



HAL
open science

Molecular mechanisms involved in the expansion of Tet2^{-/-} hematopoietic stem cells upon stresses

Anne Stolz

► **To cite this version:**

Anne Stolz. Molecular mechanisms involved in the expansion of Tet2^{-/-} hematopoietic stem cells upon stresses. Genetics. Université Paris-Saclay, 2024. English. NNT : 2024UPASL092 . tel-04955117

HAL Id: tel-04955117

<https://theses.hal.science/tel-04955117v1>

Submitted on 18 Feb 2025

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

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

Molecular mechanisms involved in the expansion of *Tet2*^{-/-} hematopoietic stem cells upon stresses

*Mécanismes moléculaires impliqués dans l'expansion des cellules souches hématopoïétiques *Tet2*^{-/-} en réponse aux stress*

Thèse de doctorat de l'université Paris-Saclay

École doctorale n° 582, Cancérologie : biologie - médecine - santé (CBMS)
Spécialité de doctorat : Sciences du cancer
Graduate School : Life Sciences and Health. Référent : Faculté de Médecine

Thèse préparée dans la unité de recherche **Cellules souches hématopoïétiques et développement des hémopathies myéloïdes (Inserm, Université Paris-Saclay)**, sous la direction de **Emilie ELVIRA-MATELOT**, Chargée de Recherche

Thèse soutenue à Paris-Saclay, le 02 décembre 2024, par
Anne STOLZ

Composition du Jury

Membres du jury avec voix délibérative

Michaela FONTENAY Directrice de recherche, Hôpital Cochin	Présidente
Laurent DELVA LEVADNY Directeur de recherche, Université Bourgogne - Franche- Comté	Rapporteur & Examinateur
Eirini TROMPOUKI Directrice de recherche, Université Côte d'Azur	Rapportrice & Examinatrice
Sandrine SARRAZIN Chargée de Recherche, Aix Marseille Université	Examinatrice
Elina ZUEVA Ingenieur de recherche, Institut Curie – PSL University	Examinatrice

Title : Molecular mechanisms involved in the expansion of *Tet2*^{-/-} hematopoietic stem cells upon stresses

Keywords : CHIP, inflammation, transposable elements, aging, epigenetic switch

Abstract : Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the expansion of hematopoietic stem cells (HSCs) harboring somatic mutations in genes commonly mutated in myeloid leukemia, without being associated with a hematologic disease. Expansion of mutated clones is notably observed in an inflammatory context such as aging, and in response to different stresses. CHIP induces a pre-leukemic state and an increased risk of developing leukemia. However, most CHIP carriers will never develop malignancies. It is thus of major interest to understand the mechanisms by which CHIP mutations trigger HSC expansion and the emergence of the pre-leukemic clone. Two non-exclusive hypotheses could explain the expansion of CHIP-mutated clones with age and in response to stress : 1- a disadvantage of non-mutated cells, and/or 2- a competitive advantage of mutated cells. It is therefore crucial to study the effect of aging and stress on both mutated and non-mutated cells. The most frequently mutated genes in CHIP encode for epigenetic factors such as TET2, which plays a role in both DNA methylation and histone modifications. Reorganization of heterochromatin is one of the most commonly reported changes in aging. Heterochromatin, through DNA methylation and the trimethylation of lysine 9 of histone H3 (H3K9me3), is also crucial in controlling transposable element (TE). When derepressed, TEs can induce DNA damage, inflammation, and transcriptomic alterations in HSCs. Our team recently showed a loss of H3K9me3, associated with an upregulation of L1Md, the most recent subfamilies of Long Interspersed Elements LINE-1, in HSC upon aging or stress inducing a premature aging such as ionizing radiation. We showed that L1Md derepression is involved in HSC loss of function through DNA damage accumulation and transcriptomic changes. The main objectives of my thesis were 1/ to investigate if loss of heterochromatin at TEs may also be involved in the loss of HSC function upon chronic inflammation, 2/ to decipher the impact of heterochromatin changes at TEs on *Tet2*^{-/-} clonal expansion upon IR and inflammatory stresses. Using H3K9me3 CUTandTag experiments, we show that chronic injections of low-dose lipopolysaccharide (LPS) reduce H3K9me3 at L1Md in WT HSCs, as previously observed upon IR. This is associated with DNA damage accumulation, as observed by H2AX foci through immunofluorescence. Using reverse transcriptase inhibitors, we further showed that LPS-induced DNA damages are dependent on L1 expression. Inversely, we showed that LPS induced an increase of H3K9me3 at L1Md in *Tet2*^{-/-} HSCs, and did not induce damages. *Tet2*^{-/-} HSCs thus seem to be protected from the effect of chronic inflammation as compared to their WT counterparts. This may explain their expansion upon inflammation. To test this hypothesis, we performed some in vitro and in vivo competitive assays between *Tet2*^{-/-} HSCs and WT HSCs transduced with either an sh-control or a sh-L1. Interestingly, we were able to show that L1 degradation in WT HSCs prevent *Tet2*^{-/-} HSCs expansion upon inflammation. Altogether, these data suggest that *Tet2*^{-/-} HSCs expansion is dependent on L1 transcripts and their deleterious effects on WT HSCs.

Titre: Mécanismes moléculaires impliqués dans l'expansion des cellules souches hématopoïétiques *Tet2*^{-/-} en réponse aux stress.

Keywords: CHIP, inflammation, éléments transposables, vieillissement, switch épigénétique

Abstract: L'hématopoïèse clonale à potentiel indéterminé (CHIP) est définie par l'expansion de cellules souches hématopoïétiques (CSH) portant des mutations somatiques dans des gènes couramment mutés dans les leucémies myéloïdes, sans être associées à une maladie hématologique. L'expansion de clones mutés est notamment observée dans un contexte inflammatoire lié à l'âge, et en réponse à différents stress. La CHIP induit un état pré-leucémique et un risque accru de développer des leucémies. Cependant, la plupart des porteurs de CHIP ne développeront jamais de tumeurs malignes. Il est donc primordial de comprendre les mécanismes par lesquels les mutations CHIP déclenchent l'expansion des CSH et l'émergence du clone pré-leucémique. Deux hypothèses non exclusives pourraient expliquer l'expansion des clones mutés CHIP avec l'âge et en réponse au stress : 1- un désavantage des cellules non mutées, et/ou 2- un avantage compétitif pour les cellules mutées. Il est donc crucial d'étudier l'effet du vieillissement et du stress sur les cellules mutées et non mutées. Les gènes les plus fréquemment mutés dans la CHIP codent pour des facteurs épigénétiques tels que TET2, qui joue un rôle à la fois dans la méthylation de l'ADN et dans les modifications des histones. La réorganisation de l'hétérochromatine est l'un des changements les plus fréquemment observés dans le vieillissement. L'hétérochromatine, par le biais de la méthylation de l'ADN et de la triméthylation de la lysine 9 de l'histone H3 (H3K9me3), joue un rôle crucial dans le contrôle des éléments transposables (TE). Lorsqu'ils sont dérégulés, les TE peuvent induire des dommages à l'ADN, une inflammation et des altérations transcriptomiques dans les CSH. Notre équipe a récemment montré une perte de H3K9me3 associée à une augmentation des L1Md, les sous-familles les plus récentes des Long Interspersed Elements LINE-1, lors du vieillissement ou suite à des stress tels que les ra-

diations ionisantes (IR), qui induisent un vieillissement prématuré dans les CSH. Nous avons montré que la dérégulation de L1Md est impliquée dans la perte de fonction des CSH par l'accumulation de dommages à l'ADN et des altérations du transcriptome.

Les principaux objectifs de ma thèse étaient les suivants : 1/étudier si la perte d'hétérochromatine au niveau des TE peut également être impliquée dans la perte de fonction des CSH dans le contexte d'une inflammation chronique, 2/étudier l'impact des changements d'hétérochromatine au niveau des TE sur l'expansion clonale des CSH *Tet2*^{-/-} en réponse à l'irradiation et à l'inflammation chronique. Par des expériences de CUTTag H3K9me3, nous montrons que des injections chroniques de lipopolysaccharide (LPS) à faible dose réduisent la marque H3K9me3 aux L1Md dans les CSH WT, comme cela a été observé précédemment lors de l'IR. Ce phénomène est associé à l'accumulation de dommages à l'ADN, observée par des foyers H2AX en immunofluorescence. En utilisant des inhibiteurs de la reverse transcriptase, nous avons montré que les dommages à l'ADN induits par le LPS dépendent des L1. Inversement, nous avons montré que le LPS induit une augmentation de H3K9me3 aux L1Md dans les CSH *Tet2*^{-/-}, et n'induit pas de dommages à l'ADN. Les CSH *Tet2*^{-/-} semblent donc être protégées de l'effet de l'inflammation chronique par rapport à leurs homologues WT. Cela pourrait expliquer leur expansion en cas d'inflammation. Pour tester cette hypothèse, nous avons réalisé des tests de compétition *in vitro* et *in vivo* entre les CSH *Tet2*^{-/-} et les CSH WT transduites avec un sh-contrôle ou un sh-L1. De manière intéressante, nous avons montré que la dégradation de L1 dans les CSH WT empêche l'expansion des CSH *Tet2*^{-/-} en réponse à l'inflammation. Dans l'ensemble, ces données suggèrent que l'expansion des CSH *Tet2*^{-/-} dépend des transcrits L1 et de leurs effets délétères sur les CSH WT.

Résumé substantiel en français

L'hématopoïèse clonale à potentiel indéterminé (CHIP) se caractérise par l'expansion de cellules souches hématopoïétiques (CSH) portant des mutations somatiques dans des gènes fréquemment mutés dans les leucémies myéloïdes, sans qu'elles ne soient associées à une pathologie hématologique. Cette expansion des clones mutés est particulièrement observée dans des contextes inflammatoires, notamment lors du vieillissement. Le CHIP est reconnu comme un état pré-leucémique, augmentant le risque de progression vers une leucémie. Les mutations les plus fréquentes affectent des gènes codant pour des facteurs épigénétiques, tels que TET2 et DNMT3A, qui, malgré leurs fonctions opposées, entraînent des altérations similaires dans les CSH. Une réorganisation spécifique de la marque d'hétérochromatine H3K9me3, jouant un rôle clé dans la répression des éléments transposables (ET), est fréquemment observée lors du vieillissement. Ces éléments transposables peuvent perturber le transcriptome et induire un état inflammatoire. Nous émettons l'hypothèse que les modifications de l'hétérochromatine et des éléments transposables jouent un rôle central dans le déclenchement de l'hématopoïèse clonale et dans le développement de la leucémie.

Les mécanismes moléculaires impliqués dans l'expansion des clones mutés CHIP demeurent encore partiellement élucidés. L'objectif principal de cette thèse était d'explorer les mécanismes moléculaires sous-jacents à l'expansion clonale des CSH *Tet2*^{-/-} sous stress. Nos travaux ont démontré que la perte d'hétérochromatine, et en particulier la perte de H3K9me3, ainsi que la réactivation des transposons, en particulier L1Md, constituent une réponse commune au vieillissement et à divers stress (radiations ionisantes, inflammation). Ces phénomènes sont particulièrement marqués lors du vieillissement des CSH. Nous avons également observé que la réactivation des L1Md est associée à une perte de fonction des CSH en réponse au stress inflammatoire et aux radiations ionisantes, se manifestant par l'accumulation de dommages à l'ADN et des altérations transcriptomiques.

Deux hypothèses non exclusives peuvent expliquer l'expansion des clones mutés CHIP avec l'âge et en réponse au stress : 1) un désavantage des cellules non mutées et/ou 2) un avantage compétitif pour les cellules mutées. Il est également intéressant de noter qu'en réponse à l'inflammation, une différence marquée a été observée entre les CSH WT et *Tet2*^{-/-} : les CSH WT ont montré une perte de H3K9me3 au niveau des transposons L1Md, alors que les CSH *Tet2*^{-/-} ont présenté une augmentation de H3K9me3 au niveau de ces mêmes transposons. Nous avons également observé que la répression des L1Md était associée à une résistance accrue des CSH *Tet2*^{-/-} aux effets du LPS.

La diminution de H3K9me3 dans les CSH WT après exposition au LPS pourrait résulter de la réduction de l'expression de SUV39H1, tandis que l'augmentation de H3K9me3 dans les CSH *Tet2*^{-/-} pourrait être liée à une surexpression de SETDB1 dans ce contexte. Enfin, nos résultats suggèrent que l'expansion des CSH *Tet2*^{-/-} en réponse à l'inflammation pourrait être due à l'activité accrue des transcrits L1, ce qui induirait des effets délétères sur les CSH WT, conférant ainsi un avantage compétitif aux CSH *Tet2*^{-/-}.

Dans l'ensemble, ces résultats soulignent l'importance des éléments L1 et de l'inflammation chronique dans le vieillissement des CSH suggèrent également que les mutations de TET2 peuvent avoir un avantage dans des contextes environnementaux tels que l'inflammation, entraînant une dérégulation synergique de l'hétérochromatine et des gènes, conduisant finalement à la sélection et à l'expansion clonale des CSH. Cette compréhension pourrait aussi aider à identifier des voies spécifiques impliquées dans l'expansion clonale en cas de

vieillesse, de stress environnemental ou de pathologies, offrant ainsi de nouvelles perspectives thérapeutiques pour mieux contrôler ces processus pathologiques.

Table of contents

Abbreviations.....	11
Table of illustrations.....	13
Acknowledgements.....	15
Introduction	
Chapter I – Hematopoiesis.....	16
I. 1 Definition of HSCs and their function in the blood system.....	16
I. 1. 1 Ontogeny of HSCs.....	16
I. 1. 2 HSC are defined by their capacity to self-renew and to differentiate into mature blood cells on a long-term.....	17
I. 1. 2. 1 HSC are defined by their capacity to reconstitute hematopoiesis on a long term.....	17
I. 1. 2. 2 HSC are characterized by their quiescence.....	18
I. 1. 2. 3 HSCs are able to self-renew.....	19
I. 1. 2. 4 HSCs are able to differentiate into mature blood cells....	20
I. 2 Global Definition of hematopoiesis: Hematopoietic Stem Cells to mature blood cells based on organism’s need.....	20
I. 3 Hierarchical models of hematopoiesis.....	21
I. 4 Mature blood cells: Myeloid and lymphoid lineages.....	23
I. 4. 1 Myeloid lineage.....	23
I. 4. 2 Lymphoid lineage.....	24
I. 5 The Impact of the Microenvironment on Hematopoiesis via Cytokines and Chemokines.....	25
I. 5. 1 The Endosteal niche: Osteoblasts and their secretion of cytokines.....	25
I. 5. 2 Adipocytes.....	27
I. 5. 3 Mesenchymal cells.....	27
Chapter II - Epigenetics: From fundamental principles to the regulation of hematopoiesis.....	29
II. 1 An introduction to epigenetics.....	29
II. 1. 1 Epigenetics: Bridging the Gap Between Genotype and Phenotype... ..	29
II. 1. 2 Chromatin conformations.....	30
II. 1. 2. 1 Open Chromatin/Euchromatin.....	31

II. 1. 2. 2 Closed chromatin/Heterochromatin.....	31
II. 1. 3 Chromatin remodeling complexes: remodelers.....	32
II. 1. 4 Histone Modifications.....	32
II. 1. 4. 1 Writers, readers and erasers of histone modifications...34	
II. 1. 4. 2 Acylation marks: Metabolically regulated and involved in gene regulation.....	34
II. 1. 4. 3 Histone Acetylation.....	35
II. 1. 4. 4 Histone Methylation.....	35
II. 1. 4. 5 H3K9me3 in heterochromatin.....	36
II. 1. 4. 6 Histone phosphorylation: γ H2AX.....	38
II. 1. 5 The dynamic balance of DNA methylation and de-methylation...39	
II. 1. 6 RNA-centered mechanisms.....	40
II. 2 Epigenetics of hematopoiesis.....	41
II. 2. 1 Chromatin state in HSCs and it's pivotal role upon differentiation..	41
II. 2. 1. 1 Epigenetics in HSC identity.....	41
II. 2. 1. 2 Epigenetics in HSC differentiation.....	42
II. 2. 1. 3 Epigenetic regulators of hematopoiesis.....	43
II. 2. 1. 3 a) H3K9me2/3 are essential for maintaining the balance between HSC self-renewal and differentiation.....	43
II. 2. 1. 3 b) The role of epigenetic regulators in HSC self- renewal and lineage regulation.....	43
Chapter III- The dioxygenase TET2: an epigenetic regulator playing a crucial role in hematopoiesis.....	45
III. 1 The structure of TET enzymes.....	45
III. 2 Distinct expression patterns of TET enzymes in vivo.....	46
III. 3 The catalytic role of TET Proteins in DNA demethylation.....	46
III. 4 The non-catalytic role of TET2.....	47
III. 4. 1 TET2 and OGT interaction: Mechanisms of histone GlcNAcylation and gene activation.....	47
III. 4. 2 TET2 and HDAC interaction: regulation of inflammatory genes in myeloid cells.....	48
III. 4. 3 TET2 and heterochromatin at transposable elements.....	48
III. 5 Regulatory mechanisms and dependencies of the TET2 enzymes.....	49

III. 5. 1 TET2 catalytic function is dependent on iron and 2-Oxoglutarate.....	49
III. 5. 2 TET2 activity can be regulated by Vitamin C.....	49
III. 5. 3 regulation of TET2 by miR-22.....	51
III. 5. 4 Modulation of TET2 Activity by IDAX.....	51
III. 6 TET2 mutations and inactivation: Implications for hematopoiesis and human hematologic disorders.....	51
III. 6. 1 TET2 loss in murine hematopoiesis.....	51
III. 6. 2 Impact of inactivation/knockdown of TET2 on human hematopoiesis.....	52
III. 6. 3 TET2 Mutations in clonal hematopoiesis and hemopathies.....	53
Chapter IV - Effect of aging on HSCs and hematopoiesis.....	55
IV. 1 Aging results in immunosenescence and a shift towards myeloid cell differentiation.....	55
IV. 1. 1 Myeloid bias and its related hematological conditions.....	55
IV. 1. 2 Immunosenescence.....	56
IV. 2 Functional changes of HSCs upon aging.....	56
IV. 3 Molecular changes of HSCs upon aging.....	58
IV. 3. 1 DNA damage accumulation in aged HSCs.....	58
IV. 3. 2 Increased ROS due to higher level of oxidative metabolism.....	59
IV. 3. 3 The role of telomers in aging of HSCs.....	60
IV. 3. 3. 1 Telomere-centric theory of cell aging.....	60
IV. 3. 3. 2 Telomeres in HSCs aging.....	61
IV. 3. 4 Epigenetic Mechanisms Driving Aging in HSC.....	61
IV. 3. 4. 1 Epigenetic changes in HSCs upon aging.....	61
IV. 3. 4. 2 ‘Loss of Heterochromatin’ theory of aging.....	63
IV. 4 Stresses as a driver of HSC Aging.....	64
IV. 4. 1 Ionizing Radiations.....	64
IV. 4. 2 Inflammation.....	65
IV. 4. 2. 1 Effect of inflammation on HSCs.....	66
IV. 4. 2. 2 Effect of inflammation on epigenetics.....	66
Chapter V- Clonal hematopoiesis of indeterminate potential (CHIP).....	67
V. 1 Definition.....	67
V. 2 CHIP is mainly driven by mutations in epigenetic factors.....	68

V. 3 Diseases associated with CHIP.....	69
V. 3. 1 The impact of CHIP on cardiovascular and systemic diseases.....	69
V. 3. 2 Clonal hematopoiesis and its risk for hematologic malignancies...	70
V. 4 Expansion of CHIP mutated HSCs.....	73
V. 4. 1 Expansion dynamics of mutated CHIP clones.....	73
V. 4. 2 Environmental factors that expand clones.....	73
V. 4. 2. 1 The role of genotoxic stresses in the expansion of mutant clones.....	73
V. 4. 2. 2 The role of Inflammation in the expansion of mutant clones.....	74
V. 4. 2. 3 The impact of aging and inflammaging on clonal hematopoiesis.....	76
Chapter VI - Transposable elements.....	78
VI. 1 Definition.....	78
VI. 2 Classifying eukaryotic transposable elements: Retrotransposons vs. DNA transposons.....	79
VI. 2. 1 Non-LTRs elements.....	80
VI. 2. 2 LTR elements.....	82
VI. 3 TEs play a critical role in evolution.....	83
VI. 3. 1 TEs are involved in shaping the size/structure of the genome, even if it's not linked to complexity.....	83
VI. 3. 2 TEs co-opted as host sequences.....	84
VI. 4 Consequences of TE Activity.....	84
VI. 4. 1 Impact of TEs on the genome/genomic instability.....	84
VI. 4. 2 TE mediated transcriptomic alterations.....	85
VI. 4. 3 Viral Mimicry/Inflammation.....	87
VI. 5 Regulation of TEs - a balancing Act: Silencing retroelements in mammalian genomes and evolutionary strategies.....	88
VI. 5. 1 Regulation of transposable elements through DNA Methylation: From retroelement silencing to host DNA protection..	86
VI. 5. 2 Regulation through histone modifications.....	91
VI. 6 Impact of heterochromatin loss and TE upregulation on HSC aging..	92

VI. 7 TEs in cancer, the 'double-edged sword' (Fig. 29).....	93
PhD Project.....	96
Materials and Methods.....	98
Results.....	103
Discussion.....	121
Concluding remarks.....	133
Bibliography.....	135
Annex.....	176

Abbreviations

0-9

2-OG: 2 oxoglutarate

5-FU: Fluorouracil

5caC: 5-carboxylcytosine

5fC: 5-formylcytosine

5hmC: 5-hydroxymethylcytosine

5mC: 5-methylcytosine

A

AGM: aorta/gonad/mesonephros

aKG: Alpha-ketoglutarate

B

BER: Base excision repair

BM: Bone marrow

bp: base pairs

C

CCUS: CHIP/Clonal Cytopenia of Undetermined Significance

cDNA: complementary DNA

CHIP: Clonal hematopoiesis of indeterminate potential

CHRS: Clonal Hematopoiesis Risk Score

CLP: Common lymphoid progenitor

CMML: Chronic myelo-monocytic leukemia

CMP: Common myeloid progenitor

CpG: C-phosphate-G

CVD: Cardiovascular diseases

CXXC4/IDAX gene: DNA-binding CXXC domain

D

d-HSC: Dormant mouse hematopoietic stem cells

DC: Dendritic cell

DDR: DNA damage response

DNMT: DNA methyltransferase

DNMT3A: DNA methyltransferase 3 alpha

DSB: double-stranded break

dsRNA: double-stranded RNA

dsDNA: double-strand DNA

E

EGR1: early growth response 1

EN: Endonuclease

ERV: Endogenous retrovirus

ESC: Embryonic stem cells

G

G-CSF: Granulocyte colony-stimulating factor

GNAT: GCN5-N-acetyltransferase

H

H3K4me1: monomethylation of lysine 4 on histone H3 protein

H3K9me3: trimethylation of lysine 9 on histone H3 protein

H3K27me3: tri-methylation of lysine 27 on histone H3 protein

HAT: Histone acetyltransferases

HDAC: Histone deacetylases

HERV: human endogenous retroviruses

HERVK: Human endogenous retrovirus K

HMGN: High Mobility Group

Nucleosome-binding

HP1: Heterochromatin Protein 1

HSC: Hematopoietic stem cell

HSCT: Hematopoietic stem cell transplantation

HSPC: Hematopoietic stem and progenitor cell

HT: horizontal transfer

HUSH: human silencing hub

I

IAP: intracisternal A particle

IFN γ : Interferon gamma

IL-6: interleukin-6

IR: ionizing radiations

J

JmjC: jumonji C

K

KAP1: KRAB domain associated protein 1

KO: Knock Out

KRAB: Krüppel-associated box

L

L1Md: evolutionary recent mouse L1s

LINE-1: long interspersed nuclear element 1

LMPP: lymphoid-primed multipotent progenitors
LPS: lipopolysaccharide
LTR: Long terminal repeat
LT-HSC: Long-term hematopoietic stem cell

N

ncRNAs: Noncoding RNAs
NK: natural killer

M

MAT: marrow adipose tissue
MDA5: melanoma differentiation-associated protein 5
MDS: Myelo-dysplastic syndrome
MEP: Myeloid-erythroid progenitor
miRNAs: micro-RNAs
MK: megakaryocytes
MLL: myeloid/lymphoid- or mixed-lineage leukemia
MN: myeloid neoplasms
MPN: Myelo-proliferative neoplasm
MPP: Multipotent progenitor
MPP8: M-phase phosphoprotein 8
MSC: Multipotent mesenchymal stem cell
MSCs: marrow stromal cells

O

OB: Osteoblast
OGT: O-linked β -N-acetylglucosamine (O-GlcNAc) transferase
ORF: Open reading frame

P

PcG: Polycomb group proteins
piRNAs: PiWi-interacting RNAs
PMP: pre-leukemic myeloproliferation
PPR: PTH/PTHrP receptors
PRC1/PRC2: Polycomb repressive complex 1/2
PTM: post-translational modifications

R

RBC: Red blood cells
RIG-I: retinoic-acid-inducible gene I

RNaseH: ribonuclease H
RNP: ribonucleoprotein
ROS: Reactive oxygen species
RP: Random permutation
RT: reverse transcriptase

S

SCF: Stem cell factor
scRNA-seq: Single-cell RNA sequencing
SETDB1: SET domain bifurcated 1
SINE: short interspersed nuclear element
ST-HSC: Short-term hematopoietic stem cell
SWI/SNF: SWItch/Sucrose Non-Fermentable

T

TDG: Thymine DNA glycosylase
TE: Transposable element
TET: Ten-Eleven translocation
TF: Transcription factor
TFBS: transcription factor binding sites
t-MN: Therapy-related myeloid neoplasms
TNF- α : tumor necrosis factor- α
TPRT: Target primed reverse transcription
TSS: transcriptional start sites

U

UTR: untranslated region

V

VAF: Variant allele frequency

W

WBC: White blood cell
WT: Wild type

X

XIST: X-inactive specific transcript

Y:

γ -H2AX: Phosphorylation of histone H2AX

Z

ZFP: zinc-finger proteins

Table of illustrations

Introduction:

- Figure 1: Ontogeny of hematopoiesis/HSCs in humans
- Figure 2: Symmetric and asymmetric divisions of HSCs
- Figure 3: Models of HSC lineage commitment
- Figure 4: The adult bone marrow HSC niche in homeostasis
- Figure 5: Open and closed chromatin
- Figure 6: Histone molecules and nucleosomes
- Figure 7: PTMs of histones H2A, H2B, H3 and H4. Post-translational modifications (PTMs) of histone amino termini affect the structure and function of chromatin
- Figure 8: Writers, readers and erasers of H3K9me3
- Figure 9: DNA methylation and demethylation are dynamically balanced
- Figure 10: TET family. Domain structure of TET proteins
- Figure 11: Expression levels of TET2 in different tissues
- Figure 12: Regulation of TET2 activity by Vitamin C
- Figure 13: Examples of TET2 mutations as background mutations followed by additional, various mutations and their progression into different malignancies (Solary et al., 2014)
- Figure 14: The aging process significantly impacts HSCs
- Figure 15: In aging mice, there is a rise in the total count of cells possessing regenerative capabilities
- Figure 16: Telomere-centric theory of cell aging
- Figure 17: CHIP describes the accumulation of somatic mutations over an individual's lifespan due to aging and stress, potentially resulting in clonal hematopoiesis of indeterminate potential (CHIP) (Adapted from Von Bonin et al., 2021)
- Figure 18: Most frequent mutations in CHIP (Bick et al, 2020)
- Figure 19: Clonal hematopoiesis is linked to various health outcomes across multiple organ systems (Beeler et al., 2022)
- Figure 20: CHIP as a precursor state for hematological neoplasms, representing a model of progression from normal hematopoiesis to CHIP, and potentially advancing to MDS or AML in certain instances. (Steensma et al., 2015)
- Figure 21: Clonal hematopoiesis risk score (CHRS) values to determine the risk of developing hematologic diseases (Weeks et al., 2023)
- Figure 22: Classification of Transposable Elements (Adapted from Garcia-Perez et al. 2016)
- Figure 23: Retro-transposition (Copy/Paste) mechanism of non-LTRs
- Figure 24: LINE-1 and its retrotransposition process
- Figure 25: The theoretical life cycle of LTR retrotransposons
- Figure 26: Different mechanisms of TE influence on gene transcription
- Figure 27: Initiation of viral mimicry by transposable elements
- Figure 28: KRAB-ZNF-KAP1 mediated silencing
- Figure 29: Ying and Yang of transposable elements

Results:

- Figure 1: Chronic LPS treatment induces a loss of H3K9me3 at L1Md and IAP, the most recent subfamilies of L1 and LTR
- Figure 2: TE derepression and upregulation is associated with DNA damage accumulation upon LPS treatment
- Figure 3: Both *Tet2*^{-/-} and WT HSCs lose their functionality upon IR
- Figure 4: LPS induces a gain of H3K9me3 at L1Md in *Tet2*^{-/-} HSCs
- Figure 5: *Tet2*^{-/-} HSCs are protected from the deleterious effects of LPS
- Figure 6: LPS induces differential transcriptomic responses in WT and *Tet2*^{-/-} HSCs
- Figure 7: L1 degradation in WT HSCs prevents *Tet2*^{-/-} HSC expansion upon LPS treatment
- Figure 8: Increased H3K9me3 at L1Mds in *Tet2*^{-/-} HSCs upon LPS treatment might be due to an increase in SETDB1

Discussion:

- Figure 1: H3K9me3 gain in *Tet2*^{-/-} HSCs upon LPS
- Figure 2: Expression of different LINE-1 families in HSC, MPPs, CMPs and GMPs (Barbieri, 2018)
- Figure 3: *Tet2*^{-/-} leads to an upregulation of TEs and inflammatory pathways
- Figure 4: *Tet2*^{-/-} HSCs exhibit amplified HSCs in response to chronic inflammatory challenge

Acknowledgement

I would like to express my sincere gratitude to Laurent Delva-Levagny and Eirini Trompouki for their willingness to review this manuscript. I also extend my thanks to the other members of the jury—President Michaela Fontenay, Elina Zueva, and Sandrine Sarrazine—for their involvement in this thesis.

Also, I would like to thank my Suivi de These Jury consisting of Iléana Antony-Debré, Christel Guillouf, David Garrick, Elina Zueva and Daniel Gautheret.

This PhD journey wouldn't have been possible without the unwavering support of my thesis supervisors, Emilie Elvira-Matelot and Françoise Porteu, who welcomed me into their lab four years ago. Emilie, I imagine you're relieved to see your first PhD student finally completing this journey. I must admit, you were often right. Thank you for mentoring me, teaching me organization, and sharing your passion for our field. Thank you also for letting me work on the best project of all.

I am also grateful to the other members of our team. Donia, thank you for your ever-present positivity, your readiness to help, and our enjoyable gym sessions. Joy, your assistance over the past year with the major experiments lightened my load significantly, allowing me to focus on reading and writing my manuscript. I'm truly grateful. The next challenge is learning Chinese for our trip to China in January! Thank you, Mathieu, the best karate kid of all times, for your funny kind especially during the CHO presentation when I really needed some support. Audrey, thank you for managing so many lab tasks and for always having an open ear for me. Aygun, Valentine, Dana, and Anaïs, thank you for your help in the lab, whether with small tasks or conversations or improving my presentations. A big thank you to Hassan for contributing to this project and assisting with my bioinformatics analysis—thanks for helping me understand bioinformatics and for our enjoyable conversations about books or "The Office" (US version).

I would also like to thank everyone in Unit U1287 for their assistance and valuable input during lab meetings, with a special thanks to Nathalie Droin for all her help with CUT&Tag, ATAC-seq, RNA-seq, and DNA methylation. Thank you to the PFIC team, especially Philippe, Flavien, Tudor, and Cyril, for the cell sortings, ensuring I always had enough cells for my experiments, and for helping with the confocal microscope. I am also thankful to the genomic platform at Gustave Roussy for their quality controls and sequencing.

Thank you, Guillaume, my mentor outside the lab, for sharing your experience and advice with me. Thanks to you, I started reading The Guardian, watching "Utopia," and exploring the world of organoids.

Fiona, thank you for always being there when I needed someone to talk to and for helping me with applications and presentations. I know that I can always count on you.

A heartfelt thank you to my best friends in Paris, Karina and Laura, for the wonderful Wednesday nights watching "The Bachelor," chatting about life, and partying together.

Finally, to my family, thank you for your unwavering support in everything I do. You are the best.

Chapter I – Hematopoiesis

I. 1 Definition of HSCs and their function in the blood system

I. 1. 1 Ontogeny of HSCs

During vertebrate embryo development, hematopoietic cells are generated in two distinct anatomical sites. The yolk sac initiates the first, transient wave of hematopoiesis, marked by the early emergence of hematopoietic progenitors with limited developmental potential that quickly differentiate into erythro-myeloid lineages. In contrast, multipotent adult-type HSCs arise independently in the aorta/gonad/mesonephros (AGM) region and adjacent vitelline and umbilical arteries during embryogenesis (Tavian et al., 2001) (Fig. 1).

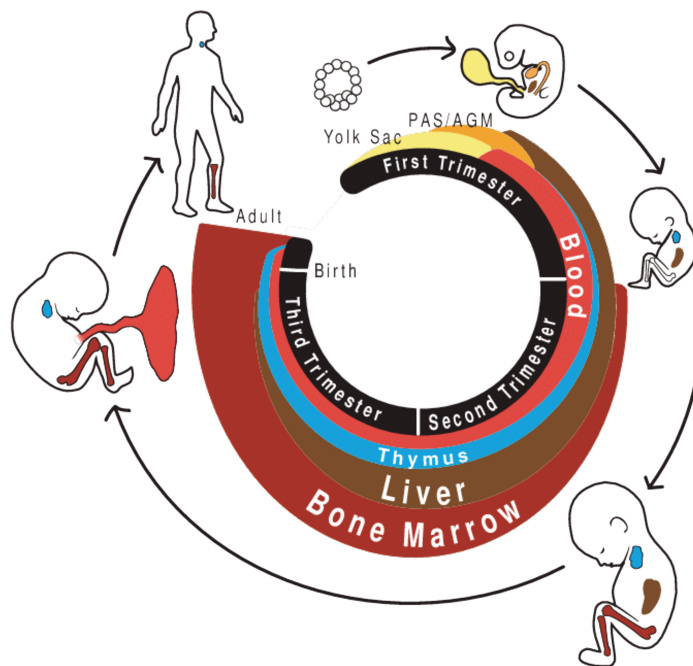


FIG. 1: Ontogeny of hematopoiesis/HSCs in humans. Changes in the anatomical location of hematopoiesis during human development are shown on a circular timeline. Hematopoiesis initially occurs in the yolk sac outside the embryo and later within the embryo in a region known as the paraaortic splanchnopleura, which subsequently develops into the aorta-gonad-mesonephros region. Around the 5th week of gestation, hematopoiesis shifts to the liver, where it remains the dominant site until mid-gestation, at which point bone marrow hematopoiesis surpasses liver activity. PAS/AGM: para-aortic splanchnopleura/aorta-gonad mesonephros (Adapted from Muench and Bárcena, 2004).

Recent studies have revealed that the placenta harbors a significant pool of HSCs. Unlike other fetal hematopoietic organs, the placenta not only supports de novo hematopoiesis but also

accumulates a large reservoir of HSCs, protecting them from signals that promote immediate differentiation (Robin et al., 2009). Indeed, Robin et al. transplanted cells isolated from human placenta throughout gestation into immunocompromised mice. PCR and flow cytometry analysis of the recipients showed the presence of engraftable human cells capable of low-level hematopoietic reconstitution as early as the first trimester and continuing until term (Robin et al., 2009). Later in gestation, the fetal liver becomes the primary site of hematopoietic activity, maintaining this role until mid-gestation. At that point, the development of blood cells is transferred to the BM, which then serves as the final and lifelong site of adult hematopoiesis (Lux et al., 2008).

I. 1. 2 HSC are defined by their capacity to self-renew and to differentiate into mature blood cells on a long-term

The life-long maintenance of blood cell production depends on the ability of HSCs to reconstitute hematopoiesis, self-renew and stay quiescent.

I. 1. 2. 1 HSC are defined by their capacity to reconstitute hematopoiesis on a long term

HSCs are identified by their functionality in in vivo repopulation experiments (McCulloch and Till, 1962). Hematopoietic stem cell transplantation (HSCT) was developed over 50 years ago and traditionally involves injecting BM into lethally irradiated recipient mice via the tail vein or retro-orbital route. Donor engraftment is then analyzed to assess the reconstitution capacity of HSCs, followed by potential secondary or tertiary engraftments to evaluate their long-term self-renewal capacity.

Long-Term and Short-Term HSCs (LT- and ST-HSCs) have different reconstitution capacities. LT-HSCs can reconstitute hematopoiesis for more than 4 months (Donnelly et al., 1999), whereas ST-HSCs can only sustain clonal populations of cells with multilineage potential for a maximum of 4 months. Multipotent progenitors (MPPs) can do so for an even shorter duration of 1-3 months (Sommerkamp et al., 2021).

These experiments allowed the identification of specific cell surface markers to differentiate these different populations. In general, the presence or absence of the following surface markers are used to identify murine HSCs: a mix of markers specifically present at the surface

of mature cells that is referred to lineage (Lin) markers: (CD11b, B220, CD3e, Ly6C/6G), Stem cell antigen-1 (Sca-1), KIT(CD117), CD34, Fms-like tyrosine kinase 3 (Flt3/Flk2), CD150 (SLAMF1) and CD48 (Table 1).

Cell types/markers	Lin	Sca-1	KIT	CD34	Flk2/Flt3	CD150	CD48
LT-HSC	-	+	+	-	-	+	-
ST-HSC	-	+	+	+	-		
MPP1	-	+	+	+	-	+	-
MPP2	-	+	+	+	-	+	+
MPP3	-	+	+	+	-	-	+
MPP4	-	+	+	+	+	-	+
LSKs	-	+	+				

Table 1: Specific murine surface markers to identify different HSC and progenitor populations

Human HSC markers include Lin, CD34, CD38, CD90 (Thy-1 membrane glycoprotein), CD45RA and CD49f (Table 2).

	CD34	CD38	CD90	CD45RA	CD49f
HSC	+	-	+	-	+
MPPs	+	-	-	-	-

Table 2: Specific human surface markers to identify different HSC and progenitor populations

I. 1. 2. 2 HSC are characterized by their quiescence

Quiescence has been proposed as a fundamental property of HSCs. Cells that enter the G0 phase, undivided cells, that didn't pass the restriction point in G1, are called quiescent or dormant (Pardee, 1974). Based on computational models, it is estimated that dormant mouse HSCs (d-HSCs) divide roughly once every 145 days, totaling about five divisions throughout their lifespan. These d-HSCs predominantly have long-term self-renewal capabilities (Wilson et al., 2008). Although they typically undergo only five divisions during normal conditions, they can be promptly activated by external or internal factors or by BM injury (Essers et al., 2009; Ito et al., 2019; Wilson et al., 2008). Quiescence is believed to prevent exhaustion

(Orford and Scadden, 2008) and safeguard the cells from acquiring mutations that could lead to malignant transformation into cancer stem cells (Lobo et al., 2007; Park and Gerson, 2005; Wang and Dick, 2005). Interestingly, after the blood system is repaired, activated HSCs return to dormancy suggesting that HSCs reversibly switch between dormancy and self-renewal at the interface between homeostasis and repair.

I. 1. 2. 3 HSCs are able to self-renew

Self-renewal is the process through which HSCs undergo division, resulting in the production of additional HSCs.

To ensure the longevity of an HSC, each division must produce at least one HSC daughter cell (Iscove and Nawa, 1997; Till et al., 1964). Asymmetric cell division results in the production of two daughter cells with distinct destinies: one remains a stem cell while the other begins to differentiate. Occasionally, symmetric renewal divisions occur, generating two identical HSC daughter cells, which can increase the number of HSCs within a clone (Iscove and Nawa, 1997). This results in greater cell production and an expanded HSC clone size in the case of emergency hematopoiesis to respond to infections or massive blood loss (Ema et al., 2005). In contrast, if divisions result in neither daughter cell being an HSC, known as extinction divisions, the HSC clone will eventually diminish as committed cells differentiate and leave the system (Till et al., 1964). Fig. 2 illustrates the processes of symmetric and asymmetric divisions in HSCs.

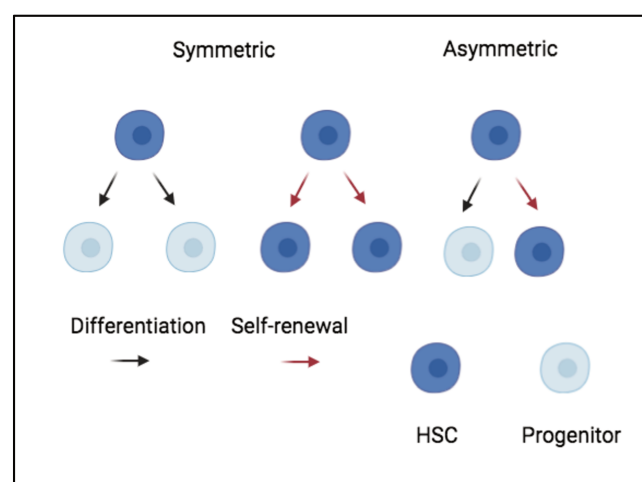


FIG. 2: Symmetric and asymmetric division of HSCs (Adapted from Sica, 2020). Symmetric division result in two identical daughter cells and asymmetric in two distinct daughter cells. In the case of HSCs. They

can differentiate into two identical progenitors or self-renew and give rise to two identical new HSCs. Asymmetric division gives rise to one HSC and one progenitor (Created with BioRender, License: PB27F4SQIN).

The core network of transcription factors (TFs)—NANOG, OCT4, SOX2, and KLF4—plays a crucial role in supporting stem cell self-renewal by orchestrating both transcriptional and epigenetic regulation of essential stem cell genes (Nichols et al., 2009; Yamanaka and Blau, 2010). While extensively studied in embryonic stem cells (ESCs), their specific functions in normal HSCs remain less understood. The reduction of OCT4, an octamer transcription factor, triggers differentiation in ESCs (Nichols et al., 1998). SOX2, shares DNA-binding sites with OCT4 and forms a heterodimer to regulate their target genes synergistically. SOX2 is involved in ESC stability through the maintenance of appropriate OCT4 expression. The OCT4/SOX2 complex regulates NANOG, which is expressed only in undifferentiated cells. NANOG is believed to prevent differentiation by suppressing differentiation genes or activating others like OCT4 (Hepburn et al., 2019). Other factors such as Klf4, also play roles in maintaining ESC pluripotency and self-renewal (Takahashi et al., 2006).

I. 1. 2. 4 HSCs are able to differentiate into mature blood cells

To initiate differentiation, stem cells have to get out of the self-renewal state, the transcriptional networks has to be dissolved, and lineages have to be chosen. How these tasks are coordinated is poorly understood. In a recent study, Parmentier et al. demonstrated that a global genome decompaction, making more than 50% of gene promoters accessible in the genome, induces stochastic activation of gene transcription in human CD34⁺ cells after stimulation with cytokines (Parmentier et al., 2022). This is followed by a gradual chromatin closure and an overall down-regulation of gene transcription. Specific regulatory actions stabilize and maintain the activity of key genes, while the rest of the genome is repressed through chromatin re-compaction. The authors propose that the fate decision process will be dependent on intrinsic and extrinsic constraints (Rommelfanger and MacLean, 2021) and the stabilization of specific regulatory networks (Xue et al., 2024). Lineage-specific transcription factors regulate gene expression by modulating chromatin accessibility, such as TCF-1, which alters chromatin accessibility at T cell-specific genes (Johnson et al., 2018).

I. 2 Global Definition of hematopoiesis: Hematopoietic Stem Cells to mature blood cells based on organism's need

Hematopoiesis is the process where hematopoietic stem cells (HSC) differentiate into immature progenitor and precursor cells finally giving rise to all the mature cells of the blood, from both the myeloid (erythrocytes, platelets, monocytes/macrophages and neutrophils) and the lymphoid (B, T, and natural killer (NK) lymphocytes) lineages (Fig. 3).

In adults, hematopoiesis occurs in the bone marrow (BM) that, in addition to hematopoietic cells (red marrow), comprises supportive stromal cells, and marrow adipose tissue (yellow marrow). Hematopoiesis is primarily located in flat bones, such as the hip bone, sternum, skull, ribs, vertebrae, and shoulder blades. Additionally, it is found in the metaphyseal and epiphyseal ends of long bones, like the femur, tibia, and humerus, where the bone is cancellous or spongy (Caon, 2020).

There is a wide range of cellular heterogeneity in the BM with various blood cells at different stages of their differentiation (Laurenti and Göttgens, 2018).

HSCs are rare cells with an estimated population of approximately 10,000 cells in mammals (Catlin et al., 2011).

I. 3 Hierarchical models of hematopoiesis

Despite the first observation of the remarkable ability of HSCs to restore the entire hematopoietic system being made over 50 years ago (Till and McCULLOCH, 1961), understanding the precise mechanisms behind this process remains a fascinating question. The classical dogma posits that hematopoiesis is a stepwise differentiation process where asymmetric division of HSCs results in one daughter cell retaining HSC characteristics and another daughter cell, known as MPP, which loses the self-renewal capacity but retains multipotent differentiation ability. According to this model, MPPs undergo sequential binary decisions until they differentiate into mature blood cell types (Akashi et al., 2000; Kondo et al., 1997) (Fig. 3A). This model, primarily derived from transplantation assays, is valuable for determining the self-renewal and lineage potential of HSCs. However, reliance on surface markers to identify HSCs may overlook the full heterogeneity of HSCs.

The advent of next-generation techniques such as single-cell RNA sequencing (scRNA-seq), combined with in vivo tracking/tracing experiments and single-cell transplantations, has challenged the classical hierarchical view of hematopoiesis (Cabezas-Wallscheid et al., 2014;

Pietras et al., 2015; Rodriguez-Fraticelli et al., 2018; Yu et al., 2017). Recent studies have identified new hematopoietic cell populations and branching points, suggesting that hematopoiesis is better represented as a continuum of differentiation rather than a strict stepwise process (Busch et al., 2015; Sun et al., 2014) (Fig. 3B). These studies have shown that HSC display heterogeneous phenotypic characteristics: quiescence and self-renewal capacity to varying degrees, differentiation bias towards a particular lineage, response to stress. These phenotypic characteristics can be transplanted into recipient mice and are therefore intrinsic to HSCs (Yu et al., 2016). Indeed, only a small fraction of HSCs produces an equal distribution of all mature blood cells, while the majority of HSCs exhibit a bias towards differentiating into specific lineages. Barcoding tools are more and more used to tackle biological questions in a non-invasive manner without the need for transplantations and the possibility to track and identify many cells. The team of Thomas Höfer developed a model called *Polylox* system to study homeostasis of the adult hematopoietic system. It is based on the introduction of long artificial substrate with unique DNA sequences flanked by LoxP sites (Pei et al., 2017). Experiments using different barcoding systems such as the Sleeping Beauty transposon-based tagging or the *Polylox* system support the idea that, except for megakaryocyte fate, MPPs likely represent the active differentiation compartment responsible for replenishing most mature blood cells (Pei et al., 2017; Rodriguez-Fraticelli et al., 2018; Sun et al., 2014). Nevertheless, despite the significant advancements brought by these single-cell techniques, they have limitations and technical challenges. Barcode-based techniques may exclude rare HSC clones due to thresholding and are not suitable to study HSC fate in native condition or in human tissues. New methods relying on endogenous clonal markers, such as the follow up of DNA methylation at single C-phosphate-G (CpG) levels, allow for lineage tracing in native conditions (Scherer et al., 2024).

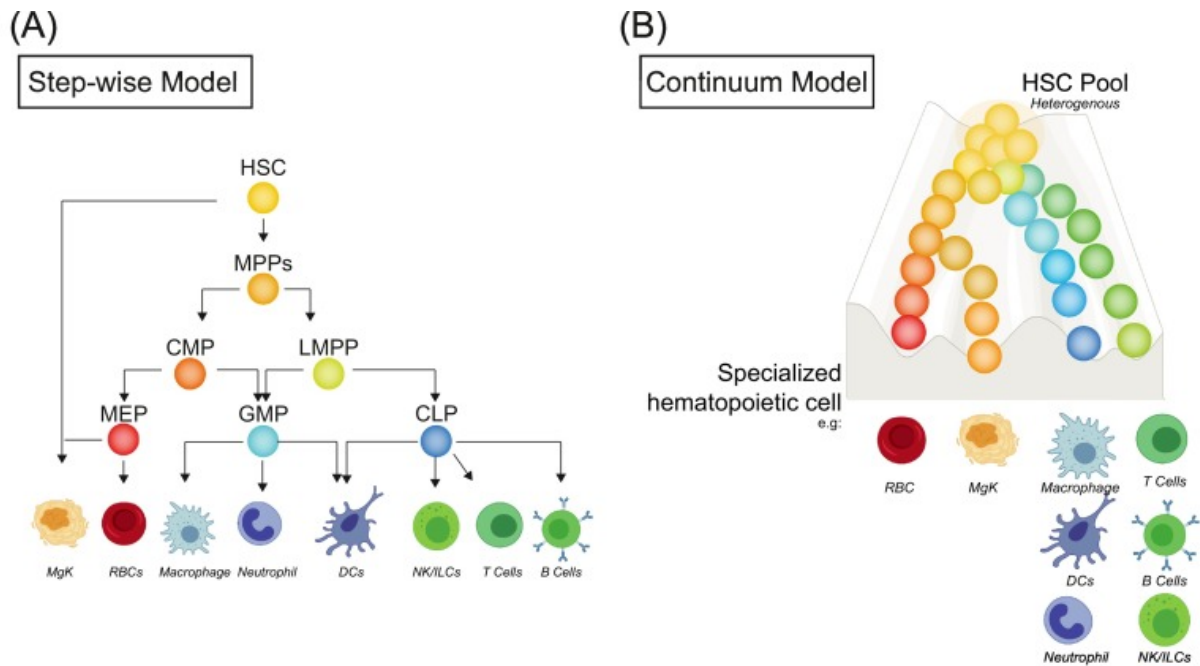


FIG. 3: Models of HSC lineage commitment (A) Stepwise model: The traditional view of hematopoiesis depicts a stepwise differentiation pattern, where HSCs are thought to contribute equally to all blood cell lineages. (B) Continuum model: hematopoiesis may rather follow a continuum of differentiation. Abbreviations: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; DCs, dendritic cells; GMP, granulocyte/macrophage progenitor; HSCs, hematopoietic stem cells; ILCs, innate lymphocyte cells; LMPP, lymphoid/myeloid progenitor; MEP, megakaryocyte/erythroid progenitor; MgK, megakaryocyte; MPP, multipotent progenitor; NK, natural killer; RBCs, red blood cells (Rodrigues et al., 2021).

I. 4 Mature blood cells: Myeloid and lymphoid lineages

I. 4. 1 Myeloid lineage

Myeloid lineage encompasses megakaryocytes, erythrocytes, and immune myeloid cells such as monocytes, macrophages, and dendritic cells (DCs), all originating from a common myeloid progenitor (CMP) in the BM. These cells are produced in the BM, and the fetal liver during embryonic development, and continue to be generated throughout adult hematopoiesis (Collin and Bigley, 2016). It is now understood that macrophages, and likely DCs, can also be derived from the embryonic yolk sac (Hoeffel et al., 2012). Myeloid cells are the earliest immune cells to develop in the body and represent a diverse group of cells that include phagocytic and granulocytic cells. These cells either quickly migrate into tissues from the bloodstream or maintain themselves locally as resident sensors, patrolling the environment. Their ability to migrate and their advanced sensory capabilities allow myeloid immune cells to detect

disturbances such as cell death or infections, thereby triggering an immediate, specific local innate immune response (Geissmann et al., 2010; Manz and Boettcher, 2014). The innate immune system is considered a non-specific defense mechanism, while the adaptive immune system is specific. The innate immune system is the one you are born with.

I. 4. 2 Lymphoid lineage

Lymphocyte development is a meticulously controlled process where immature lymphoid progenitors are derived from HSCs and mature through specific stages of differentiation. For instance, lymphoid-primed multipotent progenitors (LMPP) retain some myeloid potential but ultimately give rise to lymphoid progeny. Common lymphoid progenitors (CLPs) are descendants of LMPP that are destined to generate B lymphocytes, NKs lymphocytes and T cells.

As CLPs mature, they sequentially produce pre-pro-B cells, pro-B cells, pre-B cells, and finally, newly formed B lymphocytes (Hardy et al., 2007; Monroe and Dorshkind, 2007). B cells produce antibodies that target invading bacteria, viruses, and toxins. Maturation of B cells occur in the spleen (Petrie and Kincade, 2005).

T cells destroy the body's own cells that have been infected by viruses or have become cancerous. They mature in the thymus (Petrie and Kincade, 2005). The thymus manufactures new T cells throughout life but lacks self-renewing potential. Instead, replenishment depends on the recruitment of BM-derived progenitors that circulate in the blood (Petrie and Kincade, 2005).

Mature blood cells are predominantly short lived, that's why stem cells are required throughout life to provide multi lineage progenitors (Orkin and Zon, 2008). The high regeneration capacity of blood is one of its most important characteristics, with approximately one trillion cells arising each day in human BM (Table 3).

	RBC	WBC	Platelets	Lymphocytes
Rate/number	~200 billion	~10 billion	~400 billion	~45 million
Lifespan	~120 days	~1-3 days	~9-12 days	~1 week-few months

Table 3: Rate/number of different mature cells and their lifespan

Hematopoiesis is a complex, tightly regulated process that ensures precise adjustments in blood cell production to meet the organism's needs under normal physiological conditions and in response to external challenges.

I. 5 The Impact of the Microenvironment on Hematopoiesis via Cytokines and Chemokines

HSCs regulate themselves through cell-autonomous/intrinsic processes such as DNA damage response (DDR)/repair, reactive oxygen species (ROS), cell polarity or epigenetic modifications, but they are also heavily influenced by signals from their surrounding microenvironment (Fig.4), known as the niche. HSC regulation involves both cell-cell interactions and various secreted signals, such as cytokines and hormones (Hoffman and Calvi, 2014). These signals from the microenvironment play a crucial role in regulating HSC fate decisions, self-renewal, and residence in the marrow, and are essential for maintaining the stem cell pool.

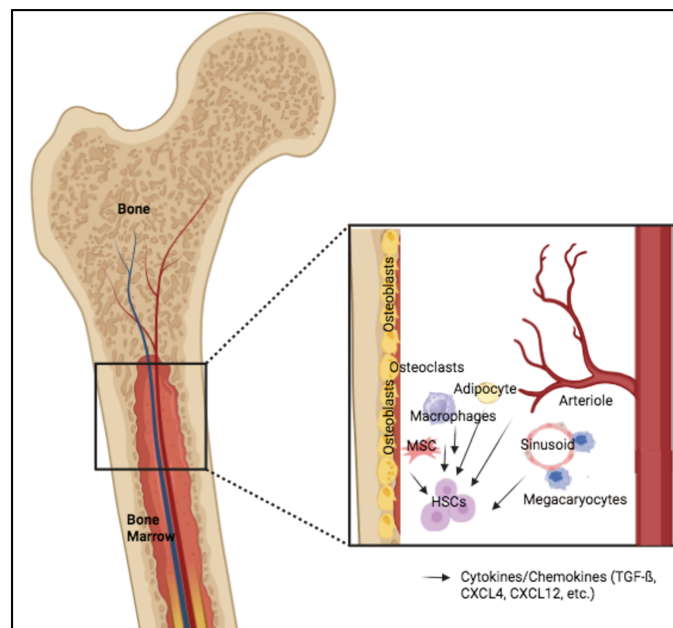


FIG. 4: The adult bone marrow HSC niche in homeostasis (Adapted from Pinho and Frenette 2019). HSCs are influenced by a variety of hematopoietic and non-hematopoietic cells including osteoblasts, mesenchymal cells, adipocytes, macrophages or megakaryocytes (Created with BioRender, License: IY27F4SZRD).

I. 5. 1 The Endosteal niche: Osteoblasts and their secretion of cytokines

The role of osteoblasts (OBs) in hematopoietic stem and progenitor cell (HSPC) maintenance and expansion within the BM has been a subject of significant research interest. Taichman and Emerson first highlighted the importance of osteoblastic lineage cells in regulating HSPC function (Taichman and Emerson, 1998). Subsequent studies using genetic models, including mice engineered to produce OB-specific, activated PTH/PTHrP receptors (Calvi et al., 2003) or mutant mice with conditional inactivation of bone morphogenetic protein (BMP) receptor type IA (Zhang et al., 2003), provided compelling evidence that activation of OBs could indeed lead to an expansion of the HSPC pool within the marrow, enhancing their long-term repopulating ability. LT-HSCs from OB-ablated mice exhibit a loss of quiescence, decreased long-term engraftment, and diminished self-renewal capacity (Bowers et al., 2015). A decrease in OB numbers favors myeloid expansion while suppressing lymphoid and erythroid expansion (Krevvata et al., 2014). Interestingly, this shift toward a myeloid bias at the expense of lymphopoiesis resembles characteristics of hematopoietic aging, that will be discussed in chapter IV. Moreover, recent investigations have shed light on the potential specialization of OBs in supporting lymphopoiesis and lymphoid tissue function (Adams et al., 2009). This emerging understanding suggests that OBs may constitute a specialized niche within the BM environment for lymphoid progenitors (Hoffman and Calvi, 2014). OBs express the chemokine CXCL12, also known as Stromal Derived Factor-1, which constitutes a signaling axis that governs HSPC self-renewal and residence within the BM. Although not a hormone, this axis serves as a central pathway through which cellular and hormonal signals from the microenvironment are conveyed to HSPCs (Nagasawa et al., 1996). These findings not only underscore the multifaceted roles of OBs in hematopoiesis but also highlight their significance in shaping the immune landscape within the BM microenvironment.

More recently, it has been proposed that the inflammatory cytokine granulocyte-colony stimulating factor (G-CSF) reprograms BM stromal cells, including CXCL12-abundant reticular cells and OBs, leading to the suppression of B lymphopoiesis in mice (Day et al., 2015). G-CSF, a mobilizing agent for HSPCs, functions by lowering CXCL12 levels in the marrow while simultaneously upregulating CXCR4 expression.

These findings highlight the capacity of nonhematopoietic cells like OBs to regulate the stem cell pool through the production of CXCL12 and by controlling HSPC cell cycle dynamics and their movement out of the marrow.

I. 5. 2 Adipocytes

While various cell types have been recognized for their positive regulatory roles in HSPCs within the marrow, adipocytes have been identified as suppressive factors. The role of adipocytes in hematopoiesis is complex and controversial, potentially mediated by multiple mechanisms. It has long been believed that adipocytes inhibit the differentiation of HSCs. This was first reported by Naveiras et al., who found that marrow adipose tissue (MAT) negatively regulates hematopoiesis, as evidenced by the reduced generation of HSCs and short-term progenitors in the adipocyte-rich caudal vertebrae compared to the adipocyte-free thoracic vertebrae of mice (Naveiras et al., 2009). Conversely, research by Zhou Bo suggests that MAT can positively regulate HSC differentiation. They found that after irradiation, the physiological HSC niche, including endothelial cells and marrow stromal cells (MSCs), is temporarily destroyed, prompting MSCs to rapidly initiate adipogenic differentiation. Newly generated adipocytes then become a temporary HSC niche, maintaining basic hematopoietic function by secreting stem cell factor (SCF) (Zhou et al., 2017).

I. 5. 3 Mesenchymal cells

Multipotent mesenchymal stem cells (MSCs), capable of differentiating into OBs, have been recognized as crucial for supporting HSPCs within the BM. These MSCs are now being utilized as an innovative therapeutic approach. Previous research, such as the study by McNiece et al. in 2004, demonstrated the capacity of human MSCs to expand HSPCs *ex vivo*. Méndez-Ferrer's group identified a stromal nestin-expressing MSC population (nestin⁺ MSC) that is closely associated with putative HSCs (McNiece et al., 2004). Nestin⁺ MSCs are strictly perivascular and typically found in the central areas of the marrow, but they are also present near the endosteum, albeit at a lower frequency. Additionally, nestin⁺ MSCs are tightly associated with adrenergic nerve fibers of the sympathetic nervous system, which regulate HSC mobilization and are responsible for the circadian oscillations in circulating HSC numbers (Katayama et al., 2006; Méndez-Ferrer et al., 2008). Notably, these MSCs express higher levels of HSC maintenance factor transcripts, including CXCL12, SCF, angiopoietin-1, interleukin-7, vascular cell adhesion molecule 1, and osteopontin, compared to any other stromal cell type, including OBs.

Other hematopoietic and non-hematopoietic cells, such as macrophages, and signals from the circadian rhythm and the sympathetic nervous system, play a role in regulating HSC quiescence

and self-renewal (Hoffman and Calvi, 2014). However, these topics will not be further discussed.

Chapter II - Epigenetics: From fundamental principles to the regulation of hematopoiesis

II. 1 An introduction to epigenetics

II. 1. 1 Epigenetics: Bridging the Gap Between Genotype and Phenotype

All somatic cells in an individual contain identical genetic information. However, their specialization and unique function are regulated by an additional layer of information applied to the DNA or histone proteins referred to as epigenetics. Originally, the term “epigenetics” referred to phenomena unexplained by genetic principles. Conrad Waddington (1905–1975) defined epigenetics as “the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being” (Waddington, 1942). Epigenetics involves heritable changes in gene expression that do not stem from alterations in the DNA sequence, unlike mutations (Gibney and Nolan, 2010). It helps elucidate how cells and organisms with identical DNA can exhibit significant phenotypic differences. Epigenetics notably helps to understand how a "stem" cell can differentiate into different cell types with different gene expression profiles, morphologies and functions while sharing the same genetic information. Epigenetics play a crucial role in HSC identity, function and fate. Hematopoietic differentiation is a model of choice for studying the role of epigenetics in cell fate commitment.

Epigenetic modifications encompass DNA methylation, post-translational modifications (PTMs) of histones, chromatin remodelers and long or small non-coding RNA (Kosan and Godmann, 2016). These modifications shape specific chromatin structures, resulting in distinct gene expression patterns in each cell: (1) heterochromatin or closed chromatin (associated with gene repression), which is highly condensed, late to replicate, and mainly contains inactive genes, and (2) euchromatin or open chromatin (permissive to transcription), which is relatively open and contains most of the active genes (Fig. 5).

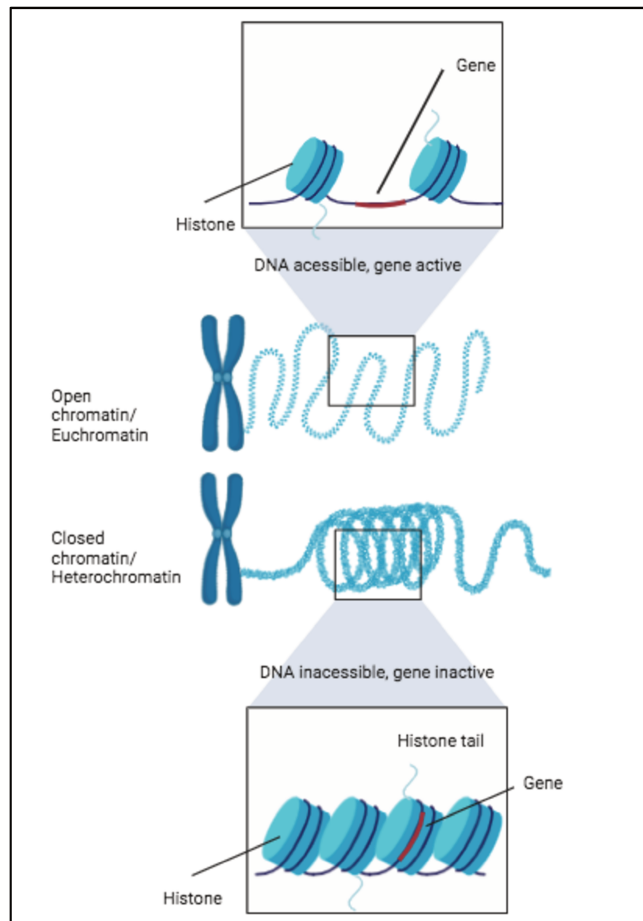


FIG. 5: Open and closed chromatin. Open chromatin, also known as euchromatin, is marked by accessible DNA, active genes, and larger spaces between nucleosomes. In contrast, closed chromatin, or heterochromatin, features inaccessible DNA, inactive genes, and a more condensed structure (Created with BioRender, License: ES27F4T654).

Epigenetic modifications play a crucial role in regulating all DNA-based processes, including transcription, DNA repair, and replication.

II. 1. 2 Chromatin conformations

The DNA of a eukaryotic cell, which can be several meters long when fully unwound, is compacted in the cell nucleus by wrapping around nucleosomes.

In each nucleosome, approximately two super helical turns of DNA encircle an octamer of core histone proteins composed of four histone partners: that are assembled in a sequential manner through deposition of a H3-H4 tetramer onto DNA followed by cooperative deposition of two H2A-H2B dimers (Böhm et al., 2011; Luger et al., 1997) (Fig. 6). The chromatin fiber consists

of a repeat of nucleosomes separated by naked DNA. This conformation is stabilized by a fifth linker histone: histone H1.

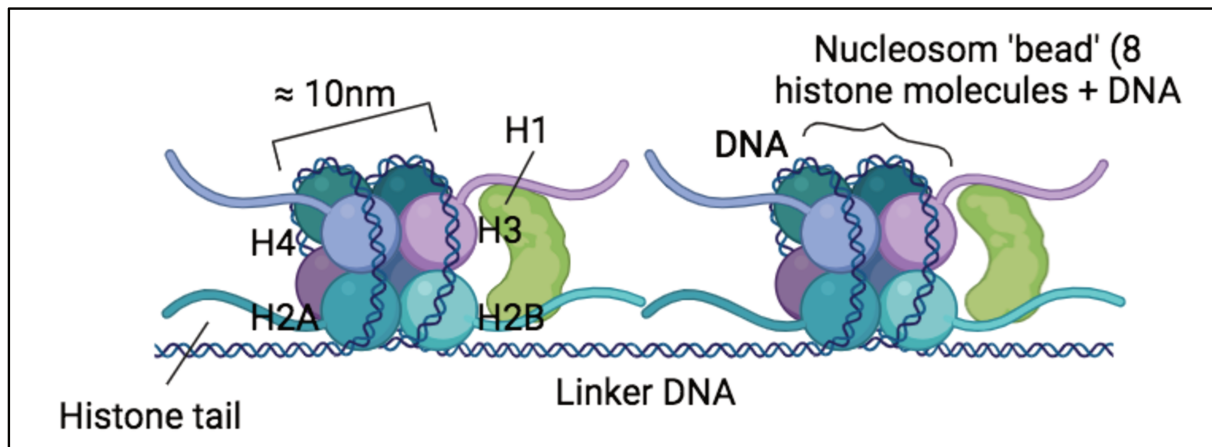


FIG. 6: Histone molecules and nucleosomes (Adapted from Chen et al.). A nucleosome contains an octamer of histone molecules, consisting of an H3-H4 tetramer and two H2A-H2B dimers. The four core histones have similar structures and an unstructured amino-terminal tail (Created with BioRender, License: IZ27F4TCII).

The position of nucleosomes, their density, and the length of time they remain in contact with chromatin play a decisive role in the accessibility of TFs to their DNA-binding sites, and thus to gene transcription.

The organization of nucleosomes is not uniform along the genome, and characterizes the different chromatin structures, more or less permissive to gene transcription.

II. 1. 2. 1 Open Chromatin/Euchromatin

Euchromatin is characterized by low DNA methylation, higher acetylation and accessibility to transcription machinery, particular histone modifications and variants that facilitate active transcription. Open chromatin is crucial for TF binding and the subsequent regulation of gene expression. It is associated with gene-rich genomic regions, with wider spacing between nucleosomes (Adam et al., 2001).

II. 1. 2. 2 Closed chromatin/Heterochromatin

Heterochromatin is a fundamental and highly conserved component of eukaryotic genomes, occupying a significant portion, ranging from approximately 25% to 90% (International Human Genome Sequencing Consortium et al., 2001a; Vicient and Casacuberta, 2017) that differs from euchromatin in its dense structure, abundance of repetitive DNA sequences, low transcription activity, low level of histone acetylation, high levels of DNA methylation at CpG dinucleotides (Epsztejn-Litman et al., 2008) and unique replication timing (Allshire and Madhani, 2018). Originally detected cytologically by the pronounced dark staining with DNA dyes (Heitz, 1928), heterochromatin has since been categorized into two main types: constitutive, which forms densely compacted regions at centromeres and telomeres across many cell types; and facultative, which is more specific to certain loci or cell types (Elgin, 1996). Constitutive heterochromatin typically consists of repeat-rich sequences and inhibits recombination between chromosomes, while facultative heterochromatin is involved in silencing cell type-inappropriate genes (Bulut-Karslioglu et al., 2014; Tachibana et al., 2001).

II. 1. 3 Chromatin remodeling complexes: remodelers

Chromatin remodeling complexes known as remodelers (Khorasanizadeh, 2004) utilize ATP hydrolysis to alter the packaging state of chromatin by moving, ejecting, or restructuring nucleosomes (Saha et al., 2006).

Chromatin remodelers are part of the RNA/DNA helicase superfamily, itself divided into four subfamilies: Imitation SWItch and Chromodomain Helicase DNA-binding, which are involved in nucleosome assembly, maturation and spacing, INO80, involved in nucleosome composition change, and finally SWItch/Sucrose Non-Fermentable (SWI/SNF), which play a key role in regulating chromatin accessibility by repositioning or ejecting nucleosomes. The recruitment and activity of these remodelers can be regulated by PTMs, such as lysine acetylation in the case of SWI/SNF (Skinotis et al., 2007; Sukanuma and Workman, 2011). SWI/SNF are involved in the control of the chromatin structure (Clapier and Cairns, 2009), gene transcription and mutations in this complex are often found in cancers (Chen et al., 2023).

II. 1. 4 Histone Modifications

Histones are small, basic proteins with a globular domain and a flexible, charged NH₂-terminus (histone "tail") (Jenuwein and Allis, 2001). These domains protrude from the nucleosome

surface and contribute to stabilizing the chromatin fiber (Garcia-Ramirez et al., 1992; Tse et al., 1998; Tse and Hansen, 1997). Interestingly, histone tails undergo multiple PTMs such as acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation (Fig. 7). Each of these histone modification plays a very specific role on gene expression according to a “histone code”. Unlike other core histones that only have N-terminal tails, H2A also has a C-terminal tail. This tail varies in length between variants and serves to regulate nucleosome formation and stability by directly binding to linker DNA (Li and Kono, 2016).

Unsurprisingly, the four core histone proteins—H2A, H2B, H3, and H4—are among the most evolutionarily conserved proteins known (van Holde, 1988). Histone modifications can influence the interaction affinities of chromatin-associated proteins, leading to dynamic shifts between transcriptionally active and silent chromatin states. For instance, acetylation and methylation of lysine residues in histones H3 and H4 have been associated with either active transcription or gene repression, depending on the specific modification (Fischle et al., 2003).

The linker histone H1 organizes nucleosomes into higher-order structures, leading to increased chromatin folding and decreased transcription (Maeshima et al., 2016; Shimada et al., 2019). In contrast, the High Mobility Group Nucleosome-binding (HMGN) family of proteins competes with histone H1, promoting chromatin decompaction, increased accessibility, and enhanced local transcription (Catez et al., 2002; Ding et al., 1997). HMGN proteins do not position randomly within the genome, they preferentially co-localize with regulatory marks at active promoters, influencing nucleosome organization, DNase hypersensitivity patterns, and PTMs (Deng et al., 2013; Trieschmann et al., 1998).

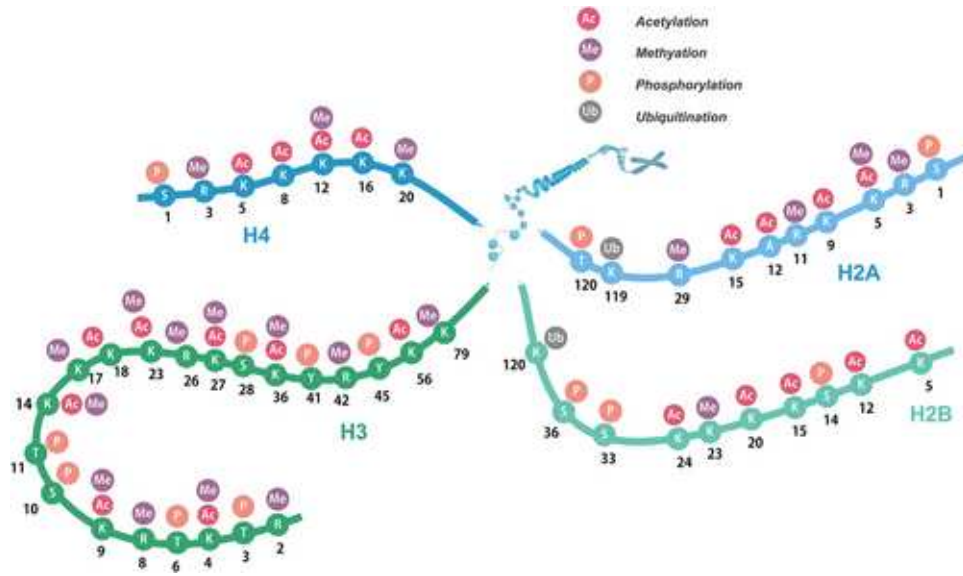


FIG. 7: PTMs of histones H2A, H2B, H3 and H4. Post-translational modifications (PTMs) of histone amino termini affect the structure and function of chromatin. In nucleosomes, DNA is wrapped around a histone octamer composed of two each of H2A, H2B, H3, and H4. Histone tails are subject to various PTMs that influence chromatin compaction and gene expression. This diagram illustrates several modifications at specific residues, including acetylation (Ac), methylation (Me), phosphorylation (P), and ubiquitination (Ub) (Liu et al., 2023).

II. 1. 4. 1 Writers, readers and erasers of histone modifications

In the realm of epigenetic regulation, there exist three key categories of participants: writers, readers, and erasers. Writers refer to chromatin-modifying enzymes responsible for adding modifications to histones. Readers, on the other hand, are proteins tasked with interpreting the signals inscribed by the writers. Lastly, erasers denote enzymes dedicated to removing the chemical information encapsulated within the PTMs (Boukas et al., 2019).

II. 1. 4. 2 Acylation marks: Metabolically regulated and involved in gene regulation

The epigenome including histone acylations are dynamically regulated and adapt in response to various metabolic changes, such as glucose deprivation or the loss of metabolic enzymes. It has been shown that alongside acetylation, other histone modifications like butyrylation, crotonylation, and propionylation are reversibly regulated and closely associated with the central glucose metabolism (Jo et al., 2020). Acetylation, butyrylation and propionylation were identified as active chromatin marks promoting transcription (Kebede et al, 2017)

II. 1. 4. 3 Histone Acetylation

The N-terminal domains of the core histones undergo reversible acetylation at specific lysine residues. Lysine acetylation is almost always associated with chromatin accessibility and transcriptional activity. Indeed, acetylation neutralizes the positive charge of lysines, weakening the interactions between histones and DNA, and contributing to a more open chromatin state (Eberharter and Becker, 2002). Direct evidence linking core histone acetylation to transcriptionally active chromatin has been demonstrated by fractionating chromatin using an antibody recognizing acetylated histones, followed by DNA probing with sequences from active genes (Hebbes et al., 1988). Histone acetylation alters nucleosomal conformation (Norton et al., 1989), potentially enhancing the accessibility of transcriptional regulatory proteins to chromatin templates (Lee et al., 1993; Vettese-Dadey et al., 1996).

Histone acetylation is modulated by the opposing activities of histone acetyltransferases (HATs) (writer) and histone deacetylases (HDACs) (eraser). HATs transfer acetyl groups from acetyl-CoA to specific lysine residues on histone proteins. These enzymes, responsible for histone and non-histone protein acetylation, encompass members from the E1A binding protein p300 /CREB-binding protein, Moz, Ybf2/Sas3, Sas2, Tip60, (family of HATs), and GCN5-N-acetyltransferase families (Jeon et al., 2014). HDACs regulate gene expression through deacetylation, serving as the catalytic core of the Sin3A, nucleosome remodeling and deacetylation, and co-repressor for element-1-silencing transcription factor complexes (Jamaladdin et al., 2014).

II. 1. 4. 4 Histone Methylation

Histone methylation occurs on all basic residues: arginines, lysines, and histidines. Lysines can undergo mono-methylation (me1), di-methylation (me2), or tri-methylation (me3) (Snowden, 2002), arginines can be monomethylated (me1), symmetrically dimethylated (me2s), or asymmetrically dimethylated (me2a) on their guanidinyll group (Turner, 2005), and histidines have been reported to be monomethylated, although this occurrence appears rare and hasn't been extensively characterized (Byvoet et al., 1972; Daitoku et al., 2021).

The most thoroughly investigated histone methylation sites include histone H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79, and H4K20. Generally, methyl groups are thought

to have slower turnover rates compared to many other PTMs, and initially, histone methylation was deemed irreversible (Byvoet et al., 1972). The identification of an H3K4 demethylase, lysine-specific demethylase 1A (also known as LSD1), revealed the reversibility of histone methylation (Shi et al., 2004). In various biological contexts, some methylation events may require stable maintenance (e.g., methylation involved in transmitting a silenced heterochromatin state through mitosis), while others may need to be adaptable (e.g., during cell differentiation or response to environmental signals).

Three enzyme families have been identified to catalyze the addition of methyl groups, derived from S-adenosylmethionine, to histones. These include SET-domain-containing proteins (Rea et al., 2000) and DOT1-like proteins (Feng et al., 2002) for lysine methylation, and members of the protein arginine N-methyltransferase family for arginine methylation (Bannister and Kouzarides, 2011). Two families of demethylases have been discovered that remove methyl-lysines: amine oxidases (Shi et al., 2004) and jumonji C (JmjC)-domain-containing, iron-dependent dioxygenases (Tsukada et al., 2006; Whetstone et al., 2006). Demethylases for arginines remain less well understood.

I will focus on the tri-methylation of histone 3 lysine 9 (H3K9me3) as I mainly focus on this epigenetic mark in my thesis.

II. 1. 4. 5 H3K9me3 in heterochromatin

Constitutive heterochromatin is characterized by di- and tri-methylation of histone 3 lysine 9 (H3K9me2 and H3K9me3). These modifications are enzymatically catalyzed by a family of SET-domain-containing methyltransferases (writers, Fig. 8), with five such enzymes identified in mammals. Among these, SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), Suppressor of variegation 3-9 histone lysine methyltransferase H1 (SUV39H1), and H2 SUV39H2 contribute to both H3K9me2 and H3K9me3, all primarily involved in methylation of H3K9me3, while GLP and G9a (also known as EHMT1 and EHMT2, respectively / writers) catalyze H3K9me1 and H3K9me2. SUV39H1 and SUV39H2 can methylate H3K9me0 but prefer H3K9me1 as a substrate to establish H3K9me3 (Loyola et al., 2009; Rea et al., 2000). In contrast, SETDB1 can mono-, di-, and tri-methylate H3K9me0 in vitro (Loyola et al., 2009; Schultz et al., 2002; Wang et al., 2003; Yang et al., 2002).

The chromodomain of Heterochromatin Protein 1 (HP1) (reader), comprising three isoforms in mammals, binds to H3K9me2/me3, promoting heterochromatin compaction and spreading of H3K9me3 by self-oligomerization and recruitment of repressive histone modifiers such as SUV39H (Bannister and Kouzarides, 2011; Groh and Schotta, 2017). The human silencing hub (HUSH) complex and KAP1 (readers) are also both involved in recruiting the methyltransferase SETDB1 and heterochromatin spreading through SETDB1-mediated H3K9me3 spreading (Tchasovnikarova et al., 2015). The HUSH complex comprises a heterotrimer, consisting of three components: transcription activation suppressor, M-phase phosphoprotein 8 (MPP8), and periphilin. MPP8 contains a chromodomain that is essential to bind H3K9me3 (Tchasovnikarova et al., 2015). HUSH recruits two effectors: MORC2, an ATP-dependent chromatin remodeler that compacts chromatin, and SETDB1 as mentioned before (Tchasovnikarova et al., 2015). SUMOylated KAP1 interacts with SETDB1 and recruits it to the chromatin, leading to the deposition of H3K9me3, consequently forming regions of condensed heterochromatin (Ivanov et al., 2007; Schultz et al., 2002).

