Interleukin-17A Signalling in Murine Hematopoietic Stem and Progenitor Cells Promotes Proliferation and Monocytic-Biased Differentiation

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Abstract

The hematopoietic system is regulated by the intrinsic programming of the hematopoietic stem and progenitor cells (HSPCs) and by the signals provided by the bone marrow microenvironment through cell-cell interactions and/or cytokines. The effect of IL17A, a proinflammatory cytokine, on hematopoiesis is indirect and mediated by bone marrow non-hematopoietic stem cell activation and G-CSF and IL-8 secretion. In blood malignancies, IL17A stimulates expansion and chemoresistance in multiple myeloma and B-cell acute lymphoblastic leukemia. These recent findings support the study of the IL17A signalling axis within the bone marrow as a potential therapeutic target. Available evidence regarding the IL17A signalling axis does not clarify the sources of IL17A within the bone marrow environment or the hematopoietic stem cell targets, as well as their fate. Here, we hypothesize that IL17A is produced in the bone marrow microenvironment by specialized cell types and regulates HSPC proliferation and differentiation.

To test this hypothesis, I measured the activation of the IL17 signalling pathway in scRNAseq datasets of mouse and human bone marrow cells. Flow cytometry and confocal microscopy were used to analyze IL17 receptor chains A/C expression in HSPCs and define IL17A-expressing cells in mouse bone marrow. IL17A levels were measured in blood and bone marrow using ELISA. *Ex vivo* mouse HSPC culture assays were performed in the presence of IL17A or control and analyzed by flow cytometry. To further test the functionality of IL17A-treated HSPCs, those were re-transplanted into lethally irradiated mice. Recipient mice were bled monthly, and the major hematopoietic lineages were measured.

Our findings show that IL-17RA/C is expressed in mice (mRNA and protein) and human HSPCs (mRNA). We identified B cells, macrophages, neutrophils, and RORyt+ cells as IL17-producing cells in the mouse bone marrow. Importantly, we observed higher levels of IL17A in bone marrow compared with blood. *Ex vivo* treatment of mouse Lin-/Sca1+/cKit+ with IL17A expands multipotent progenitors and promotes monocyte differentiation. In sorted Lin-/Sca1+/cKit+, IL17A stimulates phosphorylation of P38 and C/EBPß.

While the pathophysiological role of IL17A in hematopoiesis is not yet established, we observed that the IL17-signalling pathway transcriptional signature was up-regulated in hematopoietic stem cell samples obtained from young human donors or mice compared to old donors. Additionally, the IL17-signalling transcriptional signature was enriched in human CD34+ cells that were experimentally exposed to inflammatory stimuli, suggesting a possible role for IL17A in the context of inflammation or aging.

Overall, results from this thesis, obtained in experimental mouse models and through analysis of human HSPC transcriptional datasets, show that IL17A is produced in the bone marrow microenvironment by immune cells and that it signals bone marrow hematopoietic stem cells to drive monocytic-biased output through intracellular activation of P38 and C/EBPß. These results pave the way for a detailed study of IL17A in human pathophysiological contexts.

Résumé

Le système hématopoïétique est régulé par la programmation intrinsèque des cellules souches et progénitrices hématopoïétiques (HSPC) et par des signaux fournis par le microenvironnement de la moelle osseuse via des interactions intercellulaires et/ou des cytokines. Le rôle de l'IL17A, qui est une cytokine pro-inflammatoire, dans l'hématopoïèse serait un effet indirect par l'activation de cellules souches non hématopoïétiques de la moelle osseuse et la sécrétion de G-CSF et d'IL-8. Dans les tumeurs malignes du sang, l'IL17A stimule l'expansion et la chimiorésistance dans le myélome multiple et la leucémie lymphoblastique aiguë à cellules B. Ces découvertes récentes ont souligné l'importance d'étudier le rôle de l'axe de signalisation IL17 dans la moelle osseuse comme voie intéressante d'un point de vue thérapeutique. Cependant, jusqu'à présent, les preuves disponibles sur l'axe de signalisation de l'IL17A ne clarifient pas les sources de l'IL17A dans l'environnement de la moelle osseuse ni le sort des cellules hématopoïétiques une fois exposées à l'IL17A. Ici, nous émettons l'hypothèse que l'IL17A est produite dans le microenvironnement de la moelle osseuse par des types de cellules spécialisés et régule la prolifération et la différenciation des HSPC. Pour tester cette hypothèse, j'ai mesuré l'activation de la voie de signalisation IL17 dans des ensembles de données scRNAseq de cellules de moelle osseuse humaine et de souris. La cytométrie en flux et la microscopie confocale ont été utilisées pour analyser l'expression des chaînes A/C du récepteur IL17 dans les HSPC et définir les cellules exprimant l'IL17A dans la moelle osseuse de souris. Les taux d'IL17A ont été mesurés dans le sang et la moelle osseuse par ELISA. Des tests de culture ex vivo de HSPC de souris ont été réalisés en présence d'IL17A ou de contrôle et analysés par cytométrie en flux. Pour tester davantage la fonctionnalité des HSPC traitées à l'IL17A, elles ont été retransplantées chez des souris mortellement irradiées. Les souris receveuses ont été saignées mensuellement et les principales lignées hématopoïétiques ont été mesurées. Nos résultats montrent que l'IL-17RA/C est exprimée chez la souris (ARNm et protéines) et dans les cellules souches hématopoïétiques humaines (ARNm). Nous avons identifié les cellules B, les macrophages, les neutrophiles et les cellules RORγt+ comme cellules productrices d'IL17 dans la moelle osseuse de souris. Surtout, nous avons observé des taux d'IL17A plus élevés dans la moelle osseuse que dans le sang. Le traitement ex vivo de la LSK de souris avec l'IL17A développe les progéniteurs multipotents et favorise la différenciation des monocytes. Dans les LSK, l'IL17A stimule la phosphorylation de P38 et de C/EBPß. De plus, nous avons observé que la signature transcriptionnelle de la voie de signalisation IL17 était régulée positivement dans les échantillons de cellules souches hématopoïétiques obtenus auprès de jeunes donneurs humains par rapport aux donneurs âgés. De plus, la signature transcriptionnelle de la signalisation IL17 a été enrichie dans des cellules CD34+ humaines exposées expérimentalement à des stimuli inflammatoires, suggérant un rôle possible de l'IL17A dans le contexte de l'inflammation ou du vieillissement. Globalement, les résultats de cette thèse montrent que les cellules immunitaires produisent de l'IL17A dans le microenvironnement médullaire. De plus, l'IL17A stimule la production de monocytes grâce à l'activation intracellulaire de P38 et de C/EBPß. Ces résultats ont ouvert la voie à une étude détaillée de l'IL17A dans des contextes physiopathologiques humains.

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CONTRIBUTIONS

François E. Mercier provided supervision for this project.

Introduction (literature Review):

This chapter was performed by Cristobal González Losada and revised by François E. Mercier.

Methods and Results:

Chapters Methods and Results were written entirely by Cristobal González Lsoada. Matthew Salaciak, from Dr. Johnson laboratory, provided training in R, troubleshooting, and bioinformatic supervision of the computational analysis. Syyam Sah from John Dick Laboratory provided the computational analysis from Human Bone Marrow Map and CD34+ compartment. Christian Young provided training in flow cytometry and cell sorting. François E. Mercier guided experimental design and data interpretation. Madison Currier provided technical assistance regarding colony forming assay and animal work. Cristobal González Losada performed all other work, including bioinformatic design, cell culture, molecular biology techniques, cell isolation and purification techniques, sample preparation for flow cytometry, operation of flow cytometer and cell sorter, data analysis, writing, and generation of all figures and tables.

Discussion:

This chapter was written entirely by Cristobal González Losada.

Conclusion:

This chapter was written entirely by Cristobal González Losada.

List of Abbreviations

ACT-1: Act 1 adaptor protein
ATM: serine/threonine kinase or Ataxia-telangiectasia mutated
ATR: Rad3-related protein
BCL2: B-cell CLL/lymphoma 2
BM: Bone marrow
BRD4: bromodomain-containing protein 4
CFU: Colony-forming unit
cGAS: cyclic GMP–AMP synthase
CHK1: checkpoint kinase 1
CHK2: checkpoint kinase 2
CLP: Common lymphoid progenitor
Clustered regularly interspaced short palindromic repeats
CMP: Common myeloid progenitors
Common myeloid progenitor
DDR: DNA damage response
DN: Double negative
DP: Double Positive
ECM: extracellular matrix
EPO: Erythropoietin
FACS: Fluorescence-activated cell sorting
FBS: Fetal bovine serum
FLT3L: Fms-related tyrosine kinase 3 ligand
GDF15: growth/differentiation factor 15
GEMM: Granulocyte, erythrocyte, monocyte, megakaryocyte
GM: Granulocyte, monocyte
GM-CSF: Granulocyte-monocyte colony-stimulating factor
GMP: Granulocyte-monocyte progenitors
GSEA: Gene set enrichment analysis
HSCs: Hematopoietic stem cells
HSPCs: Hematopoietic stem and progenitor cells
IL: Interleukin
IL17A: Interleukin -17A
ILC3: Innate lymphoid cell type 3
IRF3: interferon regulatory factor 3
ITSX: Insulin-transferrin-selenium-ethanolamine
LMPP: Lymphoid-primed multipotent progenitor
LSK: Lin-/cKit+/Sca1+
LT-HSCs: Long-term hematopoietic stem cells
MAPK: Mitogen-activated protein kinase

MCP1: monocyte chemoattractant protein 1
MEP: Megakaryocyte-erythrocyte progenitors
MFI: Mean fluorescence intensity
MMP: matrix metalloproteinase
MPP2: Multipotent progenitor 2
MPP3: Multipotent progenitor 3
MPP4: Multipotent progenitor 4
mTOR: mammalian target of rapamycin
NCR: Natural cytolytic receptor
NES: Nestin
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
PBS: Phosphate-buffered saline
PRC2: polycomb repressive complex 2
rm-IL17A: rRecombiant murine interleukin -17A
RNA: Ribonucleic acid
RORyt: RAR-related orphan receptor gamma
ROS: reactive oxygen species
SASP: senescence-associated secretory patterns
SA-β-gal: senescence-associated beta-galactosidase
SCF: Stem cell factor
Sc-RNA-seq: Single-cell RNA sequencing
SEFIR: [SEF (similar expression to fibroblast growth factor genes) and IL-17R]
SLAM: Signalling lymphocyte activation molecule
STC1: stanniocalcin 1
ST-HSCs: Sort-term hematopoietic stem cells
STING: stimulator of interferon genes
TPO: Thrombopoietin
TRAF6: TNF Receptor Associated Factor 6
UMAP: Uniform manifold approximation and projection

1. Introduction: literature review

1.1. Hematopoietic stem and progenitor cells

The hematopoietic stem and progenitor cells (HSPCs) are responsible for generating approximately 10^{13} blood and immune cells within the human body (1). In humans and mice, hematopoiesis has been divided into two different "steps": 1-) primitive hematopoiesis and 2-) definitive hematopoiesis. The primitive hematopoiesis starts in the yolk sac at 3 weeks of gestation (2), and at the embryonic age of 7 days (3), in humans and mice, respectively. Due to the ethical implications of studying hematopoiesis in humans during embryogenesis as well as limitations in the current technologies (4-6), most aspects have been characterized in mice. During primitive hematopoiesis, hematopoietic cells are restricted to erythroid-myeloid progenitors (7), in charge of producing red blood cells (8), megakaryocytes (9,10), and macrophage progenitors (11,12). However, by E8 .25 hematopoietic cells with lymphoid programming, responsible for generating B and T cell subsets, can be found within the yolk sac (13,14).

Primitive myeloid cells differ from those generated during the definitive hematopoiesis. Notably, primitive erythroblasts are 1-) larger, 2-) retain their nucleus upon entry into the circulation, and 3-) have different globin expression patterns (15). Primitive macrophages can mature faster by skipping the monocyte step (11,12). These macrophages will generate tissue-resident macrophages with a long life and self-renewal capacity (16,17) and will also generate microglia (18-20). Additionally, the megakaryocyte population derived from the primitive phase is less efficient in producing platelets (21,22).

Definitive hematopoiesis starts in the aorta–gonad–mesonephros (AGM) region of the embryo at E10.5 in mice (13,14) and after 32 days of gestation in human (15). These newly generated hematopoietic stem cells (HSCs) can engraft in adult mice and are identified by the co-expression of hematopoietic and vascular markers CD144+/CD45+/CD93+/cKit+/Sca1+/CD31+ (16-18) with the equivalent phenotype in humans CD34+/CD144+/CD117+/CD91+/CD45+/CD105+ (19). However, at E9 in mice, the hematopoietic cells from the yolk sac and para-aortic splanchnopleura can engraft neonatal recipients (20,21), highlighting the existence of pre-HSCs (22) that at this point are still immature due to the microenvironment; this is demonstrated by the capacity of these cells to mature into definitive HSCs after recreating the fetal microenvironment through coculture with OP9-stromal cells (23-26).

Around E14.5 in mice, the site of hematopoiesis shifts from AGM to the fetal liver, and at E18.5 will shift again to colonize the bone marrow (27). In humans, hepatic colonization will occur at 30 days of gestation, the principal hematopoietic organ from this time to the 10^{th} week, by which time bone marrow starts to be colonized by HSCs (28) and will become the definitive site of hematopoiesis into the postnatal stage (28).

The HSC compartment is characterized by different levels of self-renewal capacity and multipotency (29). This compartment traditionally has been conceptualized as a “hematopoiesis tree model” based on hierarchical progenitor organization (30) (**Figure 1. A**). These progenitors are organized according to their self-renewal capacity and lineage potential (31). In mice, a major group of multi-potent hematopoietic progenitors within the bone marrow expresses the markers **LSK** (linage negative, c-Kit positive, and Sca-1 positive) (32) which has been identified using flow cytometry markers, gene expression profiles, and functional assays(31,33-35) (**Table 1**).

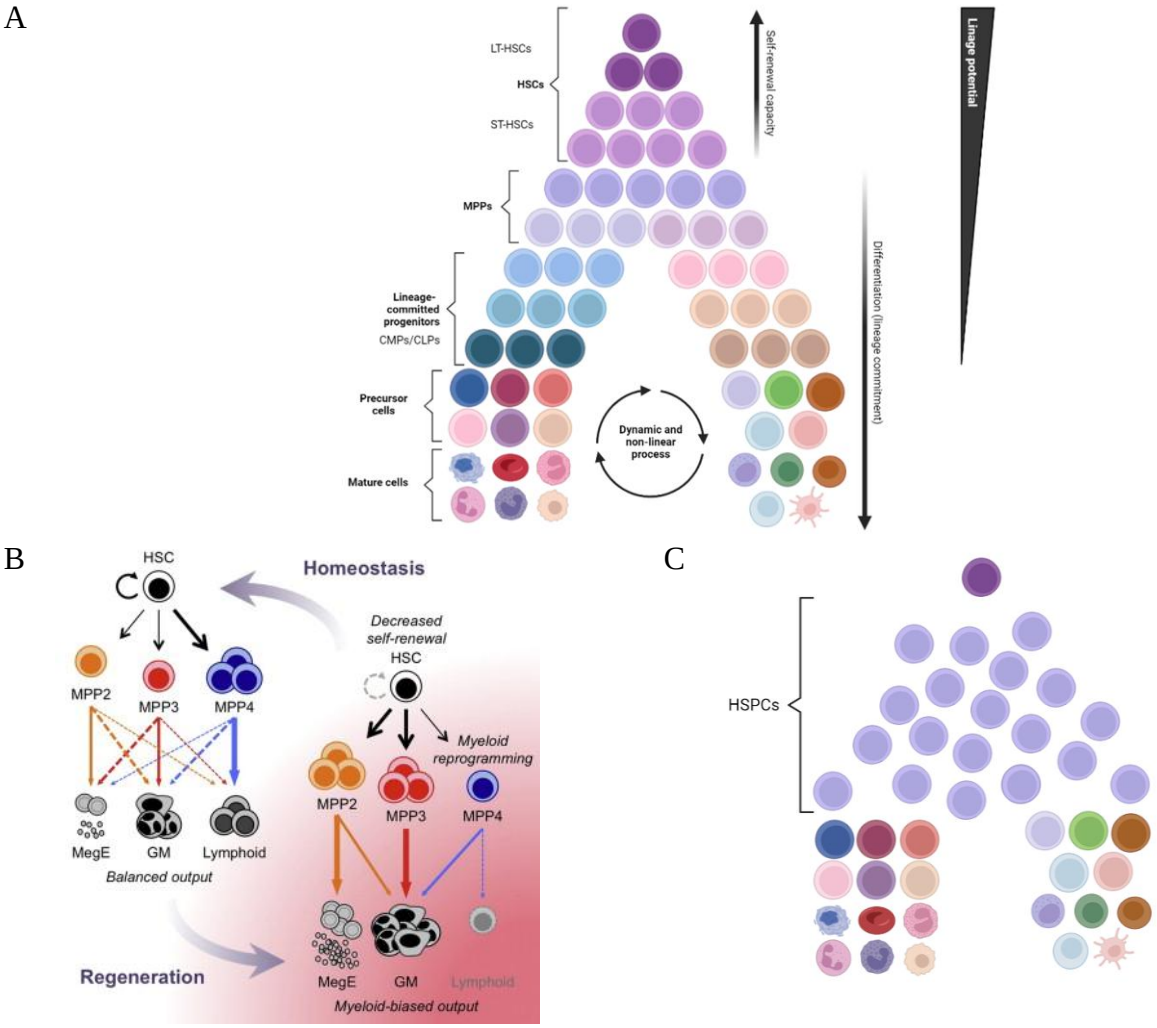

Figure 1. Hematopoietic stem and progenitor cells. A: The "classic model" of hematopoiesis is hierarchical, placing self-renewing pluripotent hematopoietic stem cells (HSCs) at the top. These cells differentiate into hematopoietic progenitor cells, and a branching lineage map illustrates the gradual narrowing of their developmental potential through successive bifurcations. **B:** The illustration depicts hematopoietic stem cell dynamics during **homeostasis** and **regeneration**. Under homeostasis, HSCs generate multipotent progenitors (MPP2, MPP3, MPP4) that produce balanced outputs across megakaryocyte/erythroid (MegE), granulocyte/monocyte (GM), and lymphoid lineages. During regeneration, HSCs exhibit reduced self-renewal and undergo myeloid reprogramming, resulting in a myeloid-biased output with diminished lymphoid differentiation (33). **C:** The pair-wise model differs from tree-like models by proposing that hematopoietic stem cells can directly commit to a specific lineage without the necessity of sequentially

eliminating alternative fates. A key feature of this model is its emphasis on the close relationships between cell lineages, highlighting the lineage options accessible to bi-potent cell populations (36). Generated in Biorender

Experimental data have shown that subsets of these progenitors are lineage-biased (31,33-35) consistent with the intrinsic program of HSCs (37). However, extrinsic signalling may induce HSPCs to switch their differentiation potential (**Figure 1. B**). Therefore, emerging experimental evidence is challenging the classical hematopoietic tree model, recognizing that HSPCs have a certain level of plasticity (38), superimposed on their pre-determined commitment potential (**Figure 1. C**). For example, a progenitor biased with lymphoid commitment might be able to generate myeloid cells under specific conditions such as inflammation (33) (Figure 1. B). HSCs indeed have this capacity to "make decisions" based on signals provided by the microenvironment through molecular messengers (39,40).

Table 1. Characterization of mouse LSK subsets

Name	Markers	Long-term self-renewal capacity	Biased-lineage potential	Hematopoiesis reconstitution*	Number of serial transplants*
LT-HSC ***(33-35)***	LSK/CD135-/CD48-/CD150+	+++	No	Yes	All
ST-HSC ***(33-35)***	LSK/CD135-/CD48-/CD150-	+	No	Yes	First one only
MPP2 ***(33-35)***	LSK/CD135-/CD48+/CD150+	-	Megakaryocytes and Erythrocytes	No	No
MPP3 ***(33-35)***	LSK/CD135-/CD48+/CD150-	-	Granulocytes and monocytes	No	No
MPP4 ***(33-35)***	LSK/CD135+/CD48+/CD150-	-	Lymphoid	No	No

* In lethally irradiated mice.
LT-HSC: long-term hematopoietic stem cells
ST-HSC: short-term hematopoietic stem cells
MPP: multipotent progenitor

1.2. The hematopoietic niche

The bone marrow is a highly organized, specialized, and dynamic organ (41), supported by a communication system that integrates every component to orchestrate a functional unit (42). The non-hematopoietic compartment (stroma), composed of mesenchymal stromal cells (43-45), extracellular matrix proteins (46), sympathetic nerve endings (47), vasculature (47), physicochemical gradients and mediators (48), is organized in a highly specialized microenvironment termed the hematopoietic niche (42,49-51) (**Figure 2)**. Despite the high vascularization in the bone marrow, this microenvironment is hypoxic, with the lowest oxygen tensions found near sinusoids in the central cavity (52).

The hematopoietic niche includes the basic regulatory unit that maintains, directs, and coordinates the fate of the hematopoietic stem cell (HSC) compartment (41,51). The correct functionality of the hematopoietic system is supported by bi-directional communication between HSCs and their niche through soluble mediators (e.g. cytokines) (41) and/or membrane-expressed mediators (integrins)(41). The bone marrow niche drives the fate of HSCs through the secretion of growth factors (53), regulation of hypoxia (52,54), and the metabolic microenvironment, for example by controlling the availability of aspartate for certain kinds of stem/progenitor cells (55). The niche also can receive feedback from the inside or outside of the bone marrow microenvironment to coordinate a hematopoietic output in response to the needs of the organism (56-58).

Despite substantial insights gained over the past twenty years through genetic knockout and reporter mouse models, advanced imaging techniques, single-cell genomics, and experimental co-culture systems, the crosstalk between niche and HSC is not fully understood (59-61). However, it is very well-known that infectious diseases (60), inflammation (62), aging (63), and epigenetics factor (64), can distort the function of the hematopoietic system (65).

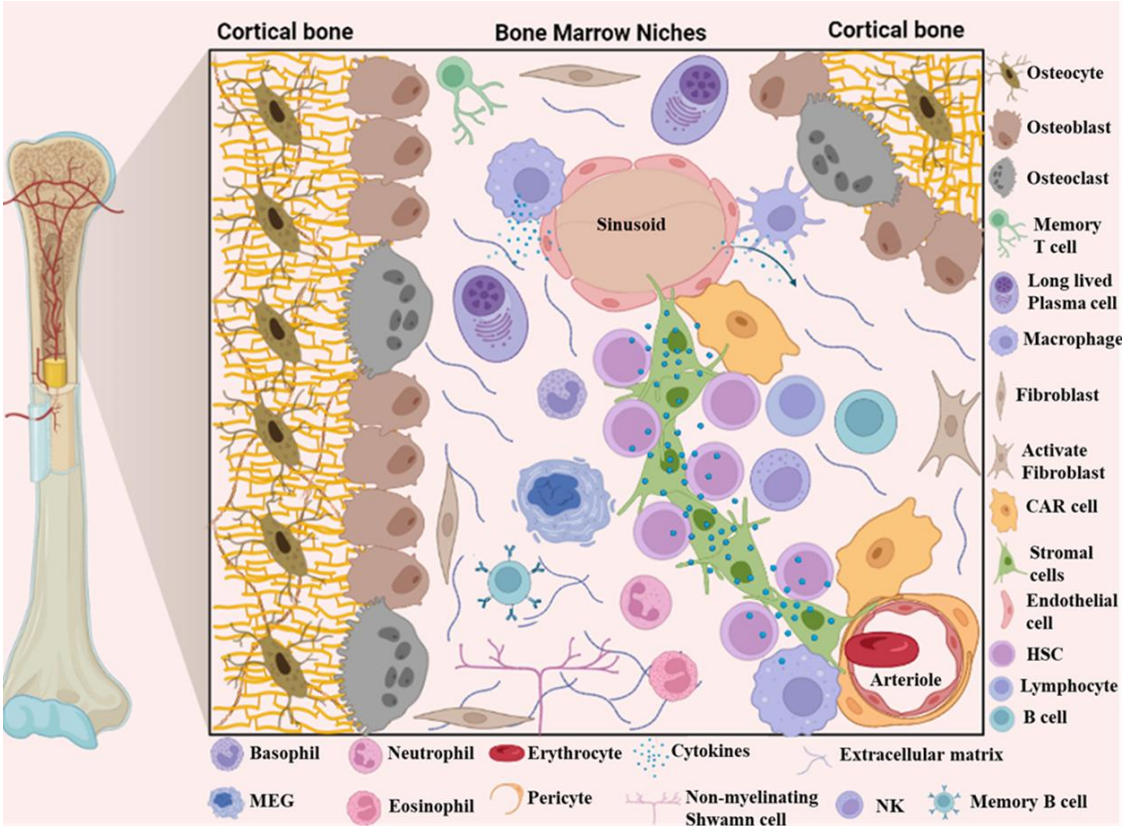

Figure 2. This image illustrates the intricate relationship between hematopoietic stem cells (HSCs) and their bone marrow microenvironment (niche) in regulating hematopoiesis. The bone marrow contains various cellular components such as stromal cells, endothelial cells, osteoblasts, osteoclasts, macrophages, CAR cells, and fibroblasts, which collectively create a supportive environment for HSC maintenance, self-renewal, and differentiation. The sinusoidal vasculature and arterioles facilitate nutrient exchange and provide spatially distinct niches that influence HSC fate through physical interactions, cytokine signalling, and extracellular matrix dynamics. These regulatory

interactions ensure the balance between quiescence and proliferation of HSCs, enabling steady-state hematopoiesis and stress-induced blood cell production. Generated in Biorender

1.2.1 Cellular components of the hematopoietic niche

Osteolineage cells were the first cell type reported in the marrow to be associated with the hematopoietic niche (66-68). The endosteum was identified as a place with HSCs with higher hematopoietic potential (68). Later studies showed *in vitro* and *in vivo* that osteoblastic cells support HSC activity and the loss of these cells in mice impaired hematopoiesis (69-72) and reduced the number of HSC (73). Osteolineage cells contribute to hematopoiesis by secreting hematopoietic factors (74) such as G-CSF (69), as well as expressing cell surface proteins essential to HSC function (Notch1) (75,76), dormancy regulation (Tie2/angiopoietin-1 signalling) (77) and/or coordination of homing (78).

Bone marrow mesenchymal stromal cells are fundamental components of the bone marrow niche (43,44). These cells can differentiate into osteoblastic cells (79), maintaining the integrity of the microenvironment. However, their impact on hematopoiesis is not only related to structural support of the microenvironment, but also, they can affect the fate of HSCs. Co-transplantation of HSCs and mesenchymal stem cells improves the bone marrow engraftment in non-human primates (80) and increases the self-renewal capacity of bone marrow HSCs (81). Additionally, the chemokine CXCL12, or stromal-derived factor-1 (SDF-1) is constitutively expressed by bone marrow stromal cells and is implicated in the regulation of HSCs quiescence (82,83), HSCs regeneration (83,84), and homing (85-87). Mesenchymal stromal cells participate in hematopoiesis by secreting hematopoietic cytokines such as IL6, GM-CSF or IL11 (88), either by as soluble factors or macrovesicles rich in mRNA during stromal cell-HSC communication (89).

Endothelial and perivascular cells colocalize with HSCs within the hematopoietic niche (90). The vasculature is required as an exit point for HSC mobilization into the bloodstream (91). Endothelial and perivascular cells also regulate hematopoiesis through secreted mediators named angiocrine factors (92,93) which can affect the balance between self-renewal and differentiation of HSCs (93). Indeed, endothelial cells are required for Notch-dependent HSC to preserve their self-renewal capacity and repopulation (94) as well as stem cell factor production (95).

Neural and glial cells, part of the bone marrow microenvironment, have been implicated in HSC regulation and fate decisions (51). Sympathetic nerve endings are in part responsible for HSCs mobilization via regulation of G-CSF and CXCL12 secretion by the bone marrow microenvironment (96) (97). On the other hand, non-myelinating Schwann cells are a major source of TGF-ß within the bone marrow (98,99). TGF-ß acts on HSCs, which are close to glial cells, to maintain their dormancy and numbers (99,100).

Megakaryocytes (Mk) contribute to the hematopoietic niche via secretion of CXCL4, TGF-ß, and TPO which are important to HSC quiescence (101-106), and the depletion of Mk result in the loss of HSCs quiescence (107).

Macrophages play a role in the retention of HSCs within the niche through Nes+ stromal cells (108-110). The bone marrow-resident macrophages can suppress the entry into the cell cycle of quiescent HSCs via Duffy antigen/chemokine receptor CD234/DARC interactions (111). A subset of these bone marrow macrophages, which express α-smooth actin, are involved in HSC maintenance via COX-2-derived prostaglandin E2 and activation of CXCL12 expression (112). Additionally, subsets of macrophages constitute a specialized niche for erythroblasts, called erythroblast islands. In this particular niche, mitochondrial transfer occurs from erythroblast to macrophages via tunnelling nanotubes (113). Depletion of macrophages in bone marrow impaired erythropoiesis (114).

A subset of **regulatory T lymphocytes (Treg)**, phenotypically characterized by $CD4^+CD25^+$ expression and with immune-modulatory function (115), plays a critical role within the bone marrow niche by controlling HSC quiescence and engraftment through the regulation of adenosine (116). Treg populations provide immune privilege to the bone marrow hematopoietic niche through IL10 secretion (117).

1.2.2 Membrane-associated protein components of the hematopoietic niche

In the bone marrow microenvironment, biochemical communication is carried out by soluble components (such as cytokines and neurohormones) and membrane-associated proteins such as chemokines and integrins (58). *Since this thesis focuses on the hematopoiesis regulation by cytokines, I briefly describe here some relevant non-soluble components within the bone marrow hematopoietic niche.*

VCAM1, an integrin expressed by endothelial cell (118), stromal cells (118) and macrophages (110), regulates mobilization of HSCs (118-120). In the same way, the chemokines **CXCL12** and **CXCL4**, expressed by stromal cells (82,83), and Mk (101-106), respectively regulate the HSC mobilization, homing, and dormancy (82,83,101-106). As previously stated, **Notch** signalling is essential for the HSC development and homing, as well as the preservation of their self-renewal capacity (75,76,94). The HSCs receive Notch signals by cell-cell communication with endothelial and perivascular cells (75,76,94).

1.2.3 Cytokine components of the hematopoietic niche

Cytokines are signalling molecules produced by different cell populations in response to changes in the microenvironment (121). These chemical messengers can communicate instructions to 1-) the same cell that secretes them (autocrine), 2-) neighbouring cells or those that belong to the same microenvironment (paracrine), or 3-) cells that are in a different anatomical zone (endocrine) (122).

