% 1.08% 0.29% 12.57% 0.39% 22.61% 0.39% 1.47% 0.21% 0.39% 1.41% 20.30% 0.29% 1.21% 0.39% 0.29% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.39% 0.21% 0.38% 0.38% 0.18% 0.18% 0.24% 0.34% 0.24%0.24% 0.24% 0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.	% of Background           0.00%           0.16%           0.00%           4.94%           8.33%           0.01%           17.11%           1.80%           0.26%           0.42%           0.00%           0.42%           0.00%           0.32%           15.78%           0.01%           0.77%           0.01%           0.03%           0.03%           0.03%           0.03%           0.03%           0.00%           0.01%           0.03%           0.01%           0.03%           1.70%           9.65%           0.01%           0.01%           0.01%           0.01%           0.01%           0.11%           0.28%           1.10%           0.22%           3.37%           0.11%           0.02%           1.10%           0.27%           9.17%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (145.6bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           183.2bp (145.8bp)           183.2bp (146.7bp)           183.2bp (145.8bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           141.7bp (141.7bp)           145.2bp (144.5bp)           150.1bp (144.2bp)           150.1bp (144.2bp)           160.4bp (142.1bp)           160.2bp (142.5bp)           162.2bp (145.2bp)           163.2bp (146.2bp)           133.2bp (146.2bp)           133.2bp (146.2bp)           133.2bp (146.2bp)           133.2bp (145.1bp)           133.2bp (145.1bp)           133.2bp (145.1bp)           133.2bp (145.5bp)           133.2bp (145.5bp)           133.2bp (145.5bp)           133.2bp (146.5bp)           133.2bp (146.5bp)           134.2bp (146.4bp)           135.2bp (147.1bp)	Best Match/Details           CDX4(Homeckou/Mys-ChP-Seq(GSE48254)Homer(0.655)           PB00321_IEG20041_11/dspar(0.575)           ZBTB12(Z)/HEK239-ZBTB12_GPP-ChP-Seq(GSE54341)Homer(0.603)           SN402/MA0745_21/sapar(0.633)           ET1V1MA0745_21/sapar(0.632)           ET1V1MA071_21/sapar(0.632)           Nanog(Homeckou/MES-Nanog-CAPI-Seq(GSE11724)Homer(0.749)           VDR:MA0603_21/sapar(0.759)           FOXE (MA1487_1.1/sapar(0.669)           Nex-6_6(wr.2)MA0503_11/sapar(0.659)           PB0032_1_Ppig1_1_Llaspar(0.659)           PB0032_1_Ppig1_1_Llaspar(0.738)           SOX10(MA0422_21/sapar(0.738)           DS0X10(MA0422_21/sapar(0.739)           PB0032_1_Ppig1_1_Llaspar(0.739)           PB0032_1_Ppig1_1_Llaspar(0.730)           PB0032_1_Ppig1_1_Llaspar(0.730)           PB0032_1_PD1_CAPE_22_laspar(0.730)           PB0050_1_DT1_CAPE_22_laspar(0.703)           PD0150_1_FD42_21/sapar(0.703)           FOXA1(Forthead)/MC7+FOXA1-CMP-Seq(GSE28431)Homer(0.780)           TFAE2QVar13(LA14885_Llaspar(0.803)           TFAE2QVar13(LA14885_Llaspar(0.803)           TFAE2QVar13(LA14885_Llaspar(0.803)           TFAE2QVar13(LA1482_Llaspar(0.561)           ZBTB18([2)]/HEX2S-2ATB18(SPC-DIP-Seq(GSE285431/Homer(0.561)           CMEXMA082_Llaspar(0.662)           CDX14MA0872_Llaspar(0.565	NTG or B27 specific yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           23           24           25           26           27           28           290           31           32           33           36           37           38           390           41           42           43           443           44	Motif AGTCGTAACGTGATAGTGAGTAACGTAAGTCAGTCAGGTCAGGTAAGGTAAGTAGTCAGTC	P-value 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-11 1.00E-10 1.00E-10 1.00E-0	log P-pvalue           5.001++01           -4.689++01           -4.689++01           -4.213++01           -4.213++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -3.208++01           -2.860++01           -2.860++01           -2.860++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.878++01           -2.188++01           -1.188++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.838++01           -1.778++01           -1.838++01           -	% of Targets 0.32% 0.22% 0.29% 8.47% 0.29% 8.47% 0.39% 0.29% 1.16% 3.3.9% 1.47% 0.21% 2.03% 0.21% 0.29% 1.2.1% 2.03% 0.29% 1.2.1% 2.03% 0.29% 0.29% 0.29% 0.29% 0.29% 0.29% 0.29% 0.21% 0.22% 0.22% 0.22% 0.22% 0.22% 0.24% 0.24% 0.16% 0.24% 0.18% 0.24% 0.24% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.25% 0.24% 0.	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.42% 0.07% 0.07% 0.01% 0.03% 0.00% 3.94% 0.02% 0.00% 0.01% 0.01% 0.01% 0.01% 0.01% 0.02% 1.10% 0.02% 0.01% 0.02% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00%	STD(8g STD)           186.6bp (131.7bp)           187.6bp (136.4bp)           187.6bp (136.4bp)           182.8bp (36.4bp)           182.8bp (36.4bp)           182.8bp (145.6bp)           183.2bp (144.5bp)           183.2bp (144.5bp)           183.2bp (144.7bp)           183.2bp (144.7bp)           141.9bp (145.4bp)           140.1bp (145.2bp)           140.2bp (142.5bp)           140.2bp (142.5bp)           140.2bp (142.5bp)           150.4bp (36.3bp)           152.2bp (145.2bp)           152.2bp (145.2bp)           153.2bp (146.2bp)           154.2bp (145.2bp)           152.2bp (145.2bp)           153.2bp (146.2bp)           124.2bp (145.2bp)           125.2bp (145.2bp)           126.2bp (145.2bp)           126.2bp (145.2bp)           126.2bp (146.2bp)           127.2bp (122.7bp)           138.0bp (0.0bp)           139.2bp (141.2bp)           130.2bp (142.7bp)           132.2bp (142.7bp)           132.2bp (142.7bp)           132.2bp (142.7bp)           132.2bp (142.7bp)           134.2bp (144.3bp)           142.2bp (144.3bp)	Best Match/Details           CDX4(Homeobox)Myby: CNP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C0041.1(Jaspar(0.655)           ZBTB12(ZI)/HEX23-ZBTB12.CFP-CNIP-Seq(GSE58341)Homer(0.603)           SNA02MA0745.2/Jaspar(0.653)           ETV1MA0715.2/Jaspar(0.653)           FV1VMA0715.2/Jaspar(0.650)           FV1VMA0715.2/Jaspar(0.650)           Nanog(Homeobox)/InES-Nanog-CNP-Seq(GSE11724)/Homer(0.749)           VDR1A06033.1/Jaspar(0.650)           P60025.1.7p8(1_1.2p8(1_2.3)           P60025.1.7p8(1_1.1/Jaspar(0.650)           P60025.1.7p8(1_1.1/Jaspar(0.730)           P80055.1.0p1(1_1.1/Jaspar(0.730)           P80055.1.0p1(1_1.1/Jaspar(0.730)           P80055.1.0p1(1_1.1/Jaspar(0.730)           P80055.1.0p1(1_1.1/Jaspar(0.730)           P80165.1.1Abspr(10.730)           P80165.1.1Abspr(10.750)           P0140.1.1P80(1_Jaspr(0.660)           TFA222(ar.3)MA0815.1.Jaspr(0.660) <td>NTG or B27 specific yes yes ro yes yes yes yes yes yes yes ro yes ro yes yes yes yes yes yes yes yes yes yes</td>	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes ro yes ro yes yes yes yes yes yes yes yes yes yes
B27	Rank           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           20           21           22           23           24           25           26           30           312           32           33           34           35           36           37           38           40           412           43           45	Metil AGTCGTAACGTAGTAGCTAACTGAGTCACTGGTCAGCGTAGCTGAGCTAGCT	P-value (0.002-21 0.002-20 1.002-10 1.002-00 1.002-	log P-pvalue           -5.001+01           -5.001+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.689e+01           -4.213e+01           -4.213e+01           -3.298e+01           -3.298e+01           -3.298e+01           -3.298e+01           -3.056e+01           -2.660e+01           -2.660e+01           -2.656e+01           -2.656e+01           -2.256e+01           -2.256e+01           -2.256e+01           -2.266e+01           -2.128e+01           -1.828e+01           -1.828e+01           -1.828e+01           -1.776*01           -1.778*04           1.338e+01           -1.33	% of Targets 0.32% 0.29% 0.29% 0.29% 0.29% 0.29% 0.29% 0.39% 2.261% 1.257% 0.39% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.39% 0.24% 0.39% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24% 0.24%0.24% 0.24% 0.24% 0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.24%0.2	% of Background           0.00%           0.16%           0.00%           4.94%           8.33%           0.01%           17.11%           1.80%           0.26%           0.42%           0.00%           0.42%           0.00%           0.32%           0.32%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.03%           0.11%           0.03%           1.170%           0.03%           1.12%           0.11%           0.02%           3.37%           0.11%           0.02%           3.37%           0.01%           0.02%           3.37%           0.01%           0.01%           0.01%           0.01%	STD(8g STD)           186.6bp (131.7bp)           128.7bp (136.4bp)           127.8bp (36.4bp)           128.2bp (36.4bp)           128.2bp (145.8bp)           138.2bp (145.8bp)           138.2bp (145.8bp)           138.2bp (145.8bp)           138.2bp (144.7bp)           141.7bp (144.7bp)           141.7bp (144.7bp)           145.2bp (144.8bp)           150.1bp (145.2bp)           150.1bp (145.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (145.2bp)           162.2bp (146.2bp)           142.2bp (145.2bp)           142.2bp (145.2bp)           142.2bp (145.2bp)           142.2bp (145.2bp)           142.2bp (146.2bp)           142.2bp (146.2bp)           142.2bp (146.2bp)           142.7bp (146.2bp)           153.2bp (151.2bp)           155.3bp (151.2bp)           155.3bp (151.2bp)           155.3bp (151.2bp)           154.2bp (151.2bp)           155.3bp (145.4bp)           154.2bp (151.2bp)           155.3bp (145.4bp)           155.3bp (151.2bp)           154.2bp (146.2bp)	Best Match/Details           CDX4(Homedool/Myc-ChP-Seq(GSE34825(H-Homer(0.655))           PB0032 1, IfScor081, 1.(Jaspar(0.6750)           ZBTB12(ZI)+EK233-ZBTB12, CFP-ChP-Seq(GSE54341)/Homer(0.603)           SNAU2/MA0745 2./Jaspar(0.633)           ETV1(MA0715 2./Jaspar(0.632)           Nanog(Homedool/MFS-Nanog-CAPF-Seq(GSE11724)/Homer(0.749)           VDR:MA053 2./Jaspar(0.632)           PB0023 1, IFSCOP 2./Jaspar(0.635)           PB0023 1, ZP091 - 2./Jaspar(0.655)           PB0023 1, ZP091 - 2./Jaspar(0.655)           PB0023 1, ZP091 - 2./Jaspar(0.739)           SOX10(MA042 - 2./Jaspar(0.739)           SOX10(MA042 - 2./Jaspar(0.739)           PB0023 1, ZP091 - 2./Jaspar(0.739)           SOX10(MA042 - 2./Jaspar(0.739)           PB0023 1, ZP041 - 2./Jaspar(0.739)           PB0025 1, LP1 - 1./Jaspar(0.739)           PB0025 1, LP1 - 1./Jaspar(0.730)           PB0025 1, LP1 - 1./Jaspar(0.730)           PB0035 1, LP1 - 2./Jaspar(0.730)           PD0150 1, TRA62 - 2./Jaspar(0.676)           PB0052 - 2.7713 - 2.771 - 2.771-2.771-2.771           PKB263 - 2.771 - 2.771 - 2.771 - 2.771 - 2.771           PKB27 - 2.771 -	NTG or B27 specific yes yes ros yes yes yes yes yes yes yes yes yes ye
827	Rankk           1           2           3           4           5           6           7           8           9           10           11           12           13           14           15           16           17           18           19           20           21           22           23           24           25           26           27           28           29           30           31           32           34           35           36           37           38           390           41           42           43           44           45           46	Motif AGTCGTAACGTGGTACGTAGGTAACGTAGGTCAGTGGTCAGGTAGGT	P-value 1.00E-20 1.00E-10 1.00E-10 1.00E-10 1.00E-10 1.00E-11 1.00E-10 1.00E-0	log P-pvalue           5:001++01           -4:001++01           -4:002++01           -4:002++01           -4:213++01           -4:213++01           -3:4702++01           -3:4702++01           -3:4702++01           -3:4702++01           -3:4702++01           -3:3054++01           -3:3054++01           -3:3054++01           -2:802++01           -2:802++01           -2:802++01           -2:802++01           -2:802++01           -2:802++01           -2:2556++01           -2:2556++01           -2:2556++01           -2:2556++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -2:162++01           -1:262++01           -1:830++01           -1:830++01           -1:830++01           -1:830++01           -1:332++01           -1:332++01           -1:332++01           -1:332++01	% of Targets 0.32% 0.22% 0.29% 8.47% 0.29% 8.47% 12.57% 3.39% 2.2.61% 3.39% 1.47% 0.39% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.47% 0.29% 1.63% 0.29% 1.63% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.21% 0.24% 0.18% 0.24%0.24% 0.24% 0.24%0.24% 0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.24%0.24% 0.	% of Background 0.00% 0.16% 0.16% 0.00% 4.94% 8.33% 0.01% 1.80% 0.26% 0.42% 0.42% 0.42% 0.00% 0.32% 15.78% 0.07% 0.07% 0.07% 0.07% 0.07% 0.02% 39.94% 0.00% 0.00% 0.02% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.02% 0.01% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00% 0.01% 0.00	STD(8g STD) 186.8bp (131.7bp) 175.7bp (136.4bp) 175.7bp (136.4bp) 128.8bp (36.4bp) 128.8bp (36.4bp) 132.8bp (145.8bp) 135.2bp (145.8bp) 132.2bp (144.7bp) 143.2bp (144.7bp) 143.8bp (144.7bp) 143.8bp (144.7bp) 140.1bp (145.2bp) 140.1bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 140.2bp (145.2bp) 150.4bp (35.2bp) 152.2bp (150.7bp) 153.3bp (146.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.8bp (146.2bp) 133.4bp (146.2bp) 133.4bp (146.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 142.7bp (145.2bp) 135.0bp (130.7bp) 135.2bp (131.7bp) 135.2bp (131.7bp) 132.2bp (148.7bp) 134.2bp (144.8bp) 144.2bp (147.7bp) 134.2bp (145.7bp) 134.2bp (145.7bp) 134.2bp (144.7bp) 142.2bp (148.7bp) 143.2bp (144.7bp) 143.2bp (144.7bp) 145	Best Match/Details           CDX4(Homeotox)/Myc-CNP-Seq(GSE48254)Homer(0.655)           PB0932.1.18C0041.1/Lisapar(0.655)           ZBTB12(ZI)/HEX23-ZBTB12.GFP-CNIP-Seq(GSE58341)Homer(0.603)           SNM2/MA0745.2/Lisapar(0.851)           ETV1MA0715.2/Lisapar(0.852)           Nanog(Homeotox)/IES-Nanog-CXIP-Seq(GSE1724)Homer(0.769)           VDR1Av0632.2/Lisapar(0.851)           PB0025.1.7Ped[1.4/Lisapar(0.852)           PB0025.1.7ped[1.4/Lisapar(0.355)           PB0025.1.7ped[1.4/Lisapar(0.356)           DS0120.1.7ped[1.4/Lisapar(0.356)           DS0120.1.7ped[1.4/Lisapar(0.739)           ZVF34/MA142.2/Lisapar(0.739)           ZVF34/MA142.2/Lisapar(0.739)           ZVF34/MA1442.2/Lisapar(0.739)           ZVF34/MA1442.2/Lisapar(0.739)           ZVF34/MA1442.2/Lisapar(0.739)           ZVF34/MA1442.2/Lisapar(0.739)           PB0050.1.Cort.1.1/Lisapar(0.739)           PD0160.1.TArkd.2.2/Lisapar(0.761)           PD1600.1.TArkd.2.2/Lisapar(0.765)           FDX34/MC471-CVA1-PS-sqc(GSE28331)Homer(0.763)           PD1601.1.TArkd.2.2/Lisapar(0.676)           PD1601.1.TArkd.2.2/Lisapar(0.676)           FD2X24/ForkMA1115.1.Lisapar(0.660)           FD424/FA11.15.1.Compare(0.656)           CDX1MA0782.1.Lisapar(0.660)           FD4242.1.Lisapar(0.660)           F	NTG or B27 specific yes yes ro yes yes yes yes yes yes yes yes yes yes

**Supplementary Table 12.** ChIPseq adult TF binding scanning related to NTG (upper panel) and B27 (lower panel) SE; Homer

Supplementary Figure 1.



**Supplementary Figure 1.** Single cell suspension was obtained from mLN of 3-5 mo-old NTG rats. Phosphorylated STAT1 MFI was assessed by flow cytometry in naive CD4<sup>+</sup> T cells after IL-27 stimulation for 10 minutes, in the presence or not of STAT1 transcriptional activator 2NP at various concentrations. Histograms correspond to the mean  $\pm$  SEM of 4 rats. (**A**) Representative dot plot of pSTAT1 expression by CD4<sup>+</sup> T cells, without stimulation, or after IL-27 stimulation with or without STAT1 activator incubation for 1h, at 50µM. (**B**) Histograms correspond to the mean  $\pm$  SEM of 4 rats. Data are shown as the mean  $\pm$  SEM of 4 independent experiments. p-value was calculated by paired t test; \*\* p<0.01.

#### **ARTICLE ORIGINAL (1)**

Supplementary Figure 2.



**Supplementary Figure 2.** Cells were sorted from (**A**,**B**) 3-4 wk-old premorbid B27-rats (n = 4 to 12) or (**C**) 3-9 moold B27-rats with established rat-SpA (n = 3) and age-matched NTG littermates. (**A**,**C**) Cocultures of splenic cDC2 with mLN naive CD4<sup>+</sup> T cells were performed in the presence of soluble anti-TCR $\alpha\beta$  mAb. (**A**) After 6 d, IL-17A concentration was assessed by ELISA in supernatants. (**B**) The absolute number of mLN cells was counted *ex vivo*; the count of naive and effector CD4<sup>+</sup> T cells was estimated after *ex vivo* cell sorting. (**C**) After 3 d, CD4<sup>+</sup> T cells were analyzed by flow cytometry for cell-surface expression of CD25 and ICOS, and intra-cellular expression of TNF $\alpha$  and IL-17A. Data are gated on live CD4<sup>+</sup> T cells that divided. (**A-C**) Histograms correspond to the mean ± SEM. Each dot represents an independent coculture. p-values were calculated by (**A**,**C**) 2-way Anova or (**B**) unpaired t-test; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

#### Supplementary Figure 3.



**Supplementary Figure 3.** mLN naive CD4<sup>+</sup> T cells were sorted from (**A**,**B**) 3-4 wk-old premorbid B27-rats (n = 4 to 6) or from (**C-E**) 3-9 mo-old B27-rats with established rat-SpA (n = 3 to 6). Age-matched (**A-E**) NTG littermates or (**E**) B7-rats were used as controls. Naive CD4<sup>+</sup> T cells were cultured in anti-CD3 coated wells, in the presence of soluble anti-CD28 mAb. (**A**,**C**) After 3 d, cells were analyzed by flow cytometry for survival (proportion of cells that did not express the Live-Dead staining) and proliferation (proportion of cells that had diluted the CTV staining). (**B**) After 6 d, IL-17A concentration was assessed by ELISA in supernatants. (**D**,**E**) After 3 d, cells were analyzed by flow cytometry for cell surface expression of CD25 and ICOS, and intra-cellular expression of TNF $\alpha$  and IL-17A. Data are gated on live CD4<sup>+</sup> T cells that had divided. Histograms correspond to the mean ± SEM of 3 to 8 rats per group. p-values were calculated by unpaired t test; \* p<0.05, \*\* p<0.01.

#### Supplementary Figure 4.



**Supplementary Figure 4.** RNA-seq was performed on naive CD4<sup>+</sup> T cells isolated from mLN of 3-mo old B27-rats with established rat-SpA, and age-matched NTG littermates (n = 7 per group). (**A**) Volcano plot displaying log<sub>2</sub> fold-change versus -log<sub>10</sub> adjusted p value for each gene expressed in naive CD4<sup>+</sup> T cells from B27-rats compared to NTG littermates. Differentially expressed genes involved in IFN/Th1 and Th17/IL-1 pathways are labeled in boxes. (**B**) The graph shows the top 20 IPA terms, which were enriched in genes differentially expressed in adult B27-rats naive CD4<sup>+</sup> T cells, as compared to NTG littermates T cells. The z-score refers to the activated (red), inhibited (blue) or undefined (white) state of the corresponding pathway in B27-rats relative to NTG littermates. Adjusted p values are expressed in -log.