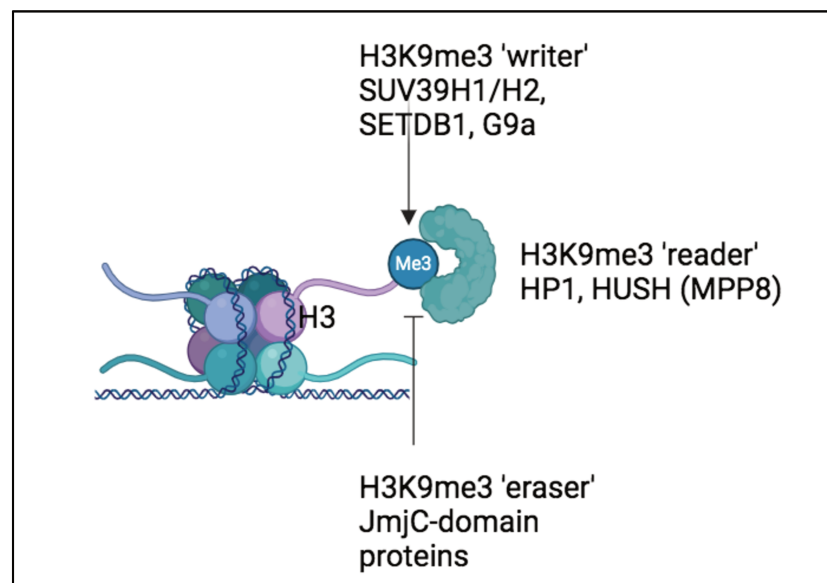


FIG. 8: Writers, readers and erasers of H3K9me3. SUV39H1/H2, SETDB1, and G9a are recognized as enzymes responsible for depositing H3K9me3 marks, referred to as 'writers'. HP1 and HUSH complexes are categorized as 'readers' of these marks, while JmjC-domain proteins function as 'erasers' to remove H3K9me3 modifications ((Adapted from Allshire and Madhani, 2018), Created with Biorender, License: LM27F4TQCL).

The pivotal role of H3K9me3 in hindering cell reprogramming (Soufi et al., 2012) and silencing lineage-specific genes (Hawkins et al., 2010) suggests that heterochromatin

maintenance is vital for cellular identity. Consequently, H3K9me3 patterns undergo reorganization during cell fate transitions in development, both in early embryos (Fadloun et al., 2013) and terminal lineage maturation, playing a crucial role in maintaining pluripotency and its exit.

Genome-wide mapping studies have elucidated the role of H3K9me3 in cell type-specific regulation of facultative heterochromatin (Hawkins et al., 2010). In differentiated human cells, H3K9me3 forms extensive contiguous domains, expanding in number and size during differentiation from pluripotency. These domains, or "patches," encompass numerous genes repressed in a cell type-specific manner (Hawkins et al., 2010). The dimethyl mark is particularly significant for silencing lineage-inappropriate genes during differentiation (Wen et al., 2009).

H3K9me3 is additionally linked with the enduring repression of transposable elements (TEs), that I will describe in detail in chapter VI. TEs are pervasive nuclear entities capable of proliferating within host genomes and inducing DNA damage and mutations in both *Drosophila* and vertebrate systems (Karimi et al., 2011; Klenov et al., 2011). TE insertions scattered across the genome often exhibit local H3K9me3 peaks within otherwise euchromatic regions. Due to its enrichment at TEs, repetitive regions, and chromosomal ends, H3K9me3-marked constitutive heterochromatin is renowned for its pivotal role in chromosome architecture and genome stability. It is essential for proper chromosome segregation and for preventing unequal recombination between repeats (Janssen et al., 2018).

II. 1. 4. 6 Histone phosphorylation: γ H2AX

Phosphorylation of H2A(X) constitutes a critical histone modification playing a pivotal role in the DDR (Rossetto et al., 2010; van Attikum and Gasser, 2005). In mammalian cells, this modification occurs on serine 139 of the H2AX variant histone, commonly known as γ H2AX (Celeste et al., 2003; Rogakou et al., 1998). This phosphorylation event takes place throughout all phases of the cell cycle and is implicated in various DDR pathways, including non-homologous end joining, homologous recombination, and replication-coupled DNA repair (Downs et al., 2000; Fernandez-Capetillo et al., 2004).

II. 1. 5 The dynamic balance of DNA methylation and de-methylation

Currently, there are four major types of DNA modifications, such as methylation at the 5th position of the pyrimidine ring of cytosine, resulting in 5-methylcytosine (5mC), or other modifications at the same position, including 5-hydroxymethyl (5hmC), or other intermediates such as 5-formyl (5fC), and 5-carboxyl (5caC) (Baylin and Jones, 2011; Wu and Zhang, 2011) (Fig. 9). 5fC and 5caC residues are less abundant than 5hmC (Ito et al., 2011) and occur on the hypomethylated promoters and enhancers of highly expressed genes in mouse ESCs (mESCs) (Neri et al., 2015). Additionally, they act as docking sites for specialized proteins with domains that specifically recognize these modifications. These readers then recruit other chromatin modifiers and remodeling enzymes, which function as the effectors of these modifications (Dawson and Kouzarides, 2012).

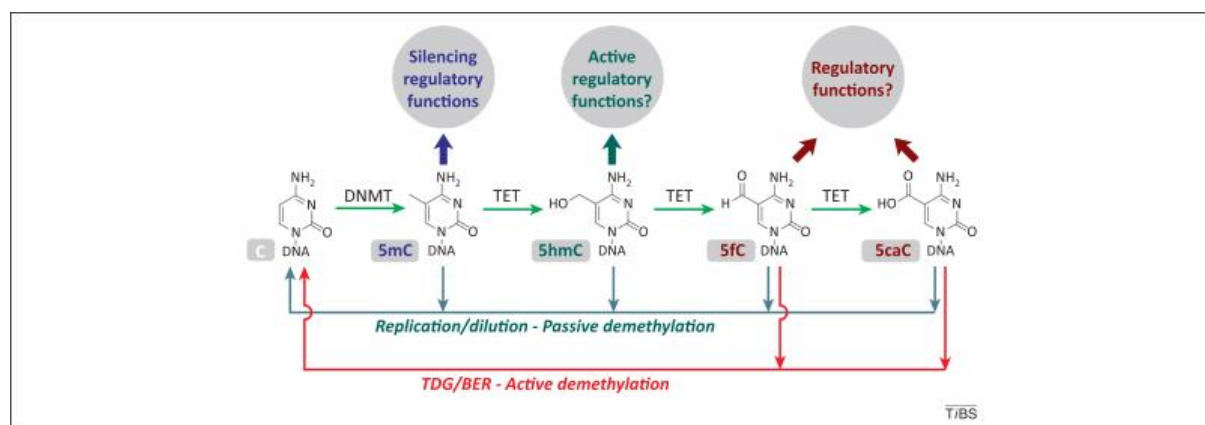


FIG. 9: The dynamic balance of DNA methylation and demethylation processes. DNA methyltransferases (DNMTs) generate 5-methylcytosine (5mC), while various pathways facilitate its demethylation. 5mC can be passively reverted to cytosine through replication-dependent dilution, occurring in the absence of DNMT activity. Additionally, TET proteins can actively oxidize 5mC to form 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), which may also undergo passive dilution during replication. Alternatively, thymine DNA glycosylase (TDG) and base excision repair (BER) can remove 5fC and 5caC, leading to active demethylation of 5mC (Song et al., 2013).

DNA methylation regulates gene expression either by attracting proteins involved in gene repression or by hindering the binding of TFs to DNA. Throughout development, the DNA methylation pattern undergoes dynamic changes due to both de novo methylation (Lienert et al., 2011) and demethylation processes. Consequently, differentiated cells acquire a stable and distinct DNA methylation pattern that governs tissue-specific gene transcription (Holliday and Pugh, 1975).

DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs), which transfer a methyl group from S-adenyl methionine to the fifth carbon of a cytosine residue to form 5mC. DNA (cytosine-5)-methyltransferase 3a (DNMT3A) and DNMT3B can establish new methylation patterns on unmodified DNA and are thus known as de novo DNMTs. In contrast, DNMT1 functions during DNA replication to copy the DNA methylation pattern from the parental DNA strand onto the newly synthesized daughter strand (Okano et al., 1999; Wu and Zhang, 2011). 5mC can be passively converted back to cytosine through replication-dependent dilution due to a lack of DNMT1 activity. TET (ten-eleven translocation) proteins catalyze the active conversion of 5mC to 5hmC, 5fC, and 5caC through three consecutive Fe (II)- and 2-oxoglutarate (2-OG)-dependent oxidation reactions which could be followed by passive replication/dilution demethylation. Alternatively, thymine DNA glycosylase (TDG) and base excision repair (BER) can remove 5fC and 5caC, leading to active demethylation of 5mC (Song et al., 2013) (Fig. 9). I will further develop the literature on TET2 in chapter III.

Generally, repetitive sequences, including transposons, centromeric, and pericentric repeats, are extensively methylated. Additionally, the gene bodies of genes that are highly expressed undergo methylation. Conversely, CpG islands, defined as CG-rich sequences ranging from 500 to 2000 base pairs commonly located in promoter regions, typically lack methylation. (Deaton and Bird, 2011).

DNA methylation plays a pivotal role in the development of mammals and is implicated in an array of biological functions. These functions encompass the regulation of transcription, the silencing of transposons, the inactivation of the X chromosome, and genomic imprinting. (Smith and Meissner, 2013).

II. 1. 6 RNA-centered mechanisms

Noncoding RNAs (ncRNAs) play diverse roles that impact nearly every biological process, spanning from DNA replication, RNA transcription, and protein translation to cell differentiation and metabolism. They can function as proto-oncogenes or tumor suppressor genes and are also involved in the epigenetic regulation of genes. Small noncoding RNAs also contribute to directing chromatin modifications, such as histone methylation. For instance, in

Schizosaccharomyces pombe, the RNAi machinery is essential for establishing and/or maintaining centromeric heterochromatin, marked by H3K9 methylation (Verdel et al., 2004).

Recent advancements in this domain suggest a critical involvement of noncoding RNA in epigenetic regulation (Rinn et al., 2007). Long ncRNAs may serve diverse roles in gene regulation, particularly in the epigenetic control of chromatin (Bernstein and Allis, 2005). An example is the silencing of the inactive X chromosome by the ncRNA X-inactive specific transcript (XIST). To balance the X chromosome copy number between male and female cells, transcription of XIST RNA from one of the two female X chromosomes recruits Polycomb group proteins (PcG) to trimethylate histone H3 on lysine 27 (H3K27me3), thereby rendering the chromosome transcriptionally silent (Plath et al., 2003).

Mounting evidence indicates that micro-RNAs (miRNAs) play crucial roles in various biological processes, including cell proliferation, differentiation, apoptosis, and hematopoiesis (Lund et al., 2004; Yi et al., 2003). Biogenesis and expression of miRNAs are subject to regulation by epigenetic modifications such as DNA methylation, RNA alterations, and histone modifications, with miRNA dysregulation being a hallmark of cancer initiation and metastasis (Humphries et al., 2019). Additionally, miRNAs control the expression of epigenetic regulators, including DNMTs and histone deacetylases (Iorio et al., 2010). miRNAs are involved in intricate double-negative feedback loops, wherein miRNA inhibition of an epigenetic regulator is reciprocally controlled at the epigenetic level by the same regulator. This miRNA–epigenetic feedback loop significantly influences gene expression levels, and disruption of this feedback loop can perturb normal physiological processes, leading to disease (Yao et al., 2019).

II. 2 Epigenetics of hematopoiesis

II. 2. 1 Chromatin state in HSCs and it's pivotal role upon differentiation

The structure of chromatin appears pivotal in defining HSC identity and in regulating gene transcription during hematopoiesis.

II. 2. 1. 1 Epigenetics in HSC identity

The role of epigenetics in defining HSC identity has notably been highlighted in mice Knock Out (KO) for the H3K9 methyltransferase SETDB1. SETDB1 KO induces the loss of HSCs and progenitors as well as ectopic expression of non-hematopoietic genes in HSCs, suggesting that the H3K9me3 mark “defines” HSC by preventing expression of non-hematopoietic genes (Koide et al., 2016).

The importance of epigenetics in defining HSC identity has been further shown in single-cell analysis (sc-ATAC-seq; sc-RNA-seq), combined with in vivo tracking/tracing experiments and single-cell transplantations. Indeed, HSC that are heterogeneous for their quiescence, self-renewal, and differentiation capacities are defined by a particular epigenetic state (DNA methylation or chromatin accessibility) at the enhancers and promoters of HSC-specific genes or genes involved in differentiation into myeloid or lymphoid lineages (Cabezas-Wallscheid et al., 2014; Lara-Astiaso et al., 2014; Weishaupt et al., 2010; Yu et al., 2017). As an example, HSCs with a lymphoid bias show weaker methylation at lymphoid enhancers and stronger methylation at myeloid enhancers, and vice versa for HSCs with a myeloid bias. The differential methylation of these enhancers does not correlate with the transcription of lymphoid or myeloid differentiation genes respectively in the HSC, but later in the cells during differentiation (Yu et al., 2017).

II. 2. 1. 2 Epigenetics in HSC differentiation

Different studies have shown that the cell fate of HSCs is programmed at the epigenetic level, with the transcriptional consequences of these epigenetic variations only being observed later during differentiation (Cui et al., 2009; Lara-Astiaso et al., 2014; Weishaupt et al., 2010).

One such example comes from Parmentier’s study, that showed that the global decompaction of the genome following cytokine stimulation precedes the stochastic activation of gene transcription in CD34⁺ human cells during the “multilineage-primed state” (Parmentier et al., 2022).

Promoter regions of many genes exhibit a compressed chromatin state in LT-HSCs and ST-HSCs compared to MPPs, indicating that only a few genes regulate HSC self-renewal, whereas many more are involved in blood cell differentiation regulation (Yu et al., 2017). ATAC-seq analyses revealed that PU.1, SPIB, and ELF5 binding motifs, which are associated with

differentiation, were less enriched in open chromatin in LT-HSCs compared to ST-HSCs and MPPs. Conversely, binding motifs for Krüppel-like factors (KLF9, KLF10, KLF14) and the ETS TF ETV2 were specifically enriched in open chromatin in LT-HSCs relative to ST-HSCs and MPPs, indicating their potential role in the maintenance of LT-HSCs (Yu et al., 2017). Many lineage-restricted promoters are linked to bivalent histone methylation, characterized by the presence of both activating and repressive marks. This bivalency would prepare genes for rapid expression (loss of the repressive mark) or repression (loss of the activating mark) during differentiation according to cell fate (Sun et al., 2014; Weishaupt et al., 2010). Indeed, bivalent promoters of genes that are expressed in T cells lose repressive marks upon differentiation (Weishaupt et al., 2010).

Lara-Astiaso's research identified TF networks that regulate chromatin dynamics and lineage specification in hematopoiesis, providing a detailed model of chromatin behavior during development. The study revealed that 60% of enhancers, defined by the presence of the H3K4me1 histone mark, are initially marked in HSCs but are activated by the addition of H3K27ac, and drive gene transcription, only within the corresponding lineage. Additionally, they found that a significant portion of dynamic enhancers are newly established during the differentiation process (Lara-Astiaso et al., 2014).

II. 2. 1. 3 Epigenetic regulators of hematopoiesis

II. 2. 1. 3 a) H3K9me2/3 are essential for maintaining the balance between HSC self-renewal and differentiation

Inhibition of G9A methyltransferase with UNC0638 (inhibitor of G9a and GLP) delays HSC differentiation by maintaining higher levels of HSC-selective genes and preventing their transition to MPP-like gene expression profiles. This is associated with reduced H3K9me2 and H3K9me3 at promoters of several upregulated genes, indicating direct regulation by G9A and that G9A promotes their silencing upon HSC differentiation. Proper H3K9 methylation by G9A is crucial for the silencing of differentiation-associated genes, highlighting its role in maintaining the balance between HSC self-renewal and differentiation (Ugarte et al., 2015).

II. 2. 1. 3 b) The role of epigenetic regulators in HSC self-renewal and lineage regulation

TET2 and DNMT3A are the most frequently mutated genes in clonal hematopoiesis of indeterminate potential (CHIP) (which I will develop in chapter VI) and in hemopathies. I will thus focus this part on these factors. Accumulating evidence suggests that these two epigenetic regulators play critical roles in both maintaining self-renewing HSCs and a balance between myeloid and lymphoid output. Mutant and KO models for *Tet2* and *Dnmt3a* show similar phenotypes including increased self-renewal and reconstitution capacity and a biased differentiation towards the myeloid lineage (myeloid bias) (Challen et al., 2014, 2012; Quivoron et al., 2011).

Different KO studies investigating the de novo methyltransferases *Dnmt3a* and *Dnmt3b* in adult HSCs revealed that their loss leads to a myeloid bias and expanding HSC numbers (Challen et al., 2014, 2012; Ostrander et al., 2020; Tadokoro et al., 2007). *Dnmt3A* is thought to play a crucial role in the choice between self-renewal and differentiation, with loss of this factor leading to HSC self-renewal rather than differentiation. Loss of *Dnmt3a* leads to the upregulation of HSC multipotency genes and a downregulation of differentiation factors. The progeny exhibited global hypomethylation and incomplete repression of HSC-specific genes (Challen et al., 2012). *Dnmt3a*-deficient HSCs also favor the erythroid differentiation program at the expense of monocyte lineage commitment (Izzo et al., 2020; Ostrander et al., 2020). The combined loss of *Dnmt3a* and *Dnmt3b* had a synergistic effect, leading to enhanced HSC self-renewal and a more severe block in differentiation compared to *Dnmt3a*-null cells, whereas the loss of *Dnmt3b* alone resulted in a mild phenotype (Challen et al., 2014).

The loss of *Tet2* leads to increased self-renewal, myelo-proliferation and an enlargement of the HSC compartment (Ko et al., 2011; Li et al., 2011; Moran-Crusio et al., 2011; Quivoron et al., 2011). Single-cell DNA methylation analyses on *Tet2*^{-/-} HSCs revealed dramatic methylation changes at CpG sites within lineage-specific TF binding motifs, resulting in disrupted transcriptional priming (Izzo et al., 2020). It was also demonstrated that 5hmC is lost and that there is a hypermethylation of enhancer elements upon the disruption of *Tet2* (Rasmussen et al., 2015).

I will develop in more details the role of TET2 in hematopoiesis in the next chapter.

Chapter III - The dioxygenase TET2: an epigenetic regulator playing a crucial role in hematopoiesis

III. 1 The structure of TET enzymes

The three enzymes of the TET family (TET1, TET2, TET3) identified in humans are evolutionarily conserved dioxygenases that catalyze DNA demethylation. I will mainly focus on TET2 in this chapter because it is the most mutated in clonal hematopoiesis and hemopathies.

The TET family was identified through the involvement of a fusion protein involving TET1 and myeloid/lymphoid- or mixed-lineage leukemia (MLL) in acute myeloid leukemia (AML) with a t(10;11) (q22;q23) translocation, hence the name Ten Eleven Translocation (TET). TET2 and TET3 were then identified by homology searches (Lorsbach et al., 2003; Ono et al., 2002). TET1 and TET3 present an amino-terminal CXXC-type zinc finger domain, and a carboxy-terminal catalytic Fe (II)- and α -ketoglutarate-dependent dioxygenase domain inserted in a cysteine-rich domain, whereas a chromosomal inversion during vertebrate evolution split TET2 gene into distinct segments encoding the catalytic domain (TET2 gene) and the DNA-binding CXXC domain (CXXC4/IDAX gene) (Fig. 10). The CXXC motif may be responsible for direct (TET1 and TET3) or indirect (TET2) DNA binding (Ko et al., 2013; Xu et al., 2012).

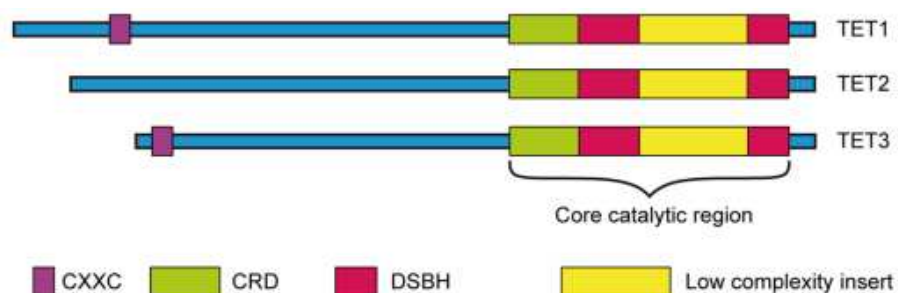


FIG. 10: TET family. Domain structure of TET proteins. All TET proteins have one core catalytic domain in the C-terminal. A CXXC domain, located in the N-terminal of TET1 and TET3, but not in TET2, confers DNA binding ability directly (Zhang, 2023). CXXC: CXXC domain, CRD: cysteine-rich domain, DSBH: double-stranded β -helix

Ko et al. (2013) showed that IDAX (CXXC4; Dvl-Binding Protein) interacts directly with the catalytic domain of TET2, leading to TET2 protein degradation through a caspase-dependent mechanism (Ko et al., 2013). The loss of IDAX in ESCs prevents TET2 degradation during ESC differentiation. shRNAs against IDAX in a human myeloid cell line upregulates TET2 and increases 5hmC levels. When the CXXC domain of IDAX is mutated, IDAX loses its ability to bind TET2 and affect 5hmC, suggesting that IDAX recruits TET2 to chromatin through its CXXC domain. This represents a unique case of epigenetic marking where IDAX recruits TET2 to the chromatin to modify DNA followed by degrading TET2 (Ko et al., 2013).

III. 2 Distinct expression patterns of TET enzymes in vivo

Different TET enzymes exhibit distinct expression patterns in vivo, with TET1 being mainly expressed in ESCs and in the brain. TET2 and TET3 are more ubiquitous, with TET2 expression predominating in a variety of differentiated tissues, especially in hematopoietic and neuronal lineages (Tahiliani et al., 2009) (Fig. 11). Besides, TET gene expression in different tissues was analyzed using the Protein Atlas database (<https://www.proteinatlas.org/>). The expression of TET2 is shown in Fig. 11, which may suggest different functions of TET2 in tissues.

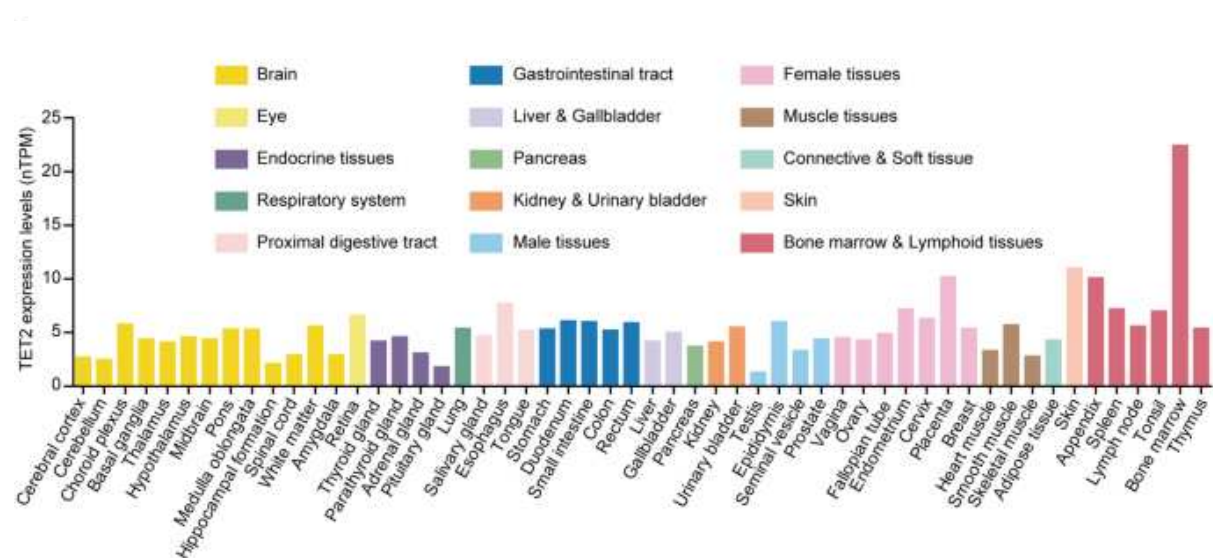


FIG. 11: Expression levels of TET2 in different tissues. The data were obtained from the protein atlas (Zhang, 2023).

III. 3 The catalytic role of TET Proteins in DNA demethylation

TET proteins (TET1-3) are Fe (II)- and α -ketoglutarate (α -KG)-dependent mammalian DNA oxidases that catalyze the conversion of 5mC to 5hmC (Tahiliani et al., 2009). The discovery of this new modification on DNA methyl cytosine has provided novel insights into DNA demethylation pathways. The TET enzymes can further oxidize 5hmC to 5fC and 5caC, which can be directly recognized and repaired by TDG-mediated BER to generate unmethylated cytosines, leading to active DNA demethylation (He et al., 2011; Ito et al., 2011) (Fig. 9). On another hand, these modified cytosines are not recognized by DNMT1 during replication, leading to passive demethylation.

III. 4 The non-catalytic role of TET2

TET2 is mostly known for its catalytic role in demethylating DNA. However, several studies have also highlighted a non-catalytic role for TET2, that involves the recruitment of histone modifiers. In this context, TET2 plays the role of either an activator or a repressor of gene transcription. TET2 mutations, which are dispersed across various exons and regions of the gene, often lead to frameshift or stop codon changes, resulting in truncated proteins and insufficient protein production, both affecting the catalytic and non-catalytic roles of TET2 (Tefferi et al., 2009).

III. 4. 1 TET2 and OGT interaction: Mechanisms of histone GlcNAcylation and gene activation

TET2 directly interacts with O-linked β -N-acetylglucosamine (O-GlcNAc) transferase (OGT), facilitating OGT-dependent histone O-GlcNAcylation through its association with chromatin. It has been shown that histones can be modified by OGT at different sites (Zhang et al., 2011), particularly at Serine 112 of histone H2B in vivo (Fujiki et al., 2011). The combined epigenetic modifications by TET2 and OGT on both DNA and histones coordinate gene transcription regulation (Chen et al., 2013). There is a significant overlap of target genes between OGT, H2B S112 GlcNAc, and TET2, with similar binding profiles to TET2 at transcriptional start sites (TSS). In wild-type ES cells, genes occupied by OGT and TET2, and enriched with H2B S112 GlcNAc, were associated with high levels of transcription. Like TET2, TET3 might also target OGT to chromatin for gene transcription regulation. Recent reports indicate that H2B GlcNAcylation is associated with active TSS and positively regulates transcription (Fujiki et al., 2011). TET2/3 co-localize with OGT on chromatin at active promoters characterized by

high levels of H3K4me3. Reduction in either TET2/3 or OGT activity results in a direct decrease in H3K4me3 and concomitant decreased transcription. Studies in *Tet2* KO mouse BM tissue support this data, showing decreases in global GlcNAcylation and H3K4me3, particularly at key regulators of hematopoiesis (Deplus et al., 2013). When TET2 and OGT bind, it is primarily for the activation of gene expression.

III. 4. 2 TET2 and HDAC interaction: regulation of inflammatory genes in myeloid cells

TET2 selectively mediates the repression of inflammatory genes during the resolution of inflammation in innate myeloid cells, including DCs and macrophages. As an example, loss of TET2 leads to the upregulation of several inflammatory mediators, including interleukin-6 (IL-6), in the late phase of the response to lipopolysaccharide (LPS) challenge. This repression mechanism, independent of DNA methylation and hydroxy-methylation, involves TET2 recruiting HDAC2 to repress *Il6* transcription via histone deacetylation (Zhang et al., 2015). In another study, Pronier et al. demonstrated in both mice and human monocytes that, even without any inflammatory challenge, downregulation of *TET2/Tet2* promotes the production of MIF (macrophage migration inhibitory factor), a key mediator of atherosclerotic lesion formation (Pronier et al., 2022). In healthy monocytes, TET2 is recruited to the *MIF* promoter and interacts with the transcription factor early growth response 1 (EGR1) and histone deacetylases. Co-immunoprecipitation experiments in human blood monocytes confirmed TET2's interaction with EGR1 and validated its ability to interact with HDAC1 and HDAC2 (Pronier et al., 2022). This provides mechanistic evidence for TET2's gene-specific transcription repression activity through histone deacetylation, preventing constant transcription activation at the chromatin level during inflammation resolution (Zhang et al., 2015).

III. 4. 3 TET2 and heterochromatin at transposable elements

Regardless of cell type, TET deficiency was broadly associated with an expected DNA hypermethylation in the euchromatic compartment, but to an unexpected DNA hypomethylation in the heterochromatic compartment (López-Moyado et al., 2019). Genomic analysis of malignant T cells lacking TET2/3 showed DNA hypomethylation in heterochromatic regions, along with the reactivation of repeat elements and an increased

frequency of single-nucleotide alterations, particularly in heterochromatic areas (López-Moyado et al., 2019).

The role of TET1 in controlling repetitive sequences has been confirmed in the study from Paul Stolz in 2022. In that study, it was demonstrated that TET1 plays a crucial role in establishing H3K9me3 at TEs, with its silencing function operating independently of its well-known role in DNA demethylation (Stolz et al., 2022). The researchers proposed that the TET1-SIN3A-HDAC complex is involved in deacetylation, which is followed by the SETDB1-KAP1 complex that deposits H3K9me3 to achieve silencing (Stolz et al., 2022). It appears that one of TET1's non-catalytic activities is to facilitate the establishment of H3K9me3 through interaction with the SIN3A/HDAC and OGT complexes. The involvement of TET1 and TET2 in depositing H3K9me3 by interacting with SIN3A/HDAC complex at TEs was also shown in the study from de la Rica et al in 2016 (De La Rica et al., 2016).

I will come back on the role of TET2 in regulating TEs in Chapter VI.

III. 5 Regulatory mechanisms and dependencies of the TET2 enzymes

III. 5. 1 TET2 catalytic function is dependent on iron and 2-Oxoglutarate

TET2 requires cofactors such as Fe (II), O₂ in addition to 2-OG (Tahiliani et al., 2009). 2-Oxoglutarate (2OG) and Fe (II)-dependent oxygenases couple substrate oxidation with 2OG decarboxylation to produce succinate and CO₂ (Berg et al., 2018).

Suppressing iron uptake or *Tet2* expression impairs the expression of erythroid genes and the differentiation of HSCs (Tseng et al., 2024). Iron is essential for dioxygenases, which support vital biological functions such as oxygen transport, energy production, DNA synthesis, and cell growth and replication. Alpha-ketoglutarate (aKG) is a critical intermediate metabolite in the tricarboxylic acid cycle, also known as the Krebs or citric acid cycle. aKG is crucial for cellular energy metabolism, amino acid synthesis, collagen synthesis, and epigenetic regulation. It acts as a cofactor for several chromatin-modifying enzymes, including TET2 (Zdzisińska et al., 2017).

III. 5. 2 TET2 activity can be regulated by Vitamin C

TET2 is enhanced in the presence of ascorbic acid (Vitamin C) (Yin et al., 2013) (Fig.12). Vitamin C interacts with the C-terminal catalytic domain of TET2, likely promoting folding and/or recycling of Fe (II). Supplementing Vitamin C to both mouse ESCs and mouse embryonic fibroblasts leads to a rapid and global increase in 5hmC with a concomitant decrease in 5mC (Blaschke et al., 2013; Minor et al., 2013). Genomic regions resistant to Vitamin C-mediated DNA demethylation exhibit higher levels of H3K9me3 (Blaschke et al., 2013), suggesting this histone mark may prevent TET2-mediated DNA demethylation pathways. Thus, Vitamin C is a direct regulator of TET2 activity and DNA methylation fidelity, providing new mechanistic insights into its biochemical role in DNA methylation.

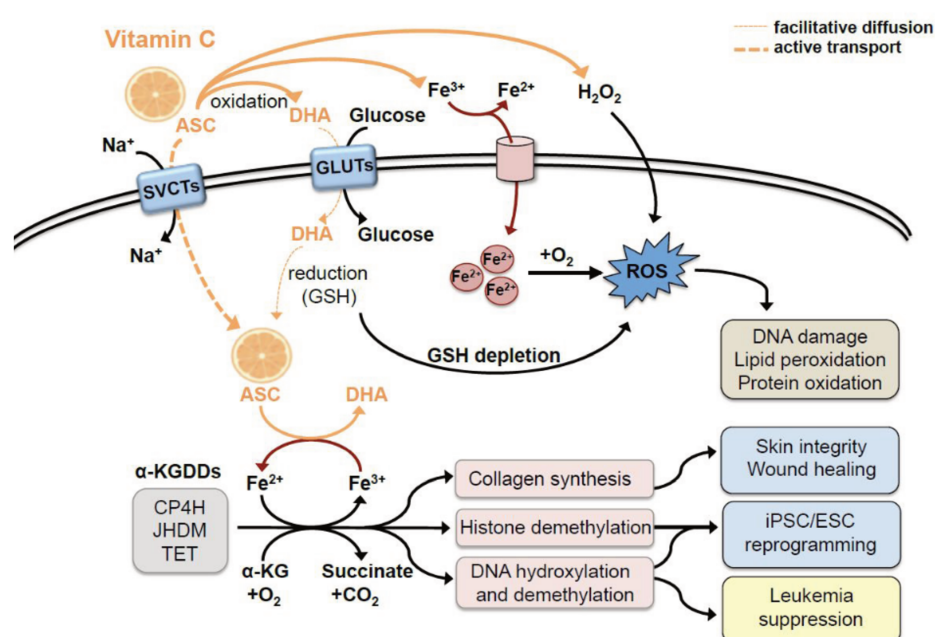


FIG. 12: Regulation of TET2 activity by Vitamin C. Vitamin C enters cells primarily in two forms: the reduced form, ascorbate (ASC), which is taken up from the plasma by sodium-dependent vitamin C transporters (SVCTs), and the oxidized form, dehydroascorbate (DHA), which can enter cells via glucose transporters (GLUTs). Once inside the cell, DHA is quickly reduced back to ASC by glutathione (GSH). Vitamin C functions as a vital cofactor for several Fe²⁺ and α -ketoglutarate-dependent dioxygenases (α -KGDDs), including collagen prolyl hydroxylases (CP4H), JmjC-histone demethylases (JHDMs), and ten-eleven translocation (TET) DNA hydroxylases. Additionally, vitamin C can enhance cellular uptake and mobilization of redox-active iron (Fe²⁺) and increase the production of reactive oxygen species (ROS), which can be leveraged in cancer therapies to induce cell death (Cimmino et al., 2018).

Vitamin C can impede the abnormal self-renewal of HSCs by enhancing the activity of Jumonji-C-domain containing histone demethylases or TET hydroxylases. As an electron donor, vitamin C reduces ROS and also facilitates the conversion of ferric iron (Fe³⁺) to ferrous

iron (Fe^{2+}), which is essential for its role as a cofactor for α -KGDDs. Vitamin C regulates various α -KGDD families, including prolyl hydroxylases (Cimmino et al., 2018). Additionally, metabolomic screening by Agathocleous et al. revealed that vitamin C levels are significantly higher in human and mouse HSCs compared to more differentiated hematopoietic cell types (Agathocleous et al., 2017).

III. 5. 3 regulation of TET2 by miR-22

A recent study identified miR-22 as an oncogene that directly targets TET2 transcripts. Conditional overexpression of miR-22 in the mouse hematopoietic compartment leads to reduced global 5hmC, increased self-renewal of HSCs, and impaired differentiation ability. Furthermore, miR-22 overexpression enhances the proliferative capacity of HSC/HPCs, causing them to outcompete their wild-type counterparts in competitive transplantation assays. Bioinformatics analysis revealed direct interaction of miR-22 with the 3'UTR of the TET2 transcript. These findings suggest that *TET2* loss contributing to the pathogenesis of myeloid malignancies can also be related to deregulation of the miR-22-TET2 pathway (Song et al., 2013).

III. 5. 4 Modulation of TET2 Activity by IDAX

As previously mentioned, Ko et al. (2013) demonstrated that IDAX, a protein dissociated from the CXXC domain, directly interacts with the catalytic domain of TET2. This interaction leads to the degradation of TET2 and a reduction in 5hmC through a caspase-dependent pathway. In ESCs, the absence of IDAX prevents TET2 degradation during ESC differentiation. Mutations in the CXXC domain of IDAX impair its ability to bind and degrade TET2, suggesting that IDAX uses its CXXC domain to recruit TET2 to chromatin (Ko et al., 2013).

III. 6 TET2 mutations and inactivation: Implications for hematopoiesis and human hematologic disorders

III. 6. 1 TET2 loss in murine hematopoiesis

It has been demonstrated that alterations in TET2 function resulted in diverse hematopoietic abnormalities and a predisposition to fatal disorders resembling human myeloid diseases with

TET2 mutations. TET2 disruption gives cells a competitive advantage, enhanced repopulating capacity, a myeloid bias and eventually leads to malignancy development (Ko et al., 2011; Li et al., 2011; Moran-Crusio et al., 2011; Quivoron et al., 2011). Additionally, *Tet2*^{+/-} mice also show increased stem cell self-renewal and extramedullary hematopoiesis, suggesting that *Tet2* haploinsufficiency contributes to hematopoietic transformation in vivo (Moran-Crusio et al., 2011). In the lab, we use *Tet2*-deficient mice that were obtained by crossing *Tet2*^{flox/flox} mice with EIIa-Cre mice. Approximately 30% of animals in this *Tet2*-deficient model developed a chronic myelo-monocytic leukemia (CMML)-like disease (Quivoron et al., 2011).

In order to decipher the impact of the catalytic vs non catalytic function of TET2 on hematopoiesis, Ito et al. (2019) compared *Tet2* KO mice, losing both the catalytic and non-catalytic function of TET2 to mice with mutation in the catalytic domain (*Tet2* mutant (Mut) mice). The increased incidence of aberrant lymphopoiesis observed in *Tet2* KO mice, but not in *Tet2* Mut mice, suggests that TET2's non-catalytic roles are involved in regulating lymphopoiesis. Their analysis conclusively establishes that the catalytic activity of TET2 is the primary regulator of myeloid lineage differentiation and the pathogenesis of myelodysplastic syndrome (MDS) through DNA demethylation. *Tet2* KO mice develop disorders affecting both the myeloid and lymphoid lineages (Ito et al., 2019).

III. 6. 2 Impact of inactivation/knockdown of TET2 on human hematopoiesis

Interestingly, knocking down TET2 in human hematopoietic cells was found to enhance hematopoietic development. Specifically, when TET2 was knocked down in hematopoietic progenitors (CD34+CD43+ cells) derived from human ESCs (hESCs) or murine primary BM cells, there was a notable increase in the number of progenitors compared to controls (Figueroa et al., 2010; Langlois et al., 2014).

In another study, genetic engineering through CRISPR-Cas9 sgRNAs against TET2 of human hematopoietic stem cells (hHSCs) and transplantation into immunodeficient mice showed that TET2 loss in hHSCs leads to distinct neutrophil heterogeneity in BM and peripheral tissues, enhancing the repopulating capacity of neutrophil progenitors and resulting in the formation of low-granule neutrophils. Neutrophils carrying TET2 mutations exhibit heightened inflammatory responses and more condensed chromatin, which is linked to increased production of compact neutrophil extracellular traps (Huerga Encabo et al., 2023).

III. 6. 3 TET2 Mutations in clonal hematopoiesis and hemopathies

Inactivating mutations of the TET2 gene were initially identified in myeloid malignancies and have since been found in B and T lymphomas, including peripheral T-cell lymphomas, suggesting that TET2 mutations occur in the stem cell compartment. TET2 mutations were present in 40 out of 86 (47%) cases of angioimmunoblastic T-cell lymphoma and in 22 out of 58 (38%) cases of peripheral T-cell lymphoma (Lemonnier et al., 2012). TET2 mutations were also detected in approximately 10% of adult T-cell leukemia/lymphoma cases (Kataoka et al., 2015; Shimoda et al., 2015). In B-cell malignancies, TET2 mutations have been reported in 0–12% of patients with diffuse large B-cell lymphoma (Asmar et al., 2013; Lohr et al., 2012; Morin et al., 2011; Quivoron et al., 2011).

TET2 is a bona fide tumor-suppressor gene in myeloid cancers. Mutations in TET2 are early events in some patients with MDS, myeloproliferative disorders, or secondary AML. The frequency of TET2 mutations in unselected patients was 20-35% with MDS (Kosmider et al., 2009), 15,5% with MPN (Chia et al., 2021), 12-34% with AML (Weissmann et al., 2012), and 30-60% with CMML (Grossmann, 2011). The prevalence of TET2 gene mutations among the three subtypes of myeloproliferative neoplasms (MPN): polycythaemia vera, essential thrombocythaemia and myelofibrosis were 16.8%, 9.8% and 15.7%, respectively (Chia et al., 2021).

TET2 mutations can also appear with age in the HSPCs of otherwise healthy individuals and give rise to CHIP (described in chapter V).

Disease emergence is dictated by the accumulation of additional mutations. For example, mutations in CTCF followed by FLT3 can lead to AML. In another rare case, a mutation in JAK2 generates MPN, which can transform into secondary myelofibrosis due to an additional ASXL1 mutation (Solary et al., 2014). TET2 can be associated with SF3B1 and JAK2 mutations, although this occurs less frequently in MPNs and MDS. However, such mutations have been observed in patients with refractory anemia with ring sideroblasts and thrombocytosis (Flach et al., 2014). In a third scenario, mutations in SRSF2 followed by KRAS can result in CMML in its proliferative form (Fig. 13). The highest incidence of TET2 genetic alterations has been observed in CMML (Solary et al., 2014).

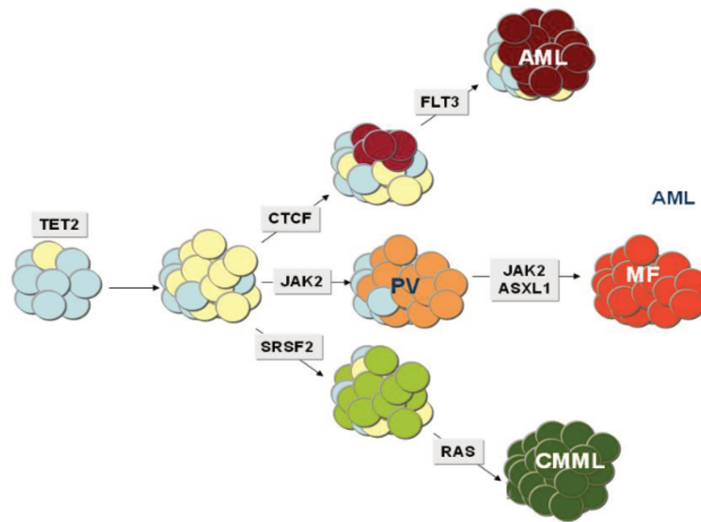


FIG. 13: Examples of TET2 mutations as background mutations followed by additional, various mutations and their progression into different malignancies (Solary et al., 2014).. AML: acute myeloid leukemia; MF: myelofibrosis; CMML: chronic myelomonocytic leukemia; PV: polycythemia vera

Chapter IV - Effect of aging on HSCs and hematopoiesis

Given that HSCs are vital for the continuous production of all blood and immune cells throughout a person's life, understanding how aging affects HSCs and hematopoiesis is crucial to comprehend the decline in hematopoietic function with age. It is widely recognized that the declining function of the immune system significantly increases the risk of infections, cancer, and autoimmune diseases in the elderly population, as extensively reviewed in the literature (Stahl and Brown, 2015). Our current comprehension of the decline in hematopoietic function encompasses a shift in the functioning of both adaptive and innate immune cells, resulting in a reduced ability to recognize new antigens (immunosenescence) and widespread chronic inflammation.

IV. 1 Aging results in immunosenescence and a shift towards myeloid cell differentiation

IV. 1. 1 Myeloid bias and its related hematological conditions

While young HSCs exhibit a balanced production of lymphoid and myeloid cells, promoting the necessary lymphopoiesis for initiating adaptive immune responses while restraining the production of potentially inflammatory myeloid cells (Yamamoto and Nakauchi, 2020), aging is marked by reduced lymphopoiesis and an increase in myeloid-biased HSCs, leading to heightened myelopoiesis (Pang et al., 2011).

Gene expression analysis has shown that aged HSCs undergo systemic downregulation of genes associated with lymphoid specification and function, while genes involved in myeloid fate and function are upregulated (Liang et al., 2005; Rossi et al., 2005).

This bias of HSC differentiation towards the myeloid lineage is associated with a higher risk of developing myeloid cancers such as MDS, MPN, mixed MDS/MPN such as CMML, and AML. MDS, previously known as myelodysplastic syndromes, are clonal hematopoietic malignancies characterized by morphological BM dysplasia and often manifesting as anemia, neutropenia, or thrombocytopenia. CMML is a clonal disorder that used to be considered a type of MDS, with TET2 and SRSF2 mutations being early initiating events (Patnaik, 2022). Given that CMML exhibits characteristics of both MDS and MPN, experts established a new 'overlap'

category for MDS/MPN neoplasms (Jaffe, 2008). MDS and CMML patients also face an increased risk of AML. Beyond MDS, chronic myeloid neoplasms (MN) encompass MPN, characterized by abnormal proliferation of both immature and mature myeloid cells, presenting with various proliferative features (Vardiman et al., 2009).

IV. 1. 2 Immunosenescence

Immunosenescence denotes the alterations in the adaptative immune system observed in aging individuals. Key features include a diminished capacity to respond to novel antigens and decreased memory response.

Aging leads to a decrease in the numbers of CLPs and reduced CLP proliferation rates in vivo (Gruver et al., 2007). T cells are particularly susceptible to the effects of aging. Various factors have been implicated in the decline of T cell function with age, yet chronic age-induced thymic atrophy and the consequent reduction in naïve T cell production appear to be the most significant contributors (Haynes et al., 2000). Moreover, the population of pre-B cells diminishes notably with age, likely due to an impediment between the pro- and pre-B cell developmental stages (Johnson, 2002). Human immunosenescence significantly impacts morbidity and mortality later in life.

IV. 2 Functional changes of HSCs upon aging

Transplantation assays using single cells and inducible lineage tracing systems have revealed that aged mice harbor a higher quantity of immunophenotypic HSCs in comparison to their younger counterparts. Aged HSCs exhibit a higher rate of cell division and proliferation compared to younger stem cells, which are more likely to remain quiescent (Pang, 2011) (Fig.14). However, despite this abundance, each individual HSC in older mice exhibits a reduced ability to generate mature hematopoietic cells (Säwen et al., 2018; Yamamoto et al., 2018). Aged HSCs are constrained in their lineage potential but also produce fewer differentiated progeny, leading to a delayed and diminished recovery process (Van Zant and Liang, 2003).

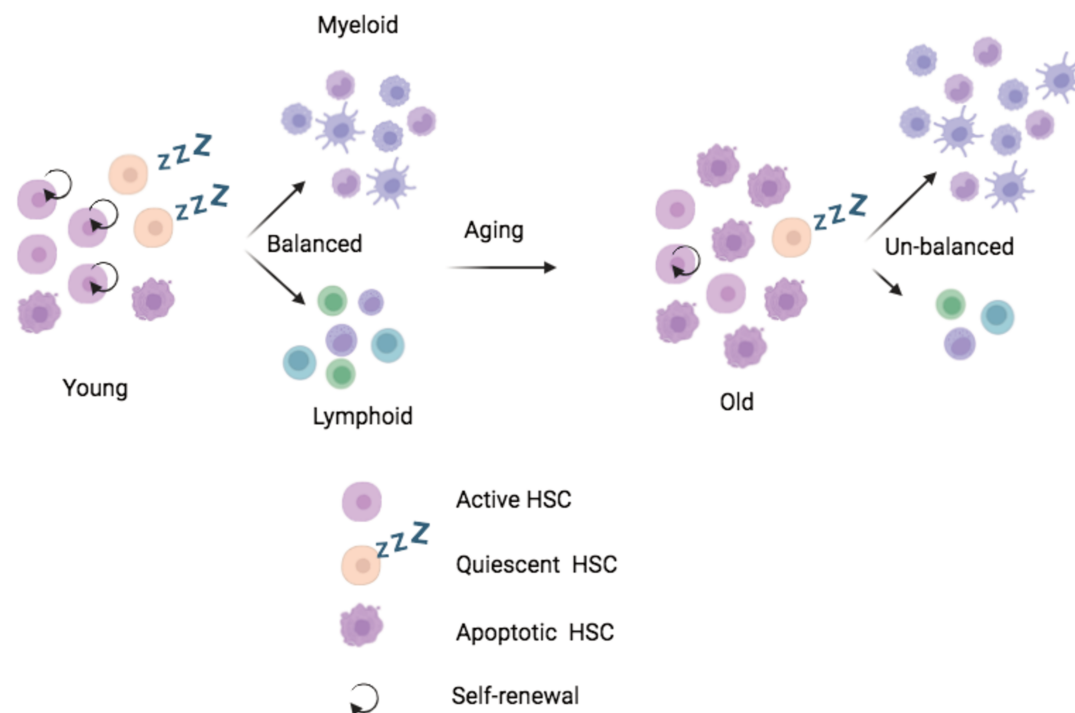


FIG. 14: The aging process significantly impacts HSCs. In younger individuals, HSCs demonstrate higher rates of self-renewal, greater quiescence, and reduced apoptosis. There exists an equilibrium between the production of lymphoid and myeloid cells. Conversely, aged HSCs exhibit decreased quiescence, reduced activity, diminished self-renewal capacity, increased apoptosis, and a shift towards generating more myeloid and fewer lymphoid cells (Created with BioRender, License: LZ27F4TZB3).

When stem cells initially obtained from aged donor mice are transplanted sequentially into young recipients, their self-renewal capacity significantly decreases upon secondary and tertiary reconstitution compared to stem cells sourced from young donors (Dykstra et al., 2011). The decrease of HSC self-renewal potential, leading to terminal differentiation or irreversible senescence, is an inevitable outcome of numerous cellular divisions.

Numerous studies have provided evidence supporting the diminished self-renewal capacity, narrowing of developmental potential, and reduced progeny output of aged stem cells under hematopoietic demands (De Haan and Lazare, 2018).

One-third of aged HSCs exhibit high levels of autophagy, maintain a low metabolic state, and possess robust long-term regeneration potential, similar to healthy young HSCs (Ho et al., 2017). Thus, it remains plausible that even in aged mice, there exist significant numbers of

highly potent, self-renewing HSCs, which may be overshadowed by a larger pool of less potent aged cells (Fig. 15).

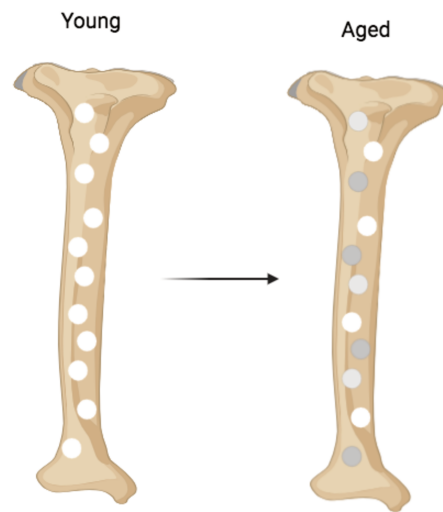


FIG. 15: In aging mice, there is a rise in the total count of cells possessing regenerative capabilities; however, the degree to which individual aged cells contribute to blood cell production varies significantly. Consequently, the proportion of stem cells exhibiting high regenerative potential (white) diminishes relative to those with lower regenerative potential (gray and dark gray) as age progresses. (Adapted from (De Haan and Lazare, 2018), Created with BioRender, License: QE27F4U67M).

IV. 3 Molecular changes of HSCs upon aging

Increased stress from cellular replication, redox imbalance, mitochondrial dysfunction, and DNA damage are recognized as key features of HSC aging. This leads to the hypothesis that the primary driver behind HSC aging is proliferation. Preserving the quiescent state becomes crucial for maintaining the stemness of adult HSCs, ensuring their low metabolic activity, stable epigenetic profile, and genomic integrity. Numerous studies have highlighted the significant role of epigenetic modifications, particularly alterations in heterochromatin associated with aging (heterochromatin theory of aging).

IV. 3. 1 DNA damage accumulation in aged HSCs

DNA damage can arise from various sources, including radiations, external genotoxic substances, and free radicals, and its accumulation with age has been consistently observed in numerous studies (Alter et al., 2012; Ju et al., 2007; Wogan et al., 2004). Phosphorylation of

histone H2AX (γ -H2AX) serves as a marker for DNA damage, and there is an accumulation of γ -H2AX foci in HSCs as they age (Rossi et al., 2007). Older HSCs also present increased replication stress due to cell cycle abnormalities, altered DNA replication dynamics, and the presence of chromosome gaps or breaks (Flach et al., 2014). However, delaying the effects of aging in mice by reducing DNA damage levels has not yet been accomplished, and a direct link between DSB formation and physiological aging remains to be established (White and Vijg, 2016).

Once old HSCs enter a quiescent state, residual replication stress persists primarily at ribosomal DNA genes, resulting in the formation of nucleolar-associated γ H2AX signals. It is thought that these signals persist due to ineffective H2AX dephosphorylation rather than ongoing DNA damage.

IV. 3. 2 Increased ROS due to higher level of oxidative metabolism

HSCs are characterized by having a low metabolic rate, being essentially glycolytic while quiescent (Chandel et al., 2016; Ito and Suda, 2014). Upon activation, young HSC change towards a more oxidative metabolism that can be reverted when they return to quiescence. However, in aged HSC, basal metabolism is shifted to a higher level of oxidative metabolism (Verovskaya et al., 2019), which increases ROS leading to oxygen related-stress, accompanied by impaired regenerative potential (Chandel et al., 2016).

The maintenance of appropriate ROS levels is critical for the maintenance of HSCs and DNA integrity, as well as for preventing ROS-mediated cell damage (Naka et al., 2008). Jang and Sharkis reported that HSCs with low levels of ROS had high potential for self-renewal, which was attenuated during aging as the ROS levels increased (Jang and Sharkis, 2007).

Recent studies have shown that in young mice, HSCs purified based on low intracellular levels of ROS displayed heightened self-renewal potential. Conversely, those with elevated ROS levels became rapidly depleted upon serial transplantation, exhibiting a myeloid-biased differentiation pattern similar to that observed in aged HSC transplantation (Jang and Sharkis, 2007). It can be concluded that intracellular ROS levels play a critical role in determining the functionality of HSCs.

While the BM, where hematopoiesis occurs, is hypoxic rather than hyperoxic, increased oxidative stress from ROS production in the BM hematopoietic niche with aging has been proposed (Latchney and Calvi, 2017).

IV. 3. 3 The role of telomers in aging of HSCs

IV. 3. 3. 1 Telomere-centric theory of cell aging

Telomeres safeguard the integrity of DNA throughout the cell cycle by acting as specialized caps on chromosomes. During cellular division, they prevent the loss of base pairs from chromosomal DNA. However, with each division, telomeres shorten until they become too short for the cell to divide, leading to cellular senescence. Indeed, the DNA polymerase, which synthesizes DNA from the 5' to the 3' end, needs a transient primer to start the process but cannot fully replicate the chromosome's 3' ends, a limitation known as the “end-replication problem” (Jang and Sharkis, 2007; Levy et al., 1992). Besides incomplete replication, telomere shortening can also result from various external and internal factors, such as radiation, infections, pollution, and other environmental factors, particularly those that stimulate excessive mitochondrial ROS production (Fig. 16). This reduction in telomere length is associated with aging and a variety of diseases. The telomere-centric theory of cell aging posits that telomere shortening is influenced not only by the number of cell divisions (mitotic clock) but also by various internal and external factors.

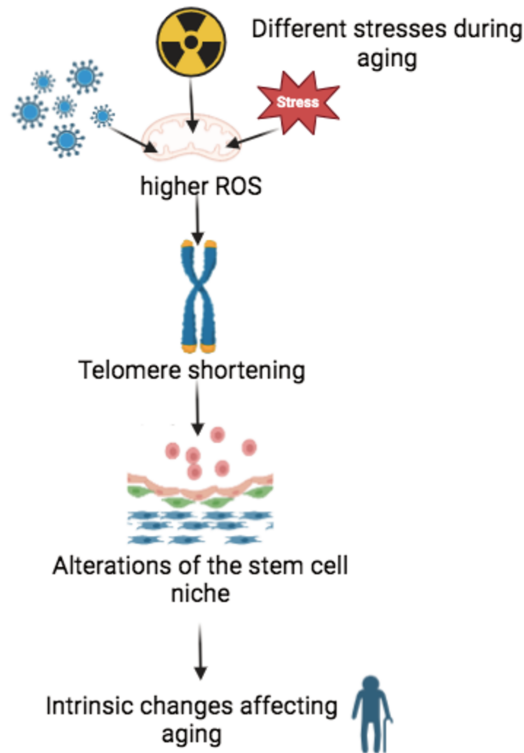


FIG. 16: Telomere-centric theory of cell aging (Adapted from Lupatov and Yarygin, 2022). Aging results from the accumulation of stresses such as radiation, infections, pollution, and other environmental factors, which can lead to telomere shortening and elevated ROS levels, ultimately causing cellular senescence and stem cell depletion (Created with BioRender, License: XP27F4UBYA).

IV. 3. 3. 2 Telomeres in HSCs aging

Over the past 25 years, numerous authors have identified telomere shortening as the primary mechanism leading to the end of HSC life (Morrison et al., 1995). There is substantial direct and indirect evidence that telomeres shorten in both human and mouse HSCs, demonstrating a progressive reduction in telomere length. HSCs, regardless of the presence of telomerase, experience telomere shortening during aging due to oxidative stress (Von Zglinicki et al., 1995).

IV. 3. 4 Epigenetic Mechanisms Driving Aging in HSC

IV. 3. 4. 1 Epigenetic changes in HSCs upon aging

Recent studies in mouse HSCs have shown that aging is linked to alterations in DNA methylation and histone modification profiles across HSC identity and self-renewal genes

(Beerman et al., 2013; D. Sun et al., 2014), suggesting that disruption of the normal epigenetic state is a key factor in the aging HSC phenotype.

A study looking at the genes that are up or down-regulated in old vs. young mice HSCs revealed that genes associated with the stress response, inflammation, and protein aggregation were up-regulated, while the down-regulated included genes involved in the preservation of genomic integrity and chromatin remodeling (Chambers et al., 2007).

In line with recently published data (Beerman et al., 2013), they observed that the overall DNA methylation pattern remains highly conserved throughout HSC aging (Taiwo et al., 2013). Comparing young and old HSCs, there is generally a significant 5% decrease in global DNA methylation (Taiwo et al., 2013) and a decrease in 5-hmC levels in aged HSCs (D. Sun et al., 2014). Increased DNA methylation upon aging generally occurs at genomic loci linked to lymphoid and erythroid lineages, while DNA methylation decreases at loci associated with the myeloid lineage. This could be linked to the myeloid bias upon aging (D. Sun et al., 2014). There is also a hypomethylation at HSC-specific genes that can explain the increased number of HSCs with age (Beerman et al., 2013; Bernitz et al., 2020; Gekas and Graf, 2013; D. Sun et al., 2014). Aging-associated differentially methylated regions that gain methylation with age preferentially appear in precursor/stem cells at specific developmental gene promoters.

Concerning histone marks, HSC aging is associated with an increase in the number of loci enriched in H3K4me3, and an expansion of this mark, particularly at HSC-specific genes. This change is correlated with an increase in gene expression, in line with the expansion of HSCs (D. Sun et al., 2014).

The number of loci marked by H3K27me3 remains constant with age, but an increase in mark intensity and coverage is observed. The increase in H3K27me3 at lymphoid differentiation genes correlates with their decreased expression and declining lymphoid differentiation potential with age (D. Sun et al., 2014).

Human HSCs also face age-associated epigenetic reprogramming, characterized by changes in DNA methylation and reductions in H3K27ac, H3K4me1, and H3K4me3 levels. This reprogramming in aged HSCs globally affects developmental and cancer-related pathways, potentially creating a predisposition to disease (Adelman et al., 2019).

These epigenetic changes may be linked to the decreased expression of key epigenetic regulators, such as *Ezh2*, the PcG complex member *Cbx2*, DNMTs, *Tet* and *Suv39H1* genes in human and mice HSCs (Djeghloul et al., 2016a; D. Sun et al., 2014).

IV. 3. 4. 2 ‘Loss of Heterochromatin’ theory of aging

The "loss of heterochromatin" model of aging, first proposed by Villeponteau (1997), suggests that heterochromatin domains established early in embryogenesis break down during aging, leading to the derepression of silenced genes and resulting in abnormal gene expression patterns (Villeponteau, 1997). This model is supported by observations of global heterochromatin loss associated with aging in both humans and animals. For instance, cells from patients with Hutchinson-Gilford Progeria Syndrome (HGPS), a condition that mimics premature aging, exhibit a decrease in the heterochromatin mark H3K9me3 and a delocalization of HP1 α and the calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia antigen from constitutive heterochromatin (Shumaker et al., 2006). Therefore, while global heterochromatin levels decrease with age, there is also an increase in localized heterochromatin formation at specific loci, a process referred to as "heterochromatin redistribution" (Sedivy et al., 2008). Another mechanism to protect from further genome instability after DNA damage through delocalization is shown in the paper of Fortuny where the methyltransferase SETDB1 coordinates histone methylation with the deposition of new histones in damaged heterochromatin after UV exposure (Fortuny et al., 2021) leading to a loss of histones in other regions. In the paper of van Meter et al. they show that during aging and in response to DNA damage there is a relocation of the heterochromatin from TEs to the site of DNA damages, leading to a loss of heterochromatin marks at some regions and a gain in others (Van Meter et al., 2014).

Loss of heterochromatin through the loss of the major heterochromatic histone methyltransferases SUV39H1 and SUV39H2 resulted in characteristic changes associated with aging such as diminished HSC function, myeloid skewing and immunosenescence, confirming the link between loss of H3K9me3 and HSC aging (Keenan et al., 2020).

Our lab participated in a study showing a loss of H3K9me3 in mouse and human HSCs, associated with loss of lymphoid differentiation. Interestingly, overexpression of SUV39H1 in

aged human HSC restores lymphoid differentiation and attenuates the myeloid bias, demonstrating the importance of heterochromatin in age-related phenotypic alterations in HSC (Djeghloul et al., 2016a).

IV. 4 Stresses as a driver of HSC Aging

Different stressors play significant roles in accelerating the aging process and contributing to the decline in hematopoietic function, including inflammation, smoking, ionizing radiations (IR) or other environmental factors. Therapies used in cancer treatment, such as chemotherapies and radiotherapies, also induce a premature aging of HSCs (Hill et al., 2020).

I will mainly focus this part on the effects of IR and inflammation on HSC aging as I used these stresses as models of premature aging in my thesis.

IV. 4. 1 Ionizing Radiations

Exposure to natural and artificial radiations, in the context of medical use such as CT-scan or radiotherapies, have harmful biological effects on hematopoiesis.

Exposure to IR is an independent risk factor for many disorder characteristics of accelerated aging. Epidemiological studies, particularly those involving atomic bomb survivors (Shuryak et al., 2010; Yoshida et al., 2019) and patients receiving medical radiation (Hernández et al., 2015), have provided valuable data on the association between IR and a wide range of age-related phenotypes, such as frailty, fatigue or hair loss, and diseases, including cancer.

The hematopoietic tissue is particularly sensitive to IR. The persistence of pan hematopoietic defects as late as 50 years after initial IR exposure in Japanese atomic bomb survivors suggests that HSCs are the primary targets of IR. Irradiation notably induces enhanced inflammatory responses decades after radiation exposure due to an increase in peripheral myeloid cells (Yoshida et al., 2006).

Therapy-related myeloid neoplasms (t-MN), such as MDS and therapy-related AML (t-MDS and t-AML), develop between 3 to 8 years after exposure to chemotherapies or radiotherapies, are frequently associated with poor overall survival, and are generally refractory to

conventional therapies (Takahashi et al., 2017a). T-MDS and t-AML constitute 15-20% of all adult AML/MDS cases. This has become a major public concern in the last decades as the number of long-term survivors of cancer treatments is continuously growing, consequently increasing the incidence of t-MN.

Our lab and others have demonstrated that total body irradiation in mice results in long-term hematopoietic defects stemming from a depletion of HSC reserves and their functions (de Laval et al., 2013a; Fleenor et al., 2015; Mohrin et al., 2010). In HSCs, IR triggers the accumulation of DNA damage, a loss of self-renewal capability, and a skewed differentiation toward the myeloid lineage. This results in elevated myeloid cell counts and a decrease in the adaptive immune response. These changes are likely contributing to many IR-induced premature aging disorders and to the high risk of developing myeloid leukemia.

Interestingly, we showed that IR-induced loss of HSC function is associated with a loss of H3K9me3 (Pelinski et al., 2022a). These data confirm the involvement of H3K9me3 in the aging phenotype of HSCs.

IV. 4. 2 Inflammation

While inflammation is a physiological process that aids in tissue repair in response to internal or external insults, a chronic inflammatory state can have detrimental consequences.

A chronic pro-inflammatory status due to the increased production of myeloid cells is a common feature of aging. This persistent low-grade inflammation, occurring without overt infection, is termed "inflammaging" and poses a significant risk factor for morbidity and mortality in the elderly as it increases the risk of cardiovascular diseases (CVD), chronic kidney disease, diabetes mellitus, cancer, depression, dementia, and sarcopenia (Kuo et al., 2006; Leonardi et al., 2018; Miller and Raison, 2016; Ruparelia et al., 2017). Unlike young individuals, older adults consistently exhibit elevated levels of inflammatory cytokines, particularly IL-6 and tumor necrosis factor- α (TNF- α) (Singh and Newman, 2011) that were shown to be linked to an increased risk of cardiovascular and neurodegenerative diseases and that are also associated with sarcopenia and frailties (Tylutka et al., 2024).

In addition to the proinflammatory state linked to the aged-induced increase in myeloid cells, chronic infections, autoimmunity, ataxia telangiectasia mutated kinase-dependent

inflammasomes and altered biomolecules from chromosome attrition can trigger chronic inflammation associated with aging. This not only increases ROS levels but also stimulates proliferation within the stem cell niche, leading to cell senescence and stem cell depletion (Lupatov and Yarygin, 2022).

IV. 4. 2. 1 Effect of inflammation on HSCs

Distinguishing between the acute and chronic effects of pro-inflammatory cytokines, as well as their impact at low or high doses, provides further insight into HSC function. Chronic low-dose LPS treatment leads to HSC expansion and myeloid skewing in a TLR4-dependent manner (Liu et al., 2015). High-dose LPS causes increased BM cell death and HSC dysfunction (Chen et al., 2010). Chronic low-dose murine endotoxemia leads to a decline in HSC function and a loss of quiescence, that has been shown by their reduced repopulation capacity in serial transplantation experiments (Esplin et al., 2011). Short-term stimulation with different cytokines, including Interferon gamma (IFN γ), Interferon alpha/beta, interleukin-1, TNF α , and IL-6, promotes HSC proliferation, but prolonged exposure to these cytokines can impair their function (Chavakis et al., 2019; Haltalli et al., 2020; Burberry et al., 2014; Khan et al., 2020).

IV. 4. 2. 2 Effect of inflammation on epigenetics

In a recent study, analysis of DNA methylation changes in monocytes from 192 healthy adults, both exposed and unexposed to inflammation showed that these changes were associated with genes frequently mutated in cancer, indicating that altered DNA methylation might connect inflammation to genetic mutations. Notably, LPS exposure caused the most significant changes, by increasing the epigenetic age/clock of the cells by 6 months linking epigenetic aging with innate immune activity (Gilchrist et al., 2024). Epigenetic clocks predict chronological age based on changes in methylation at CpGs associated with aging (Van Dongen et al., 2016).

In a separate study, it was demonstrated that the chromatin state at myeloid regulators in HSCs (De Laval et al., 2020) remains open following an initial inflammatory stimulus, enabling a quicker response to subsequent stimulation, suggesting the induction of an epigenetic memory that contributes to trained immunity.

Chapter V- Clonal hematopoiesis of indeterminate potential (CHIP)

V. 1 Definition

As other cells, HSC are not spared from accumulating somatic mutations over a lifetime. Most of these mutations are of little or no functional consequence. However, some of them may lead to a clonal expansion as compared to non-mutated cells and to a clonal hematopoiesis (CH) (Watson et al., 2020). On average, each HSC acquires about 1.3 ± 0.2 somatic exome mutations per decade (Welch et al., 2012). By the age of 70, a person would have up to 1.4 million protein-coding variants, averaging about 70 mutations per gene, in at least one HSC (Jaiswal et al., 2014).

CH is associated with a higher risk of developing leukemia. However, most individuals with CH never develop blood cancer. Additional events such as cooperative mutations in other genes may be required to induce malignant transformation. Thus, these clonal populations are considered to have “indeterminate potential.” This phenomenon is thus referred to as ‘Clonal hematopoiesis of indeterminate potential (CHIP)’ (Fig. 17). In 2015, a formal definition of CHIP was proposed with the following criteria: CHIP must occur without morphological variation in blood cells, a pre-leukemic candidate driver gene mutation should be present at a variant allele frequency of at least 2% in peripheral blood; and there should be no diagnostic criteria for hematologic malignancy (Steensma et al., 2015). According to the WHO 2022 classification (Haferlach and Heuser, 2022), CHIP is considered a myeloid precursor lesion, encompassing the same characteristics previously described by Steensma et al (Steensma et al., 2015). 10-20% of people over the age of 70 have CHIP (Bick et al., 2020; Genovese et al., 2014; Jaiswal et al., 2014; Kar et al., 2022).

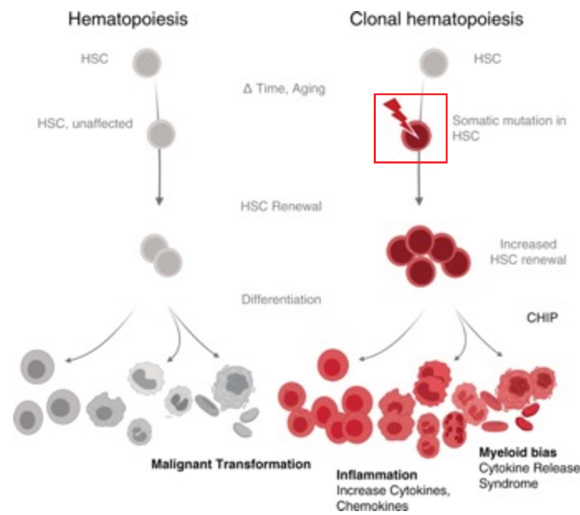


FIG. 17: CHIP describes the accumulation of somatic mutations over an individual's lifespan due to aging and stress, potentially resulting in clonal hematopoiesis of indeterminate potential (CHIP) (Adapted from Von Bonin et al., 2021).

V. 2 CHIP is mainly driven by mutations in epigenetic factors

Approximately 80% of patients with CHIP have mutations in epigenetic regulators such as DNMT3A, TET2, and ASXL1 (Bick et al., 2020; Genovese et al., 2014; Jaiswal et al., 2014; Von Bonin et al., 2021). Other mutations concern genes encoding DNA damage repair factors like PPM1D and TP53, the regulatory tyrosine kinase JAK2, which is notably associated with increased rates of thrombosis, PV and in general MPNs or mRNA spliceosome components such as SF3B1 and SRSF2 (Fig. 18).

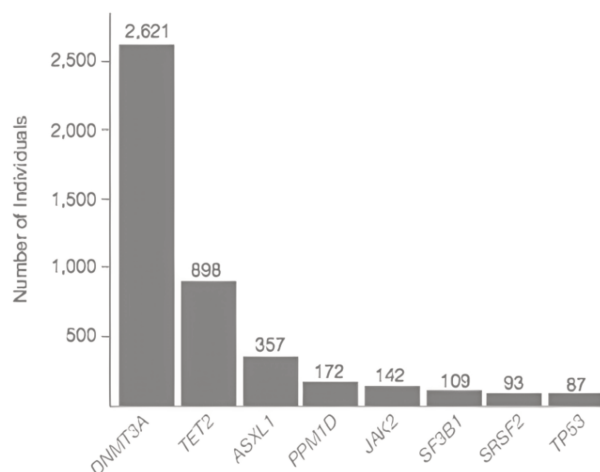


FIG. 18: Most frequent mutations in CHIP. Most frequent mutation being in epigenetic factors such as DNMT3A, followed by TET2 and ASXL1 (Bick et al., 2020).

V. 3 Diseases associated with CHIP

Clonally restricted hematopoiesis is linked to an increased risk of all-cause mortality, with most deaths resulting from non-hematologic diseases. Epidemiologic studies indicated that CHIP raises the risk of death by 40%, a rate significantly higher than what could be attributed to the risk of hematologic malignancy alone (Beeler et al., 2022; Bouzid et al., 2023; Desai et al., 2018; Genovese et al., 2014; Jaiswal et al., 2014; Tian et al., 2023).

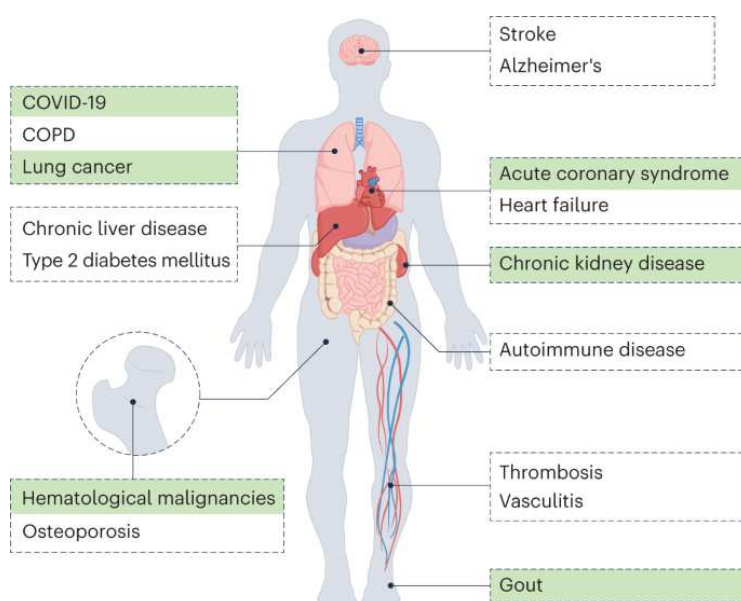


FIG. 19: Clonal hematopoiesis is linked to various health outcomes across multiple organ systems. Green-shaded boxes indicate associations between CH and diseases replicated by Kessler et al. (2022). COPD: chronic obstructive pulmonary disease (Beeler et al., 2022).

V. 3. 1 The impact of CHIP on cardiovascular and systemic diseases

CHIP is associated with a pro-inflammatory state that has been linked to various cardiovascular conditions, including coronary artery disease, myocardial infarction, and venous thromboembolic disease, as well as influencing prognosis in aortic stenosis (Dorsheimer et al., 2019) and heart failure (Mas-Peiro et al., 2020) (Fig. 19). Notably, CHIP confers a two-fold increase in cardiovascular risk, independent of traditional risk factors (Amancherla et al., 2022; Fuster et al., 2017; Marnell et al., 2021; Sano et al., 2018). CHIP has also been connected to chronic obstructive pulmonary disease (Buscarlet et al., 2017), diabetes (Fuster et al., 2020;

Jaiswal et al., 2014), psychiatric illnesses (Zink et al., 2017), early menopause (Honigberg et al., 2021), osteoporosis (Kim et al., 2021), liver disease (Wong et al., 2022) and renal dysfunction (Dawoud et al., 2020). Recently, CHIP has also been implicated in affecting both the risk and response to infections. Patients with CHIP have been found to have an increased risk of severe COVID-19, sepsis, and other infections (Dawoud et al., 2020)

The correlation between CHIP and these diseases may stem from common factors such as aging, smoking, and inflammation, but it is also possible that CHIP-driven inflammation contributes to the development of these conditions.

There are indeed several hypotheses regarding the mechanisms involved, with one suggesting that DNMT3A and TET2 converge in regulating proinflammatory pathways in monocytes and macrophages (Cobo et al., 2022; Fuster et al., 2017). *Dnmt3a*-deficiency has been associated with various potentially pro-atherogenic phenotypes across different immune cell populations, including the modest increase in IFN- γ production by T cells (Pham et al., 2013), and reduced immunosuppressive function in myeloid-derived suppressor cells (Yu et al., 2012). In murine models of atherosclerosis, *Tet2*-deficient macrophages exhibit higher levels of mRNA and protein expression of IL-1 β , IL-6, and chemokines. This was associated with larger atherosclerotic lesions in mice engrafted with *Tet2* KO as compared to non-mutated BM (Jaiswal et al., 2017). Additionally, *Jak2V617F* HSC-transplanted mice contribute to CVD, specifically atherosclerotic plaque buildup, through iron accumulation resulting from excessive erythrophagocytosis (Wang et al., 2018). TET2 has been shown to control the expression of cytokines through its non-catalytic function, by recruiting HDAC2 to specifically repress IL-6 and MIF (Pronier et al., 2022; Zhang et al., 2015). Loss of TET2 also lead to the emergence of a novel inflammatory monocytic cell population (Yeaton et al., 2022).

Finally, CHIP may also have a protective role. A recent study has shown that CH is associated with protection from Alzheimer's disease (Bouzid et al., 2023).

V. 3. 2 Clonal hematopoiesis and its risk for hematologic malignancies

CHIP is also associated with an increased risk of developing myeloid or lymphoid neoplasia (Steensma et al., 2015). CHIP is thus defined as a pre-leukemic state. Indeed, around 75% of AML patients have a CHIP mutation, years before diagnosis (Desai et al., 2018). CHIP is

associated with a 0.5-1.0% annual risk of developing leukemia. The incidence of hematopoietic malignancies in patients with CHIP is low with only 4% of individuals with CHIP in the Jaiswal study developed a hematopoietic malignancy at a median follow-up of nearly 8 years (Jaiswal et al., 2014). Follow up of BM transplantations of two healthy individuals with mutant *DNMT3A* into mice showed no expansion of the mutated clone after 8 months indicating that other factors might be required for expansion (Midic et al., 2020).

In their review, Florez et al. discuss environmental factors that are associated with CHIP expansion, including advanced age, inflammation, radiations, chemotherapy, and cigarette smoking, which promote the selection and expansion of specific CHIP mutant clones (Florez et al., 2022a). For instance, genotoxic stresses such as IR and chemotherapies drive the expansion of clones mutated for factors involved in DNA repair (p53, PPM1D), while inflammation drives the expansion of clones with epigenetic mutations. These data will be further discussed in V.4 section.

Figure 20 illustrates a model of disease progression with different evolutionary steps. Early mutations that initiate clonal expansion include TET2, DNMT3A, ASXL1, or JAK2, followed by mutations that contribute to disease characteristics, such as RUNX1, IDH1, or KRAS. The risk of developing future blood cancers and overall survival rates differ by mutation type, with DNMT3A and TET2 mutations associated with a lower risk of progression compared to PPM1D and TP53 mutations (Genovese et al., 2014; Jaiswal et al., 2014).

