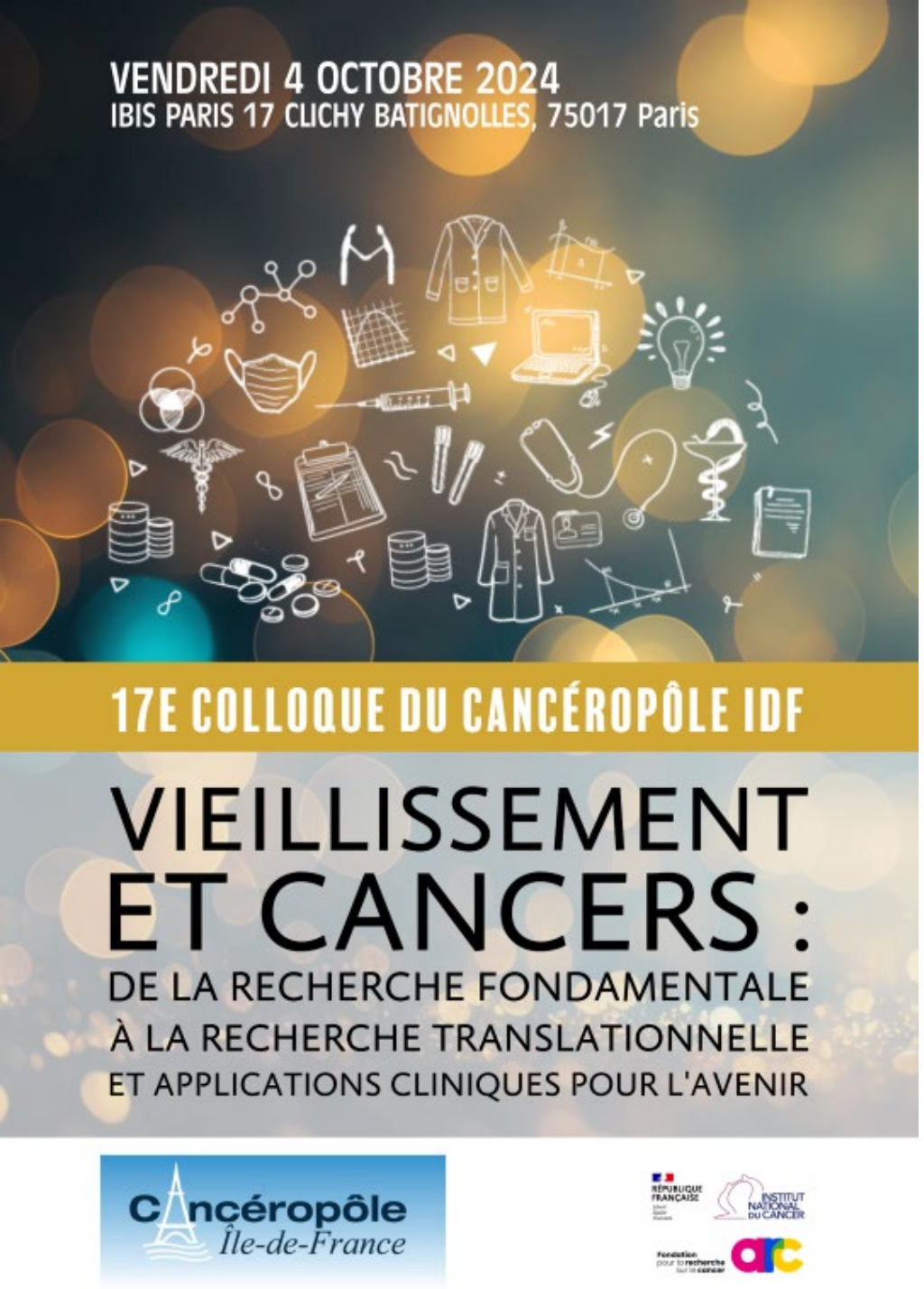

Aging of the hematopoietic stem cell and myeloid neoplasms

Estelle Duprez, DR CNRS

Team: Integrative molecular biology in hematopoiesis and leukemia