**Supplementary Figure 5.** ChIP-seq was performed with Abs specific for H3K4Me3 and H3K27Ac histone marks on mLN naive CD4<sup>+</sup> T cells sorted from (**A,B,D-F**) 3 mo-old B27-rats with established rat-SpA (n = 3), or from (**C,G**) 3 wk-old premorbid B27-rats (n = 2). Age-matched (**A-C,E-G**) NTG littermates and (**D**) B7-rats were used as controls. (**A**) Graphs show the distribution across the different genomic features of H3K4Me3 (left panel) and H3K27Ac peaks (right panel) in naive CD4<sup>+</sup> T cells. 1 representative of 3 dataset. (**B**) Volcano plots displaying log fold-change versus

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-log<sub>10</sub> adjusted p value for each H3K4Me3 peak (left panel) and H3K27Ac peak (right panel) enriched in naive CD4<sup>+</sup> T cells from B27-rats, as compared to NTG littermates T cells. Peaks considered as significantly differentially enriched (adjusted p < 0.05) are labeled in pink. (**C**) Integrated genome viewer snapshots of representative gene involved in Th17 differentiation (*ll1r1*) comparing premorbid B27-rat and NTG littermate. Graphed dataset from 1 pair of B27/NTG rats, representative of 2. (**D**) Integrated genome viewer snapshots of genes involved in Th17 differentiation (*ll1r1*) and IFN pathways (*Usp18*). Graphed dataset from 1 trio of B27/NTG/B7 rats, representative of 2. (**E**) Graph showing SE of B27-rats (left) naive CD4<sup>+</sup> T cells, as compared to those of NTG littermates (right). SE-related genes involved in IFN (blue) and Th17 (red) pathways are labeled. (**F**) Violin plot showing the mRNA normalized count extracted from RNA-seq data (expressed in log<sub>10</sub>) of genes located at proximity of typical enhancers as compared to those at proximity of SE. Median (thick line) and quartile (dotted line). p value was calculated by unpaired t test; \*\*\*\* p<0.0001. (**G**) The graph shows the 4 KEGG pathways enriched for TF predicted to bind on B27-SE of naive CD4<sup>+</sup> T cells. Adjusted p values expressed in -log<sub>10</sub>.



#### Supplementary Figure 6.

**Supplementary Figure 6.** Single cell suspension was obtained from mLN of (**A**,**C**) 3-12 mo-old or (**B**) 3 wk-old B27rats and NTG littermates, and (p)STAT MFI was assessed by flow cytometry in naive CD4<sup>+</sup> T cells. (**A**) Total STAT5 (left panel) and STAT6 (right panel). (**B**,**C**) Phosphorylated STAT3 MFI, after IL-6 stimulation for 10 minutes. Histograms correspond to the mean ± SEM of 6 to 12 rats per group. p-values were calculated by paired t test.

#### Supplementary Figure 7.



**Supplementary Figure 7.** IFN/Th1 and Th17-related genes mRNA expression was assessed by q-RT-PCR in CD4<sup>+</sup> single-positive (CD4SP) and/or CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) thymocytes sorted from the thymus of (**A-D**) 3 wk-, or (**A,C**) 10 d-old B27-rats. Age-matched (**A-C**) NTG littermates and (**D**) B7-rats were used as controls. Data are shown as the mean  $\pm$  SEM of 5 to 8 rats per condition. p-values were calculated by unpaired t test; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.

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#### Supplementary Figure 8.



**Supplementary Figure 8.** Naive CD4<sup>+</sup> T cells were sorted from the PBMC of axial SpA patients and controls. (**A**) STAT1 and STAT3 proteins MFI was assessed by flow cytometry in SpA patients (n = 39), RA patients (n = 8) and HC (n = 34). (**B**) *STAT3* gene mRNA expression was assessed by RT-q-PCR in SpA patients (n = 28), RA patients (n = 8) and HC (n = 16). Data are the mean ± SEM. p-values were calculated by 1-way Anova; \* p<0.05, \*\* p<0.01.

# TRANSITION

#### Article 1 : Perspectives

Le principal résultat mis en évidence dans ce travail est qu'il existe un défaut précoce de STAT1, à l'échelle protéique et transcriptomique, dans les lymphocytes T CD4<sup>+</sup> naïfs, et leurs précurseurs thymiques, des rats-B27. Le défaut de la voie STAT1 a un effet sur le biais de différenciation Th17. Ceci pourrait s'expliquer par une balance perturbée avec l'expression et l'activité de STAT3, TF inter-régulé avec STAT1, dont l'augmentation apparaît secondairement, concomitant au développement de la maladie (Hirahara et al., 2015).

Fait essentiel, des travaux antérieurs ont révélé que la cDC2 du rats-B27 présentait un défaut similaire de la voie de l'IFN, incluant *Stat1* (Fert et al., 2014b). Dans le cas de la cDC2, le défaut de cette voie est associé à une baisse d'expression d'IL-27 et d'IL-10 qui pourraient contribuer au biais de différenciation Th17 (Breban et al., 2021b; Fert et al., 2008b; Talpin et al., 2014b). Ceci suggère que l'expression du HLA-B27 conduirait à un défaut de la voie STAT1 dans différents sous-types cellulaires – affectant leur homéostasie respective, par des mécanismes inconnus, mais à l'issue commune.

Toutefois, avant de chercher à déterminer les mécanismes moléculaires reliant l'expression du HLA-B27 et le défaut de la voie STAT1, un enjeu central est de confirmer que l'induction de STAT1 *in vitro* et *in vivo* favorise la réversion du biais de différenciation Th17.

#### Article 2 : Induire STAT1 avec l'IL-27

Dans cette perspective, nous nous sommes intéressés à l'IL-27, cytokine capable d'induire STAT1 (**Figure 10**) (Yoshida and Hunter, 2015). Ce choix était justifié par des travaux précédents de notre équipe révélant le défaut de la voie IFN au sein de la cDC2 du rat-B27, qui était associé à un défaut d'expression de l'*Il27* et de l'*Il10* (Fert et al., 2014b).

L'IL-27 est un hétérodimère, composé d'une sous-unité p28 (ou IL-30) codée par le gène *IL27A* et de la protéine EBI3 ; elle est principalement sécrétée par les APC, dont les DC. Sa production dépend en partie de la voie JAK-STAT, notamment induite par la fixation des IFN de type I ou de type II sur leurs récepteurs respectifs. La production d'IL-27 peut également être induite lors de l'activation de la voie NFKB. L'IL-27 se fixe à un récepteur hétérodimérique, composé d'une sous-unité gp130 et IL27R $\alpha$ .

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Lorsqu'elle est libérée par la DC au cours de l'activation lymphocytaire T CD4<sup>+</sup>, elle favorise l'activation de la voie JAK et la phosphorylation de STAT1 et dans une moindre mesure celle de STAT3. L'activation de STAT1 induit à son tour un programme transcriptionnel de type Th1, marquée par l'expression du TF T-bet et la production d'IFNγ (Takeda et al., 2003). Par ailleurs, l'IL-27 a la propriété de favoriser la production d'IL-10, cytokine anti-inflammatoire. Enfin, en inhibant la transcription de RORγt et ROR $\alpha$ , l'IL-27 inhibe également la production d'IL-17 et la différenciation Th17 (Diveu et al., 2009; Hirahara et al., 2012; Murugaiyan et al., 2009; Villarino et al., 2010).

Ayant ce triple intérêt de favoriser l'induction de STAT1 et un programme Th1, en inhibant concomitamment la différenciation Th17, tout en potentialisant la production d'IL-10, l'IL-27 nous a donc semblé être une cytokine de choix pour étudier la physiopathologie du biais de différenciation Th17 et ouvrir de nouvelles perspectives thérapeutiques. Dans ce deuxième article, nous nous sommes donc attachés à caractériser les effets *in vitro* de l'IL-27 sur la fonction/différenciation des lymphocytes T CD4<sup>+</sup> de rats-B27 et de patients atteints de SpA et *in vivo* sur le développement de la SpA-rat.



Figure 10. Interleukine 27

L'interleukine (IL)-27 est produite par les cellules dendritiques, suivant l'activation de la voie JAK/STAT1 par les interférons (IFN) de type I et II. L'IL-27 sécrétée lors de la stimulation du lymphocyte T CD4<sup>+</sup> naïf, favorise la phosphorylation de STAT1, qui induit un programme de type T helper 1, caractérisé par l'expression du facteur transcriptionnel T-bet, qui favorise la production d'IFNy. L'IL-27 favorise de plus la production d'IL-10, cytokine anti-inflammatoire.

ARTICLE 2 – Effets immunomodulateurs de l'interleukine 27 *in vitro* et *in vivo* au cours de la spondyloarthrite

# Article 2 : Manuscrit

# Interleukin 27 is a novel cytokine with anti-inflammatory effects against spondyloarthritis through the suppression of Th-17 responses

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#### ABSTRACT

Spondylarthritis (SpA) development in HLA-B27/human β2-microglobulin transgenic rat (B27rat) is correlated with altered dendritic cell (DC) function that promotes a biased expansion of pro-inflammatory Th<sub>17</sub> cells and imbalance of regulatory T cells cytokines profile. DCs from B27-rat underexpress IL-27, an anti-inflammatory cytokine which boosts regulatory T cells and inhibits Th<sub>17</sub> cells. Here, we investigated whether addition of exogenous IL-27 could reverse the inflammatory pattern observed in SpA. *In vitro* addition of IL-27 to cocultures of DC and CD4<sup>+</sup> T cell subsets from B27-rats reduced IL-17 and enhanced IL-10 production by T cells. Likewise, IL-27 inhibited the production of IL-17 by T cells from SpA patients. *In vivo* treatment with IL-27 inhibited SpA development in B27-rat through the suppression of IL-17-producing CD4<sup>+</sup> T helper cells. Our results reveal a potent inhibitory effect of IL-27 and highlight this cytokine as a promising new therapeutic target in SpA.

Keywords: Ankylosing spondylitis; Dendritic cell; HLA-B27; JAK; IL-10; IL-27; Spondyloarthritis.

# INTRODUCTION

Spondyloarthritis (SpA) is a group of chronic inflammatory disorders that primarily affect axial skeleton (i.e. spine and sacroiliac joints). These cardinal features often combine with peripheral joint arthritis, enthesitis, dactylitis and specific extra-articular manifestations, i.e. uveitis, psoriasis or inflammatory bowel disease. It affects between 0.2% and 1.61%, of the population and is strongly associated with the class I major histocompatibility complex (MHC) antigen HLA-B27<sup>1,2</sup>. This association was described almost 50 years ago but the mechanisms underlying induction of SpA by HLA-B27 still remain largely unexplained<sup>3</sup>.

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The 33-3 line of HLA-B27/human  $\beta$ 2-microglobulin (h $\beta$ 2m) transgenic rats (B27-rat) has proven to be a thorough model of SpA since it develops a spontaneous inflammatory disorder combining arthritis with ulcerative colitis and psoriatic skin and nail lesions, in the presence of regular microbiota<sup>4</sup>. In this animal model, cell transfer experiments have demonstrated a fundamental role of bone-marrow derived myeloid cells expressing the HLA-B27/h $\beta$ 2m transgene and CD4<sup>+</sup> T cells in disease development<sup>5,6</sup>. Subsequent studies revealed a correlation between several dysfunctions of dendritic cells (DCs) and susceptibility to SpA<sup>7,8</sup>.

We previously showed that DCs from B27-rat promoted a biased expansion of proinflammatory T helper 17 (Th<sub>17</sub>) cells, which presumably participate to disease development<sup>9</sup>. We also reported altered DC–T cell interaction, which resulted in a decreased production of interleukin (IL)-10 and heightened production of IL-17 by CD4<sup>+</sup> regulatory T cells (Treg)<sup>10</sup>. Altered conventional type 2 DC (cDC2) functions from B27-rat were notably characterized by a defect in interferon-gamma (IFN- $\gamma$ ) signaling and a deficiency of IL-10 and IL-27 production, all of which could contribute to biased T cell stimulation<sup>11</sup>.

IL-27 is an heterodimeric member of the IL-12/IL-23 family of cytokines composed of two subunits: IL-27p28 (also known as IL-27a or IL-30) and Epstein-Barr virus induced gene 3 (EBI3 also known as IL-27b)<sup>12</sup>. It is mainly produced by activated antigen presenting cells (APCs), including DCs, and binds to IL-27R receptor also composed of two subunits: WSX-1 (alternatively named IL-27R $\alpha$  or TCCR) and gp130 (IL-6R $\beta$ ). IL-27R is expressed by numerous cell populations such as T and B cells, DCs, monocytes and endothelial cells<sup>13,14</sup>. IL-27 binding to its receptor induces activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and p38 mitogen-activated protein kinase (MAPK) pathways<sup>15</sup>.

Early studies showed that IL-27 may act as a pro-inflammatory cytokine by inducing Th<sub>1</sub> responses<sup>12</sup>. Hence, IL-27 increased T-bet expression, a Th<sub>1</sub>-specific transcription factor, and

induced IFN- $\gamma$  production by naïve T cells<sup>16</sup>. However subsequent studies revealed a more complex role of IL-27 in the regulation of T cell responses. Hence, it was shown that IL-27 could inhibit the differentiation of Th<sub>17</sub> cells by antagonizing the expression of the Th<sub>17</sub> master transcription factor, retinoic acid receptor-related orphan receptor gamma t (ROR $\gamma$ t)<sup>17,18</sup>. IL-27 was also able to induce the differentiation of type 1 Treg (Tr<sub>1</sub>) which produce IL-10 and exert immunosuppressive properties on Th<sub>17</sub> cells<sup>19,20</sup>.

Thus, we speculated that deficient production of IL-27 by DCs from B27-rat could be a key element in the emergence of Th<sub>17</sub>-driven inflammatory disease in this model. Hence, several factors that are inducible by IL-27 in Treg and directly implicated in IL-10 production by those cells, such as c-Maf, aryl hydrocarbon receptor and IL-21, were down-regulated in B27-transgenic rat Treg, consistent with such an interpretation<sup>10</sup>. Noteworthy, polymorphisms of the *IL27A* gene coding for the p28 subunit of IL-27 have been associated with SpA predisposition<sup>21</sup>. In this study our objective was to investigate the effect of IL-27 on SpA, using the B27-rat model, to determine if this cytokine would be a suitable therapy target for SpA.

# **METHODS**

**Rats.** The 33-3 B27-rats bearing 55 copies of HLA-B\*2705 and 28 copies of  $h\beta_2 m$  on a Fisher (F344) background were bred and maintained under conventional conditions, as previously described. Nontransgenic (NTG) F344 littermates were used as controls. Inducible costimulatory molecule (ICOS) knockout (ICOS<sup>-/-</sup>) Sprague-Dawley (SD) rats were produced by Dr. Ignacio Anegon, using the Talen technology <sup>22</sup>. ICOS<sup>-/-</sup> B27-rats were obtained by back-crossing ICOS<sup>-/-</sup> SD rats onto 33-3 rats for four generations. Age-and sex-matched rats (2-8
months) were used in each experiment. Study procedures were approved by the institutional animal experimentation ethical committee (APAFIS-8910).

Cell culture reagents and monoclonal antibodies. Cell culture medium was RPMI 1640 with Glutamax I (Life Technologies), supplemented with 10% fetal calf serum, streptomycin and penicillin (100 $\mu$ g/mL), 2% sodium pyruvate, 0.05 mM  $\beta$ -mercaptoethanol (only rat cells culture) and 10 mM HEPES (complete medium). Mouse recombinant (mr) IL-27, rat recombinant (rr) IL-6, IL-1β, IL-23 and human recombinant (rh) transforming growth factor-beta (TGF-β) and IL-27 were from R&D Systems. Rr and Rh IL-2 was purchased from Immunotools and Miltenyi, respectively. Purified rrIL-27 was originally produced and provided by Dr. Ahmed El Marjou from Recombinant Protein Production Facility-Curie Institute. High scale production was achieved through the service of a private company, PX-therapeutics. Mouse anti-rat CD3 and anti-rat CD28, mouse anti-human CD28 and fluorochrome-conjugated mouse anti-rat CD4 and anti-rat IFN-y were from BD Pharmingen. Flurorochrome-conjugated mouse anti-rat CD62L, ICOS, IL-17 and CD25 were from eBioscience. Mouse anti-human CD3 was from Eurobio-Mabtech. Antibodies neutralizing rat IFN-y, IL-4 and IL-10 were from eBioscience, BD Pharmingen and R&D Systems, respectively. Mouse monoclonal antibodies to human/mouse STAT1 phosphorylated at tyrosine 701 and STAT3 phosphorylated at tyrosine 705 were from BD Pharmingen. Phorbol myristate acetate (PMA), ionomycin, brefeldin A (BFA) and mitomycin C were from Sigma.

**Rat cells preparation.** Single-cell suspensions were prepared from mesenteric or popliteal lymph nodes (LN). Distinct CD4<sup>+</sup> T cell subpopulations were sorted on a FACSAria III sorter (BD Biosciences), using appropriate combinations of antibodies to identify Treg (CD4<sup>+</sup> CD25<sup>high</sup>), resting T effector (Teff) cells (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup>), activated Teff cells (CD4<sup>+</sup> CD25<sup>low</sup> CD62L<sup>-</sup>), activated T cells (CD4<sup>+</sup> CD25<sup>+</sup>) and naïve T cells (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>+</sup>). Forkhead box P3 (FoxP3)

staining using A700 anti-mouse Foxp3 was performed according to manufacturer's instructions (eBioscience) and the proportion of FoxP3<sup>+</sup> Treg among CD4<sup>+</sup> CD25<sup>high</sup> T cells was assessed by flow cytometry, showing that the majority (>90%) of them were FoxP3<sup>+</sup>. CD103<sup>+</sup> DCs were obtained from spleen as previously described<sup>23,24</sup>. Briefly, spleens were digested with Collagenase D (2mg/mL; Roche Diagnostics). After centrifugation on a 14.5% Nycodenz gradient (Nycomed), positive selection was performed by magnetic cell sorting with anti-rat CD103 microbeads with an AutoMacs Pro Separator (Miltenyi Biotec). The cDC2 were sorted from the purified CD103<sup>+</sup> DCs based on CD4<sup>+</sup> staining, on a FACSAria III sorter (BD Biosciences).

**Culture of rat CD4<sup>+</sup> T cells.** Flow cytometry-sorted activated CD4<sup>+</sup> CD25<sup>+</sup> T cells or resting CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup> Teff cells were cultured ( $10^5$  cells/well) in anti-CD3-coated (2 µg/mL) plates in the presence or not of anti-CD28 (1µg/mL) and mrIL-27 (5ng/mL). In some experiments flow cytometry-sorted activated CD4<sup>+</sup> CD25<sup>+</sup> T cells were stimulated with coated-anti-CD3 (2 µg/mL) in the presence of JAK inhibitor (tofacitinib -anti-JAK1/3- 60 mM/mL or AG490 -anti-JAK2- 10 µg/mL). After 3 days, supernatants were collected to analyze cytokines levels by enzyme-linked immunosorbent assays (ELISA) and cells were restimulated for 4 hours with PMA (10 ng/mL), ionomycin (1 µg/mL) and BFA (2 µg/mL) for intracellular cytokine staining assay performed according to manufacturer's instructions (Sigma). Stained cells were analyzed with a BD LSR Fortessa cell analyzer (BD Biosciences) using FlowJo software (TreeStar).

**Coculture of rat CD4**<sup>+</sup> **T cells with DCs.** Flow cytometry-sorted CD4<sup>+</sup> CD25<sup>high</sup> Treg or resting CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup> Teff cells were cultured (10<sup>5</sup> cells/well) with sorted CD103<sup>+</sup>splenic DCs (0.4 x 10<sup>5</sup> cells/well) pre-treated with mitomycin C (0.5 mg/mL) in anti-CD3-coated plates (2  $\mu$ g/mL) in the presence or not of mrIL-27 (5ng/mL). After 3 days, supernatants were collected to analyze cytokine levels by ELISA and cells were restimulated for 4 hours with PMA (10 ng/mL), ionomycin (1  $\mu$ g/mL) and BFA (2  $\mu$ g/mL) for intracellular cytokine staining assay. Stained cells

were analyzed as above. For RNAseq analysis, flow cytometry-sorted CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>+</sup> naive T cells were cultured (10<sup>5</sup> cells/well) with sorted splenic cDC2s (CD103<sup>+</sup> CD4<sup>+</sup>; 10<sup>4</sup> cells/well) and stimulated with coated-anti-CD3 (2 µg/mL) in the presence or not of mrIL-27 (5 ng/mL), AG490 (10 µg/mL) or tofacitinib (60 mM/mL). After 24 h of culture T cells were sorted by flow cytometry for RNA extraction.

**Rat CD4<sup>+</sup> T cell differentiation assays.** For Treg differentiation experiments, flow cytometrysorted naïve CD4<sup>+</sup> T cells (CD25<sup>-</sup> CD62L<sup>+</sup>) were cultured (5 x 10<sup>5</sup> cells/well) with CD103<sup>+</sup> splenic DCs (10<sup>5</sup> cells/well) pre-treated with mitomycin C at 0.5 mg/ml, in the presence of coated-anti-CD3 (2 µg/ml), rhTGF-β (2 ng/ml) and neutralizing anti-IFNγ and anti-IL-4 antibodies (each at 10 µg/mL). For Th<sub>17</sub> differentiation experiments, rrIL-6, rrIL-1β and rrIL-23 (each at 20 ng/mL) were added to the coculture. In some experiments, mrIL-27 (5 ng/mL) was also added to the coculture. At day 3, the medium was replaced by fresh medium supplemented with rrIL-2 (20 ng/mL). At day 6, supernatants were harvested for analysis of cytokine production by ELISA and cells were stimulated with PMA/ionomycin/BFA for intracellular cytokine staining as described above. In some experiments, 6 days after Th<sub>17</sub> differentiation, T cells were sorted by flow cytometry for RNA extraction and RNAseq analysis.

**Human cells preparation and culture.** Peripheral blood mononuclear cells (PBMC) were isolated from healthy controls and HLA-B27<sup>+</sup> SpA patients by Ficoll (GE Healthcare) density gradient centrifugation. The characteristics of SpA patients (all fulfilling Assessment of SpondyloArthritis international Society classification criteria for axial SpA<sup>25</sup>) are shown in Extended Data Table I. Written informed consent was obtained from all subjects before study which was approved by the local ethical committee. Patients were considered as resistant to conventional treatment if they had a Bath Ankylosing Spondylitis disease activity score > 3/10 despite anti-inflammatory drugs and biotherapy usage. CD14<sup>+</sup> cells were first removed from

PBMC by positive magnetic selection, using anti-human CD14 microbeads and AutoMacs Pro Separator (Miltenyi). Then, CD4<sup>+</sup> T cells were magnetically sorted from the CD14<sup>-</sup> fraction using anti-human CD4 microbeads and AutoMacs Pro Separator (Miltenyi) and cultured during 6 days with rhIL-2 (10 ng/mL), soluble anti-CD28 (2 µg/mL) and coated-anti-CD3 (10 µg/mL) in the presence or absence of rhIL-27 (40 ng/mL) in complete medium. At day 6, supernatants were harvested for measure of cytokine levels by ELISA and cells were stimulated with PMA (10ng/mL), ionomycin (1µg/mL) and BFA (2µg/mL) for 6 hours for intracellular cytokine staining.