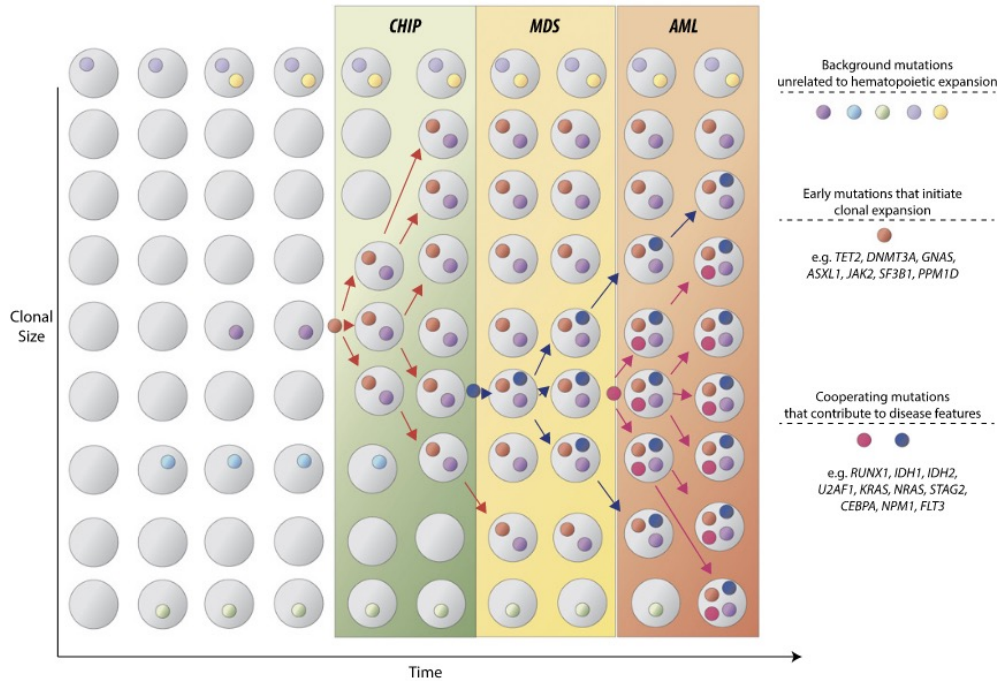


FIG. 20: CHIP as a precursor state for hematological neoplasms, representing a model of progression from normal hematopoiesis to CHIP, and potentially advancing to MDS or AML in certain instances (Steensma et al., 2015).

In the study by Weeks et al., a table is provided (Fig. 21) to calculate a score for the risk of developing hematologic diseases in CHIP. This simple prognostic model differentiates between high-risk CHIP/Clonal Cytopenia of Undetermined Significance (CCUS) and low-risk CHIP/CCUS in both population and patient cohorts. Key features influencing MN risk are divided into two groups: low-risk factors such as single *DNMT3A* mutations, and high-risk factors including: high-risk mutations such as *SRSF2*, *SF3B1*, *ZRSR2*, *JAK2*, *RUNX1*, and *IDH2*, two or more mutations, a VAF of 0.2, higher age of 65 or older, the presence of CCUS versus CHIP, and red blood cell indices.

They employed conditional probability-based random permutation analysis to identify key predictors, including high-risk mutations and single *DNMT3A* mutations, which emerged as the most significant genotypes for classification. The Clonal Hematopoiesis Risk Score (CHRS) was used to categorize individuals into low-risk (n=10,018, 88.4%), intermediate-risk (n=1,196, 10.5%), and high-risk (n=123, 1.1%) groups. In clinical cohorts, the majority of MN events occurred in high-risk patients with CHIP/CCUS.

Table 2. CHRS Values.*					
Prognostic Variable	0.5	1	1.5	2	2.5
Single <i>DNMT3A</i>	Present	Absent			
High-risk mutation		Absent			Present
Mutation number		1		≥2	
Variant allele fraction		<0.2		≥0.2	
Red cell distribution width		<15			≥15
Mean corpuscular volume		<100			≥100
Cytopenia		CHIP		CCUS	
Age (yr)		<65		≥65	

* CCUS denotes clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; and CHRS, clonal hematopoiesis risk score.

FIG. 21: Clonal hematopoiesis risk score (CHRS) values to determine the risk of developing hematologic diseases (Weeks et al., 2023). CCUS: clonal cytopenia of undetermined significance

V. 4 Expansion of CHIP mutated HSCs

V. 4. 1 Expansion dynamics of mutated CHIP clones

Several studies have investigated the expansion dynamics of mutated clones over the course of age, or in response to therapies (radio- and/or chemotherapies), or to various stresses (smoking, inflammation) (Bolton et al., 2020; Coombs et al., 2017; Fabre et al., 2022) and have shown differences in behavior between clones carrying different mutations. In a study monitoring 697 clonal hematopoiesis clones from 385 individuals aged 55 and older over a median period of 13 years, it was observed that 92.4% of the clones expanded at a consistent exponential rate. Different mutations drove significantly varied growth rates, ranging from 5% per year (*DNMT3A* and *TP53*) to over 50% per year for mutations in genes involved in splicing (*SRSF2P95H*). Growth rates of clones with the same mutation differed by approximately ±5% per year, affecting slower drivers more substantially. *DNMT3A*-mutant clones expanded preferentially during early life but exhibited slower growth in old age within a progressively competitive oligoclonal environment. Conversely, splicing gene mutations drove expansion only later in life, while *TET2*-mutant clones emerged across all ages. Finally, the study demonstrated that mutations driving faster clonal growth carry a higher risk of malignant progression (Fabre et al., 2022). The growth rate of mutated clones may depend on additional mutations in the clone. Mutations in the *TCL1A* gene reduce the expression of this gene in *TET2*-mutant clones and clone expansion (Weinstock et al., 2023).

V. 4. 2 Environmental factors that expand clones

V. 4. 2. 1 The role of genotoxic stresses in the expansion of mutant clones

Chemotherapies and radiations, used in radiotherapies for example, exert their anti-cancer effects by inducing DNA damage, disrupting DNA replication, impairing DNA repair mechanisms, blocking pro-survival signaling pathways, inhibiting transcription and translation, and triggering metabolic and cellular stress (Krisl and Doan, 2017). Certain pre-existing mutations associated with CHIP confer a clonal advantage upon therapies, allowing mutant clones to expand and persist under the selective pressure of chemotherapy and radiation. CHIP was identified in 25% of cancer patients, with 4.5% harboring presumptive leukemia driver mutations. Mutations in DDR genes such as TP53, PPM1D, and CHEK2 show the strongest correlation with prior cancer treatments, with clones harboring these mutations expanding more robustly under the stress induced by specific cytotoxic therapies (Bolton et al., 2020; Coombs et al., 2017; Gillis et al., 2017; Takahashi et al., 2017a).

Even if mutations in DNA damage pathways are the most frequently expanded upon genotoxic stresses, expansion of clones presenting mutations in epigenetic factors such as *TET2* has also been observed (Nishiyama et al., 2018; Shih et al., 2013; Takahashi et al., 2017a).

V. 4. 2. 2 The role of Inflammation in the expansion of mutant clones

Studies in both human subjects and animal models have highlighted that specific clones associated with CHIP can withstand the stress induced by inflammation, gaining a competitive advantage under these conditions (Cook et al., 2020; Florez et al., 2022a).

Expansion of *Tet2* KO HSCs upon inflammatory stress have been extensively investigated in murine models, revealing that these deficient HSCs selectively expand, particularly within the myeloid compartment, in response to acute or chronic pro-inflammatory stimuli such as TNF α and LPS (Abegunde et al., 2018; Cai et al., 2018; Caiado et al., 2023; McClatchy et al., 2023). In their study, Cai et al. demonstrate that *Tet2* KO HSPCs maintain a competitive advantage following acute inflammatory stress as compared to non-mutated cells. Similarly, TNF- α has been shown to expand *Tet2*-mutant cells in mouse models (Abegunde, 2018). In both cases, resistance of *Tet2* KO HSCs towards inflammation is associated with resistance to apoptosis, through the up-regulation of anti-apoptotic genes or down-regulation of apoptotic genes (Abegunde et al., 2018; Cai et al., 2018).

Administration of IL-1 to mice led to an IL-1 receptor 1-dependent expansion of *Tet2*^{+/-} HSPCs and mature blood cells. This expansion was driven by enhanced cell cycle progression in *Tet2*^{+/-} HSPCs, greater multilineage differentiation, and improved repopulation capacity compared to their wild-type counterparts. Consistently, IL-1 α -treated *Tet2*^{+/-} HSCs showed increased DNA replication and repair transcriptomic signatures and reduced susceptibility to IL-1 α -mediated downregulation of self-renewal genes (Caiado et al., 2023).

The study of Meisel et al. demonstrates that bacterial translocation from the intestine following disruption of the intestinal barrier or systemic bacterial stimuli like toll-like receptor 2 agonists and elevated Il-6 levels are crucial for pre-leukemic myeloproliferation (PMP) development in mice lacking TET2 in hematopoietic cells. This condition can be reversed with antibiotic treatment and does not develop in germ-free *Tet2*^{-/-} mice, highlighting the significant role of microbial signals in PMP development (Meisel et al., 2018).

In another mouse model of CHIP with hematopoietic-cell-specific *Tet2* deletion, IL1 β drives the expansion of pro-inflammatory monocytes/macrophages that correlates with impaired demethylation of enhancers and transcription factor binding sites (TFBS) related to lymphoid and erythroid lineages. Administration of IL1 β leads to significant loss of DNA methylation in WT HSPCs, a process that *Tet2*-deficient HSPCs resist. IL1 β also enhances the self-renewal capacity of *Tet2*-deficient HSPCs by upregulating self-renewal genes and preventing the demethylation of TFBS associated with terminal differentiation (McClatchy et al., 2023).

Similarly, murine clones with *Dnmt3a* loss of function are more resistant to chronic IFN γ stimulation or to TNF- α as compared to their WT counterparts (Hormaechea-Agulla et al., 2021; SanMiguel et al., 2022). *Dnmt3a*^{-/-} HSCs expand in response to infection and maintain functionality in long-term reconstitution studies. Like *Tet2* mutants, *Dnmt3a*-null HSCs stimulated with IFN γ exhibit resistance to apoptosis, characterized by significantly reduced caspase 3/7 activity compared to IFN γ -stimulated wild-type HSCs and better preserve their self-renewal capacity under inflammatory stress (Hormaechea-Agulla et al., 2021).

TET2 and *DNMT3a* mutated HSCs were also shown to have an attenuated response to inflammation in CHIP mutated individuals, as compared to non-CHIP HSCs (Jakobsen et al., 2024a). This may induce their resistance upon CHIP-induced inflammatory stress and their expansion.

Mature cells from the CHIP-mutated clones produce higher and novel inflammatory signals (Pronier et al., 2022; Yeaton et al., 2022; Zhang et al., 2015). This could induce the expansion of HSCs in a pernicious circle, as proposed by Speck (Speck, 2022). Indeed, in a zebrafish model of human *ASXL1* mutation, Avagyan et al. showed that mutated HSPCs are resistant to the inflammatory cytokines produced by their mature progeny as compared to WT HSCs through the expression of immunomodulatory genes (Avagyan et al., 2021).

Overall, research primarily conducted with mouse models supports the idea that inflammatory processes apply selective pressure that benefits the survival and expansion of HSPCs with CHIP mutations. Additionally, inflammation can arise from mature myeloid cells within the clone, creating a detrimental cycle that further promotes the expansion of mutated HSCs (Speck, 2022). This phenomenon may contribute to the progression towards hematologic malignancies.

V. 4. 2. 3 The impact of aging and inflammaging on clonal hematopoiesis

Numerous studies have demonstrated that the prevalence of CHIP increases exponentially with age in a context of low-grade inflammation called inflammaging. Indeed, there is a baseline increase in the production of pro-inflammatory cytokines such as IFN γ , TNF α , and IL-6 over time (Chung et al., 2009; Li et al., 2011; Sanada et al., 2018). This low-grade inflammation may be due to the increased production of myeloid cells upon aging as described in chapter IV.

In the study by Hong et al., they reported that aging causes a spatial re-localization of H3K9me3-marked heterochromatin in HSCs. Their findings suggest that the loss of TET2 prevents this spatial re-localization of H3K9me3-marked heterochromatin. They proposed that TET2 may play a role in regulating the subnuclear distribution of H3K9me3 in stem cells, when the DNA methylation machinery is defective due to aging or DNMT loss-of-function. This disruption could ultimately affect HSPC function. They demonstrated that *Tet2* KO HSPCs are resistant to aging compared to WT cells by maintaining perinuclear heterochromatin distribution (Hong et al., 2023).

Liao et al. found that *Dnmt3a* R878H BM cells (human equivalent: DNMT3A R882H) exhibited enhanced reconstitution ability in an aged BM environment and in response to inflammatory stress (Liao et al., 2022). It has been shown that old mice have elevated levels of TNF- α in both plasma and in the BM compartment (Henry et al., 2015). *Dnmt3a* R878H mutations protect HSPCs from chronic inflammation-induced damage, particularly from TNF α which is produced in the aged BM microenvironment. Mechanistically, they discovered that the RIPK1–RIPK3–MLKL-mediated necroptosis pathway is impaired in R878H cells when subjected to proliferative stress and TNF α exposure as compared to non-mutated cells (Liao et al., 2022).

Chapter VI - Transposable elements

VI. 1 Definition

TEs are repeated mobile DNA sequences that can independently replicate within host genomes. They usually range in length from 100 to 10,000 base pairs (bp), though some can be much larger (Arkhipova, 2017). Along with viruses, TEs are among the most complex selfish genetic elements, often encoding proteins with multiple biochemical functions and featuring intricate noncoding regulatory sequences that facilitate their transposition. The distinction between TEs and other invasive genetic elements like viruses is not always clear. TEs are defined as genetic elements capable of mobilization in the germline, thereby increasing in frequency through vertical inheritance. Although vertical inheritance through the germline is a hallmark of all TEs, horizontal transfer (HT) between species also occurs (Gilbert and Feschotte, 2018; Panaud, 2016). Although the precise mechanisms of these transfers are not fully understood, organisms engaged in close biotic interactions, such as parasitism or host-parasite relationships, appear to be more likely to exchange genetic material horizontally. HT of TEs enable TEs to bypass the host genome's silencing mechanisms, which could be crucial for their survival and spread among eukaryotes (Panaud, 2016). HTs might also drive genomic variation and biological innovation (Schaack et al., 2010).

Which vectors facilitate HTs and how DNA moves from one organism to another have long been debated. For years, only speculative hypotheses and indirect evidence were available. However, Gilbert and colleagues (2014) provided compelling evidence supporting the virus-TE vector hypothesis where viruses could serve as TE vectors between species. They identified two TEs from the cabbage looper that had been incorporated into the genome of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus during caterpillar infection. Furthermore, they demonstrated that these TEs subsequently spread to several related moth species via HT (Gilbert et al., 2014).

TEs are present in all eukaryotic genomes examined to date, with a few exceptions. In most organisms, TE content is strongly correlated with genome size, and in some species, TEs can make up as much as 85% of the genome as for example the maize reference (B73) genome

(Tenailon et al., 2011; Wegrzyn et al., 2013), with host protein-coding regions appearing as islands in a sea of TEs (Fedoroff, 2012).

VI. 2 Classifying eukaryotic transposable elements: Retrotransposons vs. DNA transposons

The most fundamental division of eukaryotic TEs, introduced by David Finnegan in 1989, distinguishes two major classes based on their way of transposition: Class I – retrotransposons, and Class II – DNA transposons (Fig. 22). Class I elements replicate via an RNA intermediate, which is then reverse transcribed back into a DNA copy and integrated into the genome (Fig. 23) (Finnegan, 1989). Because the original template element remains intact, retrotransposons are commonly referred to as “copy-and-paste” elements. In contrast, the majority of (but not all) Class II elements mobilize through a “cut-and-paste” mechanism, in which the transposon itself is excised and moved to a new genomic location.

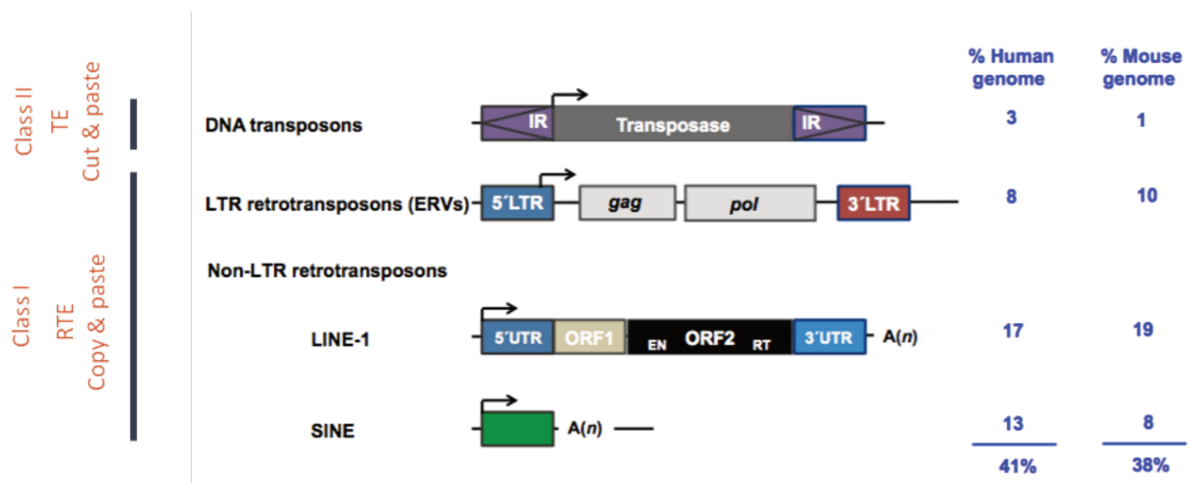


FIG. 22: Classification of Transposable Elements (Adapted from Garcia-Perez et al., 2016). TEs are classified into retrotransposons (Class I), which use a reverse-transcribed RNA intermediate, and DNA transposons (Class II). Various types of transposable elements (TEs) found in mammalian genomes are represented, including DNA transposons, endogenous retroviruses (ERVs), long interspersed elements class 1 (LINE-1), and short interspersed elements (SINEs). The proportion of each TE type in the human and mouse genomes is indicated on the right. Key features include inverted repeats (IR), untranslated regions (UTR), endonucleases (EN), reverse transcriptases (RT), long terminal repeats (LTR), and open reading frames (ORF).

Retrotransposons can be divided into three major subclasses based on their mechanisms of replication and integration: Long-terminal repeat (LTR) elements, mobilized by an integrase

(IN), "target-primed" non-LTR elements including long interspersed element class 1 (LINE-1 or L1) and short interspersed elements (SINE) (Wells and Feschotte, 2020).

VI. 2. 1 Non-LTRs elements

Among mammalian transposons, L1 retrotransposons are the only active autonomous retrotransposons in the human genome (Hancks and Kazazian, 2016). LINE-1 retrotransposons account for 17% of the human genome with approximately only 80-100 copies still capable of retro transposition. Most of the 500 000 copies present in the human genome have indeed become molecular fossils (Beck et al., 2010). In mice, LINE-1 elements make up a similar proportion of the genome, and approximately 3,000 still able to retro-transpose (Goodier et al., 2001; Richardson et al., 2015).

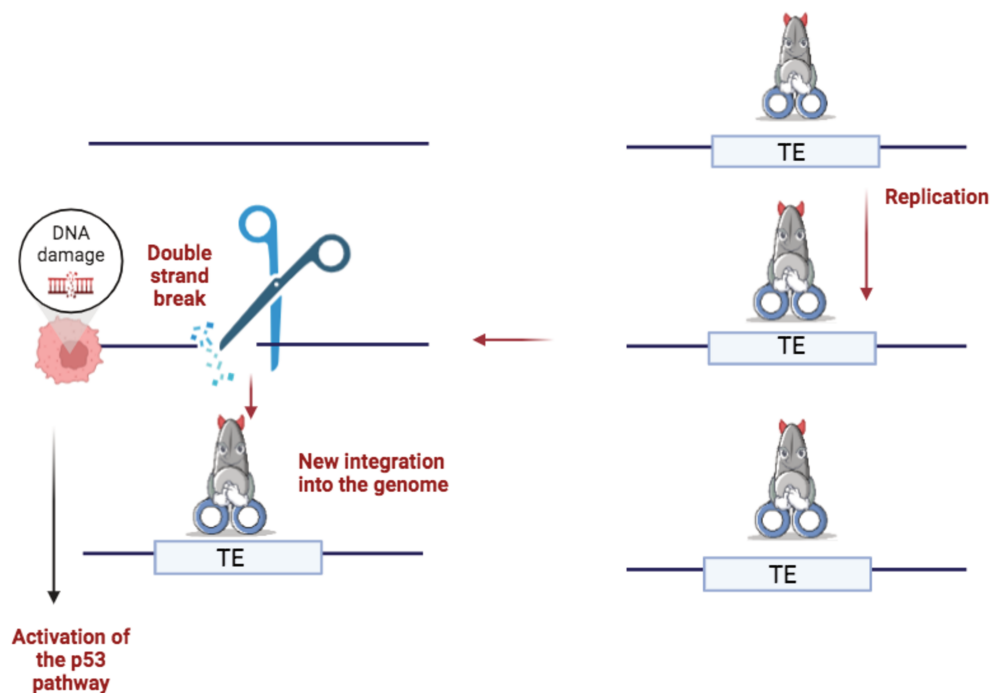


FIG. 23: Retro-transposition (Copy/Paste) mechanism of non-LTRs. Step one replication of the RTE, followed by the induction of a DSB and new integration in the genome. RTE: Retrotransposable element, DSB: double-strand break. Retro-transposition can induce DNA damage and activate the p53 pathway (Created with BioRender, License: VO27F4UICS).

LINE elements contain two open reading frames, open reading frame 1 (ORF1) and open reading frame 2 (ORF2) (Fig 22). ORF1 proteins form an oligomeric product involved in recognizing and transporting the template RNA to the nucleus (Richardson et al., 2015). ORF2 encodes both endonuclease (EN) and reverse transcriptase (RT) activities, with the latter being

crucial for target-primed reverse transcription (Luan et al., 1993). In this process, the enzyme encoded by ORF2 (ORF2p) nicks the target DNA to initiate reverse transcription of either its own RNA or other RNAs (Han, 2010) (Fig. 24). The 5' and 3' untranslated regions (UTRs) of LINE retrotransposons exhibit considerable variability. Most characterized 5' UTRs of functional non-LTR retrotransposons contain internal promoter activity (Mizrokhi et al., 1988), though promoter replacement is common (Haas et al., 2001), resulting in non-conserved promoter sequences among elements from different species. These promoters are typically transcribed by RNA polymerase II.

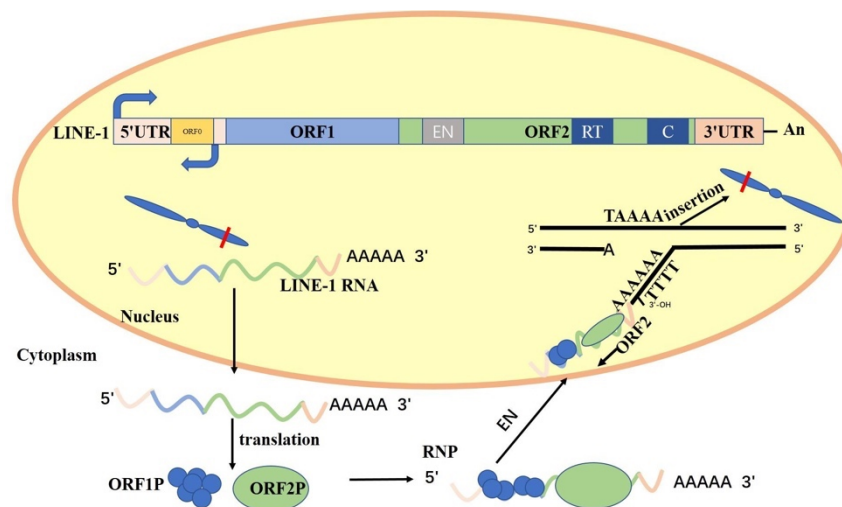


FIG. 24: LINE-1 and its retrotransposition process, highlighting the relative positions of several key regions: the 5' untranslated region (5'-UTR), the open reading frames (ORF0, ORF1, and ORF2), the 3' untranslated region (3'-UTR), and the Poly A tail. ORF2 encodes endonuclease (EN), reverse transcriptase (RT), and a cysteine-rich domain (C). LINE-1 mRNA is synthesized from the sense promoter located in the 5'-UTR. Once produced, the LINE-1 mRNA is transported to the cytoplasm, where ORF1 and ORF2 proteins are translated and assembled into a ribonucleoprotein (RNP) particle. This RNP is then imported into the nucleus. Within the nucleus, the ORF2P endonuclease in the RNP targets and cleaves specific sequences on the bottom DNA strand at the consensus site 3'AA/TTTT-5'. The resulting free 3' hydroxyl group at the cut site is used by ORF2P and the LINE-1 mRNA in the RNP as a template for reverse transcription, leading to the synthesis of complementary DNA for the LINE-1 gene (Zhang et al., 2020).

The 3' UTR of LINE elements often includes specific sequences or structures recognized by the RT ORF (Luan and Eickbush, 1995). Additionally, the 3' boundary of LINE retrotransposons may feature a polydeoxyadenosine sequence or short sequence repeats (Kajikawa and Okada, 2002). LINE-1 mRNA is synthesized from the sense promoter located in the 5'-UTR. Once produced, the LINE-1 mRNA is transported to the cytoplasm, where ORF1 and ORF2 proteins are translated and assembled into a ribonucleoprotein (RNP) particle.

This RNP is then imported into the nucleus. Within the nucleus, the ORF2P EN in the RNP targets and cleaves specific sequences on the bottom DNA strand at the consensus site 3'AA/TTTT-5'. The resulting free 3' hydroxyl group at the cut site is used by ORF2P and the LINE-1 mRNA in the RNP as a template for reverse transcription, leading to the synthesis of complementary DNA for the LINE-1 gene (Zhang et al., 2020) (Fig. 24).

Additionally, mammalian genomes contain many SINE non-LTR retrotransposons, such as Alu and SVA (SINE-VNTR-Alu) in humans (International Human Genome Sequencing Consortium et al., 2001b).

SINEs are non-autonomous retrotransposons that rely on LINE-1 enzyme machinery for mobilization. They mobilize in trans by hijacking the machinery produced by their LINE-1 autonomous counterpart which provide the necessary EN and RT functions for Alu elements. SINEs are usually derived from noncoding genes such as tRNAs, transcribed by RNA polymerase III, and trans-mobilized by the machinery of LINEs (Dewannieux et al., 2003). The estimated copy number of these elements is 1.1 million, and they are currently retrotranspositional active in the human genome (Cordaux and Batzer, 2009).

VI. 2. 2 LTR elements

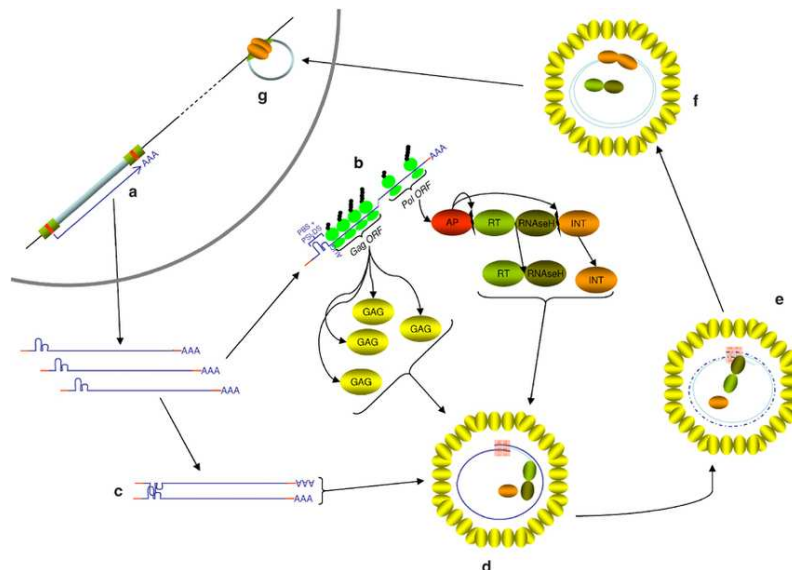


FIG. 25: The theoretical life cycle of LTR retrotransposons begins with the transcription of mRNA from the 5' R region to the 3' R region. Next, during translation, the active elements GAG and POL are synthesized. POL is then internally cleaved by aspartic proteinase (AP) into components such as RT-RNaseH and integrase (IN). Following translation, RNA dimerization occurs before or during packaging, utilizing a 'kissing-loop' mechanism. During packaging, reverse transcription starts as GAG proteins assemble into a virus-like particle (VLP). The

reverse transcription is carried out by the dual protein RT-RNaseH, which synthesizes the first strand of complementary DNA (cDNA) using the packaged RNA as a matrix. Afterward, the RNA matrix is degraded, and synthesis of the second strand of cDNA begins. The process continues with the completion of double-stranded cDNA synthesis and the linkage of integrase (IN) to the long terminal repeats (LTRs). Finally, a double-stranded break occurs, allowing the newly synthesized cDNA to integrate into a new genomic location. Key proteins involved in this cycle include reverse transcriptase (RT), ribonuclease H (RNaseH), open reading frame (ORF), aspartic proteinase (AP), and integrase (IN)(Wilhelm and Wilhelm, 2001).

The structures, coding capacity, and replication mechanisms of LTR closely resemble those of retroviruses, to which they are evolutionarily related (Eickbush and Malik, 2007) (Fig. 25). Autonomous LTR elements contain at least two distinct genes (gag and pol), generally expressed as a single polycistronic RNA transcribed from a Pol II promoter located within the LTRs. Both gag and pol encode polyproteins that are cleaved into multiple proteins by a pol-encoded protease. Pol also encodes the activities of RT, ribonuclease H (RNaseH), and IN (Wilhelm and Wilhelm, 2001). After translation, RNA dimerization takes place either before or during the packaging process, facilitated by a 'kissing-loop' mechanism. During packaging, reverse transcription begins as GAG proteins form a virus-like particle. This process is driven by the dual protein RT-RNaseH, which synthesizes the first strand of complementary DNA (cDNA) using the packaged RNA as a template. This DNA is then integrated into the host genome by the element-encoded IN. RT is a multifunctional enzyme with RNA-dependent and DNA-dependent DNA polymerase activities, as well as RNase H activity, which specifically degrades the RNA strand of RNA-DNA duplexes (Wilhelm and Wilhelm, 2001).

VI. 3 TEs play a critical role in evolution

TEs play a critical role in evolution, influencing everything from genome size and structure to the proteins encoded and their regulation.

VI. 3. 1 TEs are involved in shaping the size/structure of the genome, even if it's not linked to complexity

TEs face selective pressures for expansion. Changes in genome size can be driven by the co-evolutionary dynamics between TEs and their hosts (Ågren and Wright, 2011).

While the total TE content varies significantly among species, it has been observed that there is a very strong correlation indicating that the relative abundance/percentage of individual TE families within the genome is quite similar across species (Tenailon et al., 2011). The insertion of TEs into genes has led to the formation of large introns and, in conjunction with other factors, an abundance of pseudogenes. However, the proportion of the genome occupied by TEs does not correlate with organismal complexity (Nowoshilow et al., 2018; Nystedt et al., 2013). One example is the large genome size in conifers that appears to result from the slow and steady accumulation of a diverse array of LTR TEs (Nystedt et al., 2013).

VI. 3. 2 TEs co-opted as host sequences

TEs can be actively transcribed or serve as regulatory elements, either for their own purposes or to benefit their host.

TEs have for example influenced placental evolution by inserting themselves into genes, thereby enabling new functions and providing alternative promoters or enhancers (Keighley et al., 2023). There are TE genes (Syncytin-1, Syncytin-2, PTN and INSL4) that are unmethylated and active in the placenta while they are methylated and inactive in adult somatic tissues (Liang et al., 2005; Macaulay et al., 2011) that enable placental development. Envelope proteins produced by human endogenous retroviruses (HERVs) are essential for the differentiation and fusion of syncytiotrophoblasts which form a syncytium that acts as a interface between maternal blood and fetal tissues (Keighley et al., 2023).

Since the discovery of the RAG genes, researchers have suspected that V(D)J recombination might result from the insertion of a TE. Several observations support this hypothesis: firstly, the compact organization of the RAG locus resembles that of a TE (Schatz et al., 1989), and secondly, RAG proteins cut DNA after binding to recombination signal sequences throughout the BCR and TCR loci (Gellert, 2002). In another study they confirmed this similarity to DNA transposons based on their chromosomal translocations and deletions (Rommel et al., 2017).

VI. 4 Consequences of TE Activity

VI. 4. 1 Impact of TEs on the genome/genomic instability

Due to their ability to mobilize in the genome, TEs play a significant role in genomic instability (Cordaux and Batzer, 2009), through insertions or rearrangements within the genome.

TEs insertions can cause mutations in coding genes or disrupt gene regulatory sequences. When a TE inserts into an exon, it may alter the ORF, leading to the production of aberrant peptides or causing missense or nonsense mutations. Insertion of retrotransposons alone account for approximately 0.3% of all mutations (Cordaux and Batzer, 2009). As an example, mobilization of an L1 element in the adenomatous polyposis coli gene, together with a point mutation in the second allele, led to colorectal cancer (Scott et al., 2016). In a separate case, a somatic L1 insertion in the suppression of tumorigenicity 18 gene resulted in its up-regulation in a hepatocellular carcinoma case (Shukla et al., 2013).

L1 expression and mobilization was also associated with double-strand breaks (DSBs) and DNA damage accumulation (Belgnaoui et al., 2006) (Fig 23).

Transfection with vectors expressing L1, but not with vectors expressing L1 lacking EN activity, results in a significant increase in DSBs in DNA (Gasiior et al., 2006a). Belancio et al. demonstrated that the exogenous expression of full-length L1 or the spliced SpORF2 mRNA alone in human fibroblasts and adult stem cells triggers a senescence-like phenotype, a known response to DNA damage (Belancio et al., 2008). These data suggest that even without mobilization, the activity of the endonuclease alone is able to induce DNA damage.

Our lab showed that mouse HSCs express various families of TEs, including the most recent subfamily members of L1, L1Md, with increased expression following irradiation. By utilizing mice with an engineered human L1 retro transposition reporter cassette and inhibitors against reverse transcription, our team demonstrated that L1 retro-transposition occurs in vivo. They found that it contributes to the formation of persistent γ H2AX foci and the impairment of HSC function following irradiation (Barbieri et al., 2018a).

L1 reactivation also induced γ H2AX foci in leukemia cell lines and cell cycle exit (Gu et al., 2021a).

Reactivation and aberrant transcription of TEs have also been linked to the formation of R-loops (Zeller et al., 2016; Zhu et al., 2018), which are associated with DNA damage and DSBs (Crossley et al., 2019).

VI. 4. 2 TE mediated transcriptomic alterations

Even in the absence of transcription and mobilization, TEs have been recognized as major contributors of gene regulatory networks (Chuong et al., 2017). TEs present in intronic regions can result in alternative splicing by serving as novel splice sites, disrupting canonical splice sites, or introducing a polyadenylation signal (Deininger and Batzer, 1999; Konkel and Batzer, 2010) (Fig. 26). TEs located in intronic regions can also destabilize mRNA through providing additional splicing site leading to skipping of the downstream exon or premature termination signals, reducing gene expression (Chen et al., 2006). Similarly, TEs situated in the 5' or 3' regions of genes can affect gene expression. For instance, an Alu element can inhibit the initiation of translation of Breast Cancer gene 1 mRNA (Sobczak and Krzyzosiak, 2002).

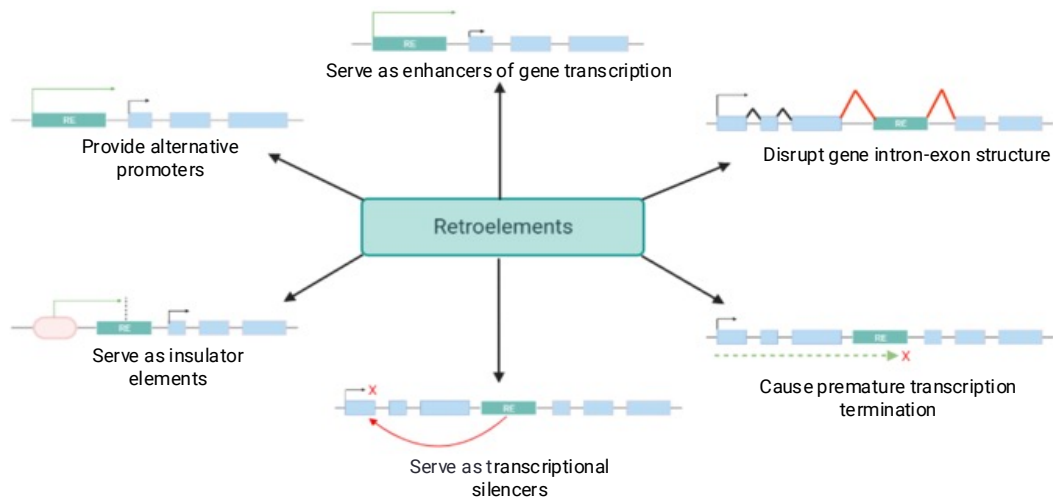


FIG. 26: Different mechanisms of TE influence on gene transcription. Green boxes: retroelements, blue boxes: gene exons, green arrow gene transcriptional start site, pink oval: enhancer element (Adapted from Gogvadze and Buzdin, 2009).

TEs can also integrate various transcriptional regulators into gene regulatory networks. They are unique in their ability to introduce regulatory sites throughout the genome during transposition, thereby coordinating the regulation of multiple genes under a shared regulatory framework as proposed by the “gene-battery” model (Sundaram and Wang, 2018). The hypothesis that TEs might serve as a readily available source of DNA for regulating gene expression stems from the observation of multiple TFBSs within TE-derived DNA (Jordan et

al., 2003). TE-derived DNA located near gene promoters is more likely to have associated TFs, indicating a more frequent role in regulating genes nearby. Many active TF binding events mapped to TEs are associated with the promoters inherent to TEs themselves (Zhou et al., 2020).

Functional TFBSs in SINEs and LINEs are found more frequently in introns than in intergenic regions. Conversely, TF binding in ERVs is more prevalent in intergenic regions (Zhou et al., 2020)

Such TE-induced changes in gene expression may disrupt regulatory networks, potentially resulting in disease conditions (Konkel and Batzer, 2010).

In the paper by Deniz et al., six ERV families with AML-associated enhancer chromatin signatures were identified. These families are enriched in the binding of key regulators of hematopoiesis and AML pathogenesis. Remarkably, the deletion or epigenetic silencing of an ERV-derived enhancer inhibits cell growth by triggering apoptosis in leukemia cell lines (Deniz et al., 2020).

Furthermore, Jang et al. showed that TEs are involved in the expression of different oncogenes in different human cancers (Jang et al., 2019).

VI. 4. 3 Viral Mimicry/Inflammation

Viral mimicry refers to a cellular state where an active antiviral response is triggered by endogenous stimuli rather than actual viral infection. This response includes both innate and adaptive immune reactions initiated by endogenous sources of cytosolic RNA or DNA (Fig. 27). Bidirectional transcription of many TEs, including ERVs, produces double-strand RNA (dsRNA), double-strand DNA (dsDNA), and RNA/DNA duplexes. These nucleic acids can induce a viral mimicry response through pathogen pattern recognition receptors, leading to NF κ B and interferon inflammatory signaling. dsRNA is detected by RIG-I-like receptors, while dsDNA triggers signaling through the cGAS/STING/TLR PRR pathway, activating potent interferon responses and inflammatory pathways, which can lead to cell death (Chiappinelli et al., 2015a; Roulois et al., 2015).

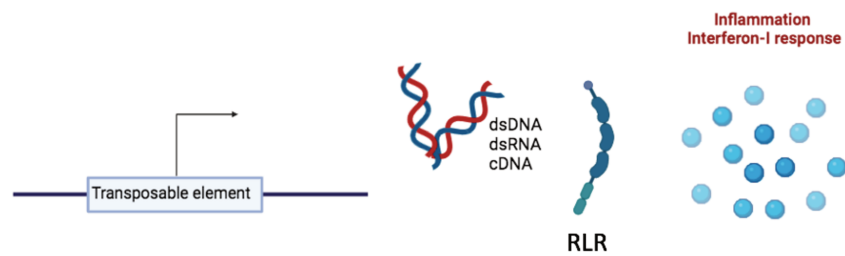


FIG. 27: Initiation of viral mimicry by transposable elements. Various classes of TEs are involved in the viral mimicry response. TE transcripts have the potential to form double-stranded RNAs (dsRNAs) or double-stranded DNA (dsDNA) (Created with BioRender, License: SD27F4UR83).

The LINE-1 element is found derepressed during aging and cellular senescence in human and mouse cell lines and tissues. The cytoplasmic accumulation of LINE-1 cDNA induces a type-I interferon (IFN-I) response. Preventing reverse transcription of these elements using RT inhibitors in aged mice prevented the aged associated inflammatory response, suggesting that L1 may be involved in the inflammaging process (De Cecco et al., 2013).

Increased expression of human endogenous retrovirus K (HERVK) has been linked to cellular senescence through the activation of innate immune responses. Suppressing HERVK and its downstream effectors using shRNA was found to reduce the associated inflammatory response (Liu et al., 2023).

Finally, abnormal TE expression upon chemotherapies induces an intrinsic sterile inflammation in HSCs through the innate immune receptor melanoma differentiation-associated protein 5 (MDA5), a member of the retinoic-acid-inducible gene I (RIG-I)-like receptor family. This leads to an exit of HSC quiescence and may be involved in HSC exhaustion upon chemotherapies (Clapes et al., 2021).

VI. 5 Regulation of TEs - a balancing Act: Silencing retroelements in mammalian genomes and evolutionary strategies

Mammalian genomes have the challenging task of distinguishing and silencing threatening retroelements without affecting those co-opted as host sequences. Retroelement families, which proliferated in distant ancestors, have been under silencing pathways control for many

generations and have acquired mutations making them less of a threat than more recently active families.

In contrast, pathways monitoring recently integrated copies of actively replicating retrotransposon families need to use targeting mechanisms to deposit epigenetic silencing modifications to the correct locations de novo.

Consequently, retroelements are under severe evolutionary pressure to devise 'escape strategies.' Thus, host strategies must be efficient and discriminative and evolutionarily flexible to adapt to new threats to the germline (Molaro and Malik, 2016).

TEs are regulated by chromatin remodelers, DNA methylation, histone modifications and small RNAs. I will only develop the parts on DNA methylation and histone modifications.

VI. 5. 1 Regulation of transposable elements through DNA Methylation: From retroelement silencing to host DNA protection

DNA methylation, specifically 5mC, is arguably the most widely adopted strategy by higher eukaryotes for maintaining TEs in a repressive state. It has been argued that the need to maintain TE silencing drove the evolution of DNA methylation as a repressive mechanism (Yoder et al., 1997), later co-opted for other contexts, such as gene imprinting. These considerations indicate that measurements of genomic 5mC levels might, in some cases, have unwittingly reported almost solely on the methylation of retroelements (Bestor, 1990). An additional hypothesis is that prokaryotic DNMTs began by protecting the host from foreign DNA integration but evolved into enzymes allowing the coexistence of foreign DNA within the host genome (Bird et al., 1995).

The integration of TEs leads not only to genome expansion and methylation of the TE DNA but also to the methylation of the flanking host DNA. Collectively, these results suggest that an unmethylated, CpG-rich TE inserted into the germline is suppressed by DNA methylation, which can subsequently spread into the surrounding DNA, leading eventually to the loss of CpG sites in neighboring DNA (Zhou et al., 2020), as the inevitable consequence of cytosine methylation is an increase in C-to-T transition mutations via deamination. Although the evolutionary cause of genome expansion is not yet fully understood, there is a notable

correlation between genome size, that could be linked to TE integration, and CpG underrepresentation. This interplay among TE insertion, DNA methylation, and 5-methylcytosine deamination could have affected the size and the structure of eukaryotic genomes (Zhou et al., 2020).

The involvement of DNA methylation in controlling TEs was shown by studying TE expression during the waves of DNA demethylation during mammalian gametogenesis and embryogenesis or using mouse models inactivated for DNMTs enzymes. DNA hypomethylation coincides with the transient upregulation of several TEs, including LINE-1 elements, during reprogramming periods of mouse germ cell development including gametogenesis and embryogenesis (Molaro et al., 2014). In mouse embryos deficient in *Dnmt1* there are elevated transcript levels of intracisternal A particle (IAP) elements, the most recent and active subfamilies of LTR in mice (Walsh et al., 1998). This highlights the crucial function of DNA methylation to suppress the expression of potentially harmful genetic elements.

Dnmt3C, a de novo DNMT gene that originated from a duplication of *Dnmt3B* in rodent genomes, was identified as crucial for methylating the promoters of evolutionarily young and active retrotransposons in the male germ line. This specialized function of DNMT3C is essential for mouse fertility and highlights the adaptability of the mammalian DNA methylation system in regulating retrotransposons (Barau et al., 2016a). Additionally, it was demonstrated that DNA methyltransferase 3-like is expressed in testis during a brief perinatal period in non-dividing precursors of spermatogonial stem cells, coinciding with the de novo methylation of retrotransposons (Bourc'his and Bestor, 2004).

Murine HSPCs expressing the human DNMT3A loss of function mutation (*Dnmt3*^{R882H}) exhibited decreased DNA methylation associated with increased expression of different families of TEs (Scheller et al., 2021).

In a mouse model of T cell lymphoma driven by TET deficiency (Tet2/3 DKO T cells), genomic analysis of the malignant T cells revealed an expected DNA hypermethylation in euchromatin regions, but a paradoxical DNA hypomethylation in the heterochromatic regions of the genome, enriched for repeated sequences, alongside the reactivation of TEs (López-Moyado et al., 2019).

VI. 5. 2 Regulation through histone modifications

TEs are mainly controlled by the repressive H3K9me3 mark. As for DNA methylation, it was suggested that this mark evolved in order to control TEs when they appeared in the first eucaryotes (Kabi and Filion, 2021).

Refined bioinformatics analyses of repeat subfamilies revealed a selective accumulation of Suv39h-dependent H3K9me3 at intact LINES and ERVs, that may keep the capacity to mobilize in the genome, as compared to degenerated and truncated LINES/ERVs, in ESCs (Bulut-Karslioglu et al., 2014).

In ESCs and neural progenitor cells, IAPs and many other ERVs are kept repressed through largely DNMT-independent mechanisms, primarily depending on host defense mechanisms involving the large family of Krüppel-associated box (KRAB) zinc-finger proteins (ZFPs) (Fig. 28), which are thought to have co-evolved with TEs (Jacobs et al., 2014). The binding of KRAB-ZFPs to TEs recruits KRAB-associated protein 1 (KAP1, also known as TRIM28), leading to the formation of repressive chromatin notably through the recruitment of SETDB1 (Hutnick et al., 2010; Karimi et al., 2011; Matsui et al., 2010).



FIG. 28: KRAB-ZNF-KAP1 mediated silencing. The removal of cytosine methylation led to rapid, widespread increases in H3K9me3 in the LTRs. During early embryogenesis, Krüppel-associated box (KRAB) proteins containing zinc fingers (ZNFs) recognize TEs in a sequence-specific manner and recruit the KAP1 cofactor. KAP1 provides a scaffold for the SET domain bifurcated 1 (SETDB1) H3K9 methyltransferase and heterochromatin protein 1 (HP1), forming a repressive chromatin structure followed by de novo DNA methylation (Deniz et al., 2019).

Recruitment of SETDB1 at TEs was also been shown to be mediated by MORC2 and subunits of the HUSH complex, specifically MPP8 and TASOR. HUSH/MORC2 complexes selectively bind to evolutionarily young, full-length L1s within transcriptionally active euchromatic regions, facilitating H3K9me3 deposition by SETDB1 and transcriptional silencing. Notably, these silencing events frequently occur within the introns of active genes, leading to down-regulation of host gene expression in a HUSH/MORC2-dependent manner (Liu et al., 2023).

TEs were also shown to be controlled by H3K27me3. Frapporti et al. observed that H3K9me3 and H3K27me3 co-occur at multiple families of TEs in an Ezh1-dependent manner in the unicellular eukaryote *Paramecium tetraurelia* (Frapporti et al., 2019). They demonstrated that the loss of these histone marks leads to a global increase in the transcriptional activity of TEs, with only modest effects on the expression of protein-coding genes. This suggests that, despite often being viewed as functionally distinct, H3K9me3 and H3K27me3 may share a common evolutionary origin and a similar ancestral role in the silencing of TEs (Frapporti et al., 2019). In the mouse, an epigenetic switch involving H3K9me3 and H3K27me3 is involved in controlling distinct TE families upon genome-wide DNA demethylation in murine ESC (Walter et al., 2016).

Interestingly, besides their role in controlling TEs by DNA methylation, TET1 and TET2 were also shown to be involved in the deposition of H3K9me3 at TEs, especially at L1Md and IAP, the most recent families of L1 and LTR respectively in mouse ES cells (De La Rica et al., 2016; Stolz et al., 2022). Loss of TET2 results in a reduction of H3K9me3 levels. However, loss of H3K9me3 was not associated with an increased expression of TEs. This could be linked DNA methylation still enriched at these TEs (De La Rica et al., 2016). TET1 and TET2 could also control TEs by recruiting HDAC complexes (De La Rica et al., 2016; Guallar et al., 2018; Stolz et al., 2022).

VI. 6 Impact of heterochromatin loss and TE upregulation on HSC aging

As discussed in Chapter IV, the "loss of heterochromatin" model of aging posits that heterochromatin domains, which are established early in embryogenesis, deteriorate with age. This breakdown leads to the derepression of previously silenced genes and TEs, resulting in abnormal gene expression patterns and genomic instability.

TE derepression due to loss of heterochromatin is proposed to be involved in the aging process in various species and cell types.

LINE-1 expression and integration may lead to accumulation of DSBs, potentially linking LINE-1 element activation to human aging processes (St. Laurent et al., 2010), even if the involvement of DNA damage in aging is debated (Best, 2009). TE activation during aging are also proposed as a significant driver of sterile inflammation that may contribute to various age-related diseases (Gorbunova et al., 2021). During cellular senescence, the LINE-1 element becomes derepressed, and its upregulation triggers the IFN-I response. This activation appears to be linked to the cytoplasmic accumulation of LINE-1 cDNA, which contributes to the sterile inflammation commonly associated with aging (De Cecco et al., 2013).

The transposon theory of aging posits that increased activation of TEs in somatic tissues during the aging process contributes to lifespan shortening (Driver and McKECHNIE, 1992).

Interestingly, our lab showed that decline in SUV39H1 levels with age in both human and mouse HSCs is associated with H3K9me3 loss and overexpression of TEs, especially L1Md and IAP (Djeghloul et al., 2016a), suggesting a role of TEs in HSCs aging.

VI. 7 TEs in cancer, the 'double-edged sword' (Fig. 29)

Through their ability to modify the cell transcriptome, to induce genomic instability and an inflammatory context, TEs are generally described as oncogenes. However, TEs are also tumor cell's Achilles's heel. Recent studies suggest that TEs, including active LINE-1 and ERVs, have been selected during evolution because of their essential role as tumor suppressor sentinels (Kelsey, 2021). Indeed, high expression of TEs induces DNA damage, senescence, and a state of "sterile inflammation", mimicking viral infection, that triggers an innate and adaptive anti-tumor immune response. Such innate immune signaling, if chronically induced, may also decrease HSC regenerative capacities.

Our lab has shown that increased expression of young active LINE-1 is partly responsible for the sustained DNA damage observed in HSCs long-time after irradiation, a phenomenon that can be reversed by RT inhibitors. The decrease in TE-induced DNA damage is associated with a significant improvement of HSC reconstitution capacities (Barbieri et al., 2018a). TE-induced

sterile inflammation upon chemotherapies might be involved in HSC activation and exit from quiescence (Clapes et al., 2021).

Tumor cells may adapt to repress TE expression to a level tolerable for their survival and proliferation.

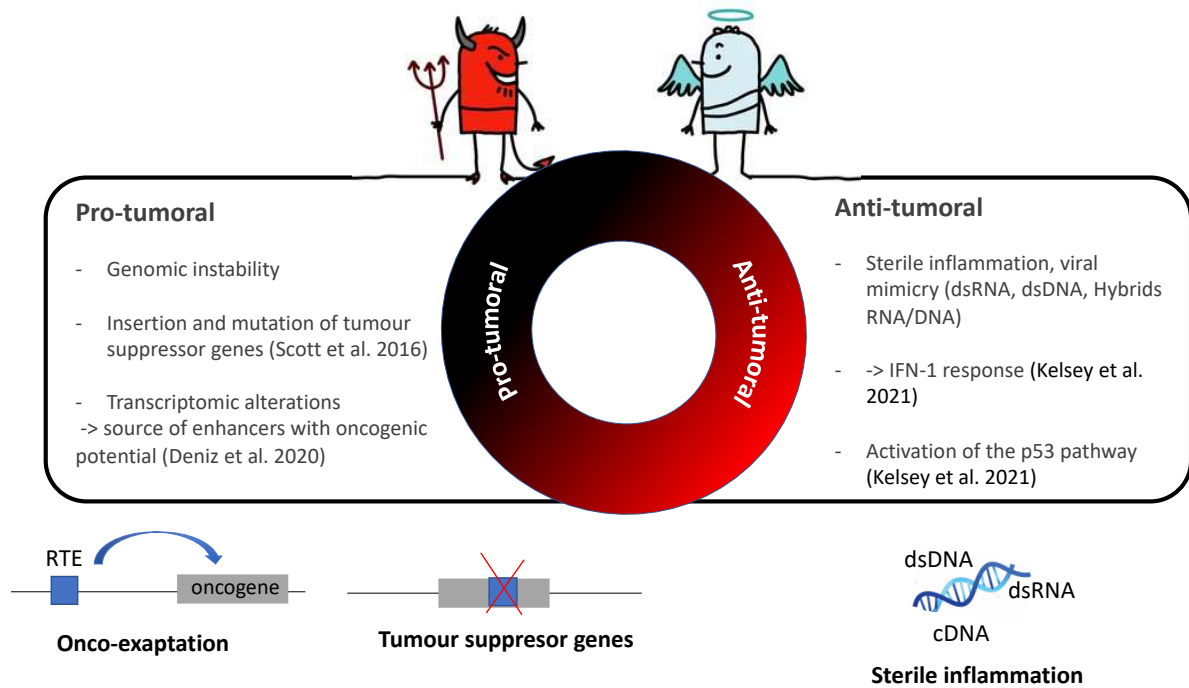


FIG. 29: Ying and Yang of transposable elements: Left: pro-tumoral including genomic instability, insertions and mutations of tumor suppressor genes and transcriptomic alterations, right: anti-tumoral including sterile inflammation, IFN-1 response and activation of the p53 pathway.

In CRISPR/Cas9 screens, two studies identified MPP8, a member of the HUSH complex, and SETDB1, as dependencies in myeloid leukemia (Cuellar et al., 2017a; Gu et al., 2021a). In both studies, it was shown that the proliferation of leukemic cell lines was dependent on the repression of TEs by H3K9me3. Reactivation of TEs leads to DNA damage accumulation and an IFN-I response finally leading to leukemic cell cycle exit and apoptosis. Interestingly, *SETDB1* expression is increased in AML and different types of cancer, suggesting a broad role of the epigenetic repression of TEs in leukemogenesis.

Suppression of TE expression and decreased inflammatory pathways were observed in leukemic stem cells (LSCs) as compared to pre-leukemic HSCs and to leukemic blasts in AML, and in CD34+ from high-risk as compared to low-risk MDS (Colombo et al., 2017a).

In a recent study, our team observed significant disorganization of heterochromatin in CD34+ cells from CMML patients, characterized by a decrease in H3K9me3 but an increase in H3K9me2, particularly at the level of TEs, as compared to aged-matched healthy controls. This is associated with decreased immune and age-associated pathways. By combining DNA hypomethylating agents (HMAs) with inhibitors of the G9A/GLP H3K9me2 methyltransferase, our team was able to reduce CMML HSPC fitness by reactivating TE-induced viral and inflammatory pathways. This approach selectively targets mutated cells while sparing wild-type HSCs, thus opening new therapeutic possibilities for CMML but also for other myeloid malignancies ((Hidaoui et al., 2024a), in preprint).

PhD Project

Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the expansion of hematopoietic stem cells (HSCs) harboring somatic mutations in genes commonly mutated in myeloid leukemia, without being associated with a hematologic disease. Mutated clones expand with age in a context of **low-grade inflammation** also referred as inflammaging, or following **genotoxic stresses**, used in the context of cancer treatment (ionizing radiations – IR in radiotherapies, or chemotherapies).

CHIP induces a pre-leukemic state and an increased risk of developing myeloid leukemia. However, most individuals with CHIP never develop blood cancer. It is thus of major importance to understand the mechanisms by which CHIP mutations trigger HSC expansion and the emergence of the pre-leukemic clone.

The most frequently mutated genes in CHIP encode for epigenetic factors such as TET2, which plays a role in both DNA methylation and histone modifications. TET2 loss of function mutations induce a competitive advantage of HSCs and imbalanced myeloid/lymphoid differentiation. However, the molecular mechanisms by which the loss of function of this epigenetic factor confers a fitness advantage to HSCs remains largely unknown.

Two non-exclusive hypotheses could explain the expansion of CHIP-mutated clones with age and in response to stress: 1- a disadvantage of non-mutated cells, and/or 2- a competitive advantage of mutated cells. It is therefore crucial to study the effect of aging and stress on both mutated and non-mutated cells.

As described in chapter IV, one theory proposes that aging may result from heterochromatin loss. As heterochromatin, mainly characterized by DNA methylation and the repressive histone mark H3K9me3, plays a major role in controlling TEs, aging may result from the derepression of TEs, through the induction of genomic instability, transcriptomic alterations, and sterile inflammation.

Upon my arrival in the lab, the team had shown a loss of H3K9me3 at, and an upregulation of L1Md and IAP, the most recent subfamilies of TEs upon aging and IR, that induces a premature aging in HSCs (Barbieri et al., 2018; Djeghloul et al., 2016a; Pelinski et al., 2022). We further showed that TE derepression is associated with HSC loss of function through DNA damage accumulation, and transcriptomic alterations (Barbieri et al., 2018; Pelinski et al., 2022).

Results showing the effect of IR on H3K9me3 at L1Md, and the impact of L1Md on HSC transcriptome and function were published in the Journal of Experimental Medicine in 2022, in an article in which I am a co-second author.

“NF-κB signaling controls H3K9me3 levels at intronic LINE-1 and hematopoietic stem cell genes in cis” [Y. Pelinski](#), D. Hidaoui*, [A. Stolz*](#), F. Hermetet, [R. Chelbi](#), M. K. Diop, [A. M. Chioukh](#), F. Porteu, E. Elvira-Matelot, Journal of Experimental Medicine. 219, e20211356 (2022). This article is included as an annex to this manuscript.

In parallel, the team of Eirini Trompouki (IRCAN-Nice) also showed the involvement of TE derepression in HSC functional changes upon chemotherapies (Clapes et al., 2021).

These data suggest that heterochromatin loss and TE derepression may be a common molecular mechanism involved in non-mutated HSC functional changes upon aging and stress.

Chronic inflammation leads to impaired HSC function and aging-like changes in hematopoiesis (Esplin et al., 2011). However, the impact of chronic inflammation on HSC heterochromatin at TEs was unknown.

The aims of my PhD project were:

1/ To investigate if derepression of TEs may be involved in the loss of HSC function upon chronic inflammation.

2/ to decipher the role of heterochromatin changes at TEs on *Tet2*^{-/-} clonal expansion upon IR and chronic inflammatory stresses.

Material and Methods

Mice strains and treatment

Wild type (WT) C57BL/6J mice (6-8 weeks-old) were from the Envigo (CD45.2) or from Charles River (CD45.1) laboratories. *Tet2*^{-/-} mice were shared by Olivier Bernard (INSERM U1170- Gustave Roussy). All the mice were housed in a specific pathogen-free environment. All procedures were reviewed and approved by the Animal Care Committee n°26 approved by the French Ministry for Research (CE #2019_078_23286 and CE #2022_066_40153). Mice were injected intraperitoneally with 6 µg LPS-B5 Ultrapure (Invivogen, tlr1-pb5lps) every other day for 30 days and control mice were injected with 1x PBS, following the protocol described by (Esplin et al., 2011). For reconstitution experiments mice were irradiated with 9.5 Gy with an X-ray irradiator in GR for BM transplantation (RX irradiator X- RAD 320), or with a cesium irradiator for lin- transplantation (GSR D1®- CEA Fontenay-Aux-Roses) and engrafted by retro-orbital injections of BM or lin- cells.

Cell harvest and culture

Bone marrow was harvested from femur, tibia and hip bones in mice. Total bone marrow was depleted of differentiated hematopoietic cells (lineage-positive cells) using Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set (BD, 558451). Magnetically sorted Lineage-negative (lin⁻) cells were kept overnight at 4°C in IMDM medium supplemented with 10% FBS (HyClone) and 1% penicillin-streptomycin (Thermofisher). Staining was performed for 20min at room temperature (RT) using CD3ε (Lin) – APC clone 145-2C11 (553066, BD), TER-119 (Lin) – APC clone Ter-119 (557909), CD45R/B220 (Lin) – APC clone RA3-6B2 (553092), Ly6G-6C (Lin)-APC clone RB6-8C5 (553129), Ly-6A/E (Sca-1) - PeCy7 clone d7 (558162), CD117 (c-Kit) – PE or PerCP-Cy5.5, clone 2B8 (553355 or 560557 respectively), CD34– FITC or AF700 clone RAM34 (560238 or 560518), CD135 (Flk2) – BV421 or PE clone A2F10.1 (562898 or 553842 respectively), all from BD Biosciences. HSCs (Lin⁻Sca⁺c-Kit⁺CD34^{low}Flk2⁻) were sorted using ARIA3, ARIA Fusion or Influx cell sorters (BD Franklin Lakes, NJ, USA) and collected in Stem Span (StemCell).

Cell cultures and treatments

Freshly isolated murine HSCs were cultured in StemSpan medium (StemCell Technologies) supplemented with 1% penicillin/streptomycin (P/S), containing the following cytokines (all from Miltenyi Biotech): FTL3-Ligand (FTL3-L, 10ng/ml), stem cell factor (SCF, 100ng/mL) interleukin 3 (IL3, 10ng/mL) and interleukin 6 (IL6, 10ng/mL), at 37°C, 5% CO₂. For treatments, LPS-B5 Ultrapure (Invivogen, tlrl-pb5lps) or lamivudine (3TC- L1295-10MG, Sigma) were added at the start of the cultures (day 0) at a concentration of 10ug/mL or 10uM respectively and for 48h.

Infected murine Lin⁻ cells were cultured in DMEM (Merck, D6429) supplemented with 1% penicillin/streptomycin (P/S), 10% FBS containing the following cytokines (all from Miltenyi Biotech): FTL3-Ligand (FTL3-L, 10ng/ml), stem cell factor (SCF, 100ng/mL) interleukin 3 (IL3, 10ng/mL) and interleukin 6 (IL6, 10ng/mL), at 37°C, 5% CO₂.

Immunofluorescence

3000-5000 HSCs were cytopsin on glass slides and immunofluorescence was performed as previously described (de Laval et al., 2013b). γ H2AX antibody was purchased from Millipore (05-636-I) and used at 1/2000. SETDB1 antibody was purchased from Santa Cruz (sc-166621) and used at 1/250. SUV39H1 antibody was purchased from Cell Signaling (8729) and used at 1/200. Detection was performed using Alexa Fluor 555-coupled anti-mouse secondary antibody (1/600) (Invitrogen, A-21425). All slides were visualized using SPE confocal microscope (Leica). Images were analyzed using CellProfiler.

sh-LINE1 virus production

shRNAs were kindly provided by Dr. Antonio Morales-Hernandez (University of Michigan). Lentivirus was prepared using a three-plasmid system: (1) shRNA transfer vector-, (2) Gag/Pol- + Rev/Tat- (pCMV), and (3) envelope plasmid (pMD2.G) by co-transfection of HEK293T cells using jetPRIME® (OZYME, POL101000046). Supernatant was collected 48 h later, cleared, titred onto HEK293T cells and stored at -80 °C. 50uL of virus were used to transduce 2 million Lin⁻ cells and incubated for 72h before detection of the GFP/mCherry signal.

CUT&Tag

CUT&Tag-IT assay kit (Active Motif) was used on 3,000 HSC according to manufacturer's instructions. Cells were incubated O/N with 0.5µg of H3K9me3 (C15410193-Diagenode). DNA fragments were amplified 18-fold by PCR using adapters supplied by the kit. PCR purification was carried out to remove remaining primers and large fragments. The quantity and quality of the libraries were assessed on Agilent 2100 Bioanalyzer (Agilent Technologies 50567-4626). Sequencing of the libraries was performed on the NovaSeq-6000 at Gustave Roussy (Illumina; 50 bp paired-end reads).

RNA-seq

HSCs from individual mice were lysed in Tri-Reagent (Zymo Research) and stored at -80°C until used. Total RNA was extracted using the Direct-Zol RNA microprep kit (Zymo research). The RNA integrity was checked on the Agilent Fragment Analyzer (Agilent) and quantified. All samples were subjected to SMARTer® Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian (Takara Bio USA, Inc., 634485). Fragmentation time is adjusted depending on the quality of the RNA input.

The quantity and quality of the libraries were evaluated on the Agilent Bioanalyzer. Libraries were pooled and sequenced in (2 x 100bp) on the NovaSeq6000 (Illumina).

CUT & Tag Analysis

Quality control was performed using FastQC (v0.11.9) and MultiQC (v1.11). Reads were aligned to the mm10 (UCSC genome) reference using Bowtie2 (v2.4.1) with the following parameters: --end-to-end --very-sensitive --no-mixed --no-discordant --phred33 -I 10 -X 700. Duplicate reads were removed with Picard (v2.26.9) using the parameters --REMOVE_DUPLICATES true --VALIDATION_STRINGENCY LENIENT. Aligned read quality scores were set to 0, retaining all reads using Samtools (v1.13) with the parameter -q 0. Aligned reads were sorted and indexed with Samtools (v1.13). A coverage track (bigWig) was generated using deeptools (v3.5.0) with parameters -bs 5 --normalizeUsing BPM. Finally, a profile plot over genomic regions (mm10.rmsk.mod.L1Md.bed) was generated using deeptools (v3.5.0) with the following parameters: --beforeRegionStartLength 1000 --regionBodyLength 5000 --afterRegionStartLength 1000.

To quantify H3K9me3 levels at TEs, the Bioconductor package Diffbind (Ross-Innes et al., 2012) v3.8.4 was employed in R v4.2.3. Paired-end mode was enabled during the read counting step using the SummarizeOverlaps method. The default mapping quality threshold (mapQCth) was adjusted to 0 for multimapping analysis. Differential binding affinity was assessed using the DBA_DESEQ2 method. The normalized H3K9me3 concentration across all TE loci within the same family or subfamily was aggregated to calculate the total H3K9me3 concentration per TE family. Differential binding at peaks was determined using a P-value threshold of 0.05.

RNA-seq analysis

Quality control was performed using FastQC (v0.11.9) and MultiQC (v1.11). UMI was extracted using umi_tools (v1.1.2). adapters were removed with Trimgalor (v0.6.5). The FASTQ file was filtered for rRNA sequences using SortMeRNA (v3.4.6). Reads were aligned to the GRCm38 mouse genome reference using STAR (v2.7.5a) and gene's quantification was performed using salmon (v1.6.0). these precedent steps were performed on the Core Cluster of the Institut Français de Bioinformatique (IFB) (ANR-11-INBS-0013). Differential expression analysis was done using DESeq2 R package with P-value <0.05

TE-transcript Analysis

Bulk RNA-seq data analysis was conducted following previously established protocols, with some modifications. In brief, reads were aligned to the mouse genome (GRCm38) using STAR (v2.7.5a) with the following parameters: `--runRNGseed 0 --outFilterMultimapNmax 100 --alignSJDBoverhangMin 1 --winAnchorMultimapNmax 100 --outFilterMismatchNmax 3 --alignEndsType EndToEnd --alignIntronMax 1 --alignMatesGapMax 350`. TEtranscripts (v2.2.1) was used to quantify reads mapping to transposable elements (TEs) with the parameter `--mode multi`. Differential expression analysis was done using DESeq2 R package with P-value <0.05

Pathway Enrichment Analysis and Data Visualization

We performed Gene Set Enrichment Analysis (GSEA) on a ranked gene list (based on fold change) using the clusterProfiler R package (v4.6.2). This approach assessed transcript abundance across a ranked list of all available genes to identify the regulation of functionally related gene sets with statistically significant enrichment. The GSEA analysis was performed

on normalized, batch-corrected counts generated in DESeq2, utilizing the mh.all.v2023.2.Mm.entrez.gmt (hallmark) and m2.all.v2023.2.Mm.entrez.gmt (curated) gene set databases.

We performed a multiple gene set enrichment analysis and displaying enrichment patterns using BubbleGUM (v1.3.19) software.

Statistical analysis

Results were statistically evaluated using GraphPad Prism version 7.0 software (GraphPad Software Inc.). The results are displayed as the means and SEM. The value of *, $P < 0.05$ was considered as significant, and **, $P < 0.01$ or ***, $P < 0.001$ as highly significant.

Results

FIGURE 1

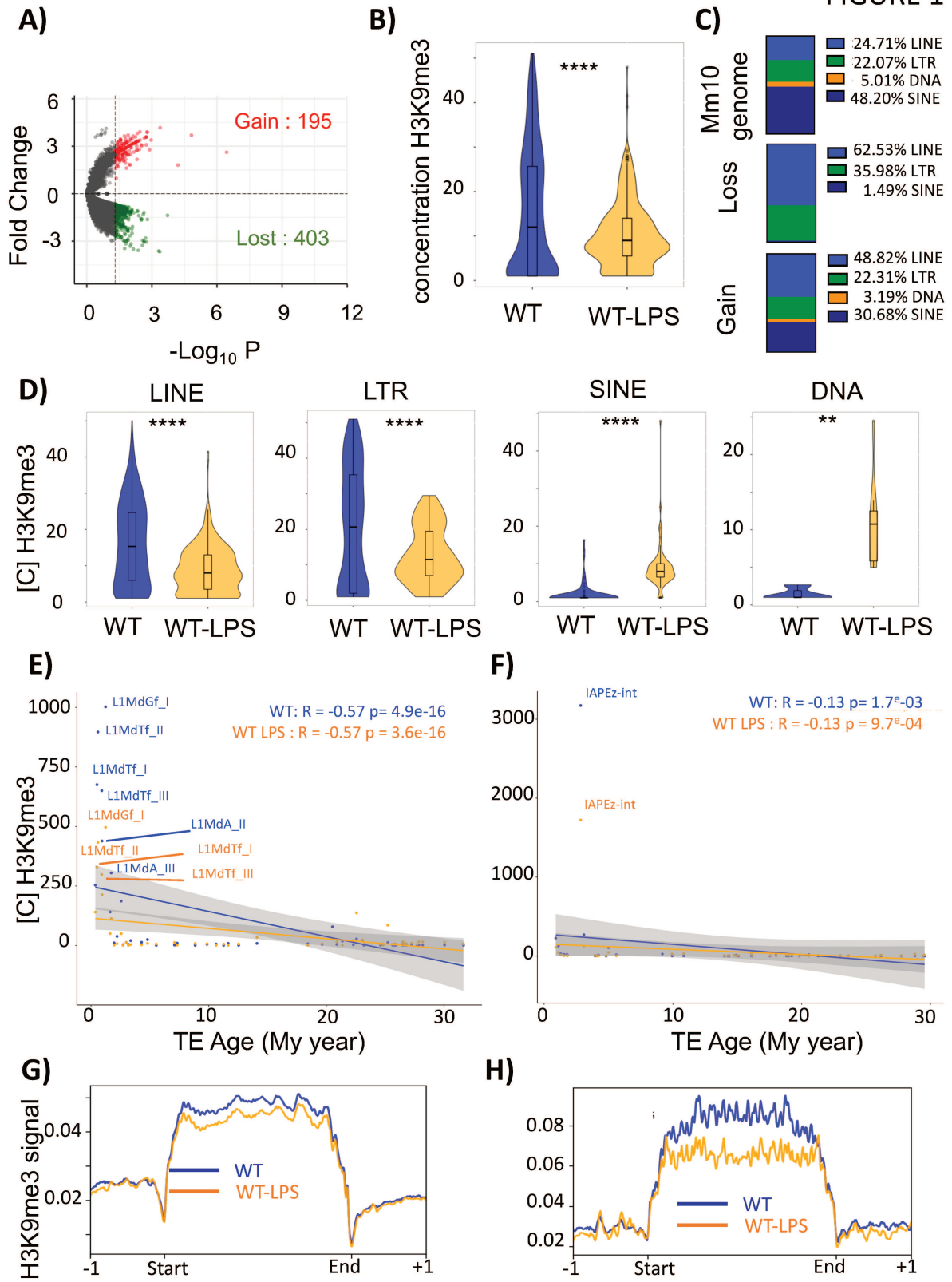


FIG.1: Chronic LPS treatment induces a loss of H3K9me3 at L1Md and IAP, the most recent subfamilies of L1 and LTR. (A) Volcano plot of the TE differentially enriched in H3K9me3 between WT-LPS and WT conditions. The horizontal axis represents the $-\log_{10}(p\text{Value})$ and the vertical axis the \log_2 fold change (FC). TE presenting a significant ($p < 0.05$) increase (red) or decrease (green) enrichment in H3K9me3 are shown. (B) Violin plot representing the distribution of H3K9me3 concentration at each TE locus retrieved in (A). (C) Distribution of the % of each family of TE among the total TE loci in the mouse mm10 genome (up) and among the TE retrieved in (A) presenting a significant decrease (middle - loss) or increase (bottom - gain) of H3K9me3. (D) Violin plot representing the distribution of H3K9me3 concentration at each locus retrieved in for LINE, LTR, SINE and DNA families of TEs in WT and WT-LPS conditions. (E-F) Correlation plot representing H3K9me3 concentration quantified at differentially enriched LINE (E) or LTR (F) elements retrieved in (A) vs their age in million years (My) in WT and WT-LPS conditions. R, Pearson correlation coefficient; p, pvalue. (G-H) Plot profile of H3K9me3 enrichment along the L1Md (G) or IAP (H) sequences +/- 1kb flanking regions in WT-LPS (orange) vs WT (blue) conditions. wilcoxon test. ** $p < 0.01$; **** $p < 0.0001$.

Chronic inflammation induces a loss of H3K9me3 at L1Md and IAP, the most recent subfamilies of L1 and LTR

To characterize the effect of chronic inflammation on non-mutated HSC heterochromatin at TEs, we used a mouse model of chronic inflammation mediated by serial injections of low doses of bacterial lipopolysaccharide (LPS), which has been shown to mimic the low-level chronic inflammation observed with age and leads to impaired HSC function and aging-like changes in hematopoiesis (Dykstra et al., 2011; Esplin et al., 2011; Rossi et al., 2005).

We performed H3K9me3 CUT&Tag experiments in HSCs sorted from mice at the end of chronic LPS treatment. We previously showed that H3K9me3 is mainly enriched at TEs, and more particularly at L1Md elements, the most recent subfamilies of LINE, in HSCs (Pelinski et al., 2022). In order to not miss out on the information at youngest TE subfamily level, we performed a multiple read analysis, as previously described (Pelinski et al., 2022). We focused our analyses at these sequences by performing a differential H3K9me3 enrichment analysis at TE genomic loci. We found 598 TE loci with significant H3K9me3 differential enrichment, 403 (67%) and 195 (33%) loci showing decreased or increased H3K9me3, respectively upon LPS treatment (Fig. 1A). The distribution of the H3K9me3 concentration at the 598 significantly differentially enriched TEs showed a significant ($p < 0.0001$) decrease in H3K9me3 at TEs (Fig. 1B). Annotation of the TEs that significantly loose H3K9me3 upon LPS treatment loci revealed that they are enriched in LINEs (62.53%) and LTR (35.98%) as compared to the distribution of these TEs in the mouse genome (24.71 and 22.07 %

respectively) (Fig. 1C). Comparing the distribution of the H3K9me3 concentration between WT-LPS and WT conditions confirmed a significant ($p < 0.0001$) loss in H3K9me3 concentration at LINEs and at LTRs, but a significant gain at SINE and DNA (Fig. 1D).

Since we and others have reported that young TE subfamilies, notably L1Md, are the most epigenetically regulated in the genome compared to old ones (Barau et al., 2016b; Pelinski et al., 2022b; Pezic et al., 2014), we further dissected H3K9me3 enrichment at LINEs and LTRs depending on their age, as calculated in (Sookdeo et al., 2013). We confirmed the significant negative correlation between the age of the LINE and H3K9me3 enrichment, showing that H3K9me3 loss mainly occur at these youngest subfamilies (Fig. 1E). The correlation between H3K9me3 enrichment and the age of LTRs is less strong, but still shows that within the LTRs the most enriched for H3K9me3 are the youngest, especially IAPEz-int. (Fig. 1F).

Finally, plot profile of H3K9me3 enrichment along L1Md and IAP sequences confirmed the global decrease of H3K9me3 (Fig. 1G-H) at these sequences.

Altogether, these data show that chronic inflammation induces the loss of H3K9me3 mainly at the youngest subfamilies of LINE and LTR, especially big and intact L1Md that keep the capacity to propagate in the genome and induce genomic instability (Barbieri et al., 2018; Belancio et al., 2010; Gasior et al., 2006).

FIGURE 2

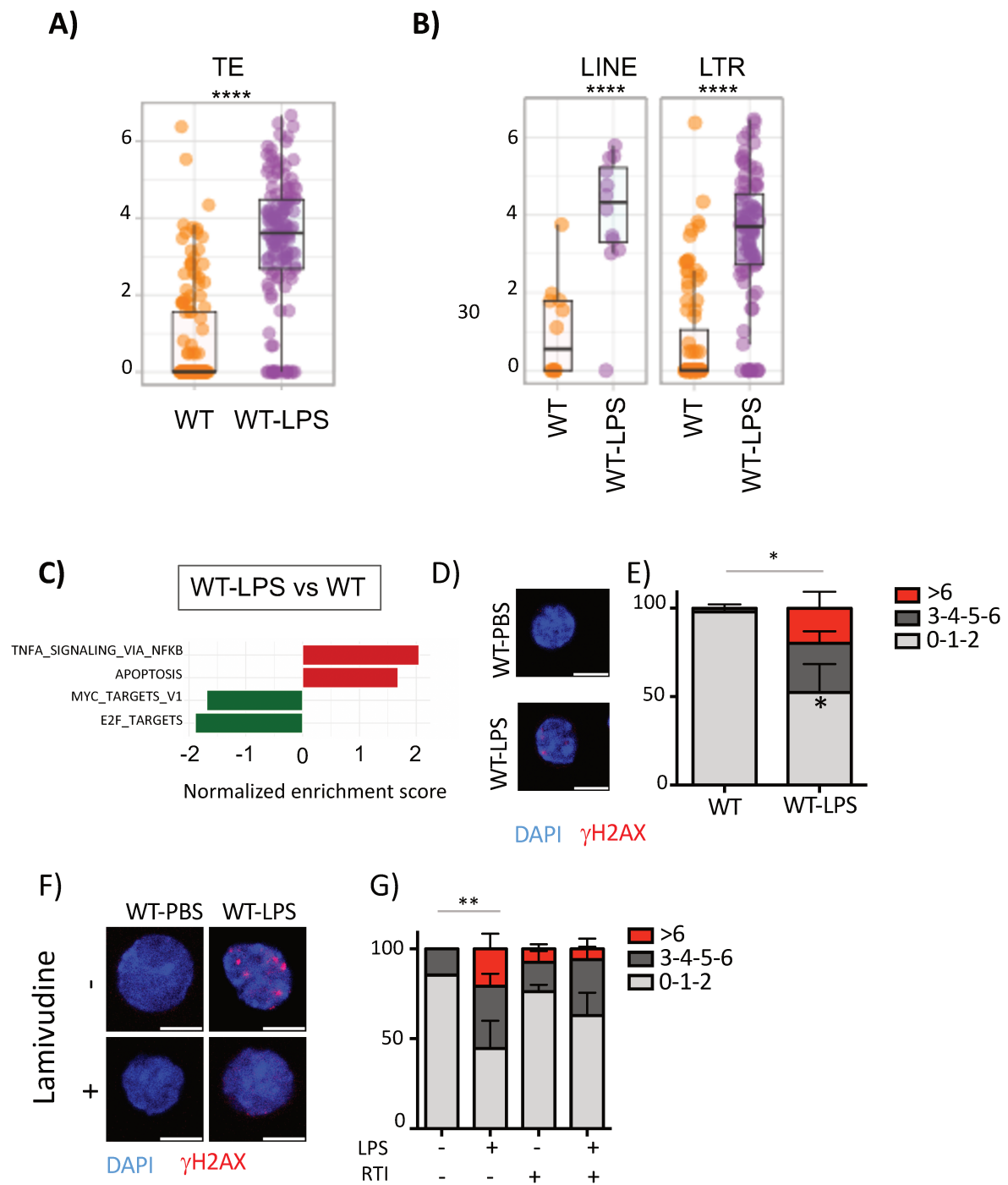


FIG. 2: TE derepression and upregulation is associated with DNA damage accumulation upon LPS treatment. (A-B) Box plot showing the level of expression of all (A) or of LINE and LTR (B) significantly ($p < 0.05$) deregulated upon LPS treatment. (C) GSEA analysis using Hallmark genes sets. graphs representing significantly ($FDR < 0.25$) enriched (red) or depleted (green) pathways in WT-LPS vs WT. (D-E) representative images (D) and quantification (E) of γ H2AX foci in WT and WT-LPS conditions in vivo. $n = 2$ independent experiments. Sidak's multiple comparison test $*p < 0.05$ for 0-1-2 foci per cell. (F-G) representative images (F) and quantification (G) of γ H2AX foci in HSCs +/- 10ug/ml LPS and +/- 10uM lamivudine (RTI) after 48h of culture in vitro. $n = 2$

*independent experiments Dunnet's multiple comparison test ** p<0.01 for 0-1-2 foci per cell. RTI: reverse transcriptase inhibitor.*

TE derepression is associated with DNA damage accumulation upon LPS treatment.