Department Onco-Hemato Immuno-Onco, OHIO

Cancer Research Center of Marseille

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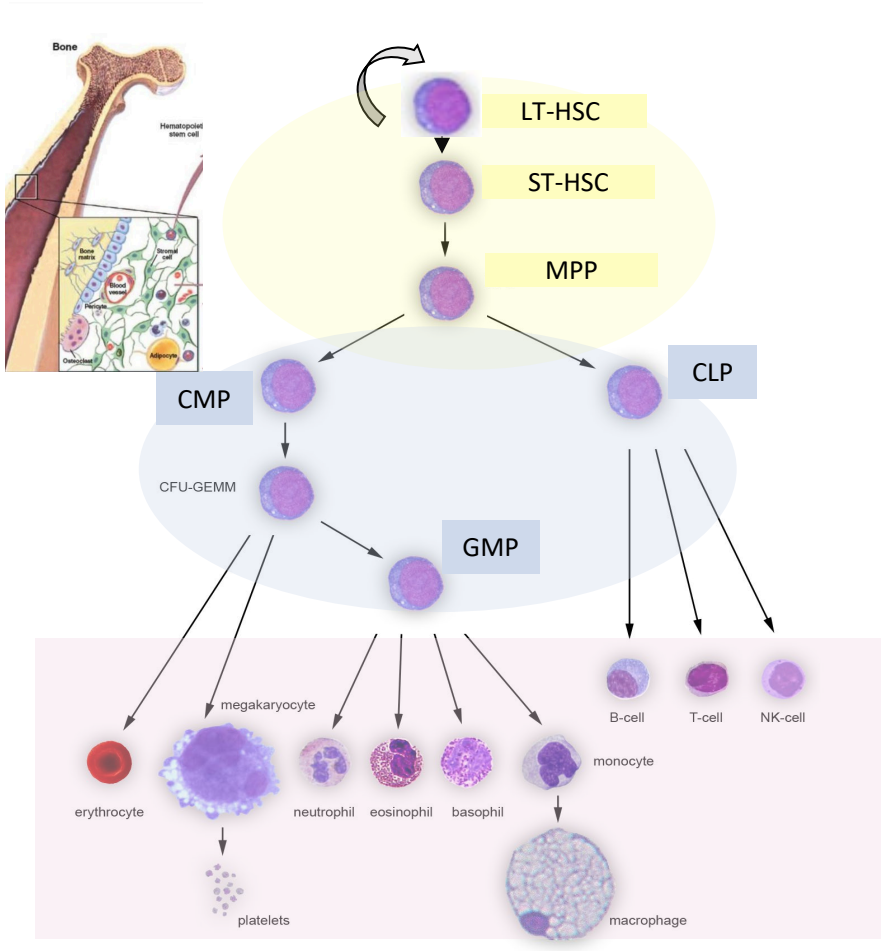
The hematopoiesis