The binding of these proteins with their functional receptor generates conformational changes that determine the course of the target cell (activation, differentiation and or proliferation) (123,124). For example, cytokines like IL1 can mediate T-cell activation (125), IL23 drives Th17 differentiation (126), and IL2 promotes T-cell proliferation (127). IL2, which promotes activation, proliferation and differentiation in competent T cells, produces all three effects listed above (128).

One single cytokine can be produced by different cell populations. For example, GM-CSF (granulocyte/monocyte-colony stimulator factor) is produced by subsets of lymphoid, myeloid and stromal cells (129). Additionally, two different cytokines can cooperate in a synergistic relationship to induce a more powerful response such as the TNF-α and IFN-γ mediated antiviral response (130,131).

Usually, the cytokine secretion patterns occur in a cascade, such that one cytokine stimulates its target cells leading to a downstream pathway activation within the target cells to induce additional cytokine production, amplifying the effect (142). Redundancy in the pathways ensures compensatory signalling mechanisms which can mitigate the compromise of any given pathway (143).

Hematopoiesis is highly regulated by cytokines (39,132-138). Cytokines instruct HSPC proliferation and lineage commitment (137,138). One of the most impressive aspects related to cytokines and hematopoiesis is their polyfunctionality. For example, **granulocyte colony stimulator factor (G-CSF)** is a hematopoietic cytokine that promotes proliferation of HSPCs, granulocytic differentiation, and bone marrow egress (139-142).

Several cytokines have been named “instructive hematopoietic cytokines” due to their effect to commit HSCs/HSPCs to one terminal phenotype. They include Erythropoietin (Epo), Thrombopoietin (TPO), M-CSF, G-CSF and GM-CSF. For example, Epo acting on MEP will promote erythroid commitment (143). TPO is the principal growth factor for megakaryocyte proliferation and differentiation and platelet formation (144-146). Despite the instruction effect on HSCs related to megakaryocyte commitment, TPO can contribute alone or in combination with other hematopoietic cytokines to increase the proliferation and differentiation of HSCs (147,148). Those hematopoietic effects are attributed in part to their effect on increasing Homeobox B4 (HOXB4) expression (149), a transcription factor essential for the self-renewal of HSCs and their recovery from stress (150-152). Additionally, TPO enhances HSC homing in the bone marrow by downregulation of matrix metalloproteinase 9 (*MMP-9*) gene expression and subsequent perturbation of the SDF-1α/CXCR4 axis. (153).

In contrast, **M-CSF** and **G-CSF** will guide myeloid progenitors to differentiate into monocytes/macrophages and granulocytes, respectively (154,155). Several pro-inflammatory cytokines have hematopoietic effects (156), such as **IL1** (157), **IL3** (158), **IL6** (159), **IL18** (160), **Type I and II interferons** (161), **TNF-α** (162), and **IL17** (163).

Stem cell factor (SCF) is a cytokine essential for preserving HSC pool (164). This cytokine, produced by stromal and endothelial cells (95,165) binds to c-Kit (the stem cell factor receptor) expressed on HSPCs to support their self-renewal capacity (166). Additionally, SCF appears to collaborate with other cytokines such as IL3, CSFs, and erythropoietin to preserve HSCs proliferation, as well as to promote myeloid and erythroid differentiation (167,168).

IL6, mainly produced by stromal cells, is a pro-inflammatory cytokine involved in myeloid differentiation during emergency hematopoiesis (169,170), a process activated by infection or inflammation, aimed at boosting the production and activation of innate immune cells to facilitate rapid pathogen elimination (171). However. IL6 promotes myeloid lineage commitment on HSCs, but it is also relevant to preserve the physiological rate between proliferation and differentiation of HSCs.

IL6-deficient mice lose control of myeloid-biased progenitor differentiation and proliferation, leading to abnormal levels of these progenitor cells as well as a slow recovery from hematopoietic ablation (172).

IL3 is a cytokine which synergizes with M-CSF and G-CSF to promote myeloid differentiation (173). As well, IL3 has been related to inducing proliferation and myeloid differentiation in HSCs (174).

FLT3L (FMS-like tyrosine kinase 3 ligand) is a growth factor for HSPCs (175) driving lymphoid/myeloid differentiation by suppressing the megakaryocyte/erythrocyte potential of the cells (176). Mouse experiments have shown that lacking FLT3L impaired NK (177), dendritic cells (177), and common lymphoid progenitors (178).

Several combinations of those cytokines are available through several culture conditions to study HSC differentiation/expansion (157,179-182).

1.3. The role of inflammation in the aging of the hematopoietic system

The hematopoietic system experiences a time-dependent decline (183) which leads to a propensity for failure of hematopoiesis and malignancies (63,184-186). Aging also can directly affect cytokine secretion patterns, shifting the balance in favour of pro-inflammatory cytokines (IL-1, IL-6, GM-CSF, IL-17, etc.) (56,63). This secretory pattern can "communicate" instructions that lead to low-grade chronic inflammation and this process can cause a myeloid-biased hematopoietic output (187).

Biological aging, organismal aging or biological senescence is the gradual deterioration of diminished replicative capacity and altered functionality of the cells in living organisms (188). The aging of the hematopoietic system in humans is associated with a reduction in self-renewal capacity, a remarkable myeloid-biased output, and an increase in the risk of blood malignancies

(183,187). Another consequence of this myeloid-biased hematopoiesis is a dysfunction of the adaptative arm of the immune system (189).

The hematopoietic niche is also affected by age, expressing morphological and functional modifications (190), such as depletion of $CD31^{high}Endomucin^{-/low}$ arterioles (191), changes in the MSCs such as depletion of periarteriolar Osteolectin+ cells (192), which, when replicated experimentally, contribute to impaired lymphopoiesis (192,193). In contrast, the architecture of marrow sinusoids is preserved which could explain why HSCs are not depleted (194).

Senescence is the process by which cells irreversibly stop dividing and enter a state of permanent growth arrest without undergoing apoptosis (195). Aging is related to cellular senescence by DNA damage accumulation (196,197). Ageing is strongly linked to increased DNA damage response (DDR) activation in both proliferating and non-proliferating cells, significantly contributing to the accumulation of senescent cells over time. Impaired DNA repair further exacerbates the buildup of DNA lesions and persistent DDR signaling, while also promoting widespread chromatin alterations observed across various cell types and at the organismal level during ageing. Additionally, DDR serves as a key driver of metabolic reprogramming, which can amplify senescence-associated secretory pattern (SASP) activity (198). Through its regulation of SASP via multiple pathways, DDR emerges as a crucial mediator of age-related inflammation (199,200).

The pathways leading to cellular senescence are driven by DDR (201,202), telomere shortening (203,204), and oncogene activation (205). DNA damage activates DDR signalling through proteins such as ATM/ATR (206), CHK1/CHK2 (207,208), and p53 (209,210), resulting in cell cycle arrest (211,212). Telomere shortening and fragility similarly trigger DDR, while oncogene activation leads to replication stress, ROS production, and mitochondrial dysfunction, culminating in DNA damage and cell cycle arrest. These mechanisms converge to induce senescence features, including prolonged cell cycle arrest (via p16/p21) (213,214), oxidative damage, increased resistance to apoptosis (BCL family proteins), metabolic changes (SA-β-gal), and the secretion of SASP (200).

SASP is a dynamic process activated alongside cell cycle arrest induced by senescence triggers. Core SASP factors include proinflammatory cytokines such as IL-6, IL-8, IL17A, and MCP1, as well as ECM-modulating enzymes like MMPs, SERPINs, and TIMPs, regulated by IL-1 signalling (215,216). (217)Recent discoveries have identified additional SASP effectors, such as GDF15, STC1, and MMP1, released as soluble molecules or in exosomes. SASP activation is driven by DDR factors, with NF-κB playing a central role (212), further enhanced by pathways like p38 MAPK and mTOR (211). Transcription factors such as NF-κB, C/EBPβ, and GATA4 regulate SASP gene expression (209,218), while chromatin regulators like BRD4 (219) compete with PRC2 to enable SASP gene activation (220). Additionally, the cGAS–STING pathway, triggered by cytosolic DNA or chromatin fragments, activates NF-κB and IRF3 (221), highlighting its role as a major SASP regulator, potentially influenced by DNase downregulation.

The stress-inducible MAPK p38 is both necessary and sufficient to induce growth arrest and SASP, even in the absence of DNA damage (222,223). p38 enhances SASP gene expression by increasing NF-κB activity (223), indicating that while DDR and p38 pathways operate independently, they converge to activate the SASP program (224).

As mentioned above, aging of the hematopoietic system affects the adaptive arm of the immune system (decreased expansion of lymphocyte progenitors as well as fully differentiated and competent lymphoid cells) (187,192,225). In turn, the aging of the immune system affects the hematopoietic system whether by blood-borne factors that act systemically on the hematopoietic system (53) and/or pro-inflammatory cytokines expressed by bone marrow stromal and immune cells (226), and mainly. Pro-inflammatory cytokines with myeloid differentiation potential (227), such as IL-1β, IL12, IL13, IL-6, IL17 and TNFα are increased within the bone marrow (162,192,228,229) .

An aging results in intrinsic changes in the HSC (230,231), characterized by the up-regulation of JAK/STAT (225), P38 MAPK (232), p65/NF-κB (233), and mTOR pathways (234). Those changes are induced by the same members of the pro-inflammatory cytokines listed above and that are increased during aging (39,162,192,228,229).

Changing the bone marrow niche by transplanting older HSC into younger mice or by sympathomimetic supplementation, restores the age-related transcriptional profiles of HSCs (235) reactivating the DNA damage response (236), and thereby improves multilineage cell production and HSC engraftment (237), respectively.

Likewise, the exogenous supplementation of P38 MAPK and mTOR inhibitors restores the repopulating capacity and maintains the quiescence of HSCs (238,239) and enhance the regenerative capacity of HSCs from aged mice (234). Inhibition of IL-11, a pro-inflammatory cytokine involved in senescence induction by stimulation of mTORC1-dependent senescence-associated secretory phenotype factors (IL-6, IL-8) (240), extends the healthspan and lifespan of mice (241,242).

All these elements highlight the therapeutic potential of research into the relationship between the hematopoietic system and the immune system during aging as a means to reduce the risk of myeloid malignancy and the inflammatory state associated with aging.

1.4. IL17 and the hematopoietic system

1.4.1. IL17 family and signalling pathway

IL-17 is a proinflammatory cytokine (243-245) discovered in 1993 (245). It is implicated in mucosal defence against extracellular pathogens (bacteria, fungi, or dysregulated commensals), tissue repair, and tertiary lymphoid tissue generation (246). IL17A is also involved in chronic inflammation and autoimmune diseases such as psoriasis (247). IL17A is the principal referent of

at least six structurally similar cytokines (organized here according to their homology with IL17A): IL17F, isoforms 1 and 2 (55 and 40% homology, respectively) (248-250), IL17B (29%) (251-253), IL17D (25%) (254,255), IL17C (23%) (256,257), and IL17E (also named IL25) (17%) (258-261).

The IL-17 receptor family consists of five subunits of homologous ubiquitous type I membrane glycoproteins that form heterodimers with other chains to bind different IL17 family members: named from IL-17RA to IL-17RE (262,263). The IL17RA is extensively expressed in comparison with the cell type expression of other IL-17R family receptors (247,264) because of the high requirement of IL17RA expression for an effective response (264-266). IL17RA, as a distinct characteristic, can modulate the signalling activation by internalizing its ligand as well as itself, which decreases the cell surface expression and the ligand availability (264,267).

IL17A signalling is driven either by the homodimer of two IL17A/IL17A or by the heterodimer of IL17A/IL17C (268,269). When one of these two variants binds to its functional receptor (IL17RA/IL17RC) (270,271), it induces dimerization of the IL17R complex that will start the cascade of signalling events through a conserved SEF/IL-17R (SEFIR) domain, which interacts with the adaptor molecule ACT-1 to start the signalling process (262,272). Additionally, the IL17 signalosome can be formed by an hexameric IL17 complex (IL17RC/IL17RA → IL17A ← IL17RA/ILRC) (273), which increases the IL17A signalling activation (273). IL17F acts similarly to IL17A, although its affinity for the IL17RA/IL17RC complex is less than IL17A. However, IL17F levels are 30-fold higher on average than IL17A in pathologies, such as psoriasis (274).

1.4.2. IL17-producing cells

T-helper cells 17 (Th17) a CD4+ T-cell subset expressing the obligate transcription factor RORγt (retinoic acid-related orphan receptors) have been identified as the main source of IL17A/F (275) (276,277). Other subsets of immune cells expressing RORyt and IL17A are innate lymphoid cells type 3 (278), subsets of γδ T cells (γδT17) (279-281), NK-T cells (282) as well as invariant NK-T (mature T cell subset with a semi-invariant T cell receptor (TCR) able to recognize lipids presented through CD1d, a non-polymorphic MHC class I-like antigen-presenting molecule) (283,284). Since all these cells express RORγt, it was initially thought to be a requirement for IL17A production (279). Its relationship was probed experimentally and explained in the following way: the RORγt unit through chromatin remodelling promotes IL17A transcription in mice and humans (285-287).

However, other RORγt-negative IL17A-producing cells were later discovered, including CD8 T cells (288,289), mesenchymal stromal cells (290) NK cells (291), B cells (292), macrophages (293), neutrophils (294), and LTi (lymphoid tissue inducer) cells (295).

The role of the IL17A/Th17 signalling axis in autoimmune diseases (296) and cancer (270,297) is increasingly recognized, particularly in leukemia (298). The plasticity of Th17 cells allows them

to further differentiate into non-classical Th1, Th2 and non-classical Treg, with a different effect on tumours (298,299).

For example, when Th17 is polarized to Th1 driven by IL12, this phenotype is capable of producing interferon-gamma, IL17A, and expressing the chemokine receptor CXCR3 (297,300). This non-classical Th1-polarized Th17 phenotype contains RORγt and T-bet and is highly implicated in the antitumor response increasing inflammation and recruiting neutrophils and other immune cells (278).

In contrast, when Th17 cells are polarized to non-classical Treg by the actions of mediators such as TGF-ß and IL6, the Treg-Th17 phenotype expresses the transcriptions factors RORγt and FOXP3 and their secretion pattern includes IL-10 and IL-1, and TGF-ß. This phenotype has a tolerogenic and protumor role in reducing antigen presentation, T cell activation and functional immune cell recruitment (297,301,302).

1.4.3. The IL17-signalling axis in the hematopoietic system

The hematopoietic potential of IL17A has been characterized using different experimental approaches such as *ex vivo* colony forming assays and *in vivo* IL17A injection followed by total bone marrow/spleen isolation and colony formation assays. However, those publications have resulted in contradictory findings. For example, IL17A injection in mice increases bone marrow granulopoiesis but not erythropoiesis (303). In spleen-derived cells, the IL17A exposure increases colony-forming units of erythroid and granulocyte/monocyte (304). IL17A *ex vivo* supplementation on mouse total bone marrow cells increased IL-6 production and granulocyte/monocyte colony-forming units (163), but in human *ex vivo* IL-17 supplementation inhibited hematopoietic progenitor proliferation (305).

The contradictory evidence might be partially technical. The absence of isolation of immunophenotypically defined bone marrow progenitor cells in older experimental designs does not allow the exclusion of indirect effects generated by the stimulation of IL-17 on the microenvironment, versus the direct effect of IL-17 on HSPC. It is known that IL-17 stimulates mesenchymal stroma cells and other cell types to produce IL-6 and GM-CSF (36). Additionally, a chimeric competitive transplantation assay that includes a functional IL17RA knockout (38, 39), a gold standard method to study HSC function (40), has never been performed.

Nonetheless, it is believed that IL17A regulates the bone marrow microenvironment by targeting bone marrow mesenchymal stem cells and stromal cells (246,262,306), which express high levels of membrane IL-17R (307,308). The downstream signalling pathway activation of IL-17R includes JAK/STAT, MAPK, NF-κB pathways (243,273,309) which are involved in the proliferation and differentiation of mesenchymal stem cells in response to IL17A (310), thereby affecting the bone marrow niche (311).

On the other hand, IL17A also affects the secretion pattern of bone marrow mesenchymal stem cells and stromal cells. IL17A signals to bone marrow stromal cells *in vitro* to produce pro-inflammatory cytokines with hematopoietic effects (134,312,313), including IL-6, TNF-α, G-CSF, GM-CSF (246,309). These cytokines can also affect the fate of the bone marrow niche changing the HSC program toward myeloid-biased output. For example, when IL-6 is generated in bone marrow stromal cells in response to IL-17 stimulus, it stimulates the RANKL, which is indispensable for the differentiation and activation of osteoclasts, and this leads to bone resorption and osteoporosis. IL-6 also induces excess production of VEGF, leading to enhanced angiogenesis (9).

Outside of bone marrow, IL17A mediates neutrophil activation and recruitment via expression of IL8 (314-316). IL17A was found as a central cytokine involved in the spontaneous development of germinal center B cell-derived autoantibodies in autoimmune mouse models (317,318), highlighting the biological effects of IL17A on bone marrow-derived cells even outside of the bone marrow microenvironment.

IL17A is also relevant in blood malignancies such as B-cell acute lymphoblastic leukemia (319,320), acute myeloid leukemia (321), and multiple myeloma (322,323), where it acts as a growth factor for the cancer cells by activating BCR-ABL, IL6/JAK/STAT3 and NF-kB signalling pathways. Additionally, IL17A promotes secretion of the chemokine CXCL16 by leukemia cells, which regulates the leukemic niche by promoting the recruitment of Th17 (320).

However, despite all the elements discussed here, there are several questions about the role of the IL17A signalling pathway within the bone marrow microenvironment (**Figure 3**). The principal questions that we address here are: What are the sources of IL17A in the bone marrow microenvironment? Can IL17A directly signal HSC? Which are the cellular subsets targeted by IL17A? What is the final output?

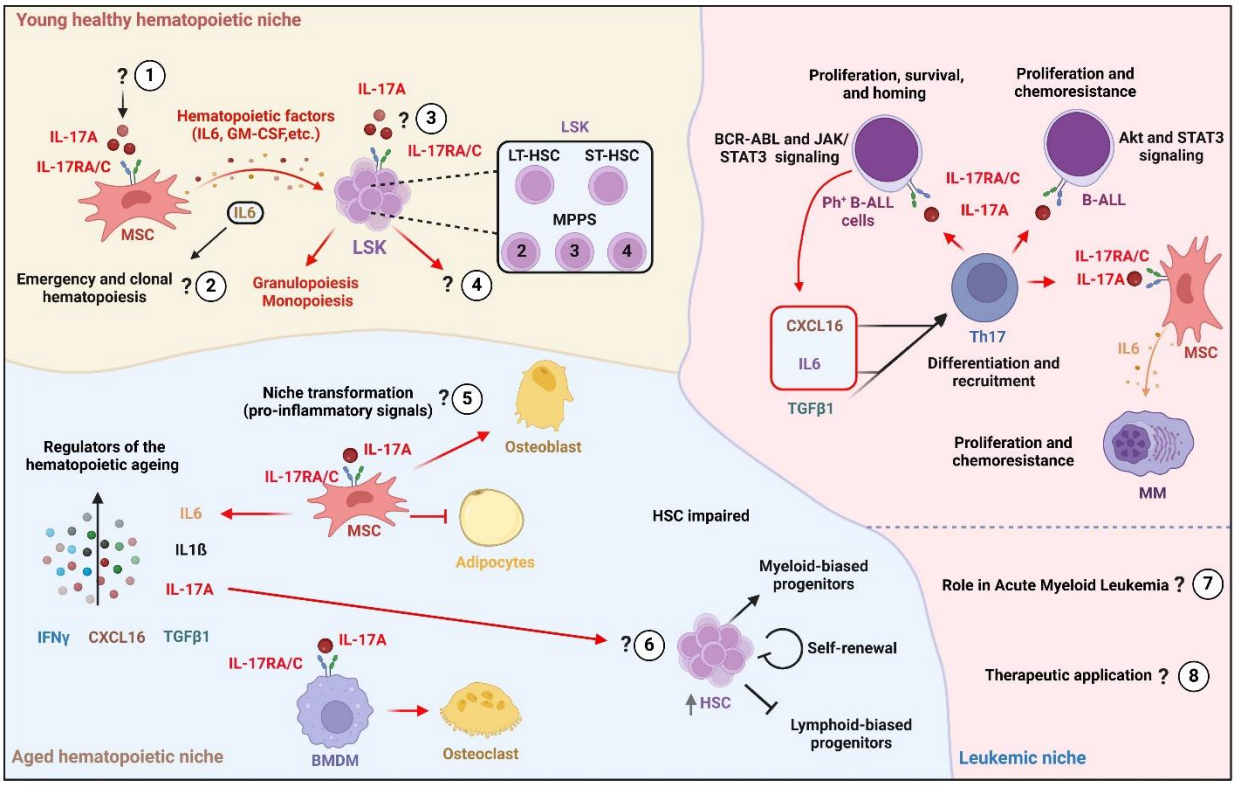

Figure 3. Summary of the current knowledge about IL17A-signalling axis within the bone marrow microenvironment. From 1 to 4, the main questions aborded in this thesis. Generated in Biorender.

2. Hypothesis and objectives

We hypothesize that IL17A is produced in the bone marrow microenvironment by immune cells and directly drives myeloid-biased output on hematopoietic stem and progenitor cells.

To study our hypothesis, we designed 2 experimental aims:

1. Assess the IL17A signalling axis in the bone marrow.
2. Investigate the functional effect of IL17A on bone marrow hematopoietic stem and progenitor cells.

3. Material and Methods

3.1 Computational analysis on publicly available single-cell RNA sequencing datasets

Using publicly available human (339) and mouse (340) bone marrow scRNAseq datasets we performed a gene expression analysis for IL-17RA, IL17RC, and ACT-1, in collaboration with Matthew Salaciak in the laboratory of Dr. Nathalie Jonhson.

In the human dataset, we performed gene-set enrichment analysis on differentially expressed genes on the HSCs clusters between young (less than 50 years old) and old (more than 50 years old) healthy donors. Since the decline of the immune system associated with SASP starts to consolidate between 45-50 years-old, we selected 50 years-old as the cutoff.

In the murine dataset, we performed a U-cell enrichment analysis (324) to explore the IL-17-signalling pathway in mouse HSPCs.

The IL17-signalling signature employed in every analysis was RACTOME_INTERLEUKIN_17_SIGNALLING (https://www.reactome.org/content/detail/R-HSA-448424)
Briefly, each dataset was annotated using the relevant gene signature from DMAP. Then, by gene-set enrichment analysis, each hematopoietic population was annotated and the visualization and dimensional reduction algorithm employed was Uniform Manifold Approximation and Projection (UMAP) (325).

In collaboration with the John Dick laboratory, we investigated the enrichment of IL17-signalling in a human bone marrow map (326) as well as the CD34+ compartment with inflammatory memory (327,328).

3.2 Donors and samples

All *in vivo* experiments were performed following McGill Animal Care Committee regulations. 6–8-week-old C57BL/6 mice were used as donors for each experiment. For *in vitro* experiments, the bone marrow sample preparation consisted of a pool of 2 male and 2 female mice.

In the case of *ex vivo* culture and *in vivo* competition assay, the sexes were tested independently (first experiment only male as a donor, rescue, and recipient; second experiment only females). Every pool was considered an independent experiment. Following euthanasia, the tibia, femur, iliac bones, and spine were dissected. Then, the bones were crushed with FACS buffer (PBS (Wisent, 311-425-CL) + 2% of FBS (Wisent, 80450) + 1% of penicillin/streptomycin (Wisent, 450-201-EL) + 2 mM glutamine (Gibco, A2916801)). The cell suspension was collected and passed through a 70-micron filter. Then, the tube was spun down at 600G for 5 minutes at 4 degrees. The supernatant was removed, and the cell pellet was resuspended in 2 ml of FACS buffer and transferred into a 15-ml conical tube with 5 ml of Ficoll-Paque solution. After 30 minutes of

centrifugation at 1200G at room temperature without acceleration or breaking, the interface of nucleated cells was collected and transferred onto a 5-ml FACS tube for staining.

To assess the IL17A levels in bone marrow plasma, both femurs per specimen were isolated, and using scissors, the distal epiphysis was shopped out. The bones were placed in a 0.6-ml Eppendorf tube with a small bottom hole within a 1.7-ml Eppendorf tube with 1 ml of PBS. The samples were spun down at 12 000 rpm for 10 minutes at 4 degrees and the supernatant was stored at -80 degrees.

Blood samples (1 ml) were collected through a cardiac puncture in a Vacutainer tube with EDTA (BD, CAT:363080). The samples were spun down at high speed, and the supernatant (plasma) was collected and stored at -80 degrees until use.

3.3 IL17A-signalling pathway

3.3.1 Hematopoietic stem and progenitor cells

We designed a multicolor-flow cytometry panel (**Figure 4**) for the identification of every subset of mouse LSK (LT-HSC, ST-HSC, MPP2, MPP3, MPP4) following the gating strategy proposed by Pietras and co-workers (33). This strategy was used to perform cell sorting during progenitor isolation, quantitative population analysis of *ex vivo* culture, and IL17-RA/RC expression.

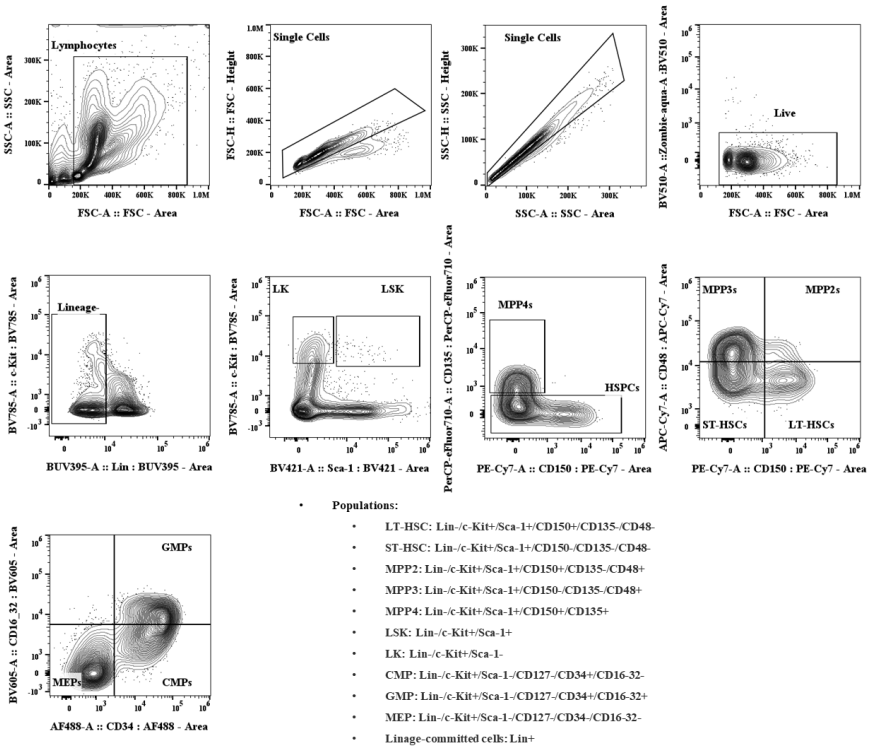

Figure 4. Panel and Gatting strategy for mouse bone marrow progenitors' analysis and sorting.

3.3.2 IL17RA and IL17RC expression analysis by flow cytometry

For IL17RA and IL17RC expression analysis, total bone marrow cells were stained with anti-mouse c-Kit-BV785 (Biolegend, 105841), Sca1-BV421 (Biolegend, 108127), Zombi-aqua (Biolegend, 423101), Biotin lineage cocktail CD11b (Biolegend, 101204), Gr1 (Biolegend, 108404), CD11c (Biolegend, 117304), TER119 (Biolegend, 116204), NK1.1 (Biolegend, 108704), F4/80 (Biolegend, 123106), CD3ε (Biolegend, 100304), CD4 (Biolegend, 100508), B220 (Biolegend, 103203), and CD8α (Biolegend, 100704) / Streptavidin-BUV395 (BD Horizont, 564176), CD16/32-BV605 (BD Biosciences, 563006), CD34-AF488 (eBioscience, 53-0341-82), CD135-PerCP-eFuor710 (eBioscience, 46-1351-80), IL17RA-PE (eBioscience, 12-7182-80) / Isotype control-PE (eBioscience, 11-4321-41), CD150-PE-Cy7 (Biolegend, 115914), IL17RC-APC (R&D System, FAB2270A-100UG) / Isotype control-APC (R&D System, IC108A), and CD48-APC-Cy7 (Biolegend, 103431).

3.3.3 IL17A uptake and IL17RA trafficking

Total bone marrow cells were collected as mentioned above. Then lineage-positive cells (CD11b+/CD11c-/NK1.1-/Gr1-/TER119-/F4/80+/CD3+/CD4+/CD8+/B220+) were depleted using a home-made biotinylated lineage cocktail and magnetic isolation tools from Miltenyi Biotec (Anti-Biotin MicroBeads, CAT: 130-090-485; QuadroMACS™, CAT:130-091-051; LD Columns, CAT: 130-042-901). We isolated lineage-negative bone marrow cells following the instructions of the company. Sample cells from each mouse donor were split into two aliquots (control and IL17A treated) and were incubated for 1 hour at 37 degrees with 5% CO_2 and 95% humidity in PBS +/- IL17A at 500ng/ml. Then cells were washed and stained for the surface marker c-Kit-BV421 (Biolegend, 105827), Zombie-NIR (Biolegend, 423105), and IL17RA-APC (eBioscience, 17-7182-80) to measure the extracellular expression of IL17RA. Then cells were washed and fixed using 4% paraformaldehyde. After fixation cells were permeabilized using 0.03% of Triton 100 for 10 minutes, then washed, and blocked for 30 minutes using PBS, 5%BSA, 10% of FBS, and 0.01% of Triton 100). Next, cells were incubated with IL17RA-PE (eBioscience, 12-7182-80) to measure the intracellular fraction of IL17RA, and IL17A-BUV395 (BD Horizon, 565246) for 30 minutes. After, cells were washed, resuspended in PBS, and analyzed using a SONY ID7000 spectral cell analyzer.