**Analysis of STAT1 and 3 phosphorylation by flow cytometry.** Single-cell suspensions of rat mesenteric LN (10<sup>6</sup> cells/tube) were stimulated with rmIL-27 (5 ng/mL) or PBS for 10 minutes at 37°C. After fixation during 10 minutes with Fix I Buffer (BD Biosciences) and permeabilization with Perm III buffer (BD Biosciences) for 30 minutes, cells were washed and stained with BD PhosFlow antibodies to pSTAT1 (pY701) and pSTAT3 (pY705) according to the manufacturer's instructions (BD Biosciences)

**Determination of STAT1 and 3 intra-cellular content by flow cytometry.** Total STAT content was determined with anti-STAT1 and anti-STAT3 (BD Biosciences) according to manufacturer's instructions Briefly, cells were stained using appropriate combinations of antibodies to identify Treg, Teff, and activated CD4<sup>+</sup> T cells as described above. After fixation in Fix I Buffer (BD Biosciences) for 10 min and permeabilization with Perm III buffer (BD Biosciences) for 30 min, cells were washed and incubated with anti-STAT1 and STAT3 antibodies for 1h. After extensive washing, the fluorescence was quantified by BD LSR Fortessa cell analyzer.

**Determination of cytokine levels by ELISA.** Rat and human IL-17, IL-10 and IFN-γ levels were determined in culture supernatants by sandwich ELISA (IL-17: eBioscience; IL-10 and IFN-γ: R&D Systems) according to the manufacturer's instructions. Concentrations were calculated according to calibration curves established from serial dilutions of recombinant standards in each assay. The sensitivity threshold of each cytokine assay was 10 pg/mL. Multiplex ELISA assay was performed using Bio-Plex Pro Rat Cytokine Panel (Biorad) according to the manufacturer's instructions.

Rat IL-27 recombinant production. The rrIL27 was prepared using a pTT3-IL-27 construction with 6 histidine signal peptide sequence. For the production the rrIL27, CHO cells were cultured in « FreeStyle™ CHO Expression Medium » (Life Technologies). Cells were thawed and subcultured three times prior to transfection. The transfection was performed using 1 µg of DNA per ml of cells culture. A volume of 2L was transfected. Cell density and viability were monitored regularly using Vi-CELLTM XR automatic cell counter and samples of supernatant collected and stored at 4°C for expression analysis by western blotting in denaturing and reducing conditions on precast Bis-Tris SDS-PAGE 4-12 % (Life Technologies). Once the viability had dropped below 75 % or 8 days after transfection, cell cultures were stopped. The supernatant was collected by centrifugation, filtered on 0.2 µm filter (Pall; PES) and loaded on 5 mL Ni Sepharose Excel prepacked column (GE Healthcare). The protein was eluted in step with PBS + 250 mM imidazole. The pool obtained from Ni Sepharose was dialyzed against sodium phosphate-NaCl buffer. The total protein concentration was determined using Bradford protocol with bovine serum albumin as a standard BCA assay (Pierce) and IL-27specific ELISA assay. To produce sufficient quantities of rrIL-27 for the preclinical assay, we requested the service of a specialized company (PX-therapeutics).

Preclinical assay. Sex-matched B27-rats and NTG F344 littermates (6-8 animals/group) aged of 4-6 weeks were randomized three days before initiation of the treatment, with a permutation table (each cage contained at least one rat from each experimental group). RrIL-27 (5 µg/injection) or vehicle (PBS) were administered intraperitoneally 3 days per week for 10 consecutive weeks (experiment 1) or every day for 12 consecutive weeks (experiment 2). The rats were examined three times per week for clinical symptoms of colitis (loose stools and/or frank diarrhea) and arthritis and were assigned for each of these symptoms a score on a scale ranging from 0 (normal) to 4, as previously described <sup>26</sup>. After 10 or 12 weeks of treatment, the rats were sacrificed. Colonic tissues were fixed in 4% phosphate-buffered formalin embedded in paraffin. Sections (4µm) were prepared and stained with hematoxylin-eosin (H&E). Tissues damages were semi-quantitatively scored blindly, following microscopic scoring criteria as previously described<sup>26</sup>. The hindpaws of rats were excised and fixed in 4% phosphate-buffered formalin. After 24 h of fixation they were immersed for 3 weeks in Decal solution (Serva). After decalcification they were sectioned and stained with H&E. Arthritis severity was quantified histologically, based on synovial hyperplasia, pannus formation, inflammatory cell infiltrate of niches and destruction of articular cartilage and bone. The intracytoplasmic cytokines production was determined by flow cytometry in CD4<sup>+</sup> T cells after PMA/ionomycin/BFA treatment.

**RNA preparation, sequencing, and RNA-seq differential expression analysis.** RNA was extracted from  $10^5 - 2 \times 10^5$  Th<sub>17</sub>-polarizing or naïve CD4<sup>+</sup> T cells cocultured with cDC2s, using the RNeasy Plus Micro Kit (Qiagen). RNA concentrations were obtained using a fluorometric Qubit RNA assay (Life Technologies). The quality of the RNA integrity number (RIN) was determined on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) as per the manufacturer's instructions. To construct the libraries, 1 µg of high-quality total RNA

sample (RIN > 8) was processed using TruSeq Stranded mRNA kit (Illumina) according to manufacturer instructions. Library Quantification Kit for Illumina Libraries (KapaBiosystems) and library profiles were assessed using the DNA High Sensitivity LabChip kit on an Agilent Bioanalyzer. Libraries were sequenced on an Illumina Nextseq 500 instrument using 75 baselengths read V2 chemistry in a paired-end mode. After sequencing, a primary analysis based on AOZAN software (Ecole Normale Supérieure, Paris) was applied to demultiplex and control the quality of the raw data (based of FastQC modules/version 0.11.5). Obtained fastq files were then aligned using STAR<sup>27</sup> algorithm (version 2.5.2b). Reads were then counted using RSEM<sup>28</sup> (v1.2.28) and the statistical analyses on the read counts were performed with the R package DESeq2<sup>29</sup> (v1.22.1) to determine the proportion of differentially expressed genes between two conditions. Differentially expressed genes identified by DESeq2 were submitted to Ingenuity Pathway Analysis (IPA) for pathway analysis using the Ingenuity Knowledge Base (Qiagen Bioinformatics)

**Statistical analysis.** Data are expressed as the mean  $\pm$  SEM. Statistical comparisons between groups were determined by a two-tailed paired Student's *t* test or 2-way analysis of variance (ANOVA) with Tukey's test. *P* values < 0.05 were considered significant.

## RESULTS

#### IL-27 reverses imbalanced production of IL-17 and IL-10 by CD4<sup>+</sup> T cell subsets from B27

#### rats.

Given that  $Th_{17}$  cells are expanded in contact with DCs<sup>9</sup> and that DCs exhibit decreased production of IL-27 in B27-rat, we sought to determine whether exogenous IL-27 could exert antagonistic effect on such T cell bias. Indeed, coculture of resting Teff cells with CD103<sup>+</sup> DCs

from B27-rat in the presence of rmIL-27 resulted in reduced IL-17 production (Fig. 1a). Increased IL-10 level was also detected in supernatants of those cocultures (Fig. 1a). To investigate next the effect of IL-27 on imbalanced production of IL-17 and IL-10 by Treg from B27-rat<sup>10</sup>, we performed cocultures of Treg (CD4<sup>+</sup> CD25<sup>high</sup>) with CD103<sup>+</sup> DCs from B27-rat. Hence, IL-27 addition decreased the percentage of IL-17<sup>+</sup> Treg and IL-17 levels produced in supernatant (Fig. 1b). In parallel, we observed a significant increase of IL-10 production by B27-rat Treg in the presence of IL-27 (Fig. 1b). Analysis by multiplex ELISA assay revealed that IL-17 and IL-10 were the only cytokines significantly modulated by IL-27 among a panel of 11 cytokines (Extended Data Fig. 1).

#### *IL-27 decreases IL-17<sup>+</sup> T cells generation from B27-rat naïve CD4<sup>+</sup> T cells.*

It was previously described that IL-27 can influence T cell differentiation by supporting Th<sub>1</sub> and Tr<sub>1</sub> differentiation and inhibiting Th<sub>17</sub> commitment<sup>14</sup>. Given that DCs from B27-rat favor IL-17 production by Treg<sup>10</sup> and Th<sub>17</sub> expansion<sup>9</sup> we tested if IL-27 could also inhibit *in vitro* differentiation of IL-17 producing-Treg from naïve CD4<sup>+</sup> T cells. A decreased proportion of IL-17<sup>+</sup> T cells was observed in the presence of rmIL-27, after coculture of naïve CD4<sup>+</sup> T cells with CD103<sup>+</sup> DCs under Treg polarizing condition (Fig. 1c). Furthermore, a decreased production of IL-17 and an increased production of IL-10 were observed in coculture supernatants in the presence of IL-27 (Fig. 1c). We observed an increased number of IFN-γ-producing T cells and higher levels of IFN-γ in the supernatant in the presence of IL-27 (Extended Data Fig. 2a) confirming the pro-Th<sub>1</sub> role of IL-27 on the differentiation of naïve CD4<sup>+</sup> T cells. Similarly, when naïve CD4<sup>+</sup> T cells were cocultured with CD103<sup>+</sup> DCs under Th<sub>17</sub>-polarizing conditions, addition of rmIL-27 reduced the proportion of IL-17 producing T cells and IL-17 production in supernatant, increasing significantly IL-10 levels (Fig. 1d). In the latter conditions IL-27 also increased IFN-γ production in culture supernatants (Extended Data Fig. 2b).

#### IL-27 exerts a direct effect on B27-rat CD4<sup>+</sup> T cells independently of DC.

Given that both DC and CD4<sup>+</sup> T cells express a functional IL-27R, we next examined which cell population(s) mediated IL-27 effects. Interestingly, we observed a direct inhibitory effect of IL-27 addition on the production of IL-17 by CD4<sup>+</sup> LN Teff cell population from B27-rat, whether in an activated (CD4<sup>+</sup> CD25<sup>low</sup>; Extended Data Fig. 3a) or resting (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup>; Extended Data Fig. 3b) state, in the absence of DCs. Alike the foregoing results observed for cocultures in the presence of DCs, IL-10 production was also increased in the presence of IL-27 in those conditions, whereas IFN-γ production was decreased (Extended Data Fig. 3a,b). Activated T cells from NTG rats produce little IL-17, as compared to those from B27-rats, however addition of IL-27 to cultures of those cells led also to a significant decrease of this cytokine in culture supernatants (Extended Data Fig. 4).

Moreover, IL-27 also inhibited the generation of IL-17 producing T cells from B27-rat naïve CD4<sup>+</sup> T cells stimulated with soluble anti-CD28 without DCs, in Th<sub>17</sub> polarizing conditions (Extended Data Fig. 5a). In these conditions, IL-27 decreased IL-17 and increased IL-10 and IFN- $\gamma$  productions (Extended Data Fig. 5b). Conversely, IL-27 treatment of DCs before coculture had no effect on the expression of co-stimulatory (CD40, CD86) or MHC (class I and II) molecules nor on the capacity of those cells to expand IL-17<sup>+</sup> T cells (data not shown).

#### Anti-IL-17 effect of IL-27 on B27-rat CD4<sup>+</sup> T cells is independent of IL-10 or ICOS

Given that IL-27 may induce the differentiation of IL-10-producing Tr<sub>1</sub> and inhibit Th<sub>17</sub> cells generation and that increased IL-10 production preceded inhibition of IL-17 in the foregoing *in vitro* studies (data no shown), decreased IL-17 production by CD4<sup>+</sup> T cells from B27-rat could be secondary to induction of IL-10 by IL-27. To test this hypothesis, we studied IL-27 effect on activated CD4<sup>+</sup> CD25<sup>+</sup> T cells from B27-rat cultured in the presence of IL-10-blocking antibody. The presence of anti-IL-10 antibody did not modify the inhibitory effect of IL-27 on IL-17

production (Fig. 2a). Moreover, IL-27 inhibited the generation of IL-17<sup>+</sup> T cells from naïve CD4<sup>+</sup> T cells under Th<sub>17</sub> polarizing conditions to a similar extent when IL-10 was blocked (Extended Data Fig. 6). Hence, the inhibitory effect of IL-27 on IL-17 production and Th<sub>17</sub> cells differentiation is IL-10-independent.

We previously showed that blockade of ICOS/ICOSL pathway resulted in increased IL-10 and decreased IL-17 productions by CD4<sup>+</sup> T cells from B27-rats<sup>10</sup>. Interestingly, in the presence of IL-27, we observed *in vitro* a decreased expression of ICOS on activated CD4<sup>+</sup>CD25<sup>+</sup> T cells and on naïve CD4<sup>+</sup> T cells cultured in Treg or Th<sub>17</sub> polarizing conditions (Fig. 2b). Therefore, to test if IL-27 effect could be secondary to ICOS down-regulation, we cultured sorted CD4<sup>+</sup> CD25<sup>+</sup> T cells from B27-rats bearing ICOS deletion (ICOS<sup>-/-</sup>) in the presence of IL-27. Addition of IL-27 reduced the proportion of IL-17<sup>+</sup> cells and IL-17 production after 3 days of culture, in CD4<sup>+</sup>CD25<sup>+</sup> cells from both ICOS<sup>+/+</sup> and ICOS<sup>-/-</sup> B27-rats to similar extent (Fig. 2c). Thus, the absence of ICOS on CD4<sup>+</sup> T cells from B27-rat did not influence the effect of IL-27 on those cells.

## IL-27 induces similar STAT1/3 phosphorylation in NTG and B27-rat CD4<sup>+</sup> T cells despite increased levels of STAT3 in B27-rat.

It has been shown that IL-27 induced the phosphorylation of STAT1 and STAT3 after binding to IL-27R and that activation of those STATs was required for inhibition of Th<sub>17</sub> differentiation and induction of IL-10 production<sup>20</sup>. To examine if such mechanism applied to IL-27 effect on B27-rat CD4<sup>+</sup> T cells, we studied the phosphorylation of STAT1 and STAT3 by flow cytometry after IL-27 stimulation in several T cells subsets. IL-27 induced the phosphorylation of STAT1 and STAT3 in approximatively 80% and 40% of Treg, respectively, without difference between NTG and B27-rat (Fig. 3a,b). Similar results were observed with activated CD4<sup>+</sup> T cells and Teff cells (Extended Data Fig. 7a). These results indicate that IL-27 stimulation has a similar capacity

to induce pSTAT1/3 in CD4<sup>+</sup> T cell subsets from NTG and B27-rats. Interestingly however, when we analyzed total STAT1/3 levels in CD4<sup>+</sup> T cells we observed an increased level of STAT3 in Treg (Fig. 3c), activated T cells and Teff cells (Extended Data Fig. 7b) from B27-rats which may contribute to the increased IL-17 production.

# JAK-STAT signaling mediates IL-10 increase induced by IL-27 in activated CD4<sup>+</sup> T cells from B27-rat.

To define potential mechanisms by which IL-27 priming inhibited Th<sub>17</sub> cell differentiation we performed RNA-sequencing (RNA-seq) in naïve CD4<sup>+</sup> T cells cocultured with CD103<sup>+</sup> DCs under Th<sub>17</sub>-polarizing condition in the presence of IL-27 or not. We found that 109 genes were down-regulated and 68 were upregulated in Th<sub>17</sub>-differenciated cells in the presence of IL-27, as compared to control condition (Fig. 3d). Interestingly, IL-27 negatively modulated several genes which have been described to be involved in the pathogenesis of SpA and/or the STAT3 pathway, a transcription factor which is increased in CD4<sup>+</sup> T cells from B27-rat (Fig. 3c) and plays a major role in the differentiation of Th<sub>17</sub> cells, including *il1r1*, *ilrl2*, *pdgfb* and *tgfbr1*<sup>30,31</sup> (Fig. 3d). On the other hand, we observed an increased expression of *maf* gene (Fig. 3d), which is downregulated in B27-rat Treg<sup>10</sup>. Interestingly, this gene codes for c-maf, a positive regulator of IL-10 production and may thereby inhibit Th<sub>17</sub> response<sup>32</sup>.

STAT3 dysfunction has been described in various chronic inflammatory diseases and JAK-STAT inhibitors are effective in treating several of these disorders, including SpA<sup>33,34</sup>. The foregoing RNAseq analysis suggested that the inhibitory effect of IL-27 on IL-17 production was associated with STAT3 pathway down-regulation. To test this hypothesis, we performed cultures of activated CD4<sup>+</sup> T cells in the presence of IL-27 and/or JAK-STAT inhibitors. Given that IL-27 induces the dimerization of gp130 and IL-27Ra that engages JAK1 and JAK2, thereby activating both STAT1 and STAT3, we performed experiments with tofacitinib, JAK1/3-STAT1

and AG490, JAK2-STAT3 inhibitors, respectively. IL-17 production was reduced in the presence of IL-27 but also in the presence of both JAK-inhibitors alone (Fig. 3e,f). Interestingly, we observed an additive effect between IL-27 and AG490, suggesting that factor(s) independent of STAT3 likely contributed to the decreased production of IL-17 induced by IL-27 (Fig. 3f). In contrast, the ability of IL-27 to induce IL-10 appeared fully dependent on JAK-STAT activation because IL-10 production induced by IL-27 was abolished in the presence of JAK-STAT inhibitors (Fig. 3e,f).

#### IL-27 modulatory effect is associated with STAT1/STAT3 balance restoration.

Interestingly, we observed by RNAseq analysis of naïve CD4<sup>+</sup> T cells cocultures that B27-rat cDC2 promoted an inflammatory bias, as compared to NTG rat cDC2, independently of the addition of polarizing cytokines (i.e. TGF- $\beta$ , IL-6, IL-1 and IL-23) (Extended Data Fig. 8a). This coculture system allowed us to study the modulatory effect of both IL-27 and AG490 on naïve CD4<sup>+</sup> T cells developing without the possible confounding effect of exogenous cytokines addition. We observed that IL-27 downregulated some genes involved in Th<sub>17</sub> response and nuclear factor-kappa B (NF $\kappa$ B) pathway, such as *il22, tnfrsf8, tnfrsf9, ccr6, il1r1*. Conversely, IL-27 upregulated several genes known to promote Th<sub>1</sub> response, including *stat1, tbx21, irf1, irf9, cxcr3, cxcl10, cxcl11* and of *socs1*, a negative regulator of cytokines signaling. These data indicate that IL-27 modulated the proinflammatory program instructed by B27-rat cDC2 to naïve CD4<sup>+</sup> T cells by downregulation of genes associated to STAT3 pathway and upregulation of genes associated to Th<sub>1</sub> response.

The presence of AG490 in cocultures also negatively modulated *il22* gene implicated in Th17 response and *il1rn and il18rap*<sup>35</sup> genes. Conversely, AG490 upregulated c-maf associated genes such as *mafg* and *maff* (Fig. 4b and Extended Data Fig. 8b). We also observed a downregulation of NFκB-associated genes only when AG490 was combined with IL-27. In this condition, the presence of IL-27 favored an upregulation of Th<sub>1</sub>-associated genes (*stat1*, *tbx21*, *irf1* and *irf9*), not observed in cells cultured only in the presence of AG490 (Fig. 4c).

#### *IL-27 decreases IL-17 production by CD4<sup>+</sup> T cells from SpA patients.*

Given that IL-27 reversed heightened production of IL-17 by several CD4<sup>+</sup> T cell subsets in the B27-rat and that increased production of IL-17 appears to be characteristic of SpA patients<sup>36</sup>, we wished to determine if IL-27 could exert similar effects on human T cells. Thus, we cultured purified CD4<sup>+</sup> T cells isolated from the peripheral blood of SpA patients and healthy controls in the presence of rhIL-27 for 6 days. Similarly to the results observed in B27-rat, the addition of rhIL-27 reduced the proportion of IL-17<sup>+</sup> CD4<sup>+</sup> human T cells (Fig. 5a) and reduced the level of IL-17 in culture supernatant to a greater extent in SpA patients than healthy controls, such that the production of IL-17 was equivalent between both groups after exposure to IL-27 (Fig. 5b). Interestingly, this anti-inflammatory effect of IL-27 was also observed on CD4<sup>+</sup> T cells from several SpA patients resistant to conventional treatment with nonsteroidal anti-inflammatory drugs and biotherapies (Fig.5a,b). However, in contrast to the results observed in B27-rat, no change of IL-10 or IFN-γ levels in supernatant was observed in the presence of IL-27 in cultures of SpA patients cells (Extended Data Fig. 9a,b), indicating that the effect of IL-27 on human CD4<sup>+</sup> T cells display some differences compared to those observed in rat.

#### *Effect of rrIL-27 on SpA development in B27-rat.*

To test *in vivo* the effect of rrIL-27 on SpA development, we treated premorbid B27-rats and control NTG rats in two consecutive experiments with rrIL-27 or vehicle. In the first experiment, rats were treated three times per week for 10 weeks whereas they were treated daily for 12 weeks in the second experiment. NTG rats, whether treated or not with IL-27, remained healthy clinically and by histology, thereby indicating that IL-27 administration was well-tolerated. As expected, vehicle-treated B27-rats developed colitis symptoms characterized by chronic

diarrhea that started at 6-7 weeks of age and progressively worsened over the treatment period. In both experiments, clinical score of colitis was reduced in the IL-27-treated B27-rats (Fig. 6a and Extended Data Fig. 10a). Histological examination of distal colon confirmed reduced inflammation in the IL-27-treated B27-rats (Fig. 6a and Extended Data Fig. 11a). In the first experiment, arthritis course was not significantly affected by IL-27 treatment (Extended Data Fig. 10b), whereas the second more intense regimen of rrIL-27 administration was associated with inhibition of arthritis development (Fig. 6b and Extended Data Fig.11b). Histological study of hindpaws exhibited inflammation and joint destruction only in the vehicletreated B27-rats (Fig. 6b) and Extended Data Fig. 11c)).