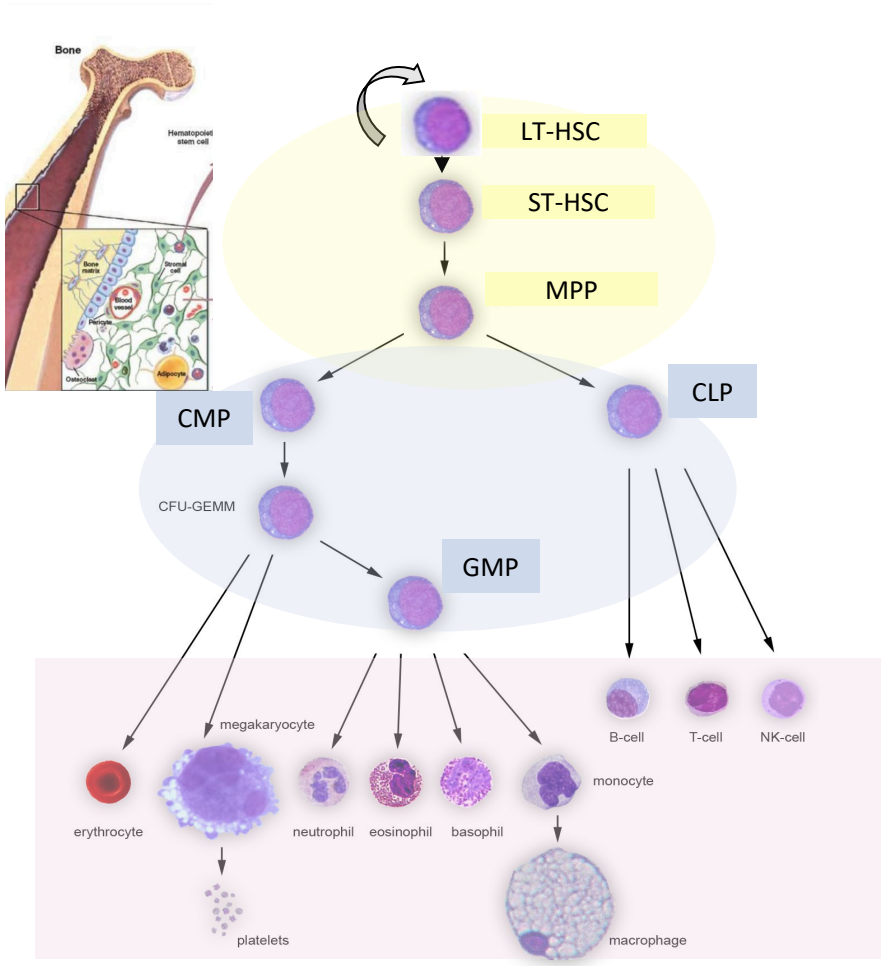
Stem Cells
- Differentiation
- Self-renewal

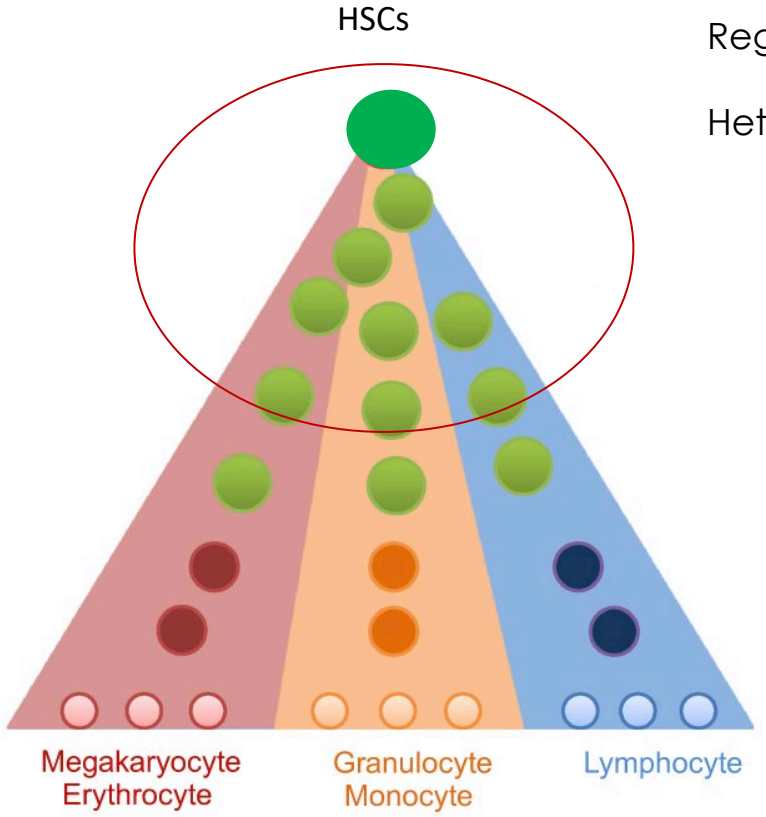
Hematopoietic Stem Cell or HSC
Multipotent Progenitors or MPP