3.3.4 Phosphorylation assay by flow cytometry on ERK1/2, P38, JNK and IKßα

Total bone marrow cells from 4-13 independent mice were equally divided into 4 aliquots to evaluate the phosphorylation response of p-ERK1/2-PE-Cy7 (Cell Signalling, 98168), p-P38-AF488 (Cell Signalling, 4551), p-JNK-AF488 (Santa Cruz Biotechnology, sc-6254), and p-IkBα-PE (eBioscience, 12-9035-42) to IL17A *ex vivo* stimulation. Summarizing, each donor has 4 samples: 1) non-IL17A, 2) + IL17A (500ng/ml), 3) non-IL17A + 1 µl/ml of GolgiStop (BD Biosciences, 554724), and 4) + IL17A (500ng/ml) + 1 µl/ml of GolgiStop. Samples were incubated for 30 minutes at 37 degrees with 5% CO_2 and 95%. Next, samples were collected, washed, stained

for extracellular markers, washed, blocked, and stained for intracellular markers (phosphorylated proteins). After, cells were washed, resuspended in PBS, and analyzed using a SONY ID7000 spectral cell analyzer.

3.3.5 IL17RA and ACT-1 expression analysis by confocal microscopy

To assess the expression of IL17RA and its intracellular molecular adapter ACT-1 we sorted 20 000 LSK directly onto an 8-well 1.5H Ibidi cover slide coated with human fibronectin 200 µL of StemPro serum-free media supplemented with 1% of Penicillin / Streptomycin, 2mM of L-Glutamine, 1% of insulin-transferrin-ethanolamine, and 25ng/ml of mouse SCF. Cells were incubated for 3 days at 37 degrees with 5% CO_2 and 95% humidity. Then, cells were rinsed with PBS and fixed with 4% PFA for 10 minutes at room temperature. Then, cells were washed with PBS and permeabilized with 200 µL of PBS with 0.03% triton X for 10 minutes at room temperature and blocked for 1 hour at room temperature with blocking buffer (PBS, 5%BSA, 10% of goat and donkey serum, 0.5% NP40).

Cells were washed 2 times and stained with rabbit anti-mouse primary antibody against IL-17RA (Invitrogen, MA5-35663) and mouse anti-mouse primary antibody against ACT-1 (Santa Cruz Biotechnology, sc-100647) for 1 hour at room temperature protected the light. After 1 hour, the cells were washed 2 times and blocked again for 30 minutes. A secondary antibody stain was done with Alexa Fluor 488 goat anti-rabbit (Invitrogen, A-11034) and Alexa Fluor 647 donkey anti-mouse (Invitrogen, A-31571) for 30 minutes at room temperature protected from the light. The samples were washed 2 times and mounting media + DAPI (Invitrogen, P36931) was added. Images were acquired in an LSM800 – Airyscan confocal microscope.

3.3.6 Western blot analysis

For western blot analysis, we isolated lineage-negative bone marrow cells (CD11b+/CD11c-/NK1.1-/Gr1-/TER119-/F4/80+/CD3+/CD4+/CD8+/B220+) using a home-made biotinylated lineage cocktail and magnetic isolation tools from Miltenyi Biotec (Anti-Biotin MicroBeads, CAT: 130-090-485; QuadroMACS™, CAT:130-091-051; LD Columns, CAT: 130-042-901). We isolated lineage-negative bone marrow cells following the company's instructions. Cells were incubated for 30 minutes at 37 degrees with 5% CO_2 and 95% humidity in PBS +/- IL17A at 500ng/ml. Then, cells were washed with cold PBS and lysed using homemade Rippa buffer (1%of Sodium deoxycholate (Thermo Fisher Scientific, CAT: 89904), 1% of NP-40 (Thermo Fisher Scientific, CAT: 85124), 150 mM of Sodium chloride (BioShop, CAT: 7647-14-5), 25 mM Tris-HCL pH=7.6 (BioShop, CAT: 77-86-1), and 0.1% of SDS (BioShop, CAT: 151-21-3)) with proteinase and phosphatase inhibitors (ThermoFisher, A32959). Protein lysates were reduced using Laemmli buffer + 2-Mercaptoethanolran, 50µg of total protein was led in each well of a Bis-Tris gel with 10% acrylamide, then transferred into a PVDF membrane (BioRad, 1620177). The image was acquired using the ChemiDoc system (BioRad, 12003153) and quantified using ImageJ which we described before (329).

The following antibodies were used to blot for Phospho-IκBα (Ser32) (14D4) Rabbit mAb #2859, phospho-p38 MAPK (Thr180/Tyr182) (12F8) Rabbit mAb #4631, phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb #3033, phospho-C/EBPβ (Thr235) Antibody #3084, phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370, Vinculin (Cell Signalling, 13901T).

3.4 IL17A in the bone marrow microenvironment

3.4.1 IL17A-producing cells identification

To evaluate the presence of IL-17-producing cells within the bone marrow microenvironment, we collected total bone marrow and performed a spectral flow cytometry characterization using the anti-mouse antibodies CD3e-BUV661 (DB Biosciences, 741479), TCRß-BV421 (Biolegend,109230), CD19-BV510 (Biolegend, 115545), TCRγδ-BV605 (Biolegend, 118129), CCR6-BV785 (Biolegend, 129823), NK1.1-AF488 (Biolegend, 108717), CD11b-BV421 (Biolegend, 101235), Gr1-FITC (Biolegend, 108718), NK1.1-BV510 (Biolegend, 108737), RORyt-PE (BD Biosciences, 562684), CD4-PE-Cy5 (Biolegend, 100513), CD8-PE-Cy7 (Biolegend, 100721), CD45-APC (Biolegend, 103111), Zombie-NIR (Biolegend, 423105). Here we follow the same strategy for fixation and permeabilization described in the microscopy section.

3.4.2 *Ex vivo* IL17A production evaluation

To assess the ability of bone marrow cells to produce IL17A, we collected total bone marrow cells and stimulated them for 2 hours with Phorbol 12-Myristate 13-Acetate (Sigma P-8139; 50 ng/ml final concentration) and Ionomycin (Sigma I-0634; 1 μg/ml final concentration) in the presence of 1uL per ml of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin). Cells were washed, fixed (following the same protocol discussed for microscopy experiments), blocked with an anti-CD16/32 in PBS with 5% BSA, and stained (extracellular staining discussed in the previous section) for 30 minutes at 4 degrees. Cells were washed and permeabilized using 0.03% Triton 100, blocked again, and stained with anti-IL17A-BUV395 (BD Horizon, 563565) and anti-RORyt (BD Biosciences, 562684). Next, samples were washed and resuspended in PBS, then analyzed using a SONY ID7000 spectral analyzer.

3.4.3 ELISA

Levels of IL17A in blood and bone marrow plasma were quantified using a quantitative ELISA sandwich kit for IL17A (homodimer) Mouse Uncoated ELISA Kit with Plates (Thermo Fisher, 88-7371-22) following the instructions of the company.

3.5 Ex vivo culture

3.5.1 Colony forming assay

Using methylcellulose-based media supplemented with IL-6, IL-3, EPO, and SCF (Methocult M3434, Stem Cell Technologies) and 1% penicillin/streptomycin, we performed a colony formation assay for myeloid differentiation on LSK cells, sorting 100 LSK per FACS tube containing 1.5 ml of M3434 at single-cell purity. After sorting the cells into the tube, a vortex was applied, and the tubes were left to sit for 30 minutes to allow bubbles to rise to the top. Next, 1.1 ml of the content was dispensed into each well of a 6-well plate using a 1-ml sterile syringe (BD Biosciences, SY012638S). The palate was swirled or tilted to uniformly distribute the media. Sterile water was added to fill inter-well spaces of the 6-well plate. Next, plates were transferred into a Square BioAssay Dish and then placed into a water-jacket incubator (37 degrees, 5% CO2, and 95% humidity). Colonies were cultured for 10 days and scored using a STEMgrid™-6 (Stem Cell Technologies, 627000).

3.5.2 Liquid culture

For expansion and differentiation assay in liquid culture, we used serum-free media (StemPro™-34 SFM (Gibco, 10639011)) supplemented with 1% of Insulin–transferrin–selenium–ethanolamine ITSX (Gibco, 51500056), 2mM of L-Glutamine (Gibco, A2916801), 1% penicillin/streptomycin (Wisent, 450-201-EL), and ß-Mepcaptoethanol (Gibco, 21985023).

A cocktail of mouse cytokines was added: 25ng/ml Flt3-L (PeproTech, 250-31L), 0/25/100/500 ng/ml IL17A (PeproTech, 210-17), 25ng/ml SCF (PeproTech, 250-03), 10 ng/ml IL-3 (PeproTech, 213-13), 10ng/ml IL-6 (PeproTech, 216-16), 25ng/ml TPO (Peprotech, AF-315-14), 25/ng/ml IL-11(Biolegend, 756104), 4 U/I EPO (Biolegend, 587604), and 10ng/ml GM-CSF (Biolegend, CAT: 576304). 2 000 LSK for expansion and differentiation assay, 10 000 SLK for EdU incorporation experiments and 1 000 LT-HSC, ST-HSC, MPP2, MPP3, and MPP4 were sorted per well in 24-well plate Flat Bottom Ultra-Low Attachment (Costar/Corning, 3473). Cells were cultured for 4 and 8 days at 37 degrees, 5% CO2, and 95% humidity. 50% of the media was replaced every day.

3.5.3 *In vivo* reconstitution assay

For this experiment, we isolated 10 000 LSK from C57/BL6 (CD45.2). Cells were cultured over 4 days using the same media recipe described above for liquid culture in the presence or absence of 100ng/ml of rm-IL17A. On day four, cells were collected and washed. Then, resuspended in a mix of 200µl of PBS + 500 000 total bone marrow cells from C57BL/6-CD45.1^{STEM}. The mix was intra-tail injected on lethally irradiated hybrid mice obtained from the cross of C57/BL6 (CD45.2) and C57BL/6-CD45.1^{STEM}. Hybrid mice received antibiotics 3 days before irradiation to prevent infections. The radiation scheme was divided into two doses of 6 G with 24 hours of differences.

After one month of transplant, peripheral blood was obtained from the saphenous vane, and after removing the red blood cells, samples were stained using anti-mouse CD45.1-FITC (Biolegend, 110705), CD45.2-BV421 (Biolegend, 109831), CD3-BUV395 (BD Biosciences, 569614), CD11b-PE-Cy7 (Biolegend, 101215), B220-PE (Biolegend, 103207), and Zombie-NIR (Biolegend, 423105). Data was acquired in a SONY ID7000 Spectral Cell Analyzer. After removing the doublet and dead cells, the fraction corresponding to the LSK cultured (CD45.2+/CD45.1-) was scored and compared between those that were stimulated with IL17A or control. The frequency of the parent was normalized via fold change relative to the control.

3.6 Statistical analysis and software

The mean fluorescent intensity was defined using the median and compared the values from the primary-labeled antibody vs its control in each case using a two-tailed unpaired t-test with Welch's correction. The absolute numbers were determined using CountBright™ (Invitrogen, C36950). Briefly, cells were gated on live cells (Zombie-NIR-) after removing the debris and doublets the following equation was used: $\mathbf{cells/\mu L} = \frac{\boldsymbol{Cells\ count}}{\boldsymbol{Beads\ count}} \boldsymbol{x} \frac{\boldsymbol{Total\ beads}}{\boldsymbol{Sample\ volume}}$, where the total beads were 20 000 and the sample volume was 200µl.

P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

Flowjo was used to analyze the flow cytometry data; GraphPad to perform the statistical analysis and graphs; Biorender was employed to generate illustrative images, ImageJ to quantify the results of the western blots; ZEN was used to analyze the immunofluorescence images.

4. Results

4.1 Human bone marrow hematopoietic stem cells expresss at RNA levels the IL17A-signalling components at the RNA level

To investigate the human bone marrow hematopoietic stem cell expression of IL17 signalling components, we downloaded a publicly available human single-cell RNA sequencing data set (330) composed of 31 healthy human donors from 10 to 89 years old with 306 286 cells. **Figures 5A** and **5B** represent the human bone marrow landscape at a single-cell resolution of the data set generated using annotations from DMAP gene signatures (Analysis performed by Mattew Salaciak).

We found expression of IL17RA and IL17RC mRNAs, which are required to form a functional IL17A receptor, in clusters belonging mainly to granulocyte-monocyte progenitors, B cell progenitors, and HSCs. However, it is interesting that we did not observe the expression of other receptors such as IL17RB and IL17RD, which could indicate that the main signalling on bone marrow is due to the IL17A and not by the other family members (**Figure 5C**).

In order to validate our results, we explored the genetic signature described for IL17 (REACTOME_INTERLEUKIN_17_SIGNALING (gsea-msigdb.org)) in a map of human bone marrow generated by members of Dr. John Dick laboratory, which contains multiple databases integrated into one (**Figure 5D**) (326). Using this approach, we observed that the IL17 signalling signature was enriched throughout the map, particularly in the lymphoid, myeloid, and HSC clusters (**Figure 5E**).

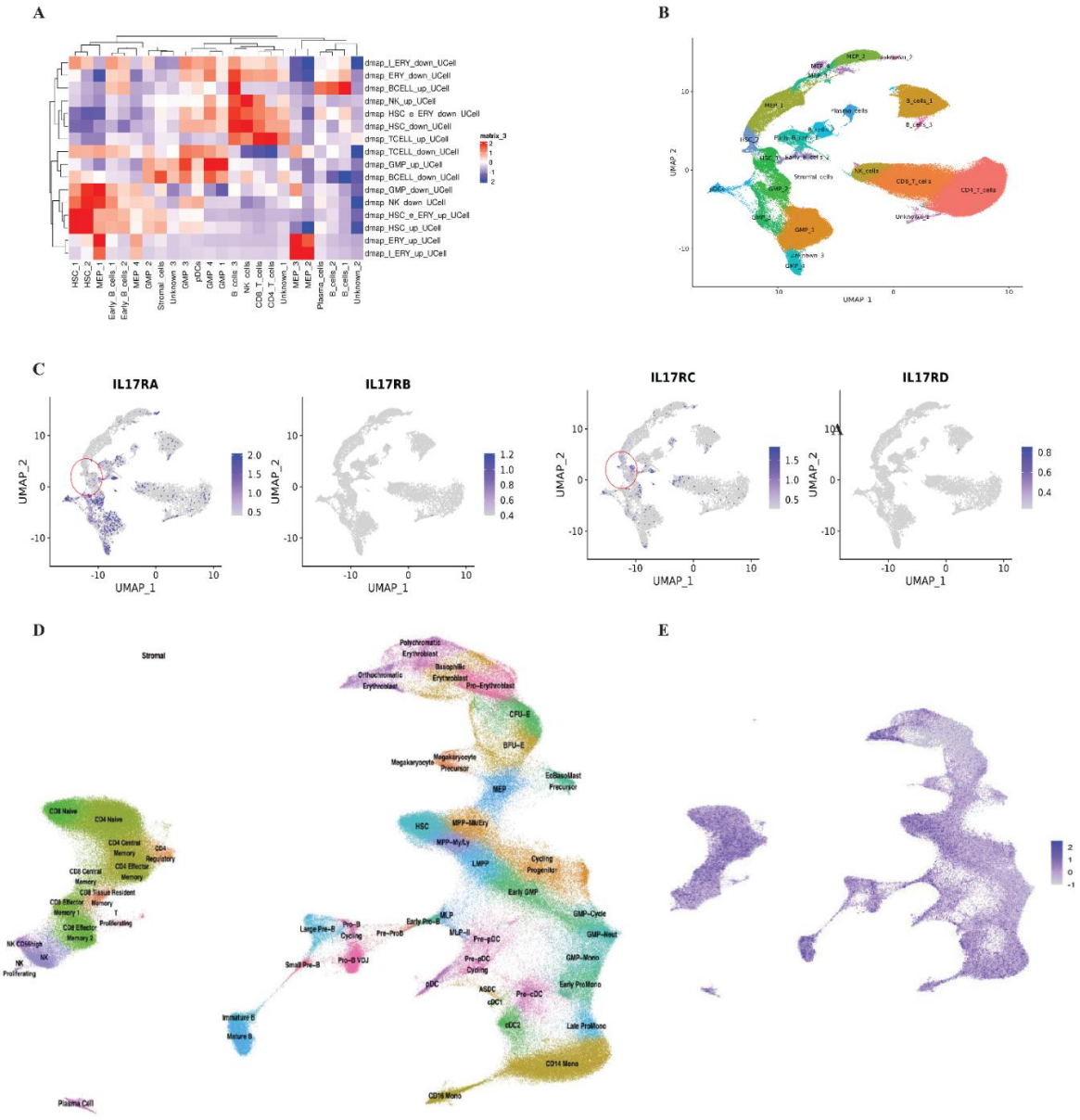

Figure 5. Human bone marrow hematopoietic stem cells express at RNA levels the IL17A-signalling components**. A:** Heatmap showing the annotation procedure generated from a gene set enrichment analysis using the DMAP signatures for the human bone marrow landscape. Here we showed how every cluster corresponds with its gene signature. **B:** Uniform Manifold Approximation and Projection (UMAP) visualization algorithm showing the human bone marrow landscape generated after annotations. **C:** The mRNA of IL17RA and IL17RC, but not IL17RB and IL17RD, were found in human bone marrow hematopoietic stem cells. **D:** A landscape of human bone marrow hematopoiesis generated by Dr. Dick laboratory (326). **E:** IL17-signalling signature expression on human bone marrow landscape.

4.2 The IL17A-signalling components are part of the changes associated with inflammation and aging in human bone marrow HSCs

As previously mentioned, IL17 is a proinflammatory cytokine involved in host defence against extracellular pathogens (273). Given the proinflammatory nature of this cytokine and its direct relationship with the induction of inflammation, we evaluated the potential relevance of the IL17 signalling signature (ATF1, ATF2, BTRC, CHUK, CREB1, CUL1, DUSP3, DUSP4, DUSP6, DUSP7, ELK1, FBXW11, FOS, IKBKB, IKBKG, IL17A, IL17C, IL17F, IL17RA, IL17RB, IL17RC, IL17RE, IL25, IRAK1, IRAK2, JUN, MAP2K1, MAP2K3, MAP2K4, MAP2K6, MAP2K7, MAP3K7, MAP3K8, MAPK1, MAPK10, MAPK11, MAPK14, MAPK3, MAPK7, MAPK8, MAPK9, MAPKAPK2, MAPKAPK3, MEF2A, MEF2C, NFKB1, NOD1, NOD2, PPP2CA, PPP2CB, PPP2R1A, PPP2R1B, PPP2R5D, RIPK2, RPS27A, RPS6KA1, RPS6KA2, RPS6KA3, RPS6KA5, SKP1, TAB1, TAB2, TAB3, TNIP2, TRAF6, UBA52, UBB, UBC, UBE2N, UBE2V1, VRK3) in normal HSC as well as the HSC subset that retains inflammatory memory (**Figure 6A**).

In this sense, we observed that IL17RA expression across subsets of human HSCs is enriched within myeloid and lymphoid progenitor cells (**Figure 6B**). This aspect corroborates our previous finding, now in a different data set as well. Interestingly, we did not see any visible differences in the expression pattern of IL17RA between normal HSC and HSC with inflammatory memory but the signature of IL17-signaling appears to be enriched in the HSC compartment that retains inflammatory memory (**Figure 6C**), suggesting a differential ability of HSCs to respond to IL-17 according to an inflammatory state.

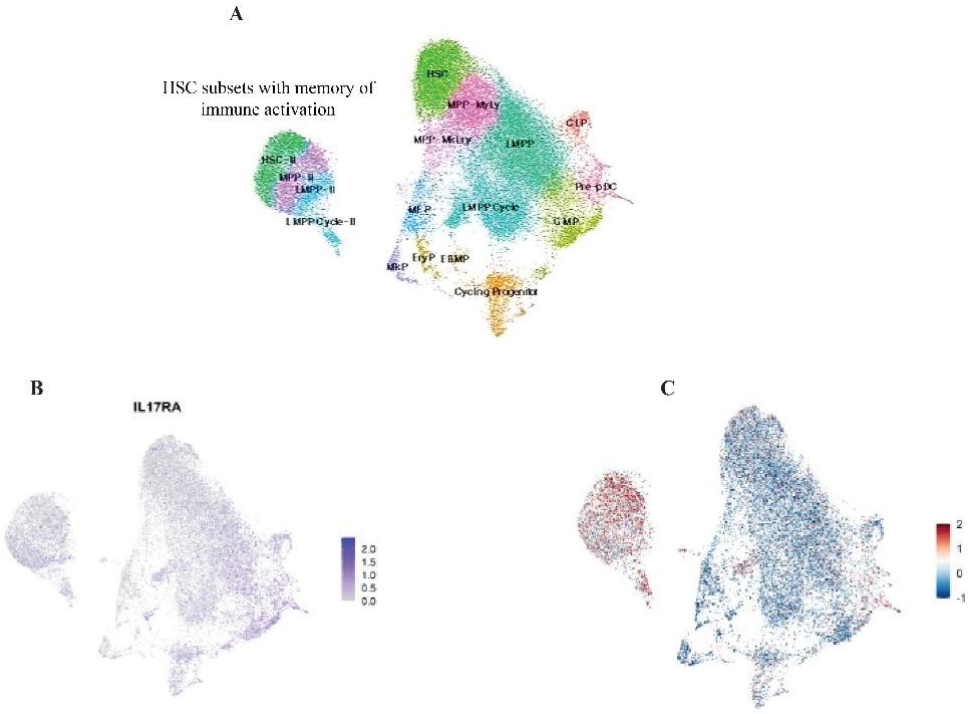

Figure 6. IL17-signalling is enriched on the new subset of human hematopoietic stem cells that retain a memory of immune activation. **A:** Human CD34+ compartment of human hematopoietic stem cells. To the left, is the normal subset of hematopoietic stem cells. To the right, the hematopoietic stem cell subsets that retain a memory of immune activation (331). **B:** The mRNA of IL17RA was found across the whole compartment, including the subsets of HSC with inflammatory memory. **C:** The IL17A signature is enriched preferentially on HSC subsets that retain a memory of immune activation.

Aging is associated with low-grade chronic inflammation and IL17A is increased in the aging bone marrow (216,217). We, therefore, explored differences in the enrichment of IL17-signaling signatures in the HSC compartment in different age groups. Since IL17A is a part of SASP and because this cytokine is increased with age, we stratified the human scRNA sequencing data set used to generate the analysis in **Figure 1A-C** into two age groups, using 50 years old as the cutoff. Next, we explored the expression of mRNA of IL17RA across cellular clusters using the same approach as in **Figure 5C**. At first glance, IL17RA mRNA expression using this approach seems to be reduced in samples derived from patients older than 50 years, particularly in GMPs and HSCs, as well as non-hematopoietic cells such as the T cell compartment (**Figure 7A**).

To better visualize differences in IL17RA expression enrichment in the bone marrow microenvironment, its expression was plotted in the whole bone marrow microenvironment (**Figure 7B**), as well as in each hematopoietic stem cell cluster and progenitors (**Figure 7C**), according to each decade of life. The results suggest that IL17RA mRNA expression seems to pick between 20 and 30 years of age in the bone marrow microenvironment and GMPs and HSCs, followed by a decreased trend from 40 to 71 years old. To gain insight into functional differences associated with the apparent decrease in IL17RA mRNA expression with age in adults, we performed a gene set enrichment analysis in HSCs using differentially expressed genes with age (cutoff age 50 years) (**Figure 7D**). In concordance with our hypothesis, IL17 signalling is upregulated on HSCs from donors less than 50 years old. These results suggest that the IL17 signalling axis is somehow relevant within the bone marrow of young adult humans. However, an important point here is the contradiction between the increment in the levels of IL17A associated with the age in the bone marrow previously described (228,229), and the downregulation of its signalling pathway, which can indicate a loss in the IL17A associated with the age.

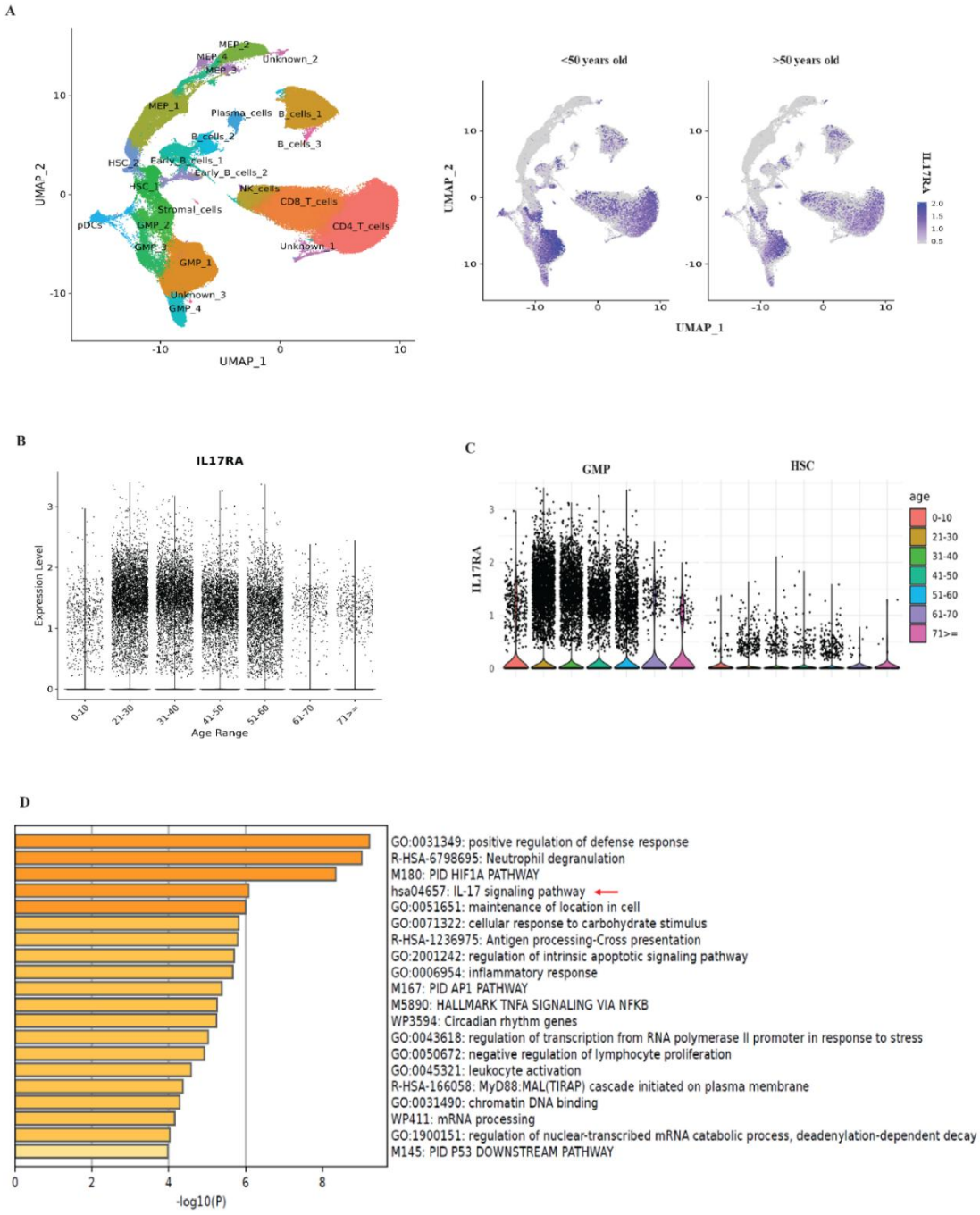

Figure 7. Age-dependent IL17-signaling in human bone marrow. **A:** UMAP visualization algorithm showing the human bone marrow landscape as well as the IL17RA mRNA expression in human bone marrow in samples from donors younger (left) or older (right) than 50 years old. **B:** Density plots representing IL17RA mRNA expression by age brackets as indicated in the x-axis where each dot represents an individual case. **C:** IL17RA mRNA expression within subsets of HSCs and progenitor cells across the age brackets. **D:** Gene-set enrichment analysis on differentially expressed genes on the HSC clusters using 50 years old as the cutoff, showing that IL17 signalling is the fourth most upregulated signature.

4.3 Mouse HSPCs express the IL17A receptor, which activates P38 and C/EBPß phosphorylation.

Considering the results obtained during our initial screening of public scRNA sequencing databases, we set out to explore the IL17A-signaling axis in the mouse bone marrow. First, we assessed if murine HSPCs express components of the IL17A signalling pathway. To this end, similar to the approach in our previous approach (Figure 5A-C), we performed a transcriptomic analysis in mouse HSPCs clusters using a public mouse scRNA sequencing data set (332), which is annotated to validate the genetic signatures available in DMAP for each HSPC population (**Figure 8A**). Mapping the bone marrow landscape in mice revealed mRNA expression of IL17RA, IL17RC, and TraF3ip2 (ACT-1) as well as enrichment of the IL17-signaling pathway in the HSPCs cluster (**Figure 8B**).

Having confirmed that IL17RA, IL17RC, and ACT-1 are expressed at mRNA levels in the murine HSPCs cluster, we assessed for expression of the IL17RA in murine bone marrow progenitors using different methodologies. We performed western blot analysis in protein extracts from murine Lin^- bone marrow cells after the elimination of terminally differentiated cells (CD4, CD8, CD3, CD11b, CD11c, CD115, F4/80, Gr1, TER119) and red blood as described in material and methods. After lineage depletion, cells were incubated in PBS for 30 minutes at 37 degrees in the presence or absence of IL17A. The results indicated that IL17RA and IL17RC, are expressed by lineage- bone marrow cells (**Figure 8C**).

To know if the LSK compartment expresses components of the IL17A receptor, we investigated the expression of IL17RA and its molecular adapter ACT-1. Immunofluorescence was performed in LSK-sorted cells as described in materials and methods. A representative image of the overlayed staining obtained with the two antibodies is shown in **Figure 8D** and it indicates that IL17RA and ACT-1A are expressed in murine LSK. However, because of the absence of an IL17RA, IL17RC, and ACT-1 deficient model, the limitation in the number of parameters allowed in microscopy; in addition to the fact that the LSK fraction is 0.01% of the bone marrow, we decided to use flow cytometry and isotype controls to characterize the IL17RA and IL17RC expression in mouse LSK subsets at single-cell resolution. We identified that in 6 murine donors, the mean fluorescence intensity of the respective anti-IL17RA or IL17RC antibody was significantly higher than its respective isotype control in LSK (**Figure 8E, 8G)**. We then, proceed to evaluate the IL17RA and IL17RC in every subset of LSK, showing that LT-HSCs, ST-HSC, MPP2, MPP3, and MPP4 expressed both IL17RA and IL17RC (**Figure 8F, 8H**). Since the murine scRNA sequencing database we used was not age-selective, we used the above approach to evaluate IL17A receptor A chain expression in mice aged 6-8 weeks and mice older than 1 year.