We next interrogated if loss of H3K9me3 at LINE and LTR could be associated with their overexpression, as we previously observed upon irradiation and aging (Barbieri et al., 2018b; Djeghloul et al., 2016b; Pelinski et al., 2022b). For this purpose we performed RNA-seq experiments in HSCs sorted from WT and WT-LPS mice, and quantified the expression of TE families using Tetrascript (Jin et al., 2015) (multiple mapping reads). We observed a significant upregulation of LINE and LTR classes of TEs ($p<0.0001$) upon LPS treatment (Fig. 2A-B)

Next, we performed Gene Set enrichment analysis (GSEA) on Hallmark gene sets. The data show a significant ($\text{padj}<0.05$) enrichment of TNFA_signaling_via_NFKB and apoptosis pathways in WT HSCs upon LPS treatment, and a decrease of proliferative pathways such as MYC_TARGETS and E2F_targets (Fig. 2C).

We and others have shown that L1 expression and mobilization is associated with double strand breaks (DSB), as measured by the presence of γ H2AX foci, notably in HSCs (Barbieri et al., 2018b; Belancio et al., 2010; Gasior et al., 2006b). Inflammation also leads to genomic instability in HSPCs (Rodriguez-Meira et al., 2023). In order to assess the impact of chronic LPS treatment on DSBs accumulation in HSCs, we assessed γ H2AX foci accumulation by immunofluorescence (IF) in vivo in HSCs sorted from mice at the end of chronic LPS treatment, or in vitro in culture in the presence or absence of 10ug.ml LPS for 48h. We show that chronic LPS treatment induced a significant increase in the number of γ H2AX positive HSCs (≥ 3 foci) in HSCs both in vitro and in vivo (Figure 2D-G).

We previously showed that DNA damage accumulation and loss of HSC self-renewal capacity upon irradiation stress is due to L1 mobilization (Barbieri et al., 2018). L1 mobilization requires its reverse transcription activity, which is carried by ORF2p (Mita and Boeke, 2016). This activity is sensitive to reverse transcription inhibitors (RTIs), including lamivudine also called 3TC, which is particularly efficient in inhibiting the reverse transcription of L1 (De Cecco et al., 2019). In order to assess if DNA damage accumulation upon chronic LPS treatment may also be due to L1 mobilization, we cultured HSCs in liquid culture in vitro with 10ug.ml LPS

and/or 10uM lamivudine and assessed γ H2AX foci by IF. Our data show that the presence of lamivudine in the medium culture prevented DNA damage accumulation upon LPS treatment (Fig. 2F-G).

Altogether, these data suggest that chronic inflammation mediated by chronic injections of LPS induces the upregulation of TEs and an increase in L1-induced DNA damage.

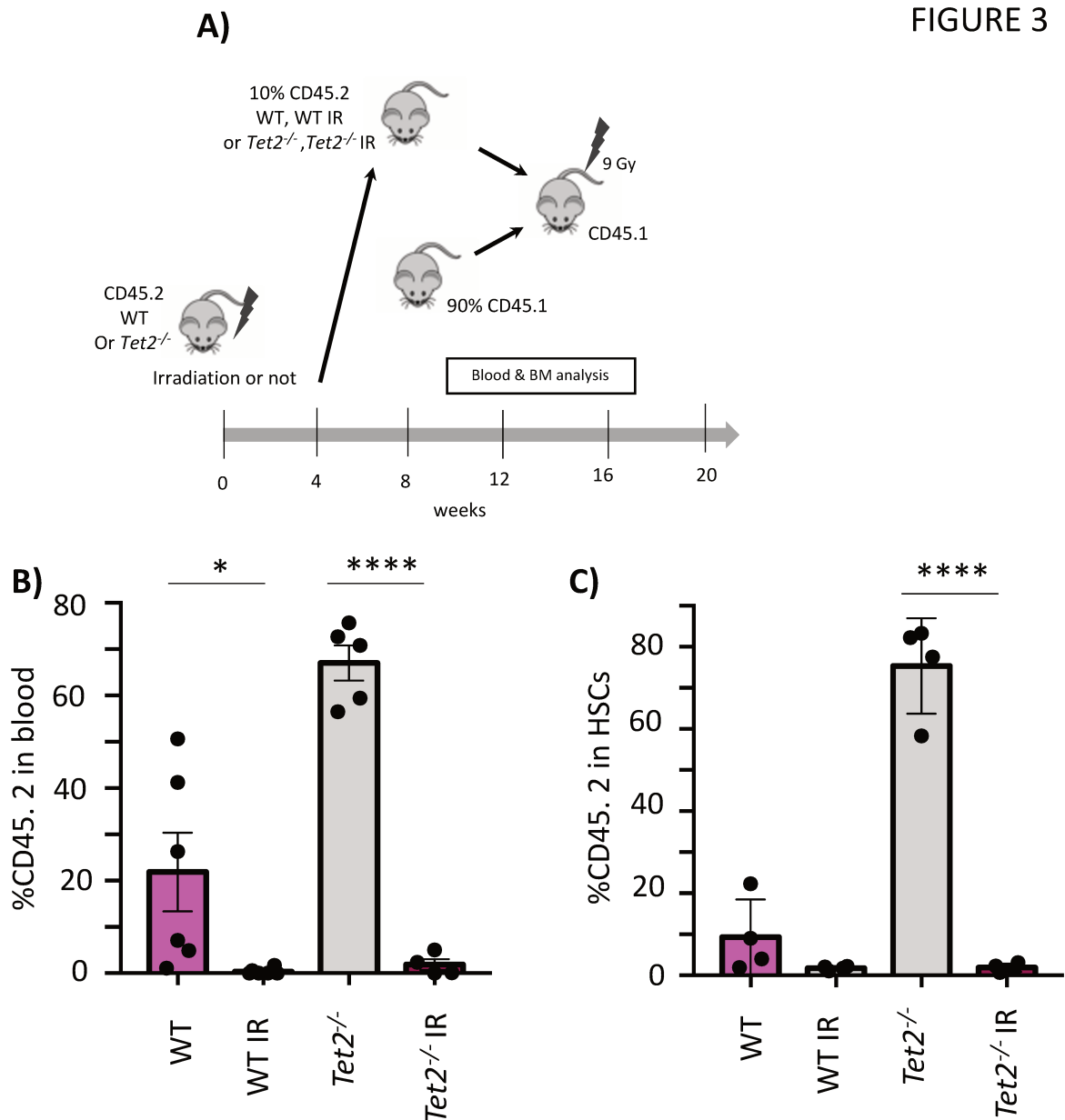


FIG. 3: Both $Tet2^{-/-}$ and WT HSC lose their functionality upon IR

(A) Reconstitution Experimental design: Irradiation (2Gray) was performed one month before engraftment of WT or $Tet2^{-/-}$. 10% CD45.2 WT, WT IR, $Tet2^{-/-}$ or $Tet2^{-/-}$ IR BM were engrafted in competition with 90% WT BM into lethally irradiated mice (9.5 Gray). (B-C) Percentage of CD45.2+ in the blood (B) or LSK CD34- Flk2- (C) 4

months after reconstitution. One-way ANOVA with Sidak multiple comparison test * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Both *Tet2*^{-/-} and WT HSCs lose their functionality upon IR

Our data show that both IR and chronic inflammatory stresses affect WT HSC function through H3K9me3 loss and TE derepression.

Even if some studies showed an effect of IR on TET2 mutated HSCs expansion in CHIP individuals (Takahashi et al., 2017b), most studies show that IR stress mainly affect HSC mutated for DNA repair factors instead of epigenetic factors, and that TET2-mutated HSCs mainly expand upon inflammatory stress, and in a context of chronic low-dose inflammation associated with aging (Florez et al., 2022b).

In order to assess the effect of IR on *Tet2*^{-/-} HSCs function, we first treated *Tet2*^{-/-} and WT mice with 2Gy IR, and one month after IR, engrafted 10% of bone marrow (BM) cells sorted from these mice in competition with 90% of BM from CD45.1 mice, in CD45.1 lethally irradiated recipient mice. BM analysis 3 months after reconstitution showed that IR drastically reduced HSC functionality of both WT and *Tet2*^{-/-} HSCs (Fig. 3B). These data suggest that IR may not affect *Tet2*^{-/-} expansion in mice.

These data, together with published data showing the preferential expansion of *TET2* mutated HSC upon aging and inflammation, prompted us to focus our analysis on the effect of chronic inflammation on *Tet2*^{-/-} HSCs expansion.

FIGURE 4

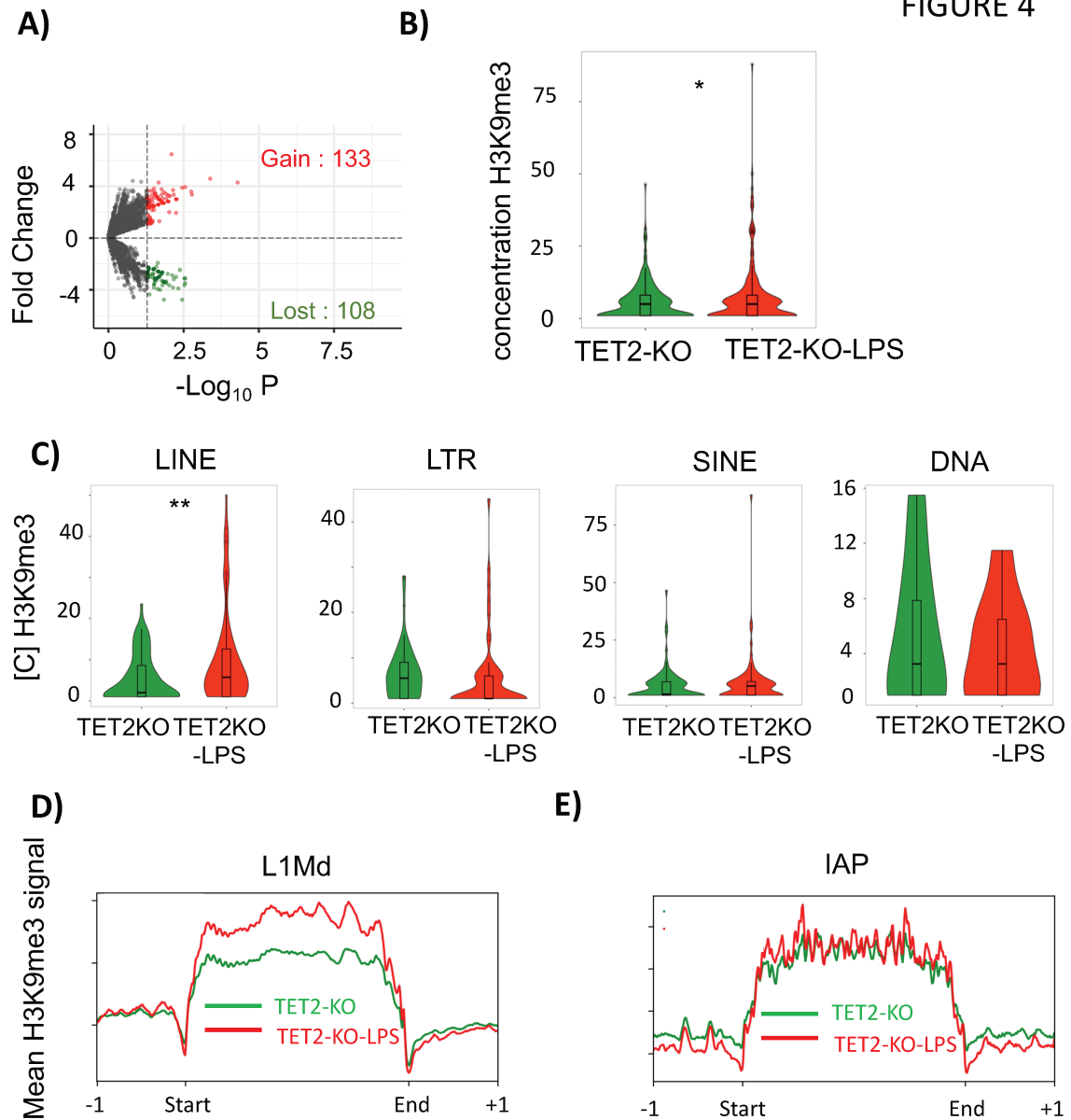


FIG. 4: LPS induces a gain of H3K9me3 at L1Md in *Tet2*^{-/-} HSCs. (A) Volcano plot of the TE differentially enriched in H3K9me3 between *Tet2*^{-/-}-LPS and *Tet2*^{-/-} conditions. The horizontal axis represents the $-\log_{10}$ (pValue) and the vertical axis the log₂ fold change (FC). TE presenting a significant ($p < 0.05$) increase (red) or decrease (green) enrichment in H3K9me3 are shown. (B) Violin plot representing the distribution of H3K9me3 concentration at each TE locus retrieved in (A). (C) Violin plots representing the distribution of H3K9me3 concentration at each locus retrieved in (A) for LINE, LTR, SINE and DNA families of TEs in *Tet2*^{-/-} and *Tet2*^{-/-}-LPS conditions. (D-E) Plot profile of H3K9me3 enrichment along the L1Md (D) or IAP (E) sequences +/- 1kb flanking regions in *Tet2*^{-/-}-LPS (red) vs *Tet2*^{-/-} (green) conditions. wilcoxon test. ** $p < 0.01$; **** $p < 0.0001$.

Chronic inflammation induces a gain of H3K9me3 at L1Md in *Tet2*^{-/-} HSCs.

We next aimed to decipher the impact of chronic inflammation on *Tet2*^{-/-} HSCs. For this purpose, we chronically treated *Tet2*^{-/-} with LPS as previously described for WT mice and assessed the effect of LPS on HSC heterochromatin in *Tet2*^{-/-} HSCs. We found 241 TE loci with significant H3K9me3 differential enrichment, 108 (45%) and 133 (55%) loci showing decreased or increased H3K9me3, respectively upon LPS treatment (Fig.4A). In contrast to what we observed in WT HSCs, the distribution of the H3K9me3 concentration showed a slight significant ($p < 0.05$) increase in H3K9me3 at TEs (Fig. 4B). Comparing the distribution of the H3K9me3 between *Tet2*^{-/-} -LPS and TET2 conditions showed that the gain of H3K9me3 specifically occurs at LINE as compared to other TE classes (Fig. 4C). Finally, plot profile of H3K9me3 enrichment along L1Md and IAP sequences showed a specific global increase of H3K9me3 at L1Md but not a IAP upon LPS treatment in *Tet2*^{-/-} HSCs (Fig. 4D-E). Surprisingly, these data show that LPS had inverse consequences on H3K9me3 enrichment at L1Md in WT and *Tet2*^{-/-} HSCs.

FIGURE 5

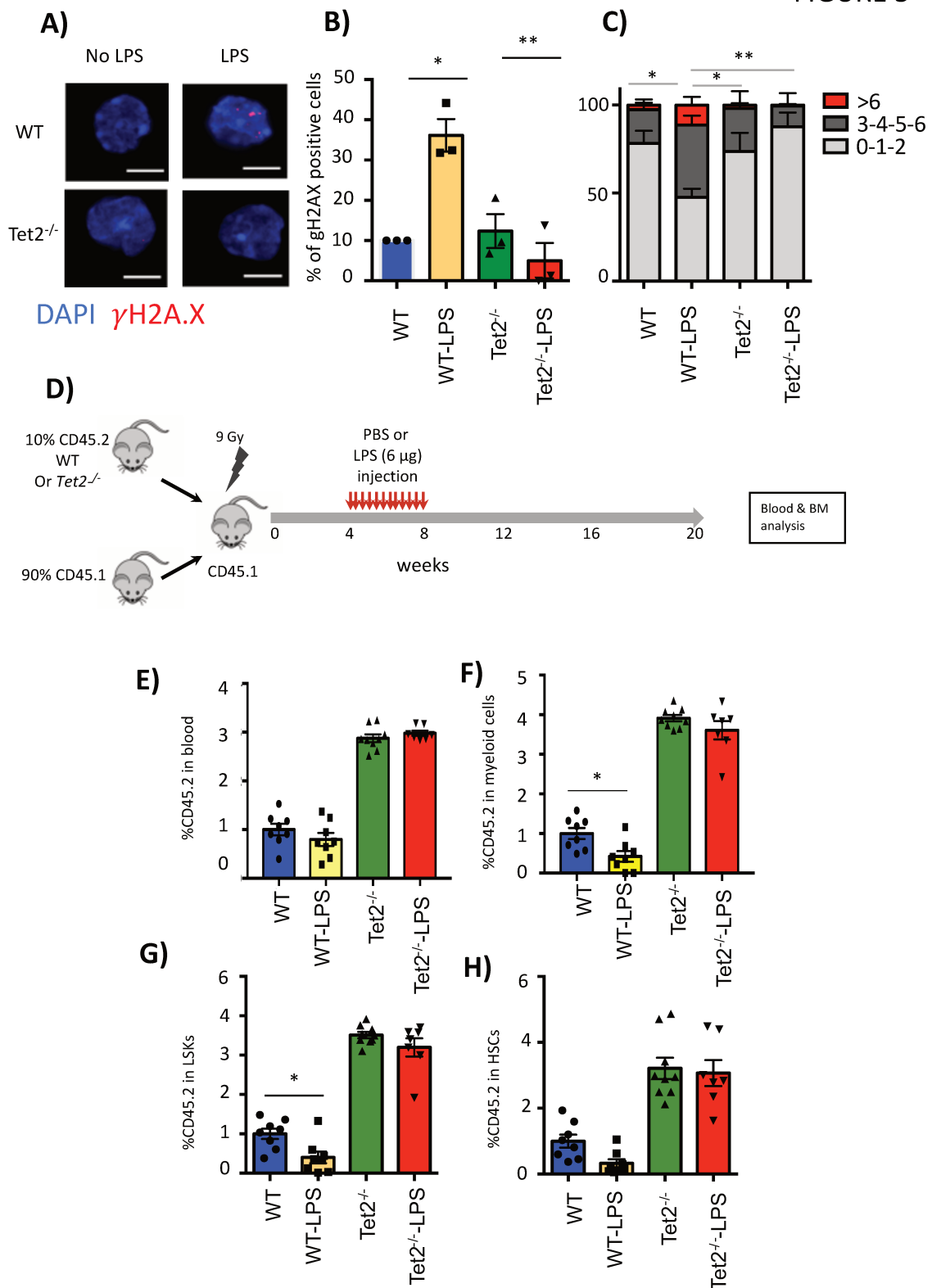


FIG. 5: Tet2^{-/-} HSCs are protected from the deleterious effects of LPS. (A-C) representative images (A) and quantification (B-C) of γH2A.X foci in WT or Tet2^{-/-} HSCs +/- 10ug/ml LPS in vitro. Sidak's multiple comparison test (B) and Tukey's multiple comparisons test (C). Light grey bars represent the comparisons for 0-2 foci, dark

grey bar for 3-6 foci. * $p < 0.05$; ** $p < 0.01$ (D) Reconstitution Experimental design: Engraftment of 10% CD45.2 WT or *Tet2*^{-/-} BM in competition with 90% WT BM into lethally irradiated mice (9.5 Gray) followed by LPS treatments (6ug per mouse) every two days for 30 days. (E-F) Percentages of CD45.2 contribution in blood (E) and in myeloid cells in blood (F) in mice 4 months after reconstitution. (G-H) Percentage of CD45.2+ in LSKs (G) or LSK CD34- Flk2- (H) 4 months after reconstitution. Sidak's multiple comparison test * $p < 0.05$.

***Tet2*^{-/-} HSCs are protected from the deleterious effects of LPS.**

These data prompted us to test the hypothesis that L1Md repression in *Tet2*^{-/-} HSCs upon chronic LPS treatment may protect them from the deleterious effect of chronic inflammation. In accordance with this hypothesis, while LPS induced a significant increase in γ H2AX positive cells in WT HSCs in vitro, it had no effect on *Tet2*^{-/-} HSCs (Fig. 5A-C).

To assess the effect of LPS on the expansion capacity of HSCs, we performed competitive reconstitution assays followed by treatments with LPS. Total BM cells isolated from mice treated were transplanted in competition with total BM cells from CD45.1 mice into lethally irradiated CD45.1 mice (Fig. 5D). Five months after reconstitution, the percentage of CD45.2 in myeloid donor cells were significantly decreased in the blood (Fig. 5F) and in LSK compartments in the bone marrow (Fig. 5G).

Altogether, these data suggest that *Tet2*^{-/-} HSCs are resistant to LPS effects as compared to WT HSCs.

FIGURE 6

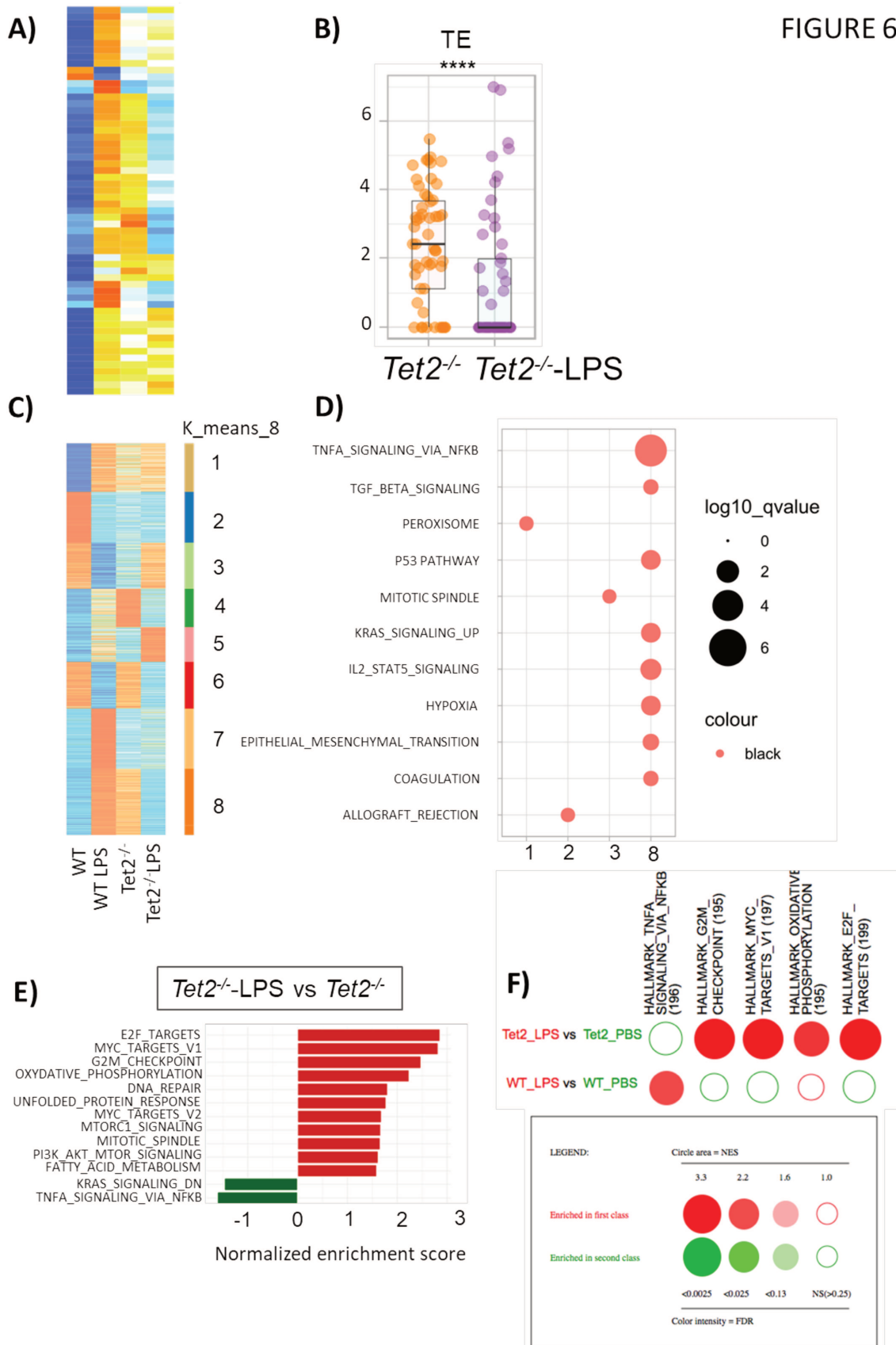


FIG. 6: LPS induces differential transcriptomic responses in WT and *Tet2*^{-/-} HSCs.

(A) Heatmap of the expression of TEs found significantly differentially expressed ($p < 0.05$) in WT-LPS vs WT in each treatment. (B) Box plot showing the level of expression of TEs found significantly ($p < 0.05$) differentially expressed in HSCs of *Tet2*^{-/-} mice treated or not with LPS. (C) Heatmap of the expression of DEGs found in WT-LPS vs WT comparison in each treatment (C) and hallmarks associated to each cluster (D). (E) GSEA analysis using Hallmark genes sets. graphs representing significantly ($FDR < 0.25$) enriched (red) or depleted (green) pathways in *Tet2*^{-/-} -LPS vs *Tet2*^{-/-} comparisons (F) BubbleGUM (GSEA Unlimited Map) analysis to identify hallmarks specific to one comparison compared to the other.

LPS induces differential transcriptomic responses in WT and *Tet2*^{-/-} HSCs.

In order to decipher the mechanisms involved in *Tet2*^{-/-} HSC resistance towards LPS as compared to WT HSCs, we performed RNA-seq experiments in HSCs sorted from *Tet2*^{-/-} and WT littermates treated or not with LPS.

We first assessed the expression of the TEs found significantly ($p < 0.05$) differentially expressed in WT-LPS vs WT (Fig. 2A) in HSC sorted from *Tet2*^{-/-} mice treated or not with LPS. Heatmap clustering of the expression levels of TEs revealed that almost all the TEs significantly overexpressed upon LPS in WT HSCs show a decreased expression in *Tet2*^{-/-} HSCs upon LPS treatment (Fig. 6A). Focusing on TEs significantly ($p < 0.05$) deregulated in *Tet2*^{-/-} HSCs upon LPS treatment, we show that LPS induces a significant decrease in TE expression in *Tet2*^{-/-} context (Fig. 6B).

We next compared transcriptomic pathways deregulated in *Tet2*^{-/-} or WT HSCs upon LPS treatment. Heatmap clustering of the expression levels of DEGs deregulated in WT-LPS vs WT conditions in WT, WT-LPS, *Tet2*^{-/-}, *Tet2*^{-/-}-LPS identified eight clusters (Fig. 6C-D). Cluster 1 is composed of genes whose expression increases in all the conditions as compared to WT HSCs. It is enriched in genes linked to the peroxisome, involved in redox signaling and fatty acids homeostasis. Cluster 2 is composed of genes whose expression decreases in all the conditions as compared to WT HSCs and is enriched for genes involved in allograft rejection. Cluster 3, which is composed of genes whose expression decreases in WT, but inversely increase in *Tet2*^{-/-} upon LPS treatment, is enriched for genes involved in mitotic spindle. Finally, cluster 8, which is composed of genes that are up-regulated in WT but inversely down-regulated in *Tet2*^{-/-} HSC upon LPS treatment are notably enriched in inflammatory (TNFA_signaling_via_NFKB, TGF_beta_signaling, IL2_STAT5_signaling) and p53

pathways (Fig. 6C-D).

Interestingly, TNFA_signaling_via_NFKB pathway was negatively enriched in *Tet2*^{-/-} HSCs upon LPS treatment, and apoptosis pathway was not enriched anymore as compared to what we observed in WT HSCs (Fig. 2C). Finally, we compared hallmarks enrichment in WT-LPS vs WT and *Tet2*^{-/-}-LPS vs *Tet2*^{-/-} conditions using BubbleGUM tool (Spinelli et al., 2015) to identify pathways significantly differentially enriched in these two comparisons. We show that TNFA_signaling_via_NFKB pathway was significantly enriched in WT-LPS vs WT comparisons, as compared to *Tet2*^{-/-}-LPS vs *Tet2*^{-/-} comparison, and proliferative pathways were inversely significantly enriched in *Tet2*^{-/-}-LPS vs *Tet2*^{-/-} as compared to WT-LPS vs WT comparisons (Fig. 6F).

Altogether, these data suggest that *Tet2*^{-/-} HSCs have an altered transcriptomic response to inflammation, as recently described in human HSCs mutated for TET2 in the context of CHIP (Jakobsen et al., 2024), associated with decreased expression of TEs, increased enrichment of proliferative pathways, and decreased enrichment of inflammatory and p53/apoptosis pathways.

FIGURE 7

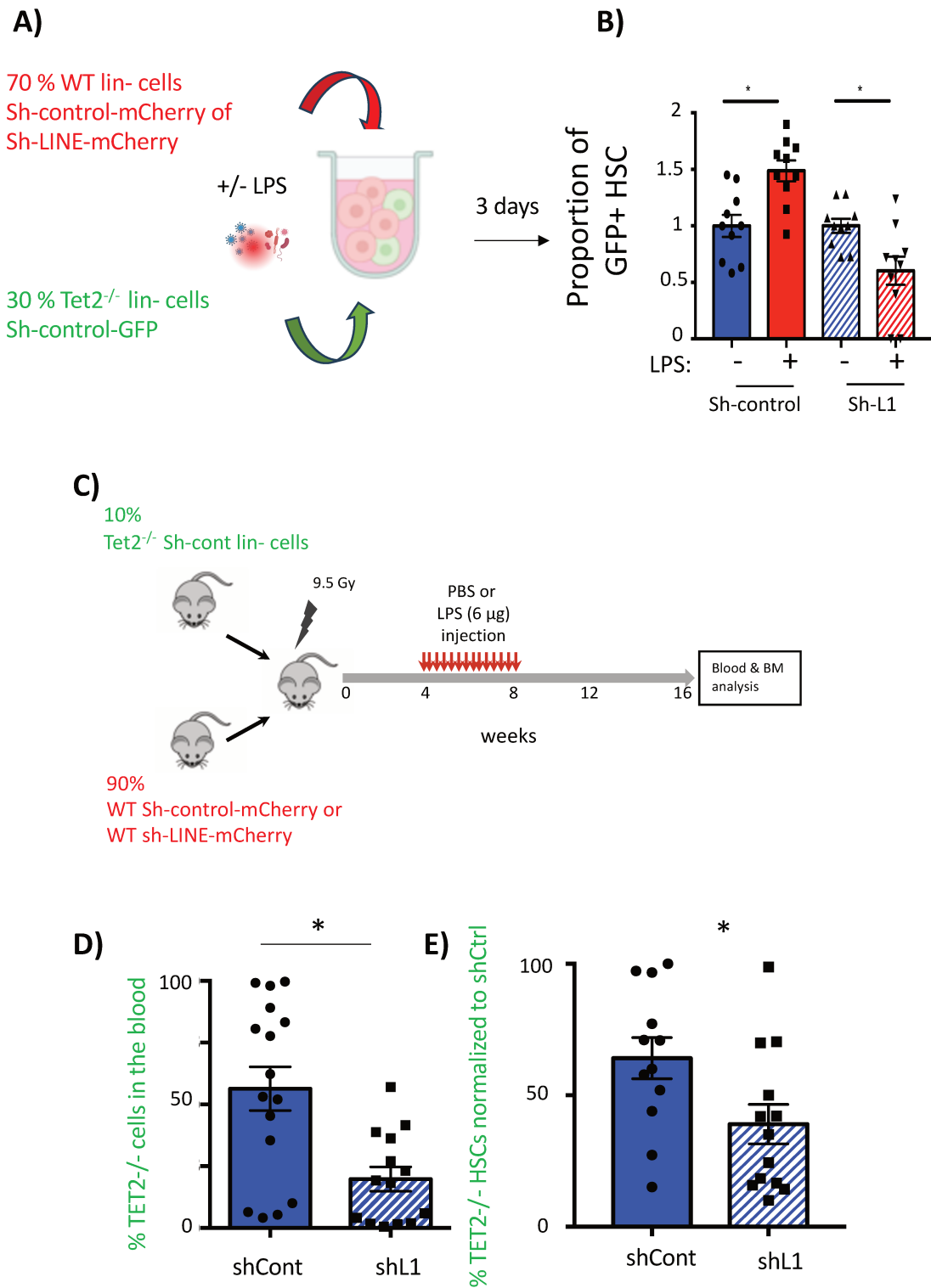


FIG. 7: L1 degradation in WT HSCs prevents Tet2^{-/-} HSC expansion upon LPS treatment. (A) experimental design for Tet2^{-/-} vs WT HSCs competition in vitro in the presence or absence of LPS. 70% of WT HSC transduced with either an sh-control or sh-LINE fused to mCherry reporter gene were put in competition in vitro with 30% of Tet2^{-/-}-HSCs transduced with an sh-control fused to GFP reporter gene in the presence or absence of LPS. (B)

Percentage of GFP+ HSCs (*Tet2*^{-/-} HSCs) was assessed 3 days after. (C) Experimental design for *Tet2*^{-/-} vs WT HSCs competition in vivo. 90% of WT lin- cells transduced with either an sh-control or sh-L1 fused to mCherry reporter gene were engrafted in competition with 10% of *Tet2*^{-/-} lin- cells transduced with an sh-control fused to GFP reporter gene in lethally irradiated recipient mice. Recipient mice were then chronically treated with LPS 1 or 2 months after engraftment. (D-F) Percentage of GFP+ cells in blood before (D) and 4 months engraftment in HSCs (E).

Resistance of *Tet2*^{-/-} HSCs towards LPS-induced DNA damage and loss of function may explain their expansion upon chronic inflammation. To test this hypothesis, we first performed competitive assays in vitro between WT HSCs transduced with either an sh-control or an sh-L1 fused to mCherry reporter gene, and *Tet2*^{-/-} HSCs transduced with an sh-control fused to GFP reporter gene (Fig. 7A). The results showed that *Tet2*^{-/-} HSCs (GFP+) significantly increased after 3 days in culture in the presence of LPS when they are in competition with WT HSCs transduced with an sh-control, as compared to the non LPS condition (Fig. 7B). However, they were no more able to expand when they are in competition with WT HSC transduced with an sh-L1 (Fig. 7B). These results suggest that *Tet2*^{-/-} expansion is dependent on the presence of L1 transcripts and their deleterious effects on WT HSCs. We next aimed at confirming these data in vivo by engrafting 10% *Tet2*^{-/-} lin- cells previously transduced with an sh-control fused to GFP with 90% of WT lin- cells transduced with either an sh-control an sh-L1 fused to mCherry (Fig. 7C).

We first assessed *Tet2*^{-/-} cells expansion in blood 1 month and after engraftment. As shown in Figure 7D, *Tet2*^{-/-} cells expanded from 10% to 56% in the blood when in competition with sh-control WT cells. These data are coherent with previous studies showing rapid expansion of *Tet2*^{-/-} cells upon engraftment (Moran-Crusio et al., 2011). Interestingly however, *Tet2*^{-/-} cells expanded only from 10 to 20% in average in blood when in competition with sh-L1 WT cells.

Pre-transplantation conditioning regimen in the context of HSC transplantation, such as ionizing radiations and/or chemotherapies, have been associated to a transient cytokine storm (Jordan et al., 2017; Weischendorff et al., 2019a). In mice, 9Gy irradiation induces increased levels of cytokines, such as IL6 and TNF α , in the plasma 6h to 10 days after irradiation (Zhang et al., 2012). These data suggest that under an inflammatory context induced by irradiation, or irradiation stress itself, the expansion of *Tet2*^{-/-} cells is significantly (p<0.01) dependent on L1 transcript in WT cells.

We next aimed at testing the effect of chronic inflammation induced by LPS on *Tet2*^{-/-} cell

expansion on a long term. For this purpose, we chronically treated recipient mice with LPS 1 month after engraftment (Fig 7C). Analysis of the proportion of *Tet2*^{-/-} GFP⁺ cells in blood or bone marrow four months after the end of LPS treatment is still ongoing.

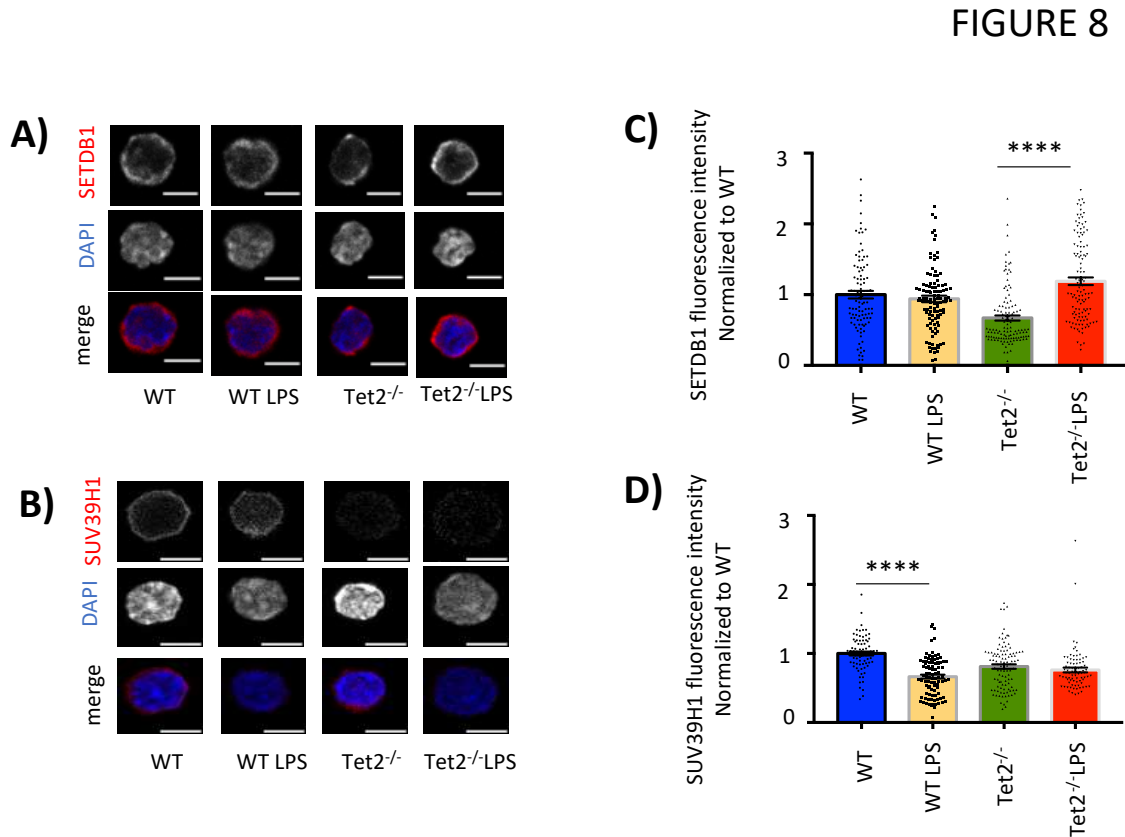


FIG. 8: Increased H3K9me3 at L1Mds in *Tet2*^{-/-} HSCs upon LPS treatment might be due to an increase in SETDB1: Representative images (A+B) and quantification of SETDB1(C) and SUV39H1(D) in HSCs (*LSK-CD34- Flk2-*) after one month of treatment with or without LPS. Bars, 5 μm. Cell profiler was used for quantification. For each cell the fluorescence intensity was normalized to the total cell surface and then to the mean intensity of WT cells. Means ± SEM from two independent experiments.

Increased H3K9me3 at L1Mds in *Tet2*^{-/-} HSCs upon LPS might be due to an increase in SETDB1

To understand the mechanism behind increased H3K9me3 at L1Md upon LPS in *Tet2*^{-/-} HSCs, we chronically treated *Tet2*^{-/-} and WT littermates with LPS and we performed IF on HSC sorted from mice the day after the end of the treatment, using antibodies directed against the H3K9me3-methyltransferases SUV39H1 and SETDB1. Additionally, we observed a significant decrease in SUV39H1 in WT HSCs treated with LPS compared to untreated WT

HSCs (Fig. 8B-D) and a significant increase in SETDB1 in *Tet2*^{-/-} HSCs treated with LPS compared to untreated *Tet2*^{-/-} HSCs (Fig. 8A+C).

These data show that H3K9me3 loss upon LPS treatment might be due to a loss of SUV39H1, as previously observed upon aging (Djeghloul et al., 2016). They also suggest that *Tet2*^{-/-} HSCs protection towards inflammation might be dependent on SETDB1.

Discussion

TET2 is one of the most frequently mutated genes in CHIP, yet not all carriers progress to leukemia. It is thus of major importance to understand the mechanisms by which CHIP mutations trigger HSC expansion and the emergence of the pre-leukemic clone. Several studies have investigated the mechanisms by which specific mutant clones gain an advantage over other, non-mutated HSCs, and have led to a better understanding of the selective environments that drive their expansion (Florez et al., 2022b).

While IR and chemotherapy are known to expand clones with mutations in DNA repair factors (*TP53* or *PPM1D*), expansion of clones mutated in epigenetic factors appear to be favored by the emergence of an inflammatory microenvironment in the bone marrow which is a characteristic feature of aging (inflammaging). Nevertheless, some studies highlighted the expansion of TET2 mutated clones upon radio-and chemotherapy (Nishiyama et al., 2018; Takahashi et al., 2017a; Wong et al., 2015).

Two non-exclusive hypotheses could explain the expansion of CHIP-mutated clones with age and in response to stress: 1- a disadvantage of non-mutated cells, and/or 2- a competitive advantage of mutated cells.

In order to decipher the molecular mechanisms involved in the expansion of *Tet2*^{-/-} HSCs upon stresses, the aims of my thesis were to study the impact of IR and inflammatory stresses on WT and *Tet2*^{-/-} HSCs.

Our team had previously shown that loss of H3K9me3 at TEs, and TE overexpression, are involved in HSC loss of function upon IR stress, through DNA damage accumulation and transcriptomic alterations (Barbieri et al., 2018; Pelinski et al., 2022).

We first aimed at deciphering if chronic inflammation could also affect HSC function through H3K9me3 loss at TEs. We could show that inflammation induces a loss of H3K9me3 at L1Md and IAP, the most recent subfamilies of L1 and LTR. This is associated with increased expression of L1 and LTR, and DNA damage accumulation. By using RTI, we further showed that L1 expression is involved in DNA damage accumulation upon LPS treatment. These data thus suggest that HSC functional changes upon chronic inflammation may be due to L1

derepression, as previously observed upon IR (Barbieri et al., 2018; Pelinski et al., 2022). To validate this hypothesis, reconstitution experiments will be needed to check if lamivudine treatment may be able to prevent loss of HSC reconstitution capacity observed upon chronic LPS treatment (Esplin et al., 2011).

We then aimed at characterizing the effect of IR and LPS on *Tet2*^{-/-} HSC. Through reconstitution experiments, we showed that IR drastically reduced HSC functionality of both WT and *Tet2*^{-/-} HSCs. These data suggest that IR may not induce *Tet2*^{-/-} expansion in mice. I thus focused the rest of my thesis on the effect of chronic inflammation on *Tet2*^{-/-} HSC.

Surprisingly, we showed that LPS had an inverse effect on H3K9me3 at TEs in *Tet2*^{-/-} HSCs as compared to WT. Indeed, LPS treatment induced an increased enrichment of H3K9me3 at L1Md. Repression of L1Md seems to protect *Tet2*^{-/-} HSCs as LPS was not able to induce DNA damage accumulation in these cells. Resistance of *Tet2*^{-/-} HSCs towards LPS-induced DNA damage may explain their expansion upon chronic inflammation. In accordance with this hypothesis, we showed that *Tet2*^{-/-} HSCs were not able to expand in response to LPS in vitro when in competition with WT HSCs where L1 transcripts were degraded by the use of an sh-L1. Preliminary data obtained in blood in vivo also suggest that *Tet2*^{-/-} HSCs are not able to expand upon irradiation-stress during reconstitution experiments when L1 was degraded in WT cells.

Altogether, these data show that *Tet2*^{-/-} HSC expansion is dependent on the epigenetic repression of L1Md upon inflammation, that protects them from their deleterious effects, as compared to WT HSCs.

Loss of *Tet2*^{-/-} HSC reconstitution capacity upon IR is associated with a drastic loss of H3K9me3

It has been shown that there is an expansion of *TET2* clones upon cytotoxic agents and IR . We thus aimed at characterizing the effect of IR on *Tet2*^{-/-} HSCs.

Through reconstitution experiments, we showed that IR drastically reduced HSC functionality of both WT and *Tet2*^{-/-} HSCs. These data suggest that IR may not affect *Tet2*^{-/-} expansion in mice (Fig. 3B+C, results). However, this should be tested by engrafting WT and *Tet2*^{-/-} BM in

CD45.1 lethally irradiated mice and treat recipient mice with 2Gy IR 1 month after irradiation, as we did when we tested the effect of chronic inflammation on *Tet2*^{-/-} HSC expansion (Fig. 5D, results).

To further characterize the effect of IR on WT and *Tet2*^{-/-} HSC heterochromatin, we performed H3K9me3 CUT&Tag in HSCs sorted from WT and *Tet2*^{-/-} mice one month after they were or not irradiated with 2Gy (Fig. 1A). Our data show a decreased enrichment of H3K9me3 at peaks in *Tet2*^{-/-} HSCs as compares to WT. We also showed that IR leads to a loss of H3K9me3 (Fig. 1A) in WT HSCs. Loss of H3K9me3 was even more pronounced in *Tet2*^{-/-} HSCs upon IR. This is in contradiction with what we observed upon LPS treatment. Indeed, H3K9me3 enrichment at peaks decreased upon LPS treatment in WT HSC, or upon *Tet2* loss, but seemed to increase upon LPS treatment in *Tet2*^{-/-} HSCs (Fig. 1B).

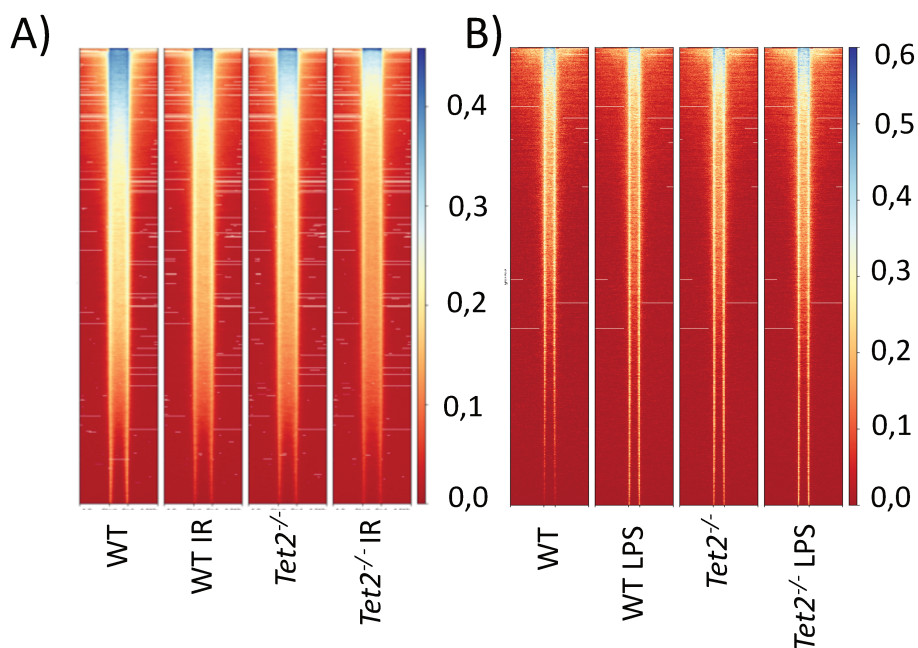


FIG. 1: H3K9me3 gain in *Tet2*^{-/-} HSCs upon LPS. Heatmaps of H3K9me3 enrichment at peaks in *Tet2*^{-/-} and WT HSCs upon IR (A) and upon LPS (B). Every line in the heatmap is a H3K9me3-peak that is more or less enriched in H3K9Me3 (blue to red).

These data suggest that H3K9me3 concentration may be linked to *Tet2*^{-/-} HSCs protection upon stresses. Indeed, while they are protected from LPS treatment (H3K9me3 increase), they are sensitive to IR (H3K9me3 decrease). Deeper analysis of H3K9me3 concentration at L1Md in *Tet2*^{-/-} IR vs NIR HSCs, and analysis of γ H2AX accumulation in *Tet2*^{-/-} IR vs NIR HSCs, in the presence or absence of RTI, could be done to test this hypothesis.

Our data suggest that 2Gi IR may not expand *Tet2*^{-/-} HSCs. Some examples in the literature show an expansion of TET2 mutated in the context of radiotherapies and/or chemotherapies (Nishiyama et al., 2018; Takahashi et al., 2017a; Wong et al., 2015). One possibility that has to be proven is that expansion of these *Tet2*^{-/-} clones may be associated with irradiation and/or chemotherapies-induced inflammation. Indeed, it has been shown that pre-transplantation conditioning regimen in the context of HSC transplantation, such as ionizing radiations and/or chemotherapies, have been associated to a transient cytokine storm, which is thought to be linked to damages in the gastro-intestinal tract and translocation of bacterial products into the blood (Jordan et al., 2017; Weischendorff et al., 2019b). In mice, 9Gy irradiation induces increased levels of cytokines, such as IL6 and TNF α , in the plasma 6h to 10 days after irradiation (Zhang et al., 2012).

Heterochromatin loss as a ‘theory of HSC aging’

The heterochromatin theory of aging, proposed by Tsurumi and Li (2012), suggests that the loss of heterochromatin underlies the aging process (Tsurumi and Li, 2012).

Our team previously demonstrated that aging and IR, that induce a premature aging in HSCs, induce a loss of H3K9me3 in HSCs (Pelinski et al., 2022a). This was measured one month after irradiation, indicating that the loss of H3K9me3 persists on a long-term, even without ongoing stimulation. Clapes et al. also showed an opening of the chromatin upon chemotherapies (Clapes et al., 2021). It thus appears that heterochromatin loss, and especially loss of H3K9me3, may be a common response to various stresses that are involved in HSC aging.

H3K9me3 loss upon aging is associated with decline in SUV39H1 levels in both human and mouse HSCs (Djeghloul et al., 2016a). It would be interesting to test the effect of stress on these factors. H3K9me3 is established by the two methyltransferases SUV39H1/H2 and SETDB1. SUV39H1 and SUV39H2 can methylate H3K9me0 but prefer H3K9me1 as a substrate to establish H3K9me3 (Loyola et al., 2009; Rea et al., 2000). In contrast, SETDB1 can mono-, di-, and tri-methylate H3K9me0 in vitro (Loyola et al., 2009; Schultz et al., 2002; Wang et al., 2003; Yang et al., 2002). We examined the expression of these methyltransferases by IF upon LPS treatment in vivo. Our preliminary data show a decrease in SUV39H1 in WT HSCs treated with LPS compared to untreated WT HSCs, and no significant difference in

SETDB1 levels (Fig. 8, results). These data suggest that loss of SUV39H1 may be a central mechanism involved in H3K9me3 loss upon aging and stresses inducing a premature aging.

Loss of H3K9me3 was also observed upon LPS treatment in monocytes. Loss of H3K9me3 in these cases is associated with KDM4 gain or G9a loss (Cao et al., 2019; Moorlag et al., 2022; Mourits et al., 2021). It would thus be also interesting to test the expression of these factors upon LPS treatment in HSCs.

Loss of H3K9me3 upon inflammation has been linked to trained immunity. Trained immunity would allow a more robust and rapid reaction to secondary insults. In HSCs, De Laval and collaborators show that myeloid enhancers stay in an open conformation after LPS treatment in HSCs. This may allow a more robust and rapid reaction to secondary stress in a similar trained immunity process (De Laval et al., 2020).

The loss of H3K9me3 after stresses such as LPS may on one hand improve the response to additional stimulation but at the end also age the cell. We propose that accumulation of stresses throughout life may induce HSC aging through heterochromatin loss.

To prevent TET2 mutated cell expansion upon inflammation, one possibility would be to target the factors involved in H3K9me3 loss upon stresses, TE derepression and the associated damage in non-mutated cells. However, this may affect the natural mechanism of trained immunity and the response to infections.

TE derepression as a ‘theory of HSC aging’

The idea that transposons may influence aging was first introduced by W. Murray in 1990 (Murray, 1990). Studies have since demonstrated that the frequency of TE transpositions increases with age across various somatic tissues (De Cecco et al., 2013; Nikitin and Woodruff, 1995; Orr, 2016; Woodruff, 1992) and suppressing this age-related increase in TE transposition can increase the lifespan of different organisms (Domingues and Hale, 2017; Driver and Vogrig, 1994; Jones et al., 2016; Lou et al., 2020).

We propose that accumulation of stresses throughout life may induce HSC aging through an increase in the frequency of TE transposition.

During my thesis, I showed that there is a loss of H3K9me3 at L1Mds and IAPs upon chronic inflammation mediated by repeated injections of LPS for one month. Our lab also showed L1Md and IAP overexpression upon IR. In another study, Clapes et al. revealed through ATAC-seq experiments that chemotherapy leads to chromatin opening, especially at TEs. In all these studies, derepression of TEs was associated with loss of HSC function, through DNA damage accumulation, transcriptomic alterations, or the induction of IFN-I response (Barbieri et al., 2018a; Clapes et al., 2021; Pelinski et al., 2022a).

We correlated the enrichment of H3K9me3 with age of all the different LINE1 elements. We showed that it is especially the evolutionary youngest subfamilies of LINE1 that are the most enriched for H3K9me3, and it's also these one that are the most affected by H3K9me3 loss upon IR (Pelinski et al., 2022a) and LPS (Figure 1E-F). This is in accordance with previous studies showing that the young families of TEs are the most epigenetically regulated in the genome (Barau et al., 2016b; Pezic et al., 2014). As these elements are the ones still able to move and damage the cells, their repression by epigenetic mechanisms may be developed by the cells as a mechanism to control them (Castro-Diaz et al., 2014).

We propose that accumulation of stresses throughout life may induce HSC aging through heterochromatin loss and TE upregulation.

Re-localisation of H3K9me3 upon aging

Our CUT&Tag data reveal a loss of H3K9me3 at LINE and LTR elements but a gain at SINEs and DNA transposons, upon LPS in HSCs, compared to untreated WT HSCs (Fig. 1D, results). This could indicate a re-localization of H3K9me3 from LINEs and LTRs to SINEs and DNA transposons. Indeed, more than a loss of heterochromatin theory of aging, Tsurumi and Li discuss the possibility of heterochromatin relocalization as a theory of aging (Tsurumi and Li, 2012). Epigenetic factors involved in H3K9me3 deposition are the same than the one involved in DNA repair (Fortuny et al., 2021; Svobodová Kovaříková et al., 2018). It has been proposed that the relocalization of heterochromatin is due to a relocalization of these epigenetic factors from TEs that they repress at steady state to sites of DNA damage that accumulate upon aging and stresses. Indeed, In the study by van Meter et al., they report that the longevity-regulating protein sirtuin 6 (SIRT6) is a potent repressor of L1 activity. However, during aging and in

response to DNA damage, SIRT6 is depleted from L1 loci, leading to the activation of these previously silenced retroelements, and enriched at DNA damage sites (Van Meter et al., 2014). This paper is an example of heterochromatin relocalization from L1 following DNA damage.

The relocalization of these factors from TEs to DNA damage upon stresses such as IR and inflammation sites might lead to DNA repair but also to the derepression of TEs and ultimately to HSC cell aging.

Hong et al. reported that aging causes a spatial re-localization of H3K9me3-marked heterochromatin in HSCs. This disruption could ultimately affect HSPC function via TE derepression and induction of an inflammatory response. Their findings suggest that the loss of TET2 prevents this spatial re-localization of H3K9me3-marked heterochromatin. They proposed that TET2 may play a role in regulating the subnuclear distribution of H3K9me3 in stem cells, when the DNA methylation machinery is defective due to aging or DNMT loss-of-function (Hong et al., 2023). *Tet2* clones may thus expand upon aging due to their resistance towards heterochromatin relocalization and TE derepression.

H3K9me3 distribution across different hematopoietic cell types have similar global levels of H3K9me3, but their spatial distribution varies significantly between cell types (Ugarte et al., 2015). Notably, GMPs exhibited the lowest amount of heterochromatin near the nuclear envelope (Ugarte et al., 2015). Spatial re-distribution/ relocalization of the heterochromatin may be involved in the differentiation process. As aging and stresses such as IR and inflammation induce a myeloid bias. It would be interesting to test the link between H3K9me3 relocalization and the induction of a myeloid program.

Importance of basal level of L1 and inflammation on cell function and self-renewal

There is a complex interaction between innate immune responses and stem cell function. Interestingly, Lefkopoulos et al showed in 2020 that TE-induced inflammation is required for HSPC development and formation. Ectopic expression of TEs enhanced HSPC formation in WT and not in *Rig-I* or *Mda5* deficient zebrafish embryos where they saw reduced inflammatory signals (Lefkopoulos et al., 2020). They could also identify TE transcripts in hemogenic endothelial cells, suggesting a role of RLR ligands in early and definitive stages of hematopoiesis.

Our team could show that there is a higher basal expression of different LINE-1 families in HSCs than in progenitor cells such as MPPs, CMPs or GMPs ((Barbieri et al., 2018a), Fig 2), suggesting a tolerance for L1 expression in HSCs, and maybe a basal role for L1 in HSC function.

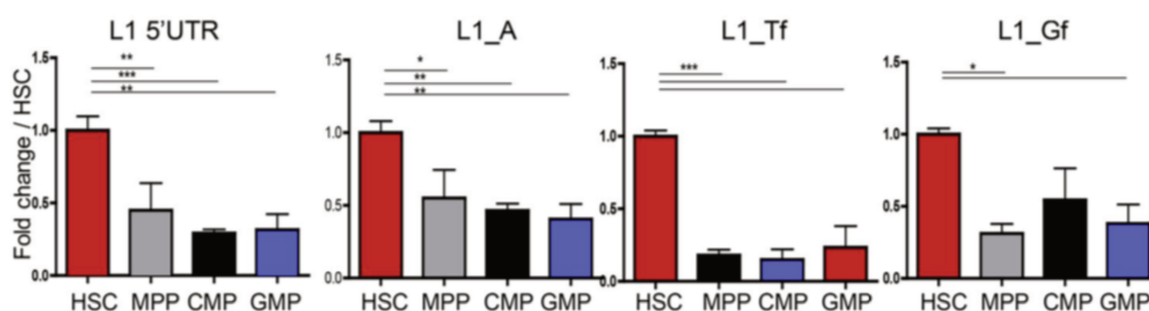


FIG. 2: Expression of different LINE-1 families in HSC, MPPs, CMPs and GMPs (Barbieri, 2018).

Research has demonstrated that knocking down LINE1 impairs the self-renewal of ESCs and triggers a transition to a 2C state (Percharde et al., 2018). LINE1 has been shown to activate rRNA synthesis, which supports rapid proliferation. LINE1 knockdown results in reduced total RNA levels, decreased nascent transcription and translation, and impaired self-renewal. They suggest that LINE1, associated with chromatin, regulates gene expression and developmental potential independently of retrotransposition activity (Percharde et al., 2018). This suggests that LINE1 play an important role on ESC self-renewal.

This implicates that a certain level of LINE1 expression might be necessary for cells such as HSCs to self-renew. However, above a certain threshold, high expression of TEs might be deleterious, as observed upon IR and inflammation.

DNA damage potentially triggers the cGAS-STING pathway in *Tet2*^{-/-} HSCs and inflammatory pathways

In a recent study, Xie et al. show that DNA damage in *Tet2*-deficient LSKs triggers the activation of the cGAS-STING pathway, which promotes enhanced self-renewal, activates the innate immune response, and drives the development of clonal hematopoiesis (Xie et al., 2023). This study also identified altered expression of several DNA repair-associated genes in *Tet2*^{-/-}

HSPCs compared to WT cells, indicating that genome instability arises due to inadequate repair mechanisms following the loss of TET2. Furthermore, inhibiting STING significantly reduced LSK expansion and myeloid skewing in both *Tet2*^{-/-} mice and BM transplant models. In fact, *Tet2* and *Sting* double knockout (DKO) donor cells restored a balanced hematopoietic hierarchy and significantly extended the lifespan of recipient mice compared to *Tet2*^{-/-} donor cells, suggesting that *Tet2*^{-/-} HSPCs rely on inflammation for expansion (Xie et al., 2023). The cGAS-STING pathway in this context, could be triggered by "self" DNA, such as dsRNA, dsDNA, or cytosolic DNA produced by TEs.

Interestingly, our RNAseq data revealed an upregulation of inflammatory pathways such as the TNFA_signaling_via_NFKB or the IFN in *Tet2*^{-/-} HSCs compared to WT HSCs (Fig. 3A).

This suggest that the increased inflammation in *Tet2*^{-/-} HSCs might be linked to the upregulation of TEs (Fig. 3B).

We could test this hypothesis by transducing shRNAs against LINE-1 in *Tet2*^{-/-} cells and check the expression of inflammatory pathways by RNAseq in sh-L1 *Tet2*^{-/-} HSCs compared to sh-control *Tet2*^{-/-} HSCs.

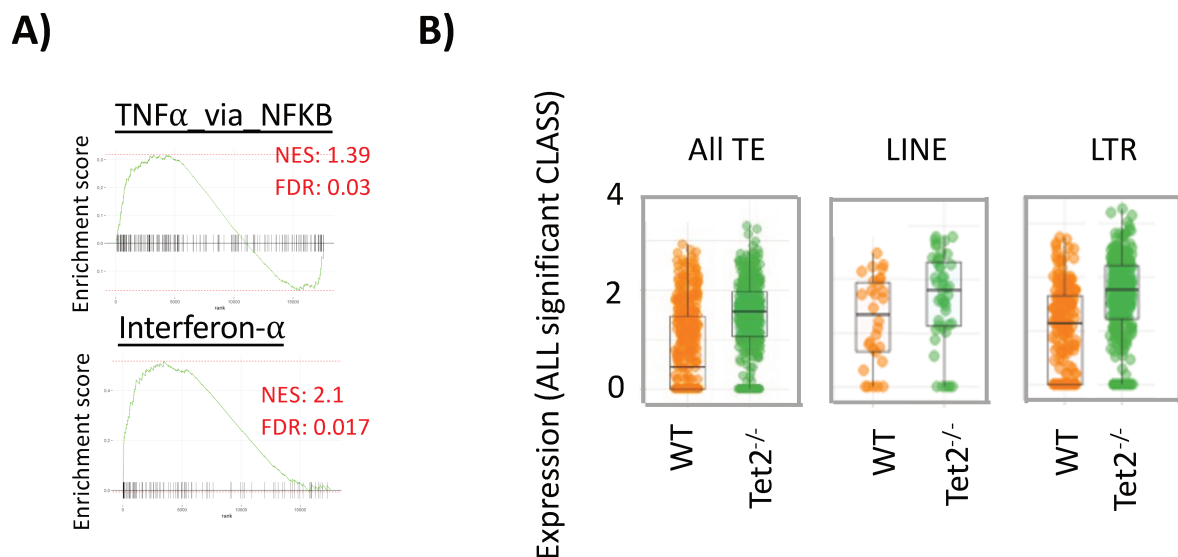


FIG. 3: *Tet2*^{-/-} leads to an upregulation of TEs and inflammatory pathways. (A) GSEA analysis of the TNFα via NFKB and the interferon-α in *Tet2*^{-/-} vs WT HSCs. (B) Box plot showing the level of expression of all TE or of the different classes of TE significantly ($p < 0.05$) deregulated in WT or *Tet2*^{-/-}.

***Tet2*^{-/-} HSCs are inflamed and resistant to further inflammation**

Interestingly, both mutant and non-mutant CHIP-mutated HSCs in the same individual show an increase in inflammatory and aging-related transcriptomic signatures compared to HSCs from non-CHIP individuals. However, within the same CHIP-mutated individual, TET2-mutant HSCs showed a reduced inflammatory response relative to WT HSCs (Jakobsen et al., 2024a). This suggests that in an inflammatory environment mediated by CHIP, TET2-mutant HSCs have an attenuated response to inflammation.

Interestingly, we show a decrease in inflammatory pathways in *Tet2*^{-/-} HSCs after chronic challenge with LPS. Our data thus also highlight an attenuated response to inflammation in *Tet2*^{-/-} HSCs. This attenuated response may be involved in the resistance of *Tet2*^{-/-} HSCs towards inflammation.

Increased proliferation pathways and frequencies in *Tet2*^{-/-} HSCs upon inflammation

Expansion of *Tet2*^{-/-} HSC may be linked to a loss of non-mutated HSC function, as suggested by our results. However, expansion of *Tet2*^{-/-} HSC may also be linked to increased self-renewal and proliferation of *Tet2*^{-/-} HSCs. Indeed, we do observe the enrichment of proliferation pathways in our RNA-seq data (Fig. 6E-F, results)

To test the effect of the expansion of *Tet2*^{-/-} HSCs, we conducted chronic LPS injections for one month followed by FACS analysis of the BM. It was observed that LPS triggers TLR4-dependent Sca-1 induction in HSCs in vivo (Demel, 2022). To quantify the number of HSCs independent of LPS's effect on Sca-1 expression, we analyzed the proportion of HSCs within the LSK population through FACS. We observed a fold change of 1.35 in WT HSCs treated with LPS and 2.14 in *Tet2*^{-/-} HSCs when analyzed within the LSK population (Fig. 4A). When examining total HSCs, we found a fold change of 1.95 in WT and 6.07 in *Tet2*^{-/-} HSCs (Fig. 4B). This suggests that *Tet2*^{-/-} HSCs expand more than WT HSCs in response to LPS. This expansion may be due to increased proliferation of HSCs. To test this possibility BrdU or Ki67 assays are necessary. BrdU is expressed during DNA replication and repair, while Ki67 marks cells that are in the proliferation stages (G1, G2, S, mitosis), not in the G0 phase.

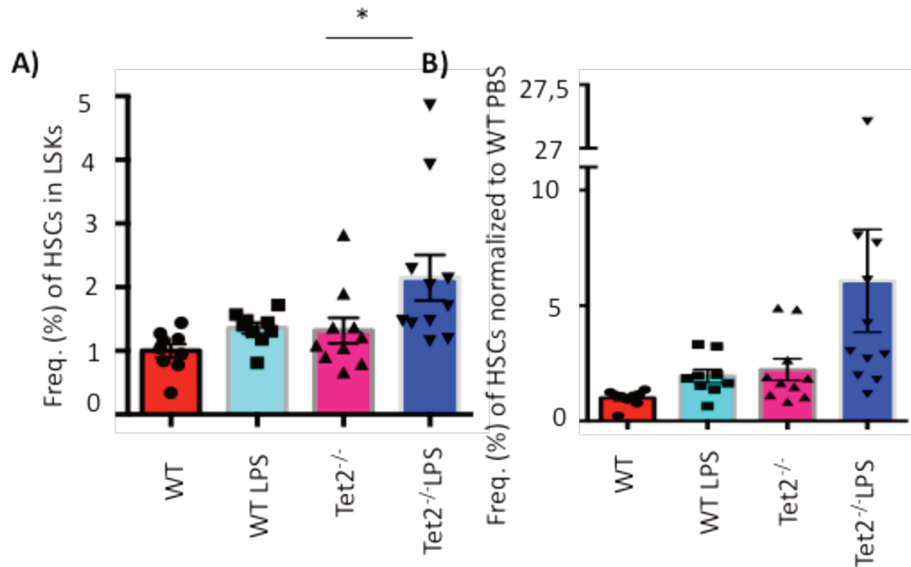


FIG.4: *Tet2*^{-/-} HSCs exhibit amplified HSCs in response to chronic inflammatory challenge. Quantification of the frequency (Freq.) of HSCs in LSKs (A) and HSCs (B) after LPS treatment in WT and *Tet2*^{-/-} mice. n = 9-10 mice per group. One-way ANOVA with Sidak multiple comparison test *p<0.05.

At this stage we cannot rule out the possibility that expansion of *Tet2*^{-/-} HSCs may also be due to increased self-renewal and increased proliferation.

Crucial role of LINE-1 suppression in LSCs and AML

To better understand the mechanism behind the epigenetic repression of L1Md in *Tet2*^{-/-} HSCs upon LPS treatment and their resistance towards inflammation, we performed IF experiments looking at the methyltransferases SUV39H1 and SETDB1 (Fig. 8). Interestingly, while our preliminary data show a decrease for SETDB1 in WT HSCs treated with LPS, they show an increase in the protein levels of SETDB1 in the *Tet2*^{-/-} HSCs treated with LPS compared to *Tet2*^{-/-} HSCs (Fig. 8).

TE repression may be necessary for CHIP-mutated clones to expand, but also for tumorigenesis. Epigenetic silencing of TEs, notably through the recruitment of SETDB1 H3K9 methyltransferase by the HUSH complex, is required for AML cell line proliferation (Cuellar et al., 2017b; Gu et al., 2021b). Interestingly, suppression of TE expression is observed in leukemic stem cells as compared to blasts in AML patients, and in leukemic stem cells of high-grade MDS patients as compared to low-grade MDS patients (Colombo et al., 2017b). SETDB1

is overexpressed in different types of malignancies such as AML, suggesting a role of SETDB1-mediated TE repression in tumorigenesis and more particularly in leukemogenesis. Our lab also recently showed an increase of the repressive H3K9me2 mark at TEs and an overexpression of G9a and GLP methyl transferases in patients with chronic myelomonocytic leukemia (CMML) (Hidaoui et al., 2024b). These data suggest that other H3K9 methyl transferases may be involved in leukemogenesis.

This raises a crucial question: How could we selectively target mutant *Tet2*^{-/-} HSCs to prevent their expansion in CHIP or upon leukemogenesis? One potential approach is to explore aberrant signaling pathways that these cells rely on for survival. Epigenetic mechanisms are reversible, and thus could be a good therapeutic target (Chiappinelli et al., 2015b; Roulois et al., 2015). Decreasing SETDB1 or HUSH leads to the reactivation of TE DNA damage, inflammation and leukemic cell death which could be used to treat different types of cancer. Donia Hidaoui from our team used a combination of HMA and G9A inhibitors to treat CMML which induced TE expression, inflammatory pathways and a selective loss of CMML HSPCs (Hidaoui et al., 2024a). Unfortunately, there is no inhibitor available for SETDB1, but we could use shRNA against SETDB1 to test if reducing SETDB1 expression may prevent *Tet2*^{-/-} HSC expansion.

Concluding remarks and summary diagram

The molecular mechanisms involved in the expansion of CHIP mutated clones are still unclear. The aim of this thesis was to study the molecular mechanism that underlie the clonal expansion of *Tet2*^{-/-} HSC upon stresses.

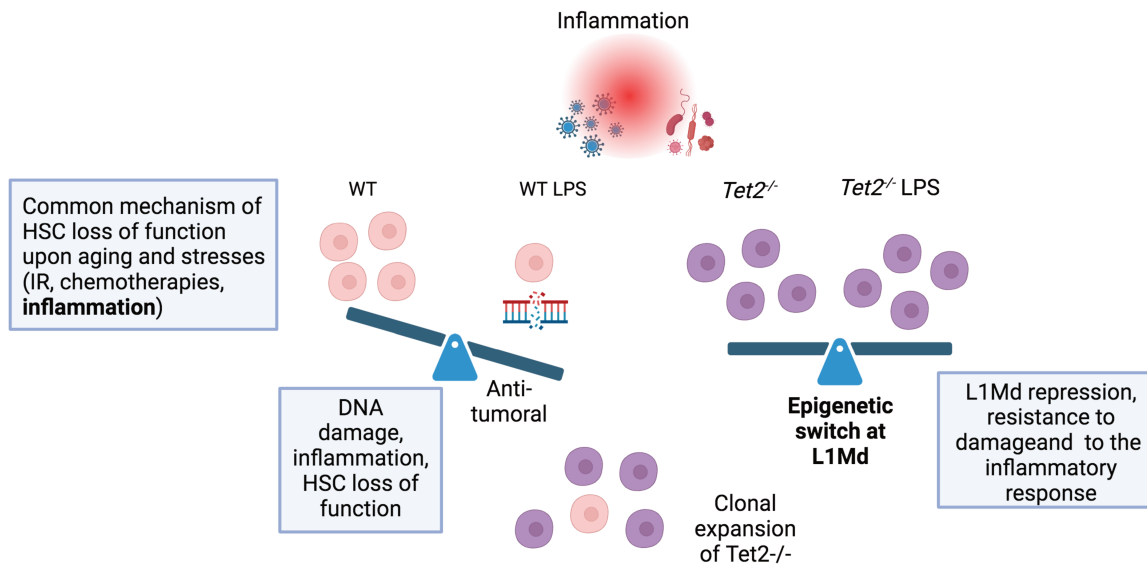
Our team could show that heterochromatin loss, and especially loss of H3K9me3, and TE derepression, especially L1Md, the most recent subfamilies of L1, are a common response to aging and various stresses (IR, inflammation) that are involved in HSC aging. We further showed that L1Md derepression is associated with loss of HSC function upon IR and inflammatory stress through DNA damage accumulation and transcriptomic alterations.

Interestingly, the impact of inflammation showed a contrast between WT HSCs, which exhibited a loss of H3K9me3 at L1Md, and *Tet2*^{-/-} HSCs, which exhibited an increase of H3K9me3 at L1Md. We further showed that repression of L1Md is associated with the resistance of *Tet2*^{-/-} HSCs towards the effects of LPS.

Decreased H3K9me3 in WT HSC upon LPS treatment may be due to decreased expression of SUV39H1, whereas increased H3K9me3 might be due to higher levels of SETDB1 in *Tet2*^{-/-} HSCs upon LPS treatment.

Finally, our data suggest that the expansion of *Tet2*^{-/-} HSCs upon inflammation appears to be driven by L1 transcript activity and its deleterious effects on WT HSCs, potentially contributing to the competitive advantage of *Tet2*^{-/-} HSCs.

Altogether, these findings underscore the role of L1 elements and chronic inflammation in shaping the differential behavior of *Tet2*^{-/-} HSCs and WT HSCs leading to clonal expansion of *Tet2*^{-/-} HSCs.



Expansion of $Tet2^{-/-}$ HSCs upon inflammation due to an epigenetic switch at L1Md

WT HSCs are sensitive to aging and stresses such as IR, chemotherapy, or chronic inflammation through L1Md derepression which can induce DNA damage, inflammation eventually leading to HSC loss of function. An epigenetic switch at the evolutionary young L1Mds happens in $Tet2^{-/-}$ HSCs upon which makes them resistant to the deleterious effects of inflammation. The different response to LPS may lead to the expansion of $Tet2^{-/-}$ HSCs (Created with BioRender, license: MQ27ESB6J4).