Progenitors
- Differentiation
- Amplification

Mature cells • Effectors

Hematopoiesis: the cloud model



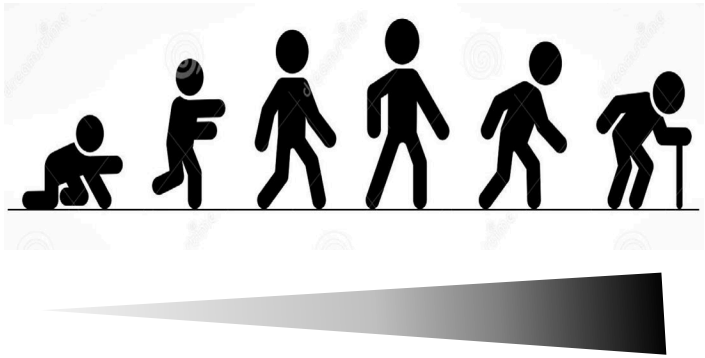


Regulators ?

Heterogeneity ?

(Adapted from Cheng, Protein cell, 2020)

Aging and Hematopoiesis



-Changes in the composition and function of blood cells

-Skewing of differentiation towards **myeloid** progenitors

Consequences

Decline of immunocompetence

===> Increase of Infection

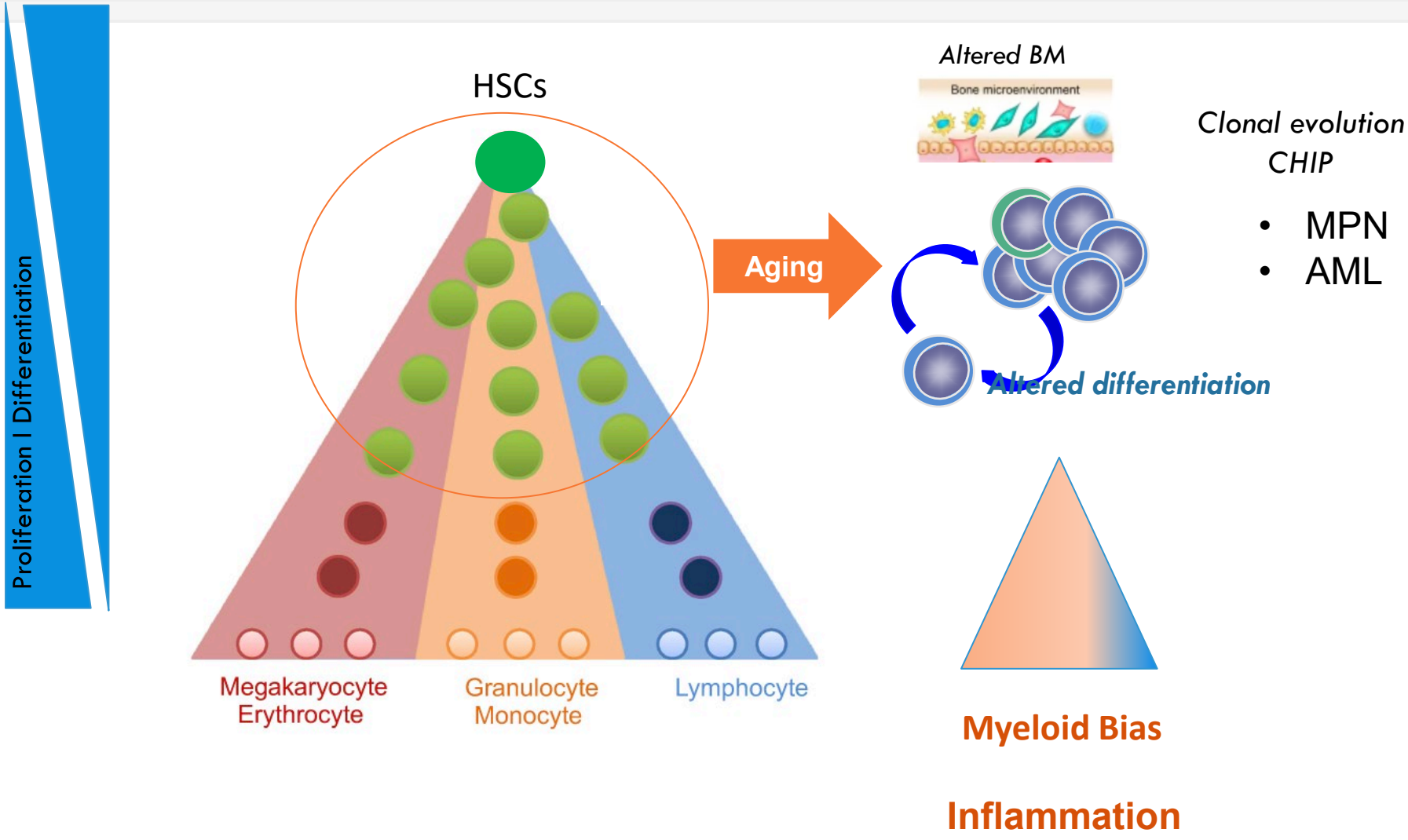
===> Chronic disease

===> Increase in autoimmune disease

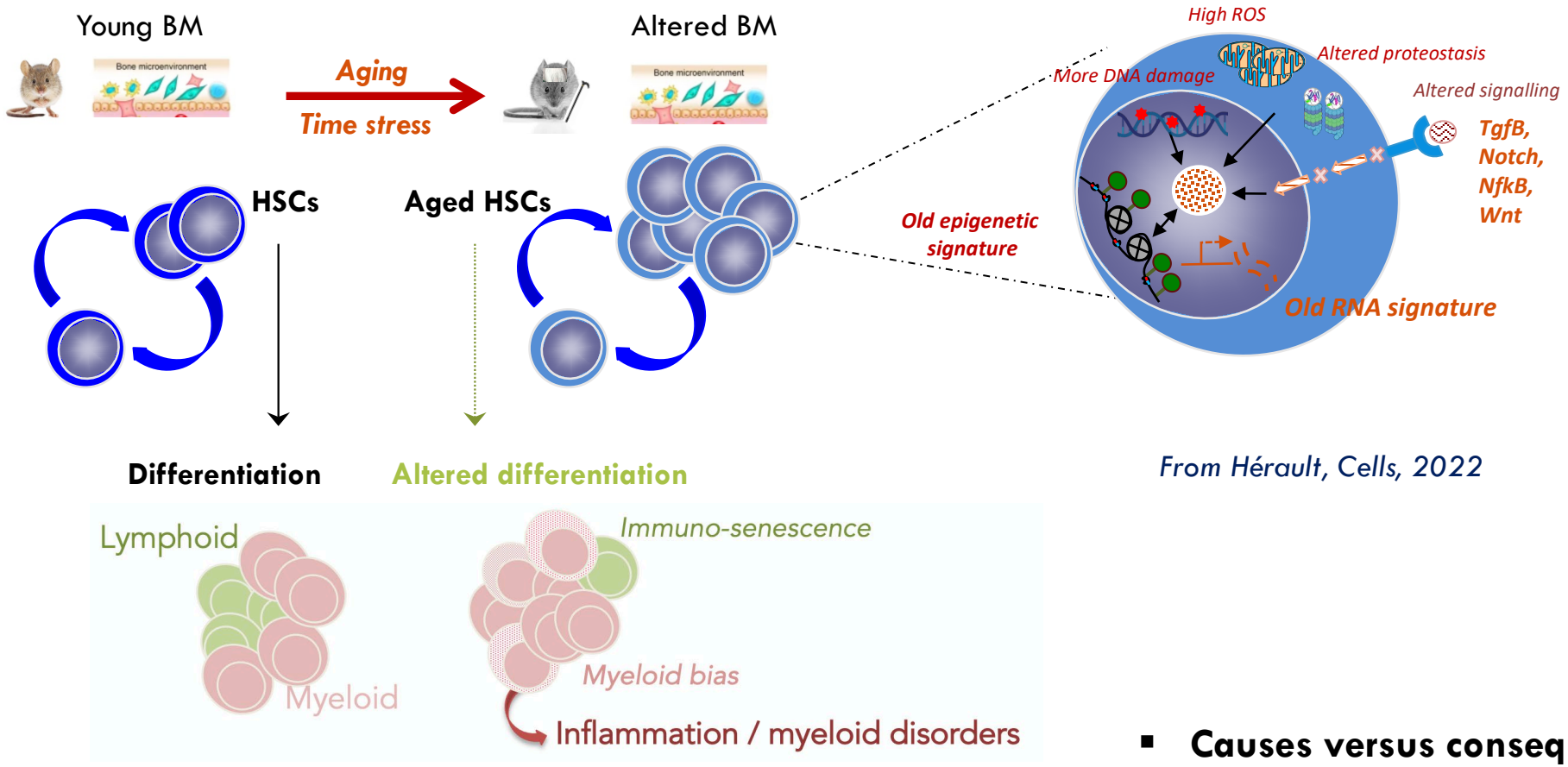
Predisposition to myeloid neoplasia

Role of the hematopoietic stem cells or HSCs ?