Consistent with our differential mRNA expression findings in human HSCs, the results in **Figure 8I** indicate that in mice there is a significant reduction of IL17A receptor A chain expression in LSKs from older donors, which is most evident in the LT-HSCs fraction.

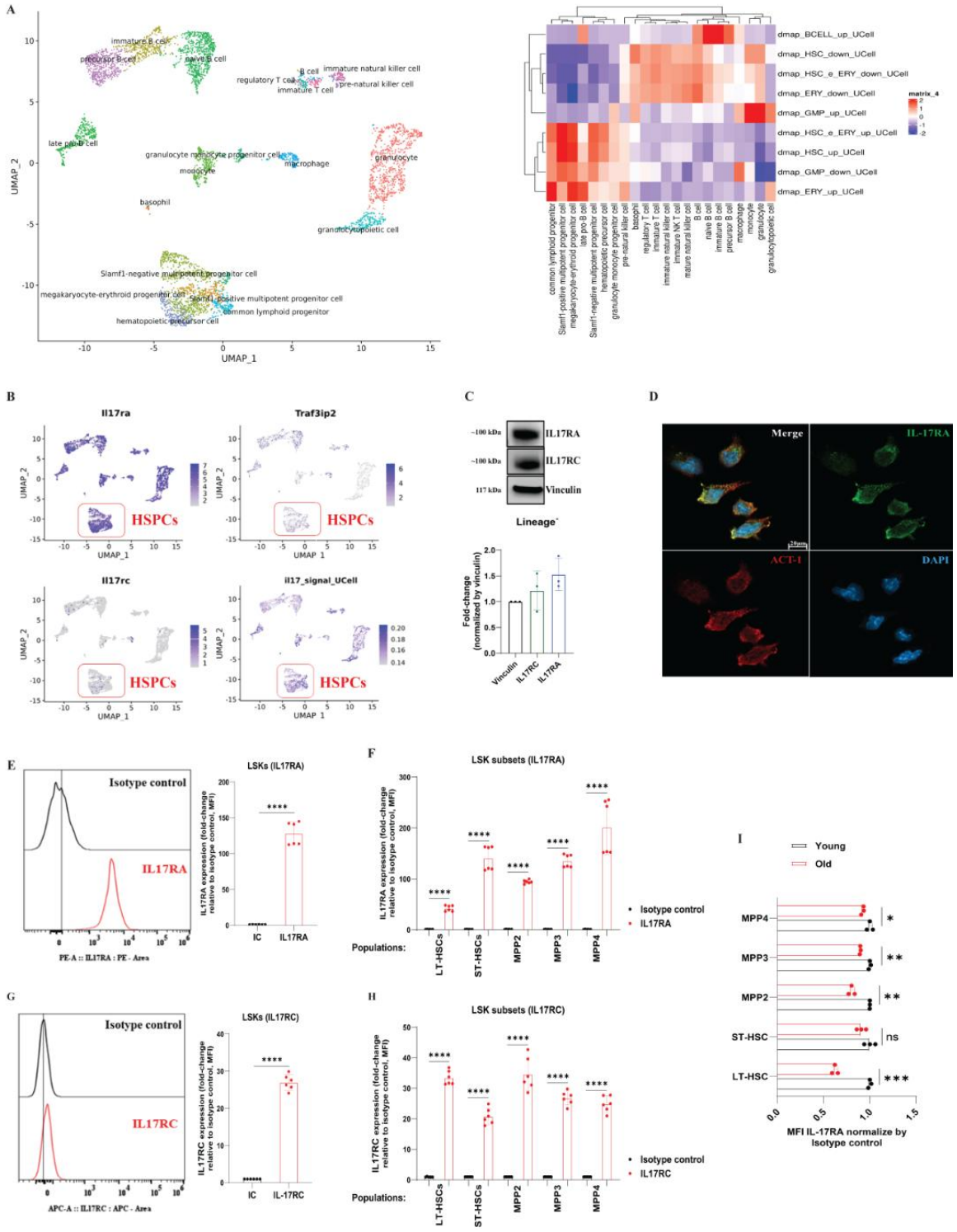

Figure 8. Mouse LSKs express the IL17A receptor**. A:** The UMAP visualization algorithm shows the mouse bone marrow landscape generated after annotations and unsupervised clustering (left). Since this dataset came with its annotation, we compared these annotations with the gene signatures from DMAP through a gene set enrichment analysis which is visualized through a heatmap (right). **B:** The mRNA of IL17RA, IL17RC, and Traf3ip2 (ACT-1) were found in mouse bone marrow hematopoietic stem and progenitor cells (HSPCs), as well as up-regulation of

IL17A-signalling signatures when evaluated by Ucell enrichment analysis. **C:** Western blot analysis was performed in mouse Lin^- bone marrow cell fraction, and **D:** immunofluorescence coupled with confocal microscopy on LSK-sorted cells. Flow cytometry analysis assessing the expression of (**E:**) IL17RA and (**G:**) IL17RC in LSK and LSK subsets (**F** and **H**). The mean fluorescent intensity was defined using the median and compared the values from the primary-labeled antibody vs its isotype control in each case using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

Having detected IL17R expression in LSK at the mRNA and protein levels, we evaluated if LSK were able to uptake IL17A during *ex vivo* supplementation, as a readout of the functional capability of the IL17A receptor, as reported in the literature (333). To carry out this experiment, we depleted lineage- cells from 4 to 13 young mouse donors. Cells were incubated in PBS at 37 degrees for 60 minutes in the presence or absence of IL17A as described in materials and methods.

The analysis shows that under IL17A stimulation, the frequency of progenitors positive for IL17RA decreased significantly compared with untreated cells and that almost 96 % of the progenitors treated with IL17A could internalize the IL17A (**Figure 9A**). We were able to validate these findings using MFI (**Figure 9B**). We also found that the decrease in the frequency of IL17RA-positive progenitors was due to a decrease in the extracellular IL17RA expression (**Figure 9B**). It is expected that IL17RA is internalized during IL17A supplementation (333) but we did not find an increase in the intracellular fraction of IL17RA in the treated cells. This could be related to an acceleration in its degradation by the proteasome in response to high concentration of the ligand (273,334,335). Under the same experimental design, future experiments could block the proteasome with MG132 to evaluate the IL17RA levels to determine if this is related to an acceleration in its degradation by the proteasome in response to the high concentration of the ligand rather than due to reduced production (288,349,350).

To gain insight into the signalling pathways activated by IL17A, we used flow cytometry and specific phospho antibodies to screen for changes in phosphorylation of downstream targets described for IL17 signalling in other cell types and those that were compatible with the IL17-signalling signature employed in our analysis (**FOS (AP.1)**, **IKBKB (IKß)**, **IL17A**, **IL17RA**, **IL17RC**, **JUN (C-Jun)**, **MAP2K1 (ERK Activator Kinase 1)**, **MAP2K4 (JNK-Activating Kinase)**, **MAP2K7 (C-Jun N-Terminal Kinase Kinase 2)**, **MAP3K7 (TAK1)**, **MAPK1 (ERK1/2)**, **MAPK10 (C-Jun N-Terminal Kinase 3)**, **MAPK11(P38ß)**, **MAPK14 (P38α)**, **MAPK3 (ERK1)**, **MAPK8 (C-Jun N-Terminal Kinase 1)**, **MAPK9 (C-Jun N-Terminal Kinase 2)**, **RPS6KA1 (S61)**, **RPS6KA2 (S62)**, **RPS6KA3 (S63)**, **RPS6KA5 (S65)**, **TAB1 (TAK1-Binding Protein 1)**, **TAB2 (TAK1-Binding Protein 2)**, **TAB3 (TAK1-Binding Protein 3)**, **TRAF6**).

Bone marrow samples collected from 4-5 young mice were stimulated for 30 min in the presence or absence of 500ng/ml of rm-IL17A. After 30 minutes of incubation, the cells were washed, fixed, and stained. In addition, to understand if the IL17A effect is direct or indirect, we blocked the

cytokine secretion by adding monensin (GolgiStop). This reagent blocks the Golgi transporter reducing the ability of cells to secrete cytokines (333).

The results show an increase in phosphorylation of P38, cJNK, IKßα, and ERK1/2 after IL17A treatment (**Figure 9C**: bars black and red). However, the effect of IL17A on P38, cJNK, IKßα, and ERK1/2 phosphorylation may be direct through IL17RA activation or indirect through IL17A-mediated secretion of other cytokines on terminally differentiated cells.

Interestingly, in the presence of monensin, IL17A failed to affect significantly ERK1/2 and Ikßα (**Figure 9C** bars green and blue), suggesting that these intracellular signalling pathways are secondary to the secretion of other cytokines, whereas P38 and cJNK are direct effects.

To gain insight into the signalling pathways activated by IL17A in bone marrow progenitors and how the terminal differentiated cells could affect the phosphorylation patterns on progenitors in response to IL17A supplementation, we depleted all the terminally differentiated cells as well as erythrocytes as commented before. A representative quality control assay of Lineage- purity is shown in **Figure 9D** where only 1.6% of immunocompetent cells are detected (Figure 9D). These cells were treated with vehicle or 500ng/ml IL17A for 30 minutes followed by western blot analysis. Representative results of 3 independent experiments in **Figure 9E**, in which we observed significant increases in the phosphorylated forms of P38 and C/EBPß-LAP. Interestingly, these results were consistent with the flow experiments in which no increase in ERK1/2 and IKßα phosphorylation was observed. Importantly, TARF6 which is part of the IL17A signaling pathway, as it binds to ACT-1 to form the complex that activates the downstream pathways, also showed a significant increase. These results suggest that IL17A in mouse progenitors (**Figure 9F**) might affect myeloid differentiation.

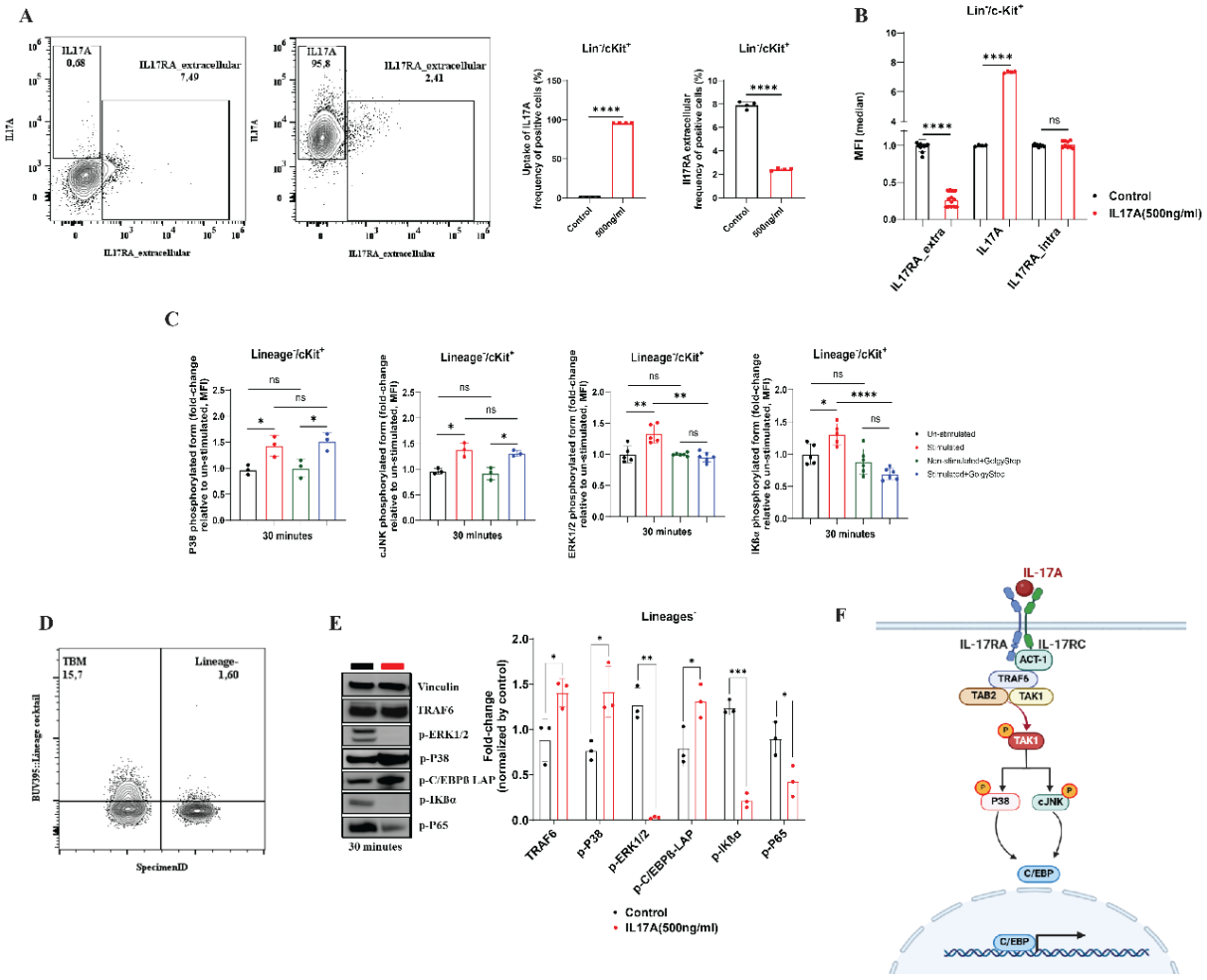

Figure 9. IL17A supplementation increases the proliferation of LSK through the phosphorylation of P38/cJN. **A:** A representative experiment of flow cytometry analysis representing the changes in the percentage of cells positives for IL17A (bar graph 1) and for IL17RA (bar graph 2) in response to IL17A *ex vivo* stimulation (500 ng/ml). **B:** MFI (median) of IL17A, IL17RA_extracellular fraction, and IL17RA_intracelular fraction normalized by fold change relative to the control. Each dot represents one donor sample. **C:** The graphs represent changes in the phosphorylation of P38, cJNK, ERK1/2, and IKßα as indicated obtained by flow cytometry after 30 minutes of incubation with or without 500ng of IL17A total bone marrow cells, without GolgiStop (bars black and red) and with GolgiStop (bars green and blue). **D:** Quality control of the Lin$^-$ fraction depletion process done by magnetic isolation and evaluated by flow cytometry. **E:** Changes in the phosphorylation status of ERK1/2, P38, C/EBPß-LAP, IKßα and P65 and expression levels of TRAF6 were analyzed by western blot analysis after *ex vivo* stimulation without IL17A (black) or with 500ng/ml of IL17A (red) for 30 minutes. Equal loading was assessed by reproving with an anti-vinculin antibody. **F:** Our proposed model of the IL17A signalling pathway in mouse HSPCs. The mean fluorescent intensity was defined using the median and compared using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

4.4 IL17A *ex vivo* supplementation increases expansion of MPP3- and MPP4-like cells and promotes monocytic differentiation affecting hematopoietic repopulation *in vivo*

Since IL17A seems to promote C/EBPß phosphorylation on HSPCs, and this transcription factor is associated with monocytic differentiation, we evaluated the effects of IL17A on proliferation, a common effect of cytokines promoting myeloid differentiation (157). To this end, we sorted LSK cells in a serum-free medium with a cocktail of cytokines that promote different myeloid phenotypes (157) to explore which lineage will be affected during *ex vivo* IL17A supplementation. After incubation for 4-8 days, we performed flow cytometry analysis using the markers that characterize each subset of LSK (Figure 10A-B).

First, we observed an increase in the frequency of LSK in response to different doses of IL17A (**Figure 10C**). In contrast, more differentiated progenitors such as the MEP and GMP fractions showed a significant decrease, more evident in the MEP fraction (Figure 10C).

Next, we tested the total number of cells within the well and found a significant increase in the total cell number after 4 days of incubation in the presence of IL17A, but this effect was not maintained over time (Figure 10D). We confirm that this increase at day 4 was due to an increase in proliferation as evaluated by EdU incorporation (Figure 10E).

Taking these results into consideration, we hypothesized that the increases in the LSK was due to an increase in some specific subsets (Figure 10F). To identify which subsets of LSK were increasing under IL17A *ex vivo* stimulation, we repeated the experimental procedure evaluating this time the absolute number of cells. Here we corroborate the expansion effect on LSK in both time points (4 and 8 days), and this expansion was accompanied by an increase in the absolute numbers of MPP3 and MPP4-like cells. Then, we evaluated if this expansion in MPP3 and MPP4-like cells could be obtained by sorting MPP3 and MPP4, to investigate if this effect was related to an expansion associated with these specific phenotypes or for differentiation of other LSK subsets such as LT-HSC into MPP3 and MPP4 like cells. We found that whether MPP3 or MPP4 cultured independently in the presence of IL17A can proliferate and increase the total number of cells (**Figure 10H-I**). Nevertheless, a maturation effect was observed in these cells when we looked for Lin-/cKit+/Sca1-/CD34-/CD16/32+ fraction (**Figure 10J**).

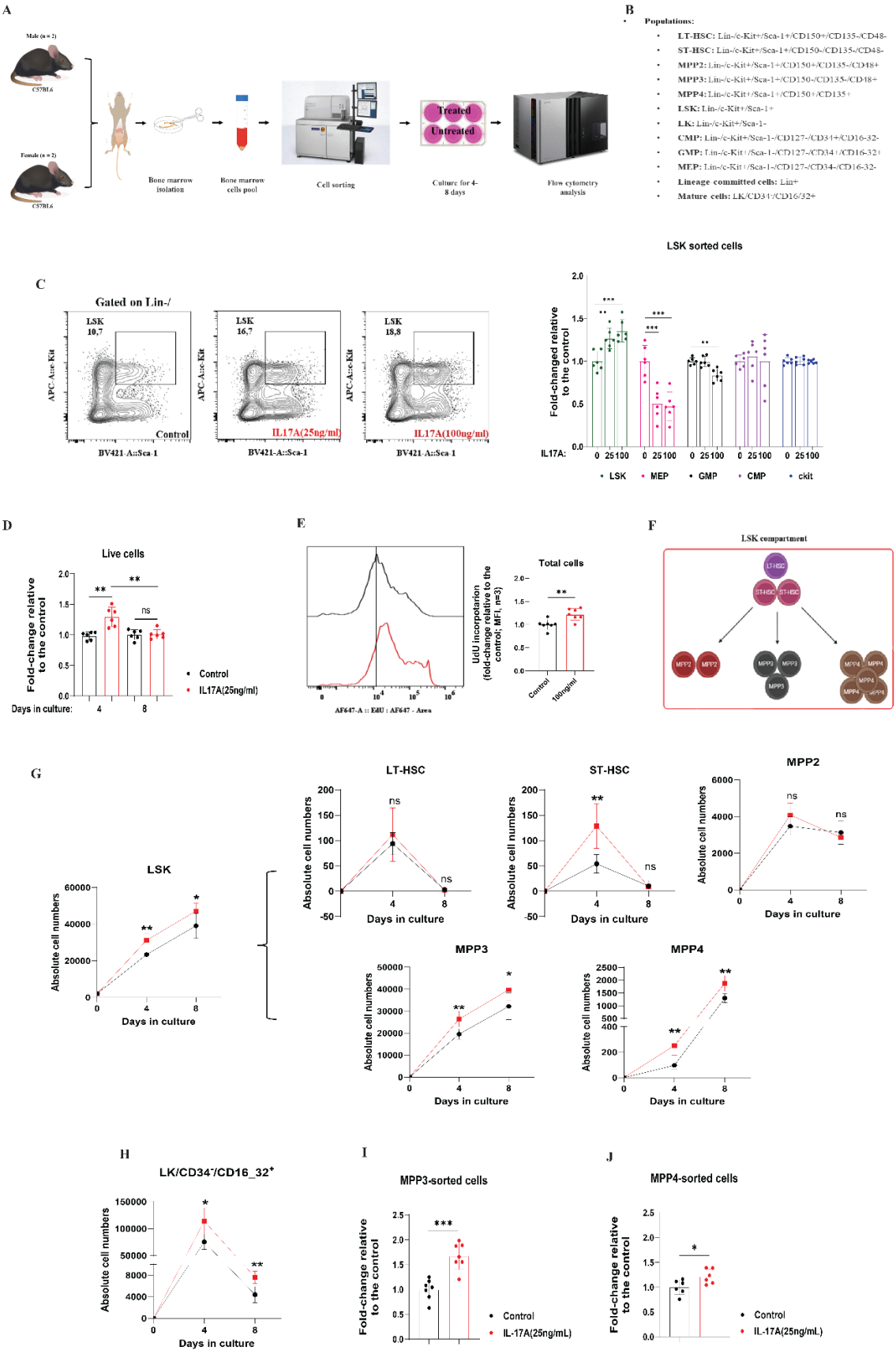
A
Male (n = 2)
C57BL6
Female (n = 2)
C57BL6
Bone marrow isolation
Bone marrow cells pool
Cell sorting
Treated
Untreated
Culture for 4-8 days
Flow cytometry analysis
B
Populations:
LT-HSC: Lin-/c-Kit+/Sca-1+/CD150+/CD135-/CD48-
ST-HSC: Lin-/c-Kit+/Sca-1+/CD150-/CD135-/CD48-
MPP2: Lin-/c-Kit+/Sca-1+/CD150+/CD135-/CD48+
MPP3: Lin-/c-Kit+/Sca-1+/CD150-/CD135-/CD48+
MPP4: Lin-/c-Kit+/Sca-1+/CD150+/CD135+
LSK: Lin-/c-Kit+/Sca-1+
LK: Lin-/c-Kit+/Sca-1-
CMP: Lin-/c-Kit+/Sca-1-/CD127-/CD34+/CD16-32-
GMP: Lin-/c-Kit+/Sca-1-/CD127-/CD34+/CD16-32+
MEP: Lin-/c-Kit+/Sca-1-/CD127-/CD34-/CD16-32-
Lineage committed cells: Lin+
Mature cells: LK/CD34+/CD16/32+
C
Gated on Lin-/
LSK 10,7
LSK 16,7
LSK 18,8
APC-Acc-Kit
BV421-A::Sca-1
Control
IL17A(25ng/ml)
IL17A(100ng/ml)
LSK sorted cells
Fold-changed relative to the control
IL17A:
0 25 100
LSK
MEP
GMP
CMP
ckit
D
Live cells
Fold-change relative to the control
Days in culture:
Control
IL17A(25ng/ml)
E
AF647-A :: EdU : AF647 - Area
UdU incorporation (fold-change relative to the control; MFI, n=3)
Total cells
Control
100ng/ml
F
LSK compartment
LT-HSC
ST-HSC
MPP2
MPP3
MPP4
G
LSK
LT-HSC
ST-HSC
MPP2
MPP3
MPP4
Absolute cell numbers
Days in culture
H
LK/CD34-/CD16_32+
I
MPP3-sorted cells
Fold-change relative to the control
Control
IL-17A(25ng/mL)
J
MPP4-sorted cells
Fold-change relative to the control
Control
IL-17A(25ng/mL)

Figure 10. IL17A supplementation expands MPP3- and MPP4 like cells *in vitro*. **A:** Experiment design for mouse LSK and LKS subsets isolation and liquid culture analysis. **B:** Established surface markers utilized to define LSK and LSK subsets **C:** Mouse LSK sorted in liquid culture (StemPro, SCF, FLT3L, TPO, EPO, IL11, IL3, IL6, GM-CSF) analyzed on day 4th of culture showed an increase in the frequency of LSK in response to IL17A (left) and reduction in MEP and GMP (right). **D:** LSK sorted cell cultures for 4 and 8 days in the absence (vehicle control) or presence of 25 mg/ml IL17A, the changes in the relative number of total live cells were assessed by flow cytometry. **E:** As described in materials and methods, the EdU incorporation assay by LSK cells was performed on day 4 after vehicle (control) or 100ng IL17A treatment. **F:** Schematic representation of the hierarchical organization of LSK subsets. **G:** Kinetic-growing analysis on LSK-sorted cells in response to IL17A (25ng/ml). After sorting LSK, the LSK fraction and the LT-HSC, ST-HSC, MPP2, MPP3, and MPP4 fractions were evaluated using flow cytometry, and the absolute numbers were compared. **H:** The kinetic of the myeloid maturation (Lin-/Sca1-/cKit+/CD34-/CD16/32+) was assessed on sorted LSK cells. **I:** MPP3 and **J:** MPP4 were isolated from mouse bone marrow using the same procedure as sections A and B, then were cultured over 8 days in the absence (control) or presence of 25ng/ml IL17A. MPP3 and MPP4 were sorted, cultured, and evaluated independently; total live cells were scored using flow cytometry. Fold change relative to the control and absolute numbers were compared using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

Because IL17A seems to bias hematopoiesis towards myeloid maturation, we assessed for changes in the frequency of myeloid cells ($CD11b^+/CD45^+$) after 4 days of treatment with 25 or 100 ng/ml IL17A to control. The results indicated that IL17A treated-LSK cells differentiated into myeloid cells since the treatment resulted in a significant increase in the $CD45^+/CD11b^+$ fraction (Figure 11A, top). Additionally, we identified that monocytes were the main subset of myeloid cells affected by IL17A (Figure 11A, left). A differentiation-kinetic analysis shows the persistence of this trend on day 8 of culture comparing absolute cell numbers (Figure 11B). Then, to investigate whether this monocytic differentiation effect is exclusive of LSk as a stem cell compartment or is visible also in each LSK subset, we sorted LT-HSC, ST-HSC, MPP2, MPP3, and MPP4 and kept them in culture under the same experimental condition until day 8. The results showed that IL127A *ex vivo* stimulation promotes monocytic differentiation in LSK and LSK subsets (Figure 11B and C). An additional validation was performed using LSK cultured in the same condition (StemPro, SCF, FLT3L, TPO, EPO, IL11, IL3, IL6, GM-CSF), but without GM-CSF to assess if the monocytic differentiation effect observed under IL17A treatment, could be related to a synergistic effect between IL17A and GM-CSF since GM-CSF is the major monocytic/granulocytic differentiator cytokine (336). Here, we show the myeloid maturation and monocytic differentiation potentials of LSK under IL17A treatment in the absence of GM-SCF (Figure 11D).

Having established the role of IL17A on myeloid/monocytic differentiation *ex vivo*, the relevance of this effect was assessed *in vivo*. To this end, we isolated LSK from pure C57/BL6 mice (CD45.2) using cell sorting; then we seeded 2,000 LSK per well in a 24-well plate with the same media and cytokine cocktail (StemPro, SCF, FLT3L, TPO, EPO, IL11, IL3, IL6, GM-CSF) and we cultured the cells for 4 days in the absence or presence of IL17A (100ng/ml IL17A) After *ex vivo* incubation, the cells were then transplanted into lethally irradiated C57BL6 hybrid (CD45.1/2) mice. At the same time, "rescue" bone marrow from C57BL6-STEM (CD45.1) donor

mice was co-transplanted to support the viability of the recipient mice while ensuring the discrimination of hematopoietic cells from the two donor mice (Figure 11E). Two independent experiments showed a significant decrease in the fraction of total blood cells one month after transplantation in mice that received IL17A-treated cells to mice that received non-IL17A stimulated cells (Figure 11F). This result together with the *in vitro* IL7A results, suggests that *ex vivo* IL17A-driven differentiation of LSK and LSK subsets depletes progenitor cell subpopulations debilitating hematopoietic repopulation in transplanted mice.

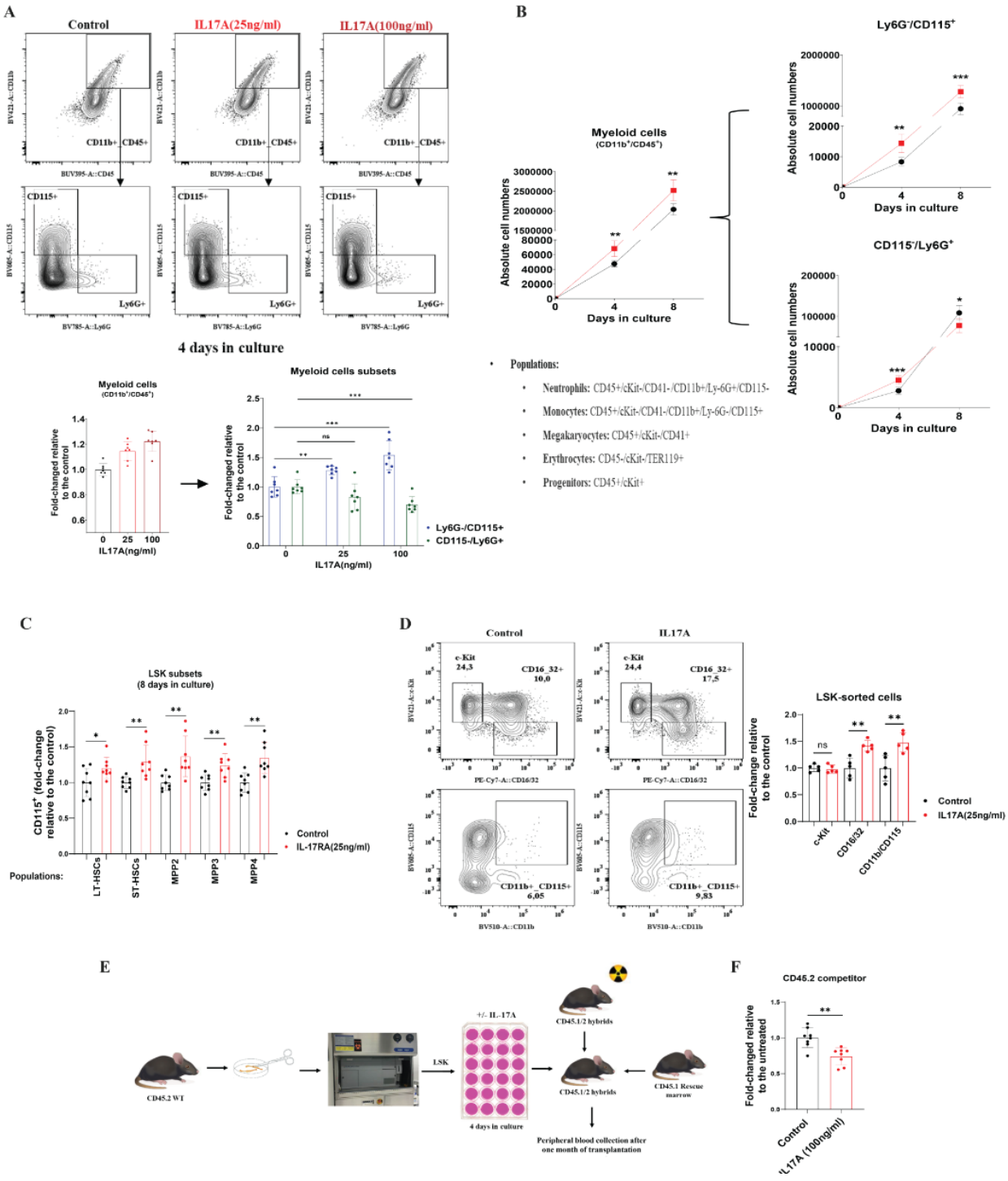

Figure 11. IL17A *ex vivo* supplementation promotes monocytic differentiation on LSK maintained in liquid culture. **A:** Mouse LSK sorted in liquid culture (StemPro, SCF, FLT3L, TPO, EPO, IL11, IL3, IL6, GM-CSF) analyzed by flow cytometry on day 4th of culture. CD45+/CD11b+ myeloid compartment (top) and monocyte and granulocyte subsets (button). **B:** Kinetic-differentiation analysis on mouse LSK-sorted cells in response to IL17A (25ng/ml) evaluated on days 4 and 8. **C:** LT-HSC, ST-HSC, MPP2, MPP3, and MPP4 sorted cells into the same media recipe and cultured for 8 days in the presence or absence of IL17A. Monocytic analysis by flow cytometry was performed.