Bibliography

- Abegunde, S.O., Buckstein, R., Wells, R.A., Rauh, M.J., 2018. An inflammatory environment containing TNF α favors Tet2-mutant clonal hematopoiesis. *Experimental Hematology* 59, 60–65. <https://doi.org/10.1016/j.exphem.2017.11.002>
- Adam, M., Robert, F., Larochele, M., Gaudreau, L., 2001. H2A.Z Is Required for Global Chromatin Integrity and for Recruitment of RNA Polymerase II under Specific Conditions. *Molecular and Cellular Biology* 21, 6270–6279. <https://doi.org/10.1128/MCB.21.18.6270-6279.2001>
- Adams, G.B., Alley, I.R., Chung, U., Chabner, K.T., Jeanson, N.T., Lo Celso, C., Marsters, E.S., Chen, M., Weinstein, L.S., Lin, C.P., Kronenberg, H.M., Scadden, D.T., 2009. Haematopoietic stem cells depend on G α s-mediated signalling to engraft bone marrow. *Nature* 459, 103–107. <https://doi.org/10.1038/nature07859>
- Adelman, E.R., Huang, H.-T., Roisman, A., Olsson, A., Colaprico, A., Qin, T., Lindsley, R.C., Bejar, R., Salomonis, N., Grimes, H.L., Figueroa, M.E., 2019. Aging Human Hematopoietic Stem Cells Manifest Profound Epigenetic Reprogramming of Enhancers That May Predispose to Leukemia. *Cancer Discovery* 9, 1080–1101. <https://doi.org/10.1158/2159-8290.CD-18-1474>
- Agathocleous, M., Meacham, C.E., Burgess, R.J., Piskounova, E., Zhao, Z., Crane, G.M., Cowin, B.L., Bruner, E., Murphy, M.M., Chen, W., Spangrude, G.J., Hu, Z., DeBerardinis, R.J., Morrison, S.J., 2017. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. *Nature* 549, 476–481. <https://doi.org/10.1038/nature23876>
- Ågren, J.A., Wright, S.I., 2011. Co-evolution between transposable elements and their hosts: a major factor in genome size evolution? *Chromosome Res* 19, 777–786. <https://doi.org/10.1007/s10577-011-9229-0>
- Akashi, K., Traver, D., Miyamoto, T., Weissman, I.L., 2000. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 404, 193–197. <https://doi.org/10.1038/35004599>
- Allshire, R.C., Madhani, H.D., 2018. Ten principles of heterochromatin formation and function. *Nat Rev Mol Cell Biol* 19, 229–244. <https://doi.org/10.1038/nrm.2017.119>
- Alter, B.P., Rosenberg, P.S., Giri, N., Baerlocher, G.M., Lansdorp, P.M., Savage, S.A., 2012. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. *Haematologica* 97, 353–359. <https://doi.org/10.3324/haematol.2011.055269>
- Amancherla, K., Wells, J.A., Bick, A.G., 2022. Clonal hematopoiesis and vascular disease. *Semin Immunopathol* 44, 303–308. <https://doi.org/10.1007/s00281-022-00913-z>
- Arkipova, I.R., 2017. Using bioinformatic and phylogenetic approaches to classify transposable elements and understand their complex evolutionary histories. *Mobile DNA* 8, 19. <https://doi.org/10.1186/s13100-017-0103-2>
- Asmar, F., Punj, V., Christensen, J., Pedersen, M.T., Pedersen, A., Nielsen, A.B., Hother, C., Ralfkiaer, U., Brown, P., Ralfkiaer, E., Helin, K., Gronbaek, K., 2013. Genome-wide profiling identifies a DNA methylation signature that associates with TET2 mutations in diffuse large B-cell lymphoma. *Haematologica* 98, 1912–1920. <https://doi.org/10.3324/haematol.2013.088740>
- Avagyan, S., Henninger, J.E., Mannherz, W.P., Mistry, M., Yoon, J., Yang, S., Weber, M.C., Moore, J.L., Zon, L.I., 2021. Resistance to inflammation underlies enhanced fitness in clonal hematopoiesis. *Science* 374, 768–772. <https://doi.org/10.1126/science.aba9304>

- Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. *Cell Res* 21, 381–395. <https://doi.org/10.1038/cr.2011.22>
- Barau, J., Teissandier, A., Zamudio, N., Roy, S., Nalesso, V., Hérault, Y., Guillou, F., Bourc’his, D., 2016a. The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* 354, 909–912. <https://doi.org/10.1126/science.aah5143>
- Barau, J., Teissandier, A., Zamudio, N., Roy, S., Nalesso, V., Hérault, Y., Guillou, F., Bourc’his, D., 2016b. The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* 354, 909–912. <https://doi.org/10.1126/science.aah5143>
- Barbieri, D., Elvira-Matlot, E., Pelinski, Y., Genève, L., De Laval, B., Yogarajah, G., Pecquet, C., Constantinescu, S.N., Porteu, F., 2018a. Thrombopoietin protects hematopoietic stem cells from retrotransposon-mediated damage by promoting an antiviral response. *Journal of Experimental Medicine* 215, 1463–1480. <https://doi.org/10.1084/jem.20170997>
- Barbieri, D., Elvira-Matlot, E., Pelinski, Y., Genève, L., de Laval, B., Yogarajah, G., Pecquet, C., Constantinescu, S.N., Porteu, F., 2018b. Thrombopoietin protects hematopoietic stem cells from retrotransposon-mediated damage by promoting an antiviral response. *Journal of Experimental Medicine* 215, 1463–1480. <https://doi.org/10.1084/jem.20170997>
- Baylin, S.B., Jones, P.A., 2011. A decade of exploring the cancer epigenome — biological and translational implications. *Nat Rev Cancer* 11, 726–734. <https://doi.org/10.1038/nrc3130>
- Beck, C.R., Collier, P., Macfarlane, C., Malig, M., Kidd, J.M., Eichler, E.E., Badge, R.M., Moran, J.V., 2010. LINE-1 Retrotransposition Activity in Human Genomes. *Cell* 141, 1159–1170. <https://doi.org/10.1016/j.cell.2010.05.021>
- Beeler, J.S., Bick, A.G., Bolton, K.L., 2022. Genetic causes and cardiovascular consequences of clonal hematopoiesis in the UK Biobank. *Nat Cardiovasc Res* 2, 13–15. <https://doi.org/10.1038/s44161-022-00198-3>
- Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A., Rossi, D.J., 2013. Proliferation-Dependent Alterations of the DNA Methylation Landscape Underlie Hematopoietic Stem Cell Aging. *Cell Stem Cell* 12, 413–425. <https://doi.org/10.1016/j.stem.2013.01.017>
- Belancio, V.P., Hedges, D.J., Deininger, P., 2008. Mammalian non-LTR retrotransposons: For better or worse, in sickness and in health. *Genome Res.* 18, 343–358. <https://doi.org/10.1101/gr.5558208>
- Belancio, V.P., Roy-Engel, A.M., Pochampally, R.R., Deininger, P., 2010. Somatic expression of LINE-1 elements in human tissues. *Nucleic Acids Research* 38, 3909–3922. <https://doi.org/10.1093/nar/gkq132>
- Belgnaoui, S.M., Gosden, R.G., Semmes, O.J., Haoudi, A., 2006. Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells. *Cancer Cell Int* 6, 13. <https://doi.org/10.1186/1475-2867-6-13>
- Berg, J.M., Tymoczko, J.L., Gatto, G.J., Stryer, L., 2018. *Stryer Biochemie*. Springer Berlin Heidelberg, Berlin, Heidelberg. <https://doi.org/10.1007/978-3-662-54620-8>
- Bernitz, J.M., Rapp, K., Daniel, M.G., Shcherbinin, D., Yuan, Y., Gomes, A., Waghray, A., Brosh, R., Lachmann, A., Ma’ayan, A., Papatsenko, D., Moore, K.A., 2020. Memory of Divisional History Directs the Continuous Process of Primitive Hematopoietic Lineage Commitment. *Stem Cell Reports* 14, 561–574. <https://doi.org/10.1016/j.stemcr.2020.03.005>

- Bernstein, E., Allis, C.D., 2005. RNA meets chromatin. *Genes Dev.* 19, 1635–1655. <https://doi.org/10.1101/gad.1324305>
- Best, B.P., 2009. Nuclear DNA Damage as a Direct Cause of Aging. *Rejuvenation Research* 12, 199–208. <https://doi.org/10.1089/rej.2009.0847>
- Bestor, T.H., 1990. DNA methylation: evolution of a bacterial immune function into a regulator of gene expression and genome structure in higher eukaryotes. *Phil. Trans. R. Soc. Lond. B* 326, 179–187. <https://doi.org/10.1098/rstb.1990.0002>
- Bick, A.G., Popadin, K., Thorball, C.W., Uddin, M.M., Zanni, M., Yu, B., Cavassini, M., Rauch, A., Tarr, P., Schmid, P., Bernasconi, E., Günthard, H.F., Libby, P., Boerwinkle, E., McLaren, P.J., Ballantyne, C.M., Grinspoon, S., Natarajan, P., Fellay, J., the Swiss HIV Cohort Study, 2020. Increased CHIP Prevalence Amongst People Living with HIV. <https://doi.org/10.1101/2020.11.06.20225607>
- Bird, A., Tate, P., Nan, X., Campoy, J., Meehan, R., Cross, S., Tweedie, S., Charlton, J., Macleod, D., 1995. Studies of DNA methylation in animals. *Journal of Cell Science* 195, 37–39. https://doi.org/10.1242/jcs.1995.Supplement_19.5
- Blaschke, K., Ebata, K.T., Karimi, M.M., Zepeda-Martinez, J.A., Goyal, P., Mahapatra, S., Tam, A., Laird, D.J., Hirst, M., Rao, A., Lorincz, M.C., Ramalho-Santos, M., 2013. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. *Nature* 500, 222–226. <https://doi.org/10.1038/nature12362>
- Böhm, V., Hieb, A.R., Andrews, A.J., Gansen, A., Rocker, A., Tóth, K., Luger, K., Langowski, J., 2011. Nucleosome accessibility governed by the dimer/tetramer interface. *Nucleic Acids Research* 39, 3093–3102. <https://doi.org/10.1093/nar/gkq1279>
- Bolton, K.L., Ptashkin, R.N., Gao, T., Braunstein, L., Devlin, S.M., Kelly, D., Patel, M., Berthon, A., Syed, A., Yabe, M., Coombs, C.C., Caltabellotta, N.M., Walsh, M., Offit, K., Stadler, Z., Mandelker, D., Schulman, J., Patel, A., Philip, J., Bernard, E., Gundem, G., Ossa, J.E.A., Levine, M., Martinez, J.S.M., Farnoud, N., Glodzik, D., Li, S., Robson, M.E., Lee, C., Pharoah, P.D.P., Stopsack, K.H., Spitzer, B., Mantha, S., Fagin, J., Boucai, L., Gibson, C.J., Ebert, B.L., Young, A.L., Druley, T., Takahashi, K., Gillis, N., Ball, M., Padron, E., Hyman, D.M., Baselga, J., Norton, L., Gardos, S., Klimek, V.M., Scher, H., Bajorin, D., Paraiso, E., Benayed, R., Arcila, M.E., Ladanyi, M., Solit, D.B., Berger, M.F., Tallman, M., Garcia-Closas, M., Chatterjee, N., Diaz, L.A., Levine, R.L., Morton, L.M., Zehir, A., Papaemmanuil, E., 2020. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat Genet* 52, 1219–1226. <https://doi.org/10.1038/s41588-020-00710-0>
- Boukas, L., Havrilla, J.M., Hickey, P.F., Quinlan, A.R., Bjornsson, H.T., Hansen, K.D., 2019. Coexpression patterns define epigenetic regulators associated with neurological dysfunction. *Genome Res.* 29, 532–542. <https://doi.org/10.1101/gr.239442.118>
- Bourc'his, D., Bestor, T.H., 2004. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* 431, 96–99. <https://doi.org/10.1038/nature02886>
- Bouزيد, H., Belk, J.A., Jan, M., Qi, Y., Sarnowski, C., Wirth, S., Ma, L., Chrostek, M.R., Ahmad, H., Nachun, D., Yao, W., NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, Bis, J., Psaty, B., Beiser, A., Bick, A.G., Bis, J.C., Fornage, M., Longstreth, W.T., Lopez, O.L., Natarajan, P., Psaty, B.M., Satizabal, C.L., Weinstock, J., Larson, E.B., Crane, P.K., Keene, C.D., Seshadri, S., Satpathy, A.T., Montine, T.J., Jaiswal, S., 2023. Clonal hematopoiesis is associated with protection from Alzheimer's disease. *Nat Med* 29, 1662–1670. <https://doi.org/10.1038/s41591-023-02397-2>

- Bowers, M., Zhang, B., Ho, Y., Agarwal, P., Chen, C.-C., Bhatia, R., 2015. Osteoblast ablation reduces normal long-term hematopoietic stem cell self-renewal but accelerates leukemia development. *Blood* 125, 2678–2688. <https://doi.org/10.1182/blood-2014-06-582924>
- Bulut-Karslioglu, A., De La Rosa-Velázquez, I.A., Ramirez, F., Barenboim, M., Onishi-Seebacher, M., Arand, J., Galán, C., Winter, G.E., Engist, B., Gerle, B., O’Sullivan, R.J., Martens, J.H.A., Walter, J., Manke, T., Lachner, M., Jenuwein, T., 2014. Suv39h-Dependent H3K9me3 Marks Intact Retrotransposons and Silences LINE Elements in Mouse Embryonic Stem Cells. *Molecular Cell* 55, 277–290. <https://doi.org/10.1016/j.molcel.2014.05.029>
- Buscarlet, M., Provost, S., Zada, Y.F., Barhdadi, A., Bourgoin, V., Lépine, G., Mollica, L., Szuber, N., Dubé, M.-P., Busque, L., 2017. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood* 130, 753–762. <https://doi.org/10.1182/blood-2017-04-777029>
- Byvoet, P., Shepherd, G.R., Hardin, J.M., Noland, B.J., 1972. The distribution and turnover of labeled methyl groups in histone fractions of cultured mammalian cells. *Archives of Biochemistry and Biophysics* 148, 558–567. [https://doi.org/10.1016/0003-9861\(72\)90174-9](https://doi.org/10.1016/0003-9861(72)90174-9)
- Cabezas-Wallscheid, N., Klimmeck, D., Hansson, J., Lipka, D.B., Reyes, A., Wang, Q., Weichenhan, D., Lier, A., von Paleske, L., Renders, S., Wünsche, P., Zeisberger, P., Brocks, D., Gu, L., Herrmann, C., Haas, S., Essers, M.A.G., Brors, B., Eils, R., Huber, W., Milsom, M.D., Plass, C., Krijgsveld, J., Trumpp, A., 2014. Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. *Cell Stem Cell* 15, 507–522. <https://doi.org/10.1016/j.stem.2014.07.005>
- Cai, Z., Kotzin, J.J., Ramdas, B., Chen, S., Nelanuthala, S., Palam, L.R., Pandey, R., Mali, R.S., Liu, Y., Kelley, M.R., Sandusky, G., Mohseni, M., Williams, A., Henao-Mejia, J., Kapur, R., 2018. Inhibition of Inflammatory Signaling in Tet2 Mutant Preleukemic Cells Mitigates Stress-Induced Abnormalities and Clonal Hematopoiesis. *Cell Stem Cell* 23, 833-849.e5. <https://doi.org/10.1016/j.stem.2018.10.013>
- Caiado, F., Kovtonyuk, L.V., Gonullu, N.G., Fullin, J., Boettcher, S., Manz, M.G., 2023. Aging drives *Tet2* +/- clonal hematopoiesis via IL-1 signaling. *Blood* 141, 886–903. <https://doi.org/10.1182/blood.2022016835>
- Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., Bringham, F.R., Milner, L.A., Kronenberg, H.M., Scadden, D.T., 2003. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425, 841–846. <https://doi.org/10.1038/nature02040>
- Cao, Y., Sun, J., Li, M., Dong, Y., Zhang, Y., Yan, J., Huang, R., Yan, X., 2019. Inhibition of G9a by a small molecule inhibitor, UNC0642, induces apoptosis of human bladder cancer cells. *Acta Pharmacol Sin* 40, 1076–1084. <https://doi.org/10.1038/s41401-018-0205-5>
- Caon, M., 2020. SkeletalSkeletal System, in: Examination Questions and Answers in Basic Anatomy and Physiology: 2900 Multiple Choice Questions and 64 Essay Topics. Springer International Publishing, Cham, pp. 185–212. https://doi.org/10.1007/978-3-030-47314-3_7
- Castro-Diaz, N., Ecco, G., Coluccio, A., Kapopoulou, A., Yazdanpanah, B., Friedli, M., Duc, J., Jang, S.M., Turelli, P., Trono, D., 2014. Evolutionally dynamic L1 regulation in embryonic stem cells. *Genes Dev.* 28, 1397–1409. <https://doi.org/10.1101/gad.241661.114>

- Catez, F., Brown, D.T., Misteli, T., Bustin, M., 2002. Competition between histone H1 and HMGN proteins for chromatin binding sites. *EMBO Reports* 3, 760–766. <https://doi.org/10.1093/embo-reports/kvf156>
- Catlin, S.N., Busque, L., Gale, R.E., Gutter, P., Abkowitz, J.L., 2011. The replication rate of human hematopoietic stem cells in vivo. *Blood* 117, 4460–4466. <https://doi.org/10.1182/blood-2010-08-303537>
- Celeste, A., Fernandez-Capetillo, O., Kruhlak, M.J., Pilch, D.R., Staudt, D.W., Lee, A., Bonner, R.F., Bonner, W.M., Nussenzweig, A., 2003. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol* 5, 675–679. <https://doi.org/10.1038/ncb1004>
- Challen, G.A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J.S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y., Liang, S., Lu, Y., Darlington, G.J., Meissner, A., Issa, J.-P.J., Godley, L.A., Li, W., Goodell, M.A., 2012. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 44, 23–31. <https://doi.org/10.1038/ng.1009>
- Challen, G.A., Sun, D., Mayle, A., Jeong, M., Luo, M., Rodriguez, B., Mallaney, C., Celik, H., Yang, L., Xia, Z., Cullen, S., Berg, J., Zheng, Y., Darlington, G.J., Li, W., Goodell, M.A., 2014. Dnmt3a and Dnmt3b Have Overlapping and Distinct Functions in Hematopoietic Stem Cells. *Cell Stem Cell* 15, 350–364. <https://doi.org/10.1016/j.stem.2014.06.018>
- Chambers, S.M., Shaw, C.A., Gatz, C., Fisk, C.J., Donehower, L.A., Goodell, M.A., 2007. Aging Hematopoietic Stem Cells Decline in Function and Exhibit Epigenetic Dysregulation. *PLoS Biol* 5, e201. <https://doi.org/10.1371/journal.pbio.0050201>
- Chandel, N.S., Jasper, H., Ho, T.T., Passequé, E., 2016. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat Cell Biol* 18, 823–832. <https://doi.org/10.1038/ncb3385>
- Chavakis, T., Mitroulis, I., Hajishengallis, G., 2019. Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. *Nat Immunol* 20, 802–811. <https://doi.org/10.1038/s41590-019-0402-5>
- Chen, C., Liu, Yu, Liu, Yang, Zheng, P., 2010. Mammalian target of rapamycin activation underlies HSC defects in autoimmune disease and inflammation in mice. *J Clin Invest* 120, 4091–4101. <https://doi.org/10.1172/JCI43873>
- Chen, J.-M., Férec, C., Cooper, D.N., 2006. LINE-1 Endonuclease-Dependent Retrotranspositional Events Causing Human Genetic Disease: Mutation Detection Bias and Multiple Mechanisms of Target Gene Disruption. *BioMed Research International* 2006, 056182. <https://doi.org/10.1155/JBB/2006/56182>
- Chen, K., Yuan, J., Sia, Y., Chen, Z., 2023. Mechanism of action of the SWI/SNF family complexes. *Nucleus* 14, 2165604. <https://doi.org/10.1080/19491034.2023.2165604>
- Chen, Q., Chen, Y., Bian, C., Fujiki, R., Yu, X., 2013. TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* 493, 561–564. <https://doi.org/10.1038/nature11742>
- Chia, Y.C., Islam, M.A., Hider, P., Woon, P.Y., Johan, M.F., Hassan, R., Ramli, M., 2021. The Prevalence of TET2 Gene Mutations in Patients with BCR-ABL-Negative Myeloproliferative Neoplasms (MPN): A Systematic Review and Meta-Analysis. *Cancers* 13, 3078. <https://doi.org/10.3390/cancers13123078>
- Chiappinelli, K.B., Strissel, P.L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N.S., Cope, L.M., Snyder, A., Makarov, V., Buhu, S., Slamon, D.J., Wolchok, J.D., Pardoll, D.M., Beckmann, M.W., Zahnow, C.A., Merghoub, T., Chan, T.A., Baylin, S.B., Strick, R., 2015a. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell* 162, 974–986. <https://doi.org/10.1016/j.cell.2015.07.011>

- Chiappinelli, K.B., Strissel, P.L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N.S., Cope, L.M., Snyder, A., Makarov, V., Buhu, S., Slamon, D.J., Wolchok, J.D., Pardoll, D.M., Beckmann, M.W., Zahnow, C.A., Merghoub, T., Chan, T.A., Baylin, S.B., Strick, R., 2015b. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell* 162, 974–986. <https://doi.org/10.1016/j.cell.2015.07.011>
- Chung, H.Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A.Y., Carter, C., Yu, B.P., Leeuwenburgh, C., 2009. Molecular inflammation: Underpinnings of aging and age-related diseases. *Ageing Research Reviews* 8, 18–30. <https://doi.org/10.1016/j.arr.2008.07.002>
- Chuong, E.B., Elde, N.C., Feschotte, C., 2017. Regulatory activities of transposable elements: from conflicts to benefits. *Nat Rev Genet* 18, 71–86. <https://doi.org/10.1038/nrg.2016.139>
- Cimmino, L., Neel, B.G., Aifantis, I., 2018. Vitamin C in Stem Cell Reprogramming and Cancer. *Trends in Cell Biology* 28, 698–708. <https://doi.org/10.1016/j.tcb.2018.04.001>
- Clapes, T., Polyzou, A., Prater, P., Sagar, Morales-Hernández, A., Ferrarini, M.G., Kehrer, N., Lefkopoulos, S., Bergo, V., Hummel, B., Obier, N., Maticzka, D., Bridgeman, A., Herman, J.S., Ilik, I., Klaeylé, L., Rehwinkel, J., McKinney-Freeman, S., Backofen, R., Akhtar, A., Cabezas-Wallscheid, N., Sawarkar, R., Rebollo, R., Grün, D., Trompouki, E., 2021. Chemotherapy-induced transposable elements activate MDA5 to enhance haematopoietic regeneration. *Nat Cell Biol* 23, 704–717. <https://doi.org/10.1038/s41556-021-00707-9>
- Clapier, C.R., Cairns, B.R., 2009. The Biology of Chromatin Remodeling Complexes. *Annu. Rev. Biochem.* 78, 273–304. <https://doi.org/10.1146/annurev.biochem.77.062706.153223>
- Cobo, I., Tanaka, T., Glass, C.K., Yeang, C., 2022. Clonal hematopoiesis driven by DNMT3A and TET2 mutations: role in monocyte and macrophage biology and atherosclerotic cardiovascular disease. *Current Opinion in Hematology* 29, 1–7. <https://doi.org/10.1097/MOH.0000000000000688>
- Collin, M., Bigley, V., 2016. Monocyte, Macrophage, and Dendritic Cell Development: the Human Perspective. *Microbiol Spectr* 4, 4.5.05. <https://doi.org/10.1128/microbiolspec.MCHD-0015-2015>
- Colombo, A.R., Zubair, A., Thiagarajan, D., Nuzhdin, S., Triche, T.J., Ramsingh, G., 2017a. Suppression of Transposable Elements in Leukemic Stem Cells. *Sci Rep* 7, 7029. <https://doi.org/10.1038/s41598-017-07356-9>
- Colombo, A.R., Zubair, A., Thiagarajan, D., Nuzhdin, S., Triche, T.J., Ramsingh, G., 2017b. Suppression of Transposable Elements in Leukemic Stem Cells. *Sci Rep* 7, 7029. <https://doi.org/10.1038/s41598-017-07356-9>
- Cook, E.K., Luo, M., Rauh, M.J., 2020. Clonal hematopoiesis and inflammation: Partners in leukemogenesis and comorbidity. *Exp Hematol* 83, 85–94. <https://doi.org/10.1016/j.exphem.2020.01.011>
- Coombs, C.C., Zehir, A., Devlin, S.M., Kishtagari, A., Syed, A., Jonsson, P., Hyman, D.M., Solit, D.B., Robson, M.E., Baselga, J., Arcila, M.E., Ladanyi, M., Tallman, M.S., Levine, R.L., Berger, M.F., 2017. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell* 21, 374–382.e4. <https://doi.org/10.1016/j.stem.2017.07.010>
- Cordaux, R., Batzer, M.A., 2009. The impact of retrotransposons on human genome evolution. *Nat Rev Genet* 10, 691–703. <https://doi.org/10.1038/nrg2640>

- Crossley, M.P., Bocek, M., Cimprich, K.A., 2019. R-Loops as Cellular Regulators and Genomic Threats. *Molecular Cell* 73, 398–411. <https://doi.org/10.1016/j.molcel.2019.01.024>
- Cuellar, T.L., Herzner, A.-M., Zhang, X., Goyal, Y., Watanabe, C., Friedman, B.A., Janakiraman, V., Durinck, S., Stinson, J., Arnott, D., Cheung, T.K., Chaudhuri, S., Modrusan, Z., Doerr, J.M., Classon, M., Haley, B., 2017a. ---Silencing of retrotransposons by SETDB1 inhibits the interferon response in acute myeloid leukemia---. *Journal of Cell Biology* 216, 3535–3549. <https://doi.org/10.1083/jcb.201612160>
- Cuellar, T.L., Herzner, A.-M., Zhang, X., Goyal, Y., Watanabe, C., Friedman, B.A., Janakiraman, V., Durinck, S., Stinson, J., Arnott, D., Cheung, T.K., Chaudhuri, S., Modrusan, Z., Doerr, J.M., Classon, M., Haley, B., 2017b. ---Silencing of retrotransposons by SETDB1 inhibits the interferon response in acute myeloid leukemia---. *Journal of Cell Biology* 216, 3535–3549. <https://doi.org/10.1083/jcb.201612160>
- Cui, K., Zang, C., Roh, T.-Y., Schones, D.E., Childs, R.W., Peng, W., Zhao, K., 2009. Chromatin Signatures in Multipotent Human Hematopoietic Stem Cells Indicate the Fate of Bivalent Genes during Differentiation. *Cell Stem Cell* 4, 80–93. <https://doi.org/10.1016/j.stem.2008.11.011>
- Daitoku, H., Someya, M., Kako, K., Hayashi, T., Tajima, T., Haruki, H., Sekiguchi, N., Uetake, T., Akimoto, Y., Fukamizu, A., 2021. siRNA screening identifies METTL9 as a histidine N π -methyltransferase that targets the proinflammatory protein S100A9. *Journal of Biological Chemistry* 297, 101230. <https://doi.org/10.1016/j.jbc.2021.101230>
- Dawoud, A.A.Z., Tapper, W.J., Cross, N.C.P., 2020. Clonal myelopoiesis in the UK Biobank cohort: ASXL1 mutations are strongly associated with smoking. *Leukemia* 34, 2660–2672. <https://doi.org/10.1038/s41375-020-0896-8>
- Dawson, M.A., Kouzarides, T., 2012. Cancer Epigenetics: From Mechanism to Therapy. *Cell* 150, 12–27. <https://doi.org/10.1016/j.cell.2012.06.013>
- Day, R.B., Bhattacharya, D., Nagasawa, T., Link, D.C., 2015. Granulocyte colony-stimulating factor reprograms bone marrow stromal cells to actively suppress B lymphopoiesis in mice. *Blood* 125, 3114–3117. <https://doi.org/10.1182/blood-2015-02-629444>
- De Cecco, M., Criscione, S.W., Peckham, E.J., Hillenmeyer, S., Hamm, E.A., Manivannan, J., Peterson, A.L., Kreiling, J.A., Neretti, N., Sedivy, J.M., 2013. Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. *Aging Cell* 12, 247–256. <https://doi.org/10.1111/accel.12047>
- De Cecco, M., Ito, T., Petrashen, A.P., Elias, A.E., Skvir, N.J., Criscione, S.W., Caligiana, A., Broccoli, G., Adney, E.M., Boeke, J.D., Le, O., Beauséjour, C., Ambati, J., Ambati, K., Simon, M., Seluanov, A., Gorbunova, V., Slagboom, P.E., Helfand, S.L., Neretti, N., Sedivy, J.M., 2019. L1 drives IFN in senescent cells and promotes age-associated inflammation. *Nature* 566, 73–78. <https://doi.org/10.1038/s41586-018-0784-9>
- De Haan, G., Lazare, S.S., 2018. Aging of hematopoietic stem cells. *Blood* 131, 479–487. <https://doi.org/10.1182/blood-2017-06-746412>
- De La Rica, L., Deniz, Ö., Cheng, K.C.L., Todd, C.D., Cruz, C., Houseley, J., Branco, M.R., 2016. TET-dependent regulation of retrotransposable elements in mouse embryonic stem cells. *Genome Biol* 17, 234. <https://doi.org/10.1186/s13059-016-1096-8>

- De Laval, B., Maurizio, J., Kandalla, P.K., Brisou, G., Simonnet, L., Huber, C., Gimenez, G., Matcovitch-Natan, O., Reinhardt, S., David, E., Mildner, A., Leutz, A., Nadel, B., Bordi, C., Amit, I., Sarrazin, S., Sieweke, M.H., 2020. C/EBP β -Dependent Epigenetic Memory Induces Trained Immunity in Hematopoietic Stem Cells. *Cell Stem Cell* 26, 793. <https://doi.org/10.1016/j.stem.2020.03.014>
- de Laval, B., Pawlikowska, P., Petit-Cocault, L., Bilhou-Nabera, C., Aubin-Houzelstein, G., Souyri, M., Pouzoulet, F., Gaudry, M., Porteu, F., 2013a. Thrombopoietin-increased DNA-PK-dependent DNA repair limits hematopoietic stem and progenitor cell mutagenesis in response to DNA damage. *Cell Stem Cell* 12, 37–48. <https://doi.org/10.1016/j.stem.2012.10.012>
- de Laval, B., Pawlikowska, P., Petit-Cocault, L., Bilhou-Nabera, C., Aubin-Houzelstein, G., Souyri, M., Pouzoulet, F., Gaudry, M., Porteu, F., 2013b. Thrombopoietin-increased DNA-PK-dependent DNA repair limits hematopoietic stem and progenitor cell mutagenesis in response to DNA damage. *Cell Stem Cell* 12, 37–48. <https://doi.org/10.1016/j.stem.2012.10.012>
- Deaton, A.M., Bird, A., 2011. CpG islands and the regulation of transcription. *Genes Dev.* 25, 1010–1022. <https://doi.org/10.1101/gad.2037511>
- Deininger, P.L., Batzer, M.A., 1999. Alu Repeats and Human Disease. *Molecular Genetics and Metabolism* 67, 183–193. <https://doi.org/10.1006/mgme.1999.2864>
- Deng, T., Zhu, Z.I., Zhang, S., Leng, F., Cherukuri, S., Hansen, L., Mariño-Ramírez, L., Meshorer, E., Landsman, D., Bustin, M., 2013. HMGN1 Modulates Nucleosome Occupancy and DNase I Hypersensitivity at the CpG Island Promoters of Embryonic Stem Cells. *Molecular and Cellular Biology* 33, 3377–3389. <https://doi.org/10.1128/MCB.00435-13>
- Deniz, Ö., Ahmed, M., Todd, C.D., Rio-Machin, A., Dawson, M.A., Branco, M.R., 2020. Endogenous retroviruses are a source of enhancers with oncogenic potential in acute myeloid leukaemia. *Nat Commun* 11, 3506. <https://doi.org/10.1038/s41467-020-17206-4>
- Deniz, Ö., Frost, J.M., Branco, M.R., 2019. Regulation of transposable elements by DNA modifications. *Nat Rev Genet* 20, 417–431. <https://doi.org/10.1038/s41576-019-0106-6>
- Desai, P., Mencia-Trinchant, N., Savenkov, O., Simon, M.S., Cheang, G., Lee, S., Samuel, M., Ritchie, E.K., Guzman, M.L., Ballman, K.V., Roboz, G.J., Hassane, D.C., 2018. Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nat Med* 24, 1015–1023. <https://doi.org/10.1038/s41591-018-0081-z>
- Dewannieux, M., Esnault, C., Heidmann, T., 2003. LINE-mediated retrotransposition of marked Alu sequences. *Nat Genet* 35, 41–48. <https://doi.org/10.1038/ng1223>
- Ding, H.-F., Bustin, M., Hansen, U., 1997. Alleviation of Histone H1-Mediated Transcriptional Repression and Chromatin Compaction by the Acidic Activation Region in Chromosomal Protein HMG-14. *Molecular and Cellular Biology* 17, 5843–5855. <https://doi.org/10.1128/MCB.17.10.5843>
- Djeghloul, D., Kuranda, K., Kuzniak, I., Barbieri, D., Naguibneva, I., Choisy, C., Bories, J.-C., Dosquet, C., Pla, M., Vanneaux, V., Socié, G., Porteu, F., Garrick, D., Goodhardt, M., 2016a. Age-Associated Decrease of the Histone Methyltransferase SUV39H1 in HSC Perturbs Heterochromatin and B Lymphoid Differentiation. *Stem Cell Reports* 6, 970–984. <https://doi.org/10.1016/j.stemcr.2016.05.007>
- Djeghloul, D., Kuranda, K., Kuzniak, I., Barbieri, D., Naguibneva, I., Choisy, C., Bories, J.-C., Dosquet, C., Pla, M., Vanneaux, V., Socié, G., Porteu, F., Garrick, D., Goodhardt, M., 2016b. Age-Associated Decrease of the Histone Methyltransferase SUV39H1 in

- HSC Perturbs Heterochromatin and B Lymphoid Differentiation. *Stem Cell Reports* 6, 970–984. <https://doi.org/10.1016/j.stemcr.2016.05.007>
- Domingues, P., Hale, B.G., 2017. Functional Insights into ANP32A-Dependent Influenza A Virus Polymerase Host Restriction. *Cell Reports* 20, 2538–2546. <https://doi.org/10.1016/j.celrep.2017.08.061>
- Donnelly, D.S., Zelterman, D., Sharkis, S., Krause, D.S., 1999. Functional activity of murine CD34⁺ and CD34⁻ hematopoietic stem cell populations. *Experimental Hematology* 27, 788–796. [https://doi.org/10.1016/S0301-472X\(99\)00032-6](https://doi.org/10.1016/S0301-472X(99)00032-6)
- Dorsheimer, L., Assmus, B., Rasper, T., Ortmann, C.A., Ecke, A., Abou-El-Ardat, K., Schmid, T., Brüne, B., Wagner, S., Serve, H., Hoffmann, J., Seeger, F., Dimmeler, S., Zeiher, A.M., Rieger, M.A., 2019. Association of Mutations Contributing to Clonal Hematopoiesis With Prognosis in Chronic Ischemic Heart Failure. *JAMA Cardiol* 4, 25. <https://doi.org/10.1001/jamacardio.2018.3965>
- Downs, J.A., Lowndes, N.F., Jackson, S.P., 2000. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* 408, 1001–1004. <https://doi.org/10.1038/35050000>
- Driver, C.J.I., McKECHNIE, S.W., 1992. Transposable Elements as a Factor in the Aging of *Drosophila melanogaster*. *Annals of the New York Academy of Sciences* 673, 83–91. <https://doi.org/10.1111/j.1749-6632.1992.tb27439.x>
- Driver, C.J.I., Vogrig, D.J., 1994. Apparent Retardation of Aging in *Drosophila melanogaster* by Inhibitors of Reverse Transcriptase. *Annals of the New York Academy of Sciences* 717, 189–197. <https://doi.org/10.1111/j.1749-6632.1994.tb12087.x>
- Dykstra, B., Olthof, S., Schreuder, J., Ritsema, M., De Haan, G., 2011. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *Journal of Experimental Medicine* 208, 2691–2703. <https://doi.org/10.1084/jem.20111490>
- Eberharter, A., Becker, P.B., 2002. Histone acetylation: a switch between repressive and permissive chromatin: Second in review series on chromatin dynamics. *EMBO Reports* 3, 224–229. <https://doi.org/10.1093/embo-reports/kvf053>
- Eickbush, T.H., Malik, H.S., 2007. Origins and Evolution of Retrotransposons, in: Craig, N.L., Craigie, R., Gellert, M., Lambowitz, A.M. (Eds.), *Mobile DNA II*. Wiley, pp. 1111–1144. <https://doi.org/10.1128/9781555817954.ch49>
- Elgin, S.C., 1996. Heterochromatin and gene regulation in *Drosophila*. *Current Opinion in Genetics & Development* 6, 193–202. [https://doi.org/10.1016/S0959-437X\(96\)80050-5](https://doi.org/10.1016/S0959-437X(96)80050-5)
- Ema, H., Sudo, K., Seita, J., Matsubara, A., Morita, Y., Osawa, M., Takatsu, K., Takaki, S., Nakauchi, H., 2005. Quantification of Self-Renewal Capacity in Single Hematopoietic Stem Cells from Normal and Lnk-Deficient Mice. *Developmental Cell* 8, 907–914. <https://doi.org/10.1016/j.devcel.2005.03.019>
- Epsztejn-Litman, S., Feldman, N., Abu-Remaileh, M., Shufaro, Y., Gerson, A., Ueda, J., Deplus, R., Fuks, F., Shinkai, Y., Cedar, H., Bergman, Y., 2008. De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat Struct Mol Biol* 15, 1176–1183. <https://doi.org/10.1038/nsmb.1476>
- Esplin, B.L., Shimazu, T., Welner, R.S., Garrett, K.P., Nie, L., Zhang, Q., Humphrey, M.B., Yang, Q., Borghesi, L.A., Kincade, P.W., 2011. Chronic exposure to a TLR ligand injures hematopoietic stem cells. *J Immunol* 186, 5367–5375. <https://doi.org/10.4049/jimmunol.1003438>
- Essers, M.A.G., Offner, S., Blanco-Bose, W.E., Waibler, Z., Kalinke, U., Duchosal, M.A., Trumpp, A., 2009. IFN α activates dormant haematopoietic stem cells in vivo. *Nature* 458, 904–908. <https://doi.org/10.1038/nature07815>

- Fabre, M.A., De Almeida, J.G., Fiorillo, E., Mitchell, E., Damaskou, A., Rak, J., Orrù, V., Marongiu, M., Chapman, M.S., Vijayabaskar, M.S., Baxter, J., Hardy, C., Abascal, F., Williams, N., Nangalia, J., Martincorena, I., Campbell, P.J., McKinney, E.F., Cucca, F., Gerstung, M., Vassiliou, G.S., 2022. The longitudinal dynamics and natural history of clonal haematopoiesis. *Nature* 606, 335–342. <https://doi.org/10.1038/s41586-022-04785-z>
- Fadloun, A., Eid, A., Torres-Padilla, M.-E., 2013. Mechanisms and dynamics of heterochromatin formation during mammalian development: closed paths and open questions. *Curr Top Dev Biol* 104, 1–45. <https://doi.org/10.1016/B978-0-12-416027-9.00001-2>
- Fedoroff, N.V., 2012. Transposable Elements, Epigenetics, and Genome Evolution. *Science* 338, 758–767. <https://doi.org/10.1126/science.338.6108.758>
- Feng, Q., Wang, H., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Struhl, K., Zhang, Y., 2002. Methylation of H3-Lysine 79 Is Mediated by a New Family of HMTases without a SET Domain. *Current Biology* 12, 1052–1058. [https://doi.org/10.1016/S0960-9822\(02\)00901-6](https://doi.org/10.1016/S0960-9822(02)00901-6)
- Fernandez-Capetillo, O., Lee, A., Nussenzweig, M., Nussenzweig, A., 2004. H2AX: the histone guardian of the genome. *DNA Repair (Amst)* 3, 959–967. <https://doi.org/10.1016/j.dnarep.2004.03.024>
- Figuroa, M.E., Abdel-Wahab, O., Lu, C., Ward, P.S., Patel, J., Shih, A., Li, Y., Bhagwat, N., Vasanthakumar, A., Fernandez, H.F., Tallman, M.S., Sun, Z., Wolniak, K., Peeters, J.K., Liu, W., Choe, S.E., Fantin, V.R., Paietta, E., Löwenberg, B., Licht, J.D., Godley, L.A., Delwel, R., Valk, P.J.M., Thompson, C.B., Levine, R.L., Melnick, A., 2010. Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation. *Cancer Cell* 18, 553–567. <https://doi.org/10.1016/j.ccr.2010.11.015>
- Finnegan, D.J., 1989. Eukaryotic transposable elements and genome evolution. *Trends in Genetics* 5, 103–107. [https://doi.org/10.1016/0168-9525\(89\)90039-5](https://doi.org/10.1016/0168-9525(89)90039-5)
- Fischle, W., Wang, Y., Jacobs, S.A., Kim, Y., Allis, C.D., Khorasanizadeh, S., 2003. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* 17, 1870–1881. <https://doi.org/10.1101/gad.1110503>
- Flach, J., Bakker, S.T., Mohrin, M., Conroy, P.C., Pietras, E.M., Reynaud, D., Alvarez, S., Diolaiti, M.E., Ugarte, F., Forsberg, E.C., Le Beau, M.M., Stohr, B.A., Méndez, J., Morrison, C.G., Passegué, E., 2014. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature* 512, 198–202. <https://doi.org/10.1038/nature13619>
- Fleenor, C.J., Higa, K., Weil, M.M., DeGregori, J., 2015. Evolved Cellular Mechanisms to Respond to Genotoxic Insults: Implications for Radiation-Induced Hematologic Malignancies. *Radiation Research* 184, 341–351. <https://doi.org/10.1667/RR14147.1>
- Florez, M.A., Tran, B.T., Wathan, T.K., DeGregori, J., Pietras, E.M., King, K.Y., 2022a. Clonal hematopoiesis: Mutation-specific adaptation to environmental change. *Cell Stem Cell* 29, 882–904. <https://doi.org/10.1016/j.stem.2022.05.006>
- Florez, M.A., Tran, B.T., Wathan, T.K., DeGregori, J., Pietras, E.M., King, K.Y., 2022b. Clonal hematopoiesis: Mutation-specific adaptation to environmental change. *Cell Stem Cell* 29, 882–904. <https://doi.org/10.1016/j.stem.2022.05.006>
- Fortuny, A., Chansard, A., Caron, P., Chevallier, O., Leroy, O., Renaud, O., Polo, S.E., 2021. Imaging the response to DNA damage in heterochromatin domains reveals core principles of heterochromatin maintenance. *Nat Commun* 12, 2428. <https://doi.org/10.1038/s41467-021-22575-5>

- Frapporti, A., Miró Pina, C., Arnaiz, O., Holoch, D., Kawaguchi, T., Humbert, A., Eleftheriou, E., Lombard, B., Loew, D., Sperling, L., Guitot, K., Margueron, R., Duhaucourt, S., 2019. The Polycomb protein Ezh1 mediates H3K9 and H3K27 methylation to repress transposable elements in *Paramecium*. *Nat Commun* 10, 2710. <https://doi.org/10.1038/s41467-019-10648-5>
- Fujiki, R., Hashiba, W., Sekine, H., Yokoyama, A., Chikanishi, T., Ito, S., Imai, Y., Kim, J., He, H.H., Igarashi, K., Kanno, J., Ohtake, F., Kitagawa, H., Roeder, R.G., Brown, M., Kato, S., 2011. GlcNAcylation of histone H2B facilitates its monoubiquitination. *Nature* 480, 557–560. <https://doi.org/10.1038/nature10656>
- Fuster, J.J., MacLauchlan, S., Zuriaga, M.A., Polackal, M.N., Ostriker, A.C., Chakraborty, R., Wu, C.-L., Sano, S., Muralidharan, S., Rius, C., Vuong, J., Jacob, S., Muralidhar, V., Robertson, A.A.B., Cooper, M.A., Andrés, V., Hirschi, K.K., Martin, K.A., Walsh, K., 2017. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 355, 842–847. <https://doi.org/10.1126/science.aag1381>
- Fuster, J.J., Zuriaga, M.A., Zorita, V., MacLauchlan, S., Polackal, M.N., Viana-Huete, V., Ferrer-Pérez, A., Matesanz, N., Herrero-Cervera, A., Sano, S., Cooper, M.A., González-Navarro, H., Walsh, K., 2020. TET2-Loss-of-Function-Driven Clonal Hematopoiesis Exacerbates Experimental Insulin Resistance in Aging and Obesity. *Cell Reports* 33, 108326. <https://doi.org/10.1016/j.celrep.2020.108326>
- García-Pérez, J.L., Widmann, T.J., Adams, I.R., 2016. The impact of transposable elements on mammalian development. *Development* 143, 4101–4114. <https://doi.org/10.1242/dev.132639>
- García-Ramírez, M., Dong, F., Ausio, J., 1992. Role of the histone “tails” in the folding of oligonucleosomes depleted of histone H1. *Journal of Biological Chemistry* 267, 19587–19595. [https://doi.org/10.1016/S0021-9258\(18\)41815-7](https://doi.org/10.1016/S0021-9258(18)41815-7)
- Gasior, S.L., Wakeman, T.P., Xu, B., Deininger, P.L., 2006a. The Human LINE-1 Retrotransposon Creates DNA Double-strand Breaks. *Journal of Molecular Biology* 357, 1383–1393. <https://doi.org/10.1016/j.jmb.2006.01.089>
- Gasior, S.L., Wakeman, T.P., Xu, B., Deininger, P.L., 2006b. The human LINE-1 retrotransposon creates DNA double-strand breaks. *J Mol Biol* 357, 1383–1393. <https://doi.org/10.1016/j.jmb.2006.01.089>
- Geissmann, F., Manz, M.G., Jung, S., Sieweke, M.H., Merad, M., Ley, K., 2010. Development of Monocytes, Macrophages, and Dendritic Cells. *Science* 327, 656–661. <https://doi.org/10.1126/science.1178331>
- Gekas, C., Graf, T., 2013. CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age. *Blood* 121, 4463–4472. <https://doi.org/10.1182/blood-2012-09-457929>
- Gellert, M., 2002. V(D)J Recombination: RAG Proteins, Repair Factors, and Regulation. *Annu. Rev. Biochem.* 71, 101–132. <https://doi.org/10.1146/annurev.biochem.71.090501.150203>
- Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., Purcell, S.M., Svantesson, O., Landén, M., Höglund, M., Lehmann, S., Gabriel, S.B., Moran, J.L., Lander, E.S., Sullivan, P.F., Sklar, P., Grönberg, H., Hultman, C.M., McCarroll, S.A., 2014. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N Engl J Med* 371, 2477–2487. <https://doi.org/10.1056/NEJMoa1409405>
- Gibney, E.R., Nolan, C.M., 2010. Epigenetics and gene expression. *Heredity* 105, 4–13. <https://doi.org/10.1038/hdy.2010.54>

- Gilbert, C., Chateigner, A., Ermenwein, L., Barbe, V., Bézier, A., Herniou, E.A., Cordaux, R., 2014. Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons. *Nat Commun* 5, 3348. <https://doi.org/10.1038/ncomms4348>
- Gilbert, C., Feschotte, C., 2018. Horizontal acquisition of transposable elements and viral sequences: patterns and consequences. *Current Opinion in Genetics & Development* 49, 15–24. <https://doi.org/10.1016/j.gde.2018.02.007>
- Gilchrist, J.J., Fang, H., Danielli, S., Tomkova, M., Nassiri, I., Ng, E., Tong, O., Taylor, C., Muldoon, D., Cohen, L.R.Z., Al-Mossawi, H., Lau, E., Neville, M., Schuster-Boeckler, B., Knight, J.C., Fairfax, B.P., 2024. Characterization of the genetic determinants of context-specific DNA methylation in primary monocytes. *Cell Genomics* 4, 100541. <https://doi.org/10.1016/j.xgen.2024.100541>
- Gillis, N.K., Ball, M., Zhang, Q., Ma, Z., Zhao, Y., Yoder, S.J., Balasis, M.E., Mesa, T.E., Sallman, D.A., Lancet, J.E., Komrokji, R.S., List, A.F., McLeod, H.L., Alsina, M., Baz, R., Shain, K.H., Rollison, D.E., Padron, E., 2017. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *The Lancet Oncology* 18, 112–121. [https://doi.org/10.1016/S1470-2045\(16\)30627-1](https://doi.org/10.1016/S1470-2045(16)30627-1)
- Gogvadze, E., Buzdin, A., 2009. Retroelements and their impact on genome evolution and functioning. *Cell. Mol. Life Sci.* 66, 3727–3742. <https://doi.org/10.1007/s00018-009-0107-2>
- Goodier, J.L., Ostertag, E.M., Du, K., Kazazian, H.H., 2001. A Novel Active L1 Retrotransposon Subfamily in the Mouse. *Genome Res.* 11, 1677–1685. <https://doi.org/10.1101/gr.198301>
- Gorbunova, V., Seluanov, A., Mita, P., McKerrow, W., Fenyö, D., Boeke, J.D., Linker, S.B., Gage, F.H., Kreiling, J.A., Petrashen, A.P., Woodham, T.A., Taylor, J.R., Helfand, S.L., Sedivy, J.M., 2021. The role of retrotransposable elements in ageing and age-associated diseases. *Nature* 596, 43–53. <https://doi.org/10.1038/s41586-021-03542-y>
- Groh, S., Schotta, G., 2017. Silencing of endogenous retroviruses by heterochromatin. *Cell. Mol. Life Sci.* 74, 2055–2065. <https://doi.org/10.1007/s00018-017-2454-8>
- Gruver, A., Hudson, L., Sempowski, G., 2007. Immunosenescence of ageing. *The Journal of Pathology* 211, 144–156. <https://doi.org/10.1002/path.2104>
- Gu, Z., Liu, Y., Zhang, Y., Cao, H., Lyu, J., Wang, X., Wylie, A., Newkirk, S.J., Jones, A.E., Lee, M., Botten, G.A., Deng, M., Dickerson, K.E., Zhang, C.C., An, W., Abrams, J.M., Xu, J., 2021a. Silencing of LINE-1 retrotransposons is a selective dependency of myeloid leukemia. *Nat Genet* 53, 672–682. <https://doi.org/10.1038/s41588-021-00829-8>
- Gu, Z., Liu, Y., Zhang, Y., Cao, H., Lyu, J., Wang, X., Wylie, A., Newkirk, S.J., Jones, A.E., Lee, M., Botten, G.A., Deng, M., Dickerson, K.E., Zhang, C.C., An, W., Abrams, J.M., Xu, J., 2021b. Silencing of LINE-1 retrotransposons is a selective dependency of myeloid leukemia. *Nat Genet* 53, 672–682. <https://doi.org/10.1038/s41588-021-00829-8>
- Guallar, D., Bi, X., Pardavila, J.A., Huang, X., Saenz, C., Shi, X., Zhou, H., Faiola, F., Ding, J., Haruehanroengra, P., Yang, F., Li, D., Sanchez-Priego, C., Saunders, A., Pan, F., Valdes, V.J., Kelley, K., Blanco, M.G., Chen, L., Wang, H., Sheng, J., Xu, M., Fidalgo, M., Shen, X., Wang, J., 2018. RNA-dependent chromatin targeting of TET2 for endogenous retrovirus control in pluripotent stem cells. *Nat Genet* 50, 443–451. <https://doi.org/10.1038/s41588-018-0060-9>
- Haas, N.B., Grabowski, J.M., North, J., Moran, J.V., Kazazian, H.H., Burch, J.B.E., 2001. Subfamilies of CR1 non-LTR retrotransposons have different 5'UTR sequences but

- are otherwise conserved. *Gene* 265, 175–183. [https://doi.org/10.1016/S0378-1119\(01\)00344-4](https://doi.org/10.1016/S0378-1119(01)00344-4)
- Haferlach, C., Heuser, M., 2022. Diagnostik bei unklaren Zytopenien – wie und wann suchen wir nach klonaler Hämatopoese? *Innere Medizin* 63, 1141–1147. <https://doi.org/10.1007/s00108-022-01402-z>
- Han, J.S., 2010. Non-long terminal repeat (non-LTR) retrotransposons: mechanisms, recent developments, and unanswered questions. *Mobile DNA* 1, 15. <https://doi.org/10.1186/1759-8753-1-15>
- Hancks, D.C., Kazazian, H.H., 2016. Roles for retrotransposon insertions in human disease. *Mobile DNA* 7, 9. <https://doi.org/10.1186/s13100-016-0065-9>
- Hardy, R.R., Kincade, P.W., Dorshkind, K., 2007. The Protean Nature of Cells in the B Lymphocyte Lineage. *Immunity* 26, 703–714. <https://doi.org/10.1016/j.immuni.2007.05.013>
- Hawkins, R.D., Hon, G.C., Lee, L.K., Ngo, Q., Lister, R., Pelizzola, M., Edsall, L.E., Kuan, S., Luu, Y., Klugman, S., Antosiewicz-Bourget, J., Ye, Z., Espinoza, C., Agarwahl, S., Shen, L., Ruotti, V., Wang, W., Stewart, R., Thomson, J.A., Ecker, J.R., Ren, B., 2010. Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell* 6, 479–491. <https://doi.org/10.1016/j.stem.2010.03.018>
- Haynes, B.F., Markert, M.L., Sempowski, G.D., Patel, D.D., Hale, L.P., 2000. The Role of the Thymus in Immune Reconstitution in Aging, Bone Marrow Transplantation, and HIV-1 Infection. *Annu. Rev. Immunol.* 18, 529–560. <https://doi.org/10.1146/annurev.immunol.18.1.529>
- He, Y.-F., Li, B.-Z., Li, Z., Liu, P., Wang, Y., Tang, Q., Ding, J., Jia, Y., Chen, Z., Li, L., Sun, Y., Li, X., Dai, Q., Song, C.-X., Zhang, K., He, C., Xu, G.-L., 2011. Tet-Mediated Formation of 5-Carboxylecytosine and Its Excision by TDG in Mammalian DNA. *Science* 333, 1303–1307. <https://doi.org/10.1126/science.1210944>
- Hebbes, T.R., Thorne, A.W., Crane-Robinson, C., 1988. A direct link between core histone acetylation and transcriptionally active chromatin. *The EMBO Journal* 7, 1395–1402. <https://doi.org/10.1002/j.1460-2075.1988.tb02956.x>
- Heitz, E., 1928. “Das” Heterochromatin der Moose. *Bornträger*.
- Henry, C.J., Casás-Selves, M., Kim, J., Zaberezhnyy, V., Aghili, L., Daniel, A.E., Jimenez, L., Azam, T., McNamee, E.N., Clambey, E.T., Klawitter, J., Serkova, N.J., Tan, A.C., Dinarello, C.A., DeGregori, J., 2015. Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. *Journal of Clinical Investigation* 125, 4666–4680. <https://doi.org/10.1172/JCI83024>
- Hepburn AC, Steele RE, Veeratterapillay R, Wilson L, Kounatidou EE, Barnard A, Berry P, Cassidy JR, Moad M, El-Sherif A, Gaughan L, Mills IG, Robson CN, Heer R. The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance. *Oncogene*. 2019 May;38(22):4412-4424. doi: 10.1038/s41388-019-0712-y. Epub 2019 Feb 11. Erratum in: *Oncogene*. 2019 May;38(22):4425. doi: 10.1038/s41388-019-0826-2.
- Hernández, L., Terradas, M., Camps, J., Martín, M., Tusell, L., Genescà, A., 2015. Aging and radiation: bad companions. *Aging Cell* 14, 153–161. <https://doi.org/10.1111/accel.12306>
- Hidaoui, D., Chelbie, R., Bohm, M., Porquet, A., Polyzou, A., Alcazer, V., Depil, S., Imanci, A., Morabito, M., Renneville, A., selimoglu-Buet, D., Thépot, S., Itzykson, R., Laplane, L., Droin, N., Trompouki, E., Elvira-Matelot, E., Solary, E., Porteu, F., 2024a. Targeting Heterochromatin Eliminates Malignant Hematopoietic Stem and Progenitor Cells in Chronic Myelomonocytic Leukemia Through Reactivation of

- Retroelements and Innate Immune pathways.
<https://doi.org/10.1101/2024.02.02.578583>
- Hidaoui, D., Chelbie, R., Bohm, M., Porquet, A., Polyzou, A., Alcazer, V., Depil, S., Imanci, A., Morabito, M., Renneville, A., selimoglu-Buet, D., Thépot, S., Itzykson, R., Laplane, L., Droin, N., Trompouki, E., Elvira-Matelot, E., Solary, E., Porteu, F., 2024b. Targeting Heterochromatin Eliminates Malignant Hematopoietic Stem and Progenitor Cells in Chronic Myelomonocytic Leukemia Through Reactivation of Retroelements and Innate Immune pathways (preprint). *Cancer Biology*.
<https://doi.org/10.1101/2024.02.02.578583>
- Hill, A., Satta, J., LaBarge, M.A., Hurria, A., 2020. How cancer therapeutics cause accelerated aging: Insights from the hallmarks of aging. *Journal of Geriatric Oncology* 11, 191–193. <https://doi.org/10.1016/j.jgo.2019.03.007>
- Ho, T.T., Warr, M.R., Adelman, E.R., Lansinger, O.M., Flach, J., Verovskaya, E.V., Figueroa, M.E., Passequé, E., 2017. Autophagy maintains the metabolism and function of young and old stem cells. *Nature* 543, 205–210.
<https://doi.org/10.1038/nature21388>
- Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., Leboeuf, M., Low, D., Oller, G., Almeida, F., Choy, S.H.Y., Grisotto, M., Renia, L., Conway, S.J., Stanley, E.R., Chan, J.K.Y., Ng, L.G., Samokhvalov, I.M., Merad, M., Ginhoux, F., 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *Journal of Experimental Medicine* 209, 1167–1181. <https://doi.org/10.1084/jem.20120340>
- Hoffman, C.M., Calvi, L.M., 2014. Minireview: Complexity of Hematopoietic Stem Cell Regulation in the Bone Marrow Microenvironment. *Molecular Endocrinology* 28, 1592–1601. <https://doi.org/10.1210/me.2014-1079>
- Holliday, R., Pugh, J.E., 1975. DNA modification mechanisms and gene activity during development. *Science* 187, 226–232.
- Hong, T., Li, J., Guo, L., Cavalier, M., Wang, T., Dou, Y., DeLaFuente, A., Fang, S., Guzman, A., Wohlan, K., Kapadia, C., Rosas, C., Yang, Y., Yin, C.C., Li, S., You, M.J., Cheng, X., Goodell, M.A., Zhou, Y., Huang, Y., 2023. TET2 modulates spatial relocalization of heterochromatin in aged hematopoietic stem and progenitor cells. *Nat Aging* 3, 1387–1400. <https://doi.org/10.1038/s43587-023-00505-y>
- Honigberg, M.C., Zekavat, S.M., Niroula, A., Griffin, G.K., Bick, A.G., Pirruccello, J.P., Nakao, T., Whitsel, E.A., Farland, L.V., Laurie, C., Kooperberg, C., Manson, J.E., Gabriel, S., Libby, P., Reiner, A.P., Ebert, B.L., NHLBI Trans-Omics for Precision Medicine Program, Natarajan, P., 2021. Premature Menopause, Clonal Hematopoiesis, and Coronary Artery Disease in Postmenopausal Women. *Circulation* 143, 410–423. <https://doi.org/10.1161/CIRCULATIONAHA.120.051775>
- Hormaechea-Agulla, D., Matatall, K.A., Le, D.T., Kain, B., Long, X., Kus, P., Jaksik, R., Challen, G.A., Kimmel, M., King, K.Y., 2021. Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFN γ signaling. *Cell Stem Cell* 28, 1428–1442.e6. <https://doi.org/10.1016/j.stem.2021.03.002>
- Huerga Encabo, H., Aramburu, I.V., Garcia-Albornoz, M., Piganeau, M., Wood, H., Song, A., Ferrelli, A., Sharma, A., Minutti, C.M., Domart, M.-C., Papazoglou, D., Gurashi, K., Llorian Sopena, M., Goldstone, R., Fallesen, T., Wang, Q., Ariza-McNaughton, L., Wiseman, D.H., Batta, K., Gupta, R., Papayannopoulos, V., Bonnet, D., 2023. Loss of TET2 in human hematopoietic stem cells alters the development and function of neutrophils. *Cell Stem Cell* 30, 781–799.e9.
<https://doi.org/10.1016/j.stem.2023.05.004>

- Humphries, B., Wang, Z., Yang, C., 2019. MicroRNA Regulation of Epigenetic Modifiers in Breast Cancer. *Cancers* 11, 897. <https://doi.org/10.3390/cancers11070897>
- Hutnick, L.K., Huang, X., Loo, T.-C., Ma, Z., Fan, G., 2010. Repression of Retrotransposal Elements in Mouse Embryonic Stem Cells Is Primarily Mediated by a DNA Methylation-independent Mechanism. *Journal of Biological Chemistry* 285, 21082–21091. <https://doi.org/10.1074/jbc.M110.125674>
- International Human Genome Sequencing Consortium, Whitehead Institute for Biomedical Research, Center for Genome Research:, Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczy, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, Christina, Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., The Sanger Centre:, Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Washington University Genome Sequencing Center, Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., US DOE Joint Genome Institute:, Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.-F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Baylor College of Medicine Human Genome Sequencing Center:, Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., RIKEN Genomic Sciences Center:, Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Genoscope and CNRS UMR-8030:, Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Department of Genome Analysis, Institute of Molecular Biotechnology:, Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., GTC Sequencing Center:, Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Beijing Genomics Institute/Human Genome Center:, Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Multimegabase Sequencing Center, The Institute for Systems Biology:, Hood, L., Rowen, L., Madan, A., Qin, S., Stanford Genome Technology Center:, Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., University of Oklahoma's Advanced Center for Genome Technology:, Roe, B.A., Chen, F., Pan, H., Max Planck Institute for Molecular Genetics:, Ramser, J., Lehrach, H., Reinhardt, R., Cold Spring Harbor Laboratory, Lita Annenberg Hazen Genome Center:, McCombie, W.R., De La Bastide, M., Dedhia, N., GBF—German Research Centre for Biotechnology:, Blöcker, H., Hornischer, K., Nordsiek, G., *Genome Analysis Group (listed in alphabetical order, also includes individuals listed under other headings):, Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.-C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G.R., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kasprzyk, A., Kennedy, S., Kent, W.J., Kitts, P.,

Koonin, E.V., Korf, I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F.A., Stupka, E., Szustakowki, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.-P., Yeh, R.-F., Scientific management: National Human Genome Research Institute, US National Institutes of Health:; Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Stanford Human Genome Center:; Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., University of Washington Genome Center:; Olson, M.V., Kaul, R., Raymond, Christopher, Department of Molecular Biology, Keio University School of Medicine:; Shimizu, N., Kawasaki, K., Minoshima, S., University of Texas Southwestern Medical Center at Dallas:; Evans, G.A., Athanasiou, M., Schultz, R., Office of Science, US Department of Energy:; Patrinos, A., The Wellcome Trust:; Morgan, M.J., 2001a. Initial sequencing and analysis of the human genome. *Nature* 409, 860–921.

<https://doi.org/10.1038/35057062>

International Human Genome Sequencing Consortium, Whitehead Institute for Biomedical Research, Center for Genome Research:; Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczy, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, Christina, Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., The Sanger Centre:; Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Washington University Genome Sequencing Center, Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., US DOE Joint Genome Institute:; Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.-F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Baylor College of Medicine Human Genome Sequencing Center:; Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., RIKEN Genomic Sciences Center:; Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Genoscope and CNRS UMR-8030:; Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Department of Genome Analysis, Institute of Molecular Biotechnology:; Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., GTC Sequencing Center:; Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Beijing Genomics Institute/Human Genome Center:; Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Multimegabase Sequencing Center, The Institute for Systems Biology:; Hood, L., Rowen, L., Madan, A., Qin, S., Stanford Genome Technology Center:; Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., University of Oklahoma's Advanced Center for Genome Technology:; Roe, B.A., Chen, F., Pan, H., Max Planck Institute for Molecular Genetics:; Ramser, J., Lehrach, H., Reinhardt, R., Cold Spring

- Harbor Laboratory, Lita Annenberg Hazen Genome Center:, McCombie, W.R., De La Bastide, M., Dedhia, N., GBF—German Research Centre for Biotechnology:, Blöcker, H., Hornischer, K., Nordsiek, G., *Genome Analysis Group (listed in alphabetical order, also includes individuals listed under other headings):, Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.-C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G.R., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kasprzyk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E.V., Korf, I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F.A., Stupka, E., Szustakowki, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.-P., Yeh, R.-F., Scientific management: National Human Genome Research Institute, US National Institutes of Health:, Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Stanford Human Genome Center:, Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., University of Washington Genome Center:, Olson, M.V., Kaul, R., Raymond, Christopher, Department of Molecular Biology, Keio University School of Medicine:, Shimizu, N., Kawasaki, K., Minoshima, S., University of Texas Southwestern Medical Center at Dallas:, Evans, G.A., Athanasiou, M., Schultz, R., Office of Science, US Department of Energy:, Patrinos, A., The Wellcome Trust:, Morgan, M.J., 2001b. Initial sequencing and analysis of the human genome. *Nature* 409, 860–921. <https://doi.org/10.1038/35057062>
- Iorio, M.V., Piovan, C., Croce, C.M., 2010. Interplay between microRNAs and the epigenetic machinery: An intricate network. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* 1799, 694–701. <https://doi.org/10.1016/j.bbagr.2010.05.005>
- Iscove, N.N., Nawa, K., 1997. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. *Current Biology* 7, 805–808. [https://doi.org/10.1016/S0960-9822\(06\)00341-1](https://doi.org/10.1016/S0960-9822(06)00341-1)
- Ito, K., Suda, T., 2014. Metabolic requirements for the maintenance of self-renewing stem cells. *Nat Rev Mol Cell Biol* 15, 243–256. <https://doi.org/10.1038/nrm3772>
- Ito, Kyoko, Lee, J., Chrysanthou, S., Zhao, Y., Josephs, K., Sato, H., Teruya-Feldstein, J., Zheng, D., Dawlaty, M.M., Ito, Keisuke, 2019. Non-catalytic Roles of Tet2 Are Essential to Regulate Hematopoietic Stem and Progenitor Cell Homeostasis. *Cell Reports* 28, 2480-2490.e4. <https://doi.org/10.1016/j.celrep.2019.07.094>
- Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C., Zhang, Y., 2011. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science* 333, 1300–1303. <https://doi.org/10.1126/science.1210597>
- Ivanov, A.V., Peng, H., Yurchenko, V., Yap, K.L., Negorev, D.G., Schultz, D.C., Psulkowski, E., Fredericks, W.J., White, D.E., Maul, G.G., Sadofsky, M.J., Zhou, M.-M., Rauscher, F.J., 2007. PHD Domain-Mediated E3 Ligase Activity Directs Intramolecular Sumoylation of an Adjacent Bromodomain Required for Gene Silencing. *Molecular Cell* 28, 823–837. <https://doi.org/10.1016/j.molcel.2007.11.012>
- Izzo, F., Lee, S.C., Poran, A., Chaligine, R., Gaiti, F., Gross, B., Murali, R.R., Deochand, S.D., Ang, C., Jones, P.W., Nam, A.S., Kim, K.-T., Kothen-Hill, S., Schulman, R.C., Ki, M., Lhoumaud, P., Skok, J.A., Viny, A.D., Levine, R.L., Kenigsberg, E., Abdel-Wahab, O., Landau, D.A., 2020. DNA methylation disruption reshapes the

- hematopoietic differentiation landscape. *Nat Genet* 52, 378–387.
<https://doi.org/10.1038/s41588-020-0595-4>
- Jacobs, F.M.J., Greenberg, D., Nguyen, N., Haeussler, M., Ewing, A.D., Katzman, S., Paten, B., Salama, S.R., Haussler, D., 2014. An evolutionary arms race between KRAB zinc-finger genes ZNF91/93 and SVA/L1 retrotransposons. *Nature* 516, 242–245.
<https://doi.org/10.1038/nature13760>
- Jaffe, R., 2008. WHO classification of tumours of haematopoietic and lymphoid tissues. *World Health Organization Classification of Tumours* 358–360.
- Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G., Lindsley, R.C., Mermel, C.H., Burt, N., Chavez, A., Higgins, J.M., Moltchanov, V., Kuo, F.C., Kluk, M.J., Henderson, B., Kinnunen, L., Koistinen, H.A., Ladenvall, C., Getz, G., Correa, A., Banahan, B.F., Gabriel, S., Kathiresan, S., Stringham, H.M., McCarthy, M.I., Boehnke, M., Tuomilehto, J., Haiman, C., Groop, L., Atzmon, G., Wilson, J.G., Neuberg, D., Altshuler, D., Ebert, B.L., 2014. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *N Engl J Med* 371, 2488–2498.
<https://doi.org/10.1056/NEJMoa1408617>
- Jaiswal, S., Natarajan, P., Silver, A.J., Gibson, C.J., Bick, A.G., Shvartz, E., McConkey, M., Gupta, N., Gabriel, S., Ardissino, D., Baber, U., Mehran, R., Fuster, V., Danesh, J., Frossard, P., Saleheen, D., Melander, O., Sukhova, G.K., Neuberg, D., Libby, P., Kathiresan, S., Ebert, B.L., 2017. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med* 377, 111–121.
<https://doi.org/10.1056/NEJMoa1701719>
- Jakobsen, N.A., Turkalj, S., Zeng, A.G.X., Stoilova, B., Metzner, M., Rahmig, S., Nagree, M.S., Shah, S., Moore, R., Usukhbayar, B., Angulo Salazar, M., Gafencu, G.-A., Kennedy, A., Newman, S., Kendrick, B.J.L., Taylor, A.H., Afinowi-Luitz, R., Gundle, R., Watkins, B., Wheway, K., Beazley, D., Murison, A., Aguilar-Navarro, A.G., Flores-Figueroa, E., Dakin, S.G., Carr, A.J., Nerlov, C., Dick, J.E., Xie, S.Z., Vyas, P., 2024a. Selective advantage of mutant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. *Cell Stem Cell* 31, 1127–1144.e17. <https://doi.org/10.1016/j.stem.2024.05.010>
- Jakobsen, N.A., Turkalj, S., Zeng, A.G.X., Stoilova, B., Metzner, M., Rahmig, S., Nagree, M.S., Shah, S., Moore, R., Usukhbayar, B., Angulo Salazar, M., Gafencu, G.-A., Kennedy, A., Newman, S., Kendrick, B.J.L., Taylor, A.H., Afinowi-Luitz, R., Gundle, R., Watkins, B., Wheway, K., Beazley, D., Murison, A., Aguilar-Navarro, A.G., Flores-Figueroa, E., Dakin, S.G., Carr, A.J., Nerlov, C., Dick, J.E., Xie, S.Z., Vyas, P., 2024b. Selective advantage of mutant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. *Cell Stem Cell* S1934590924002078. <https://doi.org/10.1016/j.stem.2024.05.010>
- Jamaladdin, S., Kelly, R.D.W., O'Regan, L., Dovey, O.M., Hodson, G.E., Millard, C.J., Portolano, N., Fry, A.M., Schwabe, J.W.R., Cowley, S.M., 2014. Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9840–9845.
<https://doi.org/10.1073/pnas.1321330111>
- Jang, H.S., Shah, N.M., Du, A.Y., Dailey, Z.Z., Pehrsson, E.C., Godoy, P.M., Zhang, D., Li, D., Xing, X., Kim, S., O'Donnell, D., Gordon, J.I., Wang, T., 2019. Transposable elements drive widespread expression of oncogenes in human cancers. *Nat Genet* 51, 611–617. <https://doi.org/10.1038/s41588-019-0373-3>
- Jang, Y.-Y., Sharkis, S.J., 2007. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood* 110, 3056–3063. <https://doi.org/10.1182/blood-2007-05-087759>

- Janssen, A., Colmenares, S.U., Karpen, G.H., 2018. Heterochromatin: Guardian of the Genome. *Annu. Rev. Cell Dev. Biol.* 34, 265–288. <https://doi.org/10.1146/annurev-cellbio-100617-062653>
- Jenuwein, T., Allis, C.D., 2001. Translating the Histone Code. *Science* 293, 1074–1080. <https://doi.org/10.1126/science.1063127>
- Jeon, J., Kwon, S., Lee, Y.-H., 2014. Histone Acetylation in Fungal Pathogens of Plants. *The Plant Pathology Journal* 30, 1–9. <https://doi.org/10.5423/PPJ.RW.01.2014.0003>
- Jin, Y., Tam, O.H., Paniagua, E., Hammell, M., 2015. TEtranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. *Bioinformatics* 31, 3593–3599. <https://doi.org/10.1093/bioinformatics/btv422>
- Jo, C., Park, S., Oh, S. et al. Histone acylation marks respond to metabolic perturbations and enable cellular adaptation. *Exp Mol Med* 52, 2005–2019 (2020). <https://doi.org/10.1038/s12276-020-00539-x>
- Johnson JL, Georgakilas G, Petrovic J, Kurachi M, Cai S, Harly C, Pear WS, Bhandoola A, Wherry EJ, Vahedi G. Lineage-Determining Transcription Factor TCF-1 Initiates the Epigenetic Identity of T Cells. *Immunity*. 2018 Feb 20;48(2):243-257.e10. doi: 10.1016/j.immuni.2018.01.012.
- Johnson, K.M., 2002. Aging and developmental transitions in the B cell lineage. *International Immunology* 14, 1313–1323. <https://doi.org/10.1093/intimm/dxf092>
- Jones, B.C., Wood, J.G., Chang, C., Tam, A.D., Franklin, M.J., Siegel, E.R., Helfand, S.L., 2016. A somatic piRNA pathway in the Drosophila fat body ensures metabolic homeostasis and normal lifespan. *Nat Commun* 7, 13856. <https://doi.org/10.1038/ncomms13856>
- Jordan, I.K., Rogozin, I.B., Glazko, G.V., Koonin, E.V., 2003. Origin of a substantial fraction of human regulatory sequences from transposable elements. *Trends in Genetics* 19, 68–72. [https://doi.org/10.1016/S0168-9525\(02\)00006-9](https://doi.org/10.1016/S0168-9525(02)00006-9)
- Jordan, K., Pontoppidan, P., Uhlving, H.H., Kielsen, K., Burrin, D.G., Weischendorff, S., Christensen, I.J., Jørgensen, M.H., Heilmann, C., Sengeløv, H., Müller, K., 2017. Gastrointestinal Toxicity, Systemic Inflammation, and Liver Biochemistry in Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 23, 1170–1176. <https://doi.org/10.1016/j.bbmt.2017.03.021>
- Ju, Z., Jiang, H., Jaworski, M., Rathinam, C., Gompf, A., Klein, C., Trumpp, A., Rudolph, K.L., 2007. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat Med* 13, 742–747. <https://doi.org/10.1038/nm1578>
- Kabi, M., Filion, G.J., 2021. Heterochromatin: did H3K9 methylation evolve to tame transposons? *Genome Biol* 22, 325, s13059-021-02550–5. <https://doi.org/10.1186/s13059-021-02550-5>
- Kajikawa, M., Okada, N., 2002. LINEs Mobilize SINEs in the Eel through a Shared 3' Sequence. *Cell* 111, 433–444. [https://doi.org/10.1016/S0092-8674\(02\)01041-3](https://doi.org/10.1016/S0092-8674(02)01041-3)
- Kar, S.P., Quiros, P.M., Gu, M., Jiang, T., Mitchell, J., Langdon, R., Iyer, V., Barcena, C., Vijayabaskar, M.S., Fabre, M.A., Carter, P., Petrovski, S., Burgess, S., Vassiliou, G.S., 2022. Genome-wide analyses of 200,453 individuals yield new insights into the causes and consequences of clonal hematopoiesis. *Nat Genet* 54, 1155–1166. <https://doi.org/10.1038/s41588-022-01121-z>
- Karimi, M.M., Goyal, P., Maksakova, I.A., Bilenky, M., Leung, D., Tang, J.X., Shinkai, Y., Mager, D.L., Jones, S., Hirst, M., Lorincz, M.C., 2011. DNA Methylation and SETDB1/H3K9me3 Regulate Predominantly Distinct Sets of Genes, Retroelements, and Chimeric Transcripts in mESCs. *Cell Stem Cell* 8, 676–687. <https://doi.org/10.1016/j.stem.2011.04.004>

- Kataoka, K., Nagata, Y., Kitanaka, A., Shiraishi, Y., Shimamura, T., Yasunaga, J., Totoki, Y., Chiba, K., Sato-Otsubo, A., Nagae, G., Ishii, R., Muto, S., Kotani, S., Watatani, Y., Takeda, J., Sanada, M., Tanaka, H., Suzuki, H., Sato, Y., Shiozawa, Y., Yoshizato, T., Yoshida, K., Makishima, H., Iwanaga, M., Ma, G., Nosaka, K., Hishizawa, M., Itonaga, H., Imaizumi, Y., Munakata, W., Ogasawara, H., Sato, T., Sasai, K., Muramoto, K., Penova, M., Kawaguchi, T., Nakamura, H., Hama, N., Shide, K., Kubuki, Y., Hidaka, T., Kameda, T., Nakamaki, T., Ishiyama, K., Miyawaki, S., Yoon, S.-S., Tobinai, K., Miyazaki, Y., Takaori-Kondo, A., Matsuda, F., Takeuchi, K., Nureki, O., Aburatani, H., Watanabe, T., Shibata, T., Matsuoka, M., Miyano, S., Shimoda, K., Ogawa, S., 2015. Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat Genet* 47, 1304–1315. <https://doi.org/10.1038/ng.3415>
- Katayama, Y., Battista, M., Kao, W.-M., Hidalgo, A., Peired, A.J., Thomas, S.A., Frenette, P.S., 2006. Signals from the Sympathetic Nervous System Regulate Hematopoietic Stem Cell Egress from Bone Marrow. *Cell* 124, 407–421. <https://doi.org/10.1016/j.cell.2005.10.041>
- Kebede AF, Nieborak A, Shahidian LZ, Le Gras S, Richter F, Gómez DA, Baltissen MP, Meszaros G, Magliarelli HF, Taudt A, Margueron R, Colomé-Tatché M, Ricci R, Daujat S, Vermeulen M, Mittler G, Schneider R. Histone propionylation is a mark of active chromatin. *Nat Struct Mol Biol.* 2017 Dec;24(12):1048-1056. doi: 10.1038/nsmb.
- Keenan, C.R., Iannarella, N., Naselli, G., Bediaga, N.G., Johanson, T.M., Harrison, L.C., Allan, R.S., 2020. Extreme disruption of heterochromatin is required for accelerated hematopoietic aging. *Blood* 135, 2049–2058. <https://doi.org/10.1182/blood.2019002990>
- Keighley, L.M., Lynch-Sutherland, C.F., Almomani, S.N., Eccles, M.R., Macaulay, E.C., 2023. Unveiling the hidden players: The crucial role of transposable elements in the placenta and their potential contribution to pre-eclampsia. *Placenta* 141, 57–64. <https://doi.org/10.1016/j.placenta.2023.05.017>
- Kelsey, M.M.G., 2021. Reconsidering LINE-1's role in cancer: does LINE-1 function as a reporter detecting early cancer-associated epigenetic signatures? *Evolution, Medicine, and Public Health* 9, 78–82. <https://doi.org/10.1093/emph/eoab004>
- Khorasanizadeh, S., 2004. The Nucleosome. *Cell* 116, 259–272. [https://doi.org/10.1016/S0092-8674\(04\)00044-3](https://doi.org/10.1016/S0092-8674(04)00044-3)
- Kim, P.G., Niroula, A., Shkolnik, V., McConkey, M., Lin, A.E., Ślabicki, M., Kemp, J.P., Bick, A., Gibson, C.J., Griffin, G., Sekar, A., Brooks, D.J., Wong, W.J., Cohen, D.N., Uddin, M.M., Shin, W.J., Pirruccello, J., Tsai, J.M., Agrawal, M., Kiel, D.P., Bouxsein, M.L., Richards, J.B., Evans, D.M., Wein, M.N., Charles, J.F., Jaiswal, S., Natarajan, P., Ebert, B.L., 2021. *Dnmt3a* -mutated clonal hematopoiesis promotes osteoporosis. *Journal of Experimental Medicine* 218, e20211872. <https://doi.org/10.1084/jem.20211872>
- Klenov, M.S., Sokolova, O.A., Yakushev, E.Y., Stolyarenko, A.D., Mikhaleva, E.A., Lavrov, S.A., Gvozdev, V.A., 2011. Separation of stem cell maintenance and transposon silencing functions of Piwi protein. *Proc. Natl. Acad. Sci. U.S.A.* 108, 18760–18765. <https://doi.org/10.1073/pnas.1106676108>
- Ko, M., An, J., Bandukwala, H.S., Chavez, L., Äijö, T., Pastor, W.A., Segal, M.F., Li, H., Koh, K.P., Lähdesmäki, H., Hogan, P.G., Aravind, L., Rao, A., 2013. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. *Nature* 497, 122–126. <https://doi.org/10.1038/nature12052>
- Ko, M., Bandukwala, H.S., An, J., Lamperti, E.D., Thompson, E.C., Hastie, R., Tsangaratou, A., Rajewsky, K., Koralov, S.B., Rao, A., 2011. Ten-Eleven-Translocation 2 (TET2)

- negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14566–14571.
<https://doi.org/10.1073/pnas.1112317108>
- Koide, S., Oshima, M., Takubo, K., Yamazaki, S., Nitta, E., Saraya, A., Aoyama, K., Kato, Y., Miyagi, S., Nakajima-Takagi, Y., Chiba, T., Matsui, H., Arai, F., Suzuki, Y., Kimura, H., Nakauchi, H., Suda, T., Shinkai, Y., Iwama, A., 2016. Setdb1 maintains hematopoietic stem and progenitor cells by restricting the ectopic activation of nonhematopoietic genes. *Blood* 128, 638–649. <https://doi.org/10.1182/blood-2016-01-694810>
- Kondo, M., Weissman, I.L., Akashi, K., 1997. Identification of Clonogenic Common Lymphoid Progenitors in Mouse Bone Marrow. *Cell* 91, 661–672.
[https://doi.org/10.1016/S0092-8674\(00\)80453-5](https://doi.org/10.1016/S0092-8674(00)80453-5)
- Konkel, M.K., Batzer, M.A., 2010. A mobile threat to genome stability: The impact of non-LTR retrotransposons upon the human genome. *Seminars in Cancer Biology* 20, 211–221. <https://doi.org/10.1016/j.semcancer.2010.03.001>
- Kosan, C., Godmann, M., 2016. Genetic and Epigenetic Mechanisms That Maintain Hematopoietic Stem Cell Function. *Stem Cells International* 2016, 1–14.
<https://doi.org/10.1155/2016/5178965>
- Kosmider, O., Gelsi-Boyer, V., Cheok, M., Grabar, S., Della-Valle, V., Picard, F., Viguié, F., Quesnel, B., Beyne-Rauzy, O., Solary, E., Vey, N., Hunault-Berger, M., Fenaux, P., Mansat-De Mas, V., Delabesse, E., Guardiola, P., Lacombe, C., Vainchenker, W., Preudhomme, C., Dreyfus, F., Bernard, O.A., Birnbaum, D., Fontenay, M., on behalf of the Groupe Francophone des Myélodysplasies, 2009. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). *Blood* 114, 3285–3291. <https://doi.org/10.1182/blood-2009-04-215814>
- Krevvata, M., Silva, B.C., Manavalan, J.S., Galan-Diez, M., Kode, A., Matthews, B.G., Park, D., Zhang, C.A., Galili, N., Nickolas, T.L., Dempster, D.W., Dougall, W., Teruya-Feldstein, J., Economides, A.N., Kalajzic, I., Raza, A., Berman, E., Mukherjee, S., Bhagat, G., Kousteni, S., 2014. Inhibition of leukemia cell engraftment and disease progression in mice by osteoblasts. *Blood* 124, 2834–2846.
<https://doi.org/10.1182/blood-2013-07-517219>
- Krisl, J.C., Doan, V.P., 2017. Chemotherapy and Transplantation: The Role of Immunosuppression in Malignancy and a Review of Antineoplastic Agents in Solid Organ Transplant Recipients. *American Journal of Transplantation* 17, 1974–1991.
<https://doi.org/10.1111/ajt.14238>
- Kuo, H.-K., Bean, J.F., Yen, C.-J., Leveille, S.G., 2006. Linking C-Reactive Protein to Late-Life Disability in the National Health and Nutrition Examination Survey (NHANES) 1999-2002. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 61, 380–387. <https://doi.org/10.1093/gerona/61.4.380>
- Langlois, T., Da Costa Reis Monte-Mor, B., Lenglet, G., Droin, N., Marty, C., Le Couédic, J.-P., Almiré, C., Auger, N., Mercher, T., Delhommeau, F., Christensen, J., Helin, K., Debili, N., Fuks, F., Bernard, O.A., Solary, E., Vainchenker, W., Plo, I., 2014. TET2 Deficiency Inhibits Mesoderm and Hematopoietic Differentiation in Human Embryonic Stem Cells. *Stem Cells* 32, 2084–2097. <https://doi.org/10.1002/stem.1718>
- Lara-Astiaso, D., Weiner, A., Lorenzo-Vivas, E., Zaretzky, I., Jaitin, D.A., David, E., Keren-Shaul, H., Mildner, A., Winter, D., Jung, S., Friedman, N., Amit, I., 2014. Chromatin state dynamics during blood formation. *Science* 345, 943–949.
<https://doi.org/10.1126/science.1256271>