Aging of HSC: myeloid bias and clonal evolution



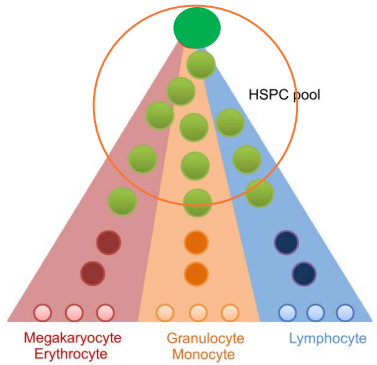
Mecanisms of HSC aging



From Hérault, Cells, 2022

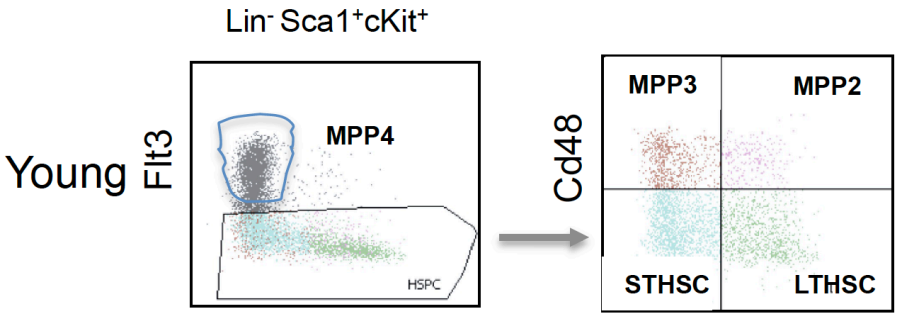
- **Causes versus consequences ?**
- **Heterogeneity ?**

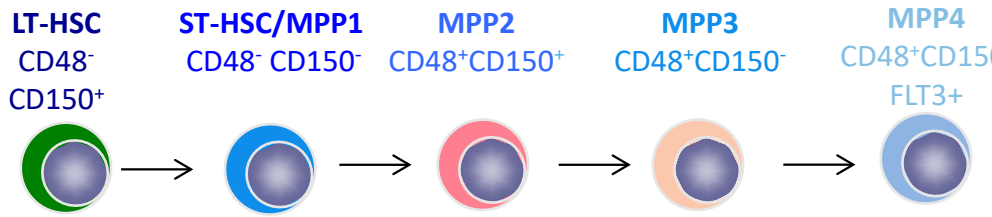
The HSC compartment changes during aging