D: Mouse LSK was sorted and cultured for 8 days using the same recipe except GM-CSF. Flow cytometry analysis was performed. **E:** *Ex vivo* treatment of mouse LSK-sorted cells in the presence or absence of IL17A for 4 days, then transplanted into lethally irradiated hybrid CD45.1/2 mice. **F:** After 1 month of transplant, the frequency of mature (blood) CD45.2 cells derived from the LSK *ex vivo* treated either with control or IL17A, were compared (of total IL17A treated LSK CD45.2 vs control treated LSK CD45.2). Fold change relative to the control and absolute numbers were compared using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

To continue validating the role of IL17A on hematopoiesis, we utilized a well-established, commercially available myeloid colony formation assay from Stem Cell Technologies (MethoCult™ M3434 Methylcellulose-Based Medium (Mouse) | STEMCELL Technologies) that enables the identification of colony-forming units derived from each myeloid population as described in Figure 12A. This semisolid media provides a good platform to validate our finding that IL17A itself can promote monocytic differentiation.

Isolated mouse LSK cells (Figure 12B) were seeded at 100 LSK/1.5 ml density in a 6 well plates M3434 medium (FBS, IL6, IL3, and Epo) and maintained in culture for 10 days in the presence or absence of 100ng/ml of IL17A. Analysis of the results of this experiment shows that IL17A significantly enhances the clonogenic capacity of monocytic colony-forming units while seeming to not affect granulocyte colony-forming units (Figure 12C), suggesting a monocytic biased output within the progenitors corroborating our previous results.

Next, we designed a sequential experiment incorporating our liquid culture platform and the colony formation assay (M3434) to provide more evidence for the effect of IL17A alone on LSK differentiation (Figure 12D). Briefly, 500 LSK isolated cells were plated in serum-free media supplemented only with SCF in the presence or absence of 100ng/ml of IL17A and maintained in culture for 24 hours. Following 24 hours of incubation, cells were washed and plated in fresh M3434 for 10 days without IL17A supplementation. Results indicate a significant increase in the clonogenic capacity of monocyte colony-forming units with a significant reduction in granulocytic colony formation (Figure 12E), suggesting that IL17A alone can bias LSK differentiation under these experimental conditions. Subsequently, cells from individual wells were mechanically dissociated, washed, and stained for flow cytometry analysis. The results in Figure 12F show an increase in the expression of F4/80 which is a pan-macrophage marker, suggesting a monocyte-macrophage maturation.

On the other hand, because our initial screening showed a significantly lower expression of IL17RA in the LT-HSC subpopulation depending on the age (**Figure 8I**), we explored whether this differential expression could be biologically relevant. Thus, we compared the ability of LT-HSCs from young donor mice (6-8 weeks old) or aging mice (> 1 year old) to form myeloid colonies after stimulation or not wiht 100ng/ml of IL17A.

The results show a decreased capacity of LT-HSCs from older mice donors to respond to IL17A, with decreased formation of monocytic, granulocytic, and multipotent colonies when compared to young donors (**Figure 12G**). These results highlight the possible relevance of IL17A during hematopoietic aging during different experimental conditions. However, future experiments should be performed to understand this role.

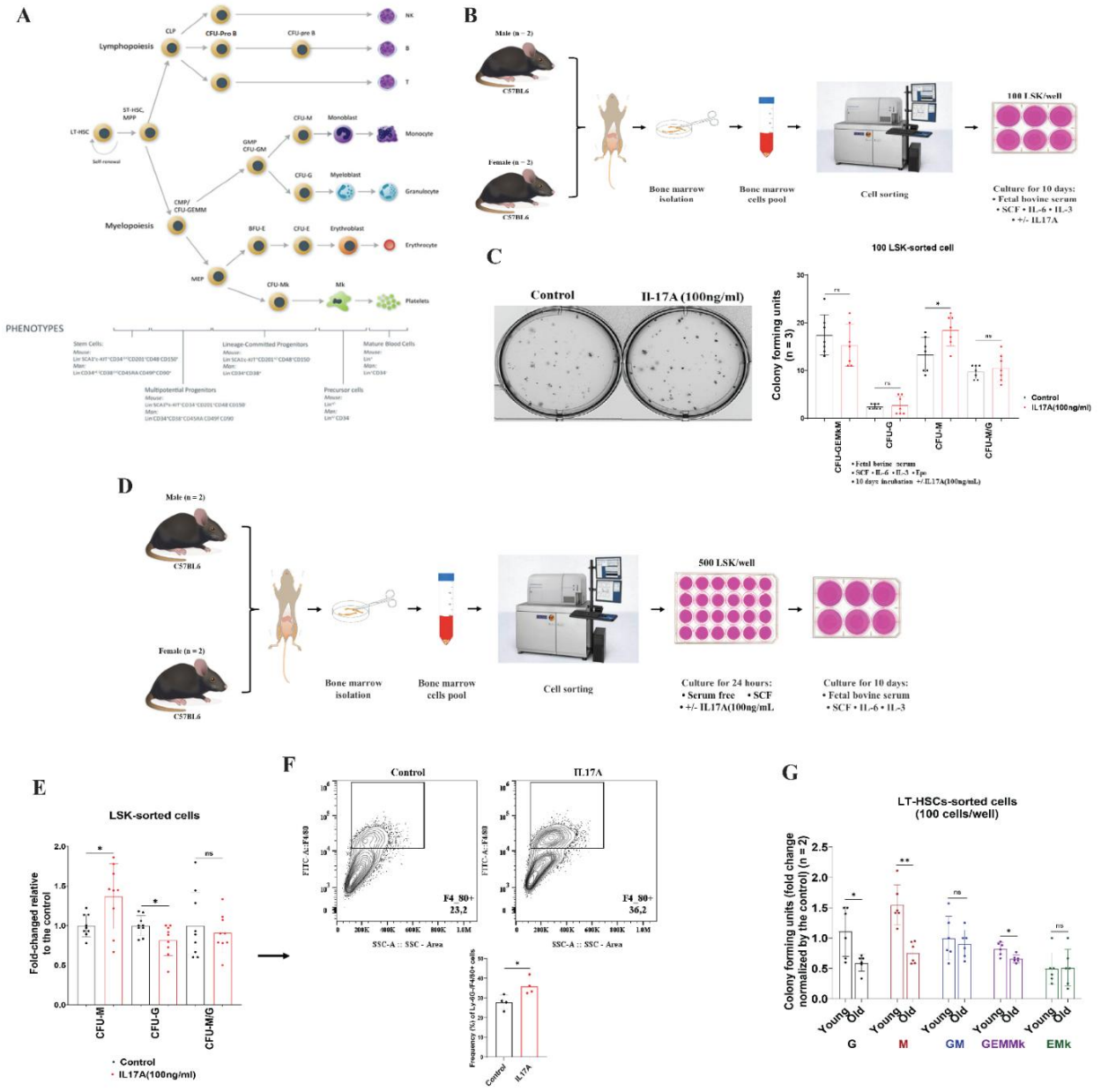

Figure 12. IL17A *ex vivo* supplementation promotes monocytic colony-forming units on LSK-sorted cells. **A:** Hematopoietic tree, phenotypic characterization by flow cytometry and colony formation assay (modified from Stem Cell Technologies). **B:** Single-cell colony-forming assay design (cell sorting performed at single-cell purity). **C:** Single-cell colony-forming assay performed in M3434 with mouse LSK-sorted cells in the presence or absence of

100ng/ml of IL17A. **D:** LSK sorted at single-cell purity on liquid culture serum-free media supplemented with stem cell factor in the presence or absence of 100ng/ml of IL17A. After 24 hours of incubation cells were washed and transplanted into M3434 IL17A-free. **E:** Colony scored after 10 days in culture. **F:** The content of each well was collected and washed after scoring the number of colonies, and then the cells were stained and analyzed by flow cytometry. **G:** Comparative study through single-cell colony-forming assay, between LSK obtained from young (6 – 8 weeks) and aged mice (more than 1 year). Each value represented was obtained after normalizing each treatment with its respective control in young and old (fold change). Fold change relative to the control and absolute numbers were compared using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

4.5 In mice, IL17A is produced by immune cells within the bone marrow

Finally, we questioned the possible origin of IL17A in bone marrow. We hypothesized that IL17A could be produced in the bone marrow by immune cells. We collected bone marrow from 10 mice between 6 and 8 weeks. After removing the red blood cells (**Figure 13A**), we characterized by flow cytometry the presence of immune cell populations as schematically shown in **Figure 13B**. Among the immune populations identified in the bone marrow, a small fraction of cells expressed the RORyt transcription factor, including Th17 and ILC3_NCR+ (**Figure 13C**) and a larger fraction of immune cells did not express RORyt, such as macrophages, neutrophils, and B cells

Considering these differences and the fact that RORyt populations have been linked to IL17A production, including NK, CD8, and B cells, we investigated the ability of these populations to produce IL17A when activated. To this end, we repeated the procedure depicted in Figure 13A, where bone marrow samples were split into equivalent aliquots treated with vehicle or stimulated with ionomycin + phorbol, respectively. To ensure the possibility of observing IL17A inside the cells, we blocked both sample fractions with monensin, a Golgi complex transport blocker (**Figure 13D**). Interestingly, all immune cells identified in our screening were able to produce IL17A (**Figure 13E**) when stimulated, in agreement with published findings (291,292,337).

The novelty of our results is that they suggest the ability of bone marrow B cells and myeloid cells to produce IL17A in the *same* compartment as susceptible LSK expressing the IL17RA. Additionally, our work indicates that within the bone marrow, there are ILC3_NCR+ capable of producing IL17A. Interestingly, these cells are the innate version of the Th17 (338). Since these cells are produced in the bone marrow and because they do not need training in the thymus (338), the IL17A axis could be controlled by these cells under physiological conditions but during immune stress, the axis seems to be activated by all the cells with immune capacity. However, future experiments must be performed to understand the contribution of each one of those cells to IL17A signalling during physiological conditions.

To strengthen our hypothesis that IL17A is a component of the bone marrow microenvironment we harvested peripheral blood and bone marrow plasma from young mice (**Figure 13F**) and

compared IL17A levels by ELISA. The results of the ELISA in 23 young mice donors showed that the mean levels of IL17A in bone marrow plasma were almost twice the mean levels of IL17A in the blood (**Figure 13G**).

Suggesting that, at least in the absence of immune activation, the bone marrow is a significant source of IL17A in the hematopoietic system. Furthermore, we also investigated if the levels of IL17A within the bone marrow could change with age. For that, we compared IL17A levels in the bone marrow plasma in aged mice (>1 year of age) and young mice (between 6-8 weeks old) utilizing the same approach. We found that IL17A levels in the bone marrow of elderly mice were significantly higher than young mice (**Figure 13H**), in line with previous reports (228,229). Further studies will be required to find if there is a causal connection between the reduction in the expression of IL17RA in LT-HSCs, the increases in the levels of IL17A within the bone marrow microenvironment, and the poor response of LSK to IL17A *ex vivo*.

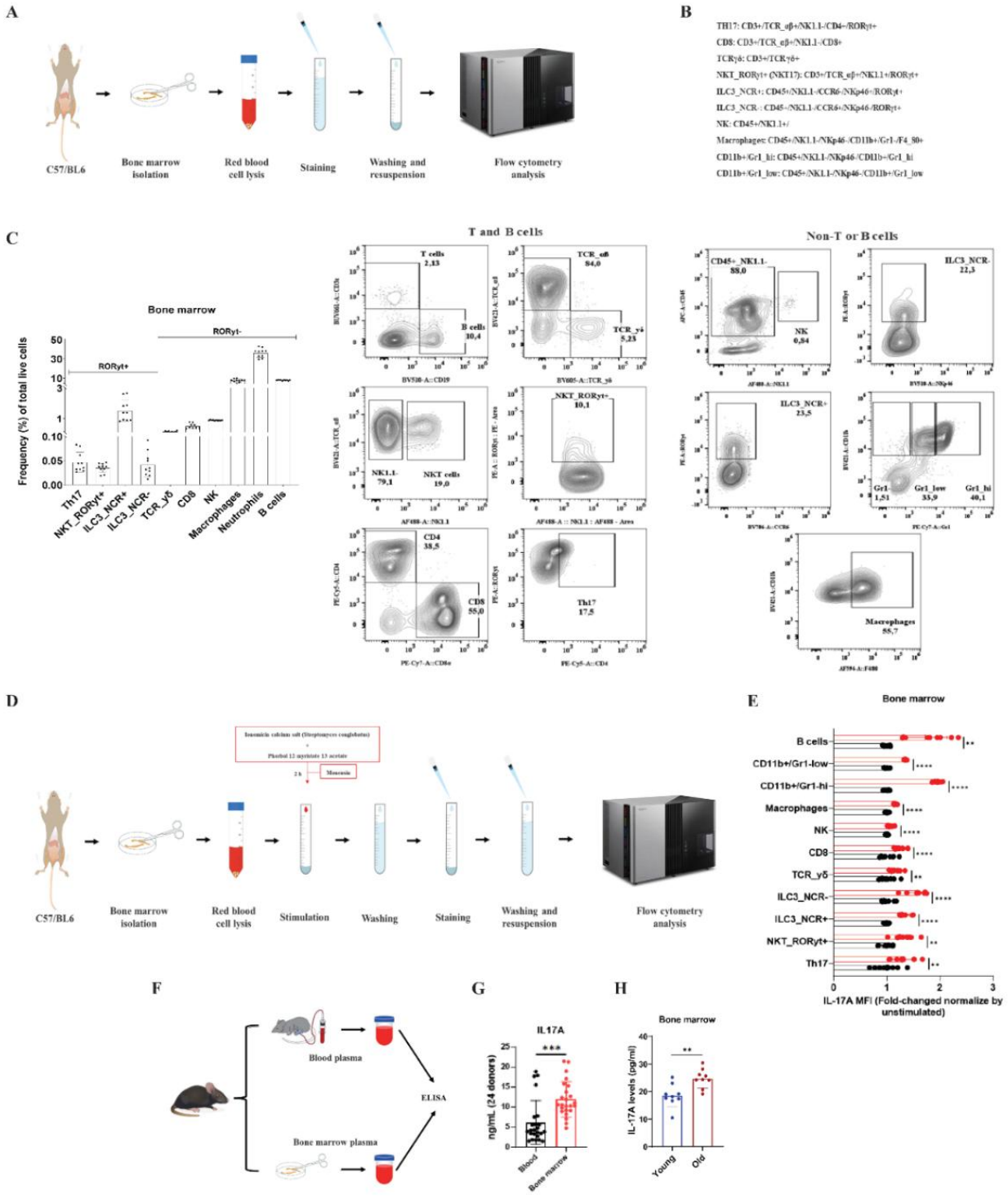

Figure 13. IL17A is present in the bone marrow microenvironment and is produced by cells of the immune system. A: Experiment design to map the immune landscape of mone marrow. **B:** Definition of each population based on relevant markers. **C:** Frequency of each immune cell population identified in the mouse bone marrow samples. **D:** *Ex vivo* stimulation experiment design on mouse total bone marrow cells and **E:** its results. **F:** Experimental protocol for blood and bone marrow plasma collection to perform an ELISA and **G:** its results. **H:** Levels of IL17A in young

and old mice after ELISA quantification. Fold change relative to the control and absolute numbers were compared using a two-tailed unpaired t-test with Welch's correction. P values lower than 0.05 were considered statistically significant ($p \geq 0.05$ → **ns**; $p < 0.05$ → *; $p \leq 0.01$ → **; $p \leq 0.001$ → ***; $p \leq 0.0001$ → ****).

5. Discussion

Hematopoiesis is highly sensitive to changes occurring not only in the bone marrow microenvironment but also in the entire body. Under basal or homeostatic conditions, the pool of HSC with self-renewal capacity mainly remains quiescent. However, during inflammatory events, there is a behavioural shift toward differentiation and the production of lineage-biased progenitors (354) in response to cytokines which exert an effect on the HSC fraction, effectively altering its transcriptional program and redirecting the cells toward a new destiny, including toward neutrophil and monocyte-macrophage lineage commitment (43,354). This is vital in immune defence since these cells are the first to invade infectious sites (355,356).

However, many factors affect the proper functioning of the hematopoietic system, among which inflammation (357,358) and aging (231,359) stand out. Both inflammation and aging of the hematopoietic system are associated with a decline in hematopoietic function and an increased predisposition to cancer (231,354,355,359). The hematopoietic system responds to inflammation with an increase in the production of immune cells, myeloid and monocytic cells in particular??, at the expense of self-renewal within the bone marrow (354). During aging, there is a marked myeloid-biased output that sacrifices the lymphoid component (199,360). Among other factors, cytokines are important mediators of these phenotypes (357,358), with variation in cytokines being observed during aging and inflammation (49,50).

Given the relevance of myeloid phenotypes in aging (231), inflammation (356), and also cancer (231,355,360), cytokines that have a myelopoietic effect are of special interest. Cytokines with classic myeloid potential, such as GM-CSF (361,362) or not so-classic potential, such as IL1 (354) or IL4, (355) and their signalling axes both within and outside the bone marrow microenvironment have been associated with tumour progression in the context of inflammation and cancer (354,355,361,362). GM-CSF, IL1 and IL4 mediate colon and lung tumour progression through the generation of myeloid phenotypes with tolerogenic capacities within the bone marrow (231,355). Additionally, in aging and inflammation, the levels of these cytokines remain elevated, prolonging their hematopoietic effect and increasing tumour progression (231).

IL17A is a proinflammatory cytokine, which has a marked role in inflammatory and autoimmune diseases (258-260,363). Additionally, IL17A has been assigned a granulopoetic role mainly in bone marrow-derived progenitors (175,318,321). However, IL17A has been identified as a neutrophil recruiter during inflammation (364,365) and within the tumour microenvironment (366). In these cases, IL17A is secreted by immune cells such as Th17 (260,363), stimulating microenvironmental cells such as stromal cells (321) to produce IL6 (363), IL1ß (327), GM-CSF (331), and IL8 (367), the latter being involved in the recruitment of neutrophils (364-366). Despite these elements, little or nothing is known about which populations of bone marrow-derived progenitors respond best to IL17A and what its effect is on these target cells.

Our transcriptomic expression analysis in public scRNA sequencing databases revealed the expression of the transcripts of both IL17A receptor chains in both HSCs and in more lineage-committed progenitors such as GMP in humans and mice. However, computational analyses based on RNA, although an important tool research tool, do not necessarily provide solid evidence. This is mainly due to factors associated not only with the extraction (low cell viability, improper cell numbers, loss of material, etc) and processing technique (data standardization, arbitrary methodologies, low RNA input, amplification bias, batch effect, etc) but rather with biological characteristics such as the affinity of the RNAs for the transcriptional machinery which mean that the amount of RNA expression not necessary means high translation into a functional protein. Therefore, to validate our sc-RNA seq findings, we performed a series of experiments that examined RNA-protein-downstream pathway functions.

We first examined IL17RA and IL17RC expression using immunoblotting techniques. We show that Il17RA and IL17RC had a similar expression on bone marrow progenitors than vinculin. However, one of the main weaknesses of our strategy and results was the absence of a knockout animal model or cell line for the proteins of interest. Therefore, we normalized the proteins of interest, IL17RA and IL17RC, to values of a well-characterized endogenous protein, vinculin (368), showing similar levels of expression. We also employed indirect immunofluorescence coupled with confocal microscopy to identify cellular subsets containing the IL17 receptor. In this case, we used an antibody from clones different from those used in the western blot, which could recognize the protein of interest in its natural state and conformation. We observed the coexpression of both IL17RA and its intracellular adaptor ACT-1 using this technique. Without the KO model, we cannot be sure that the staining is recognizing only the protein. A KO model could help to distinguish if the signal is affected by unspecific bindings.

Most convincingly, our flow cytometry results using the specific antibody for IL17RA and RC and its respective isotype control, we were able to corroborate that all the subpopulations that make up the LSK compartment (Lin-/cKit+/Sca1+) express both IL17RA and IL17RC. This expression is functional, given the capacity of these populations to internalize the receptor and incorporate the ligand into the intracellular space during *ex vivo* IL17A supplementation, which we demonstrated by *ex vivo* IL17A supplementation experiments on bone marrow progenitors. This is consistent with published reports of the function of this receptor (369). This internalization of IL17A *ex vivo* activated downstream pathways such as P38 and C/EBPß phosphorylation. While it is known that IL17A employs the TAK1/P38 signalling axis in non-bone marrow HSC, our results are novel in their characterization of these signalling pathways employed by IL17A in bone marrow-derived hematopoietic progenitors, specifically the TAK1 and C/EBP-ß pathways.

Because P38/cJNK activation is associated with cell proliferation via mTOR (339) and C/EBPß is a transcription factor involved in proliferation and myeloid differentiation, specifically monocytes and macrophages (340), our functional studies focused on these two aspects.

When the IL-17A receptor is stimulated by its ligand, a conformational change in the intracellular domains is triggered, exposing the SEFIR domain, which rapidly recruits ACT-1 and TRAF6 (341). The formation of this signalosome complex can activate several downstream signalling pathways such as ERK, P38/cJNK and IKßα (273,334,342-344). Once the complex is activated, it is unknown how the cell determines which signalling pathway to use; however, it is known that only one of these pathways is activated, that is, not two at the same time (273,334,342-344).

The TRAF6/ACT-1 complex activates TAK1 (transforming growth factor-β (TGF-β)-activated kinase 1), which is a member of the mitogen-activated protein kinase (MAPK) kinase (MAP3K) family, is one of the upstream activators of P38 and cJNK (345-347). Our results agree with these elements, demonstrating the selective activation of the signalling pathway involving P38/cJNK as well as the downstream transcription factor activated by them, C/EBPß (347).

Although we demonstrated the selective activation of this pathway (no activation of ERK, IKßα or their downstream transcription factors such as P65), we cannot conclude with total certainty that the activation of C/EBPß is exclusively due to the activation of P38 and cJNK. For this, the use of a selective blocker of the pathway involving TAK1-P38/cJNK would have been necessary. With our current results, we cannot answer whether P38/cJNK increases its total protein levels, and translocates into the nucleus to promote inflammatory gene expression, as has been previously reported (348,349) and completes the function of a complementary transcription factor to C/EBPß. Another important question that remains unanswered is the role of C/EBPß. It is known that C/EBPß (350) works with C/EBPα (350,351) and or PU.1 (350,352). The relationship between them determines the final phenotype, which could explain the different myeloid phenotypes attributed to the differentiating potential of IL17A (352,353).

The myeloid compartment encompasses the granulocytic fraction (neutrophils, eosinophils, and basophils), the monocytic fraction (macrophages and dendritic cells), and mast cells (354). This compartment is extensively studied from both hematological and immunological perspectives, with a particular emphasis on neutrophils and monocytes as key lineage representatives. Various transcription factors and signalling pathways play crucial roles in determining lineage decisions between these two cell types. Myeloid lineage specification is predominantly regulated by the C/EBP family and PU.1 transcription factors (355,356). In murine models, elevated levels of PU.1 initially dictate the commitment between lymphoid and myeloid lineages (357,358). Subsequently, C/EBPα, expressed in immature myeloid cells (359), is critical for the transition from common myeloid progenitors (CMP) to granulocyte-monocyte progenitors (GMP) (360). Within myeloblasts, PU.1 and C/EBPα exhibit reciprocal expression inhibition, with a higher C/EBPα to PU.1 ratio favouring granulopoiesis over monopoiesis (361,362). C/EBPα further directs neutrophil differentiation by activating the transcription factor GFI1 (363), whereas PU.1 activates IRF8 (364), KLF4 (365), and EGR2 (362) to facilitate monocytic differentiation.

C/EBPβ was first identified in 1990 as a basic leucine zipper (bZIP) transcription factor that binds to the interleukin 1 (IL-1)-responsive element within the IL-6 promoter, initially referred to as the nuclear factor for IL-6 (NF-IL6) (366). This factor is characterized by significant C-terminal homology with C/EBPα (366). The translation of CEBPB mRNA yields three distinct C/EBPβ isoforms: liver-enriched activating protein (LAP or C/EBPβ2) and liver-enriched inhibitory protein (LIP or C/EBPβ3) (367).

C/EBPβ isoforms are critical in the development of monocytic lineage cells (368,369), significantly influencing the regulation of hematopoiesis (370). Specifically, the liver-activated protein (LAP) isoforms of C/EBPβ have been demonstrated to inhibit cellular proliferation while regulating genes associated with differentiation (371-373). In murine bone marrow-derived progenitors, the absence of C/EBPβ markedly diminishes myelopoiesis (374). Conversely, the loss of C/EBPβ in lineage-committed cells leads to a proliferative state without impairing macrophage functionality (375,376). Before differentiation, monocytic progenitor cells upregulate the expression of C/EBPβ LAP (371), which is also reflected in an increasing LAP/LIP ratio (377). As a transcription factor, C/EBPβ regulates several genes, including CD14 (371), macrophage-2 antigen (Mac-2) (372), Fcγ receptor II (FcγRII) (372), and monocyte-specific esterase (373).

C/EBPβ supports the expression of other genes such as MD-2 (378) and chitotriosidase (CHIT1) (379) by acting as a cofactor for the transcription factor PU.1 (378,379), thereby enhancing its transcriptional capacity. Moreover, the transcription of chicken MER1-repeat-containing imprinted transcript 1 (mim-1), which is positively regulated by C/EBPβ-LAP (380), enhances c-Myb-dependent transcription (381) by inducing chromatin opening, likely through the recruitment of histone acetylating cofactors from the p300/CBP family and or the SWI/SNF chromatin remodelling complex (382,383). The presence of C/EBPβ-LAP, along with the activation of its maturation-associated target genes, is associated with reduced cellular proliferation (384), morphological changes (377,384), and an enhanced antimicrobial capacity (377). Our data is in line with these facts, showing the increment of C/EBPß-LAP and monocytic differentiation potential discussed further below.

We found that culturing LSK with IL17A increased the total cell number as well as EdU incorporation when compared to LSK in the absence of IL17A. We concluded that, under these experimental conditions, IL17A accelerated the proliferation of LSK-sorted cells on day four of culture. This was supported by the increase in the activation of P38 and C/EBPß (370,371). To clearly demonstrate that IL17A stimulation results in the activation of P38, an experiment in which, the P38 signalling pathway is blocked and the total amount of P38 is quantified, are needed.

Despite this, when the different cell populations obtained in response to IL17A were characterized at the marker level using flow cytometry, we observed that after sorting LSK and maintaining them in culture for 4 and 8 days, the fraction of progenitors experienced an increase, specifically the populations that phenotypically look like myeloid-biased progenitors and lymphoid-biased

progenitors. This finding was interesting and novel since proinflammatory cytokines with a myeloid effect do not usually expand MPPs (385,386).

Unfortunately, these results do not allow us to categorize MPP3 and MPP4 as true progenitors. The expression of surface markers is not sufficient to classify these populations when they are cultured in vitro. In this case, functional analysis exploring their potential to generate multiple differentiated phenotypes as well as their genetic signature is the gold standard for the functional characterization of these progenitors (41,43,45,46).

The MPP3 and MPP4 cells not only undergo an expansion but also a reprogramming that redirects their phenotype towards a monocytic potential, based on our flow cytometry results. Although we do not have transcriptomic analyses that allow us to identify a transcriptional change in these cells, our phenotypic analysis showed their monocytic potential when cultured in vitro using different media formulations, including the absence of GM-CSF with its well-established monocytic differentiation potential (50). Furthermore, when these cells were cultured in the presence of IL17A for four days in vitro and then transplanted into lethally irradiated mice, the cells that received treatment with IL17A experienced a decline in total reconstitution compared to untreated cells. That is, cells previously treated with IL17A for four days after one month of transplantation were significantly less successful in repopulating bone marrow. We interpreted this phenomenon as a loss of HSC/self-renewal function and increased reprogramming presumably towards a monocyte lineage commitment. Other authors have reported that during inflammation or exposure of progenitors to proinflammatory cytokines with a myeloid differentiation effect such as IL1ß (354,357) and IL4 (355) progenitor cells lose their self-renewal capacity as well as their pluripotency to facilitate the generation of the phenotypes indicated by the mediators they are signalling (354,357), in line with our findings.

We observed a maturation phenomenon characterized by monocytic differentiation in vitro in response to IL17A exposure and a defect in the repopulation of the bone marrow by the treated cells *in vivo* supporting lineage commitment. However, an *in vivo* differentiation experiment is essential to quantitatively evaluate the final phenotype of these monocytic cells obtained after 4 and 8 days of culture in the presence of IL17A (415).

Another important aspect to consider is that MAPK P38 is a new DNA damage response linked to senescence and the activation of the SASP pattern (239), which includes IL17A (416). Considering our results and the finding that IL17A induces senescence in other cell types (416), we speculate that IL17A could induce senescence in HSCs through P38 activation. This hypothesis could be supported as well by the lack of engraftment capacity of LSK treated with IL17A during ex vivo supplementation. However, further experiments should be conducted in this direction, including those aimed at evaluating senescence markers such as P53 (417), the effect on DNA damage response (214,418,419), and telomere shortening (420) in LSK under IL17A treatment in vitro or evaluating these parameters in vivo using an IL17A-KO model.