Interesting, IL-27 treatment was associated with a striking reduction of the number of CD4<sup>+</sup> T cells producing IL-17 or TNF- $\alpha$  in the mesenteric LN from B27-rats (Fig. 6a). In the popliteal LN, the numbers of IL-17<sup>+</sup> and TNF- $\alpha$ <sup>+</sup> CD4<sup>+</sup> T cells were significatively increased in the vehicle-treated but not in the IL-27-treated B27-rats, as compared to NTG controls. Together, these data show a beneficial effect of IL-27 treatment on hallmark manifestations of SpA in the B27-rat model.

## DISCUSSION

Therapeutic options to treat SpA, a disabling chronic inflammatory disorder of the joint, are still limited despite recent advances that include the development of TNFα, IL-17 and JAK blocking agents<sup>34</sup>. This is why B27-rat, considered as the most faithful animal model of SpA, is highly valuable in understanding the mechanisms of SpA and identifying new therapeutic targets <sup>37,4</sup>. This model was instrumental in highlighting CD4<sup>+</sup> Th<sub>17</sub> cells and heightened IL-17 production as major components of SpA pathophysiology<sup>9,34,38</sup>. Moreover, B27-rat DCs exhibit

aberrant features, including a decreased production of IFN- $\gamma$ , IL-10 and IL-27, that could contribute to such deregulated CD4<sup>+</sup> T cells phenotype<sup>11</sup>. IL-10 treatment alone failed to improve rat SpA<sup>26</sup>. Thus, we speculated that IL-27, a cytokine known to inhibit the differentiation of Th<sub>17</sub> and induce the differentiation of IL-10 producing Tr<sub>1</sub> could me a more suitable target<sup>12</sup>.

Here, we first showed that IL-27 selectively opposed IL-17 and stimulated IL-10 production by several subsets of B27-rat CD4<sup>+</sup> T cells, including *ex vivo* sorted Th<sub>17</sub> cells and Treg or *in vitro* polarized Th<sub>17</sub> cells and Treg, and that this effect was independent of coculture with DCs. These results indicated that in addition to reduce IL-17 production by CD4<sup>+</sup> Teff cells, IL-27 could reverse aberrant proinflammatory pattern of Treg by inhibiting excessive IL-17 and restoring IL-10 productions<sup>10</sup>. Moreover, IL-27 also reduced the production of IL-17 by CD4<sup>+</sup> T cells from SpA patients in culture, including those who were no responders to currently approved biotherapies, indicating that IL-27 could be a promising therapy by opposing Th<sub>17</sub> cells, even in case of conventional treatment failure.

Previous works demonstrated either a crucial role of IL-10 for the anti-inflammatory effect of IL-27 in a model of colitis or that IL-10 was not required for the suppression of Th<sub>17</sub> cells in the context of experimental allergic encephalomyelitis <sup>39,40</sup>. Here, we found that IL-10 secretion was not mediating the modulatory effect of IL-27 on Th<sub>17</sub> cells. It was also previously shown that the induction of Tr<sub>1</sub> cells by IL-27 relied on the induction of three components: c-maf, IL-21 and ICOS<sup>41</sup>. Here, IL-27 decreased ICOS expression on CD4<sup>+</sup> T cells from B27 rats that exhibited increased ICOS expression, a feature that could participate to Th<sub>17</sub> bias <sup>42,43</sup> (our own unpublished data). Nevertheless, using ICOS<sup>-/-</sup> B27-rats, we observed that the modulatory effect of IL-27 was independent of ICOS.

To dissect further the mechanisms by which IL-27 may oppose Th<sub>17</sub> differentiation bias favored by B27-rat DCs, we studied the effect of IL-27 on the transcriptome of naïve CD4<sup>+</sup> T cells having been differentiated in contact with B27-rat DCs in Th<sub>17</sub>-polarizing conditions. Interestingly, IL-27 modulated negatively genes associated to STAT3 signaling that are likely to control the inflammatory response involved in the pathogenesis of SpA. Conversely, *maf* and several *ifng*-associated genes, including *tbx21*, which might antagonize Th<sub>17</sub> response were upregulated<sup>44,45</sup>. This is consistent with STAT1 phosphorylation induced by IL-27 and enhanced production of IFN- $\gamma$  that was observed in this culture condition, albeit the inhibitory effect of IL-27 on Th<sub>17</sub> pathway was not mediated by IFN- $\gamma$  itself, given the presence of anti-IFN- $\gamma$  in the culture. Noteworthy, in contrast to increased production in Treg or Th<sub>17</sub> polarizing conditions, IFN- $\gamma$  production was decreased by IL-27 in differentiated CD4<sup>+</sup> T cells which is consistent with the capacity of IL-27 to limit excessive Th<sub>1</sub> response by induction of IL-10 expression<sup>46</sup>

To examine whether STAT1 and/or STAT3 phosphorylation transduced IL-27 effects in this model, we added inhibitors of JAK1/3 (tofacitinib, a molecule approved for SpA treatment) or JAK2 (AG490) that preferentially target STAT1 and STAT3, respectively. Both inhibitors reduced IL-17 and IL-10 production by activated CD4<sup>+</sup> effector T cells from B27-rat, as a likely consequence of STAT3 inhibition. This precluded us to determine if IL-27 inhibited IL-17 production through STAT signaling. However, increased production of IL-10 by IL-27 was abolished in the presence of both STAT inhibitors, showing that it was dependent on STAT signaling. Moreover, there was an additive effect of IL-27 and JAK2 inhibition on IL-17 inhibition, which might reflect the combination of STAT1 phosphorylation by IL-27 and STAT3 inhibition by AG490. Altogether, those results indicate that IL-27 exerted more thorough antiinflammatory effect on CD4<sup>+</sup> T cells from B27-rat than JAK inhibitors, by combining IL-17 inhibition to IL-10 up-regulation.

We then analyzed the effect of IL-27 and/or AG490 in a simplified coculture system where cDC2 from B27-rat drives a Th<sub>17</sub> bias in naïve CD4<sup>+</sup> T cells without exogenously added cytokines, which may underly the pathophysiology of B27-rat SpA. Using again RNAseq, we interrogated the early transcriptional events occurring after 24h of naïve CD4<sup>+</sup> T cell stimulation. This confirmed that IL-27 upregulated STAT1-IFN-pathway associated genes and downregulated genes associated to Th<sub>17</sub> program, consistent with a mechanism involving a greater phosphorylation of STAT1 than STAT3 by IL-27<sup>47</sup> (Fig. 3a). This effect of IL-27 resulted in NF $\kappa$ B pathway inhibition. AG490 also inhibited Th<sub>17</sub> pathway and NF $\kappa$ B but to a lesser degree than IL-27 and was rather inhibitory on genes of the STAT1-IFN pathway.

Given the striking *in vitro* anti-IL-17 and pro-IL-10 effect of IL-27 on CD4<sup>+</sup> T cells from B27 rats, IL-27 appeared a promising therapeutic agent in SpA. Indeed, *in vivo* treatment of B27-rats with exogenous IL-27 was able to inhibit SpA development in a dose-dependent manner and the expansion of IL-17 and TNFα producing Teff cells. Hence, a high dose of IL-27 administered daily could effectively inhibit both intestinal inflammation and arthritis, the two main pathologic features in this rat model of SpA. This result is consistent with a beneficial effect of IL-27 previously reported in others experimental models of arthritis or colitis involving Th<sub>17</sub> cells<sup>48,49</sup>. However, it is all the more remarkable that neither administration of anti-IL-17 antibody<sup>9</sup>, rmIL-10<sup>26</sup> nor low dose of rhIL-2 to stimulate Treg<sup>50</sup> were sufficient to counter the development of rat SpA.

In summary we demonstrated for the first time that exogenous IL-27 reversed the pro-Th<sub>17</sub> phenotype characteristic of the B27-rat model of SpA. Such effect was demonstrated both on the developing and differentiated IL-17 producing CD4<sup>+</sup> T cells. Moreover, similar modulatory effect was also observed in CD4<sup>+</sup> T cells from SpA patients. Finally, *in vivo* administration of rrIL-27 inhibited the development of SpA in B27-rat, in which DCs exhibit

deficient IL-27 production<sup>11</sup>. Taken together, these results highlight a critical modulatory role of IL-27 on the emergence of Th<sub>17</sub>-driven inflammatory disease in B27 rats and offer a novel therapeutic perspective for SpA patients, including those who are refractory to currently available treatments.

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### Article 2 : Figures



**Fig.1: Immuno-regulatory effect of IL-27 on IL-17 and IL-10 production by CD4**<sup>+</sup> **LN T cell subsets from B27rat.** (a) *Ex vivo* sorted resting Teff cells (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup> or (b) *ex vivo* sorted Treg (CD4<sup>+</sup> CD25<sup>high</sup>) were cocultured with CD103<sup>+</sup> DCs for 3 days; (c,d) *ex vivo* sorted naïve (CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>+</sup>) T cells were cocultured with CD103<sup>+</sup> DCs for 6 days in (c) Treg (TGF- $\beta$ ) or (d) Th<sub>17</sub> (TGF- $\beta$  + IL-6 + IL-1 + IL-23) polarizing conditions. Left panels show representative flow cytometry dot plots evaluating the percentage of IL-17-producing CD4<sup>+</sup> T cells after coculture and the mean + SEM of 5-7 independent experiments; right panels show the mean + SEM of IL-17 and IL-10 levels that were evaluated in the supernatant of cocultures by ELISA. \* p < 0.05; \*\* p < 0.01



**Fig. 2: Decreased IL-17 production induced by IL-27 is independent of IL-10 and ICOS.** (a) Left: Representative dot-plot of IL-17<sup>+</sup> cells among activated CD4<sup>+</sup>CD25<sup>+</sup> T cells from B27-rat cultured for 3 days with coated anti-CD3 in the presence or absence of IL-27 and anti-IL-10 blocking antibody. Right panel: level of IL-17 in culture supernatants (3 experiments). (b) ICOS expression on cultured activated T cells, Treg-polarizing, and Th<sub>17</sub>- polarizing cells. (c) CD4<sup>+</sup>CD25<sup>+</sup> T cells from ICOS<sup>+/+</sup> or ICOS<sup>-/-</sup> B27- rats cultured for 3 days with coated anti-CD3 in the presence or not of rmIL-27. Histograms represent the mean + SEM of 4 independent experiments. IL-17 and IL-10 concentrations were evaluated by ELISA. \* p < 0.05; \*\* p < 0.01.



**Fig. 3: JAK-STAT signaling is involved in IL-27 modulatory effect.** (a) Representative dot-plot of mesenteric LN cells stimulated by IL-27 and stained intracellularly for phosphorylated STAT1 (Tyr701) and STAT3 (Tyr705), gated on CD4+ CD25<sup>high</sup> Treg. (b) The percentage of STAT1 and STAT3 phosphorylation after IL-27 stimulation was analyzed in gated CD4<sup>+</sup> CD25<sup>high</sup> Treg from NTG and B27-rat LN. (c) STAT1 and STAT3 levels were analyzed by flow cytometry in gated CD4<sup>+</sup> CD25<sup>high</sup> Treg from in NTG and B27 rat LN. Values are the mean + SEM of 3-6 independent experiments. \*p<0.05. (d) Volcano plot representing the results of RNAseq analysis of *in vitro* differentiated Th<sub>17</sub> cells in the presence of IL-27, as compared to medium control. The data for all genes are represented by log2 (fold change) vs. log10 of adjusted p-value. The significatively different genes are highlighted in red. Values represent results from 5 independent experiments. (e,f) IL-17 and IL-10 levels were determined by ELISA in culture supernatants from CD4<sup>+</sup>CD25<sup>+</sup> activated T cells after 3 days of stimulation with coated anti-CD3 in the presence of IL-27 and/or anti-JAK1/3-tofacitinib (e) and/or anti-JAK2-AG490 (f)). Values are the mean + SEM of 6 independent experiments. \* p < 0.05.



## Fig.4: RNAseq analysis of the modulatory effect of IL-27 and/or AG490 on naïve CD4<sup>+</sup> T cells from B27 rats.

Naïve T cells that had been coculture with splenic cDC2s and coated-anti-CD3, in the presence or not of mrIL-27 (5 ng/mL), AG490 (10 µg/mL) or both for 24 h were sorted. RNA was isolated and global gene expression was determined by RNA-seq. (a) volcano plot represents IL-27 condition compared to medium control and (b) AG490 condition compared to medium control. (a-b) The data for all genes are represented by log2 (fold change) *vs* log10 of adjusted p-value. (c) A heatmap of fold change (log2) for genes differentially expressed between conditions is shown. Values represent results from of 5 independent experiments.



**Fig. 5: IL-27 decreases IL-17 production by peripheral CD4**<sup>+</sup> **T cells from SpA patients and healthy controls.** (a) Left panel: representative dot-plot and right panel: percentage of IL-17 producing-CD4<sup>+</sup> T cells from SpA patients cultured during 6 days with IL-2 in the presence or absence of IL-27 and determined after PMA/ionomycin stimulation. (b) IL-17 production was measured in culture supernatants by ELISA. The squares with symbols represent patients resistant to conventional treatment with nonsteroidal anti-inflammatory drugs and biotherapies. \* p < 0.05 \*\*p<0.005.



**Fig. 6:** *in vivo* effect of rrIL-27 on SpA development in B27-rat. (a) Clinical score of colitis (experiment 2) histological score of distal colon inflammation and absolute number of cytokines producing T cells (pooled experiments 1 and 2) were determined in mesenteric LN from NTG and B27-rats treated with vehicle or rrIL-27 for 12 weeks. (b) Clinical score of arthritis, histological score of hindpaws and the absolute number of cytokines producing T cells of experiment 2 were determined in popliteal LN from NTG and B27-rats treated with vehicle or rrIL-27 for 12 weeks. Values are the mean  $\pm$  SEM of 6-8 rats/group. \* p < 0.05 \*\*\*p<0.0005 \*\*\*\*p<0.0001.

Characteristic	SpA (n = 18)	Controls (n = 11)
Age: mean (range) in years	51 (28-72)	32 (22-47)
Sex, no (%) of males	11 (61)	7 (64)
HLA-B27+ (%)*	17 (94)	0 (0)
SpA subtype, no (%)		
AS	13 (72)	NA
Non-radiographic axial SpA	5 (28)	NA
BASDAI: mean (range)	3.4 (0-7.4)	NA
Treatments, no (%) taking		
None	6 (33)	NA
NSAID	7 (39)	NA
Sulfasalazin	2 (11)	NA
Biotherapy **	8 (44)	NA

#### Article 2 : Données complémentaires

**Extended Data table I:** SpA patients and healthy controls characteristics. SpA: spondyloarthritis; AS: ankylosing spondylitis; BASDAI: Bath Ankylosing Spondylitis Disease, Activity Index; NSAID: non-steroidal anti-inflammatory drug; NA: not applicable. \* HLA-B27 was undetermined in 1 healthy control \*\*Biotherapies: anti-TNFα (7); anti-IL-12/IL-23 (ustekinumab, 1).





**Extended Data fig. 1: Cytokines production by B27-rat Treg cocultured with B27-rat DCs in the presence or absence of rmIL-27.** A panel of 11 cytokines was studied, using multiplex ELISA assay in the supernatants of Treg and DCs cocultures. Values are the mean + SEM of 4 independent experiments.



**Extended Data fig. 2: IL-27 upregulates IFN-** $\gamma$  **production by Treg and Th17 polarizing CD4**<sup>+</sup> **LN T cells from B27-rat.** CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>+</sup> naïve T cells were polarized *in vitro* by coculture with CD103<sup>+</sup> DCs in (a) Treg (or (b) Th<sub>17</sub> conditions during 6 days in the presence or absence of IL-27 and IFN- $\gamma$  level was evaluated after PMAionomycin stimulation by flow cytometry or ELISA in the supernatants. Values are the mean + SEM of  $\geq$  4 independent experiments. \*\* p< 0.01



**Extended Data fig. 3: Anti-inflammatory effect of IL-27 on activated and resting Teff cells in the absence of DCs**. (a) Representative data of activated CD4<sup>+</sup> CD25<sup>low</sup> Teff cells from B27-rat stimulated for 3 days with anti-CD3/anti-CD28 in the presence or absence of IL-27 and restimulated with PMA-ionomycin-BFA. IL-17, IL-10 and IFN- $\gamma$  levels were evaluated by ELISA in the supernatants at the end of the culture. Values are the mean ± SEM of 7 independent experiments. \*\* p< 0.01, \*\*\* p< 0.001. (b) Representative data of resting CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup> Teff cells from B27 rat stimulated for 3 days with anti-CD3/anti-CD28 in the presence or absence of IL-27 and restimulated with PMA-ionomycin-BFA. IL-17, IL-10 and restimulated for 3 days with anti-CD3/anti-CD28 in the presence or absence of IL-27 and restimulated with PMA-ionomycin-BFA. IL-17, IL-10 and IFN- $\gamma$  levels were evaluated by ELISA in the supernatants at the end of the culture. Values are the mean ± SEM of 7 independent experiments. \*\* p< 0.01, \*\*\* p< 0.001. (b) Representative data of resting CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup> Teff cells from B27 rat stimulated for 3 days with anti-CD3/anti-CD28 in the presence or absence of IL-27 and restimulated with PMA-ionomycin-BFA. IL-17, IL-10 and IFN- $\gamma$  levels were evaluated by ELISA in the supernatants at the end of the culture. Values are the mean + SEM of 7 independent experiments. \*p<0.05



**Extended Data fig. 4: Effect of IL-27 on IL-17 production by activated T cells from NTG rats.** (a) The percentage of IL-17-producing CD4<sup>+</sup> T cells was evaluated by flow cytometry after culture of *ex vivo* sorted resting Teff cells (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>-</sup>) from NTG rat stimulated for 3 days with anti-CD3/CD28 in the presence or not of IL-27. (b) IL-17 levels were evaluated in culture supernatants by ELISA.



Extended Data fig. 5: Effect of IL-27 on the differentiation of naïve CD4<sup>+</sup> T cells from B27-rat, in the absence of DCs. (a) The percentage of IL-17-producing CD4<sup>+</sup> T cells was evaluated by flow cytometry after culture of sorted naïve CD4<sup>+</sup> T cells (CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>+</sup>) in Th<sub>17</sub> (TGF- $\beta$  + IL-6 + IL-1 + IL-23) polarizing conditions with anti-CD3/anti-CD28 stimulation. (b) IL-17, IL-10 and IFN- $\gamma$  levels were evaluated by ELISA at day 6 in the culture supernatant. Values are the mean + SEM of  $\geq$  5 independent experiments. \* p < 0.05 \*\* p < 0.01.



**Extended Data fig. 6: Decreased IL-17 production induced by IL-27 is IL-10-independent.** Left panel: representative dot-plot of IL-17<sup>+</sup> cells among B27-rat CD4<sup>+</sup> T cells cultured during 6 days in Th<sub>17</sub> polarizing conditions with coated anti-CD3 and soluble anti-CD28 in the presence or not of IL-27 and anti-IL-10 blocking antibody, starting from naïve CD4<sup>+</sup> T cells. Right panel: level of IL-17 in culture supernatants. Values are the mean + SEM of 3 independent experiments. \*\* p<0.01 \* p<0.05; ns = not significant.



**Extended Data fig. 7: IL-27 induces similar phosphorylation of STAT1 and STAT3 in NTG and B27-rat despite increased STAT3 level in B27-rat T cells. (a)** Percentage of STAT1 and STAT3 phosphorylation after IL-27 stimulation in activated CD4<sup>+</sup> CD25<sup>+</sup> T cells and CD4<sup>+</sup> CD25<sup>-</sup> Teff cells from NTG and B27-rat LN. Values are the mean + SEM of 6-7 independent experiments. **(b)** Total STAT1 and STAT3 levels were analyzed by flow cytometry in gated activated CD4<sup>+</sup> CD25<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>-</sup> Teff cells from NTG and B27-rat LN. Values are the mean + SEM of 3 independent experiments. \*p<0.05.



**Extended Data fig. 8: RNAseq analysis of naïves T cells from B27 rat after coculture with cDC2 from NTG or B27 rats in the presence or not of IL-27 and AG490, a JAK2 inhibitor:** RNA from naive CD4<sup>+</sup> CD25<sup>-</sup> CD62L<sup>+</sup> T cells from B27-rats sorted after 1 day of coculture with NTG or B27-rat cDC2 or, in the presence or not of IL-27 or JAK2 inhibitor (JAK2i, AG390), was isolated and global gene expression was determined by RNA-seq. (a) Heatmap of normalized transcript counts of genes of interest comparing cocultures with DCs from either NTG or B27-rats (b) Heatmap clustering (same experiment of Figure 4) of normalized transcript counts of genes of interest, comparing cocultures with B27-rat cDC in the presence or not of IL-27, JAK2i or IL-27 + JAK2i.



**Extended Data fig. 9: Effect of IL-27 on IL-10 and IFN-γ production by peripheral blood CD4<sup>+</sup> T cells from SpA patients and healthy controls.** (a) IL-10 and (b) IFN-γ production by CD4<sup>+</sup> T cells from SpA patient cultured during 6 days with IL-2 in the presence or absence of IL-27 was measured in supernatants by ELISA. Values are the mean + SEM of 6-13 individuals/group.