- Latchney, S.E., Calvi, L.M., 2017. The aging hematopoietic stem cell niche: Phenotypic and functional changes and mechanisms that contribute to hematopoietic aging. *Seminars in Hematology* 54, 25–32. <https://doi.org/10.1053/j.seminhematol.2016.10.001>
- Laurenti, E., Göttgens, B., 2018. From haematopoietic stem cells to complex differentiation landscapes. *Nature* 553, 418–426. <https://doi.org/10.1038/nature25022>
- Lee, D.Y., Hayes, J.J., Pruss, D., Wolffe, A.P., 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* 72, 73–84. [https://doi.org/10.1016/0092-8674\(93\)90051-Q](https://doi.org/10.1016/0092-8674(93)90051-Q)
- Lefkopoulos, S., Polyzou, A., Derecka, M., Bergo, V., Clapes, T., Cauchy, P., Jerez-Longres, C., Onishi-Seebacher, M., Yin, N., Martagon-Calderón, N.-A., Potts, K.S., Klaeylé, L., Liu, F., Bowman, T.V., Jenuwein, T., Mione, M.C., Trompouki, E., 2020. Repetitive Elements Trigger RIG-I-like Receptor Signaling that Regulates the Emergence of Hematopoietic Stem and Progenitor Cells. *Immunity* 53, 934–951.e9. <https://doi.org/10.1016/j.immuni.2020.10.007>
- Lemonnier, F., Couronné, L., Parrens, M., Jaïs, J.-P., Travert, M., Lamant, L., Tournillac, O., Rousset, T., Fabiani, B., Cairns, R.A., Mak, T., Bastard, C., Bernard, O.A., De Leval, L., Gaulard, P., 2012. Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. *Blood* 120, 1466–1469. <https://doi.org/10.1182/blood-2012-02-408542>
- Leonardi, G.C., Accardi, G., Monastero, R., Nicoletti, F., Libra, M., 2018. Ageing: from inflammation to cancer. *Immun Ageing* 15, 1. <https://doi.org/10.1186/s12979-017-0112-5>
- Levy, M.Z., Allsopp, R.C., Futcher, A.B., Greider, C.W., Harley, C.B., 1992. Telomere end-replication problem and cell aging. *Journal of Molecular Biology* 225, 951–960. [https://doi.org/10.1016/0022-2836\(92\)90096-3](https://doi.org/10.1016/0022-2836(92)90096-3)
- Li, Z., Cai, X., Cai, C.-L., Wang, J., Zhang, W., Petersen, B.E., Yang, F.-C., Xu, M., 2011. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood* 118, 4509–4518. <https://doi.org/10.1182/blood-2010-12-325241>
- Li, Z., Kono, H., 2016. Distinct Roles of Histone H3 and H2A Tails in Nucleosome Stability. *Sci Rep* 6, 31437. <https://doi.org/10.1038/srep31437>
- Liang, Y., Van Zant, G., Szilvassy, S.J., 2005. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood* 106, 1479–1487. <https://doi.org/10.1182/blood-2004-11-4282>
- Liao, M., Chen, R., Yang, Y., He, H., Xu, L., Jiang, Y., Guo, Z., He, W., Jiang, H., Wang, J., 2022. Aging-elevated inflammation promotes DNMT3A R878H-driven clonal hematopoiesis. *Acta Pharmaceutica Sinica B* 12, 678–691. <https://doi.org/10.1016/j.apsb.2021.09.015>
- Lienert, F., Wirbelauer, C., Som, I., Dean, A., Mohn, F., Schübeler, D., 2011. Identification of genetic elements that autonomously determine DNA methylation states. *Nat Genet* 43, 1091–1097. <https://doi.org/10.1038/ng.946>
- Liu, A., Wang, Y., Ding, Y., Baez, I., Payne, K.J., Borghesi, L., 2015. Cutting Edge: Hematopoietic Stem Cell Expansion and Common Lymphoid Progenitor Depletion Require Hematopoietic-Derived, Cell-Autonomous TLR4 in a Model of Chronic Endotoxin. *J Immunol* 195, 2524–2528. <https://doi.org/10.4049/jimmunol.1501231>
- Liu, R., Wu, J., Guo, H., Yao, W., Li, S., Lu, Y., Jia, Y., Liang, X., Tang, J., Zhang, H., 2023. Post-translational modifications of histones: Mechanisms, biological functions, and therapeutic targets. *MedComm* 4, e292. <https://doi.org/10.1002/mco2.292>

- Lobo, N.A., Shimono, Y., Qian, D., Clarke, M.F., 2007. The Biology of Cancer Stem Cells. *Annu. Rev. Cell Dev. Biol.* 23, 675–699. <https://doi.org/10.1146/annurev.cellbio.22.010305.104154>
- Lohr, J.G., Stojanov, P., Lawrence, M.S., Auclair, D., Chapuy, B., Sougnez, C., Cruz-Gordillo, P., Knoechel, B., Asmann, Y.W., Slager, S.L., Novak, A.J., Dogan, A., Ansell, S.M., Link, B.K., Zou, L., Gould, J., Saksena, G., Stransky, N., Rangel-Escareño, C., Fernandez-Lopez, J.C., Hidalgo-Miranda, A., Melendez-Zajgla, J., Hernández-Lemus, E., Schwarz-Cruz Y Celis, A., Imaz-Rosshandler, I., Ojesina, A.I., Jung, J., Peadamallu, C.S., Lander, E.S., Habermann, T.M., Cerhan, J.R., Shipp, M.A., Getz, G., Golub, T.R., 2012. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3879–3884. <https://doi.org/10.1073/pnas.1121343109>
- López-Moyado, I.F., Tsagaratou, A., Yuita, H., Seo, H., Delatte, B., Heinz, S., Benner, C., Rao, A., 2019. Paradoxical association of TET loss of function with genome-wide DNA hypomethylation. *Proc. Natl. Acad. Sci. U.S.A.* 116, 16933–16942. <https://doi.org/10.1073/pnas.1903059116>
- Lorsbach, R.B., Moore, J., Mathew, S., Raimondi, S.C., Mukatira, S.T., Downing, J.R., 2003. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). *Leukemia* 17, 637–641. <https://doi.org/10.1038/sj.leu.2402834>
- Lou, C., Goodier, J.L., Qiang, R., 2020. A potential new mechanism for pregnancy loss: considering the role of LINE-1 retrotransposons in early spontaneous miscarriage. *Reprod Biol Endocrinol* 18, 6. <https://doi.org/10.1186/s12958-020-0564-x>
- Loyola, A., Tagami, H., Bonaldi, T., Roche, D., Quivy, J.P., Imhof, A., Nakatani, Y., Dent, S.Y.R., Almouzni, G., 2009. The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep* 10, 769–775. <https://doi.org/10.1038/embor.2009.90>
- Luan, D.D., Eickbush, T.H., 1995. RNA Template Requirements for Target DNA-Primed Reverse Transcription by the R2 Retrotransposable Element. *Molecular and Cellular Biology* 15, 3882–3891. <https://doi.org/10.1128/MCB.15.7.3882>
- Luan, D.D., Korman, M.H., Jakubczak, J.L., Eickbush, T.H., 1993. Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: A mechanism for non-LTR retrotransposition. *Cell* 72, 595–605. [https://doi.org/10.1016/0092-8674\(93\)90078-5](https://doi.org/10.1016/0092-8674(93)90078-5)
- Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., Richmond, T.J., 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251–260. <https://doi.org/10.1038/38444>
- Lund, E., Güttinger, S., Calado, A., Dahlberg, J.E., Kutay, U., 2004. Nuclear Export of MicroRNA Precursors. *Science* 303, 95–98. <https://doi.org/10.1126/science.1090599>
- Lupatov, A.Yu., Yarygin, K.N., 2022. Telomeres and Telomerase in the Control of Stem Cells. *Biomedicines* 10, 2335. <https://doi.org/10.3390/biomedicines10102335>
- Lux, C.T., Yoshimoto, M., McGrath, K., Conway, S.J., Palis, J., Yoder, M.C., 2008. All primitive and definitive hematopoietic progenitor cells emerging before E10 in the mouse embryo are products of the yolk sac. *Blood* 111, 3435–3438. <https://doi.org/10.1182/blood-2007-08-107086>
- Macaulay, E.C., Weeks, R.J., Andrews, S., Morison, I.M., 2011. Hypomethylation of functional retrotransposon-derived genes in the human placenta. *Mamm Genome* 22, 722–735. <https://doi.org/10.1007/s00335-011-9355-1>
- Maeshima, K., Rogge, R., Tamura, S., Joti, Y., Hikima, T., Szerlong, H., Krause, C., Herman, J., Seidel, E., DeLuca, J., Ishikawa, T., Hansen, J.C., 2016. Nucleosomal arrays self-

- assemble into supramolecular globular structures lacking 30-nm fibers. *The EMBO Journal* 35, 1115–1132. <https://doi.org/10.15252/embj.201592660>
- Manz, M.G., Boettcher, S., 2014. Emergency granulopoiesis. *Nat Rev Immunol* 14, 302–314. <https://doi.org/10.1038/nri3660>
- Marnell, C.S., Bick, A., Natarajan, P., 2021. Clonal hematopoiesis of indeterminate potential (CHIP): Linking somatic mutations, hematopoiesis, chronic inflammation and cardiovascular disease. *Journal of Molecular and Cellular Cardiology* 161, 98–105. <https://doi.org/10.1016/j.yjmcc.2021.07.004>
- Mas-Peiro, S., Hoffmann, J., Fichtlscherer, S., Dorsheimer, L., Rieger, M.A., Dimmeler, S., Vasa-Nicotera, M., Zeiher, A.M., 2020. Clonal haematopoiesis in patients with degenerative aortic valve stenosis undergoing transcatheter aortic valve implantation. *European Heart Journal* 41, 933–939. <https://doi.org/10.1093/eurheartj/ehz591>
- Matsui, T., Leung, D., Miyashita, H., Maksakova, I.A., Miyachi, H., Kimura, H., Tachibana, M., Lorincz, M.C., Shinkai, Y., 2010. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. *Nature* 464, 927–931. <https://doi.org/10.1038/nature08858>
- McClatchy, J., Strogantsev, R., Wolfe, E., Lin, H.Y., Mohammadhosseini, M., Davis, B.A., Eden, C., Goldman, D., Fleming, W.H., Conley, P., Wu, G., Cimmino, L., Mohammed, H., Agarwal, A., 2023. Clonal hematopoiesis related TET2 loss-of-function impedes IL1 β -mediated epigenetic reprogramming in hematopoietic stem and progenitor cells. *Nat Commun* 14, 8102. <https://doi.org/10.1038/s41467-023-43697-y>
- McCulloch, E.A., Till, J.E., 1962. The Sensitivity of Cells from Normal Mouse Bone Marrow to Gamma Radiation in Vitro and in Vivo. *Radiation Research* 16, 822. <https://doi.org/10.2307/3571281>
- McNiece, I., Harrington, J., Turney, J., Kellner, J., Shpall, E.J., 2004. Ex vivo expansion of cord blood mononuclear cells on mesenchymal stem cells. *Cytotherapy* 6, 311–317. <https://doi.org/10.1080/14653240410004871>
- Meisel, M., Hinterleitner, R., Pacis, A., Chen, L., Earley, Z.M., Mayassi, T., Pierre, J.F., Ernest, J.D., Galipeau, H.J., Thuille, N., Bouziat, R., Buscarlet, M., Ringus, D.L., Wang, Y., Li, Y., Dinh, V., Kim, S.M., McDonald, B.D., Zurenski, M.A., Musch, M.W., Furtado, G.C., Lira, S.A., Baier, G., Chang, E.B., Eren, A.M., Weber, C.R., Busque, L., Godley, L.A., Verdú, E.F., Barreiro, L.B., Jabri, B., 2018. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature* 557, 580–584. <https://doi.org/10.1038/s41586-018-0125-z>
- Méndez-Ferrer, S., Lucas, D., Battista, M., Frenette, P.S., 2008. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature* 452, 442–447. <https://doi.org/10.1038/nature06685>
- Midic, D., Rinke, J., Perner, F., Müller, V., Hinze, A., Pester, F., Landschulze, J., Ernst, J., Gruhn, B., Matziolis, G., Heidel, F.H., Hochhaus, A., Ernst, T., 2020. Prevalence and dynamics of clonal hematopoiesis caused by leukemia-associated mutations in elderly individuals without hematologic disorders. *Leukemia* 34, 2198–2205. <https://doi.org/10.1038/s41375-020-0869-y>
- Miller, A.H., Raison, C.L., 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol* 16, 22–34. <https://doi.org/10.1038/nri.2015.5>
- Minor, E.A., Court, B.L., Young, J.I., Wang, G., 2013. Ascorbate Induces Ten-Eleven Translocation (Tet) Methylcytosine Dioxygenase-mediated Generation of 5-Hydroxymethylcytosine. *Journal of Biological Chemistry* 288, 13669–13674. <https://doi.org/10.1074/jbc.C113.464800>

- Mita, P., Boeke, J.D., 2016. How retrotransposons shape genome regulation. *Curr Opin Genet Dev* 37, 90–100. <https://doi.org/10.1016/j.gde.2016.01.001>
- Mizrokhi, L.J., Georgieva, S.G., Ilyin, Y.V., 1988. jockey, a mobile drosophila element similar to mammalian LINEs, is transcribed from the internal promoter by RNA polymerase II. *Cell* 54, 685–691. [https://doi.org/10.1016/S0092-8674\(88\)80013-8](https://doi.org/10.1016/S0092-8674(88)80013-8)
- Mohrin, M., Bourke, E., Alexander, D., Warr, M.R., Barry-Holson, K., Le Beau, M.M., Morrison, C.G., Passegué, E., 2010. Hematopoietic Stem Cell Quiescence Promotes Error-Prone DNA Repair and Mutagenesis. *Cell Stem Cell* 7, 174–185. <https://doi.org/10.1016/j.stem.2010.06.014>
- Molaro, A., Falciatori, I., Hodges, E., Aravin, A.A., Marran, K., Rafii, S., McCombie, W.R., Smith, A.D., Hannon, G.J., 2014. Two waves of de novo methylation during mouse germ cell development. *Genes Dev.* 28, 1544–1549. <https://doi.org/10.1101/gad.244350.114>
- Molaro, A., Malik, H.S., 2016. Hide and seek: how chromatin-based pathways silence retroelements in the mammalian germline. *Current Opinion in Genetics & Development* 37, 51–58. <https://doi.org/10.1016/j.gde.2015.12.001>
- Monroe, J.G., Dorshkind, K., 2007. Fate Decisions Regulating Bone Marrow and Peripheral B Lymphocyte Development, in: *Advances in Immunology*. Elsevier, pp. 1–50. [https://doi.org/10.1016/S0065-2776\(07\)95001-4](https://doi.org/10.1016/S0065-2776(07)95001-4)
- Moorlag, S.J.C.F.M., Matzaraki, V., Van Puffelen, J.H., Van Der Heijden, C., Keating, S., Groh, L., Röring, R.J., Bakker, O.B., Mourits, V.P., Koeken, V.A.C.M., De Bree, L.C.J., Smeekens, S.P., Oosting, M., Gamboa, R.A., Riksen, N.P., Xavier, R.J., Wijmenga, C., Kumar, V., Van Crevel, R., Novakovic, B., Joosten, L.A.B., Li, Y., Netea, M.G., 2022. An integrative genomics approach identifies KDM4 as a modulator of trained immunity. *Eur J Immunol* 52, 431–446. <https://doi.org/10.1002/eji.202149577>
- Moran-Crusio, K., Reavie, L., Shih, A., Abdel-Wahab, O., Ndiaye-Lobry, D., Lobry, C., Figueroa, M.E., Vasanthakumar, A., Patel, J., Zhao, X., Perna, F., Pandey, S., Madzo, J., Song, C., Dai, Q., He, C., Ibrahim, S., Beran, M., Zavadil, J., Nimer, S.D., Melnick, A., Godley, L.A., Aifantis, I., Levine, R.L., 2011. Tet2 Loss Leads to Increased Hematopoietic Stem Cell Self-Renewal and Myeloid Transformation. *Cancer Cell* 20, 11–24. <https://doi.org/10.1016/j.ccr.2011.06.001>
- Morin, R.D., Mendez-Lago, M., Mungall, A.J., Goya, R., Mungall, K.L., Corbett, R.D., Johnson, N.A., Severson, T.M., Chiu, R., Field, M., Jackman, S., Krzywinski, M., Scott, D.W., Trinh, D.L., Tamura-Wells, J., Li, S., Firme, M.R., Rogic, S., Griffith, M., Chan, S., Yakovenko, O., Meyer, I.M., Zhao, E.Y., Smailus, D., Moksa, M., Chittaranjan, S., Rimsza, L., Brooks-Wilson, A., Spinelli, J.J., Ben-Neriah, S., Meissner, B., Woolcock, B., Boyle, M., McDonald, H., Tam, A., Zhao, Y., Delaney, A., Zeng, T., Tse, K., Butterfield, Y., Birol, I., Holt, R., Schein, J., Horsman, D.E., Moore, R., Jones, S.J.M., Connors, J.M., Hirst, M., Gascoyne, R.D., Marra, M.A., 2011. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature* 476, 298–303. <https://doi.org/10.1038/nature10351>
- Morrison, S.J., Uchida, N., Weissman, I.L., 1995. The Biology of Hematopoietic Stem Cells. *Annu. Rev. Cell Dev. Biol.* 11, 35–71. <https://doi.org/10.1146/annurev.cb.11.110195.000343>
- Mourits, V.P., Van Puffelen, J.H., Novakovic, B., Bruno, M., Ferreira, A.V., Arts, R.J., Groh, L., Crişan, T.O., Zwaag, J., Jenthoe, E., Kox, M., Pickkers, P., Van De Veerdonk, F.L., Weis, S., Oosterwijk, E., Vermeulen, S.H., Netea, M.G., Joosten, L.A., 2021. Lysine methyltransferase G9a is an important modulator of trained immunity. *Clin & Trans Imm* 10, e1253. <https://doi.org/10.1002/cti2.1253>

- Muench, M.O., Bárcena, A., 2004. Stem Cell Transplantation in the Fetus. *Cancer Control* 11, 105–118. <https://doi.org/10.1177/107327480401100217>
- Murray, B.E., 1990. The life and times of the Enterococcus. *Clin Microbiol Rev* 3, 46–65. <https://doi.org/10.1128/CMR.3.1.46>
- Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., Kishimoto, T., 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 382, 635–638. <https://doi.org/10.1038/382635a0>
- Naka, K., Muraguchi, T., Hoshii, T., Hirao, A., 2008. Regulation of Reactive Oxygen Species and Genomic Stability in Hematopoietic Stem Cells. *Antioxidants & Redox Signaling* 10, 1883–1894. <https://doi.org/10.1089/ars.2008.2114>
- Naveiras, O., Nardi, V., Wenzel, P.L., Hauschka, P.V., Fahey, F., Daley, G.Q., 2009. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature* 460, 259–263. <https://doi.org/10.1038/nature08099>
- Neri, F., Incarnato, D., Krepelova, A., Rapelli, S., Anselmi, F., Parlato, C., Medana, C., Dal Bello, F., Oliviero, S., 2015. Single-Base Resolution Analysis of 5-Formyl and 5-Carboxyl Cytosine Reveals Promoter DNA Methylation Dynamics. *Cell Reports* 10, 674–683. <https://doi.org/10.1016/j.celrep.2015.01.008>
- Nichols, J., Silva, J., Roode, M., Smith, A., 2009. Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. *Development* 136, 3215–3222. <https://doi.org/10.1242/dev.038893>
- Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Schöler H, Smith A. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*. 1998 Oct 30;95(3):379-91. doi: 10.1016/s0092-8674(00)81769-9.
- Nikitin, A.G., Woodruff, R.C., 1995. Somatic movement of the manner transposable element and lifespan of *Drosophila* species. *Mutation Research/DNAging* 338, 43–49. [https://doi.org/10.1016/0921-8734\(95\)00010-4](https://doi.org/10.1016/0921-8734(95)00010-4)
- Nishiyama, T., Ishikawa, Y., Kawashima, N., Akashi, A., Adachi, Y., Hattori, H., Ushijima, Y., Kiyoi, H., 2018. Mutation analysis of therapy-related myeloid neoplasms. *Cancer Genetics* 222–223, 38–45. <https://doi.org/10.1016/j.cancergen.2018.02.006>
- Norton, V.G., Imai, B.S., Yau, P., Bradbury, E.M., 1989. Histone acetylation reduces nucleosome core particle linking number change. *Cell* 57, 449–457. [https://doi.org/10.1016/0092-8674\(89\)90920-3](https://doi.org/10.1016/0092-8674(89)90920-3)
- Nowoshilow, S., Schloissnig, S., Fei, J.-F., Dahl, A., Pang, A.W.C., Pippel, M., Winkler, S., Hastie, A.R., Young, G., Roscito, J.G., Falcon, F., Knapp, D., Powell, S., Cruz, A., Cao, H., Habermann, B., Hiller, M., Tanaka, E.M., Myers, E.W., 2018. The axolotl genome and the evolution of key tissue formation regulators. *Nature* 554, 50–55. <https://doi.org/10.1038/nature25458>
- Nystedt, B., Street, N.R., Wetterbom, A., Zuccolo, A., Lin, Y.-C., Scofield, D.G., Vezzi, F., Delhomme, N., Giacomello, S., Alexeyenko, A., Vicedomini, R., Sahlin, K., Sherwood, E., Elfstrand, M., Gramzow, L., Holmberg, K., Hällman, J., Keech, O., Klasson, L., Koriabine, M., Kucukoglu, M., Käller, M., Luthman, J., Lysholm, F., Niittylä, T., Olson, Å., Rilakovic, N., Ritland, C., Rosselló, J.A., Sena, J., Svensson, T., Talavera-López, C., Theißen, G., Tuominen, H., Vanneste, K., Wu, Z.-Q., Zhang, B., Zerbe, P., Arvestad, L., Bhalerao, R., Bohlmann, J., Bousquet, J., Garcia Gil, R., Hvidsten, T.R., De Jong, P., MacKay, J., Morgante, M., Ritland, K., Sundberg, B., Lee Thompson, S., Van De Peer, Y., Andersson, B., Nilsson, O., Ingvarsson, P.K., Lundeberg, J., Jansson, S., 2013. The Norway spruce genome sequence and conifer genome evolution. *Nature* 497, 579–584. <https://doi.org/10.1038/nature12211>

- Okano, M., Bell, D.W., Haber, D.A., Li, E., 1999. DNA Methyltransferases Dnmt3a and Dnmt3b Are Essential for De Novo Methylation and Mammalian Development. *Cell* 99, 247–257. [https://doi.org/10.1016/S0092-8674\(00\)81656-6](https://doi.org/10.1016/S0092-8674(00)81656-6)
- Ono, R., Taki, T., Taketani, T., Taniwaki, M., Kobayashi, H., Hayashi, Y., 2002. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11)(q22;q23). *Cancer Res* 62, 4075–4080.
- Orford, K.W., Scadden, D.T., 2008. Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. *Nat Rev Genet* 9, 115–128. <https://doi.org/10.1038/nrg2269>
- Orkin, S.H., Zon, L.I., 2008. Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. *Cell* 132, 631–644. <https://doi.org/10.1016/j.cell.2008.01.025>
- Orr, W.C., 2016. Tightening the connection between transposable element mobilization and aging. *Proc. Natl. Acad. Sci. U.S.A.* 113, 11069–11070. <https://doi.org/10.1073/pnas.1613350113>
- Ostrander, E.L., Kramer, A.C., Mallaney, C., Celik, H., Koh, W.K., Fairchild, J., Haussler, E., Zhang, C.R.C., Challen, G.A., 2020. Divergent Effects of Dnmt3a and Tet2 Mutations on Hematopoietic Progenitor Cell Fitness. *Stem Cell Reports* 14, 551–560. <https://doi.org/10.1016/j.stemcr.2020.02.011>
- Panaud, O., 2016. Horizontal transfers of transposable elements in eukaryotes: The flying genes. *Comptes Rendus. Biologies* 339, 296–299. <https://doi.org/10.1016/j.crvi.2016.04.013>
- Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D.J., Schrier, S.L., Weissman, I.L., 2011. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20012–20017. <https://doi.org/10.1073/pnas.1116110108>
- Pardee, A.B., 1974. A Restriction Point for Control of Normal Animal Cell Proliferation. *Proc. Natl. Acad. Sci. U.S.A.* 71, 1286–1290. <https://doi.org/10.1073/pnas.71.4.1286>
- Park, Y., Gerson, S.L., 2005. DNA Repair Defects in Stem Cell Function and Aging. *Annu. Rev. Med.* 56, 495–508. <https://doi.org/10.1146/annurev.med.56.082103.104546>
- Parmentier, R., Racine, L., Moussy, A., Chantalat, S., Sudharshan, R., Papili Gao, N., Stockholm, D., Corre, G., Fourel, G., Deleuze, J.-F., Gunawan, R., Paldi, A., 2022. Global genome decompaction leads to stochastic activation of gene expression as a first step toward fate commitment in human hematopoietic cells. *PLoS Biol* 20, e3001849. <https://doi.org/10.1371/journal.pbio.3001849>
- Patnaik, M.M., 2022. How I diagnose and treat chronic myelomonocytic leukemia. *Haematologica* 107, 1503–1517. <https://doi.org/10.3324/haematol.2021.279500>
- Pei, W., Feyerabend, T.B., Rössler, J., Wang, X., Postrach, D., Busch, K., Rode, I., Klapproth, K., Dietlein, N., Quedenau, C., Chen, W., Sauer, S., Wolf, S., Höfer, T., Rodewald, H.-R., 2017. Polylox barcoding reveals haematopoietic stem cell fates realized in vivo. *Nature* 548, 456–460. <https://doi.org/10.1038/nature23653>
- Pelinski, Y., Hidaoui, D., Stolz, A., Hermetet, F., Chelbi, R., Diop, M.K., Chioukh, A.M., Porteu, F., Elvira-Matelot, E., 2022a. NF-κB signaling controls H3K9me3 levels at intronic LINE-1 and hematopoietic stem cell genes in cis. *Journal of Experimental Medicine* 219, e20211356. <https://doi.org/10.1084/jem.20211356>
- Pelinski, Y., Hidaoui, D., Stolz, A., Hermetet, F., Chelbi, R., Diop, M.K., Chioukh, A.M., Porteu, F., Elvira-Matelot, E., 2022b. NF-κB signaling controls H3K9me3 levels at intronic LINE-1 and hematopoietic stem cell genes in cis. *Journal of Experimental Medicine* 219, e20211356. <https://doi.org/c>
- Percharde, M., Lin, C.-J., Yin, Y., Guan, J., Peixoto, G.A., Bulut-Karslioglu, A., Biechele, S., Huang, B., Shen, X., Ramalho-Santos, M., 2018. A LINE1-Nucleolin Partnership

- Regulates Early Development and ESC Identity. *Cell* 174, 391-405.e19. <https://doi.org/10.1016/j.cell.2018.05.043>
- Petrie, H.T., Kincade, P.W., 2005. Many roads, one destination for T cell progenitors. *The Journal of Experimental Medicine* 202, 11–13. <https://doi.org/10.1084/jem.20050990>
- Pezic, D., Manakov, S.A., Sachidanandam, R., Aravin, A.A., 2014. piRNA pathway targets active LINE1 elements to establish the repressive H3K9me3 mark in germ cells. *Genes Dev* 28, 1410–1428. <https://doi.org/10.1101/gad.240895.114>
- Pham, D., Yu, Q., Walline, C.C., Muthukrishnan, R., Blum, J.S., Kaplan, M.H., 2013. Opposing Roles of STAT4 and Dnmt3a in Th1 Gene Regulation. *The Journal of Immunology* 191, 902–911. <https://doi.org/10.4049/jimmunol.1203229>
- Pietras, E.M., Reynaud, D., Kang, Y.-A., Carlin, D., Calero-Nieto, F.J., Leavitt, A.D., Stuart, J.M., Göttgens, B., Passegué, E., 2015. Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. *Cell Stem Cell* 17, 35–46. <https://doi.org/10.1016/j.stem.2015.05.003>
- Plath, K., Fang, J., Mlynarczyk-Evans, S.K., Cao, R., Worringer, K.A., Wang, H., De La Cruz, C.C., Otte, A.P., Panning, B., Zhang, Y., 2003. Role of Histone H3 Lysine 27 Methylation in X Inactivation. *Science* 300, 131–135. <https://doi.org/10.1126/science.1084274>
- Pronier, E., Imanci, A., Selimoglu-Buet, D., Badaoui, B., Itzykson, R., Roger, T., Jego, C., Naimo, A., Francillette, M., Breckler, M., Wagner-Ballon, O., Figueroa, M.E., Aglave, M., Gautheret, D., Porteu, F., Bernard, O.A., Vainchenker, W., Delhommeau, F., Solary, E., Droin, N.M., 2022. Macrophage migration inhibitory factor is overproduced through EGR1 in TET2^{low} resting monocytes. *Commun Biol* 5, 110. <https://doi.org/10.1038/s42003-022-03057-w>
- Quivoron, C., Couronné, L., Della Valle, V., Lopez, C.K., Plo, I., Wagner-Ballon, O., Do Cruzeiro, M., Delhommeau, F., Arnulf, B., Stern, M.-H., Godley, L., Opolon, P., Tilly, H., Solary, E., Duffourd, Y., Dessen, P., Merle-Beral, H., Nguyen-Khac, F., Fontenay, M., Vainchenker, W., Bastard, C., Mercher, T., Bernard, O.A., 2011. TET2 Inactivation Results in Pleiotropic Hematopoietic Abnormalities in Mouse and Is a Recurrent Event during Human Lymphomagenesis. *Cancer Cell* 20, 25–38. <https://doi.org/10.1016/j.ccr.2011.06.003>
- Rasmussen, K.D., Jia, G., Johansen, J.V., Pedersen, M.T., Rapin, N., Bagger, F.O., Porse, B.T., Bernard, O.A., Christensen, J., Helin, K., 2015. Loss of *TET2* in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. *Genes Dev.* 29, 910–922. <https://doi.org/10.1101/gad.260174.115>
- Rea, S., Eisenhaber, F., O’Carroll, D., Strahl, B.D., Sun, Z.-W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., Jenuwein, T., 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593–599. <https://doi.org/10.1038/35020506>
- Richardson, S.R., Doucet, A.J., Kopera, H.C., Moldovan, J.B., Garcia-Perez, J.L., Moran, J.V., 2015. The Influence of LINE-1 and SINE Retrotransposons on Mammalian Genomes. *Microbiol Spectr* 3, 3.2.15. <https://doi.org/10.1128/microbiolspec.MDNA3-0061-2014>
- Rinn, J.L., Kertesz, M., Wang, J.K., Squazzo, S.L., Xu, X., Bruggmann, S.A., Goodnough, L.H., Helms, J.A., Farnham, P.J., Segal, E., Chang, H.Y., 2007. Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs. *Cell* 129, 1311–1323. <https://doi.org/10.1016/j.cell.2007.05.022>
- Robin, C., Bollerot, K., Mendes, S., Haak, E., Crisan, M., Cerisoli, F., Lauw, I., Kaimakis, P., Jorna, R., Vermeulen, M., Kayser, M., Van Der Linden, R., Imanirad, P., Verstegen,

- M., Nawaz-Yousaf, H., Papazian, N., Steegers, E., Cupedo, T., Dzierzak, E., 2009. Human Placenta Is a Potent Hematopoietic Niche Containing Hematopoietic Stem and Progenitor Cells throughout Development. *Cell Stem Cell* 5, 385–395. <https://doi.org/10.1016/j.stem.2009.08.020>
- Rodrigues, C.P., Shvedunova, M., Akhtar, A., 2021. Epigenetic Regulators as the Gatekeepers of Hematopoiesis. *Trends in Genetics* 37, 125–142. <https://doi.org/10.1016/j.tig.2020.09.015>
- Rodriguez-Fraticelli, A.E., Wolock, S.L., Weinreb, C.S., Panero, R., Patel, S.H., Jankovic, M., Sun, J., Calogero, R.A., Klein, A.M., Camargo, F.D., 2018. Clonal analysis of lineage fate in native haematopoiesis. *Nature* 553, 212–216. <https://doi.org/10.1038/nature25168>
- Rodriguez-Meira, A., Norfo, R., Wen, S., Chédeville, A.L., Rahman, H., O’Sullivan, J., Wang, G., Louka, E., Kretzschmar, W.W., Paterson, A., Brierley, C., Martin, J.-E., Demeule, C., Bashton, M., Sousos, N., Moralli, D., Subha Meem, L., Carrelha, J., Wu, B., Hamblin, A., Guermouche, H., Pasquier, F., Marzac, C., Girodon, F., Vainchenker, W., Drummond, M., Harrison, C., Chapman, J.R., Plo, I., Jacobsen, S.E.W., Psaila, B., Thongjuea, S., Antony-Debré, I., Mead, A.J., 2023. Single-cell multi-omics identifies chronic inflammation as a driver of TP53-mutant leukemic evolution. *Nat Genet* 55, 1531–1541. <https://doi.org/10.1038/s41588-023-01480-1>
- Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., Bonner, W.M., 1998. DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139. *Journal of Biological Chemistry* 273, 5858–5868. <https://doi.org/10.1074/jbc.273.10.5858>
- Rommel, P.C., Oliveira, T.Y., Nussenzweig, M.C., Robbiani, D.F., 2017. RAG1/2 induces genomic insertions by mobilizing DNA into RAG1/2-independent breaks. *Journal of Experimental Medicine* 214, 815–831. <https://doi.org/10.1084/jem.20161638>
- Rommelfanger, M.K., MacLean, A.L., 2021. A single-cell resolved cell-cell communication model explains lineage commitment in hematopoiesis. *Development* 148, dev199779. <https://doi.org/10.1242/dev.199779>
- Rossetto, D., Truman, A.W., Kron, S.J., Côté, J., 2010. Epigenetic modifications in double-strand break DNA damage signaling and repair. *Clin Cancer Res* 16, 4543–4552. <https://doi.org/10.1158/1078-0432.CCR-10-0513>
- Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., Weissman, I.L., 2007. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729. <https://doi.org/10.1038/nature05862>
- Rossi, D.J., Bryder, D., Zahn, J.M., Ahlenius, H., Sonu, R., Wagers, A.J., Weissman, I.L., 2005. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9194–9199. <https://doi.org/10.1073/pnas.0503280102>
- Roulois, D., Loo Yau, H., Singhania, R., Wang, Y., Danesh, A., Shen, S.Y., Han, H., Liang, G., Jones, P.A., Pugh, T.J., O’Brien, C., De Carvalho, D.D., 2015. DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. *Cell* 162, 961–973. <https://doi.org/10.1016/j.cell.2015.07.056>
- Ruparelia, N., Chai, J.T., Fisher, E.A., Choudhury, R.P., 2017. Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nat Rev Cardiol* 14, 133–144. <https://doi.org/10.1038/nrcardio.2016.185>
- Saha, A., Wittmeyer, J., Cairns, B.R., 2006. Chromatin remodelling: the industrial revolution of DNA around histones. *Nat Rev Mol Cell Biol* 7, 437–447. <https://doi.org/10.1038/nrm1945>

- Sanada, F., Taniyama, Y., Muratsu, J., Otsu, R., Shimizu, H., Rakugi, H., Morishita, R., 2018. Source of Chronic Inflammation in Aging. *Front. Cardiovasc. Med.* 5, 12. <https://doi.org/10.3389/fcvm.2018.00012>
- SanMiguel, J.M., Eudy, E., Loberg, M.A., Young, K.A., Mistry, J.J., Mujica, K.D., Schwartz, L.S., Stearns, T.M., Challen, G.A., Trowbridge, J.J., 2022. Distinct Tumor Necrosis Factor Alpha Receptors Dictate Stem Cell Fitness versus Lineage Output in *Dnmt3a* - Mutant Clonal Hematopoiesis. *Cancer Discovery* 12, 2763–2773. <https://doi.org/10.1158/2159-8290.CD-22-0086>
- Sano, S., Oshima, K., Wang, Y., Katanasaka, Y., Sano, M., Walsh, K., 2018. CRISPR-Mediated Gene Editing to Assess the Roles of Tet2 and Dnmt3a in Clonal Hematopoiesis and Cardiovascular Disease. *Circulation Research* 123, 335–341. <https://doi.org/10.1161/CIRCRESAHA.118.313225>
- Säwen, P., Eldeeb, M., Erlandsson, E., Kristiansen, T.A., Laterza, C., Kokaia, Z., Karlsson, G., Yuan, J., Soneji, S., Mandal, P.K., Rossi, D.J., Bryder, D., 2018. Murine HSCs contribute actively to native hematopoiesis but with reduced differentiation capacity upon aging. *eLife* 7, e41258. <https://doi.org/10.7554/eLife.41258>
- Schaack, S., Gilbert, C., Feschotte, C., 2010. Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. *Trends in Ecology & Evolution* 25, 537–546. <https://doi.org/10.1016/j.tree.2010.06.001>
- Schatz, D.G., Oettinger, M.A., Baltimore, D., 1989. The V(D)J recombination activating gene, RAG-1. *Cell* 59, 1035–1048. [https://doi.org/10.1016/0092-8674\(89\)90760-5](https://doi.org/10.1016/0092-8674(89)90760-5)
- Scheller, M., Ludwig, A.K., Göllner, S., Rohde, C., Krämer, S., Stäble, S., Janssen, M., Müller, J.-A., He, L., Bäumer, N., Arnold, C., Gerß, J., Schönung, M., Thiede, C., Niederwieser, C., Niederwieser, D., Serve, H., Berdel, W.E., Thiem, U., Hemmerling, I., Leuschner, F., Plass, C., Schlesner, M., Zaugg, J., Milsom, M.D., Trumpp, A., Pabst, C., Lipka, D.B., Müller-Tidow, C., 2021. Hotspot DNMT3A mutations in clonal hematopoiesis and acute myeloid leukemia sensitize cells to azacytidine via viral mimicry response. *Nat Cancer* 2, 527–544. <https://doi.org/10.1038/s43018-021-00213-9>
- Scherer, M., Singh, I., Braun, M., Szu-Tu, C., Kardorff, M., Rühle, J., Frömel, R., Beneyto-Calabuig, S., Raffel, S., Rodriguez-Fraticelli, A., Velten, L., 2024. Somatic epimutations enable single-cell lineage tracing in native hematopoiesis across the murine and human lifespan. <https://doi.org/10.1101/2024.04.01.587514>
- Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., Rauscher, F.J., 2002. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev.* 16, 919–932. <https://doi.org/10.1101/gad.973302>
- Scott, E.C., Gardner, E.J., Masood, A., Chuang, N.T., Vertino, P.M., Devine, S.E., 2016. A hot L1 retrotransposon evades somatic repression and initiates human colorectal cancer. *Genome Res.* 26, 745–755. <https://doi.org/10.1101/gr.201814.115>
- Sedivy, J.M., Banumathy, G., Adams, P.D., 2008. Aging by epigenetics—A consequence of chromatin damage? *Experimental Cell Research* 314, 1909–1917. <https://doi.org/10.1016/j.yexcr.2008.02.023>
- Shi, Yujiang, Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., Casero, R.A., Shi, Yang, 2004. Histone Demethylation Mediated by the Nuclear Amine Oxidase Homolog LSD1. *Cell* 119, 941–953. <https://doi.org/10.1016/j.cell.2004.12.012>
- Shih, A.H., Chung, S.S., Dolezal, E.K., Zhang, S.-J., Abdel-Wahab, O.I., Park, C.Y., Nimer, S.D., Levine, R.L., Klimek, V.M., 2013. Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia. *Haematologica* 98, 908–912. <https://doi.org/10.3324/haematol.2012.076729>

- Shimada, M., Chen, W.-Y., Nakadai, T., Onikubo, T., Guermah, M., Rhodes, D., Roeder, R.G., 2019. Gene-Specific H1 Eviction through a Transcriptional Activator→p300→NAP1→H1 Pathway. *Molecular Cell* 74, 268-283.e5. <https://doi.org/10.1016/j.molcel.2019.02.016>
- Shimoda, K., Shide, K., Kameda, T., Hidaka, T., Kubuki, Y., Kamiunten, A., Sekine, M., Akizuki, K., Shimoda, H., Yamaji, T., Nakamura, K., Abe, H., Miike, T., Iwakiri, H., Tahara, Y., Sueta, M., Yamamoto, S., Hasuike, S., Nagata, K., Kitanaka, A., 2015. TET2 Mutation in Adult T-Cell Leukemia/Lymphoma. *JCEH* 55, 145–149. <https://doi.org/10.3960/jslrt.55.145>
- Shukla, R., Upton, K.R., Muñoz-Lopez, M., Gerhardt, D.J., Fisher, M.E., Nguyen, T., Brennan, P.M., Baillie, J.K., Collino, A., Ghisletti, S., Sinha, S., Iannelli, F., Radaelli, E., Dos Santos, A., Rapoud, D., Guettier, C., Samuel, D., Natoli, G., Carninci, P., Ciccarelli, F.D., Garcia-Perez, J.L., Faivre, J., Faulkner, G.J., 2013. Endogenous Retrotransposition Activates Oncogenic Pathways in Hepatocellular Carcinoma. *Cell* 153, 101–111. <https://doi.org/10.1016/j.cell.2013.02.032>
- Shumaker, D.K., Dechat, T., Kohlmaier, A., Adam, S.A., Bozovsky, M.R., Erdos, M.R., Eriksson, M., Goldman, A.E., Khuon, S., Collins, F.S., Jenuwein, T., Goldman, R.D., 2006. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8703–8708. <https://doi.org/10.1073/pnas.0602569103>
- Shuryak, I., Sachs, R.K., Brenner, D.J., 2010. Cancer Risks After Radiation Exposure in Middle Age. *JNCI Journal of the National Cancer Institute* 102, 1628–1636. <https://doi.org/10.1093/jnci/djq346>
- Singh, T., Newman, A.B., 2011. Inflammatory markers in population studies of aging. *Ageing Research Reviews* 10, 319–329. <https://doi.org/10.1016/j.arr.2010.11.002>
- Skiniotis, G., Moazed, D., Walz, T., 2007. Acetylated Histone Tail Peptides Induce Structural Rearrangements in the RSC Chromatin Remodeling Complex. *Journal of Biological Chemistry* 282, 20804–20808. <https://doi.org/10.1074/jbc.C700081200>
- Smith, Z.D., Meissner, A., 2013. DNA methylation: roles in mammalian development. *Nat Rev Genet* 14, 204–220. <https://doi.org/10.1038/nrg3354>
- Sobczak, K., Krzyzosiak, W.J., 2002. Structural Determinants of BRCA1 Translational Regulation. *Journal of Biological Chemistry* 277, 17349–17358. <https://doi.org/10.1074/jbc.M109162200>
- Solary, E., Bernard, O.A., Tefferi, A., Fuks, F., Vainchenker, W., 2014. The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. *Leukemia* 28, 485–496. <https://doi.org/10.1038/leu.2013.337>
- Sommerkamp, P., Romero-Mulero, M.C., Narr, A., Ladel, L., Hustin, L., Schönberger, K., Renders, S., Altamura, S., Zeisberger, P., Jäcklein, K., Klimmeck, D., Rodriguez-Fraticelli, A., Camargo, F.D., Perié, L., Trumpp, A., Cabezas-Wallscheid, N., 2021. Mouse multipotent progenitor 5 cells are located at the interphase between hematopoietic stem and progenitor cells. *Blood* 137, 3218–3224. <https://doi.org/10.1182/blood.2020007876>
- Song, S.J., Ito, K., Ala, U., Kats, L., Webster, K., Sun, S.M., Jongen-Lavrencic, M., Manova-Todorova, K., Teruya-Feldstein, J., Avigan, D.E., Delwel, R., Pandolfi, P.P., 2013. The Oncogenic MicroRNA miR-22 Targets the TET2 Tumor Suppressor to Promote Hematopoietic Stem Cell Self-Renewal and Transformation. *Cell Stem Cell* 13, 87–101. <https://doi.org/10.1016/j.stem.2013.06.003>
- Sookdeo, A., Hepp, C.M., McClure, M.A., Boissinot, S., 2013. Revisiting the evolution of mouse LINE-1 in the genomic era. *Mob DNA* 4, 3. <https://doi.org/10.1186/1759-8753-4-3>

- Soufi, A., Donahue, G., Zaret, K.S., 2012. Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell* 151, 994–1004. <https://doi.org/10.1016/j.cell.2012.09.045>
- Speck, N.A., 2022. A Pernicious Cycle Affecting Premalignant Stem Cells. *N Engl J Med* 386, 596–598. <https://doi.org/10.1056/NEJMcibr2117528>
- Spinelli, L., Carpentier, S., Montañana Sanchis, F., Dalod, M., Vu Manh, T.-P., 2015. BubbleGUM: automatic extraction of phenotype molecular signatures and comprehensive visualization of multiple Gene Set Enrichment Analyses. *BMC Genomics* 16, 814. <https://doi.org/10.1186/s12864-015-2012-4>
- St. Laurent, G., Hammell, N., McCaffrey, T.A., 2010. A LINE-1 component to human aging: Do LINE elements exact a longevity cost for evolutionary advantage? *Mechanisms of Ageing and Development* 131, 299–305. <https://doi.org/10.1016/j.mad.2010.03.008>
- Stahl, E.C., Brown, B.N., 2015. Cell Therapy Strategies to Combat Immunosenescence. *Organogenesis* 11, 159–172. <https://doi.org/10.1080/15476278.2015.1120046>
- Steensma, D.P., Bejar, R., Jaiswal, S., Lindsley, R.C., Sekeres, M.A., Hasserjian, R.P., Ebert, B.L., 2015. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* 126, 9–16. <https://doi.org/10.1182/blood-2015-03-631747>
- Stolz, P., Mantero, A.S., Tvardovskiy, A., Ugur, E., Wange, L.E., Mulholland, C.B., Cheng, Y., Wierer, M., Enard, W., Schneider, R., Bartke, T., Leonhardt, H., Elsässer, S.J., Bultmann, S., 2022. TET1 regulates gene expression and repression of endogenous retroviruses independent of DNA demethylation. *Nucleic Acids Research* 50, 8491–8511. <https://doi.org/10.1093/nar/gkac642>
- Suganuma, T., Workman, J.L., 2011. Signals and Combinatorial Functions of Histone Modifications. *Annu. Rev. Biochem.* 80, 473–499. <https://doi.org/10.1146/annurev-biochem-061809-175347>
- Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., Wang, H., Le, T., Faull, K.F., Chen, R., Gu, H., Bock, C., Meissner, A., Göttgens, B., Darlington, G.J., Li, W., Goodell, M.A., 2014. Epigenomic Profiling of Young and Aged HSCs Reveals Concerted Changes during Aging that Reinforce Self-Renewal. *Cell Stem Cell* 14, 673–688. <https://doi.org/10.1016/j.stem.2014.03.002>
- Sun, J., Ramos, A., Chapman, B., Johnnidis, J.B., Le, L., Ho, Y.-J., Klein, A., Hofmann, O., Camargo, F.D., 2014. Clonal dynamics of native haematopoiesis. *Nature* 514, 322–327. <https://doi.org/10.1038/nature13824>
- Sundaram, V., Wang, T., 2018. Transposable Element Mediated Innovation in Gene Regulatory Landscapes of Cells: Re-Visiting the “Gene-Battery” Model. *BioEssays* 40, 1700155. <https://doi.org/10.1002/bies.201700155>
- Svobodová Kovaříková, A., Legartová, S., Krejčí, J., Bártová, E., 2018. H3K9me3 and H4K20me3 represent the epigenetic landscape for 53BP1 binding to DNA lesions. *Aging (Albany NY)* 10, 2585–2605. <https://doi.org/10.18632/aging.101572>
- Tachibana, M., Sugimoto, K., Fukushima, T., Shinkai, Y., 2001. SET Domain-containing Protein, G9a, Is a Novel Lysine-preferring Mammalian Histone Methyltransferase with Hyperactivity and Specific Selectivity to Lysines 9 and 27 of Histone H3. *Journal of Biological Chemistry* 276, 25309–25317. <https://doi.org/10.1074/jbc.M101914200>
- Tadokoro, Y., Ema, H., Okano, M., Li, E., Nakauchi, H., 2007. De novo DNA methyltransferase is essential for self-renewal, but not for differentiation, in hematopoietic stem cells. *The Journal of Experimental Medicine* 204, 715–722. <https://doi.org/10.1084/jem.20060750>

- Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., Rao, A., 2009. Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1. *Science* 324, 930–935. <https://doi.org/10.1126/science.1170116>
- Taichman, R.S., Emerson, S.G., 1998. The Role of Osteoblasts in the Hematopoietic Microenvironment. *STEM CELLS* 16, 7–15. <https://doi.org/10.1002/stem.160007>
- Taiwo, O., Wilson, G.A., Emmett, W., Morris, T., Bonnet, D., Schuster, E., Adejumo, T., Beck, S., Pearce, D.J., 2013. DNA methylation analysis of murine hematopoietic side population cells during aging. *Epigenetics* 8, 1114–1122. <https://doi.org/10.4161/epi.26017>
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug 25;126(4):663-76. doi: 10.1016/j.cell.2006.07.024. Epub 2006 Aug 10. PMID: 16904174.
- Takahashi, K., Wang, F., Kantarjian, H., Doss, D., Khanna, K., Thompson, E., Zhao, L., Patel, K., Neelapu, S., Gumbs, C., Bueso-Ramos, C., DiNardo, C.D., Colla, S., Ravandi, F., Zhang, J., Huang, X., Wu, X., Samaniego, F., Garcia-Manero, G., Futreal, P.A., 2017a. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *The Lancet Oncology* 18, 100–111. [https://doi.org/10.1016/S1470-2045\(16\)30626-X](https://doi.org/10.1016/S1470-2045(16)30626-X)
- Takahashi, K., Wang, F., Kantarjian, H., Doss, D., Khanna, K., Thompson, E., Zhao, L., Patel, K., Neelapu, S., Gumbs, C., Bueso-Ramos, C., DiNardo, C.D., Colla, S., Ravandi, F., Zhang, J., Huang, X., Wu, X., Samaniego, F., Garcia-Manero, G., Futreal, P.A., 2017b. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *The Lancet Oncology* 18, 100–111. [https://doi.org/10.1016/S1470-2045\(16\)30626-X](https://doi.org/10.1016/S1470-2045(16)30626-X)
- Tavian, M., Robin, C., Coulombel, L., Péault, B., 2001. The Human Embryo, but Not Its Yolk Sac, Generates Lympho-Myeloid Stem Cells. *Immunity* 15, 487–495. [https://doi.org/10.1016/S1074-7613\(01\)00193-5](https://doi.org/10.1016/S1074-7613(01)00193-5)
- Tchasovnikarova, I.A., Timms, R.T., Matheson, N.J., Wals, K., Antrobus, R., Göttgens, B., Dougan, G., Dawson, M.A., Lehner, P.J., 2015. Epigenetic silencing by the HUSH complex mediates position-effect variegation in human cells. *Science* 348, 1481–1485. <https://doi.org/10.1126/science.aaa7227>
- Tefferi, A., Pardanani, A., Lim, K.-H., Abdel-Wahab, O., Lasho, T.L., Patel, J., Gangat, N., Finke, C.M., Schwager, S., Mullally, A., Li, C.-Y., Hanson, C.A., Mesa, R., Bernard, O., Delhommeau, F., Vainchenker, W., Gilliland, D.G., Levine, R.L., 2009. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. *Leukemia* 23, 905–911. <https://doi.org/10.1038/leu.2009.47>
- Tenaillon, M.I., Hufford, M.B., Gaut, B.S., Ross-Ibarra, J., 2011. Genome Size and Transposable Element Content as Determined by High-Throughput Sequencing in Maize and *Zea luxurians*. *Genome Biology and Evolution* 3, 219–229. <https://doi.org/10.1093/gbe/evr008>
- Tian, R., Wiley, B., Liu, J., Zong, X., Truong, B., Zhao, S., Uddin, M.M., Niroula, A., Miller, C.A., Mukherjee, S., Heiden, B.T., Luo, J., Puri, V., Kozower, B.D., Walter, M.J., Ding, L., Link, D.C., Amos, C.I., Ebert, B.L., Govindan, R., Natarajan, P., Bolton, K.L., Cao, Y., 2023. Clonal Hematopoiesis and Risk of Incident Lung Cancer. *JCO* 41, 1423–1433. <https://doi.org/10.1200/JCO.22.00857>
- Till, J.E., McCULLOCH, E.A., 1961. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14, 213–222.

- Till, J.E., McCulloch, E.A., Siminovitch, L., 1964. A STOCHASTIC MODEL OF STEM CELL PROLIFERATION, BASED ON THE GROWTH OF SPLEEN COLONY-FORMING CELLS. *Proc. Natl. Acad. Sci. U.S.A.* 51, 29–36. <https://doi.org/10.1073/pnas.51.1.29>
- Trieschmann, L., Martin, B., Bustin, M., 1998. The chromatin unfolding domain of chromosomal protein HMG-14 targets the N-terminal tail of histone H3 in nucleosomes. *Proc. Natl. Acad. Sci. U.S.A.* 95, 5468–5473. <https://doi.org/10.1073/pnas.95.10.5468>
- Tse, C., Hansen, J.C., 1997. Hybrid Trypsinized Nucleosomal Arrays: Identification of Multiple Functional Roles of the H2A/H2B and H3/H4 N-Termini in Chromatin Fiber Compaction. *Biochemistry* 36, 11381–11388. <https://doi.org/10.1021/bi970801n>
- Tse, C., Sera, T., Wolffe, A.P., Hansen, J.C., 1998. Disruption of Higher-Order Folding by Core Histone Acetylation Dramatically Enhances Transcription of Nucleosomal Arrays by RNA Polymerase III. *Molecular and Cellular Biology* 18, 4629–4638. <https://doi.org/10.1128/MCB.18.8.4629>
- Tseng, Y.-J., Kageyama, Y., Murdaugh, R.L., Kitano, A., Kim, J.H., Hoegenauer, K.A., Tiessen, J., Smith, M.H., Uryu, H., Takahashi, K., Martin, J.F., Samee, M.A.H., Nakada, D., 2024. Increased iron uptake by splenic hematopoietic stem cells promotes TET2-dependent erythroid regeneration. *Nat Commun* 15, 538. <https://doi.org/10.1038/s41467-024-44718-0>
- Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., Zhang, Y., 2006. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 439, 811–816. <https://doi.org/10.1038/nature04433>
- Tsurumi, A., Li, W., 2012. Global heterochromatin loss: A unifying theory of aging? *Epigenetics* 7, 680–688. <https://doi.org/10.4161/epi.20540>
- Turner, B.M., 2005. Reading signals on the nucleosome with a new nomenclature for modified histones. *Nat Struct Mol Biol* 12, 110–112. <https://doi.org/10.1038/nsmb0205-110>
- Tylutka, A., Walas, Ł., Zembron-Lacny, A., 2024. Level of IL-6, TNF, and IL-1 β and age-related diseases: a systematic review and meta-analysis. *Front. Immunol.* 15, 1330386. <https://doi.org/10.3389/fimmu.2024.1330386>
- Ugarte, F., Sousae, R., Cinquin, B., Martin, E.W., Krietsch, J., Sanchez, G., Inman, M., Tsang, H., Warr, M., Passegué, E., Larabell, C.A., Forsberg, E.C., 2015. Progressive Chromatin Condensation and H3K9 Methylation Regulate the Differentiation of Embryonic and Hematopoietic Stem Cells. *Stem Cell Reports* 5, 728–740. <https://doi.org/10.1016/j.stemcr.2015.09.009>
- van Attikum, H., Gasser, S.M., 2005. The histone code at DNA breaks: a guide to repair? *Nat Rev Mol Cell Biol* 6, 757–765. <https://doi.org/10.1038/nrm1737>
- Van Dongen, J., Nivard, M.G., Willemsen, G., Hottenga, J.-J., Helmer, Q., Dolan, C.V., Ehli, E.A., Davies, G.E., Van Iterson, M., Breeze, C.E., Beck, S., BIOS Consortium, Hoen, P.A.C. 't, Pool, R., Van Greevenbroek, M.M.J., Stehouwer, C.D.A., Kallen, C.J.H.V.D., Schalkwijk, C.G., Wijmenga, C., Zhernakova, S., Tigchelaar, E.F., Beekman, M., Deelen, J., Van Heemst, D., Veldink, J.H., Van Den Berg, L.H., Van Duijn, C.M., Hofman, B.A., Uitterlinden, A.G., Jhamai, P.M., Verbiest, M., Verkerk, M., Van Der Breggen, R., Van Rooij, J., Lakenberg, N., Mei, H., Bot, J., Zhernakova, D.V., Van't Hof, P., Deelen, P., Nooren, I., Moed, M., Vermaat, M., Luijk, R., Bonder, M.J., Van Dijk, F., Van Galen, M., Arindrarto, W., Kielbasa, S.M., Swertz, M.A., Van Zwet, E.W., Isaacs, A., Franke, L., Suchiman, H.E., Jansen, R., Van Meurs, J.B., Heijmans, B.T., Slagboom, P.E., Boomsma, D.I., 2016. Genetic and

- environmental influences interact with age and sex in shaping the human methylome. *Nat Commun* 7, 11115. <https://doi.org/10.1038/ncomms11115>
- van Holde, K.E., 1988. *Chromatin*, Springer Series in Molecular and Cell Biology. Springer New York.
- Van Meter, M., Kashyap, M., Rezazadeh, S., Geneva, A.J., Morello, T.D., Seluanov, A., Gorbunova, V., 2014. SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. *Nat Commun* 5, 5011. <https://doi.org/10.1038/ncomms6011>
- Van Zant, G., Liang, Y., 2003. The role of stem cells in aging. *Experimental Hematology* 31, 659–672. [https://doi.org/10.1016/S0301-472X\(03\)00088-2](https://doi.org/10.1016/S0301-472X(03)00088-2)
- Vardiman, J.W., Thiele, J., Arber, D.A., Brunning, R.D., Borowitz, M.J., Porwit, A., Harris, N.L., Le Beau, M.M., Hellström-Lindberg, E., Tefferi, A., Bloomfield, C.D., 2009. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 114, 937–951. <https://doi.org/10.1182/blood-2009-03-209262>
- Verdel, A., Jia, S., Gerber, S., Sugiyama, T., Gygi, S., Grewal, S.I.S., Moazed, D., 2004. RNAi-Mediated Targeting of Heterochromatin by the RITS Complex. *Science* 303, 672–676. <https://doi.org/10.1126/science.1093686>
- Verovskaya, E.V., Dellorusso, P.V., Passegué, E., 2019. Losing Sense of Self and Surroundings: Hematopoietic Stem Cell Aging and Leukemic Transformation. *Trends in Molecular Medicine* 25, 494–515. <https://doi.org/10.1016/j.molmed.2019.04.006>
- Vettese-Dadey, M., Grant, P.A., Hebbes, T.R., Crane-Robinson, C., Allis, C.D., Workman, J.L., 1996. Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. *EMBO J* 15, 2508–2518.
- Vicient, C.M., Casacuberta, J.M., 2017. Impact of transposable elements on polyploid plant genomes. *Annals of Botany* 120, 195–207. <https://doi.org/10.1093/aob/mcx078>
- Villeponteau, B., 1997. The heterochromatin loss model of aging. *Experimental Gerontology* 32, 383–394. [https://doi.org/10.1016/S0531-5565\(96\)00155-6](https://doi.org/10.1016/S0531-5565(96)00155-6)
- Von Bonin, M., Jambor, H.K., Teipel, R., Stölzel, F., Thiede, C., Damm, F., Kroschinsky, F., Schetelig, J., Chavakis, T., Bornhäuser, M., 2021. Clonal hematopoiesis and its emerging effects on cellular therapies. *Leukemia* 35, 2752–2758. <https://doi.org/10.1038/s41375-021-01337-8>
- Von Zglinicki, T., Saretzki, G., Döcke, W., Lotze, C., 1995. Mild Hyperoxia Shortens Telomeres and Inhibits Proliferation of Fibroblasts: A Model for Senescence? *Experimental Cell Research* 220, 186–193. <https://doi.org/10.1006/excr.1995.1305>
- Waddington, C.H., 1942. The Epigenotype. *Int. J. Epidemiol.* 41, 10–13. <https://doi.org/10.1093/ije/dyr184>
- Walsh, C.P., Chaillet, J.R., Bestor, T.H., 1998. Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat Genet* 20, 116–117. <https://doi.org/10.1038/2413>
- Walter, M., Teissandier, A., Pérez-Palacios, R., Bourc’his, D., 2016. An epigenetic switch ensures transposon repression upon dynamic loss of DNA methylation in embryonic stem cells. *Elife* 5, e11418. <https://doi.org/10.7554/eLife.11418>
- Wang, H., An, W., Cao, R., Xia, L., Erdjument-Bromage, H., Chatton, B., Tempst, P., Roeder, R.G., Zhang, Y., 2003. mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol Cell* 12, 475–487. <https://doi.org/10.1016/j.molcel.2003.08.007>
- Wang, J.C.Y., Dick, J.E., 2005. Cancer stem cells: lessons from leukemia. *Trends in Cell Biology* 15, 494–501. <https://doi.org/10.1016/j.tcb.2005.07.004>

- Wang, W., Liu, W., Fidler, T., Wang, Y., Tang, Y., Woods, B., Welch, C., Cai, B., Silvestre-Roig, C., Ai, D., Yang, Y.-G., Hidalgo, A., Soehnlein, O., Tabas, I., Levine, R.L., Tall, A.R., Wang, N., 2018. Macrophage Inflammation, Erythrophagocytosis, and Accelerated Atherosclerosis in *Jak2*^{V617F} Mice. *Circulation Research* 123. <https://doi.org/10.1161/CIRCRESAHA.118.313283>
- Watson, C.J., Papula, A.L., Poon, G.Y.P., Wong, W.H., Young, A.L., Druley, T.E., Fisher, D.S., Blundell, J.R., 2020. The evolutionary dynamics and fitness landscape of clonal hematopoiesis. *Science* 367, 1449–1454. <https://doi.org/10.1126/science.aay9333>
- Weeks, L.D., Niroula, A., Neuberg, D., Wong, W., Lindsley, R.C., Luskin, M.R., Berliner, N., Stone, R.M., DeAngelo, D.J., Soiffer, R.J., Uddin, M.M., Griffin, G., Vlasschaert, C., Gibson, C.J., Jaiswal, S., Bick, A.G., Malcovati, L., Natarajan, P., Ebert, B.L., 2023. Prediction of Risk for Myeloid Malignancy in Clonal Hematopoiesis. *NEJM Evidence* 2. <https://doi.org/10.1056/EVIDoa2200310>
- Wegrzyn, J.L., Lin, B.Y., Zieve, J.J., Dougherty, W.M., Martínez-García, P.J., Koriabine, M., Holtz-Morris, A., deJong, P., Crepeau, M., Langley, C.H., Puiu, D., Salzberg, S.L., Neale, D.B., Stevens, K.A., 2013. Insights into the Loblolly Pine Genome: Characterization of BAC and Fosmid Sequences. *PLoS ONE* 8, e72439. <https://doi.org/10.1371/journal.pone.0072439>
- Weinstock, J.S., Gopakumar, J., Burugula, B.B., Uddin, M.M., Jahn, N., Belk, J.A., Bouzid, H., Daniel, B., Miao, Z., Ly, N., Mack, T.M., Luna, S.E., Prothro, K.P., Mitchell, S.R., Laurie, C.A., Broome, J.G., Taylor, K.D., Guo, X., Sinner, M.F., Von Falkenhausen, A.S., Käab, S., Shuldiner, A.R., O’Connell, J.R., Lewis, J.P., Boerwinkle, E., Barnes, K.C., Chami, N., Kenny, E.E., Loos, R.J.F., Fornage, M., Hou, L., Lloyd-Jones, D.M., Redline, S., Cade, B.E., Psaty, B.M., Bis, J.C., Brody, J.A., Silverman, E.K., Yun, J.H., Qiao, D., Palmer, N.D., Freedman, B.I., Bowden, D.W., Cho, M.H., DeMeo, D.L., Vasan, R.S., Yanek, L.R., Becker, L.C., Kardia, S.L.R., Peyser, P.A., He, J., Rienstra, M., Van Der Harst, P., Kaplan, R., Heckbert, S.R., Smith, N.L., Wiggins, K.L., Arnett, D.K., Irvin, M.R., Tiwari, H., Cutler, M.J., Knight, S., Muhlestein, J.B., Correa, A., Raffield, L.M., Gao, Y., De Andrade, M., Rotter, J.I., Rich, S.S., Tracy, R.P., Konkle, B.A., Johnsen, J.M., Wheeler, M.M., Smith, J.G., Melander, O., Nilsson, P.M., Custer, B.S., Duggirala, Ravindranath, Curran, J.E., Blangero, J., McGarvey, S., Williams, L.K., Xiao, S., Yang, M., Gu, C.C., Chen, Y.-D.I., Lee, W.-J., Marcus, G.M., Kane, J.P., Pullinger, C.R., Shoemaker, M.B., Darbar, D., Roden, D.M., Albert, C., Kooperberg, C., Zhou, Y., Manson, J.E., Desai, P., Johnson, A.D., Mathias, R.A., NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, Abe, N., Abecasis, Gonçalo R., Aguet, F., Albert, C., Almasy, L., Alonso, A., Ament, S., Anderson, P., Anugu, P., Applebaum-Bowden, D., Ardlie, K., Arking, D., Arnett, D.K., Ashley-Koch, A., Aslibekyan, S., Assimes, T., Auer, P., Avramopoulos, D., Ayas, N., Balasubramanian, A., Barnard, J., Barnes, K.C., Barr, R.G., Barron-Casella, E., Barwick, L., Beaty, T., Beck, G., Becker, D., Becker, L.C., Beer, R., Beitelshees, A., Benjamin, E., Benos, T., Bezerra, M., Bielak, L., Bis, J., Blackwell, T.W., Blangero, J., Boerwinkle, E., Bowden, D.W., Bowler, R., Brody, J.A., Broeckel, U., Broome, J.G., Brown, D., Bunting, K., Burchard, E., Bustamante, C., Buth, E., Cade, B.E., Cardwell, J., Carey, V., Carrier, J., Carty, C., Casaburi, R., Casas Romero, J.P., Casella, J., Castaldi, P., Chaffin, M., Chang, C., Chang, Y.-C., Chasman, D., Chavan, S., Chen, B.-J., Chen, W.-M., Chen, Y.-D.I., Cho, M.H., Choi, S.H., Chuang, L.-M., Chung, M., Chung, R.-H., Clish, C., Comhair, S., Conomos, M., Cornell, E., Correa, A., Crandall, C., Crapo, J., Cupples, L.A., Curran, J.E., Curtis, J., Custer, B.S., Damcott, C., Darbar, D., David, S., Davis, C., Daya, M., De Andrade, M., De Las Fuentes, L., De Vries, P.,

DeBaun, M., Deka, R., DeMeo, D.L., Devine, S., Dinh, H., Doddapaneni, H., Duan, Q., Dugan-Perez, S., Duggirala, Ravi, Durda, J.P., Dutcher, S.K., Eaton, C., Ekunwe, L., Boueiz, A.E., Ellinor, P., Emery, L., Erzurum, S., Farber, C., Farek, J., Fingerlin, T., Flickinger, M., Fornage, M., Franceschini, N., Frazar, C., Fu, M., Fullerton, S.M., Fulton, L., Gabriel, S., Gan, W., Gao, S., Gao, Y., Gass, M., Geiger, H., Gelb, B., Geraci, M., Germer, S., Gerszten, R., Ghosh, A., Gibbs, R., Gignoux, C., Gladwin, M., Glahn, D., Gogarten, S., Gong, D.-W., Goring, H., Graw, S., Gray, K.J., Grine, D., Gross, C., Gu, C.C., Guan, Y., Guo, X., Gupta, N., Haas, D.M., Haessler, J., Hall, M., Han, Y., Hanly, P., Harris, D., Hawley, N.L., He, J., Heavner, B., Heckbert, S.R., Hernandez, R., Herrington, D., Hersh, C., Hidalgo, B., Hixson, J., Hobbs, B., Hokanson, J., Hong, E., Hoth, K., Hsiung, C.A., Hu, J., Hung, Y.-J., Huston, H., Hwu, C.M., Irvin, M.R., Jackson, R., Jain, D., Jaquish, C., Johnsen, J.M., Johnson, A.D., Johnson, C., Johnston, R., Jones, K., Kang, Hyun Min, Kaplan, R., Kardia, S.L.R., Kelly, S., Kenny, E.E., Kessler, M., Khan, A., Khan, Z., Kim, W., Kimoff, J., Kinney, G., Konkle, B.A., Kooperberg, C., Kramer, H., Lange, C., Lange, E., Lange, L., Laurie, C., Laurie, C.A., LeBoff, M., Lee, J., Lee, S., Lee, W.-J., LeFaive, J., Levine, D., Levy, D., Lewis, J.P., Li, X., Li, Y., Lin, Henry, Lin, Honghuang, Lin, X., Liu, S., Liu, Yongmei, Liu, Yu, Loos, R.J.F., Lubitz, S., Lunetta, K., Luo, J., Magalang, U., Mahaney, M., Make, B., Manichaikul, A., Manning, A., Manson, J.E., Martin, L., Marton, M., Mathai, S., Mathias, R.A., May, S., McArdle, P., McDonald, M.-L., McFarland, S., McGarvey, S., McGoldrick, D., McHugh, C., McNeil, B., Mei, H., Meigs, J., Menon, V., Mestroni, L., Metcalf, G., Meyers, D.A., Mignot, E., Mikulla, J., Min, N., Minear, M., Minster, R.L., Mitchell, B.D., Moll, M., Momin, Z., Montasser, M.E., Montgomery, C., Muzny, D., Mychaleckyj, J.C., Nadkarni, G., Naik, R., Naseri, T., Natarajan, P., Nekhai, S., Nelson, S.C., Neltner, B., Nessner, C., Nickerson, D., Nkechinyere, O., North, K., O'Connell, J., O'Connor, T., Ochs-Balcom, H., Okwuonu, G., Pack, A., Paik, D.T., Palmer, N.D., Pankow, J., Papanicolaou, G., Parker, C., Peloso, G., Peralta, J.M., Perez, M., Perry, J., Peters, U., Peyser, P.A., Phillips, L.S., Pleiness, J., Pollin, T., Post, W., Becker, J.P., Boorgula, M.P., Preuss, M., Psaty, B.M., Qasba, P., Qiao, D., Qin, Z., Rafaels, N., Raffield, L.M., Rajendran, M., Ramachandran, V.S., Rao, D.C., Rasmussen-Torvik, L., Ratan, A., Redline, S., Reed, R., Reeves, C., Regan, E., Reiner, A.P., Sefuiva Reupena, M., Rice, K., Rich, S.S., Robillard, R., Robine, N., Roden, D.M., Roselli, C., Rotter, J.I., Ruczinski, I., Runnels, A., Russell, P., Ruuska, S., Ryan, K., Sabino, E.C., Saleheen, D., Salimi, S., Salvi, S., Salzberg, S., Sandow, K., Sankaran, V.G., Santibanez, J., Schwander, K., Schwartz, D., Sciruba, F., Seidman, C., Seidman, J., Sériès, F., Sheehan, V., Sherman, S.L., Shetty, Amol, Shetty, Aniket, Sheu, W.H.-H., Shoemaker, M.B., Silver, B., Silverman, E.K., Skomro, R., Smith, Albert Vernon, Smith, Jennifer, Smith, Josh, Smith, N.L., Smith, T., Smoller, S., Snively, B., Snyder, M., Sofer, T., Sotoodehnia, N., Stilp, A.M., Storm, G., Streeten, E., Su, J.L., Sung, Y.J., Sylvia, J., Szpiro, A., Taliun, D., Tang, H., Taub, M., Taylor, K.D., Taylor, M., Taylor, S., Telen, M., Thornton, T.A., Threlkeld, M., Tinker, L., Tirschwell, D., Tishkoff, S., Tiwari, H., Tong, C., Tracy, R.P., Tsai, M., Vaidya, D., Berg, D.V.D., VandeHaar, P., Vrieze, S., Walker, T., Wallace, R., Walts, A., Wang, F.F., Wang, H., Wang, J., Watson, K., Watt, J., Weeks, D.E., Weinstock, J.S., Weir, B., Weiss, S.T., Weng, L.-C., Wessel, J., Willer, C., Williams, K., Williams, L.K., Wilson, C., Wilson, J., Winterkorn, L., Wong, Q., Wu, J., Xu, H., Yanek, L.R., Yang, I., Yu, K., Zekavat, S.M., Zhang, Y., Zhao, S.X., Zhao, W., Zhu, X., Zody, M., Zoellner, S., Blackwell, T.W., Abecasis, Goncalo R., Smith, Albert V., Kang, Hyun M., Satpathy, A.T., Natarajan, P., Kitzman, J.O., Whitsel, E.A., Reiner, A.P., Bick, A.G., Jaiswal, S.,

2023. Aberrant activation of TCL1A promotes stem cell expansion in clonal haematopoiesis. *Nature* 616, 755–763. <https://doi.org/10.1038/s41586-023-05806-1>
- Weischendorff, S., Kielsen, K., Nederby, M., Schmidt, L., Burrin, D., Heilmann, C., Ifversen, M., Sengeløv, H., Mølgaard, C., Müller, K., 2019a. Reduced Plasma Amino Acid Levels During Allogeneic Hematopoietic Stem Cell Transplantation Are Associated with Systemic Inflammation and Treatment-Related Complications. *Biol Blood Marrow Transplant* 25, 1432–1440. <https://doi.org/10.1016/j.bbmt.2019.03.018>
- Weischendorff, S., Kielsen, K., Nederby, M., Schmidt, L., Burrin, D., Heilmann, C., Ifversen, M., Sengeløv, H., Mølgaard, C., Müller, K., 2019b. Reduced Plasma Amino Acid Levels During Allogeneic Hematopoietic Stem Cell Transplantation Are Associated with Systemic Inflammation and Treatment-Related Complications. *Biology of Blood and Marrow Transplantation* 25, 1432–1440. <https://doi.org/10.1016/j.bbmt.2019.03.018>
- Weishaupt, H., Sigvardsson, M., Attema, J.L., 2010. Epigenetic chromatin states uniquely define the developmental plasticity of murine hematopoietic stem cells. *Blood* 115, 247–256. <https://doi.org/10.1182/blood-2009-07-235176>
- Weissmann, S., Alpermann, T., Grossmann, V., Kowarsch, A., Nadarajah, N., Eder, C., Dicker, F., Fasan, A., Haferlach, C., Haferlach, T., Kern, W., Schnittger, S., Kohlmann, A., 2012. Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia* 26, 934–942. <https://doi.org/10.1038/leu.2011.326>
- Welch, J.S., Ley, T.J., Link, D.C., Miller, C.A., Larson, D.E., Koboldt, D.C., Wartman, L.D., Lamprecht, T.L., Liu, F., Xia, J., Kandoth, C., Fulton, R.S., McLellan, M.D., Dooling, D.J., Wallis, J.W., Chen, K., Harris, C.C., Schmidt, H.K., Kalicki-Veizer, J.M., Lu, C., Zhang, Q., Lin, L., O’Laughlin, M.D., McMichael, J.F., Delehaunty, K.D., Fulton, L.A., Magrini, V.J., McGrath, S.D., Demeter, R.T., Vickery, T.L., Hundal, J., Cook, L.L., Swift, G.W., Reed, J.P., Alldredge, P.A., Wylie, T.N., Walker, J.R., Watson, M.A., Heath, S.E., Shannon, W.D., Varghese, N., Nagarajan, R., Payton, J.E., Baty, J.D., Kulkarni, S., Klco, J.M., Tomasson, M.H., Westervelt, P., Walter, M.J., Graubert, T.A., DiPersio, J.F., Ding, L., Mardis, E.R., Wilson, R.K., 2012. The Origin and Evolution of Mutations in Acute Myeloid Leukemia. *Cell* 150, 264–278. <https://doi.org/10.1016/j.cell.2012.06.023>
- Wells, J.N., Feschotte, C., 2020. A Field Guide to Eukaryotic Transposable Elements. *Annu. Rev. Genet.* 54, 539–561. <https://doi.org/10.1146/annurev-genet-040620-022145>
- Wen, B., Wu, H., Shinkai, Y., Irizarry, R.A., Feinberg, A.P., 2009. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet* 41, 246–250. <https://doi.org/10.1038/ng.297>
- Whetstine, J.R., Nottke, A., Lan, F., Huarte, M., Smolikov, S., Chen, Z., Spooner, E., Li, E., Zhang, G., Colaiacovo, M., Shi, Y., 2006. Reversal of Histone Lysine Trimethylation by the JMJD2 Family of Histone Demethylases. *Cell* 125, 467–481. <https://doi.org/10.1016/j.cell.2006.03.028>
- White, R.R., Vijg, J., 2016. Do DNA Double-Strand Breaks Drive Aging? *Molecular Cell* 63, 729–738. <https://doi.org/10.1016/j.molcel.2016.08.004>
- Wilhelm, M., Wilhelm, F.-X., 2001. Reverse transcription of retroviruses and LTR retrotransposons: CMLS, *Cell. Mol. Life Sci.* 58, 1246–1262. <https://doi.org/10.1007/PL00000937>
- Wilson, A., Laurenti, E., Oser, G., Van Der Wath, R.C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C.F., Eshkind, L., Bockamp, E., Lió, P., MacDonald, H.R., Trumpp, A., 2008. Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal during Homeostasis and Repair. *Cell* 135, 1118–1129. <https://doi.org/10.1016/j.cell.2008.10.048>

- Wogan, G.N., Hecht, S.S., Felton, J.S., Conney, A.H., Loeb, L.A., 2004. Environmental and chemical carcinogenesis. *Seminars in Cancer Biology* 14, 473–486. <https://doi.org/10.1016/j.semcancer.2004.06.010>
- Wong, T.N., Ramsingh, G., Young, A.L., Miller, C.A., Touma, W., Welch, J.S., Lamprecht, T.L., Shen, D., Hundal, J., Fulton, R.S., Heath, S., Baty, J.D., Klco, J.M., Ding, L., Mardis, E.R., Westervelt, P., DiPersio, J.F., Walter, M.J., Graubert, T.A., Ley, T.J., Druley, T.E., Link, D.C., Wilson, R.K., 2015. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature* 518, 552–555. <https://doi.org/10.1038/nature13968>
- Wong, W.J., Emdin, C., Bick, A., Zekavat, S.M., Niroula, A., Pirruccello, J., Dichtel, L., Griffin, G., Uddin, M.M., Gibson, C.J., Kovalcik, V., Lin, A.E., McConkey, M.E., Vromman, A., Sellar, R.S., Kim, P.G., Agrawal, M., Weinstock, J., Long, M.T., Yu, B., Banerjee, R., Nicholls, R.C., Kelly, M., Loh, P.-R., McCarroll, S., Boerwinkle, E., Vasani, R., Jaiswal, S., Johnson, A., Chung, R.T., Corey, K., Levy, D., Ballantyne, C., NHLBI TOPMed Hematology Working Group, Ebert, B.L., Natarajan, P., 2022. Clonal hematopoiesis and risk of chronic liver disease. <https://doi.org/10.1101/2022.01.17.22269409>
- Woodruff, R.C., 1992. Transposable DNA elements and life history traits: I. Transposition of P DNA elements in somatic cells reduces the lifespan of *Drosophila melanogaster*. *Genetica* 86, 143–154. <https://doi.org/10.1007/BF00133717>
- Wu, H., Zhang, Y., 2011. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. *Genes Dev.* 25, 2436–2452. <https://doi.org/10.1101/gad.179184.111>
- Xie, J., Sheng, M., Rong, S., Zhou, D., Wang, C., Wu, W., Huang, Jingru, Sun, Y., Wang, Yin, Chen, P., Wu, Y., Wang, Yuanxian, Wang, L., Zhou, B.O., Huang, X., Walsh, C.P., Bohlander, S.K., Huang, Jian, Wang, X., Xu, G.-L., Gao, H., Shi, Y., 2023. STING activation in TET2-mutated hematopoietic stem/progenitor cells contributes to the increased self-renewal and neoplastic transformation. *Leukemia* 37, 2457–2467. <https://doi.org/10.1038/s41375-023-02055-z>
- Xu, Y., Xu, C., Kato, A., Tempel, W., Abreu, J.G., Bian, C., Hu, Y., Hu, D., Zhao, B., Cerovina, T., Diao, J., Wu, F., He, H.H., Cui, Q., Clark, E., Ma, C., Barbara, A., Veenstra, G.J.C., Xu, G., Kaiser, U.B., Liu, X.S., Sugrue, S.P., He, X., Min, J., Kato, Y., Shi, Y.G., 2012. Tet3 CXXC Domain and Dioxygenase Activity Cooperatively Regulate Key Genes for *Xenopus* Eye and Neural Development. *Cell* 151, 1200–1213. <https://doi.org/10.1016/j.cell.2012.11.014>
- Xue, G., Zhang, X., Li, W., Zhang, Lu, Zhang, Z., Zhou, X., Zhang, D., Zhang, Lei, Li, Z., 2024. A logic-incorporated gene regulatory network deciphers principles in cell fate decisions. *eLife* 12, RP88742. <https://doi.org/10.7554/eLife.88742>
- Yamamoto, R., Nakauchi, H., 2020. In vivo clonal analysis of aging hematopoietic stem cells. *Mechanisms of Ageing and Development* 192, 111378. <https://doi.org/10.1016/j.mad.2020.111378>
- Yamamoto, R., Wilkinson, A.C., Ooehara, J., Lan, X., Lai, C.-Y., Nakauchi, Y., Pritchard, J.K., Nakauchi, H., 2018. Large-Scale Clonal Analysis Resolves Aging of the Mouse Hematopoietic Stem Cell Compartment. *Cell Stem Cell* 22, 600–607.e4. <https://doi.org/10.1016/j.stem.2018.03.013>
- Yamanaka, S., Blau, H.M., 2010. Nuclear reprogramming to a pluripotent state by three approaches. *Nature* 465, 704–712. <https://doi.org/10.1038/nature09229>
- Yang, X., Zhang, F., Kudlow, J.E., 2002. Recruitment of O-GlcNAc Transferase to Promoters by Corepressor mSin3A. *Cell* 110, 69–80. [https://doi.org/10.1016/S0092-8674\(02\)00810-3](https://doi.org/10.1016/S0092-8674(02)00810-3)

- Yao, Q., Chen, Y., Zhou, X., 2019. The roles of microRNAs in epigenetic regulation. *Current Opinion in Chemical Biology* 51, 11–17. <https://doi.org/10.1016/j.cbpa.2019.01.024>
- Yeaton, A., Cayanan, G., Loghavi, S., Dolgalev, I., Leddin, E.M., Loo, C.E., Torabifard, H., Nicolet, D., Wang, J., Corrigan, K., Paraskevopoulou, V., Starczynowski, D.T., Wang, E., Abdel-Wahab, O., Viny, A.D., Stone, R.M., Byrd, J.C., Guryanova, O.A., Kohli, R.M., Cisneros, G.A., Tsigos, A., Eisfeld, A.-K., Aifantis, I., Guillaumot, M., 2022. The Impact of Inflammation-Induced Tumor Plasticity during Myeloid Transformation. *Cancer Discovery* 12, 2392–2413. <https://doi.org/10.1158/2159-8290.CD-21-1146>
- Yi, R., Qin, Y., Macara, I.G., Cullen, B.R., 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* 17, 3011–3016. <https://doi.org/10.1101/gad.1158803>
- Yin, R., Mao, S.-Q., Zhao, B., Chong, Z., Yang, Y., Zhao, C., Zhang, D., Huang, H., Gao, J., Li, Z., Jiao, Y., Li, C., Liu, S., Wu, D., Gu, W., Yang, Y.-G., Xu, G.-L., Wang, H., 2013. Ascorbic Acid Enhances Tet-Mediated 5-Methylcytosine Oxidation and Promotes DNA Demethylation in Mammals. *J. Am. Chem. Soc.* 135, 10396–10403. <https://doi.org/10.1021/ja4028346>
- Yoder, J.A., Walsh, C.P., Bestor, T.H., 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends in Genetics* 13, 335–340. [https://doi.org/10.1016/S0168-9525\(97\)01181-5](https://doi.org/10.1016/S0168-9525(97)01181-5)
- Yoshida, K., French, B., Yoshida, N., Hida, A., Ohishi, W., Kusunoki, Y., 2019. Radiation exposure and longitudinal changes in peripheral monocytes over 50 years: the Adult Health Study of atomic-bomb survivors. *Br J Haematol* 185, 107–115. <https://doi.org/10.1111/bjh.15750>
- Yoshida, T., Yao-Ming Ng, S., Zuniga-Pflucker, J.C., Georgopoulos, K., 2006. Early hematopoietic lineage restrictions directed by Ikaros. *Nat Immunol* 7, 382–391. <https://doi.org/10.1038/ni1314>
- Yu, Q., Zhou, B., Zhang, Y., Nguyen, E.T., Du, J., Glosston, N.L., Kaplan, M.H., 2012. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 109, 541–546. <https://doi.org/10.1073/pnas.1103803109>
- Yu, V.W.C., Yusuf, R.Z., Oki, T., Wu, J., Saez, B., Wang, X., Cook, C., Baryawno, N., Ziller, M.J., Lee, E., Gu, H., Meissner, A., Lin, C.P., Kharchenko, P.V., Scadden, D.T., 2016. Epigenetic Memory Underlies Cell-Autonomous Heterogeneous Behavior of Hematopoietic Stem Cells. *Cell* 167, 1310–1322.e17. <https://doi.org/10.1016/j.cell.2016.10.045>
- Yu, X., Wu, C., Bhavanasi, D., Wang, H., Gregory, B.D., Huang, J., 2017. Chromatin dynamics during the differentiation of long-term hematopoietic stem cells to multipotent progenitors. *Blood Advances* 1, 887–898. <https://doi.org/10.1182/bloodadvances.2016003384>
- Zdzisińska, B., Żurek, A., Kandefer-Szerszeń, M., 2017. Alpha-Ketoglutarate as a Molecule with Pleiotropic Activity: Well-Known and Novel Possibilities of Therapeutic Use. *Arch. Immunol. Ther. Exp.* 65, 21–36. <https://doi.org/10.1007/s00005-016-0406-x>
- Zeller, P., Padeken, J., Van Schendel, R., Kalck, V., Tijsterman, M., Gasser, S.M., 2016. Histone H3K9 methylation is dispensable for *Caenorhabditis elegans* development but suppresses RNA:DNA hybrid-associated repeat instability. *Nat Genet* 48, 1385–1395. <https://doi.org/10.1038/ng.3672>
- Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.-G., Ross, J., Haug, J., Johnson, T., Feng, J.Q., Harris, S., Wiedemann, L.M., Mishina, Y., Li, L., 2003. Identification of

- the haematopoietic stem cell niche and control of the niche size. *Nature* 425, 836–841. <https://doi.org/10.1038/nature02041>
- Zhang, M., Yin, L., Zhang, K., Sun, W., Yang, S., Zhang, B., Salzman, P., Wang, W., Liu, C., Vidyasagar, S., Zhang, Lei, Ju, S., Okunieff, P., Zhang, Lurong, 2012. Response patterns of cytokines/chemokines in two murine strains after irradiation. *Cytokine* 58, 169–177. <https://doi.org/10.1016/j.cyto.2011.12.023>
- Zhang, Q., Zhao, K., Shen, Q., Han, Y., Gu, Y., Li, X., Zhao, D., Liu, Y., Wang, C., Zhang, X., Su, X., Liu, J., Ge, W., Levine, R.L., Li, N., Cao, X., 2015. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature* 525, 389–393. <https://doi.org/10.1038/nature15252>
- Zhang, S., Roche, K., Nasheuer, H.-P., Lowndes, N.F., 2011. Modification of Histones by Sugar β -N-Acetylglucosamine (GlcNAc) Occurs on Multiple Residues, Including Histone H3 Serine 10, and Is Cell Cycle-regulated. *Journal of Biological Chemistry* 286, 37483–37495. <https://doi.org/10.1074/jbc.M111.284885>
- Zhang, X., Zhang, R., Yu, J., 2020. New Understanding of the Relevant Role of LINE-1 Retrotransposition in Human Disease and Immune Modulation. *Front. Cell Dev. Biol.* 8, 657. <https://doi.org/10.3389/fcell.2020.00657>
- Zhou, B.O., Yu, H., Yue, R., Zhao, Z., Rios, J.J., Naveiras, O., Morrison, S.J., 2017. Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF. *Nat Cell Biol* 19, 891–903. <https://doi.org/10.1038/ncb3570>
- Zhou, W., Liang, G., Molloy, P.L., Jones, P.A., 2020. DNA methylation enables transposable element-driven genome expansion. *Proc. Natl. Acad. Sci. U.S.A.* 117, 19359–19366. <https://doi.org/10.1073/pnas.1921719117>
- Zhu, Q., Hoong, N., Aslanian, A., Hara, T., Benner, C., Heinz, S., Miga, K.H., Ke, E., Verma, S., Soroczynski, J., Yates, J.R., Hunter, T., Verma, I.M., 2018. Heterochromatin-Encoded Satellite RNAs Induce Breast Cancer. *Molecular Cell* 70, 842–853.e7. <https://doi.org/10.1016/j.molcel.2018.04.023>
- Zink, F., Stacey, S.N., Norddahl, G.L., Frigge, M.L., Magnusson, O.T., Jonsdottir, I., Thorgeirsson, T.E., Sigurdsson, A., Gudjonsson, S.A., Gudmundsson, J., Jonasson, J.G., Tryggvadottir, L., Jonsson, T., Helgason, A., Gylfason, A., Sulem, P., Rafnar, T., Thorsteinsdottir, U., Gudbjartsson, D.F., Masson, G., Kong, A., Stefansson, K., 2017. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* 130, 742–752. <https://doi.org/10.1182/blood-2017-02-769869>

ARTICLE

NF- κ B signaling controls H3K9me3 levels at intronic LINE-1 and hematopoietic stem cell genes in cis

Yanis Pelinski^{1,2}, Donia Hidaoui^{1,2*}, Anne Stolz^{1,2*}, François Hermetet^{1,2}, Rabie Chelbi^{1,2}, M'boyba Khadija Diop^{2,3}, Amir M. Chioukh^{1,2}, Françoise Porteu^{1,2**}, and Emilie Elvira-Matelot^{1,2**}

Ionizing radiations (IR) alter hematopoietic stem cell (HSC) function on the long term, but the mechanisms underlying these effects are still poorly understood. We recently showed that IR induces the derepression of LIMd, the mouse young subfamilies of LINE-1/L1 retroelements. L1 contributes to gene regulatory networks. However, how LIMd are derepressed and impact HSC gene expression are not known. Here, we show that IR triggers genome-wide H3K9me3 decrease that occurs mainly at LIMd. Loss of H3K9me3 at intronic LIMd harboring NF- κ B binding sites motifs but not at promoters is associated with the repression of HSC-specific genes. This is correlated with reduced NFKB1 repressor expression. TNF- α treatment rescued all these effects and prevented IR-induced HSC loss of function in vivo. This TNF- α /NF- κ B/H3K9me3/LIMd axis might be important to maintain HSCs while allowing expression of immune genes during myeloid regeneration or damage-induced bone marrow ablation.