We show that IL17A affects hematopoietic cells by committing them to a monocytic phenotype and impairing their self-renewal capacity. We were also interested in knowing the levels of IL17A in the bone marrow and the cells that produce it in both basal conditions and conditions of stress, as well as the origin of IL17A in the marrow. We found that IL17A is present in bone marrow at levels higher than those in blood, suggesting a local production in bone marrow. We also showed that a variety of cells in bone marrow is capable of producing IL17A during *ex vivo* stimulation in contrast to the basal state when only 0.6% of cells seem to express IL17A. However, we do not have experiments to evaluate the kinetics of IL17A secretion under basal conditions in the bone marrow microenvironment nor the cells responsible for maintaining the signal under these basal conditions.

In the context of B-ALL, the Th17 population in bone marrow increases due to the increased expression of CXCL16, which is a Th17 recruiting factor (334,335). During aging there is also an increase in factors that promote Th17 maturation such as IL1, IL6 and TGF-ß which are key regulators of hematopoietic aging (421), as well as CXCL16 (243). Our quantification of IL17A showed that aged mice had higher levels of IL17A in the bone marrow compared to young mice, as well as a decrease in IL17RA expression by progenitors, which could be associated with a hyper signalling phenomenon as has been reported for this cytokine (243,244).

Our computational results showed that the IL17 signalling pathway is relevant during inflammation and undergoes certain age-related changes. This is not surprising considering that IL17A is a proinflammatory cytokine (258-260,363) and that aging is considered from an immunological point of view as a chronic low-grade inflammatory disease (422). Our results do not answer primordial? questions regarding the role of IL17A in hematopoiesis, including 1) What is the cause and effect of the downregulation of the IL17A signalling pathway associated with the age and the increments in its levels in blood and bone marrow? 2) Is the IL17A signalling pathway in HSC necessary for normal hematopoiesis or only pertinent during inflammation? 3) Which progenitors require IL17A to complete their maturation, and which are increased by its absence? 4) What changes occur in the bone marrow microenvironment that affect IL17A levels and what impact does this have on hematopoietic output?

The success of monoclonal therapies approved for use in humans as a treatment for autoimmune diseases such as psoriasis and IBD? may extend beyond the inhibitory effect on IL17A at the tissue level and may impact on hematopoiesis. The data generated from our experiments begins to elucidate the role of IL17A signalling in the bone marrow compartment and the effects of inflammation and aging on this pathway within the marrow space (423).

6. Conclusions

Overall, our results suggest that IL17A is produced in the bone marrow microenvironment by non-hematopoietic cells, primarily by ILC type 3, B cells and myeloid cells.

Once IL17A binds to its receptor expressed on LSK, it leads to downstream activation of P38-cJNK and C/EBPß (**Figure 14A**). Additionally, in myeloid differentiation conditions, IL17A *ex vivo* supplementation signals LSK progenitors expanding MPP3- and MPP4-like progenitors and reprograming them to monocyte-biased output (**Figure 14B**).

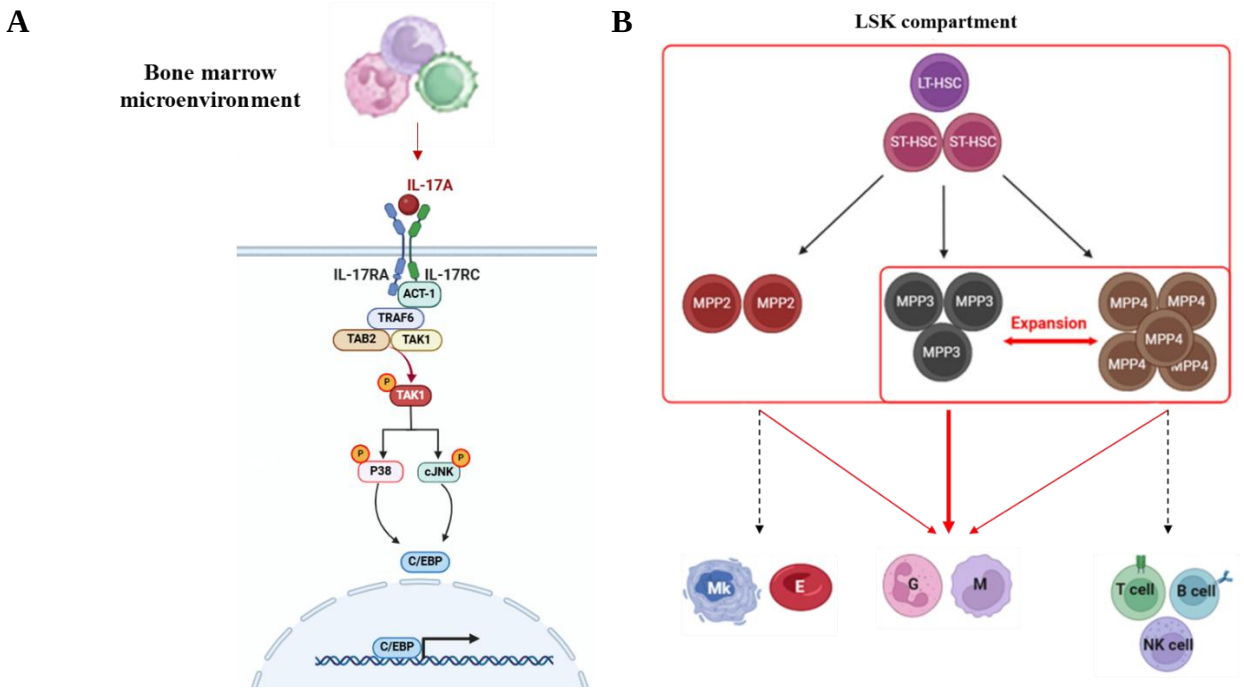

Figure 14. Graphical abstract conclusions. **A:** Our model of IL17A-signalling pathway in murine bone marrow LSK. **B:** The IL17A effect on murine bone marrow LSK during *ex vivo* supplementation and myeloid differentiation culture conditions. Generate in Biorender

7. Reference

1. Cosgrove J, Hustin LSP, de Boer RJ, Perié L. Hematopoiesis in numbers. Trends in Immunology **2021**;42:1100-12
2. Bloom W, Bartelmez G. Hematopoiesis in young human embryos. American Journal of Anatomy **1940**;67:21-53
3. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development **1999**;126:5073-84
4. Lovell-Badge R, Anthony E, Barker RA, Bubela T, Brivanlou AH, Carpenter M, Charo RA, Clark A, Clayton E, Cong Y. ISSCR guidelines for stem cell research and clinical translation: the 2021 update. Stem cell reports **2021**;16:1398-408
5. Rivron NC, Arias AM, Pera MF, Moris N, M'hamdi HI. An ethical framework for human embryology with embryo models. Cell **2023**;186:3548-57
6. Clark AT, Brivanlou A, Fu J, Kato K, Mathews D, Niakan KK, Rivron N, Saitou M, Surani A, Tang F. Human embryo research, stem cell-derived embryo models and in vitro gametogenesis: Considerations leading to the revised ISSCR guidelines. Stem Cell Reports **2021**;16:1416-24
7. Atkins MH, Scarfò R, McGrath KE, Yang D, Palis J, Ditadi A, Keller GM. Modeling human yolk sac hematopoiesis with pluripotent stem cells. J Exp Med **2022**;219
8. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development **1999**;126:5073-84
9. Tober J, Koniski A, McGrath KE, Vemishetti R, Emerson R, de Mesy-Bentley KKL, Waugh R, Palis J. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. Blood **2007**;109:1433-41
10. Tober J, Koniski A, McGrath KE, Vemishetti R, Emerson R, de Mesy-Bentley KK, Waugh R, Palis J. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. Blood **2007**;109:1433-41
11. Naito M, Yamamura F, Nishikawa S, Takahashi K. Development, differentiation, and maturation of fetal mouse yolk sac macrophages in cultures. J Leukoc Biol **1989**;46:1-10
12. Takahashi K, Yamamura F, Naito M. Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. J Leukoc Biol **1989**;45:87-96
13. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell **1996**;86:897-906

14. de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. The EMBO journal **2000**;19:2465-74
15. Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. The Journal of experimental medicine **2011**;208:2417
16. De Bruijn MF, Ma X, Robin C, Ottersbach K, Sanchez M-J, Dzierzak E. Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. Immunity **2002**;16:673-83
17. Bertrand JY, Giroux S, Golub R, Klaine M, Jalil A, Boucontet L, Godin I, Cumano A. Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proceedings of the National Academy of Sciences **2005**;102:134-9
18. Taoudi S, Morrison AM, Inoue H, Gribi R, Ure J, Medvinsky A. Progressive divergence of definitive haematopoietic stem cells from the endothelial compartment does not depend on contact with the foetal liver. **2005**
19. Ivanovs A, Rybtsov S, Anderson RA, Turner ML, Medvinsky A. Identification of the niche and phenotype of the first human hematopoietic stem cells. Stem cell reports **2014**;2:449-56
20. Yoder MC, Hiatt K, Dutt P, Mukherjee P, Bodine DM, Orlic D. Characterization of definitive lymphohematopoietic stem cells in the day 9 murine yolk sac. Immunity **1997**;7:335-44
21. Yoder MC, Hiatt K, Mukherjee P. In vivo repopulating hematopoietic stem cells are present in the murine yolk sac at day 9.0 postcoitus. Proceedings of the National Academy of Sciences **1997**;94:6776-80
22. Lux CT, Yoshimoto M, McGrath K, Conway SJ, Palis J, Yoder MC. All primitive and definitive hematopoietic progenitor cells emerging before E10 in the mouse embryo are products of the yolk sac. Blood, The Journal of the American Society of Hematology **2008**;111:3435-8
23. Rybtsov S, Batsivari A, Bilotkach K, Paruzina D, Senserrich J, Nerushev O, Medvinsky A. Tracing the origin of the HSC hierarchy reveals an SCF-dependent, IL-3-independent CD43− embryonic precursor. Stem cell reports **2014**;3:489-501
24. Rybtsov S, Sobiesiak M, Taoudi S, Souilhol C, Senserrich J, Liakhovitskaia A, Ivanovs A, Frampton J, Zhao S, Medvinsky A. Hierarchical organization and early hematopoietic specification of the developing HSC lineage in the AGM region. The Journal of experimental medicine **2011**;208:1305
25. Taoudi S, Gonneau C, Moore K, Sheridan JM, Blackburn CC, Taylor E, Medvinsky A. Extensive hematopoietic stem cell generation in the AGM region via maturation of VE-cadherin+ CD45+ pre-definitive HSCs. Cell stem cell **2008**;3:99-108

26. Hadland BK, Varnum-Finney B, Poulos MG, Moon RT, Butler JM, Rafii S, Bernstein ID. Endothelium and NOTCH specify and amplify aorta-gonad-mesonephros–derived hematopoietic stem cells. The Journal of clinical investigation **2015**;125:2032-45
27. Kumaravelu P, Hook L, Morrison AM, Ure J, Zhao S, Zuyev S, Ansell J, Medvinsky A. Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. **2002**
28. Baron MH, Isern J, Fraser ST. The embryonic origins of erythropoiesis in mammals. Blood **2012**;119:4828-37
29. Yu VW, Scadden DT. Hematopoietic Stem Cell and Its Bone Marrow Niche. Curr Top Dev Biol **2016**;118:21-44
30. Laurenti E, Göttgens B. From haematopoietic stem cells to complex differentiation landscapes. Nature **2018**;553:418-26
31. Bujanover N, Goldstein O, Greenshpan Y, Turgeman H, Klainberger A, Scharff Ye, Gazit R. Identification of immune-activated hematopoietic stem cells. Leukemia **2018**;32:2016-20
32. Kumar R, Fossati V, Israel M, Snoeck HW. Lin-Sca1+kit- bone marrow cells contain early lymphoid-committed precursors that are distinct from common lymphoid progenitors. J Immunol **2008**;181:7507-13
33. Pietras EM, Reynaud D, Kang YA, Carlin D, Calero-Nieto FJ, Leavitt AD, Stuart JM, Göttgens B, Passegué E. Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. Cell Stem Cell **2015**;17:35-46
34. Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, Panero R, Patel SH, Jankovic M, Sun J, Calogero RA, Klein AM, Camargo FD. Clonal analysis of lineage fate in native haematopoiesis. Nature **2018**;553:212-6
35. Sommerkamp P, Romero-Mulero MC, Narr A, Ladel L, Hustin L, Schönberger K, Renders S, Altamura S, Zeisberger P, Jäcklein K, Klimmeck D, Rodriguez-Fraticelli A, Camargo FD, Perié L, Trumpp A, Cabezas-Wallscheid N. Mouse multipotent progenitor 5 cells are located at the interphase between hematopoietic stem and progenitor cells. Blood **2021**;137:3218-24
36. Brown G, Tsapogas P, Ceredig R. The changing face of hematopoiesis: a spectrum of options is available to stem cells. Immunology & Cell Biology **2018**;96:898-911
37. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature **2008**;453:306-13
38. Kucinski I, Campos J, Barile M, Severi F, Bohin N, Moreira PN, Allen L, Lawson H, Haltalli MLR, Kinston SJ, O'Carroll D, Kranc KR, Göttgens B. A time- and single-cell-resolved model of murine bone marrow hematopoiesis. Cell Stem Cell **2024**;31:244-59.e10
39. Metcalf D. Hematopoietic cytokines. Blood **2008**;111:485-91

40. Zhu J, Emerson SG. Hematopoietic cytokines, transcription factors and lineage commitment. Oncogene **2002**;21:3295-313
41. Lucas D. Structural organization of the bone marrow and its role in hematopoiesis. Curr Opin Hematol **2021**;28:36-42
42. Liggett LA, Sankaran VG. Unraveling Hematopoiesis through the Lens of Genomics. Cell **2020**;182:1384-400
43. Maridas DE, Rendina-Ruedy E, Le PT, Rosen CJ. Isolation, Culture, and Differentiation of Bone Marrow Stromal Cells and Osteoclast Progenitors from Mice. J Vis Exp **2018**
44. Gao Q, Wang L, Wang S, Huang B, Jing Y, Su J. Bone Marrow Mesenchymal Stromal Cells: Identification, Classification, and Differentiation. Front Cell Dev Biol **2021**;9:787118
45. Inoue T, Kurosaki T. Memory B cells. Nature Reviews Immunology **2024**;24:5-17
46. Zanetti C, Krause DS. "Caught in the net": the extracellular matrix of the bone marrow in normal hematopoiesis and leukemia. Experimental Hematology **2020**;89:13-25
47. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature **2014**;505:327-34
48. Aasebø E, Birkeland E, Selheim F, Berven F, Brenner AK, Bruserud Ø. The Extracellular Bone Marrow Microenvironment-A Proteomic Comparison of Constitutive Protein Release by In Vitro Cultured Osteoblasts and Mesenchymal Stem Cells. Cancers (Basel) **2020**;13
49. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature **2012**;481:457-62
50. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature **2014**;505:327-34
51. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells **1978**;4:7-25
52. Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, Zaher W, Mortensen LJ, Alt C, Turcotte R, Yusuf R, Côté D, Vinogradov SA, Scadden DT, Lin CP. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature **2014**;508:269-73
53. Comazzetto S, Shen B, Morrison SJ. Niches that regulate stem cells and hematopoiesis in adult bone marrow. Dev Cell **2021**;56:1848-60
54. Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, Mahoney JE, Park SY, Lu J, Protopopov A, Silberstein LE. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nat Cell Biol **2013**;15:533-43
55. van Gastel N, Spinelli JB, Sharda A, Schajnovitz A, Baryawno N, Rhee C, Oki T, Grace E, Soled HJ, Milosevic J, Sykes DB, Hsu PP, Vander Heiden MG, Vidoudez C, Trauger SA, Haigis MC, Scadden DT. Induction of a Timed Metabolic Collapse to Overcome Cancer Chemoresistance. Cell Metab **2020**;32:391-403.e6

56. Hayashi Y, Sezaki M, Takizawa H. Development of the hematopoietic system: Role of inflammatory factors. Wiley Interdiscip Rev Dev Biol **2019**;8:e341
57. Brunner KFaC. Mechanisms Controlling Hematopoiesis. In: Lawrie DC, editor. Hematology - Science and

Practice: InTech; 2012.
58. Pinho S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche. Nature Reviews Molecular Cell Biology **2019**;20:303-20
59. Niazi V, Parseh B, Ahani M, Karami F, Gilanchi S, Atarodi K, Soufi M, Soleimani M, Ghafouri-Fard S, Taheri M, Zali H. Communication between stromal and hematopoietic stem cell by exosomes in normal and malignant bone marrow niche. Biomedicine & Pharmacotherapy **2020**;132:110854
60. Chen K, Jiao Y, Liu L, Huang M, He C, He W, Hou J, Yang M, Luo X, Li C. Communications Between Bone Marrow Macrophages and Bone Cells in Bone Remodeling. Front Cell Dev Biol **2020**;8:598263
61. Porter RL, Calvi LM. Communications between bone cells and hematopoietic stem cells. Arch Biochem Biophys **2008**;473:193-200
62. Glatman Zaretsky A, Engiles JB, Hunter CA. Infection-induced changes in hematopoiesis. J Immunol **2014**;192:27-33
63. Groarke EM, Young NS. Aging and Hematopoiesis. Clin Geriatr Med **2019**;35:285-93
64. Rice KL, Hormaeche I, Licht JD. Epigenetic regulation of normal and malignant hematopoiesis. Oncogene **2007**;26:6697-714
65. Bousounis P, Bergo V, Trompouki E. Inflammation, Aging and Hematopoiesis: A Complex Relationship. Cells **2021**;10
66. Lord BI, Testa NG, Hendry JH. The relative spatial distributions of CFUs and CFUc in the normal mouse femur. Blood **1975**;46:65-72
67. Gong JK. Endosteal marrow: a rich source of hematopoietic stem cells. Science **1978**;199:1443-5
68. Haylock DN, Williams B, Johnston HM, Liu MC, Rutherford KE, Whitty GA, Simmons PJ, Bertoncello I, Nilsson SK. Hemopoietic stem cells with higher hemopoietic potential reside at the bone marrow endosteum. Stem Cells **2007**;25:1062-9
69. Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J Exp Med **1994**;179:1677-82
70. Taichman RS, Reilly MJ, Emerson SG. Human osteoblasts support human hematopoietic progenitor cells in vitro bone marrow cultures. Blood **1996**;87:518-24
71. Visnjic D, Kalajzic I, Gronowicz G, Aguila HL, Clark SH, Lichtler AC, Rowe DW. Conditional ablation of the osteoblast lineage in Col2.3deltatk transgenic mice. J Bone Miner Res **2001**;16:2222-31
72. Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood **2004**;103:3258-64

73. Lymperi S, Ersek A, Ferraro F, Dazzi F, Horwood NJ. Inhibition of osteoclast function reduces hematopoietic stem cell numbers in vivo. Blood **2011**;117:1540-9
74. Marusić A, Kalinowski JF, Jastrzebski S, Lorenzo JA. Production of leukemia inhibitory factor mRNA and protein by malignant and immortalized bone cells. J Bone Miner Res **1993**;8:617-24
75. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature **2003**;425:841-6
76. Weber JM, Forsythe SR, Christianson CA, Frisch BJ, Gigliotti BJ, Jordan CT, Milner LA, Guzman ML, Calvi LM. Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. Bone **2006**;39:485-93
77. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell **2004**;118:149-61
78. Jung Y, Wang J, Song J, Shiozawa Y, Wang J, Havens A, Wang Z, Sun YX, Emerson SG, Krebsbach PH, Taichman RS. Annexin II expressed by osteoblasts and endothelial cells regulates stem cell adhesion, homing, and engraftment following transplantation. Blood **2007**;110:82-90
79. Park D, Spencer JA, Koh BI, Kobayashi T, Fujisaki J, Clemens TL, Lin CP, Kronenberg HM, Scadden DT. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. Cell Stem Cell **2012**;10:259-72
80. Masuda S, Ageyama N, Shibata H, Obara Y, Ikeda T, Takeuchi K, Ueda Y, Ozawa K, Hanazono Y. Cotransplantation with MSCs improves engraftment of HSCs after autologous intra-bone marrow transplantation in nonhuman primates. Exp Hematol **2009**;37:1250-7.e1
81. Ahn JY, Park G, Shim JS, Lee JW, Oh IH. Intramarrow injection of beta-catenin-activated, but not naive mesenchymal stromal cells stimulates self-renewal of hematopoietic stem cells in bone marrow. Exp Mol Med **2010**;42:122-31
82. Nie Y, Han YC, Zou YR. CXCR4 is required for the quiescence of primitive hematopoietic cells. J Exp Med **2008**;205:777-83
83. Tzeng YS, Li H, Kang YL, Chen WC, Cheng WC, Lai DM. Loss of Cxcl12/Sdf-1 in adult mice decreases the quiescent state of hematopoietic stem/progenitor cells and alters the pattern of hematopoietic regeneration after myelosuppression. Blood **2011**;117:429-39
84. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity **2006**;25:977-88
85. Kawabata K, Ujikawa M, Egawa T, Kawamoto H, Tachibana K, Iizasa H, Katsura Y, Kishimoto T, Nagasawa T. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. Proc Natl Acad Sci U S A **1999**;96:5663-7

86. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science **1999**;283:845-8
87. Bonig H, Priestley GV, Nilsson LM, Jiang Y, Papayannopoulou T. PTX-sensitive signals in bone marrow homing of fetal and adult hematopoietic progenitor cells. Blood **2004**;104:2299-306
88. Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP, Gerson SL. Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. J Hematother Stem Cell Res **2000**;9:841-8
89. Xie H, Sun L, Zhang L, Liu T, Chen L, Zhao A, Lei Q, Gao F, Zou P, Li Q, Guo AY, Chen Z, Wang H. Mesenchymal Stem Cell-Derived Microvesicles Support Ex Vivo Expansion of Cord Blood-Derived CD34(+) Cells. Stem Cells Int **2016**;2016:6493241
90. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell **2005**;121:1109-21
91. Storb R, Graham TC, Epstein RB, Sale GE, Thomas ED. Demonstration of hemopoietic stem cells in the peripheral blood of baboons by cross circulation. Blood **1977**;50:537-42
92. Chute JP, Muramoto GG, Dressman HK, Wolfe G, Chao NJ, Lin S. Molecular profile and partial functional analysis of novel endothelial cell-derived growth factors that regulate hematopoiesis. Stem Cells **2006**;24:1315-27
93. Kobayashi H, Butler JM, O'Donnell R, Kobayashi M, Ding BS, Bonner B, Chiu VK, Nolan DJ, Shido K, Benjamin L, Rafii S. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. Nat Cell Biol **2010**;12:1046-56
94. Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M, Witte L, May C, Shawber C, Kimura Y, Kitajewski J, Rosenwaks Z, Bernstein ID, Rafii S. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell Stem Cell **2010**;6:251-64
95. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature **2012**;481:457-62
96. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell **2006**;124:407-21
97. Méndez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature **2008**;452:442-7
98. Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A, Nakauchi H. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell **2011**;147:1146-58

99. Yamazaki S, Iwama A, Takayanagi S, Eto K, Ema H, Nakauchi H. TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. Blood **2009**;113:1250-6
100. Barker JE. Early transplantation to a normal microenvironment prevents the development of Steel hematopoietic stem cell defects. Exp Hematol **1997**;25:542-7
101. Bruns I, Lucas D, Pinho S, Ahmed J, Lambert MP, Kunisaki Y, Scheiermann C, Schiff L, Poncz M, Bergman A, Frenette PS. Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. Nat Med **2014**;20:1315-20
102. Zhao M, Perry JM, Marshall H, Venkatraman A, Qian P, He XC, Ahamed J, Li L. Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nat Med **2014**;20:1321-6
103. Nakamura-Ishizu A, Takubo K, Fujioka M, Suda T. Megakaryocytes are essential for HSC quiescence through the production of thrombopoietin. Biochem Biophys Res Commun **2014**;454:353-7
104. Qian H, Buza-Vidas N, Hyland CD, Jensen CT, Antonchuk J, Månsson R, Thoren LA, Ekblom M, Alexander WS, Jacobsen SE. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. Cell Stem Cell **2007**;1:671-84
105. Zhang Y, Lin CHS, Kaushansky K, Zhan H. JAK2V617F Megakaryocytes Promote Hematopoietic Stem/Progenitor Cell Expansion in Mice Through Thrombopoietin/MPL Signaling. Stem Cells **2018**;36:1676-84
106. Nakamura-Ishizu A, Takubo K, Kobayashi H, Suzuki-Inoue K, Suda T. CLEC-2 in megakaryocytes is critical for maintenance of hematopoietic stem cells in the bone marrow. J Exp Med **2015**;212:2133-46
107. Pinho S, Marchand T, Yang E, Wei Q, Nerlov C, Frenette PS. Lineage-Biased Hematopoietic Stem Cells Are Regulated by Distinct Niches. Dev Cell **2018**;44:634-41.e4
108. Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, Poulton IJ, van Rooijen N, Alexander KA, Raggatt LJ, Lévesque JP. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood **2010**;116:4815-28
109. Chow A, Lucas D, Hidalgo A, Méndez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Tanaka M, Merad M, Frenette PS. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J Exp Med **2011**;208:261-71
110. Li D, Xue W, Li M, Dong M, Wang J, Wang X, Li X, Chen K, Zhang W, Wu S, Zhang Y, Gao L, Chen Y, Chen J, Zhou BO, Zhou Y, Yao X, Li L, Wu D, Pan W. VCAM-1(+) macrophages guide the homing of HSPCs to a vascular niche. Nature **2018**;564:119-24
111. Hur J, Choi JI, Lee H, Nham P, Kim TW, Chae CW, Yun JY, Kang JA, Kang J, Lee SE, Yoon CH, Boo K, Ham S, Roh TY, Jun JK, Lee H, Baek SH, Kim HS. CD82/KAI1 Maintains the Dormancy of Long-Term Hematopoietic Stem Cells through Interaction with DARC-Expressing Macrophages. Cell Stem Cell **2016**;18:508-21

112. Ludin A, Itkin T, Gur-Cohen S, Mildner A, Shezen E, Golan K, Kollet O, Kalinkovich A, Porat Z, D'Uva G, Schajnovitz A, Voronov E, Brenner DA, Apte RN, Jung S, Lapidot T. Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. Nat Immunol **2012**;13:1072-82
113. Yang C, Endoh M, Tan DQ, Nakamura-Ishizu A, Takihara Y, Matsumura T, Suda T. Mitochondria transfer from early stages of erythroblasts to their macrophage niche via tunnelling nanotubes. Br J Haematol **2021**;193:1260-74
114. Chow A, Huggins M, Ahmed J, Hashimoto D, Lucas D, Kunisaki Y, Pinho S, Leboeuf M, Noizat C, van Rooijen N, Tanaka M, Zhao ZJ, Bergman A, Merad M, Frenette PS. $CD169^{+}$ macrophages provide a niche promoting erythropoiesis under homeostasis and stress. Nat Med **2013**;19:429-36
115. Zou L, Barnett B, Safah H, Larussa VF, Evdemon-Hogan M, Mottram P, Wei S, David O, Curiel TJ, Zou W. Bone marrow is a reservoir for CD4+CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals. Cancer Res **2004**;64:8451-5
116. Hirata Y, Furuhashi K, Ishii H, Li HW, Pinho S, Ding L, Robson SC, Frenette PS, Fujisaki J. CD150(high) Bone Marrow Tregs Maintain Hematopoietic Stem Cell Quiescence and Immune Privilege via Adenosine. Cell Stem Cell **2018**;22:445-53.e5
117. Fujisaki J, Wu J, Carlson AL, Silberstein L, Putheti P, Larocca R, Gao W, Saito TI, Lo Celso C, Tsuyuzaki H, Sato T, Côté D, Sykes M, Strom TB, Scadden DT, Lin CP. In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. Nature **2011**;474:216-9
118. Pinho S, Wei Q, Maryanovich M, Zhang D, Balandrán JC, Pierce H, Nakahara F, Di Staulo A, Bartholdy BA, Xu J, Borger DK, Verma A, Frenette PS. VCAM1 confers innate immune tolerance on haematopoietic and leukaemic stem cells. Nature Cell Biology **2022**;24:290-8
119. Craddock CF, Nakamoto B, Andrews RG, Priestley GV, Papayannopoulou T. Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice. Blood **1997**;90:4779-88
120. Papayannopoulou T, Priestley GV, Nakamoto B. Anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the kit/mkit ligand pathway. Blood **1998**;91:2231-9
121. Dumonde DC, Wolstencroft RA, Panayi GS, Matthew M, Morley J, Howson WT. "Lymphokines": non-antibody mediators of cellular immunity generated by lymphocyte activation. Nature **1969**;224:38-42
122. Lázár-Molnár E, Hegyesi H, Tóth S, Falus A. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. Cytokine **2000**;12:547-54
123. Kourilsky P, Truffa-Bachi P. Cytokine fields and the polarization of the immune response. Trends Immunol **2001**;22:502-9
124. Yoshimura A, Ito M, Chikuma S, Akanuma T, Nakatsukasa H. Negative Regulation of Cytokine Signaling in Immunity. Cold Spring Harb Perspect Biol **2018**;10

125. Lichtman AH, Chin J, Schmidt JA, Abbas AK. Role of interleukin 1 in the activation of T lymphocytes. Proc Natl Acad Sci U S A **1988**;85:9699-703
126. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol **2014**;14:585-600
127. Cheng LE, Öhlén C, Nelson BH, Greenberg PD. Enhanced signaling through the IL-2 receptor in CD8⁺ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8⁺ T cells rather than promotion of cell death. Proceedings of the National Academy of Sciences **2002**;99:3001-6
128. Ross SH, Cantrell DA. Signaling and Function of Interleukin-2 in T Lymphocytes. Annu Rev Immunol **2018**;36:411-33
129. Mu X, Liu K, Li H, Wang F-S, Xu R. Granulocyte-macrophage colony-stimulating factor: an immunotarget for sepsis and COVID-19. Cellular & Molecular Immunology **2021**;18:2057-8
130. Wong GH, Goeddel DV. Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons. Nature **1986**;323:819-22
131. Bartee E, McFadden G. Cytokine synergy: an underappreciated contributor to innate anti-viral immunity. Cytokine **2013**;63:237-40
132. Alexander WS. Cytokines in hematopoiesis. Int Rev Immunol **1998**;16:651-82
133. de Haan G, Dontje B, Nijhof W. Concepts of hemopoietic cell amplification. Synergy, redundancy and pleiotropy of cytokines affecting the regulation of erythropoiesis. Leuk Lymphoma **1996**;22:385-94
134. Jovcić G, Bugarski D, Petakov M, Stanković J, Stojanović N, Milenković P. Effect of IL-17 on in vitro hematopoietic progenitor cells growth and cytokine release in normal and post-irradiated murine bone marrow. Growth Factors **2001**;19:61-71
135. Schwarzmeier JD. The role of cytokines in haematopoiesis. Eur J Haematol Suppl **1996**;60:69-74
136. Velazquez L, Cheng AM, Fleming HE, Furlonger C, Vesely S, Bernstein A, Paige CJ, Pawson T. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. J Exp Med **2002**;195:1599-611
137. Zhang CC, Lodish HF. Cytokines regulating hematopoietic stem cell function. Curr Opin Hematol **2008**;15:307-11
138. de Kruijf EFM, Fibbe WE, van Pel M. Cytokine-induced hematopoietic stem and progenitor cell mobilization: unraveling interactions between stem cells and their niche. Ann N Y Acad Sci **2020**;1466:24-38
139. Bernitz JM, Daniel MG, Fstkchyan YS, Moore K. Granulocyte colony-stimulating factor mobilizes dormant hematopoietic stem cells without proliferation in mice. Blood **2017**;129:1901-12