**Extended Data fig. 10**: *In vivo* effect of rrIL-27 on SpA development in B27-rat. (a) Clinical score of colitis and (b) arthritis from NTG and B27-rats treated three times per week with vehicle or rrIL-27, during 10 weeks (experiment 1). Values are the mean + SEM of 6-8 rats/group. \* p < 0.05 (c) Peak arthritic score of B27-rats treated daily with vehicle or rrIL-27, during 12 weeks (experiment 2; mean + SEM). (d) Distal colon and (e) hindpaw histopathology of B27-rats treated with vehicle or rrIL-27 daily, during 12 weeks (experiment 2). Left panels: original magnification x 40 (bars =  $300\mu$ m). Right panels selected area are shown at higher magnification (bars =  $100\mu$ m). Arrows: infiltration of the *lamina propria* (d) and synovial tissue (e) by inflammatory cells. Arrowheads: crypts abscess (d) and cartilage damage (arrow), (arrowhead).
# DISCUSSION

L'ensemble de ce travail doctoral a permis d'établir :

**1.** L'existence d'un défaut précoce de la voie STAT1/IFN au sein des lymphocytes T CD4<sup>+</sup> naïfs du rat-B27 ;

2. L'association de ce défaut de la voie STAT1 à un biais de différenciation Th17 ;

3. L'implication d'une empreinte épigénétique précoce prédisposant au biais Th17 ;

**4.** La potentialisation du biais Th17 par les cDC2, qui présentent elles aussi un défaut similaire de la voie STAT1/IFN, associé à un défaut d'expression du gène de l'IL-27 ;

**5.** Un potentiel impact de ces résultats chez les patients atteints de SpA, présentant une diminution de *STAT1* et du ratio *STAT1/STAT3* dans leurs lymphocytes T CD4<sup>+</sup> naïfs ;

**6.** L'intérêt de l'IL-27, qui rétablit *in vitro* l'équilibre entre les voies STAT1/IFN et STAT3/Th17, et qui limite *in vivo* le développement de la SpA-rat.

Nous résumons ces résultats dans la Figure 11, et discutons ici chacun de ces points.



# *Figure 11. Défaut de STAT1, nouvelle pièce du SpAzzle ?*

Le défaut de la voie STAT1 dans les lymphocytes T CD4<sup>+</sup> naïfs du rat-B27 précède la maladie et favorise un biais de différenciation Th17, médié par une activation accrue de STAT3. Ce phénomène est lié à une empreinte épigénétique, marquée par l'activité de superenhancers (SE) définissant un programme Th17. Ce biais est potentialisé par la cellule dendritique (DC), qui présente elle-même un défaut de la voie STAT1 et d'expression d'interleukine (IL)-27. Rétablir l'équilibre entre les voies STAT1 et STAT3 en traitant le rat-B27 par l'IL-27 permet de limiter la réponse lymphocytaire T CD4<sup>+</sup> de type Th17 et de réduire la sévérité de la maladie, articulaire et intestinale.

Le(s) mécanisme(s) possiblement responsable(s) du défaut de la voie STAT1 dans les lymphocytes T CD4<sup>+</sup> naïfs du rat-B27 pourrait être :

- un effet direct de l'expression du HLA-B27 sur le lymphocyte T CD4<sup>+</sup> naïf,
- un effet indirect via les signaux émis par la DC ;
- un effet corrélé à la dysbiose, notamment intestinale.

# Défaut de STAT1

La coculture des cDC2 avec les lymphocytes T CD4<sup>+</sup> naïfs de rats-B27 ou NTG a révélé que ces deux sous-types cellulaires contribuaient de façon additive au développement d'un profil activé et Th17 lors de la stimulation lymphocytaire. Ceci nous a incité à étudier le transcriptome des lymphocytes T CD4<sup>+</sup> naïfs du rats-B27 *ex vivo*. Même avant le début de la maladie, nous avons mis en évidence une diminution d'expression de gènes de la voie de l'IFN/Th1 et une augmentation d'expression de gènes de la voie Th17. De façon intéressante, cette signature transcriptomique était prédite comme liée à une balance perturbée entre les TF STAT1 et STAT3. Nous avons alors mis en évidence à l'échelle protéique que le défaut de STAT1 précédait le développement de la maladie et persistait dans le temps, alors que l'excès de STAT3 apparaissait comme un évènement secondaire, n'étant observé que chez les rats avec une maladie établie. Autre point essentiel, nous avons mis en évidence que le défaut de signature de la voie STAT1/IFN était observé même dans les thymocytes du rat-B27, de façon plus marquée au stade simple positif CD4<sup>+</sup>, après le réarrangement complet du TCRαβ.

Des travaux précédents ont clairement démontré que les cDC2 du rat-B27 – à l'instar des monocytes dérivés de macrophages de patients atteints de SpA – présentent un défaut similaire de la voie IFN, incluant un défaut de *Stat1* ; cet effet est spécifique du HLA-B27 (Fert et al., 2014b). Cette empreinte commune entre cellules myéloïdes et thymocytes/lymphocytes T CD4<sup>+</sup> naïfs évoque un effet ubiquitaire de l'expression du HLA-B27.

D'un point de vue mécanistique, le lien entre l'expression du HLA-B27 et le défaut de STAT1/IFN reste à expliquer. Nous ne pouvons tout d'abord à ce stade affirmer s'il est direct ou indirect. Tenant compte des données récemment publiées grâce au modèle de la drosophile transgénique pour le HLA-B27, cet effet pourrait être associé à une perturbation de la voie TGFβ par l'expression du HLA-B27 (Grandon et al., 2019). Connectant ces résultats à notre problématique, un travail a mis en évidence que le TGFβ était capable d'inhiber la voie Fas ; or celle-ci est associée au blocage de l'activation de STAT1 en induisant sa séquestration nucléaire, en favorisant ainsi un biais Th17 (Mayer-Barber and Yan, 2017; Meyer zu Horste et al., 2018). Nous pouvons alors émettre l'hypothèse qu'en perturbant la voie TGFβ, le HLA-B27 déverrouille ce mécanisme régulateur résultant en une séquestration nucléaire chronique de STAT1. Une autre hypothèse pourrait être liée à l'effet du HLA-B27 sur la régulation du métabolisme lipidique, comme cela est suggéré par les anomalies du tissu adipeux dans la

## **DISCUSSION et CONCLUSION**

moelle osseuse des patients atteints de SpA et chez le rat-B27, ou encore des données récentes non publiées du laboratoire portant sur des DC dérivées de macrophages de patients (Furesi et al., 2021). Connectant ces résultats à notre problématique, plusieurs travaux ont mis en évidence des interactions entre la voie de l'IFN – notamment dans un contexte de réponse antivirale – et le métabolisme lipidique (Kanno et al., 2021; Monson et al., 2018; York et al., 2015). Il semble également exister un effet propre de la β2m dans la régulation positive de la voie de l'IL-1, décrite par ailleurs comme inter-régulée avec la voie IFN – comme nous semblons d'ailleurs l'observer chez le rat-B27 (Hofbauer et al., 2021; Mayer-Barber and Yan, 2017).

Pour répondre à certaines de ces questions, nous pourrions utiliser la drosophile transgénique pour le HLA-B27 et évaluer notamment comment la voie des STAT interagit avec la voie BMP/TGFβ, et/ou le métabolisme lipidique. Nous pourrions également évaluer l'effet du HLA-B27 sur la voie IFN/STAT1 à l'aide de lignées cellulaires de patients B27+ ou B27-. Il serait intéressant d'étudier le profil d'expression de la voie IFN/STAT1 dans d'autres sous-types cellulaires, impliqués dans la réponse immune de type 3 observée chez les patients, notamment les ILC3, MAIT, lymphocytes T γδ ou NK RORγt<sup>+</sup>. Le profil transcriptomique des T γδ ou NK comparativement aux cellules de patients atteints de polyarthrite rhumatoïde a été précédemment étudié mais l'existence d'un défaut de la voie STAT1/IFN n'était pas rapportée (Venken et al., 2019). Enfin, il serait intéressant d'approfondir l'étude du phénotype des lymphocytes T CD4<sup>+</sup> naïfs des rats-B27 par des données transcriptomique sur cellule unique, pour déterminer si l'effet observé dans notre travail est en lien avec une sous-population particulière de lymphocytes T CD4<sup>+</sup> naïfs, dont nous pourrions alors mieux définir l'ontogénie et les fonctions.

# Du défaut de STAT1 au biais Th17

Nous avons mis en évidence que chez le rat-B27, le défaut de STAT1 dans les lymphocytes T CD4<sup>+</sup> naïfs et leurs progéniteurs thymiques – similaire à celui de la DC – était associé à un biais de différenciation Th17. Ce phénomène est inversé par l'induction de STAT1 avec une petite molécule modulant l'activité transcriptionnelle de STAT1, ou avec l'IFNγ, ou encore avec l'IL-27. Nous avons approfondi les mécanismes impliqués dans l'inter-régulation entre les différenciations Th1 et Th17, en nous focalisant sur les effets de l'IL-27. *In vitro*, l'IL-27 présente un effet anti-IL-17 sur différentes populations lymphocytaires exprimant l'IL-17 en excès chez le rat-B27 (Th17, T<sub>REG</sub>,, Th avant ou en cours de différenciation), ainsi que sur les lymphocytes T CD4<sup>+</sup> de patients atteints de SpA. L'effet de l'IL-27 sur le profil Th17 ne dépendait pas de la signalisation d'ICOS, ni de la production d'IL-10. En revanche, à l'échelle transcriptomique, il était associé à l'inhibition de la signalisation Th17 (ainsi que de la voie NFκB), et à l'activation de la voie Th1. Ceci soulève l'hypothèse d'une balance inversée et pathogène entre STAT1 et STAT3, en faveur du deuxième chez le rat-B27, résultant en un biais Th17. Les programmes de différenciation Th1 et Th17 sont en effet inter-régulés. Ceci est notamment lié à l'inhibition de RORγt par T-BET – induit par STAT1, *via* les protéines RUNX1 ou AHR (Lazarevic et al., 2011; Villarino et al., 2010; Yokosawa et al., 2017). Fait intéressant d'ailleurs, deux études utilisant le modèle murin d'arthrite induite au collagène ont montré une exacerbation de l'activité inflammatoire lorsque *T-bet* est muté/KO ou à l'inverse une inhibition des arthrites lorsqu'il est surexprimé dans les lymphocytes T CD4<sup>+</sup>ou (Kondo et al., 2012; Shimizu et al., 2021). Par ailleurs, la régulation de la balance Th1/Th17 est également dépendante des programmes transcriptionnels respectifs de STAT1 et de STAT3, qui s'inter-régulent (Hirahara et al., 2015; Kim et al., 2015; Tsai et al., 2019; Villarino et al., 2010).

L'étude des déficits immunitaires monogéniques humains permet également de mettre en lumière des mécanismes physiologiques de la différenciation lymphocytaire T CD4<sup>+</sup> (Mogensen, 2019). C'est le cas notamment des mutations gain de fonction de *STAT1* qui sont associés à un déficit chronique en Th17, associé à un surrisque infectieux vis-à-vis notamment de *candida albicans*, et associé chez certains patients à une maladie inflammatoire par excès des réponses dépendant de l'IFN de type I (Liu et al., 2011; Okada et al., 2020). Le déficit de *STAT1* est, quant à lui, associé à un déficit vis-à-vis des infections à mycobactéries et à une maladie inflammatoire systémique mais le profil Th17 n'a pas été directement étudié dans les données descriptives d'immunophénotypage des patients (Boehmer et al., 2020).

Par ailleurs, nous avons mis en évidence que l'expression de *Stat1* était négativement corrélée à celle de l'*ll1r1* et que l'expression accrue d'*ll1r1* était une caractéristique précoce des lymphocytes T CD4<sup>+</sup> naïfs et leurs progéniteurs thymocytaires CD4<sup>+</sup>. De plus, l'addition d'IL1β amplifiait spécifiquement le biais de différenciation Th17 chez les rats-B27, mais pas chez les rats non transgéniques possiblement du fait de cet excès d'expression d'*ll1r1*. L'inter-régulation entre les voies de l'IFN et de l'IL1 pourrait, dans notre système, être un mécanisme amplificateur du biais Th17 (Mayer-Barber and Yan, 2017). Il serait d'ailleurs intéressant de déterminer à quel stade et dans quel contexte, un excès d'IL-1 pourrait être produit chez le rat-B27. De plus, la SpA semble associée à une dérégulation de la voie de l'IL-1, comme en témoignent : (i) le lien entre SpA et variant du gène *MEFV*, connu pour induire une activation de l'inflammasome et la production d'IL-1 ; (ii) l'association de la SpA à des polymorphismes des gènes de l'*IL1R1* et *IL1R2*, mis en évidence par les GWAS ; (iii) le chevauchement phénotypique entre la SpA et l'ostéomyélite chronique, dont la physiopathologie repose sur un rôle clef de l'IL-1, comme en témoigne le modèle de souris *Pstpip2*(cmo) ; (iv) l'efficacité au moins partielle du blocage de l'IL-1 $\beta$  chez les patients atteints de SpA – bien que ces essais pilotes n'aient pas été poursuivis par des études à plus grande échelle (Chitu et al., 2009; Cosan et al., 2010; Guggino et al., 2021; Haibel et al., 2005; International Genetics of Ankylosing Spondylitis Consortium (IGAS) et al., 2013; Lukens et al., 2014; Tan et al., 2004).

## Régulation épigénétique

Nous avons mis en évidence que la signature transcriptomique favorisant un biais Th17 était imprimé précocement au niveau épigénétique. Cela a été évalué par l'étude pangénomique de l'enrichissement de marques activatrices comme H3K4Me3 et H3K27Ac. Cette deuxième marque nous a permis de déterminer les SE, régions régulatrices promptes à déterminer le devenir cellulaire. De façon intéressante, les gènes intégrés dans les SE des lymphocytes T CD4<sup>+</sup> naïfs de rats-B27 étaient liés à un programme pro-Th17, ce même avant le début de la maladie. En étudiant les TF prédits comme régulateurs du programme des lymphocytes T CD4<sup>+</sup> de rats-B27, on retrouvait d'ailleurs IRF4, BATF et STAT3. A l'inverse, une signature IFN était plus marquée dans les SE des lymphocytes T CD4<sup>+</sup> naïfs de rats témoins, laissant supposer que l'absence de ce programme favoriserait par défaut un biais vers une signature STAT3.

Pour déterminer les mécanismes épigénétiques à l'origine de ce profil d'activation biaisé des SE dans les lymphocytes T CD4<sup>+</sup> naïfs de rats-B27, il serait essentiel d'évaluer par ChIP-seq si le profil de fixation de STAT1 (et STAT3) sur l'ensemble du génome diverge chez le rat-B27. Ceci pourrait en effet corroborer l'hypothèse du caractère causal d'anomalies épigénétiques, éventuellement impactées par l'expression du HLA-B27 ou s'y additionnant.

De façon intéressante, des données s'accumulent pour soutenir l'hypothèse d'un rôle pathogène d'anomalies épigénétiques au cours de la SpA, et dans d'autres maladies

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inflammatoires (Cherqaoui et al., 2020; Farh et al., 2015; Ohkura et al., 2020). Il a notamment été mis en évidence que la reprogrammation épigénétique de lymphocytes T CD4<sup>+</sup> de patients atteints de SpA induisait la modification de leur profil transcriptomique immuno-métabolique, tout en inhibant le profil Th17 (Cribbs et al., 2020).

## Rôle de la DC et du microbiote

Nous avons mis en évidence que la cDC2 était capable de potentialiser le biais de différenciation Th17. Ceci dépendait d'un impact transcriptomique fort, marqué par la modification d'expression de gènes ayant trait au métabolisme, à la structure chromatinienne, mais également à l'activation et à la différenciation lymphocytaire. Ces résultats renforcent les données précédemment publiées dans le modèle du rat-B27 révélant le défaut fonctionnel des DC, qui ont un rôle dans l'expansion des Th17, possiblement en lien avec un défaut de la voie IFN/STAT1 (Breban et al., 2021b). Une question importante à ce stade serait de déterminer si la DC est intrinsèquement programmée à développer un phénotype pro-inflammatoire ou si celui-ci est induit ou aggravé par la dysbiose intestinale (Breban et al., 2021c). Tenant compte du fait que le défaut de STAT1 dans les lymphocytes CD4<sup>+</sup> apparaît dès le stade de thymocyte CD4<sup>+</sup>, on pourrait émettre l'hypothèse que leur stimulation anormale par les DC survienne à ce stade du développement thymocytaire. En effet, l'éducation thymocytaire est en partie conditionnée par des DC ayant capté des antigènes au niveau intestinal puis migrant dans le thymus (Zegarra-Ruiz et al., 2021).

Le défaut intrinsèque de STAT1 pourrait favoriser une dysbiose, notamment intestinale – comme observée chez le rat-B27 et chez les patients atteints de SpA – qui à son tour induirait un biais dans les réponses lymphocytaires T CD4<sup>+</sup>. C'est une hypothèse d'ailleurs étayée dans un travail démontrant que des souris déficitaire en *Stat1* développent une maladie inflammatoire systémique, incluant une colite, médiée par un biais de différenciation Th17, qui dépend de la dysbiose intestinale induite par le défaut de *Stat1* (Marié et al., 2021). Par ailleurs, une étude a également révélé qu'un biais de différenciation lymphocytaire T CD4<sup>+</sup> vers un profil Th17 pouvait être induit par une dysbiose maternelle, transmise à sa progéniture, *via* un priming épigénétique persistant (Kim et al., 2022).

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## STAT1 : Nouveau biomarqueur de SpA ?

Comme chez le rat-B27, les lymphocytes T CD4<sup>+</sup> naïfs de patients atteints de SpA ont une diminution d'expression de *STAT1*, également associée à une baisse du rapport *STAT1/STAT3*. Cette observation semble caractéristique de la SpA, car absente chez les patients atteints de polyarthrite rhumatoïde, corroborant l'hypothèse d'un mécanisme pathogène spécifique. Le développement de nouveaux outils pour diagnostiquer plus tôt la SpA sont indispensables pour réduire le long délai diagnostique et optimiser la prise en charge thérapeutique (Brown et al., 2020). On pourrait envisager dans un premier temps d'évaluer la signature *STAT1/STAT3* dans une cohorte plus large de patients, incluant notamment des patients nouvellement diagnostiqués, en comparaison avec des patients souffrants de lombalgies mécaniques en évaluant prospectivement le lien entre cette signature initiale et le diagnostic avéré de SpA.

# **Corriger le défaut de STAT1**

Après avoir décrit les effets in vitro de l'IL-27, nous avons démontré in vivo son efficacité dosedépendante sur l'atteinte articulaire et intestinale de la SpA-rat. Ceci s'associait à la réduction des lymphocytes T effecteurs IL17<sup>+</sup> et TNFα<sup>+</sup>. L'utilisation de l'IL-27 présente de plus l'avantage supplémentaire de favoriser la production d'IL-10; au moins chez le rat-B27, l'IL-27 permet ainsi d'induire une réponse anti-inflammatoire endogène. Le rat-B27 est un bon modèle préclinique – du fait de sa fidélité tant anatomoclinique que physiopathologique à la SpA humaine (Breban et al., 2021b). Corroborant le rôle pathogène d'un défaut d'IL-27 au cours de la SpArat, une souris IL-27RA<sup>-/-</sup> p53<sup>R172H/+</sup> développe un phénotype de type SpA axiale (Dibra et al., 2018). Compte tenu de ces résultats, il semblerait pertinent à ce stade d'optimiser la galénique de l'IL-27 – qui présente du fait de sa structure une instabilité problématique en termes pharmacodynamique. Cela permettrait ensuite d'envisager un essai thérapeutique de phase I, dans l'optique de compléter l'arsenal thérapeutique de la SpA. Celui-ci s'est d'ailleurs enrichi cette dernière décennie par les inhibiteurs spécifiques de l'IL-17 et de l'IL-23, à la lumière des solides données physiopathologiques soulignant le rôle de l'activation inappropriée de l'axe IL-23/IL-17 au cours de la SpA (Chergaoui et al., 2021). Toutefois, l'efficacité dissociée et non anticipée d'une part des anti-IL-17 sur l'atteinte articulaire mais pas sur les atteintes extraarticulaires, et d'autre part des anti-IL-23 sur les atteintes extra-articulaires mais pas sur

l'atteinte articulaire, a souligné un défaut dans la modélisation de ce système complexe (Sieper et al., 2019). D'introduction plus récente dans l'arsenal thérapeutique de la SpA, l'efficacité des JAKi implique d'autres enjeux. D'une part, ne s'agissant pas d'anticorps monoclonaux, ils présentent l'avantage d'être stables et de pouvoir s'administrer oralement plutôt que par voie injectable, mais cet avantage est contrebalancé par l'inconvénient d'une spécificité non contrôlée vis-à-vis de leur cible. De plus, tenant compte de l'effet redondant de la voie JAK/STAT, une JAK donnée répond à différentes cytokines, et agit sur différents effecteurs d'aval, dont certains possiblement bénéfiques pour les réponses immunes (Salas et al., 2020). Enfin, des données récentes de phase IV ont mis en garde contre un potentiel surrisque oncologique et cardiovasculaire induit par un anti-JAK1/JAK3 (Ytterberg et al., 2022). Mieux comprendre les mécanismes les plus en amont dans le développement d'une réponse biaisée de type 3 permettrait d'une part de mieux anticiper le résultat de la modulation d'une cible donnée, d'autre part de découvrir et évaluer de nouvelles cibles.

# CONCLUSIONS

La SpA est une maladie complexe, hétérogène aux plans clinique, biologique, et physiopathologique. De nombreux acteurs cellulaires, relevant de mécanismes immunitaires innés ou adaptatifs, et agissant sur différents sites – notamment médullaire osseux, synovial, intestinal – participent à l'exacerbation d'une réponse immune de type 3. Une caractéristique forte de la SpA est son association au HLA-B27, dont la preuve du rôle de pathogène a été établie à l'aide du modèle du rat-B27. Le modèle du rat-B27 nous a permis ici de mettre en lumière un défaut précoce de la voie STAT1/IFN dans les lymphocytes T CD4<sup>+</sup> naïfs, précédant le développement de la SpA-rat, favorisant secondairement un biais de différenciation Th17, potentialisé par les cDC2 et imprimé au plan épigénétique. Ce phénomène est réversible en induisant la voie STAT1. Il reste à comprendre aujourd'hui les mécanismes qui relient la voie STAT1 au HLA-B27, à la fonction des DC et des lymphocytes T CD4<sup>+</sup>, en lien avec la dysbiose intestinale.