Introduction

Exposure to ionizing radiations (IR), in the context of medical use such as radiotherapies, is an independent risk factor for many disorders characteristic of an accelerated aging. The hematopoietic tissue is particularly sensitive to IR. We and others have shown that total body irradiation (TBI) in mice leads to long-term defects in hematopoiesis due to loss of hematopoietic stem cell (HSC) reserves and functions (Fleenor et al., 2015; de Laval et al., 2013; Mohrin et al., 2010). In HSCs, IR induces DNA damage accumulation, loss of self-renewal, and a biased differentiation toward the myeloid lineage leading to increased myeloid cell counts and decline of the adaptive immune response. These changes are likely contributing to many IR-induced premature aging disorders and to the high risk of developing myeloid leukemia. Understanding the molecular mechanisms leading to HSC loss of function upon IR is necessary to modulate its adverse effects. It may also help identifying the first events leading to hematologic malignancies.

IR induces DNA double strand breaks (DSBs). DNA damage is thought to be one of the main driving forces of aging. However, delaying the effects of age in mice by decreasing the levels of DNA damage has never been achieved, and a direct link between DSB formation and physiological aging is still lacking (White and Vijg, 2016). Although IR has been shown to induce chromosomal abnormalities in progenitors (de Laval et al., 2013; de Laval et al.,

2014; Mohrin et al., 2010), in fact HSCs are quite resilient toward accumulating DNA mutations in response to DNA damage (Moehrle et al., 2015).

In addition to DSBs, IR has been shown to induce changes in chromatin state, mainly at the level of DNA methylation, in different tissues and cell lines (Miousse et al., 2017b). Epigenetic alterations have been observed in aged HSCs in the absence of mutations in epigenetic factors (Djeghloul et al., 2016; Sun et al., 2014). Reorganization of heterochromatin is among the most commonly reported changes in aging and senescence, supporting its essential role in maintaining proper cellular function (Tsurumi and Li, 2012). Maintenance of HSC identity is dependent on the heterochromatin mark H3K9me3 (Koide et al., 2016). Decreased H3K9me3 in HSCs due to loss of *Suv39h2* and/or *Suv39h1* methyltransferases occurs with age and is associated with the loss of B cell differentiation (Djeghloul et al., 2016) and hematopoietic changes archetypal of aging (Keenan et al., 2020). However, whether the long-term effects of IR on HSCs are linked to IR-induced changes in heterochromatin remains to be addressed.

Heterochromatin plays also a major role in the maintenance of the genome stability by repressing transposable elements (TEs), including DNA transposons and retro-TEs (RTEs), further

¹INSERM UMR1287, Gustave Roussy, Villejuif, France; ²Université Paris-Saclay, Gif-sur-Yvette, France; ³Bioinformatics Platform UMS AMMICA INSERM US23/CNRS 3655, Gustave Roussy, Villejuif, France.

*D. Hidaoui and A. Stolz contributed equally as second author; **F. Porteu and E. Elvira-Matelot contributed equally as last author and are co-corresponding authors. Correspondence to Emilie Elvira-Matelot: emilie.elvira-matelot@inserm.fr; Françoise Porteu: francoise.porteu@gustaveroussy.fr.

© 2022 Pelinski et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

classified as LTR sequences that characterize endogenous retroviruses (ERVs), and non-LTR elements such as long or short interspersed elements (LINE-1/L1; SINE). Propagation of RTEs in the genome has been recognized as a great source of genomic instability. Even without propagating, RTEs have also been recently recognized as major contributors of gene regulatory networks (Chuong et al., 2017). Indeed, they qualitatively and quantitatively control gene expression, providing alternative enhancers, promoters, splicing, or polyadenylation signals, and also serving as cis-regulatory elements in a cell-specific fashion. Basal L1 expression in early mouse embryo is necessary for its proper development (Jachowicz et al., 2017). RTEs are involved in T cell differentiation by regulating genes involved in immune processes (Adoue et al., 2019). Abnormal RTE expression has been observed in cancers, including acute myeloid leukemia (AML), and may be involved in the pathogenesis through the alteration of host gene expression (Chuong et al., 2017) and the expression of oncogenes (Deniz et al., 2020; Jang et al., 2019).

We recently showed that evolutionary recent mouse L1s (L1Md) are highly expressed in HSCs and that their expression is further increased and maintained at high levels up to 1 mo after TBI (Barbieri et al., 2018). TE expression is also increased in HSCs after chemotherapies (Clapes et al., 2021) and the decreased H3K9me3 in aged HSCs is associated with increased L1Md expression (Djehoul et al., 2016). However, the impact of L1Md derepression on the HSC transcriptome remains to be addressed. In addition, the mechanisms and signaling pathways by which IR specifically triggers L1Md expression in HSCs are currently unknown. We and others previously showed that the NF- κ B signaling pathway is required to prevent IR-induced HSC injury (Hu et al., 2021; de Laval et al., 2014). In addition, TNF- α -induced NF- κ B supports HSC survival during inflammation and chemotherapeutic stress induced by 5-fluorouracil (Yamashita and Passequé, 2019). On the other hand, basal activation of NF- κ B is required for HSC homeostasis and self-renewal potential and the expression of key HSC maintenance genes are severely impaired in mice deficient for NF- κ B pathway factors (Fang et al., 2018; Hu et al., 2021; Stein and Baldwin, 2013). We show here that IR induces a major loss of H3K9me3 in HSCs, which mainly affects L1Md subfamilies, and more specifically intronic L1Md displaying NF- κ B binding sites. By controlling the levels of H3K9me3 at selected intronic L1Md located in genes belonging to the HSC signature, this pathway plays a crucial role to preserve HSC-specific gene expression and HSC function during IR stress.

Results

H3K9me3 is mainly enriched at recent L1Md subfamilies in HSCs

To assess the effect of IR on H3K9me3 in HSCs and more particularly at TE sequences, we performed H3K9me3 chromatin immunoprecipitation sequencing (ChIP-seq) experiments in HSCs (Lin⁺Sca⁺Kit⁺CD34⁺Flk2⁻) sorted from mice 1 mo after TBI (2 Gy) or not, as previously described (Barbieri et al., 2018;

Fig. 1, A and B). Deep characterization of H3K9me3 enrichment in HSCs, notably at TE sequences, has never been performed. We thus first characterized H3K9me3 genomic coverage in HSCs at steady state.

Analysis of genomic repeats is still a bioinformatics challenge. The assignment of reads complementary to sequences that are repeated in the genome and that present low sequence variation is largely compromised. These multiple mapping reads are often discarded in ChIP-seq studies. However, when using only unique mapping reads, one can induce a bias of representation of the TE subfamilies toward the oldest ones as the youngest subfamilies, such as L1Md, present a very low mappability due to quasi-identical copies (Teissandier et al., 2019). Therefore, while young families of TEs are the most epigenetically regulated in the genome (Barau et al., 2016; Pezic et al., 2014), they are severely underestimated in unique read analysis.

To maximize the output information on these sequences, we considered all reads that mapped to the genome without mismatches and randomly assigned them at one of their best possible positions in the genome (Fig. 1 A). We combined both unique and multiple mapping reads analyses (U-MRA and M-MRA, respectively) to finally get a compromise between precise assignment of the unique reads and global information at youngest TE subfamily level.

Quality control of the resulting reads indicated high genomic coverage in both U- and M-MRA and in non-irradiated (NIR) and IR conditions (Table S1). Peak calling followed by reproducibility measurement between replicates (irreproducible discovery rate [IDR]) identified 456 peaks on average in the NIR conditions in the U-MRA. As expected, with 7,769 peaks, M-MRA gave a substantial increased number of peaks (Fig. 1 C and Table S1). Our peak calling retrieved the SUV39h1- and -2-dependent H3K9me3 peaks that were previously described at young TEs such as intracisternal A particle (IAP) ERVs (Fig. S1 A) using this strategy in mouse embryonic stem cells (mESCs; Bulut-Karslioglu et al., 2014). Of note, these elements were not covered by U-MRA.

After annotation of the peaks using annotateR, both U-MRA and M-MRA showed that the majority of the H3K9me3-enriched peaks occur at TEs (41.9 and 52%, respectively; Fig. 1 D). Interestingly, M-MRA shows a gain in LINE representation, with 32.7% of the total H3K9me3 enriched peaks vs. 16.5% in U-MRA. By contrast, M-MRA shows reduced SINE and DNA representation while LTR representation was not affected. This suggests that H3K9me3 enrichment occurs mainly at LINES in HSCs. Heatmap representation of the average concentration (i.e., H3K9me3 enrichment normalized to input) of H3K9me3 at peaks retrieved from M-MRA, followed by RTE genomic coverage, further confirmed that peaks enriched in H3K9me3 at basal are mostly covering LINES compared to LTR, SINEs, or DNA, and more especially the young subfamilies of LINES (L1Md) compared to older ones (Lx5; Fig. 1 E).

Altogether, these data demonstrate that H3K9me3 is mainly enriched at repetitive sequences, and more particularly at young L1Md elements in HSCs.

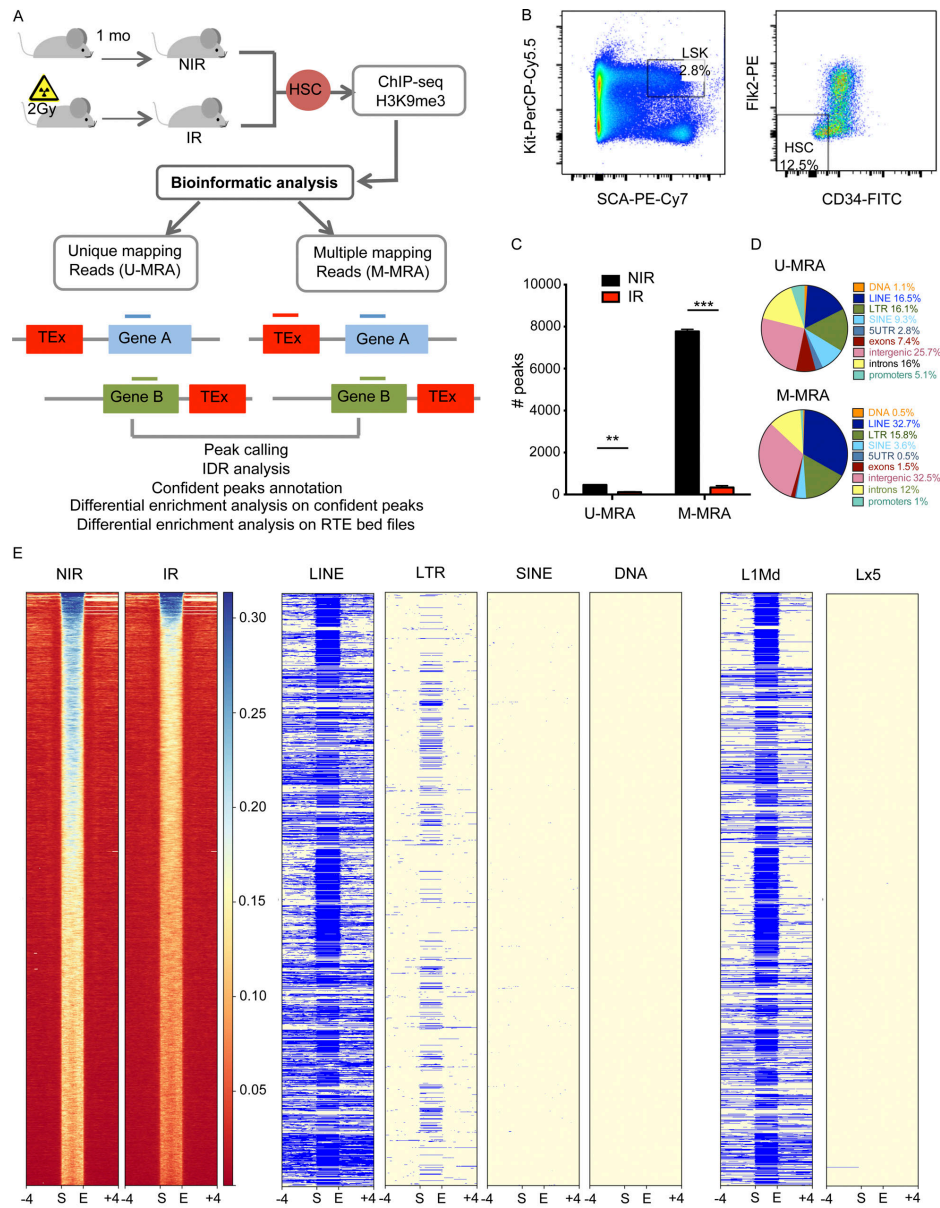


Figure 1. **H3K9me3 is mainly enriched at recent L1Md subfamilies in HSCs.** (A) Experimental and bioinformatic analysis design for H3K9me3 ChIP-seq. Mice were subjected to 2 Gy total body irradiation (IR) or left untreated (NIR). Analysis was done on both U- and M-MRA. (B) Gating strategy for HSCs (LSK CD34-Flk2-) sorting. (C) Number of peaks with IDR score <0.05 in U- and M-MRA and in NIR and IR conditions. *******, $P < 0.001$; ******, $P < 0.01$, *t* test. (D) Repartition of confident peak annotations in each genomic feature using annotateR in NIR conditions for U- and M-MRA. (E) Left columns: Heatmap of

H3K9me3 enrichment at M-MRA confident peaks in NIR and IR conditions. Each row represents one-scaled H3K9me3 peak with ± 4 kb flanking regions. The color scale represents H3K9me3 enrichment over the input (H3K9me3 concentration) with the red corresponding to lower enrichment, and the blue to stronger enrichment. Center and right columns: Genomic coverage of the H3K9me3 peaks showed in the heatmap at different TE families and subfamilies (LINE, LTR, SINE, DNA, L1Md, Lx5) are also represented. Blue, TE is present; yellow, TE is absent in the peak (row).

Irradiation induces a loss of H3K9me3 that mainly affects the recent L1Md subfamilies

We next investigated changes in H3K9me3 that occur upon IR. We first compared the number of H3K9me3 peaks identified in IR vs. NIR conditions. Only 115 peaks in average were found in IR in the U-MRA, compared to 456 peaks in NIR condition, and 283 compared to 7,769 in the M-MRA (Fig. 1 C). Heatmap representation further showed a loss of H3K9me3 enrichment at peaks retrieved from M-MRA upon IR (Fig. 1 E).

Differential H3K9me3 enrichment analysis performed at H3K9me3 peaks identified in both IR and NIR conditions further revealed a strong decrease in H3K9me3 enrichment in IR (Fig. 2 A). We found 13 and 253 peaks showing significant ($P < 0.05$) differential H3K9me3 enrichment upon IR in U- and M-MRA, respectively. All of them in U-MRA and 252/253 peaks in M-MRA showed decreased H3K9me3 upon IR (Fig. 2 A). These data reveal a major loss of H3K9me3 genomic coverage upon IR, regarding both the number of peaks and the concentration of H3K9me3 at conserved peaks.

We performed the same analysis at TE genomic loci instead of peaks and found 2,559 loci with significant H3K9me3 differential enrichment, 1,667 and 892 loci showing decreased or increased H3K9me3, respectively, upon IR (Fig. 2 B). Annotation of these loci showed that they are enriched in LINES (66.7%) and LTR (29.23%) compared to the distribution of these TEs in the mouse genome (24.71 and 22.07%, respectively; Fig. 2 C). The distribution of the H3K9me3 concentration showed a significant ($P < 0.0001$) decrease in H3K9me3 at both LINES and LTR upon IR (Fig. 2 D). Comparing the distribution of the \log_2 fold change between IR and NIR conditions in H3K9me3 concentration showed that this decrease is significantly ($P < 0.0001$) more pronounced at LINES than at LTRs (Fig. 2 E).

Since young TE subfamilies, notably L1Md, were previously reported to be the most epigenetically regulated in the genome compared to old LINES in ESCs and testis (Barau et al., 2016; Pezic et al., 2014), we further dissected H3K9me3 enrichment at TEs depending on their age, as calculated in Sookdeo et al. (2013). We observed a significant negative correlation between the age of the LINE and H3K9me3 enrichment, with the youngest, typically L1Md_A and L1Md_Tf, showing the highest enrichment (Fig. 2 F), as previously observed for DNA methylation (Miousse et al., 2017a). IR induced a significantly ($P < 0.0001$) more pronounced loss of H3K9me3 at young L1Md compared to other LINES (Fig. 2 G).

Altogether these data reveal that 1 mo after IR, HSCs display a major loss of H3K9me3, which mainly occurs at L1Md, the subfamilies of TEs the most enriched in H3K9me3 at steady state. This prompted us to further focus our analysis on L1Md.

Plot profile of H3K9me3 enrichment along L1Md sequences showed asymmetric distribution of H3K9me3 along the L1 body, with the highest enrichment and the highest loss upon IR at the

5' end of these elements (Fig. 2 H and Fig. S1 B). Finally, we confirmed the global decrease of H3K9me3 at L1Md, and more particularly at L1Md_A, using ChIP-quantitative PCR (qPCR) experiments with primers recognizing either all L1Md or specifically L1Md_A promoter (Fig. 2 I), as described previously (Barbieri et al., 2018).

Irradiation induces a strong deregulation of the HSC transcriptome

In order to unravel the consequences of IR-induced H3K9me3 loss on the HSC transcriptome, we performed RNA-seq of HSCs 1 mo after TBI. Comparison of IR vs. NIR revealed 1,067 differentially expressed genes (DEGs; $P < 0.05$), with 602 (56.4%) genes downregulated and 465 (43.6%) genes upregulated upon IR (Fig. 3, A and B; and Table S2). Differences in gene expression are very strong, as almost 80% of the DEGs between IR and NIR present a fold change above 10 and more than 50% present a fold change above 50 (Fig. 3 B). Gene set enrichment analysis (GSEA) on Hallmark gene sets indicated significant enrichments in DNA repair, G2/M checkpoint, and oxidative phosphorylation pathways, as expected upon IR (Fig. 3 C), whereas the main pathways lost in IR are related to cell signaling (Fig. 3 D). Among these, the most significant decrease concerns genes regulated by NF- κ B in response to TNF- α (Fig. 3, D and E). A recent report showed that TNF- α induces a specific prosurvival gene signature in HSCs (Yamashita and Passegué, 2019). Interrogating this gene signature, composed of 62 genes representing both core regulators of the NF- κ B pathway and TNF- α -induced HSC-specific survival genes, we observed its significant loss upon IR (Fig. 3 F). IR also induced the loss of the different HSC TNF- α signatures taken individually: the two in vitro signatures obtained 3 and 12 h after TNF- α treatment and the in vivo signature obtained 3 h after TNF- α treatment in mice (Fig. S2 A). By contrast, IR had no effect on the granulocyte/monocyte progenitor (GMP) TNF- α gene signature (Fig. 3 G; Yamashita and Passegué, 2019).

Given the importance of the NF- κ B pathway at maintaining HSC survival and self-renewal or during chemotherapeutic and IR stress (Hu et al., 2021; de Laval et al., 2014; Yamashita and Passegué, 2019), we next interrogated different published HSC signatures that are enriched in low-output/self-renewing and functional long-term regeneration HSCs compared to differentiating HSCs, or in dormant vs. activated HSCs (Cabezas-Wallscheid et al., 2017; Chambers et al., 2007; Pietras et al., 2015; Rodriguez-Fraticelli et al., 2020). We found a significant loss of all these signatures upon IR (Fig. 3 H and Fig. S2 B). Likewise, the megakaryocyte (MK)-biased output HSC signature (Rodriguez-Fraticelli et al., 2020), representing platelet-primed HSCs that were also previously described to be at the apex of the HSC hierarchy (Sanjuan-Pla et al., 2013), was also enriched in NIR vs. IR HSCs (Fig. S2 B). Conversely, the high-output and multilineage signatures (Rodriguez-Fraticelli et al., 2020),

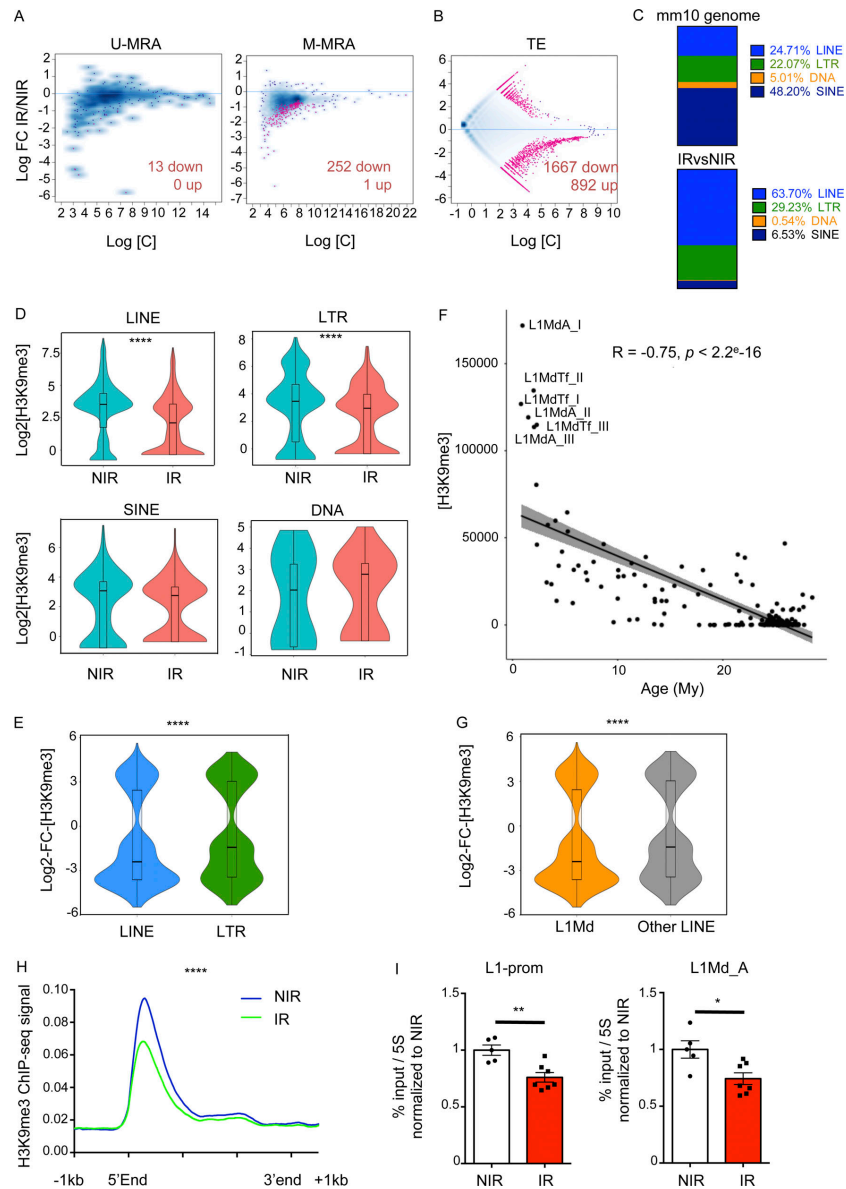


Figure 2. Irradiation induces a loss of H3K9me3 that mainly affects the recent L1Md subfamilies. (A and B) MA-plots showing nonsignificant (blue dots) and significant ($P < 0.05$, pink dots) differential H3K9me3 enrichment at confident peaks between NIR and IR conditions analyzed both in U and M-MRA (A) and TE loci (B). The number of peaks showing a significant decreased (down) or increased (up) in H3K9me3 enrichment upon IR is indicated in the plot. (C) Distribution of the percent of each family of TE among the total TE loci in the mouse mm10 genome (up) and among the significantly differentially enriched TE (bottom) retrieved in B. (D) Violin plot representing the distribution of H3K9me3 concentration at each locus retrieved in B for LINE, LTR, SINE, and DNA families of TEs in NIR and IR conditions. (E) Violin plot representing the distribution of the \log_2 fold change (FC) in H3K9me3 concentration at each locus

retrieved in B for LINE and LTR. **(F)** Correlation plot representing H3K9me3 concentration quantified at all LINE elements in M-MRA vs. their age in million years (My). R, Pearson correlation coefficient. **(G)** Violin plot representing the distribution of the \log_2 fold change in H3K9me3 concentration at each locus retrieved in B for L1Md or other LINE. **(H)** Plot profile of H3K9me3 enrichment along the L1Md sequences (>5 kb) \pm 1 kb flanking regions in IR (green) vs. NIR (blue) conditions. Wilcoxon test. **(I)** H3K9me3 enrichment at L1Md promoters analyzed by ChIP-qPCR normalized to H3K9me3 enrichment at repetitive 5S rRNA. $n = 3$ independent experiments. Each dot represents pools of three (NIR) or four (IR) mice. Results are expressed as fold change from the mean value of the NIR condition and represented as means \pm SEM. t test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

which mark differentiating HSCs, showed a significant enrichment upon IR (Fig. S2 C). Altogether, these data show that IR induces a loss of transcriptional signatures involved in HSC quiescence, long-term potency, and self-renewal capacity, and a gain in gene signatures involved in HSC differentiation, thus recapitulating the HSC loss of self-renewal previously reported upon IR (de Laval et al., 2013).

IR-induced downregulation of gene expression is associated with the presence of L1Md in their introns

In order to check if gene deregulation upon IR might be associated with H3K9me3 enrichment changes at gene promoters, we quantified H3K9me3 enrichment over promoter regions (-2 kb; +1 kb around transcription start site [TSS]; U-MRA). We found 239 promoter sequences showing a significant ($P < 0.05$) H3K9me3 differential enrichment upon IR, 211 and 28 showing decreased and increased H3K9me3, respectively (Fig. S2 D). However, these variations are not correlated with gene upregulation, and only very poorly correlated with gene downregulation (Fig. 4 A and Fig. S2 E).

Since the loss of H3K9me3 upon IR mainly occurs at L1Md, we sought to determine if L1Md derepression might be involved in the gene deregulation observed after IR. Most of the information concerning H3K9me3 enrichment at young RTE such as L1Md is obtained through M-MRA. However, reads from the M-MRA are arbitrary assigned and cannot be precisely localized. Thus, it is impossible to determine if L1Md derepression is associated with gene deregulation by crossing our ChIP-seq and RNA-seq data. To overcome this issue, we crossed the list of the 1,067 DEGs ($P < 0.05$) in IR vs. NIR from our RNA-seq data with the list of genes hosting one or several L1Md (reconstructed repeatMasker database; Fig. 4 B and Table S3). The vast majority of these L1Md are located in introns (99%; Fig. S2 F). We found that 377 DEGs in IR host one or several L1Md, in majority located in introns. This is significantly ($P < 0.0001$) more than one would expect by chance, as revealed by a permutation test using 10,000 lists of 1,067 genes randomly extracted from the refseq database (Fig. 4, B and C). These results reveal a strong and significant association between gene deregulation upon IR and the presence of an intronic L1Md in these genes. This is specific for L1Md as no significant association was observed between DEGs and the presence of Lx5, an older LINE subfamily (Fig. 4 D). Surprisingly, this association is specific for genes that are downregulated upon IR (Fig. 4 E) and was not found for upregulated genes (Fig. 4 F and Table S3).

Interestingly, 50% of the genes from long-term (LT)-HSC signatures (Chambers et al., 2007; Pietras et al., 2015) whose expression is significantly decreased upon IR (Fig. 4, G and H) contain one or several L1Md in their introns. Similarly, we found

that 31 and 41% of the genes from the low-output and MK-biased signatures (Rodriguez-Fraticelli et al., 2020) host one or several L1Md (Fig. S2, G and H). Altogether, these data highlight a significant association between genes whose expression is repressed upon IR, notably those belonging to the HSC signature, and the presence of intronic L1Md.

Gene repression upon IR is associated with loss of H3K9me3 at selected intronic L1Md loci harboring NF- κ B binding sites

We next investigated if the loss of H3K9me3 induced by IR at intronic L1Md in a given gene could be associated with its decreased expression in cis. For this purpose, we chose six candidate genes whose expression is significantly reduced by IR in our RNA-seq data: *Mecom*, *Pkia*, *Ttc8*, *Rbms3*, *Rmdn2*, and *Akt3* (Table S2), five of them (*Mecom*, *Pkia*, *Ttc8*, *Rbms3*, *Rmdn2*) being part of the different HSC signatures, as well as four negative controls, also harboring at least one intronic L1Md but whose expression remains unchanged upon IR (0.7 < fold change < 1.3, $P > 0.05$): *Snx27*, *Mapre2*, *Celf2*, and *Pdcd1lg2*. 1 mo after TBI, a significant downregulation of *Mecom*, *Pkia*, and *Rmdn2*, but not of *Snx27*, *Mapre2*, *Celf2*, was observed (Fig. 5, A and C). To assess H3K9me3 enrichment specifically at intronic L1Md of these genes, we performed H3K9me3 ChIP-qPCR experiments using primer pairs located both in the intron and in the 5' end of the L1Md, thus allowing unique and specific amplicon production (Fig. 5, A and B). Apart for *Rmdn2*, we chose the longest L1Md (>5 kb), displaying higher enrichment at the 5' end (Fig. 2 H), to detect significant basal H3K9me3. We first confirmed that H3K9me3 is indeed present at intronic L1Md, as compared to *Spil* and 5S negative controls (Fig. S3, A and B). Of note, H3K9me3 levels at tested L1Md are similar to those found globally at L1Md_A promoters (Fig. S3 B). IR induced a significant decrease in H3K9me3 specifically at all the chosen candidate intronic L1Md of genes downregulated upon IR (Fig. 5 D and Fig. S3 C). Of note, we randomly chose four negative candidates that fit our criteria, and for the four candidates selected, no loss of H3K9me3 was detected. This indicates that IR-induced H3K9me3 loss at intronic L1Md is not a general event but rather that it seems to occur only in specific genes whose expression is reduced upon IR. This suggests that the presence of the H3K9me3 mark at this location may play a role in the regulation of gene expression in cis upon IR.

In order to test this hypothesis, we chose to delete specifically the intronic L1Md of *Mecom* through CRISPR/Cas9 and guide RNAs targeting each side of the L1Md (5'gRNA and 3'gRNA; Fig. 5 E) and tested *Mecom* expression upon IR. We electroporated LSK cells with the Cas9/guide RNA (gRNA) ribonucleoprotein particles (RNP) complex (Gundry et al., 2016) together with a siglo green electroporation indicator and we sorted

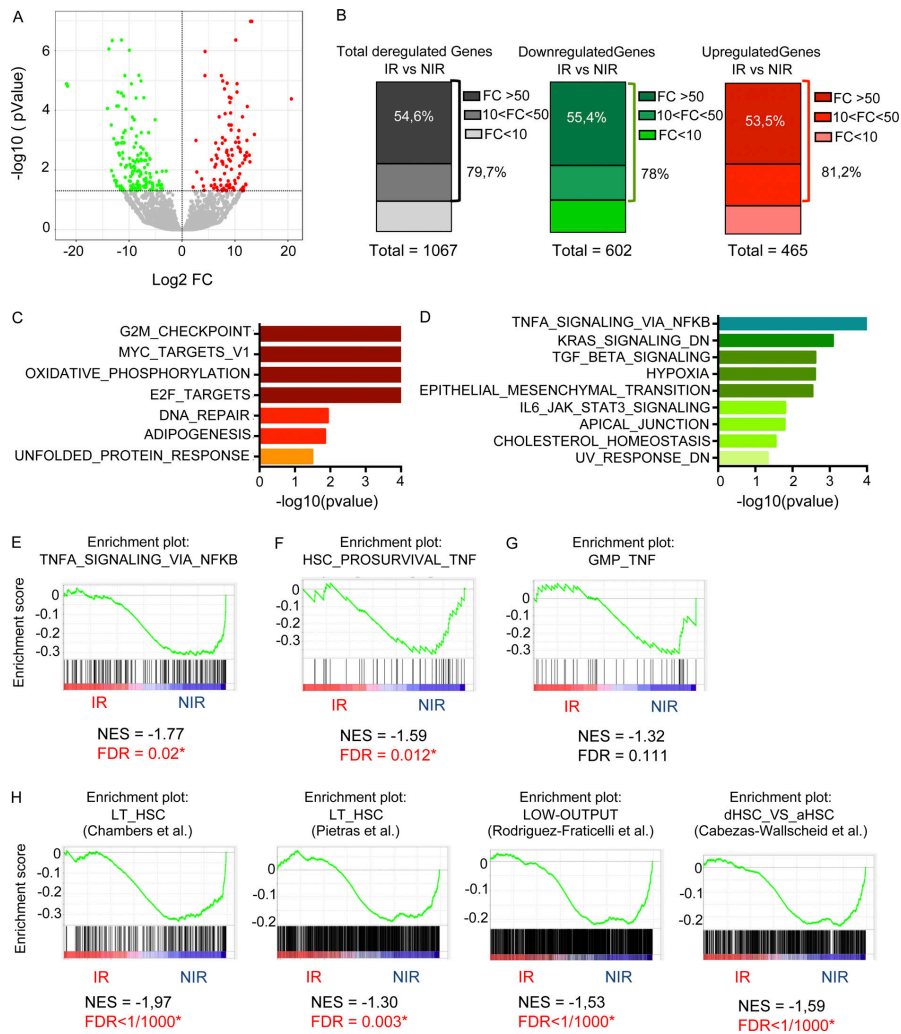


Figure 3. Irradiation induces a strong deregulation of the HSC transcriptome. (A) Volcano plot of the DEGs between IR and NIR conditions. The horizontal axis represents the \log_2 fold change (FC) and the vertical axis the $-\log_{10}(P$ value). Significantly ($P < 0.05$) upregulated (red) and downregulated (green) genes are shown. (B) Repartition of total ($P < 0.05$) DEG (gray) and downregulated (green) or upregulated (red) genes in IR vs. NIR according to their fold change. (C–H) GSEA analysis using Hallmark gene sets. Significant ($P < 0.05$) gene sets gained (C) or lost (D) in the IR condition compared to NIR. $\text{Log}_{10}(P$ value) is set to 4 when $P < 0.001$. Enrichment plots for TNF- α signaling via NF- κ B hallmark (E), HSC prosurvival TNF- α (F), and GMP TNF- α (G) gene signatures. (H) Enrichment plots for LT-HSC; low-output and dormant vs. activated HSC signatures. FDR, false discover rate; NES, normalized enrichment score.

electroporated (FITC+) HSCs for analysis (Fig. 5 F and Fig. S3 D). We first tested different combinations of 5' and 3' gRNAs (Table S4). Only one of these combinations was efficient in deleting the LIMd (Fig. S3 E). Using this combination, we then validated that LIMd deletion occurred in *Mecom*, but not in *Pkia*, through qRT-PCR using the ChIP-qPCR primers (Fig. S3 F). We also validated

that in mock electroporated cells IR could induce the specific downregulation of *Mecom* and *Pkia* but not of *Snx27* expression in vitro, as observed in vivo (Fig. 5 G). Deletion of its intronic LIMd leads to a reduction in *Mecom* gene expression, without affecting *Pkia* or *Snx27* expression, suggesting that the presence of the LIMd is important for the proper regulation of *Mecom*

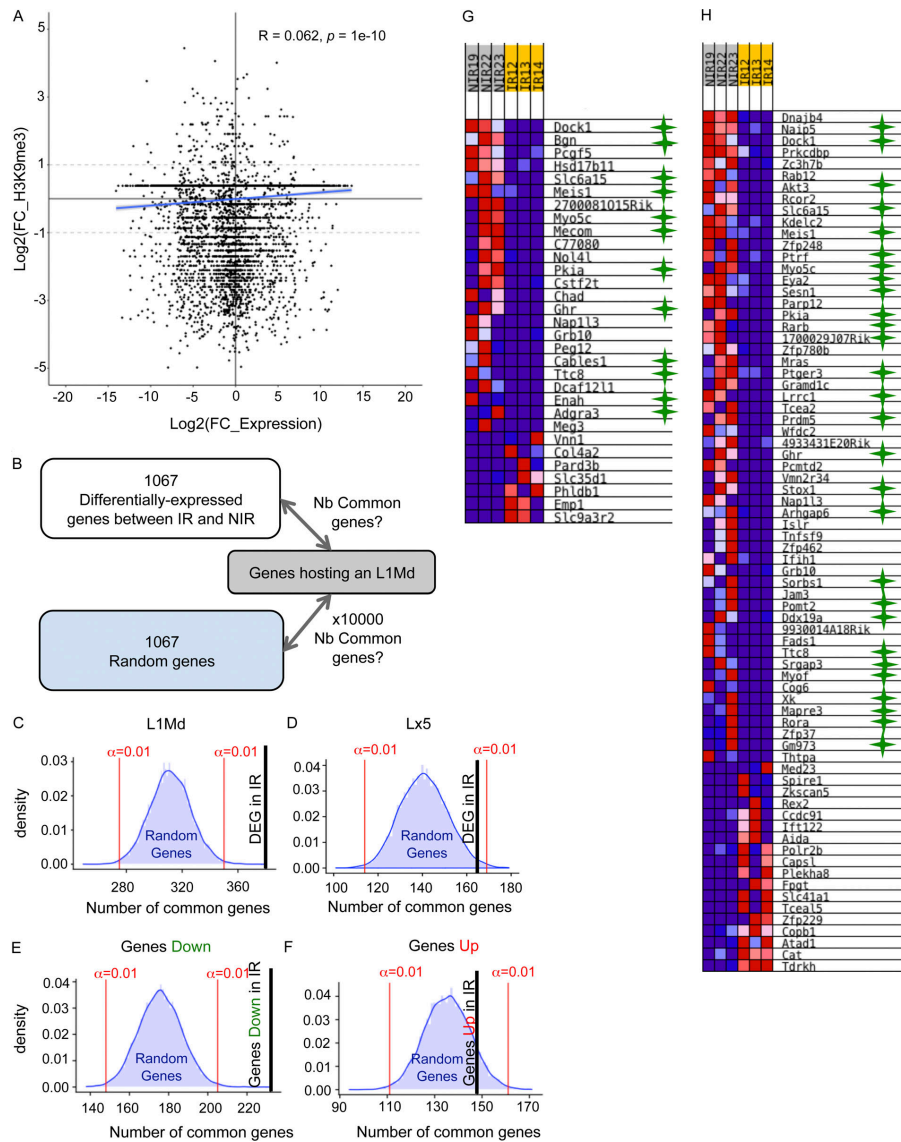


Figure 4. Gene repression upon IR is associated with intronic L1Md. (A) Correlation plot representing the $\log_2(\text{fold change})$ in H3K9me3 concentration at gene promoters vs. $\log_2(\text{fold change})$ in gene expression. (B-F) Permutation test comparing of the number of genes found in common between the list of DEGs or 10,000 lists of random genes and the list of genes hosting one or several L1Md. Blue curve: Distribution of the number of genes found in common between random genes and genes hosting an L1. Black vertical line: number of genes found in common between DEGs and genes hosting an L1Md (C) or an Lx5 (D); or between genes downregulated (E) or upregulated (F) upon IR and hosting an L1Md. Significance bars ($P < 0.01$) are shown in red. (G and H) Heatmaps of the expression of genes from two LT-HSC signatures that are significantly up- (red) or down- (blue) regulated in IR vs. NIR. Green stars indicate the presence of an intronic L1Md in the downregulated gene.

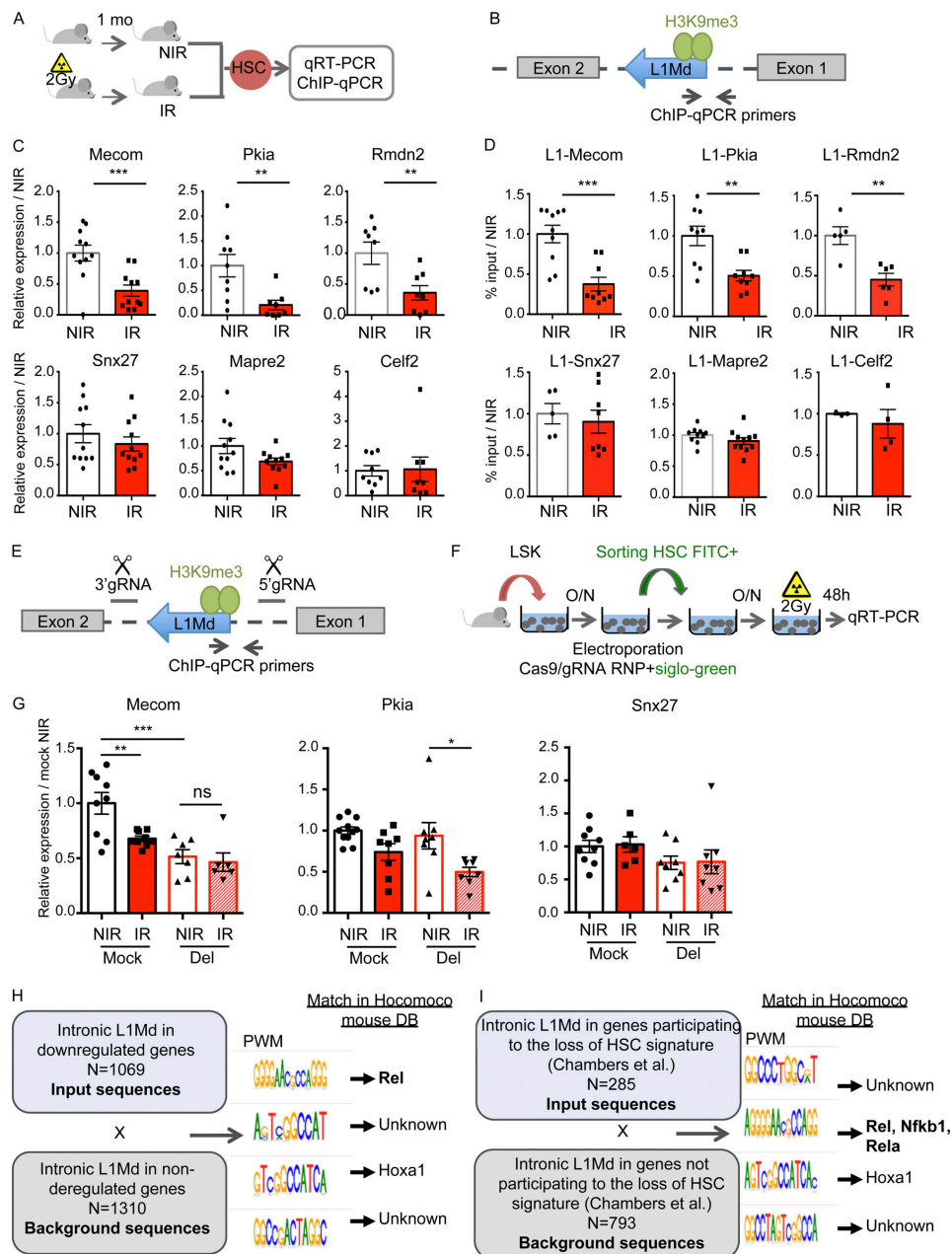


Figure 5. Gene repression upon IR is associated with the loss of H3K9me3 at intronic L1Md loci harboring NF-κB binding sites. (A and C) Experimental design and mRNA expression assessed by qRT-PCR in HSCs 1 mo after TBI. Ct values were normalized to RPL32 and HPRT. Results are expressed as fold change

from the mean value of the NIR condition. Each dot represents a pool of three (NIR) or four (IR) mice. Means \pm SEM from three to four independent experiments. **(B and D)** Experimental design and H3K9me3 ChIP-qPCR enrichment 1 mo after TBI. The primer positioning at the intronic LIMd allowing the amplification of a unique and specific product is shown (B). Each dot represents a pool of three (NIR) or four (IR) mice from two to four independent experiments. Results are means \pm SEM of the percentage of input normalized to the NIR condition. *t* test. **(E–G)** CRISPR/Cas9-induced deletion intronic LIMd in *Mecom* gene. gRNA positioning around LIMd sequence (E), experimental design (F), and mRNA expression assessed by qRT-PCR in Cas9-gRNA RNP electroporated HSCs 48 h after irradiation in vitro (G). Ct values were normalized to Rpl32. mRNA expression was normalized to mock NIR values. Each dot represents a pool of electroporated HSCs from three to five independent experiments. One-way ANOVA with Sidak's multiple comparison test. **(H and I)** De novo motif discovery analysis performed with the BAMMmotif tool on LIMd sequences located in introns of downregulated genes vs. nonderegulated genes (H) or the genes participating vs. not participating to the loss of the LT-HSC signature (I). Enriched motifs were matched to known motifs using the Hocomoco mouse database. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

expression. While *Pkia* expression remained significantly reduced upon IR after *Mecom* intronic LIMd deletion, *Mecom* expression was not affected anymore. This suggests that *Mecom* intronic LIMd acts in cis, and is responsible for the specific gene downregulation upon IR (Fig. 5 G).

To unravel what makes the specificity of H3K9me3 loss at LIMd upon IR, we interrogated the potential enrichment for binding motifs for transcription factors in the LIMd located in genes downregulated in IR (*P* < 0.05, 1069 LIMd = input sequences) vs. nonderegulated genes (*P* > 0.05, 0.7 < fold change < 1.3, 1310 LIMd = background sequences) through de novo motif search using BAMMmotif (<https://bammotif.soedinglab.org/>; Kiesel et al., 2018). Of the four motifs found significantly enriched in LIMd located in downregulated genes, two were not retrieved in the mouse Hocomoco motif database, one motif corresponds to the binding site of Hoxa1, and one to the binding site of Rel, a member of the NF- κ B transcription factor family (Fig. 5 H). De novo motif search in the LIMd located in downregulated genes vs. upregulated genes also retrieved Rel motif (Fig. S3 G). Furthermore, de novo search for motifs specifically enriched in LIMd located in genes participating (284 LIMd) vs. genes not participating (793 LIMd) to the loss of the HSC signature (Fig. 3 H) also revealed significant enrichment for NF- κ B transcription factors Rel, RelA, and Nfkb1 (Fig. 5 I). Similar results were found for the low-output/self-renewing LT-HSC signatures (Fig. S3, H and I). Of note, this motif is present in the intronic LIMd of IR-regulated genes *Mecom*, *Pkia*, *Ttc8*, *Rbms3*, *Akt3*, and *Rmdn2* which lose H3K9me3 upon IR (Fig. 5 D, Fig. S2 C, and Table S3), supporting the possibility that it may regulate the presence of H3K9me3 at these loci.

Performing de novo search on promoter sequences of genes downregulated by IR (*P* < 0.05, 3,893 promoter sequences) vs. nonderegulated genes (0.7 < fold change < 1.3, *P* > 0.05, 14,208 promoter sequences from which we randomly extracted 3,893 sequences) did not show specific enrichment in motifs for NF- κ B members. Instead, motifs for different transcription factors such as Egr, Znf, Foxj, and Foxq were found (Fig. S3 J).

Altogether, these data suggest that the NF- κ B pathway may control HSC gene expression by regulating the presence of H3K9me3 mark at LIMd located in introns, and not by affecting promoters.

TNF- α treatment prevents loss of H3H9me3 at intronic LIMd and HSC gene repression during IR stress

In mammals, the NF- κ B family is composed of five members: RELA (p65), RELB, c-REL, and the precursor proteins NFKB1

(p105) and NFKB2 (p100), which are processed into their active forms, p50 and p52, respectively, and are active as homo- or heterodimers (Cartwright et al., 2016). The canonical NF- κ B pathway involves p50, p65, and c-Rel. P50 lacks transactivation domain. Thus, while p50/p65 or c-Rel heterodimers act as transcriptional activators, p50/p50 (NFKB1) homodimers are generally described as transcriptional repressors. NFKB1 notably represses the expression of proinflammatory genes through the recruitment of chromatin modifiers and H3K9 methylation (Ea et al., 2012; Elsharkawy et al., 2010). In addition, p50 has been shown to shuttle between the nucleus and cytoplasm and to bind to a large number of genes in unstimulated cells (Schreiber et al., 2006). This makes of this factor a good candidate to regulate H3K9me3 levels at LIMd presenting NF- κ B sites and IR-induced HSC gene expression changes. Supporting this possibility, HSCs sorted from mice 1 mo after TBI showed a significant decrease in both NFKB1 mRNA, as well as protein expression tested with two different antibodies (Fig. 6, A–C; and Fig. S4 A). Processing of p105 to p50 is regulated both independently of the NF- κ B activation pathway and during activation of the canonical pathway induced by proinflammatory cytokines such as TNF- α (Cartwright et al., 2016). As shown above, IR induces a loss of the TNFA_signaling_Via_NFKB signature (Fig. 3, D and E). Thus, we asked whether TNF- α stimulation could prevent IR effects by rescuing the levels of p50 homodimers. NFKB1 protein expression decreased after 48 h of culture of purified HSCs that have been irradiated in vitro (Fig. 6, D and E; and Fig. S4 B). Addition of TNF- α to the cell medium prior to IR prevented this effect. As after TBI, the loss of NFKB1 induced by IR in vitro was associated with a specific decrease of *Mecom*, *Pkia*, and *Ttc8* but not of *Celf2*, *Snx27*, and *Mapre2* mRNAs (Fig. 6 F and Fig. S4 C). Importantly, H3K9me3 ChIP-qPCR at intronic LIMd of the selected genes correlated with gene expression with a significant and specific reduction at *Mecom* and *Pkia* intronic LIMd but not at *Snx27* and *Celf2* (Fig. 6 G). This shows that IR-induced H3K9me3 loss at intronic LIMd and gene expression changes in HSCs are direct and short-term and that TNF- α stimulation can rescue both effects. Supporting a role of NFKB1 in these effects, *Nfkb1*^{-/-} HSCs showed reduced expression of *Mecom* and *Pkia* but not of *Snx27* mRNA when compared to WT HSCs (Fig. 6 H). In addition, TNF- α was unable to rescue HSC gene expression or H3K9me3 levels at their intronic LIMd in *Nfkb1*^{-/-} HSCs (Fig. 6, I and J).

We then investigated if TNF- α stimulation could prevent IR effects in vivo. WT mice received two injections with 2 μ g TNF- α at 12-h intervals and were irradiated 1 h after the last injection. We then performed H3K9me3 CUT&Tag and RNA-seq in HSCs

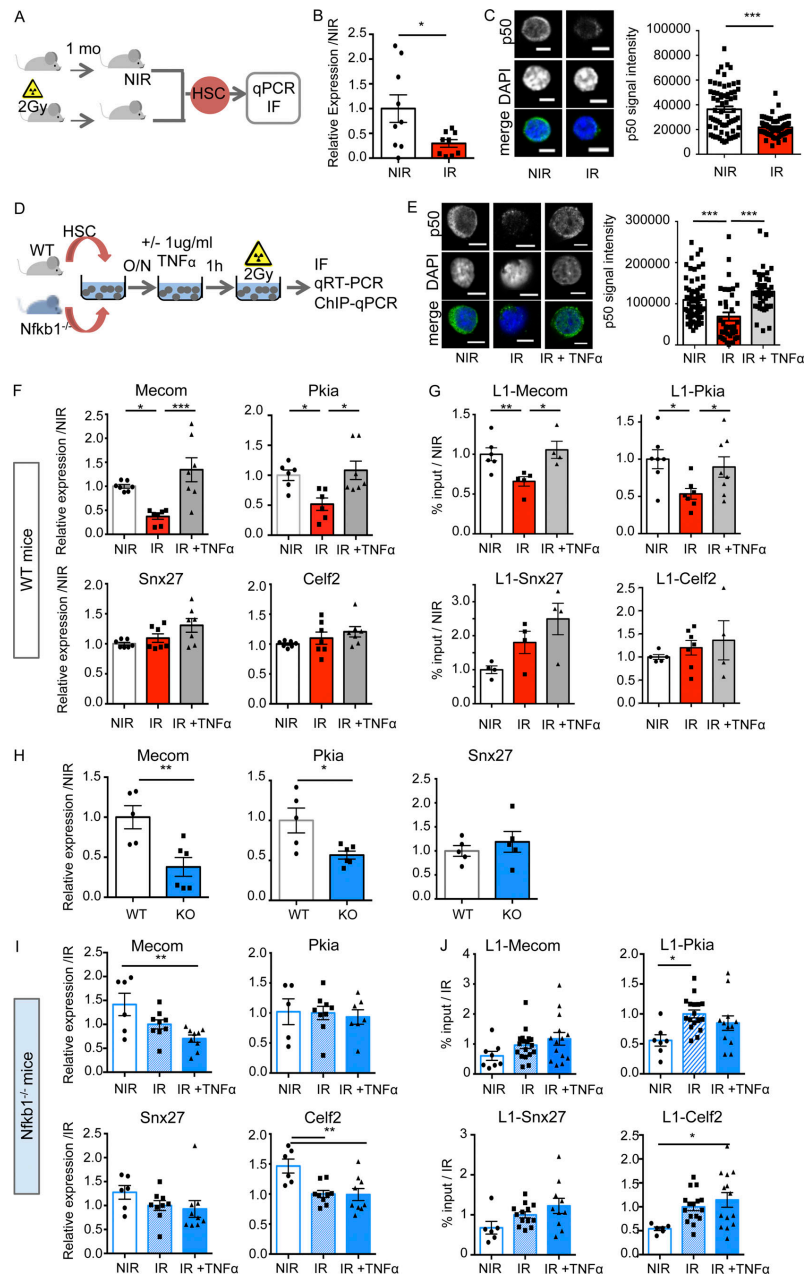


Figure 6. **TNF- α treatment prevents loss of H3H9me3 at intronic L1Md and HSC gene repression in vitro.** (A–C) NFKB1 expression in HSCs 1 mo after TBI. (B) mRNA expression measured by qRT-PCR. Ct values were normalized to mean of RPL32 and HPRT. Results are expressed as fold change from the mean

Pelinski et al.
Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine 11 of 22
<https://doi.org/10.1084/jem.20211356>

Downloaded from http://rupress.org/jem/article-pdf/219/6/e20211356/1435569/jem_20211356.pdf by Institut Gulbena Foussy user on 21 June 2023

value of the NIR condition. Means \pm SEM. Each dot represents a pool of three (NIR) or four (IR) mice from three independent experiments. *t* test. (C) Representative images and quantification of NFKB1 protein mean immunofluorescence (IF) intensity. Bars, 5 μ M. Each dot represents a cell. Results are expressed as fold change from the mean value of the NIR condition from two independent experiments and represented as means \pm SEM. *t* test. (D) Experimental design analyzing the effects of IR and TNF- α in vitro in WT and *Nfkb1*^{-/-} mice. (E) Representative images and quantification of NFKB1 staining. Bars, 5 μ M. Each dot represents a cell. Results are represented as mean \pm SEM of NFKB1 IF intensity. One-way ANOVA with Tukey's multiple comparison test. (F) Gene expression evaluated by qRT-PCR in WT mice. Means \pm SEM from two independent experiments. One-way ANOVA with Tukey's multiple comparison test. (G) H3K9me3 enrichment at intronic LIMd evaluated by ChIP-qPCR. Results are expressed as in legend to Fig. 5 D. Means \pm SEM from two to three independent experiments. One-way ANOVA with Bonferroni's multiple comparison test. (H and I) mRNA expression measured by qRT-PCR in HSCs sorted from *Nfkb1*^{-/-} mice (KO) vs. WT without IR (H) or after IR and with or without prior TNF- α treatment in vitro (I). Ct values were normalized to the mean of RPL32 and HPRT. (J) H3K9me3 enrichment at intronic LIMd evaluated by ChIP-qPCR. For each experiment (H–J), a pool of 14–16 mice was used and divided in two to four culture replicates. Each dot represents one replicate of two to five independent experiments. Means \pm SEM, *t* test (H); one-way ANOVA with Tukey's multiple comparison test (I and J). *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

sorted 1 mo after IR (Fig. 7 A). CUT&Tag gave an efficient profiling of H3K9me3 with exceptionally low background as observed at the SUV39h-1- and -2-dependent H3K9me3 peaks (Bulut-Karslioglu et al., 2014; Fig. S5 A) and as previously described (Kaya-Okur et al., 2019). Plot profile of H3K9me3 enrichment along LIMd sequences confirmed the loss of H3K9me3 at LIMd upon IR and showed it could be prevented by TNF- α (Fig. 7 B). Notably, TNF- α also inhibited the specific loss of H3K9me3 enrichment at the intronic LIMd of *Mecom* and *Pkia* but not *Mapre2* (Fig. 7 C) and restored the corresponding gene expression (Fig. 7 D). RNA-seq analysis further showed that TNF- α injection in vivo prevented IR-induced loss of both TNF- α via NF- κ B (Fig. 7 E) and long-term HSC signatures (Fig. 7 F and Fig. S5 B).

TNF- α treatment prevents HSC loss of function during IR stress independently of their level of DNA damage

To confirm the importance of this pathway in HSC maintenance, we analyzed the effect of TNF- α treatment on HSC function upon IR. Total bone marrow (BM) cells isolated from mice treated with TNF- α before TBI or not were transplanted in competition with total BM cells from mice ubiquitously expressing GFP (ubi-GFP mice) into lethally irradiated ubi-GFP mice (Schaefer et al., 2001; Fig. 7 A). 7 wk and 3 mo after reconstitution, as expected, the percentage of GFP negative IR donor cells in the blood was greatly decreased. TNF- α treatment before TBI significantly prevented this effect (Fig. 8, A and B; and Fig. S5 C). It also significantly prevented IR-induced Lin-Sca+Kit+ (LSK) and LT-HSC loss (Fig. 8, C and D; and Fig. S5 D). Secondary transplants showed that the self-renewal function of HSCs after IR could be restored by TNF- α treatment (Fig. 8 E). TNF- α treatment could similarly rescue blood reconstitution and LT-HSCs in the BM when injected after TBI, either in one dose at 6 h or in two doses at 1 and 13 h (Fig. 8, F–J).

Contrary to what we previously observed with thrombopoietin (de Laval et al., 2013), TNF- α treatment was not able to prevent γ H2AX foci formation upon IR or to enhance the DSB repair as we observed no effect of TNF- α on the number of γ H2AX foci at short (30 min) or long (24 h) term after IR in vitro (Fig. 8 K). In addition, TNF- α treatment had no effect on the number of γ H2AX foci in HSCs 1 mo after IR (Fig. 8 L). Altogether, these data suggest that TNF- α treatment rescues HSC reconstitution ability upon IR independently of their level of DNA damage by preventing IR-induced decrease in NFKB1

repressor expression, specific derepression of LIMd harboring NF- κ B binding sites in the introns of HSC genes, and thereby their repression (Fig. 9).

Discussion

H3K9me3 alterations are a hallmark of aging and cellular senescence in model organisms (Criscione et al., 2016; Ocampo et al., 2016). Although H3K9me3 has been shown to be crucial for HSC identity (Koide et al., 2016), H3K9me3 changes in HSCs have, to our knowledge, never been studied in the context of stress such as IR. We show here that IR stress profoundly affects HSC heterochromatin by significantly reducing H3K9me3 enrichment, without affecting the expression of factors controlling H3K9 tri-methylation (SETDB1, KAP1, or MPP8 from the human silencing hub complex). This loss mainly occurs at evolutionary recent LIMd elements. This effect was observed both after short time in vitro and long time after TBI in vivo, suggesting that heterochromatin alterations may explain the long-term effect of IR on HSC function.

Heterochromatin alterations are associated with a strong deregulation of the HSC transcriptome. H3K9me3 enrichment at promoters has recently emerged as a key player in the repression of lineage-inappropriate genes (Koide et al., 2016). Surprisingly, we found here that gene deregulation is not associated with H3K9me3 changes at gene promoters, but is rather associated with the loss of H3K9me3 at intronic LIMd. H3K9me3 enriched at intronic LIMd was previously shown to be involved in the tight regulation of gene transcription in ESCs (Liu et al., 2018). Some ERVs also play the role of AML enhancers with a driving role in leukemia cell phenotype (Deniz et al., 2020). However, our study is the first showing the involvement of LIMd on the regulation of HSC gene expression.

With CRISPR/Cas9 targeted deletion, we show that the presence of intronic LIMd in *Mecom* is required for *Mecom* gene-specific downregulation upon IR and suggests that intronic LIMd can act in cis to regulate gene expression. Although surprising at first glance, repression of genes following derepression of intragenic LI was previously reported in cancers (Aporntewan et al., 2011). This may be due to transcriptional interference (Han et al., 2004; Kaer et al., 2011; Ninova et al., 2020). We cannot test directly this hypothesis due to the repetitive nature of these sequences. However, we could observe downregulation of *Rmdn2* gene whereas the LIMd located in its

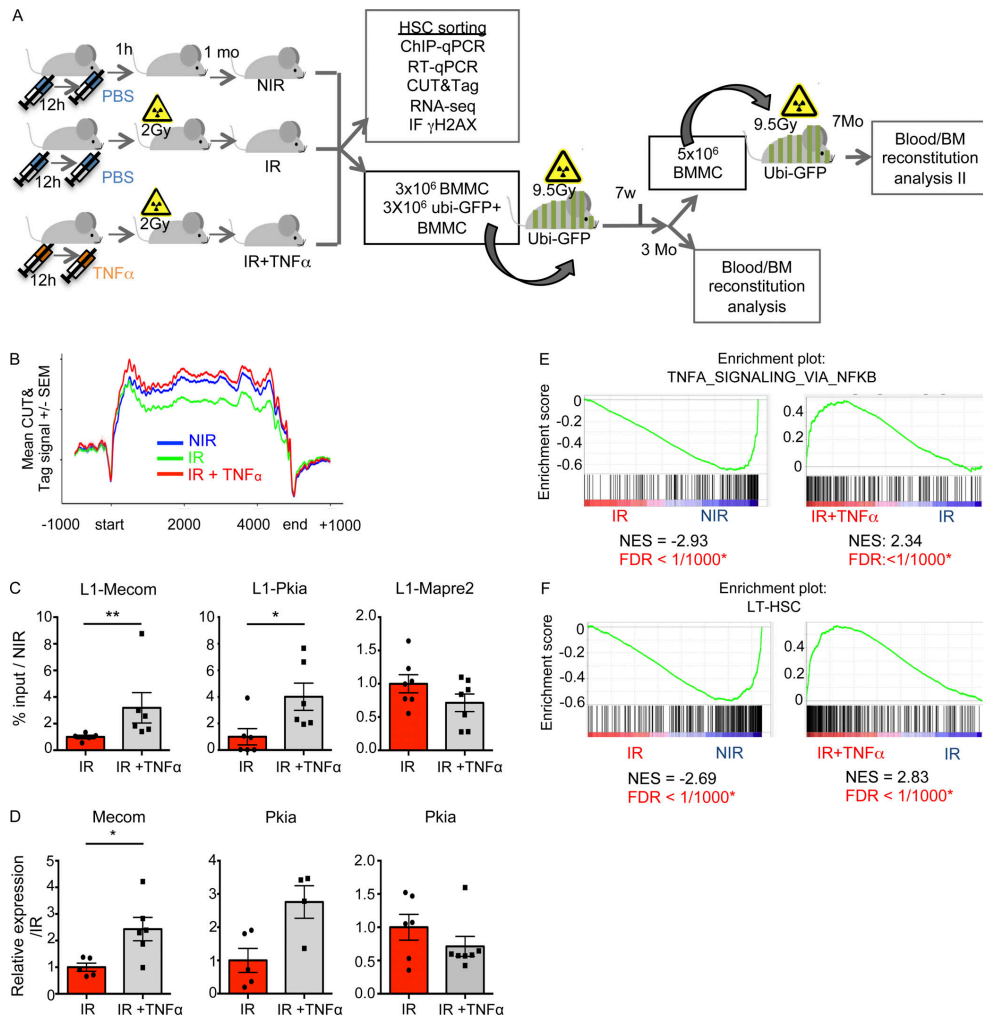


Figure 7. TNF- α treatment prevents loss of H3H9me3 at intronic LIMd and HSC gene repression. (A) Experimental design for TNF- α treatment in vivo, molecular analysis, and reconstitution experiments using HSCs sorted from mice 1 mo after TBI and previously treated with TNF- α (IR + TNF α) or not (IR), or left untreated (NIR); BMMC, bone marrow mononuclear cells. **(B)** Plot profile of H3K9me3 enrichment along the LIMd sequences \pm 1 kb flanking regions in NIR (blue), IR (green), and IR + TNF- α (red) conditions. Each line represents the merged CUT&Tag signal from two (NIR) to three (IR and IR + TNF- α) mice \pm SEM. **(C)** H3K9me3 enrichment at intronic LIMd evaluated by ChIP-qPCR. **(D)** mRNA expression measured by qRT-PCR. Ct values were normalized to RPL32 and HPRT. **(C and D)** Results are expressed as fold change from the mean value of the IR condition. Each dot represents a pool of four mice. Means \pm SEM from two independent experiments, *t* test. **(E and F)** GSEA analysis using gene sets. Enrichment plots for TNF- α signaling via NF- κ B hallmark (E) and LT-HSC signature (F). *, *P* < 0.05; **, *P* < 0.01.

intron lacks 5'UTR promoter sequences (Fig. 5 B), suggesting that gene repression may not be exclusively due to intronic LIMd transcription. Instead of blocking transcription, DNA methylation or H3K9me3 within gene bodies is a feature of transcribed gene (Jones, 2012; Ninova et al., 2020; Vakoc et al.,

2005). H3K9me3 loss in gene bodies was previously shown to be associated with gene repression (Ninova et al., 2020). The presence of H3K9me3 islands in the body of the genes has been proposed to slow-down RNA Polymerase II (RNAP II) elongation rate (Saint-André et al., 2011; Vakoc et al., 2005). This was

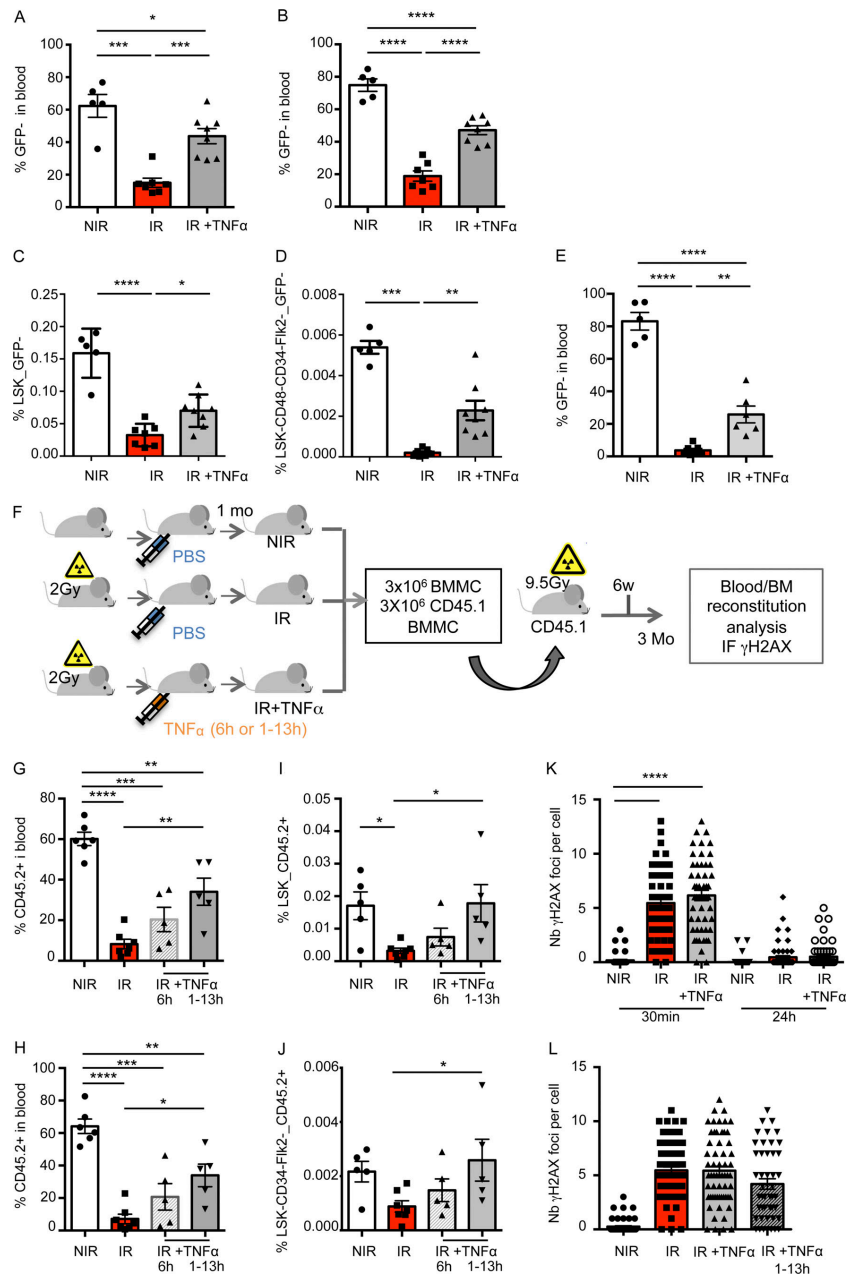


Figure 8. **TNF- α treatment prevents loss of H3H9me3 HSC function in vivo independently of their level of DNA damage.** (A and B) Percentage of GFP-negative donor contribution in blood in mice transplanted with NIR, IR, or IR + TNF- α cells at 7 (A) and 14 wk (B) after reconstitution. (C and D) LSK GFP- (C) or

Pelinski et al.
Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine 14 of 22
<https://doi.org/10.1084/jem.20211356>

Downloaded from http://rupress.org/jem/article-pdf/219/6/e20211356/1435569/jem_20211356.pdf by Institut Gulabie Roussey user on 21 June 2023

LSK CD34⁺Flk2⁻CD48⁻ (D) GFP-negative donor HSC contribution in the BM 14 wk after reconstitution. One-way ANOVA Tukey's multiple comparison test. (E) Percentage of GFP-negative donor contribution in blood in mice secondary transplanted with pool of NIR, IR, or IR + TNF- α mice from the primary reconstitution. One-way ANOVA Tukey's multiple comparison test. (F) Experimental design for TNF- α treatment after IR in vivo and reconstitution experiments using HSCs sorted from mice 1 mo after TBI and treated with TNF- α 6 h after IR (IR + TNF α 6 h), treated with TNF 1 and 13 h after IR (IR + TNF α 1-13 h), irradiated but non-treated with TNF- α (IR), or non irradiated and non treated (NIR); BMMC, bone marrow mononuclear cells. (G and H) Percentage of CD45.2 donor contribution in blood in mice transplanted with NIR, IR, or IR + TNF- α cells at 6 (G) and 11 wk (H). (I and J) Percentage of LSK⁺CD45.2 (I) or LSK⁺CD34⁺Flk2⁻CD45.2⁺ (J) donor HSC contribution in the BM 11 wk after reconstitution. One-way ANOVA Tukey's (I) or Dunnett's (J) multiple comparison test. (K and L) γ H2AX foci number 30 min and 24 h after IR in vitro with or without prior TNF- α treatment (K) or 1 mo after IR in vivo with or without TNF- α treatment before IR (IR + TNF- α) or after IR (IR + TNF- α 1-13 h; L). One-way ANOVA Tukey's multiple comparison test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

shown to help the recognition of true vs. cryptic RNA processing sites, controlling alternative splicing (de la Mata et al., 2003), polyadenylation, and finally transcript stability. This is particularly relevant in the case of genes bearing long introns.