140. Richards MK, Liu F, Iwasaki H, Akashi K, Link DC. Pivotal role of granulocyte colony-stimulating factor in the development of progenitors in the common myeloid pathway. Blood **2003**;102:3562-8
141. Panopoulos AD, Watowich SS. Granulocyte colony-stimulating factor: Molecular mechanisms of action during steady state and 'emergency' hematopoiesis. Cytokine **2008**;42:277-88
142. Schuettpelz LG, Borgerding JN, Christopher MJ, Gopalan PK, Romine MP, Herman AC, Woloszynek JR, Greenbaum AM, Link DC. G-CSF regulates hematopoietic stem cell activity, in part, through activation of Toll-like receptor signaling. Leukemia **2014**;28:1851-60
143. Grover A, Mancini E, Moore S, Mead AJ, Atkinson D, Rasmussen KD, O'Carroll D, Jacobsen SE, Nerlov C. Erythropoietin guides multipotent hematopoietic progenitor cells toward an erythroid fate. J Exp Med **2014**;211:181-8
144. Kelemen E, Cserhati I, Tanos B. Demonstration and some properties of human thrombopoietin in thrombocythaemic sera. Acta Haematol **1958**;20:350-5
145. Drachman JG, Griffin JD, Kaushansky K. The c-Mpl ligand (thrombopoietin) stimulates tyrosine phosphorylation of Jak2, Shc, and c-Mpl. J Biol Chem **1995**;270:4979-82
146. Kaushansky K, Lok S, Holly RD, Broudy VC, Lin N, Bailey MC, Forstrom JW, Buddle MM, Oort PJ, Hagen FS, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. Nature **1994**;369:568-71
147. Sitnicka E, Lin N, Priestley GV, Fox N, Broudy VC, Wolf NS, Kaushansky K. The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells. Blood **1996**;87:4998-5005
148. Ku H, Yonemura Y, Kaushansky K, Ogawa M. Thrombopoietin, the ligand for the Mpl receptor, synergizes with steel factor and other early acting cytokines in supporting proliferation of primitive hematopoietic progenitors of mice. Blood **1996**;87:4544-51
149. Kirito K, Fox N, Kaushansky K. Thrombopoietin stimulates Hoxb4 expression: an explanation for the favorable effects of TPO on hematopoietic stem cells. Blood **2003**;102:3172-8
150. Antonchuk J, Sauvageau G, Humphries RK. HOXB4 overexpression mediates very rapid stem cell regeneration and competitive hematopoietic repopulation. Exp Hematol **2001**;29:1125-34
151. Thorsteinsdottir U, Sauvageau G, Humphries RK. Enhanced in vivo regenerative potential of HOXB4-transduced hematopoietic stem cells with regulation of their pool size. Blood **1999**;94:2605-12
152. Buske C, Feuring-Buske M, Abramovich C, Spiekermann K, Eaves CJ, Coulombel L, Sauvageau G, Hogge DE, Humphries RK. Deregulated expression of HOXB4 enhances the primitive growth activity of human hematopoietic cells. Blood **2002**;100:862-8
153. Liu Y, Ding L, Zhang B, Deng Z, Han Y, Wang S, Yang S, Fan Z, Zhang J, Yan H, Han D, He L, Yue W, Wang H, Li Y, Pei X. Thrombopoietin enhances hematopoietic stem and

progenitor cell homing by impeding matrix metalloproteinase 9 expression. STEM CELLS Translational Medicine **2020**;9:661-73
154. Metcalf D, Burgess AW. Clonal analysis of progenitor cell commitment of granulocyte or macrophage production. J Cell Physiol **1982**;111:275-83
155. Rieger MA, Hoppe PS, Smejkal BM, Eitelhuber AC, Schroeder T. Hematopoietic cytokines can instruct lineage choice. Science **2009**;325:217-8
156. Mirantes C, Passegué E, Pietras EM. Pro-inflammatory cytokines: Emerging players regulating HSC function in normal and diseased hematopoiesis. Experimental Cell Research **2014**;329:248-54
157. Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk LV, Zhang S, Lakshminarasimhan R, Chin CP, Techner J-M, Will B, Nerlov C, Steidl U, Manz MG, Schroeder T, Passegué E. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. Nature Cell Biology **2016**;18:607-18
158. Weber GF, Chousterman BG, He S, Fenn AM, Nairz M, Anzai A, Brenner T, Uhle F, Iwamoto Y, Robbins CS, Noiret L, Maier SL, Zönnchen T, Rahbari NN, Schölch S, Klotzsche-von Ameln A, Chavakis T, Weitz J, Hofer S, Weigand MA, Nahrendorf M, Weissleder R, Swirski FK. Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis. Science **2015**;347:1260-5
159. Hirano T, Taga T, Matsuda T, Hibi M, Suematsu S, Tang B, Murakami M, Kishimoto T. Interleukin 6 and its receptor in the immune response and hematopoiesis. Int J Cell Cloning **1990**;8 Suppl 1:155-66; discussion 66-7
160. Radujkovic A, Kordelas L, Bogdanov R, Müller-Tidow C, Beelen DW, Dreger P, Luft T. Interleukin-18 and Hematopoietic Recovery after Allogeneic Stem Cell Transplantation. Cancers (Basel) **2020**;12
161. Morales-Mantilla DE, King KY. The Role of Interferon-Gamma in Hematopoietic Stem Cell Development, Homeostasis, and Disease. Curr Stem Cell Rep **2018**;4:264-71
162. Yamashita M, Passegué E. TNF-α Coordinates Hematopoietic Stem Cell Survival and Myeloid Regeneration. Cell Stem Cell **2019**;25:357-72.e7
163. Jovči G, Bugarski D, Petakov M, Stanković J, Stojanović N, Milenković P. Effect of IL-17 on in vitro hematopoietic progenitor cells growth and cytokine release in normal and post-irradiated murine bone marrow. Growth Factors **2001**;19:61-71
164. McNiece IK, Briddell RA. Stem cell factor. J Leukoc Biol **1995**;58:14-22
165. Barker JE. Sl/Sld hematopoietic progenitors are deficient in situ. Exp Hematol **1994**;22:174-7
166. Rybtsov S, Batsivari A, Bilotkach K, Paruzina D, Senserrich J, Nerushev O, Medvinsky A. Tracing the origin of the HSC hierarchy reveals an SCF-dependent, IL-3-independent CD43(-) embryonic precursor. Stem Cell Reports **2014**;3:489-501

167. McNiece IK, Langley KE, Zsebo KM. Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and epo to stimulate human progenitor cells of the myeloid and erythroid lineages. Exp Hematol **1991**;19:226-31
168. Dawson MA, Schwarer AP, Muirhead JL, Bailey MJ, Bollard GM, Spencer A. Successful mobilization of peripheral blood stem cells using recombinant human stem cell factor in heavily pretreated patients who have failed a previous attempt with a granulocyte colony-stimulating factor-based regimen. Bone Marrow Transplantation **2005**;36:389-96
169. Dorronsoro A, Lang V, Ferrin I, Fernández-Rueda J, Zabaleta L, Pérez-Ruiz E, Sepúlveda P, Trigueros C. Intracellular role of IL-6 in mesenchymal stromal cell immunosuppression and proliferation. Scientific Reports **2020**;10:21853
170. Tie R, Li H, Cai S, Liang Z, Shan W, Wang B, Tan Y, Zheng W, Huang H. Interleukin-6 signaling regulates hematopoietic stem cell emergence. Experimental & Molecular Medicine **2019**;51:1-12
171. Forte E. A cell-intrinsic regulator of emergency hematopoiesis. Nature Cardiovascular Research **2023**;2:8-
172. Bernad A, Kopf M, Kulbacki R, Weich N, Koehler G, Gutierrez-Ramos JC. Interleukin-6 is required in vivo for the regulation of stem cells and committed progenitors of the hematopoietic system. Immunity **1994**;1:725-31
173. Lachmann N, Ackermann M, Frenzel E, Happle C, Klimenkova O, Brennig S, Lüttge D, Buchegger T, Janciauskiene S, Skokowa J, Dilloo D, Moritz T. IL-3 Specifies Early Hematopoietic Development from Human iPSCs and Synergizes with M-CSF and G-CSF on Myeloid Differentiation. Blood **2014**;124:4308-
174. Nitsche A, Junghahn I, Thulke S, Aumann J, Radonić A, Fichtner I, Siegert W. Interleukin-3 promotes proliferation and differentiation of human hematopoietic stem cells but reduces their repopulation potential in NOD/SCID mice. Stem Cells **2003**;21:236-44
175. Small D, Levenstein M, Kim E, Carow C, Amin S, Rockwell P, Witte L, Burrow C, Ratajczak MZ, Gewirtz AM, et al. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. Proc Natl Acad Sci U S A **1994**;91:459-63
176. Tsapogas P, Swee LK, Nusser A, Nuber N, Kreuzaler M, Capoferri G, Rolink H, Ceredig R, Rolink A. In vivo evidence for an instructive role of fms-like tyrosine kinase-3 (FLT3) ligand in hematopoietic development. Haematologica **2014**;99:638-46
177. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, Maliszewski CR, Lynch DH, Smith J, Pulendran B, Roux ER, Teepe M, Lyman SD, Peschon JJ. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood **2000**;95:3489-97
178. Sitnicka E, Bryder D, Theilgaard-Mönch K, Buza-Vidas N, Adolfsson J, Jacobsen SE. Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. Immunity **2002**;17:463-72

179. Losada CG, Poon WWL, Salaciak M, Amghar S, Johnson NA, Sharif-Askari B, Mercier FE. Interleukin-17A Directly Signals to Bone Marrow-Derived Hematopoietic Stem and Progenitor Cells to Promote Their Expansion and Differentiation in Vitro. Blood **2023**;142:5587-
180. Antonchuk J, Sauvageau G, Humphries RK. HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell **2002**;109:39-45
181. Ema H, Takano H, Sudo K, Nakauchi H. In vitro self-renewal division of hematopoietic stem cells. J Exp Med **2000**;192:1281-8
182. Ando K, Yahata T, Sato T, Miyatake H, Matsuzawa H, Oki M, Miyoshi H, Tsuji T, Kato S, Hotta T. Direct evidence for ex vivo expansion of human hematopoietic stem cells. Blood **2006**;107:3371-7
183. Cheng J, Türkel N, Hemati N, Fuller MT, Hunt AJ, Yamashita YM. Centrosome misorientation reduces stem cell division during ageing. Nature **2008**;456:599-604
184. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. Nat Med **1996**;2:1011-6
185. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, Weissman IL. Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci U S A **2005**;102:9194-9
186. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. Science **2019**;366
187. Nishi K, Sakamaki T, Kao KS, Sadaoka K, Fujii M, Takaori-Kondo A, Miyanishi M. Age-Associated Myeloid Biased Hematopoiesis Depends on Relative Decrease of Short-Term Hematopoietic Stem Cell. Blood **2019**;134:2481-
188. Singh AK, Althoff MJ, Cancelas JA. Signaling Pathways Regulating Hematopoietic Stem Cell and Progenitor Aging. Curr Stem Cell Rep **2018**;4:166-81
189. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell **2008**;132:631-44
190. MacLean AL, Rudolph KL. Hematopoietic stem cell aging by the niche. Blood **2024**;144:347-8
191. Kusumbe AP, Ramasamy SK, Itkin T, Mäe MA, Langen UH, Betsholtz C, Lapidot T, Adams RH. Age-dependent modulation of vascular niches for haematopoietic stem cells. Nature **2016**;532:380-4
192. Ho YH, Del Toro R, Rivera-Torres J, Rak J, Korn C, García-García A, Macías D, González-Gómez C, Del Monte A, Wittner M, Waller AK, Foster HR, López-Otín C, Johnson RS, Nerlov C, Ghevaert C, Vainchenker W, Louache F, Andrés V, Méndez-Ferrer S. Remodeling of Bone Marrow Hematopoietic Stem Cell Niches Promotes Myeloid Cell Expansion during Premature or Physiological Aging. Cell Stem Cell **2019**;25:407-18.e6
193. Shen B, Tasdogan A, Ubellacker JM, Zhang J, Nosyreva ED, Du L, Murphy MM, Hu S, Yi Y, Kara N, Liu X, Guela S, Jia Y, Ramesh V, Embree C, Mitchell EC, Zhao YC, Ju LA, Hu Z, Crane GM, Zhao Z, Syeda R, Morrison SJ. A mechanosensitive peri-arteriolar niche for osteogenesis and lymphopoiesis. Nature **2021**;591:438-44

194. Saçma M, Pospiech J, Bogeska R, de Back W, Mallm JP, Sakk V, Soller K, Marka G, Vollmer A, Karns R, Cabezas-Wallscheid N, Trumpp A, Méndez-Ferrer S, Milsom MD, Mulaw MA, Geiger H, Florian MC. Haematopoietic stem cells in perisinusoidal niches are protected from ageing. Nat Cell Biol **2019**;21:1309-20
195. Lee KA, Flores RR, Jang IH, Saathoff A, Robbins PD. Immune Senescence, Immunosenescence and Aging. Front Aging **2022**;3:900028
196. Mi B, Xiong Y, Knoedler S, Alfertshofer M, Panayi AC, Wang H, Lin S, Li G, Liu G. Ageing-related bone and immunity changes: insights into the complex interplay between the skeleton and the immune system. Bone Research **2024**;12:42
197. Starling S. Senescent immune cells promote skeletal ageing. Nature Reviews Endocrinology **2021**;17:706-
198. Rodier F, Coppé JP, Patil CK, Hoeijmakers WA, Muñoz DP, Raza SR, Freund A, Campeau E, Davalos AR, Campisi J. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol **2009**;11:973-9
199. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest **2013**;123:958-65
200. Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nature Reviews Molecular Cell Biology **2021**;22:75-95
201. Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, Kaplunov JM, Bucci G, Dobreva M, Matti V, Beausejour CM, Herbig U, Longhese MP, d'Adda di Fagagna F. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat Cell Biol **2012**;14:355-65
202. Rossiello F, Aguado J, Sepe S, Iannelli F, Nguyen Q, Pitchiaya S, Carninci P, d'Adda di Fagagna F. DNA damage response inhibition at dysfunctional telomeres by modulation of telomeric DNA damage response RNAs. Nat Commun **2017**;8:13980
203. Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nat Commun **2012**;3:708
204. Bae NS, Baumann P. A RAP1/TRF2 complex inhibits nonhomologous end-joining at human telomeric DNA ends. Mol Cell **2007**;26:323-34
205. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science **2008**;319:1352-5
206. Adamowicz M, Vermezovic J, d'Adda di Fagagna F. NOTCH1 Inhibits Activation of ATM by Impairing the Formation of an ATM-FOXO3a-KAT5/Tip60 Complex. Cell Rep **2016**;16:2068-76
207. Ito T, Teo YV, Evans SA, Neretti N, Sedivy JM. Regulation of Cellular Senescence by Polycomb Chromatin Modifiers through Distinct DNA Damage- and Histone Methylation-Dependent Pathways. Cell Rep **2018**;22:3480-92

208. Aird KM, Iwasaki O, Kossenkov AV, Tanizawa H, Fatkhutdinov N, Bitler BG, Le L, Alicea G, Yang TL, Johnson FB, Noma KI, Zhang R. HMGB2 orchestrates the chromatin landscape of senescence-associated secretory phenotype gene loci. J Cell Biol **2016**;215:325-34
209. Mazzucco AE, Smogorzewska A, Kang C, Luo J, Schlabach MR, Xu Q, Patel R, Elledge SJ. Genetic interrogation of replicative senescence uncovers a dual role for USP28 in coordinating the p53 and GATA4 branches of the senescence program. Genes Dev **2017**;31:1933-8
210. Davalos AR, Kawahara M, Malhotra GK, Schaum N, Huang J, Ved U, Beausejour CM, Coppe JP, Rodier F, Campisi J. p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. J Cell Biol **2013**;201:613-29
211. Astle MV, Hannan KM, Ng PY, Lee RS, George AJ, Hsu AK, Haupt Y, Hannan RD, Pearson RB. AKT induces senescence in human cells via mTORC1 and p53 in the absence of DNA damage: implications for targeting mTOR during malignancy. Oncogene **2012**;31:1949-62
212. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol **2008**;6:2853-68
213. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell **2004**;14:501-13
214. Coppé JP, Rodier F, Patil CK, Freund A, Desprez PY, Campisi J. Tumor suppressor and aging biomarker p16(INK4a) induces cellular senescence without the associated inflammatory secretory phenotype. J Biol Chem **2011**;286:36396-403
215. Park MD, Le Berichel J, Hamon P, Wilk CM, Belabed M, Yatim N, Saffon A, Boumelha J, Falcomatà C, Tepper A, Hegde S, Mattiuz R, Soong BY, LaMarche NM, Rentzeperis F, Troncoso L, Halasz L, Hennequin C, Chin T, Chen EP, Reid AM, Su M, Cahn AR, Koekkoek LL, Venturini N, Wood-Isenberg S, D'Souza D, Chen R, Dawson T, Nie K, Chen Z, Kim-Schulze S, Casanova-Acebes M, Swirski FK, Downward J, Vabret N, Brown BD, Marron TU, Merad M. Hematopoietic aging promotes cancer by fueling IL-1α-driven emergency myelopoiesis. Science **2024**;386:eadn0327
216. De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NJ, Criscione SW, Caligiana A, Brocculi G, Adney EM, Boeke JD, Le O, Beauséjour C, Ambati J, Ambati K, Simon M, Seluanov A, Gorbunova V, Slagboom PE, Helfand SL, Neretti N, Sedivy JM. L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature **2019**;566:73-8
217. Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J. Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci U S A **2009**;106:17031-6

218. Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. Science **2015**;349:aaa5612
219. Wang S, Pike AM, Lee SS, Strong MA, Connelly CJ, Greider CW. BRD4 inhibitors block telomere elongation. Nucleic Acids Res **2017**;45:8403-10
220. Tasdemir N, Banito A, Roe JS, Alonso-Curbelo D, Camiolo M, Tschaharganeh DF, Huang CH, Aksoy O, Bolden JE, Chen CC, Fennell M, Thapar V, Chicas A, Vakoc CR, Lowe SW. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. Cancer Discov **2016**;6:612-29
221. Dou Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangensteen KJ, Simithy J, Lan Y, Lin Y, Zhou Z, Capell BC, Xu C, Xu M, Kieckhaefer JE, Jiang T, Shoshkes-Carmel M, Tanim K, Barber GN, Seykora JT, Millar SE, Kaestner KH, Garcia BA, Adams PD, Berger SL. Cytoplasmic chromatin triggers inflammation in senescence and cancer. Nature **2017**;550:402-6
222. Özcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G, Galderisi U. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging (Albany NY) **2016**;8:1316-29
223. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. Embo j **2011**;30:1536-48
224. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, Peeper DS. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell **2008**;133:1019-31
225. Kirschner K, Chandra T, Kiselev V, Flores-Santa Cruz D, Macaulay IC, Park HJ, Li J, Kent DG, Kumar R, Pask DC, Hamilton TL, Hemberg M, Reik W, Green AR. Proliferation Drives Aging-Related Functional Decline in a Subpopulation of the Hematopoietic Stem Cell Compartment. Cell Rep **2017**;19:1503-11
226. Valletta S, Thomas A, Meng Y, Ren X, Drissen R, Sengül H, Di Genua C, Nerlov C. Micro-environmental sensing by bone marrow stroma identifies IL-6 and TGFβ1 as regulators of hematopoietic ageing. Nat Commun **2020**;11:4075
227. He H, Wang J. Inflammation and hematopoietic stem cells aging. Blood Sci **2021**;3:1-5
228. Kovtonyuk LV, Caiado F, Garcia-Martin S, Manz EM, Helbling P, Takizawa H, Boettcher S, Al-Shahrour F, Nombela-Arrieta C, Slack E, Manz MG. IL-1 mediates microbiome-induced inflammaging of hematopoietic stem cells in mice. Blood **2022**;139:44-58
229. Zhang L, Liu M, Liu W, Hu C, Li H, Deng J, Cao Q, Wang Y, Hu W, Li Q. Th17/IL-17 induces endothelial cell senescence via activation of NF-κB/p53/Rb signaling pathway. Laboratory Investigation **2021**;101:1418-26
230. Zhang L, Mack R, Breslin P, Zhang J. Molecular and cellular mechanisms of aging in hematopoietic stem cells and their niches. Journal of Hematology & Oncology **2020**;13:157

231. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nature Reviews Immunology **2013**;13:376-89
232. Li X, Zeng X, Xu Y, Wang B, Zhao Y, Lai X, Qian P, Huang H. Mechanisms and rejuvenation strategies for aged hematopoietic stem cells. Journal of Hematology & Oncology **2020**;13:31
233. Stein SJ, Baldwin AS. Deletion of the NF-κB subunit p65/RelA in the hematopoietic compartment leads to defects in hematopoietic stem cell function. Blood **2013**;121:5015-24
234. Chen C, Liu Y, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci Signal **2009**;2:ra75
235. Kuribayashi W, Iwama A, Oshima M. Incomplete rejuvenation of aged HSCs in young bone marrow niche. Experimental Hematology **2019**;76:S72
236. Ramalingam P, Gutkin MC, Poulos MG, Tillery T, Doughty C, Winiarski A, Freire AG, Rafii S, Redmond D, Butler JM. Restoring bone marrow niche function rejuvenates aged hematopoietic stem cells by reactivating the DNA Damage Response. Nature Communications **2023**;14:2018
237. Maryanovich M, Zahalka AH, Pierce H, Pinho S, Nakahara F, Asada N, Wei Q, Wang X, Ciero P, Xu J, Leftin A, Frenette PS. Adrenergic nerve degeneration in bone marrow drives aging of the hematopoietic stem cell niche. Nat Med **2018**;24:782-91
238. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, Suda T. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med **2006**;12:446-51
239. Jung H, Kim DO, Byun JE, Kim WS, Kim MJ, Song HY, Kim YK, Kang DK, Park YJ, Kim TD, Yoon SR, Lee HG, Choi EJ, Min SH, Choi I. Thioredoxin-interacting protein regulates haematopoietic stem cell ageing and rejuvenation by inhibiting p38 kinase activity. Nat Commun **2016**;7:13674
240. Herranz N, Gallage S, Mellone M, Wuestefeld T, Klotz S, Hanley CJ, Raguz S, Acosta JC, Innes AJ, Banito A, Georgilis A, Montoya A, Wolter K, Dharmalingam G, Faull P, Carroll T, Martínez-Barbera JP, Cutillas P, Reisinger F, Heikenwalder M, Miller RA, Withers D, Zender L, Thomas GJ, Gil J. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol **2015**;17:1205-17
241. Widjaja AA, Lim W-W, Viswanathan S, Chothani S, Corden B, Dasan CM, Goh JWT, Lim R, Singh BK, Tan J, Pua CJ, Lim SY, Adami E, Schafer S, George BL, Sweeney M, Xie C, Tripathi M, Sims NA, Hübner N, Petretto E, Withers DJ, Ho L, Gil J, Carling D, Cook SA. Inhibition of IL-11 signalling extends mammalian healthspan and lifespan. Nature **2024**;632:157-65
242. Miller RA. Blocking an inflammatory protein slows the pace of ageing. Nature **2024**;632:35-6
243. Ge Y, Huang M, Yao YM. Biology of Interleukin-17 and Its Pathophysiological Significance in Sepsis. Front Immunol **2020**;11:1558

244. Miossec P. Local and systemic effects of IL-17 in joint inflammation: a historical perspective from discovery to targeting. Cellular & Molecular Immunology **2021**;18:860-5
245. Rouvier E, Luciani MF, Mattéi MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J Immunol **1993**;150:5445-56
246. Xu S, Cao X. Interleukin-17 and its expanding biological functions. Cellular & Molecular Immunology **2010**;7:164-74
247. Iznardo H, Puig L. Dual inhibition of IL-17A and IL-17F in psoriatic disease. Ther Adv Chronic Dis **2021**;12:20406223211037846
248. Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, Liu MC, Krishnaswamy G, Germino G, Huang SK. Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. J Immunol **2001**;167:4430-5
249. Hymowitz SG, Filvaroff EH, Yin JP, Lee J, Cai L, Risser P, Maruoka M, Mao W, Foster J, Kelley RF, Pan G, Gurney AL, de Vos AM, Starovasnik MA. IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. Embo j **2001**;20:5332-41
250. Chang SH, Dong C. IL-17F: regulation, signaling and function in inflammation. Cytokine **2009**;46:7-11
251. Gaffen SL, Kramer JM, Yu JJ, Shen F. The IL-17 Cytokine Family. Vitamins & Hormones: Academic Press; 2006. p 255-82.
252. Laprevotte E, Cochaud S, du Manoir S, Lapierre M, Dejou C, Philippe M, Giustiniani J, Frewer KA, Sanders AJ, Jiang WG, Michaud HA, Colombo PE, Bensussan A, Alberici G, Bastid J, Eliaou JF, Bonnefoy N. The IL-17B-IL-17 receptor B pathway promotes resistance to paclitaxel in breast tumors through activation of the ERK1/2 pathway. Oncotarget **2017**;8:113360-72
253. Bie Q, Song H, Chen X, Yang X, Shi S, Zhang L, Zhao R, Wei L, Zhang B, Xiong H, Zhang B. IL-17B/IL-17RB signaling cascade contributes to self-renewal and tumorigenesis of cancer stem cells by regulating Beclin-1 ubiquitination. Oncogene **2021**;40:2200-16
254. Huang J, Lee HY, Zhao X, Han J, Su Y, Sun Q, Shao J, Ge J, Zhao Y, Bai X, He Y, Wang X, Wang X, Dong C. Interleukin-17D regulates group 3 innate lymphoid cell function through its receptor CD93. Immunity **2021**;54:673-86.e4
255. Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. J Immunol **2002**;169:642-6
256. Swedik S, Madola A, Levine A. IL-17C in human mucosal immunity: More than just a middle child. Cytokine **2021**;146:155641
257. Li H, Chen J, Huang A, Stinson J, Heldens S, Foster J, Dowd P, Gurney AL, Wood WI. Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proc Natl Acad Sci U S A **2000**;97:773-8

258. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, McIlgorm A, Jolin HE, McKenzie AN. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J Exp Med **2006**;203:1105-16
259. Owyang AM, Zaph C, Wilson EH, Guild KJ, McClanahan T, Miller HR, Cua DJ, Goldschmidt M, Hunter CA, Kastelein RA, Artis D. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J Exp Med **2006**;203:843-9
260. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, Muchamuel T, Hurst SD, Zurawski G, Leach MW, Gorman DM, Rennick DM. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity **2001**;15:985-95
261. Lee J, Ho WH, Maruoka M, Corpuz RT, Baldwin DT, Foster JS, Goddard AD, Yansura DG, Vandlen RL, Wood WI, Gurney AL. IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. J Biol Chem **2001**;276:1660-4
262. Mojsilović S, Jauković A, Santibañez JF, Bugarski D. Interleukin-17 and its implication in the regulation of differentiation and function of hematopoietic and mesenchymal stem cells. Mediators Inflamm **2015**;2015:470458
263. Krstic J, Obradovic H, Kukolj T, Mojsilovic S, Okic-Dordevic I, Bugarski D, Santibanez JF. An Overview of Interleukin-17A and Interleukin-17 Receptor A Structure, Interaction and Signaling. Protein Pept Lett **2015**;22:570-8
264. Kurte M, Luz-Crawford P, Vega-Letter AM, Contreras RA, Tejedor G, Elizondo-Vega R, Martinez-Viola L, Fernández-O'Ryan C, Figueroa FE, Jorgensen C, Djouad F, Carrión F. IL17/IL17RA as a Novel Signaling Axis Driving Mesenchymal Stem Cell Therapeutic Function in Experimental Autoimmune Encephalomyelitis. Front Immunol **2018**;9:802
265. Shen F, Hu Z, Goswami J, Gaffen SL. Identification of common transcriptional regulatory elements in interleukin-17 target genes. J Biol Chem **2006**;281:24138-48
266. Maitra A, Shen F, Hanel W, Mossman K, Tocker J, Swart D, Gaffen SL. Distinct functional motifs within the IL-17 receptor regulate signal transduction and target gene expression. Proc Natl Acad Sci U S A **2007**;104:7506-11
267. Lindemann MJ, Hu Z, Benczik M, Liu KD, Gaffen SL. Differential regulation of the IL-17 receptor by gammac cytokines: inhibitory signaling by the phosphatidylinositol 3-kinase pathway. J Biol Chem **2008**;283:14100-8
268. Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, Ross JF, Qiu Y, Whitters MJ, Tomkinson KN, Dunussi-Joannopoulos K, Carreno BM, Collins M, Wolfman NM. Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Biol Chem **2007**;282:13447-55
269. Chang SH, Dong C. A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. Cell Res **2007**;17:435-40