# REVUE 1 – Spondyloarthrite axiale : nouvelles cibles thérapeutiques

EXPERT OPINION ON THERAPEUTIC TARGETS https://doi.org/10.1080/14728222.2021.1973429

## REVIEW

## Axial spondyloarthritis: emerging drug targets

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## ABSTRACT

**Introduction:** Axial spondyloarthritis (AxSpA) is an inflammatory disorder that affects the joints, entheses, and bone tissues and is sometimes associated with psoriasis, anterior uveitis, and gut inflammation. Its pathogenesis is not wholly understood and treatment strategies require optimization. Data concerning AxSpA pathogenesis support a critical role of abnormal CD4<sup>+</sup> T cell differentiation and exacerbated type 3 immune response. This knowledge boosted the development of interleukin (IL)-17 and Janus kinase inhibitors for AxSpA treatment beyond tumor necrosis factor- $\alpha$  inhibition.

Areas covered: Emerging drug targets in animal and cellular models and with phase-II clinical trials have been evaluated. We also reflect on key issues for preclinical and clinical research going forward. Expert opinion: Some of the most promising approaches include: (i) modulation of transforming growth factor- $\beta$  family that could exert a specific role on bone formation; (ii) blockade of granulocyte-macrophage colony-stimulating factor that could reduce type 3 immune responses, and (iii) rebalancing of biased immune response by cytokines such as IL-2 or IL-27 that could favor anti-inflammatory response and sustained drug-free remission. Multiomics tools and artificial intelligence could contribute to identification of optimal targets and help stratify patients for the most appropriate treatment options.

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## 1. Introduction

## 1.1. Clinical description

Axial spondyloarthritis (AxSpA) is a chronic inflammatory rheumatism, predominantly affecting spine and sacroiliac joints [1]. It is generally accepted that there is a continuum from nonradiographic to radiographic AxSpA, so-called ankylosing spondylitis (AS) [2]. The prevalence of AxSpA varies from 0.1 to 1.4% in Northern countries [3]. Sex distribution is balanced for nonradiographic AxSpA patients, whereas AS is more frequent in male, with a ratio of 2–3:1 [4]. In average, AxSpA starts during the third decade, and in about 10% of patients before the age of 16 years, but in less than 5% after 50 years [5]. The key features of AxSpA are summarized in Figure 1.

Patients affected with AxSpA complain of chronic back/ buttock pain, with inflammatory characteristics (i.e. responsible for night awakening and morning stiffness, improved by exercise and worsened by inactivity), entheses pain, peripheral arthralgia, and chronic fatigue. Clinical examination can evidence spine and/or sacroiliac joint pain on pressure, axial skeleton stiffness, peripheral arthritis, dactylitis, peripheral enthesitis. AxSpA is often associated with extra-articular manifestations: acute anterior uveitis in 5–40%, psoriasis in 4–20%, inflammatory bowel disease (IBD) in 4–10% of the cases [6]. Family history of SpA or extra-articular related features is reported in 20–40% of the patients [7]. Advanced radiographic structural changes of the sacroiliac joints are considered mandatory to ascertain AS, but their absence does not exclude AxSpA, particularly in the early phase and in females. Sacroiliac joints abnormalities can be assessed by plain radiograph, magnetic resonance imaging (MRI), or computed tomography scanning [8,9]. One of the hallmarks of AxSpA is a combination of bone resorption with exaggerated ossification – especially at entheses, i.e. syndesmophytes when affecting the vertebra.

Importantly, the human leukocyte antigen (HLA)-B27, a class I surface molecule encoded by the HLA-B locus of the major histocompatibility complex, is present in 70–90% of AxSpA patients, as compared to 8% in the Caucasian population. Its presence increases by 40-fold the risk of developing AxSpA [10]. However, given its relatively high prevalence in the general population, its specificity is insufficient to allow screening for AxSpA in the absence of suggestive context [11]. Increased levels of C reactive protein or erythrocyte sedimentation rate may also contribute to the diagnosis, but with limited specificity and sensitivity.

The diagnosis of AxSpA remains challenging, especially in early disease. Indeed, it relies on a combination of suggestive clinical manifestations and supportive biological and imaging abnormalities that lack specificity and exclusion of alternative diagnosis, including degenerative spine disease, diffuse

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#### Article highlights

- Recent data from translational research and animal models have aided the identification of new drug targets for AxSpA.
- Reversal of type 3 immune cell response by conterbalancing STAT3
- or RORY activation, is a promising approach to dampening uncontrolled inflammation in SpA. Fine-tuning the immune response by using cytokines such as IL-2 or IL-27 is an appealing direction to correcting SpA-associated inflam-
- IL-27 is an appealing direction to correcting SpA-associated inflammatory response.
   Emerging data highlight TGF-β and GM-CSF as targets of particular
- interest.Targeting nonimmune cells or metabolic bone change is a more
- distant goal for the future.
   Integrating multiomics data could improve knowledge and appreciation of disease heterogeneity and tailor suitable therapeutic targets at patient level.

idiopathic skeletal hyperostosis, or fibromyalgia [12]. Overall, the diagnosis delay is often long, i.e. 7–11 years in average [13].

AxSpA has a major impact on the social life of patients, causing physical and psychological burden, work productivity loss, reduced employment, and life expectancy [14]. All of this is responsible for heavy social burden, incremented by the cost of care. Thus, global burden of the disease would be reduced if long-term remission was quickly reached, and if structural damage was limited [15].

## 1.2. Pathogenesis of AxSpA

Understanding the mechanism of AxSpA may help to improve diagnosis, define therapeutic targets, and monitor disease evolution [16]. An overview of current knowledge on AxSpA pathogenesis is displayed in Figure 2.

A remarkable aspect of AxSpA is its high heritability, involving multiple genetic factors dominated by HLA-B27, discovered almost 50 years ago. In recent years, genome-wide association studies revealed numerous additional susceptibility genetic factors, including polymorphisms in more than 100 loci, several of which are involved in immune response, such as *ERAP1, IL23R*, and *RUNX3*. However, each of these novel findings explains only a small fraction of disease predisposition [17–19].

The precise mechanism by which HLA-B27 predisposes to AxSpA remains incompletely understood [11,20]. However, its pathogenic role has been demonstrated in animal model. Hence, HLA-B27 and human  $\beta$ -microglobulin (h $\beta$ 2 m) transgenic rat lines, develop a spontaneous inflammatory disease that strikingly recapitulates the spectrum of SpA, by combining peripheral arthritis with ulcerative colitis-like IBD and psoriasis [21]. In this model, altered interactions between antigen-presenting cells - including dendritic cells (DC) and CD4<sup>+</sup> T cells contribute to chronic inflammation [21-24]. Interestingly, a possible implication of CD4<sup>+</sup> T cells, harboring a biased TCR repertoire, and of antigenpresenting cells, has also been evidenced in AxSpA patients [25,26]. Moreover, excessive activation of type 3 immune response, including a bias of CD4<sup>+</sup> T cells differentiation toward T helper 17 (Th17) cells characterized by the production of interleukin-17 (IL-17) among other cytokines- has been reported in both animal models and AxSpA patients [23,27-34]. Disease development is thus associated with increased production of proinflammatory cytokines,



Figure 1. The clinical picture of axial spondyloarthritis

Axial spondyloarthritis (AxSpA) is a complex and heterogeneous disease, especially in terms of prevalence, clinical features, and drug response. AxSpA is predominantly responsible of spine and sacroiliac joints inflammation, and to a lesser extent of peripheral limbs arthritis, enthesitis or dactylitis. Extra-articular features might also occur, including acute anterior uveitis, psoriasis, and inflammatory bowel disease. IL17i: Interleukin 17 inhibitors; JAKi: Janus kinase inhibitors; NSAIDs: Nonsteroidal inflammatory drugs; TNFi: Tumor necrosis factor α inhibitors



## Figure 2. Pathogenesis of axial spondyloarthritis.

The cause of AxSpA that remains incompletely understood, involves a combination of genetic and environmental factors. This combination leads to innate immunity, and bone-resident cells dysregulation. It implicates the abnormal secretion of cytokines, such as IL-23. Moreover, adaptive immunity is inappropriately activated, leading to an expansion of IL-17-producing lymphoid cells, such as pathogenic T helper 17 cells. These abnormal immune responses might occur locally, or at distant sites. SNP: single-nucleotide polymorphism

including IL-17, IL-23, and tumor necrosis factor (TNF)- $\alpha$  [35,36], cytokines that have been targeted by biotherapies.

In addition, to better understand the particular pattern of bone pathology observed in AxSpA and its link with type 3 immune responses, the relationship between osteoclast/ osteoblast balance and functions, mesenchymal cells, and immune cells has been examined [34,37]. Interestingly, in this context, there might be differential mechanisms operating in trabecular bone on one hand – associated with bone loss, and in enthesis on the other hand – associated with bone formation [27,38–40].

In addition to the strong genetic predisposition, environmental factors, such as mechanical stress and the microbiome play a critical role on AxSpA pathogenicity. Gut microbiota dysbiosis has been observed in AxSpA patients and in HLA-B27 transgenic rat model, and might partly explain Th17 differentiation bias [41–43]. Remarkably, those rats are protected from disease development when raised in germ-free conditions [44]. However, the precise mechanism by which microbiota might contribute to disease development remains unknown [45].

## 1.3. Current treatments

### 1.3.1. Overview

AxSpA requires a multidisciplinary management coordinated by the rheumatologist, following codified evidence-based and updated strategies [46]. Goals of the medical treatment are mainly to: (i) reduce musculoskeletal symptoms, while improving function and social participation; (ii) prevent or slow down structural osteo-articular damage and bone formation; (iii) prevent or reduce extra-articular manifestations (uveitis, psoriasis, IBD); (iv) limit disease-secondary comorbidities, including osteoporosis and cardiovascular events [47].

## 1.3.2. Immunomodulatory drugs

Current drugs validated for AxSpA care are summarized in Table 1. Treatment usually starts with nonsteroidal antiinflammatory drugs (NSAIDs). Noteworthy, NSAIDs efficacy on spine/buttock pain is also considered evocative of AxSpA. NSAIDs exert pleiotropic effects, mediated by the inhibition of arachidonic acid/cyclo-oxygenase/prostaglandin pathway.

In patients with persistent disease activity despite optimal NSAIDs intake, TNF- $\alpha$  inhibitors (TNFi) are recommended as first biotherapy. TNFi act on broad innate/adaptive immune cell types, partially through nuclear-factor-kappa B (NFkB) pathway modulation [35]. Used in the clinic since the late 1990's, TNFi benefit from a wide experience with respect of their long-term safety profile. Approximately 60–70% of AxSpA patients exhibit a sustained response to TNFi, achieving partial

In case of therapeutic failure with one TNFi, either primarily or following initial success, switching to another TNFi or to an anti-IL-17 blocking antibody (IL-17i) should be considered. Indeed, several IL-17i have been successfully developed to treat AxSpA since 2010's, a result consistent with the pathological role of excessive type 3 immune response in this disease [48].

Otherwise, Janus kinase (JAK) inhibitors (JAKi) are a family of small molecules, that exhibited clear efficacy to treat several immune-mediated inflammatory disorders, during the last 20 years, and have been more recently used in AxSpA [49].

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Table 1. Current treatments for AxSpA.

Drug		Administration	Efficiency on extra-	Main adverse effects
class	Name	mode	articular involvement	CI during pregnancy
NSAIDs	-	0	No	- Gastrointestinal ulceration
				- Renal dysfunction
				- High blood pressure
				- Cardiovascular events
				- CI during the 3rd trimester
TNF-α	Etanercept	SC	- Psoriasis	<ul> <li>Infection (including reactivation of tuberculosis)</li> </ul>
inhibitors	Adalimumab		- IBD	- Cephalalgia
	Golimumab		- Uveitis	- Cytopenia
	Certolizumab		- Psoriasis	- Hepatic reaction
				<ul> <li>No absolute CI during pregnancy with any TNFi. The choice of Certolizumab is preferred if pregnancy is planned since it is the only one that does not cross the placenta barrier</li> </ul>
	Infliximab	IV, SC		
IL-17	Secukinumab	SC	- Psoriasis	- Infection
inhibitors	Ixekizumab		- Avoid in case of	(including mycosis)
			active IBD	- Neutropenia
				- Injection site cutaneous reaction
				- CI during pregnancy due to lack of information
JAK	Tofacitinib	0	- Psoriasis	- Infection
inhibitors	(JAK1/3)		- UC	(including varicella-zoster virus)
	Upadacitinib		- Psoriasis	- Cephalalgia
	(JAK1)		- IBD	- Cytopenia
				- Hepatic reaction
				- Possible increased risk of cancer and cardiovascular events
				- CL during pregnancy

- CL during\_pregnancy. Despite negative trials in AxSpA, sulfasalazine is still considered as a potential drug of interest to treat peripheral arthritis manifestations when biologics are contraindicated or unavailable. Noteworthy, intra-articular injection(s) of glucocorticoid may be helpful, and adjuvant analgesics can be used on demand. The importance of nonpharmacological strategies in AxSpA should also be highlighted, including exercise on a regular basis, physical therapy, smoking discontinuation, overweight correction, albeit this is not the scope of this review. CI: Contraindicated; D: day; IBD: Inflammatory bowel disease; IL: Interleukin; IV: Intravenous; JAK: Janus kinase; NSAID: Non-steroidal inflammatory drugs; O: Oral; SC: Subcutaneous; TNF: Tumor necrosis factor; UC: Ulcerative colitis; W: Week.

JAK signaling pathway is used by an array of cytokine and growth factor receptors, including IL-6, IL-12, IL-23, IL-21, interferon (IFN)- $\gamma$ , granulocyte-macrophage colony-stimulating factor (GM-CSF), several of them being implicated in the pathogenesis of SpA [50,51]. optimize drug development. Current advances regarding AxSpA pathogenesis led to propose mechanistic hypotheses, resulting in putative drug targets, as highlighted in Figure 3.

## 1.4. Mechanistic hypotheses driving drug development

Basic and translational research is critical to improve our knowledge concerning AxSpA pathogenesis, and to

## 2. Emerging drug targets

Compounds that are currently in phase I or II clinical trials for AxSpA treatment are summarized in Table 2.



Figure 3. New targets in axial spondyloarthritis.

Figure 2: New targets in taxit sponsyloation. Two kinds of strategies and translational data revealed critical pathways involved in AxSpA pathogenesis. This opens new targets for drug development. Two kinds of strategies can be identified: either inhibiting proinflammatory cytokines – by directly inhibiting IL-17 or GM-CSF for instance, or indirectly limiting their production through JAK-STAT pathway modulation; or favoring immuno-regulatory responses – by inducing regulatory T cells, or indirectly increasing the production of IL-10 for instance. GM-CSF: Granulocyte macrophage colony stimulating factor, IL: Interedivin, JAK Janus Kinase; p: phosphorylated STAT; STAT: Signal Transducers and Activators of Transcription; TGF-β: Transforming Growth Factor β; TNF-α: Tumor Necrosis Factor α; TYK2: Tyrosine Kinase 2

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Table 2. Drugs in	phase I/II for AxSpA.				
Compound	Mechanism of action	Company	Structure	Indication	Stage of development For AS indication
Bimekizumab	Anti-IL17A and F	UCB	Humanized monoclonal antibody	<ul> <li>Psoriasis</li> <li>Active radiographic AxSpA after NSAID failure</li> </ul>	- Phase IIb - Ongoing phase III
Netakimab	Anti-IL17A	Biocad	Humanized monoclonal antibody	<ul> <li>Psoriasis</li> <li>Active radiographic AxSpA after NSAID failure</li> </ul>	- Phase IIb - Ongoing phase III - Approved in Russia
Vuakizumab (SHR1314)	Anti-IL17A	Creative Biolabs	Humanized monoclonal antibody	- Psoriasis - Active AxSpA after NSAID failure	- Ongoing phase I/II
Brodalumab (KHK4827)	Anti-IL17RA	Leo Pharma	Humanized monoclonal antibody	- Psoriasis, PsA - Active AxSpA after NSAID failure	- Completed phase III
Tildrakizumab	Anti-IL23	Sun Pharma	Humanized monoclonal antibody	- Psoriasis - Active AxSpA after NSAID failure	- Phase II/III
Namilumab	Anti-GM-CSF	Takeda Izana	Humanized monoclonal antibody	<ul> <li>Psoriasis: fail to achieve primary end point</li> <li>Active AxSpA after NSAID failure</li> </ul>	- Ongoing Phase Ilb (Namaste)
SHR0302	Anti-JAK1	-	-	- Active AS after NSAID failure	- Ongoing recruitment
NDI-031407	Anti-TYK2	Nimbus therapeutics	-	- AxSpA	<ul> <li>Preclinical study (SKG mouse model of AS)</li> </ul>
IPH4102	KIR3DL receptor inhibitor	Theradis Pharma	Monoclonal antibody	- Active AxSpA	- Phase I

AxSpA: axial spondyloarthritis; AS: ankylosing spondylitis; GM-CSF: Granulocyte macrophage stimulating colony stimulating factors; IBD: inflammatory bowel disease; IL: interleukin; JAK: Janus kinase; NSAIDs: Nonsteroidal inflammatory drugs; TGF-β: transforming growth factor; PsA: psoriatic arthritis.

## 2.1. JAK/STAT pathway modulation

## 2.1.1. JAK inhibitors

The efficacy of JAK inhibition (JAKi) in AxSpA, including upadacitinib and tofacitinib, has been demonstrated in several clinical trials. The potential interest of other selective inhibitors of the pathway is still under study, including baricitinib – preferentially JAK1/2i, peficitinib – preferentially JAK3i over JAK 1/2 and TYK2, filgotinib – preferentially JAK1i.

Activation of JAK is triggered by receptor dimerization, resulting in the phosphorylation of signal transducer and activator of transcription (STAT), subsequently translocated to the nucleus as dimer, where it acts as transcription factor. Thus, JAK-STAT pathway is complex and exerts pro- and antiinflammatory effects, regulating a variety of critical cellular processes, such as cell growth, survival, and activation [51].

The specific target of JAKi is not clearly defined in AxSpA, but the mechanism of their anti-inflammatory effect has been recently investigated. JAKi decrease STAT1, 3, and 5 phosphorylation, inducing inhibition of Th17 responses and GM-CSF production, especially in the synovial fluid [52]. Interestingly, in psoriasis, a condition related to SpA, tofacitinib effect was partly attributed to a decrease in DC number and a type 3 immune response inhibition [53].

## 2.1.2. STAT3 inhibition

STAT3 is a critical transcription factor for Th17 differentiation. Inhibition of STAT3 phosphorylation has been evaluated using SpA mouse model (curdlan-challenged SKG mice). Treatment with PIAS3, a protein inhibitor of activated STAT3, reduced disease severity. This beneficial effect was associated with lower levels of IL-17A and TNF- $\alpha$  in the peripheral joint, axial bone, and small intestine/colonic tissues [54]. In this model, PIAS3-dependent reduced STAT3 phosphorylation and Th17 differentiation were associated with increased STAT5 phosphorylation and regulatory T cells differentiation. Noteworthy, PIAS3 also negatively regulates osteoclastogenesis, an effect that could be beneficial for AS-associated osteoporosis [55].

## 2.2. T cells differentiation reprogramming

## 2.2.1. Interleukin 2

Low-dose IL-2 supports the development of regulatory T cells in the thymus and is a key factor for regulatory T cells survival, stability, and function in the periphery. At higher doses, IL-2 is also critical for CD4<sup>+</sup> T cells proliferation, and terminal differentiation of CD8<sup>+</sup> T cells. This led to study the effects of low dose IL-2 (0.25-3 millions IU per day), in order to specifically and safely expand regulatory T cells [56]. Administration of low-dose IL-2 for 6-months was evaluated in several immune-mediated inflammatory disorders, including 10 patients with AS, in an uncontrolled open-label trial [57]. IL-2 treatment was associated with a transient increase in circulating regulatory T cells and a sustained increase in regulatory T cells over effector T cells ratio, until 8 months. In the AS group, a 25% improvement of BASDAI was achieved after 6 months, which is in the range of the placebo effect routinely observed in controlled trials. Otherwise, the overall safety profile of low-dose IL-2 appeared acceptable [56].

Conversely, in HLA-B27 transgenic rat, low-dose IL-2 treatment was inefficient to reverse effector/regulatory CD4<sup>+</sup> T cells imbalance and to prevent SpA development, albeit an increase in regulatory T cells was observed in the IL-2 treated wild-type littermates [58]. The prospect of low-dose IL-2 treatment for AXSpA remains uncertain but might be improved by adapting drug dose or stability. Moreover, mutated IL-2 proteins with enhanced regulatory T cell-selectivity – due to increased dependence on the IL-2-receptor component CD25 that is expressed at higher levels on those cells than on effector T cells – have shown efficacy in murine model of autoimmunity and are currently tested in patients with autoimmune arthritis [59].

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### 2.2.2. Interleukin 27

IL-27 is a heterodimeric cytokine, mainly produced by activated antigen-presenting cells, macrophages and fibroblasts, that signals via a specific heterodimeric receptor [60]. IL-27 displays broad biologic effects, depending on context and temporal aspects, and feedback modulation. Initially, it was considered as proinflammatory, by enhancing IFN-y production and Th1 differentiation, through STAT1 and T-bet activation [61]. More recent work evidenced that it could display anti-inflammatory properties by negatively regulating Th17mediated inflammatory response, through STAT3 and RORa/ RORyt repression, and that this could be mediated by STAT1 and suppressor of cytokine signaling protein 1 induction [62]. Moreover, it can promote regulatory T cells function by upregulating IL-10 production [61]. Hence, IL-27 reduced arthritis severity and gut inflammation in several animal models by inhibiting Th17 while promoting Th1 responses, potentially reducing innate cell recruitment and IL-6/IL-17A-production [63-65].