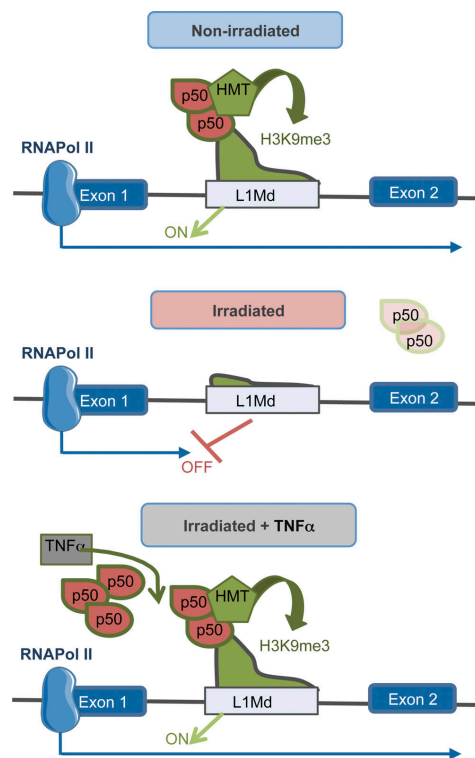


Figure 9. **Model.** At basal, the NF- κ B pathway, possibly through its repressor NFKB1 (p50/p50 homodimers), is involved in the recruitment of H3K9 methylases (HMT) at intronic L1Md enriched in NF- κ B binding sites motifs, and apposition of the repressive histone mark H3K9me3. H3K9me3 "islands" into the body of transcribed genes may help the processing of RNAP II (RNAPol II) and transcript stability. Upon irradiation, loss of the TNF- α -NF- κ B pathway leads to a loss of H3K9me3 at the intronic L1Md, gene repression, and transcript stability. This is prevented by prior TNF- α treatment.

Interestingly, the genes downregulated upon IR in HSCs are significantly longer than by chance (data not shown). Slowing down RNAP II elongation rate in these long intron genes might also prevent R-loop formation and genomic instability (Aguilera and Gaillard, 2014). Thus, even in the absence of intronic L1Md transcription, loss of H3K9me3 islands enriched at intronic L1Md in the body of the genes might lead to gene repression. In accordance with this hypothesis, we showed that deletion of the L1Md in the intron of *Mecom* resulted in decreased *Mecom* gene expression (Fig. 5 G). *Mecom* repression in this case may be due to the absence of H3K9me3 islands in its gene body, as observed upon IR-mediated loss of H3K9me3. However, the precise mechanisms involved in HSC gene regulation through their intronic L1Md will require further investigations.

Our RNA-seq analysis indicates a strong downregulation of the TNF- α -NF- κ B gene signature 1 mo after TBI. IR specifically reduced the recently described HSC-prosurvival TNF- α -NF- κ B signature required to maintain HSCs during inflammation or cytotoxic BM ablation (Yamashita and Passegué, 2019). This suggests that loss of long-term regenerating HSCs and TNF- α -NF- κ B gene expression upon IR may be linked. Supporting this possibility, treatment of HSCs with TNF- α before IR in vitro, or its injection to mice before or after TBI, restored HSC gene expression and their reconstitution ability. These results strongly support previous data showing that TNF- α -NF- κ B signaling is required to regulate HSC function under stress (Hu et al., 2021; de Laval et al., 2014; Yamashita and Passegué, 2019). TNF- α promotes HSC survival through p65/RelA NF- κ B subunit (Yamashita and Passegué, 2019). This factor has also been found to control the expression of genes involved in HSC maintenance (Stein and Baldwin, 2013). However, the promoters of HSC genes downregulated upon IR are not enriched in NF- κ B binding sites. We show that L1Md associated with gene repressed upon IR are specifically and significantly enriched in NF- κ B binding sites, and that this pathway regulates gene expression by controlling the level of H3K9me3 at these sequences. Interestingly, H3K9me3 enriched genomic regions specific to human ESCs as compared to more differentiated cells are enriched in NF- κ B binding sites, suggesting their importance in establishing and maintaining the pluripotent state (Whitaker et al., 2015).

Whereas most of the NF- κ B members can form active transcription factors, NFKB1 p50 subunit lacks transactivation domain and p50:p50 homodimers have been shown to act as stimulus-specific repressors, notably during the resolution phase of inflammation, by recruiting H3K9 methyltransferases

and histone deacetylases at both NF- κ B and type-I IFN response genes (Cartwright et al., 2018; Ea et al., 2012; Elsharkawy et al., 2010). p50:p65 heterodimers are the most abundant form of NF- κ B generated upon inflammatory stimuli. By contrast, p50 homodimers predominate in unstimulated cells where they can be prebound to the chromatin (Cartwright et al., 2016; Schreiber et al., 2006), suggesting that this factor may also play a role under noninflammatory conditions. Indeed, we found that NFKB1 is present in both the cytoplasm and the nucleus of resting HSCs. Deletion of *Nfkb1*, or downregulation of p50/NFKB1 gene and protein as induced by IR, correlates with the decrease of both gene expression and H3K9me3 levels at their intronic LIMd harboring an NF- κ B binding sites. Conversely, increasing p50 production upon TNF- α stimulation rescued H3K9me3 levels at the specific intronic LIMd and gene expression in WT but not in *Nfkb1*^{-/-} HSCs. This strongly supports the possibility that p50:p50 homodimers are the active repressor promoting the enrichment of H3K9me3 at LIMd located in HSC genes and the cis regulation of the host gene.

A growing body of evidence indicates that TEs have been coopted for transcriptional regulation in different cell and tissue types (Chuong et al., 2017). TEs are reservoirs of functional transcription factor binding sites. Since these sequences are widespread in the genome, they are largely contributing to the innovation of regulatory networks in a tissue-specific fashion (Chuong et al., 2017; Hermant and Torres-Padilla, 2021; Sundaram and Wang, 2018). Although LTRs dominate this relationship, a search for binding motifs in young LI in human and mouse has revealed the presence of various TF motifs, including CTCF, YY1, and MYC (Sun et al., 2018; Sundaram and Wang, 2018). Our results show that NF- κ B motifs are specifically enriched in most of the intronic LIMd sequences of genes downregulated during IR stress, and involve as much as 96% of these genes (Table S3). The presence of NF- κ B binding sites in TEs is reminiscent of a study reporting that, in the human genome, 11% of NF- κ B-binding sites reside in specific Alu SINES, and that the vast majority of sites bound by NF- κ B do not correlate with changes in gene expression (Antonaki et al., 2011). Although it is not known how many of these NF- κ B motifs present in intronic LIMd have a functional role, the ability of TNF- α to restore both H3K9me3 levels at the LIMd and the expression specifically of genes including NF- κ B motif-enriched intronic LIMds strongly suggests that at least some of these NF- κ B-TE associations can influence gene expression.

TEs have rewired the antiviral gene regulatory network and they have been shown to play a key role in the regulatory evolution of immune response. Strong but opposing forces are driving the coevolution of TEs and antiviral defense (Chuong et al., 2016; Moelling and Broecker, 2019). Many IFN/NF- κ B-target genes are viral restriction factors and contribute to the immune control of both endogenous (i.e., TEs) and exogenous genomic parasites (Gázquez-Gutiérrez et al., 2021; Schneider et al., 2014). We and others have previously shown that IFN-I signaling controls young LIMd expression and LI retrotransposition in HSCs and various tissues (Barbieri et al., 2018; Goodier et al., 2015; Yu et al., 2015). However, through the formation of double-strand (ds)RNA or cytoplasmic cDNA

resembling viral nucleic acids, TEs are sensed by the cells as invading viruses and promote the activation of IRF3 and NF- κ B transcription factors and the major antiviral immune pathways (Gázquez-Gutiérrez et al., 2021; Volkman and Stetson, 2014). Notably, TE-derived dsRNAs have been shown to provide the inflammatory signal necessary for HSC generation during embryonic development (Lefkopoulos et al., 2020). Intriguingly, beside HSC maintenance genes, many genes involved in IFN and NF- κ B immune response pathway are found among genes downregulated in IR presenting an intronic LIMd with an NF- κ B binding site (Table S3). These include target genes of IFN and NF- κ B, such as *Eif2ak2* and *Oaslq*, that are known to control LI retrotransposition and/or levels, and whose activity are triggered by virus- or TE-derived dsRNAs; *Jak2*, *Tyk2*, *Tnfrsf9*, and *Birc2* involved in IFN and TNF responses, respectively, as well as T-cell suppressing activity genes, *CD274* (PD-L1) and *CD86*. This further reinforces the causal relationships between TEs and immune genes and their coevolution. Interestingly, a higher TE occurrence has been found in immune gene-associated genomic regions and young TEs are specifically enriched in blood cells, as compared to other tissues (Trizzino et al., 2018; Ye et al., 2020). Using BAMMmotif for de novo motif search, we have found that the NF- κ B motif is specifically enriched in LIMd that are present in genes of the HSC signature, and in the myeloid-leucocyte-mediated-immunity signature (GO:0002444) as compared to genes enriched in pancreas, testis, kidney, liver, placenta, and salivary gland (Su et al., 2002; Fig. S3 K), or in genes from the immune system process (GO:0002376) as compared to genes from the reproductive process (GO:0022414; Fig. S3 L). This suggests that NF- κ B binding sites in LIMd might have been actively selected in introns of key HSC genes because of the immune-linked maintenance. This regulation might be important to expand the NF- κ B and TNF- α activity by engaging more genes, including HSC maintenance genes into the NF- κ B regulatory networks. Such activity could be important to maintain HSCs while allowing expression of immune gene during TNF- α -NF- κ B-induced myeloid regeneration or damage-induced bone marrow ablation, and further highlights the complex role of inflammation-induced pathways in HSCs. TNF- α levels are increased in patients with hematopoietic malignancies and the HSC-specific TNF- α signature is upregulated in myelodysplastic syndrome/AML malignant HSCs (Yamashita and Passegué, 2019). Exploring the mechanisms controlling TE expression and how inflammatory signals and aging impact them in normal and malignant HSC could lead to the identification of new selective dependencies of AML and new treatment strategies.

Materials and methods

Mice strains and treatments

WT C57BL/6J mice (6–8 wk old) were from the Envigo Laboratories. *Nfkb1*^{-/-} mice were from The Jackson Laboratory (B6.Cg-Nfkb1^{tm1Bal}/J; Stock No:006097). All the mice were housed in a specific pathogen-free environment. All procedures were reviewed and approved by the Animal Care Committee no. 26 approved by the French Ministry for Research (#2019_078_23286; CE). Mice were injected retro-orbitally with

Pelinski et al.

Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine

<https://doi.org/10.1084/jem.20211356>

16 of 22

Downloaded from <http://rupress.org/jem/article-pdf/121/9/16/20211356/1435699/jem.20211356.pdf> by Institut Galvane Roussey user on 21 June 2023

2 μg TNF- α (Biolegend-Ozyme) before or after sublethal TBI (2 Gy; RX irradiator X- RAD 320).

Cell harvest and culture

Bone marrow was harvested from femur, tibia, and hip bones in mice. Total bone marrow was depleted of differentiated hematopoietic cells (lineage-positive cells) using Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set (BD). Magnetically sorted Lineage-negative (lin⁻) cells were kept overnight (O/N) at 4°C in IMDM medium supplemented with 10% FBS (HyClone) and 1% penicillin-streptomycin (Thermo Fisher Scientific). Staining was performed for 20 min at room temperature (RT) using CD3 ϵ (Lin)-APC clone 145-2C11 (553066; BD), TER-119 (Lin)-APC clone Ter-119 (557909; BD), CD45R/B220 (Lin)-APC clone RA3-6B2 (553092; BD), Ly6G-6C (Lin)-APC clone RB6-8C5 (553129; BD), Ly-6A/E (Sca-1)-PeCy7 clone d7 (558162; BD), CD117 (c-Kit)-PE or PerCP-Cy5.5, clone 2B8 (553355 or 560557, respectively; BD), CD34-FITC or AF700 clone RAM34 (560238 or 560518; BD), CD135 (Flk2)-BV421 or PE clone A2F10.1 (562898 or 553842, respectively; BD). HSCs (Lin⁻Sca⁺c-Kit⁺CD34^{low}Flk2⁻) were sorted using ARIA3, ARIA Fusion, or Influx cell sorters (BD) and collected in Stem Span (StemCell).

When the cells were irradiated in vitro, HSCs were cultured in medium containing Flt3-Ligand, IL-3, IL-6, SCF as described (de Laval et al., 2013) in the presence or absence of TNF- α . TNF- α was added to the medium at 1 $\mu\text{g}/\text{ml}$ 1 h before IR.

CRISPR-Cas9 deletion

gRNAs were designed to generate specific deletion of the intronic LIMd of Mecom using CRISPOR (<http://crispor.tefor.net/>; Table S4). 1 μg total gRNAs (0.5 μg 5'-gRNA + 0.5 μg 3'-gRNA; Dharmacon) were incubated with 1 μg Cas9 (CASI2205; Dharmacon) during 15 min at RT and the Cas9-gRNA RNP was then co-electroporated with an equimolar siglo-green transfection indicator (D-001630-01-05) in 100 000 LSK after an O/N culture in medium containing Flt3-Ligand, IL-3, IL-6, SCF, as described (de Laval et al., 2013), and using a Neon transfection system (Thermo Fisher Scientific) with the optimized electroporation condition 1700 V, 20 ms, 1 pulse as previously described (Gundry et al., 2016). Just after electroporation, FITC + HSC were sorted and collected in Stem Span (StemCell) containing Flt3-Ligand, IL-3, IL-6, SCF, and left O/N in culture before irradiation. Cells were finally collected 48 h after irradiation for further experiments.

DNA extraction and genomic deletion verification

DNA from electroporated HSC was extracted using the tissue XS kit (Macherey-Nagel) according to the manufacturer's instructions, and the specific deletion of Mecom intronic LIMd was checked through qRT-PCR using the ChIP-qPCR primers (Table S4).

qRT-PCR

HSCs were lysed in Tri-Reagent (Zymo Research) and stored at -80°C until used. Total RNA was extracted using the Direct-Zol RNA microprep kit (Zymo research) and reverse-transcribed with EZ Dnase VILO (Invitrogen). Real-time PCR was

performed using the SYBR pPCR premix Ex Taq (Takara) or LUNA Universal qPCR Master Mix (NEB) on a 7500 real-time PCR machine (Applied Biosystems). Samples were tested for qPCR before reverse transcription to rule out detection of contaminating DNA. qPCR primers used were designed in different exons so as to minimize possible gDNA amplification. All data were normalized to the mean expression of RPL32 and hypoxanthine phosphoribosyltransferase (HPRT). Primer sequences are shown in Table S4.

When necessary, 1.25 μl of cDNA was preamplified for 14 PCR cycles in a multiplex reaction using Preamp Master-Mix (100-5580; Fluidigm) and primer mix (200 μM of each primer). To rule out primer dimerization or hairpin formation in the pre-amplification mix, primer sequences were previously analyzed using MFE3.0 PCR Primer Quality Control Software (Wang et al., 2019).

ChIP-qPCR

10,000 HSCs were harvested in 1 ml IMDM medium supplemented with 10% FBS and cross-linked using 1% formaldehyde (Invitrogen) for 10 min at RT. ChIP-qPCR experiments were performed using the True Micro-ChIP Kit (Diagenode) according to the manufacturer's instructions. Cells were sonicated using the Bioruptor Pico (Diagenode) sonication device for 10 cycles (20 s ON/40 s OFF). Chromatin was incubated O/N at 4°C using 0.25 μg of H3K9me3 (C15410193; Diagenode) per IP. ChIP DNA was eluted and purified using the MicroChIP Diapure Columns (Diagenode). Subsequent qPCR was performed as above. ChIP-qPCR primers for intronic LIMd were designed such that one primer is located in the 5' region of the LIMd, and the other primer is located in the intron of the host gene to allow the amplification of unique and specific product (Table S4).

Immunofluorescence

3,000-5,000 HSCs were cytospun on glass slides and immunofluorescence was performed as previously described (de Laval et al., 2013). Two different monoclonal anti-NFKB1 (p50) antibodies were used at 1/200: clone E10 was purchased from Santa Cruz Biotechnology, and clone D4P4D from Cell Signaling. γH2AX antibody was purchased from Millipore (05-636-1) and used at 1/2000. Detection was performed using Alexa Fluor 488-coupled antimouse secondary antibody (1/600). All slides were visualized using SPE confocal microscope (Leica). Pictures were analyzed using CellProfiler.

Statistical analysis

Results were statistically evaluated using either the one-way ANOVA or unpaired *t* test using GraphPad Prism version 6.0 software (GraphPad Software Inc.). The results are displayed as the means and SEM. The value of *, $P < 0.05$ was considered as significant, and **, $P < 0.01$ or ***, $P < 0.001$ as highly significant.

ChIP-seq

The ChIP-seq experiment has been conducted by Diagenode ChIP-seq (Cat# G02010000; Diagenode). The chromatin was prepared using the True MicroChIP Kit (Cat# C01010130; Diagenode). Chromatin was sheared using Bioruptor Pico sonication

Pelinski et al.

Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine

<https://doi.org/10.1084/jem.20211356>

17 of 22

Downloaded from http://press.oxfordjournals.org/jem/article-pdf/121/9/16/20211356/1435569/jem_20211356.pdf by Institut Galvane Roussey user on 21 June 2023

device (Cat# B01060001; Diagenode) combined with the Bioruptor Water cooler for seven cycles using 30" (ON) 30" (OFF) settings. Shearing was performed in 0.65 ml Bioruptor Pico Microtubes (Cat# C30010011; Diagenode) with the following cell numbers: 10,000 cells in 100 μ l for batch #1 and 20,000 in 100 μ l for batch #2. 30 μ l of this chromatin was used to assess the size of the DNA fragments obtained by High Sensitivity NGS Fragment Analysis Kit (DNF-474) on a Fragment Analyzer (Advanced Analytical Technologies, Inc.). ChIP was performed using IP-Star Compact Automated System (Cat# B03000002; Diagenode) following the protocol of the aforementioned kit. Chromatin corresponding to 7,000 or 18,000 cells was immunoprecipitated using 0.5 μ g of H3K9me3 antibody (C15410193; Diagenode antibody). Chromatin corresponding to 10% was set apart as Input.

For each sample, a library preparation was performed on 500 pg of DNA using the MicroPLEX v2 protocol. The ChIP samples were processed together and a control library was processed in parallel of the samples using the same amount of a control Diagenode ChIP'd DNA. According to the protocol, 12 cycles of amplification were performed to amplify the libraries. After amplification, 1 μ l of each library was loaded on Fragment Analyzer to check if enough material was generated. If not, additional cycles were performed until having enough material. The libraries were amplified for two to seven additional cycles, and then 1 μ l of the libraries was analyzed on the Fragment Analyzer. Using the quantification values from the Qubit and the size measurement generated by the Fragment Analyzer, the molar concentration of each library was calculated. Then, the different libraries were diluted to reach the final concentration each and pooled together. Batch #1 was sequenced into two lanes of a HiSeq 4000 (75 bp, paired end), and batch #2 was sequenced into one lane of a NovaSeq (150 bp, paired-end).

RNA-seq

HSCs from individual mice were lysed in Tri-Reagent (Zymo Research) and stored at -80°C until used. Total RNA was extracted using the Direct-Zol RNA microprep kit (Zymo research). For the IR vs. NIR analysis, the RNA integrity (RNA Integrity Score ≥ 7.0) was checked on the Agilent Fragment Analyzer (Agilent) and quantified. All samples were subjected to SMARTer cDNA synthesis using SMARTer Ultra Low Input RNA Kit for Sequencing - v3. Double-stranded cDNA (ds-cDNA) was sheared using Covaris to obtain ds-cDNA in the 200–500 bp size range. ds-cDNA fragments were end-repaired, extended with an "A" base on the 3' end, ligated with indexed paired-end adaptors (NEXTflex; Bioo Scientific) using the Bravo Platform (Agilent), amplified by PCR for 6 cycles and purified with AMPure XP beads (Beckman Coulter). For TNF- α analysis, all samples were subjected to SMARTer cDNA synthesis using SMARTer stranded total RNA-seq kit v3 following the manufacturer's instructions. Fragmentation time is adjusted depending on the quality of the RNA input.

The final libraries were pooled and sequenced using the on-board cluster method, as paired-end sequencing (2×100 bp reads) on Illumina NovaSeq-6000 sequencer at Gustave Roussy (Illumina).

Pelinski et al.

Cis-regulation of HSC genes by intronic LINE-1

CUT&Tag

CUT&Tag-IT assay kit (Active Motif) was used on 3,000 HSCs according to the manufacturer's instructions. Cells were incubated O/N with 0.5 μ g of H3K9me3 (C15410193; Diagenode).

Genomic analysis

RNA-seq

Reads quality. Quality of RNA-seq reads was assessed with Fastqc v0.11.8, Fastq-screen (Wingett and Andrews, 2018) v0.13.0, and MultiQC (Ewels et al., 2016) v1.7.

RNA quantification. Salmon (Patro et al., 2017) tool v0.14.1 was used to quantify mm10 NCBI RNA reference sequences (O'Leary et al., 2016; RefSeq Curated, last updated 2017-11-16) downloaded from the UCSC Table Browser (Karolchik et al., 2004). Salmon was launched with the following parameters: `--numBootstraps 60 --libType A --validateMappings`.

For the second RNA-seq performed in NIR, IR, and IR + TNF- α conditions, we used nf-core/rnaseq (version 3.3) pipeline for RNAseq analysis (<https://doi.org/10.5281/zenodo.1400710>), with the following additional parameters: `--genome mm10 --clip_r2 14`, and performed on the Core Cluster of the Institut Français de Bioinformatique (ANR-11-INBS-0013).

Differential gene expression analysis. Statistical analysis was performed using R v3.5.1. Transcript expression levels were aggregated in gene expression levels using tximport Bioconductor package (Soneson et al., 2015) v1.13.16. Deseq2 (Love et al., 2014) v1.22.2 method was used to identify differentially expressed genes between groups with a P value threshold of 0.05.

Permutation test. To create the list of genes hosting an LiMd, browser extensible data (BED) files containing LiMd genomic localizations (reconstructed Replibase from Walter et al. [2016]) were intersected with the refseq_curated database from UCSC. Permutation test ($n = 10,000$) between lists of genes hosting an LiMd and DEG in IR vs. NIR, or the same number of random genes (randomly extracted from Refseq without DEG) was performed using R studio and considered significant if $P < 0.01$.

Motif enrichment analysis. Motif enrichment analysis was performed using BaMM! Web interface (Kiesel et al., 2018; Siebert and Söding, 2016) and de novo motif discovery module (pattern = 10, $P < 0.001$). Query motif was matched to known motifs using the Hocomoco mouse database.

GSEA analysis. GSEA analysis was performed using Hallmark Gene Sets V7. To plot graphs, $-\log_{10}$ P value is set to 4 when $P < 0.0001$.

ChIP-seq

Alignment. Human sequences were found in mouse ChIP-seq reads. The contamination was removed with Xenome (Conway et al., 2012) v1.0.0. After contamination removal, ChIP-seq sequence reads were mapped to the Mouse genome build mm10 by using Burrows-Wheeler Aligner MEM algorithm (Li and Durbin, 2009; BWA v0.7.17). The read group ID was attached to every read in the resulting alignment file (bam file) with the -R parameter, and shorter split hits were marked as secondary with -M. Samtools (Li et al., 2009) fixmate v1.9 was used to check mate-pair information between mates and fixed if needed on a

Journal of Experimental Medicine

<https://doi.org/10.1084/jem.20211356>

18 of 22

Downloaded from <http://press.oxfordjournals.org/> by Institut Gustave Roussy user on 21 June 2023

name-sorted bam file. The duplicate reads were tagged by samtools markduplicates using a position sorted bam file. Secondary alignments and unmapped reads have been filtered out and only properly paired reads have been kept. Two types of downstream analysis have been performed, with multimapped reads (mapping quality score ≥ 0) and one with uniquely mapped reads (mapping quality score ≥ 1). Cross-correlation scores (normalized or relative strand cross-correlation coefficient) have been calculated by phantompeakqualtools package (Kharchenko et al., 2008; Landt et al., 2012) v1.2. DeepTools (Ramírez et al., 2016) bamCoverage v3.3.0 has been used to generate normalized bigwig files with the following parameters: --binSize 1 --normalizeUsing BPM --extendReads --ignoreDuplicates. Then, deepTools bigwigCompare was used to subtract input signal from chip signal.

Peak calling. Areas in the genome enriched with aligned reads (also called peaks) were identified with MACS2 (Zhang et al., 2008) callpeak v2.1.2 with the following parameters: -f BAMPE -g mm10 -q 0.05 --broad --broad-cutoff 0.05 for H3K9me3 broad mark.

Irreproducible discovery rate (IDR) analysis. To measure the reproducibility between replicate experiments, we used the IDR method (Li et al., 2011) v2.0.4.2 with the following parameters: --rank q.value --random-seed 12345 --plot. Peaks with a global IDR score < 0.05 were selected and used for downstream analysis.

Peak annotation. Annotatr 1.8.0 (R3.5.1) was used for peak annotation.

H3K9me3 quantification and differential binding. To quantify H3K9me3 concentration at TE or promoters (-2 kb; $+1$ kb TSS), the Bioconductor package Diffbind (Ross-Innes et al., 2012) v2.10 was used in R v3.5.1. Paired-end mode was activated for read counting step with SummarizeOverlaps method. The default mapping quality threshold (mapQCth) was modified in 0 for multimapping analysis or 1 for unique mapping analysis. DBA_DESEQ2_BLOCK method was used to consider unwanted variable during normalization. Normalized H3K9me3 concentration at all TE loci from a same family/subfamily was summed to get a total H3K9me3 concentration per TE family. The age of a TE was calculated as in Sookdeo et al. (2013): divergences were converted to time assuming a neutral rodent genomic substitution rate of 1.1%/million yr.

Differential binding at peaks was identified with a P value threshold of 0.05.

Heatmaps. To plot heatmaps of H3K9me3 enrichment at peaks, deeptools package v3.2.0 was used in R v3.5.1. The peaks (IDR < 0.05) files obtained for NIR and IR conditions were first fused using bedops. A matrix was then built using ComputeMatrix tool in the scale-regions mode between the generated fused bed file and the corresponding normalized bigwig files after input subtraction. A body length of 2.5 kb (mean size of the peaks) was selected, as well as a 4-kb distance upstream and downstream of the start and the end of the peak. We asked for a “-outFileSortedRegions” that gives the sorted bed file used for the heatmap. This sorted bed file was then used for genome coverage analysis, i.e., identification of the presence of a given

TE in each row after computing a matrix with the TE genome coverage bigwig.

TE genome coverage. To generate TE genome coverage, bedtools package v2.27.1 was used. -bga option on the genomeCoverageBed tool was used. The bedGraphs generated were then converted to bigwig files using the bedGraphToBigWig tool.

CUT&Tag

CUT&Tag was analyzed as described as in https://yezhenstat.github.io/CUTTag_tutorial/index.html with the following parameters: Quality Control was performed using FastQC (0.11.9) and MutiQC (1.10.1); Bowtie2 (2.4.1) alignment to mm10 (UCSC genome) was performed with the following parameters: --end-to-end --very-sensitive --no-mixed --no-discordant --phred33 -I 10 -X 700; duplicate reads were removed using Picar (2.26.9) with the following parameters: --REMOVE_DUPLICATES true --VALIDATION_STRINGENCY LENIENT; aligned read quality score was set to 0 to keep all reads by using samtools (1.13) with the following parameters: -q 0; aligned reads were sorted and indexed using samtools (1.13); a coverage track (bigWig) was generated using deeptools (3.5.0) with the following parameters: -bs 5 --normalizeUsing BPM; peak calling was performed using macs2 (2.2.7.1) with the following parameters: -B --broad --broad-cutoff 0.1 -f BAMPE -g mm --max-gap 2000 --min-length 200; profile plot for scores over genomic regions (mm10.rmsk.mod.LIMd.bed) were performed using deeptools (3.5.0) with the following parameters: --beforeRegionStartLength 1000 --regionBodyLength 5000 --afterRegionStartLength 1000 \; statistics of the CUT and TAG signal were performed using the R package Rseab 0.2.0: using as input a score matrix computed by deeptools's computeMatrix, we plotted the mean density profile of all condition with the SEM.

Online supplemental material

Fig. S1 relates to Figs. 1 and 2 showing additional information on ChIP-seq data. Fig. S2 relates to Figs. 3 and 4 showing additional information on RNA-seq data and the comparison between the ChIP-seq and the RNA-seq data. Fig. S3 relates to Fig. 5 showing ChIP-qPCR at intronic LIMd of additional target genes, the gating strategy for sorting HSC electroporated with the Cas9/gRNA RNP complex for intronic LIMd deletion, the results of the tests for gRNAs efficiency, and additional de novo motif search. Fig. S4 relates to Fig. 6 showing NFKB1 immunofluorescence data using another antibody (sc-8414, clone E10) and the effect of TNF- α on gene expression upon IR at additional target genes. Fig. S5 relates to Figs. 7 and 8 showing comparison of ChIP-seq and CUT&Tag profiles, additional gene signatures affected by IR and TNF- α treatment, and the gating strategies for analyzing HSC reconstitution capacity in blood and BM after IR and with or without TNF- α treatment. Table S1 shows quality control of the reads and peak calling data for the ChIP-seq analysis, Table S2 shows information on differentially expressed genes upon IR for RNA-seq analysis, Table S3 gives information on intragenic LIMd, and Table S4 lists primers and gRNAs used for the study.

Data availability

The dataset generated from the ChIP-seq for Figs. 1 and 2 are available in ArrayExpress accession no. E-MTAB-11865, from the RNA-seq for Figs. 3 and 4 in ArrayExpress accession no. E-MTAB-11866, and from RNA-seq and CUT&Tag for Fig. 7 in ArrayExpress accession nos. E-MTAB-11867 and E-MTAB-11864, respectively.

Acknowledgments

We thank the animal facility, the Genomic and the Imaging and Cytometry Platforms of Gustave Roussy for RNA-seq, confocal, and cell sorting analysis, respectively; S. Gregoricchio and Drs. C. Lobry and C. Guillouf (INSERM U1170, Gustave Roussy) for their advice for ChIP-seq analysis; D. Dubray for statistical analysis; and Drs. M. Goodhardt and D. Garrick (INSERM UMRS-1126, Paris) for helpful discussions. We also thank A. Teissandier and D. Bourc'his for providing us with the reconstructed repeatMasker database.

This work was supported by INSERM and grants from Ligue Nationale Contre le Cancer (LNCC; Equipe labellisée EL2020) and Institut National du Cancer (PLBIO No. 2020-095) to F. Porteu, ARC Foundation (No. 20161204988), GEFLUC Paris-Ile de France (2017), LNCC (2018–2019), and Agence National de la Recherche (ANRJC2C-02-CE14-0018-01) to E. Elvira-Matelot. Y. Pelinski and D. Hidaoui are recipients of fellowship from the Ministère de l'Enseignement Supérieur de la Recherche et de l'Innovation. A. Stolz is recipient of a fellowship from LNCC. F. Hermetet is supported by Institut National du Cancer (PLBIO no. 2020-095 to F. Porteu).

Author contributions: Y. Pelinski, A. Stolz, and E. Elvira-Matelot performed the RNA-seq, ChIP-seq, CUT&Tag, and ChIP-qPCR experiments and analyzed the results. M.K. Diop, R. Chelbi, A.M. Chioukh, and E. Elvira-Matelot performed bioinformatic analyses. D. Hidaoui, F. Hermetet, and A. Stolz performed IF, qPCR, and reconstitution experiments and analyzed the results. F. Porteu and E. Elvira-Matelot designed and supervised the study, analyzed the results and wrote the manuscript.

Disclosures: The authors declare no competing interests exist.

Submitted: 23 June 2021

Revised: 23 December 2021

Accepted: 21 June 2022

References

Adoue, V., B. Binet, A. Malbec, J. Fourquet, P. Romagnoli, J.P.M. van Meerwijk, S. Amigorena, and O.P. Joffre. 2019. The histone methyltransferase SETDB1 controls T helper cell lineage integrity by repressing endogenous retroviruses. *Immunity*. 50:629–644.e8. <https://doi.org/10.1016/j.immuni.2019.01.003>

Aguilera, A., and H. Gaillard. 2014. Transcription and recombination: When RNA meets DNA. *Cold Spring Harbor Perspect. Biol.* 6:a016543. <https://doi.org/10.1101/cshperspect.a016543>

Antonaki, A., C. Demetriades, A. Polyzos, A. Banos, G. Vatsellas, M.D. Lavigne, E. Apostolou, E. Mantouvalou, D. Papadopoulou, G. Mosialos, and D. Thanos. 2011. Genomic analysis reveals a novel nuclear factor- κ B

(NF- κ B)-binding site in Alu-repetitive elements. *J. Biol. Chem.* 286: 38768–38782. <https://doi.org/10.1074/jbc.M111.234161>

Aporntewan, C., C. Phokaew, J. Piriyaopongsa, C. Ngamphiw, C. Ittiwut, S. Tongshima, and A. Mutirangura. 2011. Hypomethylation of intragenic LINE-1 represses transcription in cancer cells through AGO2. *PLoS One*. 6:e17934. <https://doi.org/10.1371/journal.pone.0017934>

Barau, J., A. Teissandier, N. Zamudio, S. Roy, V. Nalesso, Y. Héroult, F. Guillouf, and D. Bourc'his. 2016. The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science*. 354:909–912. <https://doi.org/10.1126/science.aah5143>

Barbieri, D., E. Elvira-Matelot, Y. Pelinski, L. Genève, B. de Laval, G. Yogarajah, C. Pecquet, S.N. Constantinescu, and F. Porteu. 2018. Thrombopoietin protects hematopoietic stem cells from retrotransposon-mediated damage by promoting an antiviral response. *J. Exp. Med.* 215:1463–1480. <https://doi.org/10.1084/jem.20170997>

Bulut-Karslioglu, A., I.A. De La Rosa-Velázquez, F. Ramirez, M. Barenboim, M. Onishi-Seebacher, J. Arand, C. Galán, G.E. Winter, B. Engist, B. Gerle, et al. 2014. Suv39h-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. *Mol. Cell*. 55: 277–290. <https://doi.org/10.1016/j.molcel.2014.05.029>

Cabezas-Wallscheid, N., F. Buettner, P. Sommerkamp, D. Klimmeck, L. Ladel, F.B. Thalheimer, D. Pastor-Flores, L.P. Roma, S. Renders, P. Zeisberger, et al. 2017. Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. *Cell*. 169:807–823.e19. <https://doi.org/10.1016/j.cell.2017.04.018>

Cartwright, T., N.D. Perkins, and C. L. Wilson. 2016. NFKB1: A suppressor of inflammation, ageing and cancer. *FEBS J.* 283:1812–1822. <https://doi.org/10.1111/febs.13627>

Cartwright, T.N., J.C. Worrell, L. Marchetti, C.M. Dowling, A. Knox, P. Kiely, J. Mann, D.A. Mann, and C.L. Wilson. 2018. HDAC1 interacts with the p50 NF- κ B subunit via its nuclear localization sequence to constrain inflammatory gene expression. *Biochim. Biophys. Acta Gene Regul. Mech.* 1861:962–970. <https://doi.org/10.1016/j.bbagr.2018.09.001>

Chambers, S.M., C.A. Shaw, C. Gatz, C.J. Fisk, L.A. Donehower, and M.A. Goodell. 2007. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol.* 5:e201. <https://doi.org/10.1371/journal.pbio.0050201>

Chuong, E.B., N.C. Elde, and C. Feschotte. 2016. Regulatory evolution of innate immunity through co-option of endogenous retroviruses. *Science*. 351:1083–1087. <https://doi.org/10.1126/science.125497>

Chuong, E.B., N.C. Elde, and C. Feschotte. 2017. Regulatory activities of transposable elements: From conflicts to benefits. *Nat. Rev. Genet.* 18: 71–86. <https://doi.org/10.1038/nrg.2016.139>

Clapes, T., A. Polyzou, P. Prater, Sagar, A. Morales-Hernández, M.G. Ferrarini, N. Kehrler, S. Lefkopoulos, V. Bergo, B. Hummel, et al. 2021. Chemotherapy-induced transposable elements activate MDA5 to enhance haematopoietic regeneration. *Nat. Cell Biol.* 23:704–717. <https://doi.org/10.1038/s41556-021-00707-9>

Conway, T., J. Wazny, A. Bromage, M. Tymms, D. Sooraj, E.D. Williams, and B. Beresford-Smith. 2012. Xenome: A tool for classifying reads from xenograft samples. *Bioinformatics*. 28:1172–1178. <https://doi.org/10.1093/bioinformatics/bts236>

Criscione, S.W., Y.V. Teo, and N. Neretti. 2016. The chromatin landscape of cellular senescence. *Trends Genet.* 32:751–761. <https://doi.org/10.1016/j.tig.2016.09.005>

Deniz, O., M. Ahmed, C.D. Todd, A. Rio-Machin, M.A. Dawson, and M.R. Branco. 2020. Endogenous retroviruses are a source of enhancers with oncogenic potential in acute myeloid leukaemia. *Nat. Commun.* 11:3506. <https://doi.org/10.1038/s41467-020-17206-4>

Djehghoul, D., K. Kuranda, I. Kuzniak, D. Barbieri, I. Naguibneva, C. Choisy, J.-C. Bories, C. Dosquet, M. Pla, V. Vanneaux, et al. 2016. Age-associated decrease of the histone methyltransferase SUV39H1 in HSC perturbs heterochromatin and B lymphoid differentiation. *Stem Cell Rep.* 6: 970–984. <https://doi.org/10.1016/j.stemcr.2016.05.007>

Ea, C.-K., S. Hao, K.S. Yeo, and D. Baltimore. 2012. EHMT1 protein binds to nuclear factor- κ B p50 and represses gene expression. *J. Biol. Chem.* 287: 31207–31217. <https://doi.org/10.1074/jbc.M112.365601>

Elsharkawy, A.M., F. Oakley, F. Lin, G. Packham, D.A. Mann, and J. Mann. 2010. The NF- κ B p50:p50:HDAC-1 repressor complex orchestrates transcriptional inhibition of multiple pro-inflammatory genes. *J. Hepatol.* 53:519–527. <https://doi.org/10.1016/j.jhep.2010.03.025>

Ewels, P., M. Magnusson, S. Lundin, and M. Källér. 2016. MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*. 32:3047–3048. <https://doi.org/10.1093/bioinformatics/btw354>

Pelinski et al.

Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine
<https://doi.org/10.1084/jem.20211356>

20 of 22

- Fang, J., T. Muto, M. Kleppe, L.C. Bolanos, K.M. Hueneman, C.S. Walker, L. Sampson, A.M. Wellendorf, K. Chetal, K. Choi, et al. 2018. TRAF6 mediates basal activation of NF- κ B necessary for hematopoietic stem cell homeostasis. *Cell Rep.* 22:1250–1262. <https://doi.org/10.1016/j.celrep.2018.01.013>
- Fleener, C.J., A.I. Rozhok, V. Zaberezhnyy, D. Mathew, J. Kim, A.-C. Tan, L.D. Bernstein, and J. DeGregori. 2015. Contrasting roles for C/EBP α and Notch in irradiation-induced multipotent hematopoietic progenitor cell defects. *Stem Cell.* 33:1345–1358. <https://doi.org/10.1002/stem.1936>
- Gázquez-Gutiérrez, A., J. Witteveldt, S. R. Heras, and S. Macías. 2021. Sensing of transposable elements by the antiviral innate immune system. *RNA.* 27:735–752. <https://doi.org/10.1261/rna.078721.121>
- Goodier, J.L., G.C. Pereira, L.E. Cheung, R.J. Rose, and H.H. Kazazian. 2015. The broad-spectrum antiviral protein ZAP restricts human retrotransposition. *PLoS Genet.* 11:e1005252. <https://doi.org/10.1371/journal.pgen.1005252>
- Gundry, M.C., L. Brunetti, A. Lin, A.E. Mayle, A. Kitano, D. Wagner, J.I. Hsu, K.A. Hoegenauer, C.M. Rooney, M.A. Goodell, and D. Nakada. 2016. Highly efficient genome editing of murine and human hematopoietic progenitor cells by CRISPR/Cas9. *Cell Rep.* 17:1453–1461. <https://doi.org/10.1016/j.celrep.2016.09.092>
- Han, J.S., S.T. Szak, and J.D. Boeke. 2004. Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. *Nature.* 429:268–274. <https://doi.org/10.1038/nature02536>
- Hermant, C., and M.-E. Torres-Padilla. 2021. TFs for TEs: The transcription factor repertoire of mammalian transposable elements. *Genes Dev.* 35: 22–39. <https://doi.org/10.1101/gad.344473.120>
- Hu, M., Y. Lu, H. Zeng, Z. Zhang, S. Chen, Y. Qi, Y. Xu, F. Chen, Y. Tang, M. Chen, et al. 2021. MicroRNA-21 maintains hematopoietic stem cell homeostasis through sustaining the NF- κ B signaling pathway in mice. *Haematologica.* 106:412–423. <https://doi.org/10.3324/haematol.2019.236927>
- Jachowicz, J.W., X. Bing, J. Pontabry, A. Bošković, O.J. Rando, and M.-E. Torres-Padilla. 2017. LINE-1 activation after fertilization regulates global chromatin accessibility in the early mouse embryo. *Nat. Genet.* 49: 1502–1510. <https://doi.org/10.1038/ng.3945>
- Jang, H.S., N.M. Shah, A.Y. Du, Z.Z. Dailey, E.C. Pehrsson, P.M. Godoy, D. Zhang, D. Li, X. Xing, S. Kim, et al. 2019. Transposable elements drive widespread expression of oncogenes in human cancers. *Nat. Genet.* 51: 611–617. <https://doi.org/10.1038/s41588-019-0373-3>
- Jones, P.A. 2012. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13:484–492. <https://doi.org/10.1038/nrg3230>
- Kaer, K., J. Branovets, A. Hallikma, P. Nigumann, and M. Speek. 2011. Intronic L1 retrotransposons and nested genes cause transcriptional interference by inducing intron retention, exonization and cryptic polyadenylation. *PLoS One.* 6:e26099. <https://doi.org/10.1371/journal.pone.0026099>
- Karolchik, D., A.S. Hinrichs, T.S. Furey, K.M. Roskin, C.W. Sugnet, D. Haussler, and W.J. Kent. 2004. The UCSC Table Browser data retrieval tool. *Nucleic Acids Res.* 32:D493–D496. <https://doi.org/10.1093/nar/gkh103>
- Kaya-Okur, H.S., S.J. Wu, C.A. Codomo, E.S. Pledger, T.D. Bryson, J.G. Henikoff, K. Ahmad, and S. Henikoff. 2019. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* 10: 1930. <https://doi.org/10.1038/s41467-019-09982-5>
- Keenan, C.R., N. Iannarella, G. Naselli, N.G. Bediaga, T.M. Johanson, L.C. Harrison, and R.S. Allan. 2020. Extreme disruption of heterochromatin is required for accelerated hematopoietic aging. *Blood.* 135:2049–2058. <https://doi.org/10.1182/blood.2019002990>
- Kharchenko, P.V., M.Y. Tolstorukov, and P.J. Park. 2008. Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nat. Biotechnol.* 26: 1351–1359. <https://doi.org/10.1038/nbt.1508>
- Kiesel, A., C. Roth, W. Ge, M. Wess, M. Meier, and J. Söding. 2018. The BaMM web server for de-novo motif discovery and regulatory sequence analysis. *Nucleic Acids Res.* 46:W215–W220. <https://doi.org/10.1093/nar/gky431>
- Koide, S., M. Oshima, K. Takubo, S. Yamazaki, E. Nitta, A. Saraya, K. Aoyama, Y. Kato, S. Miyagi, Y. Nakajima-Takagi, et al. 2016. Setdb1 maintains hematopoietic stem and progenitor cells by restricting the ectopic activation of nonhematopoietic genes. *Blood.* 128:638–649. <https://doi.org/10.1182/blood-2016-01-694810>
- De La Mata, M., C.R. Alonso, S. Kadener, J.P. Fededa, M. Blaustein, F. Pelisch, P. Cramer, D. Bentley, and A.R. Kornblihtt. 2003. A slow RNA polymerase II affects alternative splicing in vivo. *Mol. Cell.* 12:525–532. <https://doi.org/10.1016/j.molcel.2003.08.001>
- Landt, S.G., G.K. Marinov, A. Kundaje, P. Kheradpour, F. Pauli, S. Batzoglou, B.E. Bernstein, P. Bickel, J.B. Brown, P. Cayting, et al. 2012. ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res.* 22:1813–1831. <https://doi.org/10.1101/gr.136184.111>
- de Laval, B., P. Pawlikowska, D. Barbieri, C. Besnard-Guerin, A. Cico, R. Kumar, M. Gaudry, V. Baud, and F. Porteu. 2014. Thrombopoietin promotes NHEJ DNA repair in hematopoietic stem cells through specific activation of Erk and NF- κ B pathways and their target, IEX-1. *Blood.* 123: 509–519. <https://doi.org/10.1182/blood-2013-07-515874>
- de Laval, B., P. Pawlikowska, L. Petit-Cocault, C. Billhou-Nabera, G. Aubin-Houzelstein, M. Souyri, F. Pouzoulet, M. Gaudry, and F. Porteu. 2013. Thrombopoietin-increased DNA-PK-dependent DNA repair limits hematopoietic stem and progenitor cell mutagenesis in response to DNA damage. *Cell Stem Cell.* 12:37–48. <https://doi.org/10.1016/j.stem.2012.10.012>
- Lefkopoulou, S., A. Polyzou, M. Derecka, V. Berge, T. Clapes, P. Cauchy, C. Jerez-Longres, M. Onishi-Seebacher, N. Yin, N.-A. Martagon-Calderón, et al. 2020. Repetitive elements trigger RIG-I-like receptor signaling that regulates the emergence of hematopoietic stem and progenitor cells. *Immunity.* 53:934–951.e9. <https://doi.org/10.1016/j.immuni.2020.10.007>
- Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, and 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics.* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- Li, Q., J.B. Brown, H. Huang, and P.J. Bickel. 2011. Measuring reproducibility of high-throughput experiments. *Ann. Appl. Stat.* 5:1752–1779. <https://doi.org/10.1214/11-AOAS466>
- Liu, N., C.H. Lee, T. Swigut, E. Grow, B. Gu, M.C. Bassik, and J. Wysocka. 2018. Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. *Nature.* 553:228–232. <https://doi.org/10.1038/nature25179>
- Love, M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15: 550. <https://doi.org/10.1186/s13059-014-0550-8>
- Miousse, L.R., J. Chang, L. Shao, R. Pathak, É. Nzabarushimana, K.R. Kutanzi, R.D. Landes, A.J. Tackett, M. Hauer-Jensen, D. Zhou, and I. Koturbash. 2017a. Inter-strain differences in LINE-1 DNA methylation in the mouse hematopoietic system in response to exposure to ionizing radiation. *Int. J. Mol. Sci.* 18:1430. <https://doi.org/10.3390/ijms18071430>
- Miousse, L.R., K.R. Kutanzi, and I. Koturbash. 2017b. Effects of ionizing radiation on DNA methylation: From experimental biology to clinical applications. *Int. J. Radiat. Biol.* 93:457–469. <https://doi.org/10.1080/09553002.2017.1287454>
- Moehrl, B.M., K. Nattamai, A. Brown, M.C. Florian, M. Ryan, M. Vogel, C. Bliederhaeuser, K. Soller, D.R. Prows, A. Abdollahi, et al. 2015. Stem cell-specific mechanisms ensure genomic fidelity within HSCs and upon aging of HSCs. *Cell Rep.* 13:2412–2424. <https://doi.org/10.1016/j.celrep.2015.11.030>
- Moelling, K., and F. Broecker. 2019. Viruses and evolution—viruses first? A personal perspective. *Front. Microbiol.* 10:523. <https://doi.org/10.3389/fmicb.2019.00523>
- Mohrin, M., E. Bourke, D. Alexander, M.R. Warr, K. Barry-Holson, M.M. Le Beau, C.G. Morrison, and E. Passequé. 2010. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. *Cell Stem Cell.* 7:174–185. <https://doi.org/10.1016/j.stem.2010.06.014>
- Ninova, M., Y.A. Chen, B. Godneeva, A.K. Rogers, Y. Luo, K. Fejes Tóth, and A.A. Aravin. 2020. Su(var)2-10 and the SUMO pathway link piRNA-guided target recognition to chromatin silencing. *Mol. Cell.* 77: 556–570.e6. <https://doi.org/10.1016/j.molcel.2019.11.012>
- Ocampo, A., P. Reddy, P. Martínez-Redondo, A. Platero-Luengo, F. Hatanaka, T. Hishida, M. Li, D. Lam, M. Kurita, E. Beyret, et al. 2016. In vivo amelioration of age-associated hallmarks by partial reprogramming. *Cell.* 167:1719–1733.e12. <https://doi.org/10.1016/j.cell.2016.11.052>
- O’Leary, N.A., M.W. Wright, J.R. Brister, S. Ciufu, D. Haddad, R. McVeigh, B. Rajput, B. Robertse, B. Smith-White, D. Ako-Adjei, et al. 2016. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.* 44:D733–D745. <https://doi.org/10.1093/nar/gkv1189>
- Patro, R., G. Duggal, M.I. Love, R.A. Irizarry, and C. Kingsford. 2017. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods.* 14:417–419. <https://doi.org/10.1038/nmeth.4197>

- Pezic, D., S.A. Manakov, R. Sachidanandam, and A.A. Aravin. 2014. piRNA pathway targets active LINE1 elements to establish the repressive H3K9me3 mark in germ cells. *Genes Dev.* 28:1410–1428. <https://doi.org/10.1101/gad.240895.114>
- Pietras, E.M., D. Reynaud, Y.-A. Kang, D. Carlin, F.J. Calero-Nieto, A.D. Leavitt, J.M. Stuart, B. Göttgens, and E. Passegué. 2015. Functionally distinct subsets of lineage-biased multipotent progenitors control blood production in normal and regenerative conditions. *Cell Stem Cell.* 17: 35–46. <https://doi.org/10.1016/j.stem.2015.05.003>
- Ramírez, F., D.P. Ryan, B. Grünig, V. Bhardwaj, F. Kilpert, A.S. Richter, S. Heyne, F. Dündar, and T. Manke. 2016. deepTools2: A next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.* 44: W160–W165. <https://doi.org/10.1093/nar/gkw257>
- Rodríguez-Fraticelli, A.E., C. Weinreb, S.W. Wang, R.P. Migueles, M. Janovic, M. Usart, A.M. Klein, S. Lowell, and F.D. Camargo. 2020. Single-cell lineage tracing unveils a role for TCF15 in haematopoiesis. *Nature.* 583:585–589. <https://doi.org/10.1038/s41586-020-2503-6>
- Ross-Innes, C.S., R. Stark, A.E. Teschendorff, K.A. Holmes, H.R. Ali, M.J. Dunning, G.D. Brown, O. Gojis, I.O. Ellis, A.R. Green, et al. 2012. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature.* 481:389–393. <https://doi.org/10.1038/nature10730>
- Saint-André, V., E. Batsché, C. Rachez, and C. Muchardt. 2011. Histone H3 lysine 9 trimethylation and HP1 favor inclusion of alternative exons. *Nat. Struct. Mol. Biol.* 18:337–344. <https://doi.org/10.1038/nsmb.1995>
- Sanjuan-Pla, A., I.C. Macaulay, C.T. Jensen, P.S. Woll, T.C. Luis, A. Mead, S. Moore, C. Carella, S. Matsuoka, T. Bouriez Jones, et al. 2013. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature.* 502:232–236. <https://doi.org/10.1038/nature12495>
- Schaefer, B.C., M.L. Schaefer, J.W. Kappler, P. Marrack, and R.M. Kedl. 2001. Observation of antigen-dependent CD8+ T-cell/dendritic cell interactions in vivo. *Cell Immunol.* 214:110–122. <https://doi.org/10.1006/cimm.2001.1895>
- Schneider, W.M., M.D. Chevillotte, and C.M. Rice. 2014. Interferon-stimulated genes: A complex web of host defenses. *Annu. Rev. Immunol.* 32:513–545. <https://doi.org/10.1146/annurev-immunol-032713-120231>
- Schreiber, J., R.G. Jenner, H.L. Murray, G.K. Gerber, D.K. Gifford, and R.A. Young. 2006. Coordinated binding of NF-kappaB family members in the response of human cells to lipopolysaccharide. *Proc. Natl. Acad. Sci. USA.* 103:5899–5904. <https://doi.org/10.1073/pnas.0510996103>
- Siebert, M., and J. Söding. 2016. Bayesian markov models consistently outperform PWMs at predicting motifs in nucleotide sequences. *Nucleic Acids Res.* 44:6055–6069. <https://doi.org/10.1093/nar/gkw521>
- Soneson, C., M.I. Love, and M.D. Robinson. 2015. Differential analyses for RNA-seq: Transcript-level estimates improve gene-level inferences. *F1000Res.* 4:1521. <https://doi.org/10.12688/f1000research.7563.2>
- Sookdeo, A., C.M. Hepp, M.A. McClure, and S. Boissinot. 2013. Revisiting the evolution of mouse LINE-1 in the genomic era. *Mob. DNA.* 4:3. <https://doi.org/10.1186/1759-8753-4-3>
- Stein, S.J., and A.S. Baldwin. 2013. Deletion of the NF-κB subunit p65/RelA in the hematopoietic compartment leads to defects in hematopoietic stem cell function. *Blood.* 121:5015–5024. <https://doi.org/10.1182/blood-2013-02-486142>
- Su, A.I., M.P. Cooke, K.A. Ching, Y. Hakak, J.R. Walker, T. Wiltshire, A.P. Orth, R.G. Vega, L.M. Sapinoso, A. Moqrich, et al. 2002. Large-scale analysis of the human and mouse transcriptomes. *Proc. Natl. Acad. Sci. USA.* 99:4465–4470. <https://doi.org/10.1073/pnas.012025199>
- Sun, D., M. Luo, M. Jeong, B. Rodriguez, Z. Xia, R. Hannah, H. Wang, T. Le, K.F. Faull, R. Chen, et al. 2014. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell Stem Cell.* 14:673–688. <https://doi.org/10.1016/j.stem.2014.03.002>
- Sun, X., X. Wang, Z. Tang, M. Grivainis, D. Kahler, C. Yun, P. Mita, D. Fenyő, and J.D. Boeke. 2018. Transcription factor profiling reveals molecular choreography and key regulators of human retrotransposon expression. *Proc. Natl. Acad. Sci. USA.* 115:E5526–E5535. <https://doi.org/10.1073/pnas.1722565115>
- Sundaram, V., and T. Wang. 2018. Transposable element mediated innovation in gene regulatory landscapes of cells: Re-visiting the “Gene-Battery” model. *Bioessays.* 40. <https://doi.org/10.1002/bies.201700155>
- Teissandier, A., N. Servant, E. Barillot, and D. Bourc’his. 2019. Tools and best practices for retrotransposon analysis using high-throughput sequencing data. *Mob. DNA.* 10:52. <https://doi.org/10.1186/s13100-019-0192-1>
- Trizzino, M., A. Kapusta, and C.D. Brown. 2018. Transposable elements generate regulatory novelty in a tissue-specific fashion. *BMC Genom.* 19: 468. <https://doi.org/10.1186/s12864-018-4850-3>
- Tsurumi, A., and W.X. Li. 2012. Global heterochromatin loss: A unifying theory of aging?. *Epigenetics.* 7:680–688. <https://doi.org/10.4161/epi.20540>
- Vakoc, C.R., S.A. Mandat, B.A. Olenchock, and G.A. Blobel. 2005. Histone H3 lysine 9 methylation and HP1gamma are associated with transcription elongation through mammalian chromatin. *Mol. Cell.* 19:381–391. <https://doi.org/10.1016/j.molcel.2005.06.011>
- Volkman, H.E., and D.B. Stetson. 2014. The enemy within: Endogenous retroelements and autoimmune disease. *Nat. Immunol.* 15:415–422. <https://doi.org/10.1038/ni.2872>
- Walter, M., A. Teissandier, R. Pérez-Palacios, and D. Bourc’his. 2016. An epigenetic switch ensures transposon repression upon dynamic loss of DNA methylation in embryonic stem cells. *Elife.* 5:e11418. <https://doi.org/10.7554/eLife.11418>
- Wang, K., H. Li, Y. Xu, Q. Shao, J. Yi, R. Wang, W. Cai, X. Hang, C. Zhang, H. Cai, and W. Qu. 2019. MFEprimer-3.0: Quality control for PCR primers. *Nucleic Acids Res.* 47:W610–W613. <https://doi.org/10.1093/nar/gkz351>
- Whitaker, J.W., Z. Chen, and W. Wang. 2015. Predicting the human epigenome from DNA motifs. *Nat. Methods.* 12:265–272. <https://doi.org/10.1038/nmeth.3065>
- White, R.R., and J. Vijg. 2016. Do DNA double-strand breaks drive aging?. *Mol. Cell.* 63:729–738. <https://doi.org/10.1016/j.molcel.2016.08.004>
- Wingett, S.W., and S. Andrews. 2018. FastQ screen: A tool for multi-genome mapping and quality control. *F1000Res.* 7:1338. <https://doi.org/10.12688/f1000research.15931.2>
- Yamashita, M., and E. Passegué. 2019. TNF-α Coordinates hematopoietic stem cell survival and myeloid regeneration. *Cell Stem Cell.* 25:357–372.e7. <https://doi.org/10.1016/j.stem.2019.05.019>
- Ye, M., C. Goudot, T. Hoyler, B. Lemoine, S. Amigorena, and E. Zueva. 2020. Specific subfamilies of transposable elements contribute to different domains of T lymphocyte enhancers. *Proc. Natl. Acad. Sci. USA.* 117: 7905–7916. <https://doi.org/10.1073/pnas.1912008117>
- Yu, Q., C.J. Carbone, Y.V. Katlinskaya, H. Zheng, K. Zheng, M. Luo, P.J. Wang, R.A. Greenberg, and S.Y. Fuchs. 2015. Type I interferon controls propagation of long interspersed element-1. *J. Biol. Chem.* 290: 10191–10199. <https://doi.org/10.1074/jbc.M114.612374>
- Zhang, Y., T. Liu, C.A. Meyer, J. Eeckhoutte, D.S. Johnson, B.E. Bernstein, C. Nusbaum, R.M. Myers, M. Brown, W. Li, and X.S. Liu. 2008. Model-based analysis of ChIP-seq (MACS). *Genome biology.* 9:R137. <https://doi.org/10.1186/gb-2008-9-9-r137>

Supplemental material

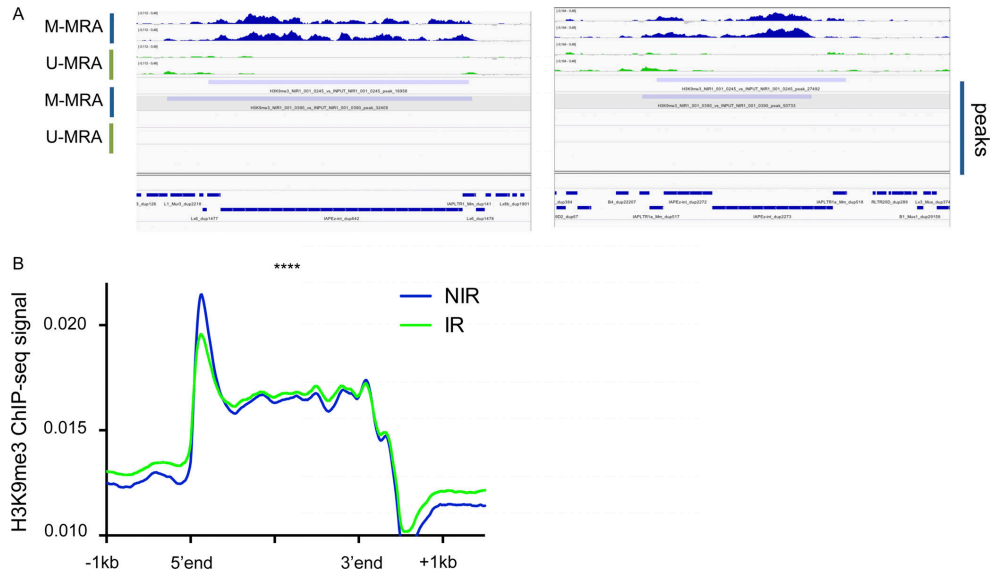


Figure S1. **H3K9me3 is lost at recent LIMd subfamilies in HSC.** (A) Integrative genomic viewer visualization of H3K9me3 enrichment and peaks at two described loci with M-MRA or U-MRA analysis, as indicated: (left) chr2:39209585-39320316; (right) chr6:5271421-5288640 (Bulut-Karslioglu et al., 2014). (B) Plot profile representing H3K9me3 enrichment along LIMd sequences \pm 1 kb flanking regions in NIR (blue) vs. IR (green) conditions. ****, $P < 0.0001$, Wilcoxon test.

Downloaded from http://press.oxfordjournals.org/jem/article-pdf/21/9/6/20211356/1435569/jem_20211356.pdf by Institut Gustave Roussy user on 21 June 2023

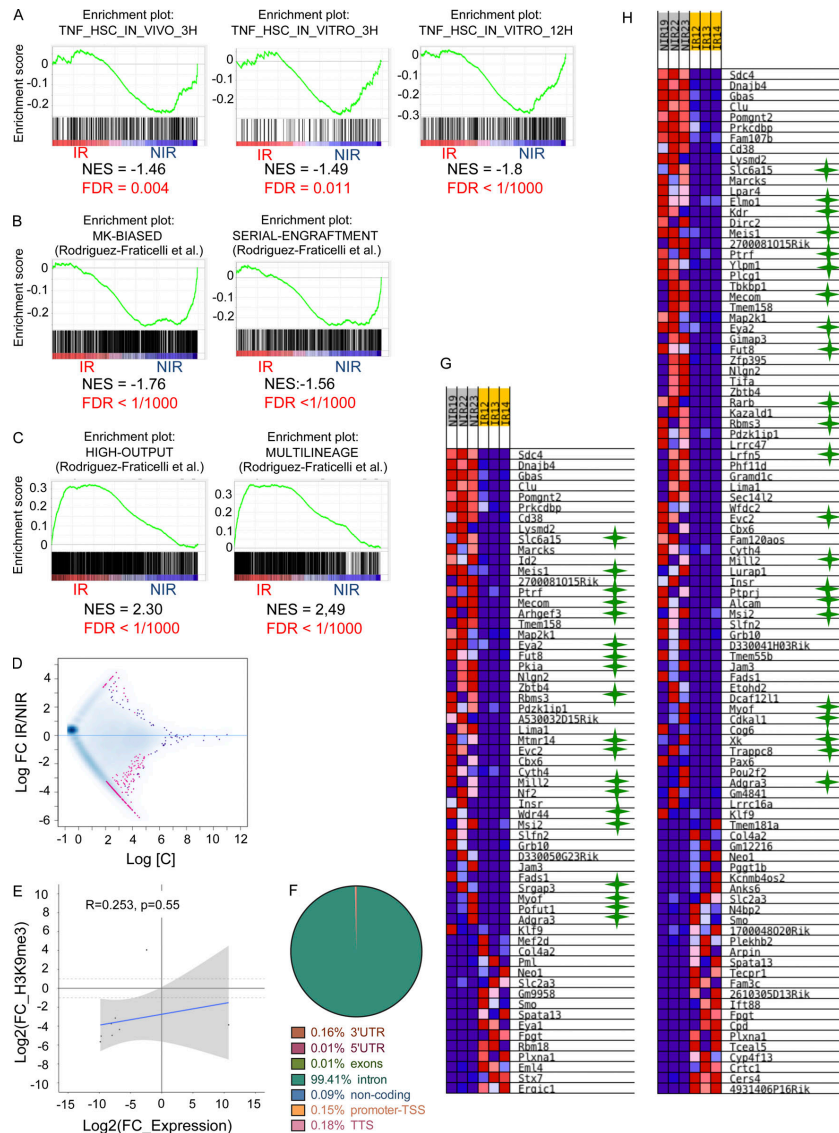


Figure S2. **Gene repression upon IR is associated with intronic L1Md.** (A–C) Enrichment plots in IR vs. NIR conditions obtained from GSEA for TNF signatures induced in HSCs after TNF- α treatment 3 h in vivo and 3 and 12 h in vitro (A); MK-biased and serial engraftment HSC signatures (B); high output and multilineage differentiation signatures (C). (D) Quantitative analysis of H3K9me3 enrichment at promoters (–2 kb;+1 kb TSS) performed by U-MRA. Non-significant (blue dots) and significant ($P < 0.05$, pink dots) differential H3K9me3 enrichment at genes promoters are shown. (E) Correlation plot between H3K9me3 concentration at gene promoters vs. gene expression at genes presenting both significant deregulation and differential H3K9me3 enrichment at their promoters upon IR ($P < 0.05$). (F) Repartition of intragenic L1Md localization in the genes. TTS, transcriptional termination site. (G and H) Heatmaps of the expression of genes from MK-biased (G) and the low-output (H) HSC signatures that are significantly either upregulated (red) or downregulated (blue) in IR vs. NIR. Green stars indicate the presence of an intronic L1Md in the downregulated genes. FC, fold change; FDR, false discovery rate; NES, normalized enrichment score.

Pelinski et al.
Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine 52
<https://doi.org/10.1084/jem.20211356>

Downloaded from http://rupress.org/jem/article-pdf/219/6/e20211356/1435659/jem_20211356.pdf by Institut Gustave Roussy user on 21 June 2023

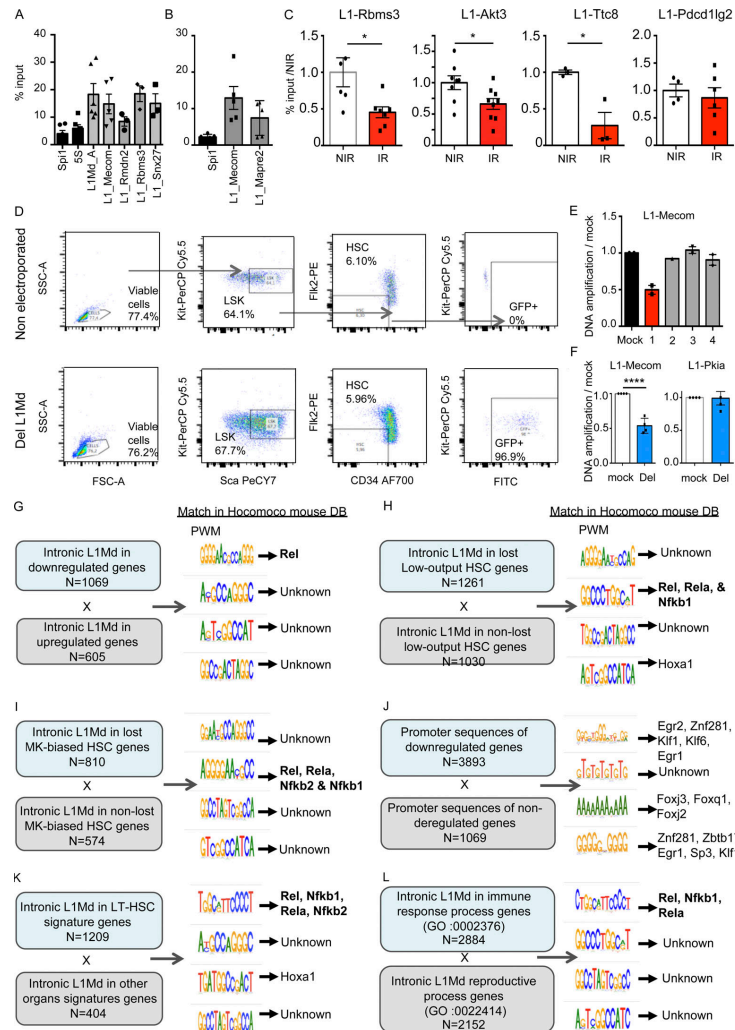


Figure S3. Gene repression upon IR is associated with the loss of H3K9me3 at intronic L1Md loci harboring NF-κB binding sites. (A and B) H3K9me3 enrichment in the NIR conditions evaluated by ChIP-qPCR at long (>5 kb) intronic L1Md promoters of the indicated genes compared to *Spi1* (A) and L1_A promoter compared to repetitive 5S ribosomal RNA (B). Results are expressed as the means ± SEM of the percentage of input. Each dot represents a pool of three mice from two to four independent experiments. (C) H3K9me3 ChIP-qPCR enrichment 1 mo after TBI. Each dot represents a pool of three (NIR) or four (IR) mice from two to three independent experiments. Results are means ± SEM of the percentage of input normalized to the NIR condition. *t* test. (D) Gating strategy for electroporated siglo+ HSCs in del L1Md condition using nonelectroporated cells as a control for designing FITC gate. (E) To test the efficiency of the different combinations of gRNAs, DNA amplification of *Mecom* L1Md was assessed and normalized to the amplification of *Snx27* L1Md by qRT-PCR. In red, the couple of guides that were selected for further analysis. (F) DNA amplification assessed by qRT-PCR and in Cas9-gRNA RNP electroporated HSCs 48 h after irradiation in vitro. Ct values were normalized to L1-Snx27. *t* test. (G-L) De novo motif discovery analysis performed with the BaMMotif tool on L1Md sequences located in: introns of downregulated genes vs. upregulated genes (G); genes participating vs. not participating to the loss of the low-output or MK-biased HSC signatures (H and I); promoter sequences (−2 kb; +1 kb TSS) from downregulated vs. nonderegulated genes (J); L1Md located in genes from the HSC signature vs. genes from other organs signatures (kidney + liver + pancreas + testis + salivary gland + placenta) from Su et al. (2002) (K). (L) L1Md located in genes from the Gene Ontology (GO) term immune response process (GO:0002376) vs. genes from the GO term reproductive process (GO: 0022414). Enriched motifs were matched to known motifs using the Hocomoco mouse database. *, *P* < 0.05; ****, *P* < 0.0001.

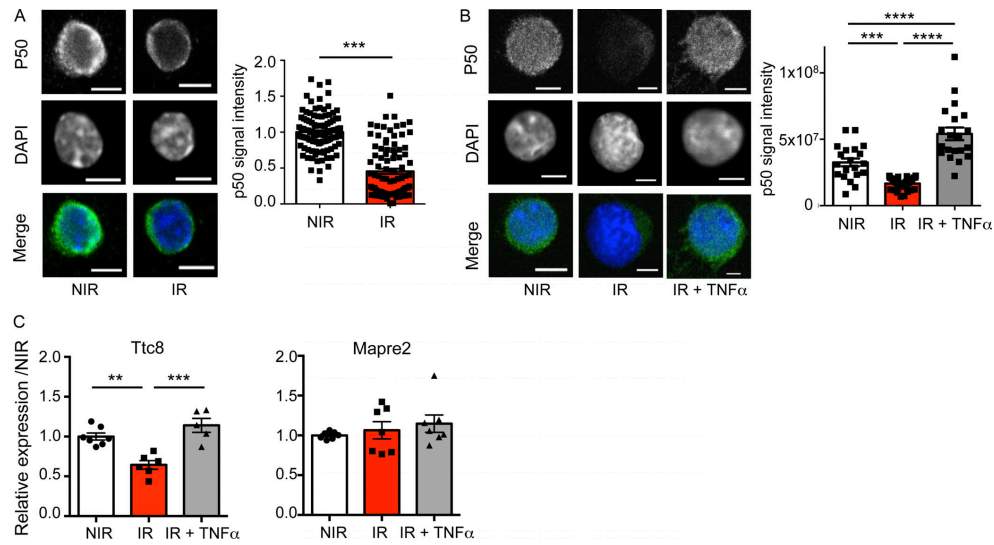


Figure S4. **TNF- α treatment prevents loss of NFKB1 and HSC gene repression in vitro.** (A and B) Representative images and quantification of NFKB1 protein mean IF intensity using the monoclonal anti-NFKB1 (p50) antibody clone from Santa Cruz Biotechnology. Bars, 5 μ M. Each dot represents a cell. Results are expressed as fold change from the mean value of the NIR condition from two independent experiments and represented as means \pm SEM. t test for A or one-way ANOVA with Tukey's multiple comparison test for B. (C) *Ttc8* and *Mapre2* mRNA expression evaluated by qRT-PCR. Ct values were normalized to *Rpl32* and *Hprt*. Results are expressed as fold change from the mean value of the NIR condition and represented as means \pm SEM from two independent experiments. One-way ANOVA Tukey's multiple comparison test. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

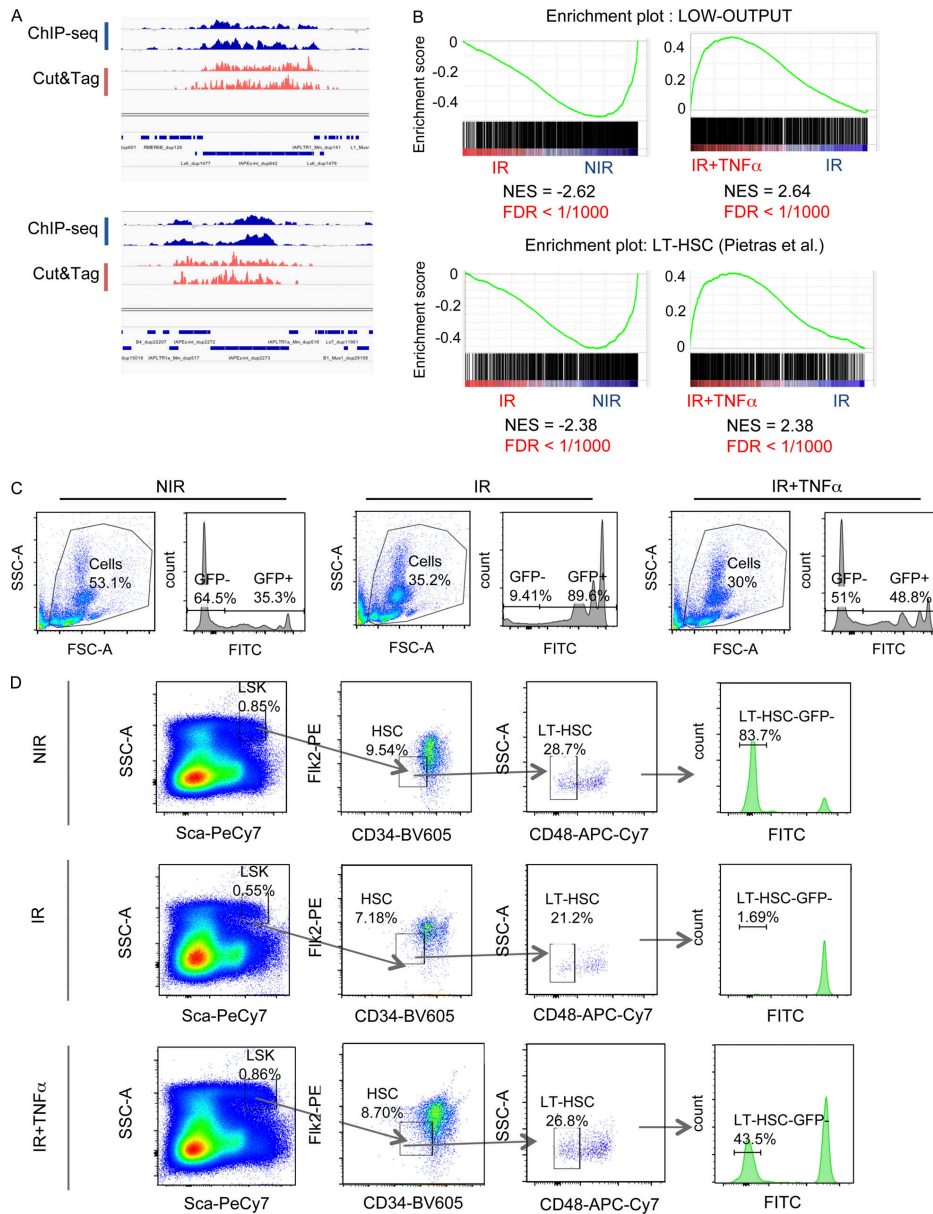


Figure S5. **TNF- α treatment prevents loss of H3H9me3 at intronic LIMd, HSC gene repression, and HSC loss of function upon IR.** (A) Integrative genomic viewer visualization of H3K9me3 enrichment obtained by ChIP-seq and CUT&Tag M-MRA analysis at two described loci as indicated: (left) chr2: 39209585–39320316; (right) chr6:5271421–5288640 (Bulut-Karslioglu et al. 2014). (B) Enrichment plots in IR vs. NIR and in IR + TNF- α vs. IR conditions obtained from GSEA for low-output and LT-HSC signatures. (C and D) Gating strategy to evaluate donor-derived GFP⁻ vs. recipient GFP⁺ cells in peripheral blood (C) and bone marrow HSCs (D). FDR, false discovery rate; NES, normalized enrichment score.

Pelinski et al.
Cis-regulation of HSC genes by intronic LINE-1

Journal of Experimental Medicine 55
<https://doi.org/10.1084/jem.20211356>

Downloaded from http://rupress.org/jem/article-pdf/211/9/6/20211356/1435569/jem_20211356.pdf by Institut Gulabie Roussy user on 21 June 2023

Provided online are four tables. Table S1 shows quality control of the reads and peak calling data for the ChIP-seq analysis, Table S2 shows information on differentially expressed genes upon IR for RNA-seq analysis, Table S3 gives information on intragenic L1Md, and Table S4 lists primers and gRNAs used for the study.

Downloaded from http://rupress.org/jem/article-pdf/219/6/2021.1356/1435569/jem_20211356.pdf by Institut Gustave Roussy user on 21 June 2023