270. Gorczynski RM. IL-17 Signaling in the Tumor Microenvironment. Adv Exp Med Biol **2020**;1240:47-58
271. Mitra A, Raychaudhuri SK, Raychaudhuri SP. IL-17 and IL-17R: an auspicious therapeutic target for psoriatic disease. Actas Dermosifiliogr **2014**;105 Suppl 1:21-33
272. Monin L, Gaffen SL. Interleukin 17 Family Cytokines: Signaling Mechanisms, Biological Activities, and Therapeutic Implications. Cold Spring Harb Perspect Biol **2018**;10
273. Goepfert A, Barske C, Lehmann S, Wirth E, Willemsen J, Gudjonsson JE, Ward NL, Sarkar MK, Hemmig R, Kolbinger F, Rondeau JM. IL-17-induced dimerization of IL-17RA drives the formation of the IL-17 signalosome to potentiate signaling. Cell Rep **2022**;41:111489
274. Kolbinger F, Loesche C, Valentin MA, Jiang X, Cheng Y, Jarvis P, Peters T, Calonder C, Bruin G, Polus F, Aigner B, Lee DM, Bodenlenz M, Sinner F, Pieber TR, Patel DD. β-Defensin 2 is a responsive biomarker of IL-17A-driven skin pathology in patients with psoriasis. J Allergy Clin Immunol **2017**;139:923-32.e8
275. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol **2005**;6:1123-32
276. Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell **2006**;126:1121-33
277. Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, Wang YH, Schluns KS, Broaddus RR, Zhu Z, Dong C. Regulation of inflammatory responses by IL-17F. J Exp Med **2008**;205:1063-75
278. Bugaut H, Aractingi S. Major Role of the IL17/23 Axis in Psoriasis Supports the Development of New Targeted Therapies. Front Immunol **2021**;12:621956
279. Kumar R, Theiss AL, Venuprasad K. RORγt protein modifications and IL-17-mediated inflammation. Trends Immunol **2021**;42:1037-50
280. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity **2009**;31:331-41
281. Ribot JC, deBarros A, Pang DJ, Neves JF, Peperzak V, Roberts SJ, Girardi M, Borst J, Hayday AC, Pennington DJ, Silva-Santos B. CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. Nat Immunol **2009**;10:427-36
282. Bird L. NKT cells join the IL-17 gang. Nature Reviews Immunology **2008**;8:324-
283. Price AE, Reinhardt RL, Liang HE, Locksley RM. Marking and quantifying IL-17A-producing cells in vivo. PLoS One **2012**;7:e39750
284. Michel ML, Keller AC, Paget C, Fujio M, Trottein F, Savage PB, Wong CH, Schneider E, Dy M, Leite-de-Moraes MC. Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia. J Exp Med **2007**;204:995-1001

285. Jetten AM, Kurebayashi S, Ueda E. The ROR nuclear orphan receptor subfamily: critical regulators of multiple biological processes. Prog Nucleic Acid Res Mol Biol **2001**;69:205-47
286. Xiao S, Yosef N, Yang J, Wang Y, Zhou L, Zhu C, Wu C, Baloglu E, Schmidt D, Ramesh R, Lobera M, Sundrud MS, Tsai PY, Xiang Z, Wang J, Xu Y, Lin X, Kretschmer K, Rahl PB, Young RA, Zhong Z, Hafler DA, Regev A, Ghosh S, Marson A, Kuchroo VK. Small-molecule RORγt antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. Immunity **2014**;40:477-89
287. Wang X, Zhang Y, Yang XO, Nurieva RI, Chang SH, Ojeda SS, Kang HS, Schluns KS, Gui J, Jetten AM, Dong C. Transcription of Il17 and Il17f is controlled by conserved noncoding sequence 2. Immunity **2012**;36:23-31
288. Huber M, Heink S, Pagenstecher A, Reinhard K, Ritter J, Visekruna A, Guralnik A, Bollig N, Jeltsch K, Heinemann C, Wittmann E, Buch T, Prazeres da Costa O, Brüstle A, Brenner D, Mak TW, Mittrücker HW, Tackenberg B, Kamradt T, Lohoff M. IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis. J Clin Invest **2013**;123:247-60
289. Kondo T, Takata H, Matsuki F, Takiguchi M. Cutting edge: Phenotypic characterization and differentiation of human CD8+ T cells producing IL-17. J Immunol **2009**;182:1794-8
290. Yang R, Liu Y, Kelk P, Qu C, Akiyama K, Chen C, Atsuta I, Chen W, Zhou Y, Shi S. A subset of IL-17(+) mesenchymal stem cells possesses anti-Candida albicans effect. Cell Res **2013**;23:107-21
291. Mills KHG. IL-17 and IL-17-producing cells in protection versus pathology. Nature Reviews Immunology **2023**;23:38-54
292. León B, Lund FE. IL-17-producing B cells combat parasites. Nature Immunology **2013**;14:419-21
293. Pavlov O, Selutin A, Pavlova O, Selkov S. Macrophages are a source of IL-17 in the human placenta. Am J Reprod Immunol **2018**;80:e13016
294. Katayama M, Ohmura K, Yukawa N, Terao C, Hashimoto M, Yoshifuji H, Kawabata D, Fujii T, Iwakura Y, Mimori T. Neutrophils are essential as a source of IL-17 in the effector phase of arthritis. PLoS One **2013**;8:e62231
295. Sécca C, Bando JK, Fachi JL, Gilfillan S, Peng V, Di Luccia B, Cella M, McDonald KG, Newberry RD, Colonna M. Spatial distribution of LTi-like cells in intestinal mucosa regulates type 3 innate immunity. Proceedings of the National Academy of Sciences **2021**;118:e2101668118
296. Zambrano-Zaragoza JF, Romo-Martínez EJ, Durán-Avelar Mde J, García-Magallanes N, Vibanco-Pérez N. Th17 cells in autoimmune and infectious diseases. Int J Inflam **2014**;2014:651503
297. Bailey SR, Nelson MH, Himes RA, Li Z, Mehrotra S, Paulos CM. Th17 Cells in Cancer: The Ultimate Identity Crisis. Frontiers in Immunology **2014**;5

298. Xi Y, Jingying D, Chenglong L, Hong Z, Rong Z, Xiaodong W, Chunsen W, Xiaobing H. Epigenetic Therapy Promotes the Ratio of Th1/Th17 Lineage to Reverse Immune Evasion and Treat Leukemia Relapse Post-allogeneic Stem Cell Transplantation in Non-APL AML Patients. Front Mol Biosci **2020**;7:595395
299. Wu B, Wan Y. Molecular control of pathogenic Th17 cells in autoimmune diseases. Int Immunopharmacol **2020**;80:106187
300. Zhao J, Lu Q, Liu Y, Shi Z, Hu L, Zeng Z, Tu Y, Xiao Z, Xu Q. Th17 Cells in Inflammatory Bowel Disease: Cytokines, Plasticity, and Therapies. J Immunol Res **2021**;2021:8816041
301. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol **2008**;9:1297-306
302. Sehrawat S, Rouse BT. Interplay of Regulatory T Cell and Th17 Cells during Infectious Diseases in Humans and Animals. Front Immunol **2017**;8:341
303. Jovčić G, Bugarski D, Petakov M, Krstić A, Vlaški M, Stojanović N, Milenković P. In vivo effects of interleukin-17 on haematopoietic cells and cytokine release in normal mice. Cell Proliferation **2004**;37:401-12
304. Jovcić G, Bugarski D, Krstić A, Vlaski M, Petakov M, Mojsilović S, Stojanović N, Milenković P. The effect of interleukin-17 on hematopoietic cells and cytokine release in mouse spleen. Physiol Res **2007**;56:331-9
305. Broxmeyer HE, Starnes T, Ramsey H, Cooper S, Dahl R, Williamson E, Hromas R. The IL-17 cytokine family members are inhibitors of human hematopoietic progenitor proliferation. Blood **2006**;108:770-
306. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med **1996**;183:2593-603
307. Silva WA, Jr., Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, Santos AR, Zago MA. The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells **2003**;21:661-9
308. Huang H, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, Lee Y, Kim HH. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death Differ **2009**;16:1332-43
309. Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs MK. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity **1995**;3:811-21
310. Huang H, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, Lee Y, Kim HH. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death & Differentiation **2009**;16:1332-43
311. Liao C, Zhang C, Jin L, Yang Y. IL-17 alters the mesenchymal stem cell niche towards osteogenesis in cooperation with osteocytes. J Cell Physiol **2020**;235:4466-80

312. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol **1998**;160:3513-21
313. Chen K, Kolls JK. Interluekin-17A (IL17A). Gene **2017**;614:8-14
314. Paquissi FC, Abensur H. The Th17/IL-17 Axis and Kidney Diseases, With Focus on Lupus Nephritis. Front Med (Lausanne) **2021**;8:654912
315. Normanton M, Marti LC. Current data on IL-17 and Th17 cells and implications for graft versus host disease. Einstein (Sao Paulo) **2013**;11:237-46
316. Laan M, Prause O, Miyamoto M, Sjöstrand M, Hytönen AM, Kaneko T, Lötvall J, Lindén A. A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha. Eur Respir J **2003**;21:387-93
317. Hsu H-C, Yang P, Wang J, Wu Q, Myers R, Chen J, Yi J, Guentert T, Tousson A, Stanus AL. Interleukin 17–producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. Nature immunology **2008**;9:166-75
318. Doreau A, Belot A, Bastid J, Riche B, Trescol-Biemont M-C, Ranchin B, Fabien N, Cochat P, Pouteil-Noble C, Trolliet P, Durieu I, Tebib J, Kassai B, Ansieau S, Puisieux A, Eliaou J-F, Bonnefoy-Bérard N. Interleukin 17 acts in synergy with B cell–activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus. Nature Immunology **2009**;10:778-85
319. Bi L, Wu J, Ye A, Wu J, Yu K, Zhang S, Han Y. Increased Th17 cells and IL-17A exist in patients with B cell acute lymphoblastic leukemia and promote proliferation and resistance to daunorubicin through activation of Akt signaling. J Transl Med **2016**;14:132
320. Wang F, Li Y, Yang Z, Cao W, Liu Y, Zhao L, Zhang T, Zhao C, Yu J, Yu J, Zhou J, Zhang X, Li P-P, Han M, Feng S, Ng BW-L, Hu Z-W, Jiang E, Li K, Cui B. Targeting IL-17A enhances imatinib efficacy in Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia. Nature Communications **2024**;15:203
321. Bassani B, Simonetti G, Cancila V, Fiorino A, Ciciarello M, Piva A, Khorasani AM, Chiodoni C, Lecis D, Gulino A, Fonzi E, Botti L, Portararo P, Costanza M, Brambilla M, Colombo G, Schwaller J, Tzankov A, Ponzoni M, Ciceri F, Bolli N, Curti A, Tripodo C, Colombo MP, Sangaletti S. ZEB1 shapes AML immunological niches, suppressing CD8 T cell activity while fostering Th17 cell expansion. Cell Rep **2024**;43:113794
322. Prabhala RH, Fulciniti M, Pelluru D, Rashid N, Nigroiu A, Nanjappa P, Pai C, Lee S, Prabhala NS, Bandi RL, Smith R, Lazo-Kallanian SB, Valet S, Raje N, Gold JS, Richardson PG, Daley JF, Anderson KC, Ettenberg SA, Di Padova F, Munshi NC. Targeting IL-17A in multiple myeloma: a potential novel therapeutic approach in myeloma. Leukemia **2016**;30:379-89
323. Wang S, Ma Y, Wang X, Jiang J, Zhang C, Wang X, Jiang Y, Huang H, Hong L. IL-17A Increases Multiple Myeloma Cell Viability by Positively Regulating Syk Expression. Transl Oncol **2019**;12:1086-91

324. Andreatta M, Carmona SJ. UCell: Robust and scalable single-cell gene signature scoring. Comput Struct Biotechnol J **2021**;19:3796-8
325. Becht E, McInnes L, Healy J, Dutertre C-A, Kwok IWH, Ng LG, Ginhoux F, Newell EW. Dimensionality reduction for visualizing single-cell data using UMAP. Nature Biotechnology **2019**;37:38-44
326. Zeng AGX, Iacobucci I, Shah S, Mitchell A, Wong G, Bansal S, Gao Q, Kim H, Kennedy JA, Minden MD, Haferlach T, Mullighan CG, Dick JE. Precise single-cell transcriptomic mapping of normal and leukemic cell states reveals unconventional lineage priming in acute myeloid leukemia. bioRxiv **2023**
327. Kaufmann KB, Zeng AGX, Coyaud E, Garcia-Prat L, Papalexi E, Murison A, Laurent EMN, Chan-Seng-Yue M, Gan OI, Pan K, McLeod J, Boutzen H, Zandi S, Takayanagi SI, Satija R, Raught B, Xie SZ, Dick JE. A latent subset of human hematopoietic stem cells resists regenerative stress to preserve stemness. Nat Immunol **2021**;22:723-34
328. Zeng AGX, Nagree MS, Jakobsen NA, Shah S, Murison A, Cheong J-G, Turkalj S, Lim INX, Jin L, Araújo J, Aguilar-Navarro AG, Parris D, McLeod J, Kim H, Lee HS, Zhang L, Boulanger M, Wagenblast E, Flores-Figueroa E, Wang B, Schwartz GW, Shultz LD, Josefowicz SZ, Vyas P, Dick JE, Xie SZ. Identification of a human hematopoietic stem cell subset that retains memory of inflammatory stress. bioRxiv **2023**:2023.09.11.557271
329. Jankovic M, Poon WWL, Gonzales-Losada C, Vazquez GG, Sharif-Askari B, Ding Y, Craplet-Desombre C, Ilie A, Shi J, Wang Y, Jayavelu AK, Orthwein A, Mercier F. The E3 ubiquitin ligase Herc1 modulates the response to nucleoside analogs in acute myeloid leukemia. Blood Adv **2024**;8:5315-29
330. Setty M, Kiseliovas V, Levine J, Gayoso A, Mazutis L, Pe'er D. Characterization of cell fate probabilities in single-cell data with Palantir. Nat Biotechnol **2019**;37:451-60
331. Zeng AGX, Nagree MS, Murison A, Lim I, Shah S, Aguilar-Navarro A, Araújo J, Parris D, McLeod JL, Jin L, Wagenblast E, Flores-Figueroa E, Dick JE, Xie SZ. Discovery of a New Human Hematopoietic Stem Cell Involved with Aging That Retains Memory of Immune Activation. Blood **2022**;140:2218-9
332. Schaum N, Karkanias J, Neff NF, May AP, Quake SR, Wyss-Coray T, Darmanis S, Batson J, Botvinnik O, Chen MB, Chen S, Green F, Jones RC, Maynard A, Penland L, Pisco AO, Sit RV, Stanley GM, Webber JT, Zanini F, Baghel AS, Bakerman I, Bansal I, Berdnik D, Bilen B, Brownfield D, Cain C, Chen MB, Chen S, Cho M, Cirolia G, Conley SD, Darmanis S, Demers A, Demir K, de Morree A, Divita T, du Bois H, Dulgeroff LBT, Ebadi H, Espinoza FH, Fish M, Gan Q, George BM, Gillich A, Green F, Genetiano G, Gu X, Gulati GS, Hang Y, Hosseinzadeh S, Huang A, Iram T, Isobe T, Ives F, Jones RC, Kao KS, Karnam G, Kershner AM, Kiss BM, Kong W, Kumar ME, Lam JY, Lee DP, Lee SE, Li G, Li Q, Liu L, Lo A, Lu W-J, Manjunath A, May AP, May KL, May OL, Maynard A, McKay M, Metzger RJ, Mignardi M, Min D, Nabhan AN, Neff NF, Ng KM, Noh J, Patkar R, Peng WC, Penland L, Puccinelli R, Rulifson EJ, Schaum N, Sikandar SS, Sinha R, Sit RV, Szade K, Tan W, Tato C, Tellez K, Travaglini KJ, Tropini C, Waldburger L, van

Weele LJ, Wosczyna MN, Xiang J, Xue S, Youngyunpipatkul J, Zanini F, Zardeneta ME, Zhang F, Zhou L, Bansal I, Chen S, Cho M, Cirolia G, Darmanis S, Demers A, Divita T, Ebadi H, Genetiano G, Green F, Hosseinzadeh S, Ives F, Lo A, May AP, Maynard A, McKay M, Neff NF, Penland L, Sit RV, Tan W, Waldburger L, Youngyunpipatkul J, Batson J, Botvinnik O, Castro P, Croote D, Darmanis S, DeRisi JL, Karkanias J, Pisco AO, Stanley GM, Webber JT, Zanini F, Baghel AS, Bakerman I, Batson J, Bilen B, Botvinnik O, Brownfield D, Chen MB, Darmanis S, Demir K, de Morree A, Ebadi H, Espinoza FH, Fish M, Gan Q, George BM, Gillich A, Gu X, Gulati GS, Hang Y, Huang A, Iram T, Isobe T, Karnam G, Kershner AM, Kiss BM, Kong W, Kuo CS, Lam JY, Lehallier B, Li G, Li Q, Liu L, Lu W-J, Min D, Nabhan AN, Ng KM, Nguyen PK, Patkar R, Peng WC, Penland L, Rulifson EJ, Schaum N, Sikandar SS, Sinha R, Szade K, Tan SY, Tellez K, Travaglini KJ, Tropini C, van Weele LJ, Wang BM, Wosczyna MN, Xiang J, Yousef H, Zhou L, Batson J, Botvinnik O, Chen S, Darmanis S, Green F, May AP, Maynard A, Pisco AO, Quake SR, Schaum N, Stanley GM, Webber JT, Wyss-Coray T, Zanini F, Beachy PA, Chan CKF, de Morree A, George BM, Gulati GS, Hang Y, Huang KC, Iram T, Isobe T, Kershner AM, Kiss BM, Kong W, Li G, Li Q, Liu L, Lu W-J, Nabhan AN, Ng KM, Nguyen PK, Peng WC, Rulifson EJ, Schaum N, Sikandar SS, Sinha R, Szade K, Travaglini KJ, Tropini C, Wang BM, Weinberg K, Wosczyna MN, Wu SM, Yousef H, Barres BA, Beachy PA, Chan CKF, Clarke MF, Darmanis S, Huang KC, Karkanias J, Kim SK, Krasnow MA, Kumar ME, Kuo CS, May AP, Metzger RJ, Neff NF, Nusse R, Nguyen PK, Rando TA, Sonnenburg J, Wang BM, Weinberg K, Weissman IL, Wu SM, Quake SR, Wyss-Coray T, The Tabula Muris C, Overall c, Logistical c, Organ c, processing, Library p, sequencing, Computational data a, Cell type a, Writing g, Supplemental text writing g, Principal i. Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. Nature **2018**;562:367-72

333. Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor–based signaling and implications for disease. Nature Immunology **2019**;20:1594-602
334. Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. Cytokine **2013**;64:477-85
335. Knizkova D, Pribikova M, Draberova H, Semberova T, Trivic T, Synackova A, Ujevic A, Stefanovic J, Drobek A, Huranova M, Niederlova V, Tsyklauri O, Neuwirth A, Tureckova J, Stepanek O, Draber P. CMTM4 is a subunit of the IL-17 receptor and mediates autoimmune pathology. Nat Immunol **2022**;23:1644-52
336. Becher B, Tugues S, Greter M. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. Immunity **2016**;45:963-73
337. Zeng B, Shi S, Ashworth G, Dong C, Liu J, Xing F. ILC3 function as a double-edged sword in inflammatory bowel diseases. Cell Death & Disease **2019**;10:315
338. Zhou W, Sonnenberg GF. Activation and Suppression of Group 3 Innate Lymphoid Cells in the Gut. Trends in Immunology **2020**;41:721-33

339. Geng H, Li R, Feng D, Zhu Y, Deng L. Role of the p38/AKT Pathway in the Promotion of Cell Proliferation by Serum Heat Inactivation. Int J Mol Sci **2023**;24
340. Sato A, Yokota A, Hayashi Y, Kamio N, Sagai S, Maekawa T, Hirai H. C/EBPβ Isoforms Regulate Proliferation and Differentiation of Regenerating Hematopoietic Stem/Progenitor Cells. Blood **2019**;134:3713-
341. Goepfert A, Barske C, Lehmann S, Wirth E, Willemsen J, Gudjonsson JE, Ward NL, Sarkar MK, Hemmig R, Kolbinger F, Rondeau J-M. IL-17-induced dimerization of IL-17RA drives the formation of the IL-17 signalosome to potentiate signaling. Cell Reports **2022**;41
342. Wu L, Wang C, Boisson B, Misra S, Rayman P, Finke JH, Puel A, Casanova JL, Li X. The differential regulation of human ACT1 isoforms by Hsp90 in IL-17 signaling. J Immunol **2014**;193:1590-9
343. Zhang C-J, Wang C, Jiang M, Gu C, Xiao J, Chen X, Martin BN, Tang F, Yamamoto E, Xian Y, Wang H, Li F, Sartor RB, Smith H, Husni ME, Shi F-D, Gao J, Carman J, Dongre A, McKarns SC, Coppieters K, Jørgensen TN, Leonard WJ, Li X. Act1 is a negative regulator in T and B cells via direct inhibition of STAT3. Nature Communications **2018**;9:2745
344. Zhou Y, Toh M-L, Zrioual S, Miossec P. IL-17A versus IL-17F induced intracellular signal transduction pathways and modulation by IL-17RA and IL-17RC RNA interference in AGS gastric adenocarcinoma cells. Cytokine **2007**;38:157-64
345. Ajibade AA, Wang HY, Wang R-F. Cell type-specific function of TAK1 in innate immune signaling. Trends in Immunology **2013**;34:307-16
346. Xu YR, Lei CQ. TAK1-TABs Complex: A Central Signalosome in Inflammatory Responses. Front Immunol **2020**;11:608976
347. Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol **2019**;20:1594-602
348. Maik-Rachline G, Lifshits L, Seger R. Nuclear P38: Roles in Physiological and Pathological Processes and Regulation of Nuclear Translocation. International Journal of Molecular Sciences **2020**;21:6102
349. Maik-Rachline G, Zehorai E, Hanoch T, Blenis J, Seger R. The nuclear translocation of the kinases p38 and JNK promotes inflammation-induced cancer. Science Signaling **2018**;11:eaao3428
350. Poli V. The Role of C/EBP Isoforms in the Control of Inflammatory and Native Immunity Functions *. Journal of Biological Chemistry **1998**;273:29279-82
351. Roos AB, Berg T, Barton JL, Didon L, Nord M. Airway epithelial cell differentiation during lung organogenesis requires C/EBPα and C/EBPβ. Dev Dyn **2012**;241:911-23
352. Jules J, Li YP, Chen W. C/EBPα and PU.1 exhibit different responses to RANK signaling for osteoclastogenesis. Bone **2018**;107:104-14

353. Bach C, Staber PB, Ye M, Zhang P, Friedman AD, Delwel R, Tenen DG. PU.1 Is a Downstream Target of C/EBPα in Normal Hematopoiesis and Acute Myeloid Leukemia. Blood **2011**;118:1353-
354. Janeway C, Travers P, Walport M, Shlomchik M. Immunobiology: the immune system in health and disease. Garland Pub. New York; 2001.
355. Lawrence SM, Corriden R, Nizet V. The Ontogeny of a Neutrophil: Mechanisms of Granulopoiesis and Homeostasis. Microbiol Mol Biol Rev **2018**;82
356. Huber R, Pietsch D, Günther J, Welz B, Vogt N, Brand K. Regulation of monocyte differentiation by specific signaling modules and associated transcription factor networks. Cell Mol Life Sci **2014**;71:63-92
357. DeKoter RP, Singh H. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science **2000**;288:1439-41
358. Pang SHM, de Graaf CA, Hilton DJ, Huntington ND, Carotta S, Wu L, Nutt SL. PU.1 Is Required for the Developmental Progression of Multipotent Progenitors to Common Lymphoid Progenitors. Front Immunol **2018**;9:1264
359. Bjerregaard MD, Jurlander J, Klausen P, Borregaard N, Cowland JB. The in vivo profile of transcription factors during neutrophil differentiation in human bone marrow. Blood **2003**;101:4322-32
360. Johansen LM, Iwama A, Lodie TA, Sasaki K, Felsher DW, Golub TR, Tenen DG. c-Myc is a critical target for c/EBPalpha in granulopoiesis. Mol Cell Biol **2001**;21:3789-806
361. Dahl R, Walsh JC, Lancki D, Laslo P, Iyer SR, Singh H, Simon MC. Regulation of macrophage and neutrophil cell fates by the PU.1:C/EBPalpha ratio and granulocyte colony-stimulating factor. Nat Immunol **2003**;4:1029-36
362. Laslo P, Spooner CJ, Warmflash A, Lancki DW, Lee HJ, Sciammas R, Gantner BN, Dinner AR, Singh H. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. Cell **2006**;126:755-66
363. Hock H, Hamblen MJ, Rooke HM, Traver D, Bronson RT, Cameron S, Orkin SH. Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differentiation. Immunity **2003**;18:109-20
364. Schönheit J, Kuhl C, Gebhardt ML, Klett FF, Riemke P, Scheller M, Huang G, Naumann R, Leutz A, Stocking C, Priller J, Andrade-Navarro MA, Rosenbauer F. PU.1 level-directed chromatin structure remodeling at the Irf8 gene drives dendritic cell commitment. Cell Rep **2013**;3:1617-28
365. Feinberg MW, Wara AK, Cao Z, Lebedeva MA, Rosenbauer F, Iwasaki H, Hirai H, Katz JP, Haspel RL, Gray S, Akashi K, Segre J, Kaestner KH, Tenen DG, Jain MK. The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation. Embo j **2007**;26:4138-48
366. Kishimoto T, Akira S, Taga T. IL-6 receptor and mechanism of signal transduction. Int J Immunopharmacol **1992**;14:431-8

367. Spike AJ, Rosen JM. C/EBPß Isoform Specific Gene Regulation: It's a Lot more Complicated than you Think! J Mammary Gland Biol Neoplasia **2020**;25:1-12
368. Williams SC, Cantwell CA, Johnson PF. A family of C/EBP-related proteins capable of forming covalently linked leucine zipper dimers in vitro. Genes Dev **1991**;5:1553-67
369. Katz S, Kowenz-Leutz E, Müller C, Meese K, Ness SA, Leutz A. The NF-M transcription factor is related to C/EBP beta and plays a role in signal transduction, differentiation and leukemogenesis of avian myelomonocytic cells. Embo j **1993**;12:1321-32
370. Sato A, Hirai H, Yokota A, Tamura A, Shoji T, Kashiwagi T, Iwasa M, Fujishiro A, Miura Y, Maekawa T. C/EBPβ Isoforms Distinctively and Collaboratively Regulate the Behavior of Hematopoietic Stem and Progenitor Cells in Regenerative Conditions. Blood **2015**;126:3580-
371. Pan Z, Hetherington CJ, Zhang D-E. CCAAT/Enhancer-binding Protein Activates the CD14 Promoter and Mediates Transforming Growth Factor β Signaling in Monocyte Development *. Journal of Biological Chemistry **1999**;274:23242-8
372. Gorgoni B, Maritano D, Marthyn P, Righi M, Poli V. C/EBP beta gene inactivation causes both impaired and enhanced gene expression and inverse regulation of IL-12 p40 and p35 mRNAs in macrophages. J Immunol **2002**;168:4055-62
373. Ji Y, Studzinski GP. Retinoblastoma protein and CCAAT/enhancer-binding protein beta are required for 1,25-dihydroxyvitamin D3-induced monocytic differentiation of HL60 cells. Cancer Res **2004**;64:370-7
374. Hirai H, Zhang P, Dayaram T, Hetherington CJ, Mizuno S-i, Imanishi J, Akashi K, Tenen DG. C/EBPβ is required for 'emergency' granulopoiesis. Nature Immunology **2006**;7:732-9
375. Screpanti I, Romani L, Musiani P, Modesti A, Fattori E, Lazzaro D, Sellitto C, Scarpa S, Bellavia D, Lattanzio G, et al. Lymphoproliferative disorder and imbalanced T-helper response in C/EBP beta-deficient mice. Embo j **1995**;14:1932-41
376. Gutsch R, Kandemir JD, Pietsch D, Cappello C, Meyer J, Simanowski K, Huber R, Brand K. CCAAT/enhancer-binding protein beta inhibits proliferation in monocytic cells by affecting the retinoblastoma protein/E2F/cyclin E pathway but is not directly required for macrophage morphology. J Biol Chem **2011**;286:22716-29
377. Gutsch R, Kandemir JD, Pietsch D, Cappello C, Meyer J, Simanowski K, Huber R, Brand K. CCAAT/Enhancer-binding Protein β Inhibits Proliferation in Monocytic Cells by Affecting the Retinoblastoma Protein/E2F/Cyclin E Pathway but Is Not Directly Required for Macrophage Morphology*. Journal of Biological Chemistry **2011**;286:22716-29
378. Tissières P, Araud T, Ochoda A, Drifte G, Dunn-Siegrist I, Pugin J. Cooperation between PU.1 and CAAT/Enhancer-binding Protein β Is Necessary to Induce the Expression of the MD-2 Gene*. Journal of Biological Chemistry **2009**;284:26261-72
379. Pham T-H, Langmann S, Schwarzfischer L, El Chartouni C, Lichtinger M, Klug M, Krause SW, Rehli M. CCAAT Enhancer-binding Protein β Regulates Constitutive Gene

Expression during Late Stages of Monocyte to Macrophage Differentiation *. Journal of Biological Chemistry **2007**;282:21924-33

380. Burk O, Mink S, Ringwald M, Klempnauer KH. Synergistic activation of the chicken mim-1 gene by v-myb and C/EBP transcription factors. Embo j **1993**;12:2027-38
381. Mink S, Jaswal S, Burk O, Klempnauer KH. The v-Myb oncoprotein activates C/EBPbeta expression by stimulating an autoregulatory loop at the C/EBPbeta promoter. Biochim Biophys Acta **1999**;1447:175-84
382. Plachetka A, Chayka O, Wilczek C, Melnik S, Bonifer C, Klempnauer KH. C/EBPbeta induces chromatin opening at a cell-type-specific enhancer. Mol Cell Biol **2008**;28:2102-12
383. Kowenz-Leutz E, Pless O, Dittmar G, Knoblich M, Leutz A. Crosstalk between C/EBPbeta phosphorylation, arginine methylation, and SWI/SNF/Mediator implies an indexing transcription factor code. Embo j **2010**;29:1105-15
384. Marcinkowska E, Garay E, Gocek E, Chrobak A, Wang X, Studzinski GP. Regulation of C/EBPbeta isoforms by MAPK pathways in HL60 cells induced to differentiate by 1,25-dihydroxyvitamin D3. Exp Cell Res **2006**;312:2054-65
385. Pietras EM. Inflammation: a key regulator of hematopoietic stem cell fate in health and disease. Blood **2017**;130:1693-8
386. Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk LV, Zhang S, Lakshminarasimhan R, Chin CP, Techner JM, Will B, Nerlov C, Steidl U, Manz MG, Schroeder T, Passegué E. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. Nat Cell Biol **2016**;18:607-18