In AxSpA patients, serum levels of IL-27 were correlated with disease activity; however, the precise role of this cytokine in this context remains to be established [66]. Interestingly, IL-27 expression was decreased in DC from HLA-B27 transgenic rat lines developing SpA [22]. Moreover, *in vitro* coculture of CD4<sup>+</sup> T cells with DC in the presence of IL-27 resulted in an inhibition of IL-17 and an increase of IL-10 production in this model, suggesting that it might exert immunoregulatory effects [67].

Worth mentioning, IL-27 appeared as a negative regulator of vertebral bone loss and cartilage ossification of intervertebral discs, two features of AxSpA, as shown in a genetically modified mouse model combining IL-27 receptor  $\alpha$  deletion with p53 heterozygous dominant negative mutation, which also develops neutrophilic dermatitis [68].

## 2.2.3. RORyt inhibition

RORyt is the hallmark transcription factor for Th17 differentiation. Several studies reported an increased expression of its coding gene – *RORC* – in peripheral blood or arthritic joint lymphoid cells, including CD4<sup>+</sup> T cells,  $\gamma\delta$ -T cells, invariant natural killer T cells, and type 3 innate lymphoid cells from SpA patients [69]. The  $\gamma\delta$ -T cells and invariant natural killer T cells isolated from SpA patients joint were a major source of IL-17 and pharmacological inhibition of RORyt reversed selectively the abnormal secretion profile of those cell subsets *in vitro* [69]. However, such strategy has not yet been translated into clinical application.

## 2.2.4. Targeting epigenetic

Cell fate is tightly regulated by transcriptional program, which depends on epigenetic changes – modulating the expression of specific genes core. Epigenomic have been extensively studied in the context of T cell differentiation, notably of Th17 cells; and epigenomic abnormalities might be involved in AxSpA pathophysiology [70–72].

Reconditioning epigenomic landscape to reverse pathogenic process has thus emerged as a new potential therapeutic goal, initially in the field of cancerology, but extending now to immune-mediated inflammatory disorders, especially to reprogram T cell differentiation [73–75]. Hence, histone H3K27 demethylase KDM6 appears critical for Th17 differentiation [76]. A KDM6 inhibitor (GSK-J4) was tested on *in vitro* differentiated Th17 cells from AxSpA patients and shown to induce transcriptional and metabolic reprogramming associated with suppression of IL-17 production.

Despite such promises, no clinical study of epigenetic modulation has been initiated yet for immune-mediated inflammatory disorder treatment. Importantly, as far as the main preclinical and clinical research in the field has been developed for cancer, safety profile remains difficult to extrapolate to other types of diseases.

## 2.3. Other targeted cytokines

**2.3.1.** Granulocyte-macrophage colony-stimulating factor Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor and a cytokine, mainly involved in myeloid cells differentiation. It is secreted by T cells, innate lymphoid cells, macrophages, fibroblasts, chondrocytes, endothelial, and epithelial cells, in the context of a local inflammatory process, especially in response to IL-1 $\beta$ , IL-6 and TNFa. Its receptor, that signals through JAK2 activation, is expressed by myeloid cells – monocyte/macrophages, neutrophils, eosinophils, DC – and fibroblasts [77].

Recent studies have highlighted the role of this cytokine as a target of interest in SpA. Indeed, in curdlan-challenged SKG mice, hematopoietic stem cells were shown to upregulate GM-CSF-receptor, leading to an increased myelopoiesis and accumulation of granulocyte/monocyte progenitors in the bone marrow and at extramedullary sites, such as the joint [78]. In that model, GM-CSF promoted a tissue-toxic neutrophil accumulation and GM-CSF blockade substantially decreased disease severity. GM-CSF is also involved in cartilage destruction and bone resorption in arthritis mouse models [79].

Moreover, GM-CSF production by lymphoid cells and synovial T cells from AxSpA patients was shown to be increased [80]. In that study, GM-CSF was mainly produced by CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells,  $\gamma\delta$  T cells and innate lymphoid cells. Another study revealed that type 3 innate lymphoid cells, with a specific capacity to express GM-CSF and IL-22 after stimulation, were expanded in AxSpA arthritic joint [81].

Phase II clinical trials using neutralizing monoclonal antibodies to target GM-CSF (otilimab, namilumab) or its receptor (mavrilimumab) have been undertaken in rheumatoid arthritis, showing preliminary evidence of rapid and sustained efficacy with a satisfactory safety profile [82,83]. A phase II study is ongoing in AxSpA. However, the role of GM-CSF in systemic immune response, especially regarding its immunoregulatory effects, is not well defined and should raise caution [84,85].

## 2.3.2. Transforming growth factor $\beta$ pathway

The transforming growth factor (TGF)- $\beta$  superfamily consists of more than 30 mediators, including TGF- $\beta$ , bone morphogenetic proteins (BMP) and activins. TGF- $\beta$  is mainly produced by activated fibroblasts, epithelial cells and DC [86]. Upon activation, TGF- $\beta$  binds to its receptor, leading to SMAD family members 2/3 phosphorylation, and to a lesser extent, to mitogen-activated protein kinases, Rho-GTPases and/or phosphoinositide 3-kinase activation. TGF- $\beta$  is crucial for various biological functions, including embryogenesis. It has been extensively studied for its anti-proliferative effects and its role in anti-inflammatory responses, especially the promotion of regulatory T cells maintenance/function, but also in Th17 differentiation [87]. Interestingly, TGF- $\beta$  also contributes to the maintenance of intestinal epithelium barrier function and some commensal gut bacteria exert a TGF- $\beta$ -dependent immunomodulatory effect [88].

The HLA-B27 and hβ<sub>2</sub>m transgenic Drosophila model designed to investigate the non-canonical effects of HLA-B27 - linked HLA-B27 expression to signaling disruption of the BMP pathway at the level of its type I receptors [89]. Interestingly, mammalian orthologs of the receptor impacted by HLA-B27 transgene in Drosophila, i.e. ALK1 and ALK2 are involved in activin, and TGF- $\beta$  signaling regulation. Consistently, T cells from AxSpA patients exhibited an increased phosphorylation of SMAD2/3 in response to activin A and TGF- $\!\beta$  stimulation. Thus, this deregulated pathway could contribute to skew Th17 differentiation and ossification process, two hallmarks of AxSpA. With this regard, it is remarkable that genetically determined dysregulation of ALK2 is responsible for Fibrodysplasia Ossificans Progressiva, a rare genetic disorder that leads to exuberant ectopic ossification dependent on excessive activin signaling [90]. An anti-activin -A antibody, Garetosmab, has been developed to treat this condition, that could offer an opportunity for AS treatment [91].

### 2.3.3. Interleukin 1

IL-1 is the prototypic pro-inflammatory cytokine. There are two forms of IL-1: IL-1 $\beta$  and  $\alpha$ . IL-1 $\beta$  is produced by monocytes/ macrophages and neutrophils in context of pathogen-induced or cellular-sensed stress signals. IL-1 $\beta$  precursor becomes active via an intracellular inflammasome-dependent cleavage or *via* the action of proteases. IL-1 $\alpha$  is produced by epithelial cells, acting mainly locally, and can be released extracellularly especially in the context of necrosis. IL-1 receptor is widely expressed on myeloid cells, T cells, chondrocytes, and osteo-clasts. Binding of IL-1 results in NFkB pathway activation, IL-23 and GM-CSF production, Th17 differentiation, osteoclast stimulation, among others [92–94]. Several natural mechanisms counterbalance these effects, such as endogenous antagonist receptors.

IL-1 could play a role in AxSpA, as shown in the HLA-B27 transgenic rat model [95]. Interestingly, genetic association has been described between AxSpA and polymorphisms in *IL1B* and *IL1R* genes [96–98]. Moreover, rare polymorphism of the *MEFV* gene responsible for familial Mediterranean fever – a recessive IL-1-mediated monogenic disease – has been associated with the risk of developing AxSpA and several other IL-1-mediated monogenic clasease are characterized by manifestations closely related to AxSpA, including chronic osteomyelitis, arthritis, and/or IL-17/23-mediated psoriasis [99–102].

Efficacy of the commercially available natural antagonist of IL-1 (IL-1RA), anakinra, was tested in patients with rather longstanding AxSpA in two small-size open-label uncontrolled trials, showing disparate efficacy but no safety issue

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[103,104]. One of them reported significant improvement of clinical parameters, serum C-reactive protein levels and MRI scoring [102], whereas improvement was weaker and non-significant in the other, albeit still considered as greater than expected from a placebo effect [101]. Considering the rather short half-life of anakinra that may limit its biological activity, there is still a potential therapeutic interest for IL-1 blockade in AxSpA, in particular for stable blocking agent, such as anti-IL-1 antibody.

## 2.4. Acting on nonimmune mechanisms

## 2.4.1. Mesenchymal stem cells

As discussed previously, better understanding of bone pathogenesis in AxSpA might help to develop treatments capable of opposing syndesmophytes and bony ankylosis development. In that purpose, it was shown that mesenchymal stem cells (MSC) - precursor of chondrocytes and osteoblasts - isolated from AxSpA patients spinal enthesis supported an accelerated mineralization process in vitro that was associated with activation of a metabolic pathway, implicating HLA-B27 and the tissue-nonspecific alkaline phosphatase (TNAP) [105]. Inhibitors of TNAP - including pamidronate - reversed exaggerated AxSpA MSC mineralization and blocked in vivo bony appositions in a mouse in which AxSpA or control MSC were implanted in the right lamina of lumbar spine. Consistently, serum bone-specific TNAP level positively correlated with the risk for radiographic progression in AxSpA patients [105]. Thus, this work opens new therapeutic strategy to halt AxSpA ossification.

## 2.4.2. Unfolded protein response

Protein folding and quality control are controlled events occurring in the endoplasmic reticulum and critical for cell homeostasis [106]. Internal stressors might be recognized during this process and induce the unfolded protein response (UPR). This UPR then stimulate the production of several proteins, including chaperones and proinflammatory cytokine, such as IL-23. HLA-B27 appears to be especially prone to misfolding, being thus a potential activator of UPR, possibly linking HLA-B27 to skewed Th17 response. Indeed, UPR has been shown to occur in bone marrow macrophages from HLA-B27 transgenic rat, but not yet in AxSpA patients cells [28,105,107,108]. UPR therapeutic modulation has already emerged in cancer field and could thus be applied to AxSpA [109]. Importantly, it remains uncertain in how inhibiting such critical biological process might adversely impact systemic homeostasis.

### 2.4.3. Intervention on microbiota dysbiosis

Other strategies might emerge from new evidence linking AxSpA pathogenesis to gut microbiota changes, i.e. dysbiosis. Hence, manipulating specific bacterial subpopulations could improve disease in animal models of SpA [45]. Pilot interventions on microbiota conducted in the context of IBD, using direct (i.e. probiotics or fecal microbiota transplant) or indirect (i.e. prebiotic diet) approaches have paved the way in clinic and are now becoming translated to AxSpA treatment [110] [ClinicalTrials-NCT03092427: Probiotic Treatment in Juvenile

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Idiopathic Arthritis; NCT04713995: Microbiome and Axial Spondyloarthritis].

## 2.4.4. Neuromodulation

Acting on the neurologic control of inflammation and pain appears as a promising way of improving patients' symptoms. With this regard, ongoing study is aiming at evaluating the efficacy of meditation techniques in AxSpA [ClinicalTrials-NCT02744014: Add-on Therapy for AS], whereas another one will evaluate the efficacy of noninvasive *vagus* nerve stimulation in AxSpA patients unresponsive to biologics [ClinicalTrials-NCT04286373: Efficacy of noninvasive Vagus Nerve Stimulation for Treatment of AS Resistant to Biotherapies] [111].

## 3. Conclusion

Beyond the breakthrough represented by TNFi two decades ago, continuous effort contributed to the emergence of several other targeted treatments for AxSpA. These include IL-17i and JAKi, with efficacy roughly comparable to TNFi. Overall, the safety profile of these drugs appears reassuring, even if long-term data are only available for TNFi. However, there is still a need for more innovative therapeutics, including safer and more precise ways to reset the inflammatory process underlying AxSpA. This will require sustained fundamental and translational research. Current drug target development aims specially to reprogram abnormal differentiation of CD4<sup>+</sup> T cells. However, promising data point toward the potential interest of delivering cytokines able to exert immunoregulatory properties - such as IL-2 or IL-27 -, of blocking proinflammatory mediators with pleiotropic biological functions – such as GM-CSF and IL-1 – or of modulating TGF- $\beta$ pathway.

### 4. Expert opinion

Improving the research strategy performance for AxSpA relies on identification of key inflammatory pathways – that may depend or not of the presence of HLA-B27 – involved in disease onset and flaring. It is also important to clarify which cellular subtype(s) and/or tissue(s) should preferentially be targeted. All those aspects should take into account specific extra-articular manifestations risk that depends on the patient. The ultimate goal is to achieve drug-free remission or induce self-sustaining changes in the chronic inflammatory process, in order to avoid disease relapse – occurring in a majority of AxSpA patients after treatment discontinuation [112]. Finally, one should aim at limiting unwanted immunosuppression – an usual collateral effect of drugs inhibiting chronic inflammation – that might favor infectious events or cancer development.

TNFi were the first successful targeted therapies for AxSpA. Remarkably, all the five TNFi that have reached the market (Table 1) display high level of longstanding efficacy and safety profile in AxSpA, in such a way that they have not been surpassed by more recent drugs. Importantly, one of the limitations of TNFi is the high rate of adverse immunization that they provoke, leading to anti-TNFi antibodies development and treatment escapement, especially in females. Thus, engineering newer generation of TNFi devoid of such caveat would be tremendously useful. Moreover, developing TNFi selective for one of the two distinct receptors (TNFa receptor I or II) could provide an advantage over the current nonselective TNFi, in terms of efficacy on the different aspects of AxSpA manifestations and safety.

Recent progress in understanding AxSpA pathogenesis helped to define several new relevant drug targets. Targeting pathogenic type 3 immune response appears central to those developments. This concerns primarily the cytokines that drive this type of response (IL-1, IL-6, IL-21, IL-23) and the pro-inflammatory mediators produced by the corresponding effectors, exemplified by Th17 cells (IL-17A, IL-17F, TNFa, GM-CSF). Indeed, as cytokines are circulating mediators with potential systemic actions, they appear as optimal drug targets. However, precisely understanding the complete context of production and action of a given cytokine is mandatory to predict the effects of its blockade. Remarkably, among the drivers of Th17 cell differentiation. II-6 or IL-23 inhibition failed to improve established AxSpA, although it appeared efficacious on rheumatoid arthritis as for IL-6i and on psoriatic arthritis, psoriasis and IBD as for IL-23i [33]. It is still possible that the timing of administration could explain the disappointing result of IL-6i and IL-23i and that treating early AxSpA could be more beneficial. Moreover, unexpectedly, IL-17i had no beneficial effect on gut inflammation, highlighting altogether the difficulties of bench to bedside translation. Besides, deciphering extensively how a cytokine systemically acts should help to anticipate its potential side effects, especially regarding immunosuppression and its consequences on infection and cancer development. Noteworthy, a recent notification from the Food and Drug Administration warned against a potential increased risk of serious cardiovascular events and cancer cases in rheumatoid arthritis patients treated with tofacitinib.

Alternatively to cytokine blocking strategies, emerging data argue for the potential benefit of administering immunoregulatory cytokines. This is the case for IL-2, IL-10, TGF $\beta$ , and IL-27, which may allow to counter-regulate the inflammatory processes and bring the expectation of achieving drugfree remission. Until now however, neither IL-2 [58], nor IL-10 [113] showed promising efficacy in pre-clinical model of SpA.

Suitable therapeutic strategies based on identification of key cellular actors and/or metabolic changes that underlie AxSpA pathogenesis seem still a more distant goal. However, modulating cell differentiation is likely a relevant approach. This may concern for instance inhibition of Th17 development *via* inactivation of STAT3 – which is also a target of JAKi – or through epigenetic reprogramming. However, the landscape of epigenetic changes and regulation remains poorly defined in the context of AxSpA preventing any therapeutic intervention to be envisioned in the near future [72]. Besides CD4<sup>+</sup> T cells that have been under focus until now, recent data highlighted the potential implication of innate lymphoid, innate NK and  $\gamma\delta$ -T cells that should also be more thoroughly investigated in the future. Otherwise, modulating the

deleterious role of other critical cell partners involved in AxSpA, such as antigen-presenting cells and mesenchymal stem cells, remains challenging. Furthermore, targeting new bone formation remains a matter of concern and will require a more comprehensive understanding of dysregulated bone metabolism in AxSpA.

An important issue that must also be raised is the current difficulty to ascertain AxSpA diagnosis early, to evaluate disease activity, and stratify patients with regard to prognosis and outcome drug response or risk of relapse [114–116]. Addressing those questions would be of great interest to optimize bench to bedside translation. This paradigm to favor face-to-face protocols and for comparison of several drug options as second line of treatment.

Multi-omics tools and artificial intelligence might help in the future to identify optimal drug targets, and stratify patients, based on biological predictors, risk of flare after treatment tapering or discontinuation, and to evaluate drug efficacy in real life [117–119]. These tools might also help in integrating the complex environmental influences on AxSpA pathogenesis.

## List of abbreviations

AxSpA: Axial spondyloarthritis AS: Ankylosing spondylitis BMP: Bone morphogenetic protein DC: Dendritic cell GM-CSF: Granulocyte-macrophage colony-stimulating factor hβ2m: Human-β-2 microglobulir HLA: Human leukocyte antigen IBD: Inflammatory bowel disease IFN: Interferon IL: Interleukin IL-17i: Interleukin 17 inhibitor JAK: Janus kinase JAKi: Janus kinase inhibitor MRI: Magnetic resonance imaging NFkB: nuclear-factor-kappa B NSAID: Non-steroidal anti-inflammatory drug STAT: Signal transducer and activator of transcription TGF-β: Transforming growth factor β Th17: T helper 17 cell TNF: Tumour necrosis factor TNFi: Tumour necrosis factor inhibitor

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# REVUE 2 - Epigénétique au cours de la spondyloarthrite

## Joint Bone Spine 87 (2020) 565-571



## Review

## Epigenetics of spondyloarthritis

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## ABSTRACT

Spondyloarthritis (SpA) is a chronic inflammatory disorder resulting from a combination of genetic predisposition and environmental factors. Despite recent advances, a substantial fraction of its genetic basis remains poorly understood. Several mechanisms have been proposed to account for this unexplained heritability, including epigenetics which can play a role at the interface between genetic and environmental susceptibility factors. Epigenetics refers to changes in gene expression that are not encoded in the DNA sequence itself. Such mechanisms may include DNA methylation, histone modifications and non-coding RNAs. Disruption of one of these systems can lead to inappropriate gene expression, which in turn might favour the development of disease. Thanks to recent technological progress, there has been a growing interest in the field of epigenetics in complex diseases, including SpA. However, epigenetic studies face some methodological limitations that hamper interpretation of their results: small sample size, absence of biological replication, lack of adequate controls for potential confounders, studies not performed in the most relevant cell/tissues. In the future, integration of epigenetics with other "omics" data will probably be necessary to improve our understanding of SpA pathogenesis. These issues need to be addressed before considering the use of epigenetic marks in clinical routine, as biomarkers or as drug targets.

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## 1. Introduction

Complex diseases such as spondyloarthritis (SpA) result from a combination of genetic predisposition and environmental factors. SpA has a strong genetic component dominated by the HLA-B27 allele of the major histocompatibility complex (MHC). Several large genome-wide association studies (GWAS) have uncovered more than 40 other susceptibility loci outside the MHC [1]. However, only about 25% of the heritability is likely to be explained by currently known risk loci [2]. Several mechanisms have been proposed to account for the unexplained heritability, including epigenetic variations. Notably, epigenetic effects may be affected by the envi-ronment and thus potentially represent a major interface between genetic and environmental susceptibility factors.

Introduced in the early 1940s by Conrad Waddington, the term epigenetics initially refers to "the branch of biology which studies

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the causal interactions between genes and their products which bring the phenotype into being" [3]. Over the years, the meaning of the word has gradually narrowed and may be defined today as "all meiotically and mitotically heritable changes in gene expres-sion that are not coded in the DNA sequence itself" [4]. Epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNAs, thereby regulating gene expression (Fig. 1). Epigenetic modifications can occur during development, under endogenous stimulations, or be environmentally induced. They are also reversible and can be inherited during cell divisions Epigenetic modifications are key regulators of gene activation

states. Thus, disruption of one of these interacting systems can lead to inappropriate gene expression, which in turn might favour the development of diseases. Aberrant epigenetic profiles have been described, mainly in cancer [6], and more recently in several chronic inflammatory diseases [7]. Investigating epigenetic modifications is expected to provide new insights into SpA pathogenesis that can also lead to the development of innovative diagnostic or prognostic tools as well as the identification of new drug targets. In this review, we summarize and discuss the current state of research and knowledge on epigenetic modifications occurring in SpA.

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Fig. 1. Epigenetic mechanisms and their influence on gene expression. (A) DNA methylation refers to the addition of a methyl group to a cytosine. It is described as a 'silencing' epigenetic mark. (B) Nucleosomes, composed of DNA wrapped around histone proteins, can change conformation to modulate DNA accessibility. Histone tails are post-translationally modified by the addition or removal of chemical groups (also called 'histones marks', such as H3K27Me3 – associated with chromatin compaction and repression of transcription, or H3K4Me3 – associated with chromatin opening and enabling of transcription). (C) Noncoding RNAs, especially micro-RNA, can mediate the recognition and binding of RNAs that become targeted for degradation.

## 2. Epigenetic processes

### 2.1. DNA methylation

DNA methylation refers to the addition of a methyl group to a cytosine to form 5-methylcytosine. It predominantly occurs on cytosine phosphate guanine (CpG)-paired bases, often clustered in particular regions of the genome, called CpG islands, located in the promoter of nearly 60% of genes [8]. The process of methylation establishment and maintenance is carried out by the DNA methyltransferase(DNMT) proteins family: DNMT3A, DNMT3B and DNMT3L for de novo methylation and DNMT1 to maintain DNA methylation during cellular replication.

DNA methylation is frequently described as a 'silencing' epigenetic mark (Fig. 1A). However, it has been recently shown that the effect of DNA methylation on gene expression varies with context and with the position of the methylation relative to the transcriptional unit. Methylation in the immediate vicinity of the transcription start site blocks transcription initiation, leading to a decrease of messenger RNA (mRNA) production. On the contrary, methylation in the gene body has been correlated with transcription elongation and might be involved in splicing mechanism [8].

## 2.2. Post-translational modifications of histones

The basic element/unit of chromatin is the nucleosome, a nucleoprotein complex composed by DNA wrapped around a histone octamer (two copies of H2A, H2B, H3 and H4). The poorly structured N-terminal 'tails' of histone proteins that protrude from the nucleosome are subjected to addition of a diversity of chemical groups also termed as histones marks [9]. Methylation, acetylation, phosphorylation and ubiquitination are among these modifications. They can act as synergistic, complementary or antagonistic signals to dynamically modulate transcription outcome in the cell by altering chromatin structure and regulating DNA accessibility to transcription factors (Fig. 1B) [10]. Histone marks can be either deposited or removed by different sets of histone-modifying enzymes called "writers" and "erasers" respectively [11]. This particular aspect implies that histone marks are reversible in order to activate or silence gene expression. Moreover, histones can bear multiple marks on different residues influencing mutually each other. This observation early suggested the existence of crosstalk that finetunes transcription regulation in a context-dependent manner. It is the interplay between these modifications that inspired Jenuwein and Allis to propose almost 20 years ago the hypothesis of the histone code [12].

## 2.3. MicroRNAs

Among non-coding RNAs, microRNAs (miRNAs) have been the most studied – especially in translational research – and are predominantly involved in gene expression modulation. Other types of non-coding RNAs, including long non-coding (IncRNAs), have been recently involved in the pathogenesis of inflammatory diseases [13].

MiRNAs are small noncoding, single stranded, endogenously expressed RNAs molecules [14]. The canonical biogenesis of miRNA is predominant and requires several steps. First DNA sequence is transcribed into primary miRNA (pri-miRNA) which is secondary cleaved into precursor miRNAs (pre-miRNA) by the microprocessor complex comprised of Drosha and DGCR8. The pre-miRNA is then exported to the cytoplasm and processed by Dicer, resulting in a mature miRNA duplex which is loaded into the Argonaute family of proteins to form the minimal miRNA-induced silencing complex (miRISC). Finally miRISC can interact with complementary sequences on the 3'UTR of target mRNAs to induce translational repression through mRNA deadenylation and decapping leading to 5'–3' degradation. [15]. Generally, miRNAs do not completely silence their target genes, but rather decrease their expression.

A given miRNA may have hundreds of different mRNA targets, and a given target might be regulated by multiple miRNAs. This leads to a complex dynamic modulation of gene expression, which has been shown to play a critical role in numerous cellular processes including development, proliferation, invasion, and apoptosis. Moreover, numerous studies have demonstrated that miRNAs can be released into extracellular fluids, and might act as autocrine, paracrine, or endocrine regulators to modulate cellular activities [16].

#### 3. Factors influencing epigenetic modifications

Epigenetic marks can be affected by a variety of factors described below. These factors are important to keep in mind when considering an experimental design to identify specific alterations of the epigenome in a given disease to avoid spurious findings.

### 3.1. Cell type

On the contrary to the primary sequence of the human genome which is largely preserved in all human cell types and tissue, the epigenome varies considerably among cell types, as extensively studied by the NIH Roadmap Epigenomics Program and ENCyclopedia Of DNA Elements (ENCODE) project [17,18].

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This cell specificity must be considered in analytic perspectives, especially to critically evaluate epigenomic studies, taking into account that a high proportion has been performed in peripheral blood with a mixture of different cell types. Thus, differences observed between conditions might be related to a difference in the proportion of different cell types rather than to the studied condition.

## 3.2. Individual characteristics

Among demographic factors, age seems to have a strong influence on epigenetic marks [19]. Epigenome is also influenced by ethnicity [20,21] and by sex [22,23].

### 3.3. Lifestyle and environmental factors

All epigenetic marks can be altered by exposure to lifestyle factors such as smoking, medication, alcohol and diet and environmental factors such as air pollution [24,25].

## 3.4. Interindividual variations of the DNA sequence

Interindividual variations of the DNA sequence (such as single nucleotide polymorphism) are a substantial determinant of epigenetic marks. Indeed, DNA polymorphism can influence both DNA methylation state [26], miRNA expression [27] and histone posttranslational modifications magnitude [28]. It may be particularly useful to identify the functional consequences of disease associated variants [29].

## 4. Measurement and analysis of epigenetic marks

## 4.1. DNA methylation

DNA methylation information is erased by standard molecular biology techniques, such as polymerase chain reaction (PCR). Therefore, almost all DNA methylation analysis techniques rely on a 2-step process: a methylation-dependent pre-treatment of the DNA, followed by amplification or hybridization [30]. DNA pretreatment relies on three main approaches: methylation-sensitive endonuclease digestion, affinity enrichment or bisulphite conversion (considered as the gold standard). Once genomic DNA has been treated with one of the methylation-dependent steps, probe hybridization or sequencing can be used to reveal the location of the methylated residues. The combination of these two steps has resulted in the development of numerous techniques, details of which can be found elsewhere [30]. All these techniques were initially restricted to limited regions, but many of them can now be used on a genome-wide scale. To date, the most common method of DNA methylation assessment relies on Illumina® BeadChip® arrays because they offer a good compromise between cost and number of measured sites.

## 4.2. Post-translational modifications of histones

Chromatin immunoprecipitation followed by sequencing (ChIPseq) extensively contributed to our understanding of the relationship between histone marks and transcription regulation occurring during cell differentiation [31]. Histone mark enrichment profiles, often referred as epigenome maps, are used to segment and annotate whole-genome regions into distinct "chromatin states", which represent more detailed characteristic epigenetic signatures (e.g., weak transcription and poised promoter) [32]. Analysis of the distribution of either H3K4me3 active or H3K27me3 repressive marks allows reliable prediction of transcription states at promoters genome-wide. But most of all, histone marks allow *ab initio* prediction of noncoding regulatory sequences such as enhancers. Enhancers are key elements involved in cell-specific transcription regulation through long distance interactions with promoter sequences [33]. They have been involved in many genetic disorders and developmental diseases as their malfunction can lead to aberrant gene expression. If their prediction remains highly challenging, enhancers are known to be associated with certain epigenetic signatures, the best known being H3K27ac and H3K27ac/H3K4me1 that respectively describe primed and active elements [34].

### 4.3. Micro-RNAs

MiRNAs can be isolated from cells, tissues – but also body fluids (such as serum, plasma, tears, or urine), from which they are much harder to extract (due to a lower quantity). A significant improvement has been observed in miRNAs isolation methods, from the traditional phenol-chloroform extraction followed by RNA precipitation, with high level of contaminants, to column-based RNA adsorption method and complementary specific techniques – including protocol for separation of small RNAs [35].

Detection of miRNA is challenging because of their small size (21 to 24 nucleotides), low levels of expression and high sequence similarity with other expressed miRNAs. A suitable method should be chosen based on the requirements of the investigation (e.g. single-target investigation or genome-wide study) and the experimental conditions (amount of RNA, level of expression, sample type...). Current methods used for detecting miRNAs include quantitative PCR (qPCR) or in situ hybridization for genome-wide detection.

Identifying target genes and functional effects of a defined miRNA is another challenge. Different methods can be used, each with varying benefit/limitation ratio: *in silico*-based target prediction; transient transfection of a miRNA mimic or inhibitor followed by whole transcriptome sequencing; CRISPR/Cas9 strategy; cross-linking of RNA-protein complexes followed by immunoprecipitation and RNA sequencing to detect miRNA/mRNA binding [35].

## 5. Epigenetic variations in spondyloarthritis

To date, most of the epigenetic studies in SpA have been focused on miRNAs. Less than 10 studies investigating DNA methylation have been published and even less investigated histone modifications.

## 5.1. DNA methylation profile in SpA

Variants in *DNMT3A*, *DNMT3B* and *DNMT3L* genes have been recently associated with ankylosing spondylitis (AS) [2], implying that DNA methylation could be important for AS pathogenesis. However, only a few studies have investigated this epigenetic mechanism in SpA, all of them focused on AS. Their designs and main results are summarized in Table 1.

Only two such studies were performed genome-wide [43,44]. The first one published in 2017 compared a limited number of AS patients with healthy controls (5 in each group) and identified 1915 differentially methylated positions (DMPs). The most significant DMP was located in *HLA-DQB1*, a MHC class II locus often in linkage disequilibrium with *HLA-B* [43]. Thus, given the HLA-B27 frequency bias between cases and controls, it cannot be excluded that such a difference is linked to HLA-B27 rather than to the disease status itself. More recently, the influence of the HLA-B27 on the genome-wide DNA methylation profile was studied by comparing three groups of patients: 12 HLA-B27 + AS, 12 HLA-B27 – AS patients

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# Table 1 Design and main results of DNA methylation studies in SpA.

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Study	Region	Population	Methods	Sample type	Main results
Hao et al. [36]	China	5 AS 5 HC	Genome-wide Illumina® array	РВМС	1,915 DMPs Best association in <i>HLADQB1</i> gene
Coit et al. [37]	Europe	12 HLA-B27+ AS 12 HLA-B27– AS 12 OA	Genome-wide Illumina® array	Whole blood	AS vs. OA: 67 DMPs HLA-B27+ vs. HLA-B27– AS: 159 DMPs
Lai et al. [38]	Taiwan	43 HLA-B27+ AS 6 HLA-B27+ HC	Candidate gene qRT-PCR	Serum	Hypermethylation of SOCS1 in AS vs. HC
Aslani et al. [39]	Iran	40 AS 40 HC	Candidate gene qRT-PCR	PBMC	Hypermethylation of DNMT1 promoter and lower DNMT1 mRNA expression in AS vs. HC
Karami et al. [40]	Iran	50 AS 50 HC	Candidate gene qRT-PCR	РВМС	Hypermethylation of <i>BCL11B</i> promoter and lower BCL11B mRNA expression in AS vs. HC
Chen et al. [41]	China	99 AS 99 HC	Candidate gene NGS-based	РВМС	Hypermethylation of <i>IRF8</i> promoter and lower <i>IRF8</i> mRNA expression in AS vs. HC
Zhang et al. [42]	China	99 AS 99 HC	Candidate gene qRT-PCR	РВМС	Hypermethylation of two <i>IL12B</i> CpG islands and higher IL12B mRNA expression in AS vs. HC

As: ankylosing spondylitis; HC: healthy controls; PBMC: peripheral blood mononuclear cells; DMPs: differentially methylated positions; OA: osteoarthritis; qRT-PCR: quantitative real-time polymerase chain reaction.

## Table 2

Correlations between miRNA and SpA-related clinical, biological or radiological parameters.

Study	miRNA	Region	SpA subtype	Sample size	Sample type	Disease-related parameters	Sense of correlation
Wang et al. [56] Qian et al. [57] Kook et al. [58] Lai et al. [52] Li et al. [59] Prajzlerova et al. [60]	miR-199a-5p miR-155 miR-214 miR-221 miR-221-3p miR-181a-5p miR-181a-5p miR-196-5p miR-190-5p miR-193a-3p miR-625-3p miR-855-5p	China China Korea China China Czech Republic	AS AS AS AS nr-axSpA AS	41 80 65 27 26 48	CD3+ cells Serum CD3+ cells PBMC Serum	ASDAS, m-SASSS BASDAI ASDAS BASRI ASDAS, CRP CRP BASDAI	Negative Positive Positive Positive Negative Positive Positive
Perez-Sanchez et al. [54]	miR-146a-5p miR-125a-5p miR-22-3p	Spain	AS	53	Serum	CRP, TNF- $\alpha$ levels	Positive
Fogel et al. [51]	mir-146a-5p	France	AS	81	CD14 <sup>+</sup> PBMC	CRP	Negative

AS: ankylosing spondylitis; ASDAS: ankylosing spondylitis disease activity score; m-SASSS: modified stoke ankylosing spondylitis spinal score; BASDAI: Bath ankylosing spondylitis radiologic index; nr-axSpA: non radiographic axial spondyloarthritis; PBMC: peripheral blood mononuclear cell; CRP; C-reactive protein; TNF: tumor necrosis factor.

and 12 patients with HLA-B27– osteoarthritis (OA) [44]. Interestingly, more DMPs were detected between HLA-B27-positive and -negative AS patients (n = 159) than between AS and OA (n = 67), suggesting a strong influence of HLA-B27 on the DNA methylation profile.

Other studies were centred on known AS-genetically associated regions and/or inflammatory-related genes [36,37,45–47]. Almost all of them examined correlation between methylation levels and parameters such as disease activity or clinical manifestations, but due their small sample sizes they were rather underpowered. Regarding studies of previously reported AS-associated genes, it is noteworthy that none of them investigated whether the associated SNPs at these *loci* operated through effects on methylation.

## 5.2. Histone modifications in SpA

As for DNA methylation, only few studies have addressed so far alterations of histone marks in SpA and none of them has been performed at genome-wide level. Most of them concerned AS-associated SNPs whose positions correlated with enhancers

signature. By integrating prediction of candidate causal variants based on dense genotyping data with enhancer mapping in different immune cell types using H3K27ac distribution, Farh et al. detected enrichment of associated SNPs in active enhancers from Th0, Th1 and Th17 cells [42]. Studying H3K4me1 mark allowed the identification of an enhancer close to the IL23R gene that encodes a component of the heterodimeric IL-23 receptor. This could potentially account for the effect of the AS-risk allele of SNP rs11209032 on increasing Th1 proliferation [41]. Surprisingly however, if the level of H3K4me1 in this region was decreased in patients homozygous for the risk allele, no change in IL23R mRNA was observed, suggesting a more complex regulatory mechanism involving this enhancer. A similar strategy led to explain the putative role of rs4648889, an AS-associated SNP that falls in a H3K27ac/H3K4me1-enriched enhancer of RUNX3 [40]. Both H3K4me1 and RUNX3 mRNA levels were consistently decreased in CD8+ T cells from AS patients carrying the risk allele. As RUNX3 is known to enhance CD8 T-cell development, the lower expression of RUNX3 induced by rs4648889 might explain the lower number of CD8+ T cells previously reported in AS patients [39]. Despite the

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lack of comprehensive data from large cohort of patients, these data suggest a strong association between SpA and cis-regulatory genetic polymorphisms inducing chromatin remodeling.

Mutations in histone modifying enzymes that regulate global level of histone modifications were pointed out in numerous diseases, such as cancers, due to their impact on the expression level of many genes simultaneously [29]. To date, only few of them were correlated with SpA. The global level of acetylated histones in the cell depends on a balance between Histone Acetyl Transferase (HAT) and Histone Deacetylase (HDAC) that regulate transcription factors and RNA polymerase recruitment on promoters. A HAT/HDAC activity change was assayed in AS patients treated with TNF $\alpha$  inhibitor [38]. Treatment was associated with a significant increase in HAT activity that can explain similarity between the anti-inflammatory effects of  $\text{TNF}\alpha$  inhibitors and that of some HDAC inhibitors [48]. An increased level of HDAC3 that regulates the c-Jun N-terminal kinase pathway and NF-KB activity was also detected in peripheral blood mononuclear cells from AS patients [49].

## 5.3. MicroRNAs in SpA

There is growing evidence that aberrant miRNA expression is linked to inflammatory diseases, such as rheumatoid arthritis (RA) or inflammatory bowel diseases. During the last decade, involvement of miRNAs has been studied in the context of AS [50] with two main approaches: (i) miRNAs as actors of SpA pathogenesis, as discussed in the paragraph below or (ii) miRNAs as diagnostic, prognostic or therapeutic biomarkers, as discussed in the next section.

MiRNA expression profile of SpA patients has been studied in nearly 40 studies to date. More than two third of them concerned bulk samples (whole blood, peripheral blood mononuclear cells, serum, plasma) with non overlapping thus inconclusive results. The others focused on sorted cell subsets, such as the circulating CD14<sup>+</sup> monocytes or CD4<sup>+</sup> T cells, two cell types thought to be critically involved in disease pathogenesis. In SpA patients, both cell types were shown to harbor significant modifications in miRNAs expression profile (with 13 and 11 differential expressed miR-NAs, respectively). These miRNAs were predicted to be involved in disease relevant pathways such as inflammation, intestinal permeability, or bone formation [51,52].

Some variations are consistent with the pro-inflammatory cytokine environment reported in SpA. For instance, IL-23 was shown to induce a differential miRNAs expression profile in T cells from AS patients, including overexpression of miR-29b-1-5p [53]. Moreover, Perez-Sanchez et al. identified several miRNAs overexpressed in SpA patient with an influence on the production of inflammatory cytokines such as IL-1 [54]. The role of T helper 17 (Th17) cells is well described in SpA pathophysiology. Interestingly, miR-10b was significantly increased in AS Th17 cells, as compared to cells from healthy controls. This overexpression seemed to be induced by TNF- $\alpha$  and IL-6 and inhibited the production of IL17A [55].

Several studies searched for a correlation between miRNAs expression profile and markers of disease-activity (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], Bath AS Disease Activity [BASDAI], AS Disease Activity Score [ASDAS]), inflammatory cytokines production, or ossification related-parameters. The most significant results are summarized in Table 2. Of note, one study compared miRNAs between HLA-B27 positive and -negative axial SpA patients and observed significantly different expression profiles [48].

Despite the strong influence of DNA sequence on miRNA expression, few studies have tried to determine whether miR-NAs deregulation might be an independent genetic determinant

#### Table 3

Methodological points to consider when conducting or interpreting epigenetic studies.

Торіс	Potential issue	Points to consider
Cohort selection	Spurious associations Lack of reproducibility	Match age, sex and ethnicity between cases and controls Consider potential confounding factors: smoking, treatment, HLA-B27 distribution Discuss the possibility to include patients with other inflammatory diseases as controls
Sample type	Mix of cells with different proportions between groups No disease-relevance of sample type	Whenever possible, use of sorted cells is preferable Choose the most relevant cell types considering disease pathogenesis (tissue resident and/or circulating cells)
Sample size	Lack of statistical power	Estimate the sample size requirement depending on the number of tests performed and of the expected effect size
Replication/ validation	Lack of reproducibility	Depending on the method of detection initially used, consider the interest of a technical validation (same samples assessed with a different method, most often PCR) Replication of the results in an independent cohort is highly recommended

of AS/SpA. Niu et al. and Xu et al. tried to identify a specific association between miR-146a polymorphisms, known to be involved in immune responses, and SpA [61,62]. However, no strong association has been clearly identified. Morevoer, none of the GWAS hits map to a miRNA gene [2].

#### 6. Future directions for epigenetic studies in SpA

### 6.1. How to improve quality of studies?

Studies examining DNA methylation, miRNA and histone modifications in SpA have suffered from several methodological limitations, which hamper interpretation of their results: small sample size, absence of biological replication, lack of adequate controls for potential confounders, studies not performed in the most relevant cell/tissues. These issues need to be addressed to increase their reliability and to consider the potential use of epigenetic marks in clinical routine. Table 3 summarizes key points which should be taken into account in the design of future epigenetic studies in the context of SpA.

#### 6.2. Integration with other -omics data

No single technology can capture the complexity of the molecular mechanisms that lead to human disease. Thus, the combination of several technologies such as dense genotyping, transcriptomics, epigenomics, metabolomics and proteomics could help to provide a deeper comprehension of the disease pathogeny. However, this multi-omics approach introduces new computational and interpretation challenges [63]. To date no systematic multi-omics approach has been applied to SpA but efforts have been made in this direction, for example by linking together AS associated variants and epigenetic marks [43–45]. Integration with microbiota data could also be very useful due to the strong pathophysiologic link between gut and joint in SpA [64].

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#### 6.3. From bench to bedside

Epigenetic marks provide new insights into disease pathogenesis but might also be useful in clinical practice, with two main domains of application: biomarkers and drug development.

Epigenetic biomarkers refer to any epigenetic mark or altered epigenetic mechanism which can be measured in the body fluids or tissues and which is able to contribute to diagnose or predict the outcome of a disease or to predict/monitor responses to therapy [65]. In SpA, the lack of relevant studies in the field strongly hampers their translation into clinical application. Some tried to use circulating miRNAs expression profiling as a diagnosis tool for AS but performance on validation cohorts still need to be assessed [54,57]. Few studies also tried to define the relation between miR-NAs expression profile and treatment response in the perspective of using miRNAs as a therapeutic strategy tool but the process of validation has not been performed yet [66,67].

During the last 20 years, the perspective of epigenome reversible intervention to treat human diseases has emerged, historically beginning in the field of cancerology. Some encouraging results and promising developments have been observed in numerous indications, including haematopoietic and solid cancers: for example, DNMT and HDAC inhibitors, and more recently small molecules targeting specific genetic alterations [68]. More recent studies have been carried for the discovery of epigenetic drugs to treat inflammatory disease, especially in RA, systemic lupus and systemic-onset juvenile idiopathic arthritis [69]. Advances in SpA epigenetic treatment strategy is ongoing: an example of promising targetedstrategy drug development has been recently published by Cribbs et al., showing the potential in vitro effect on Th17 cells differentiation of an H3K27 demethylases inhibitor [70].

#### Disclosure of interest

The authors declare that they have no competing interest.

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