Antibody staining
Flow Cytometry/FACS

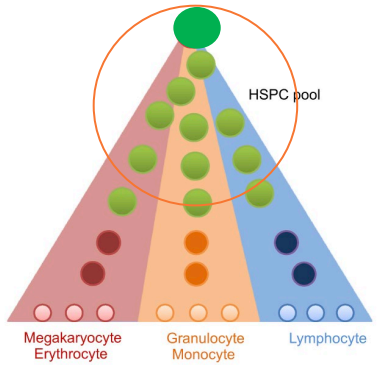
Lin- Sca1+ ckit+





Mouse HSPCs

The HSC compartment changes during aging



Antibody staining
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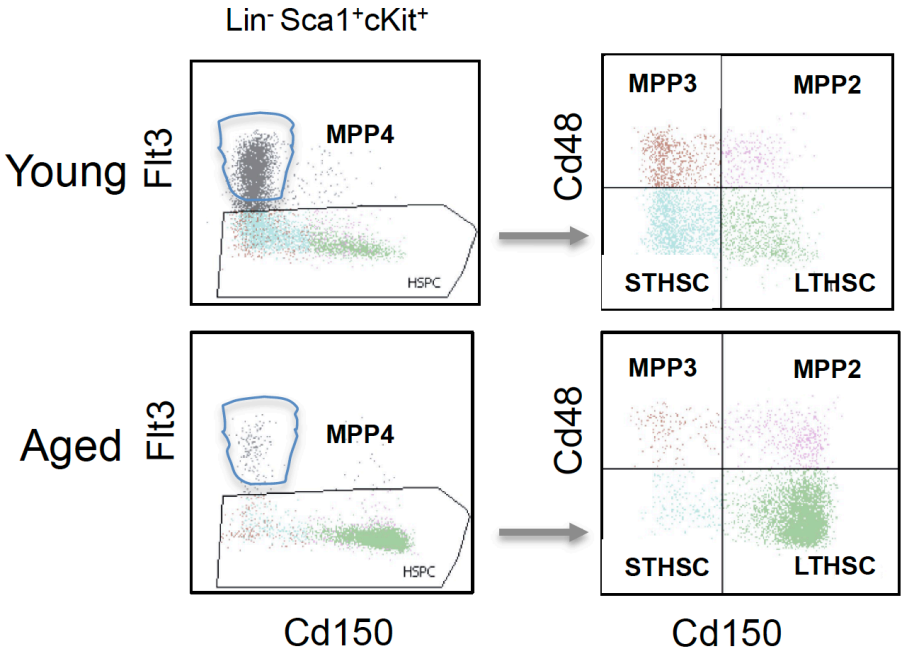
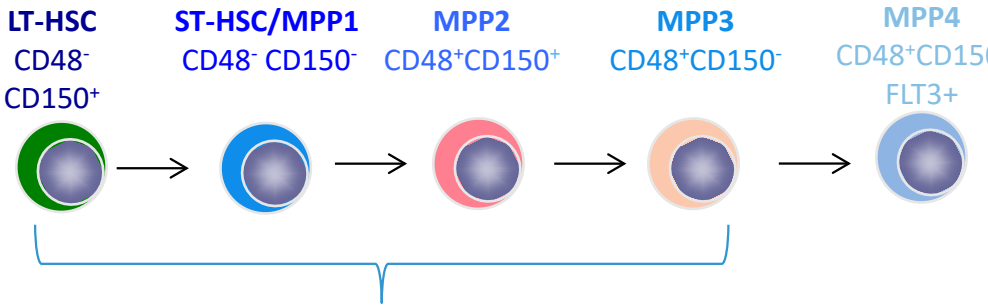
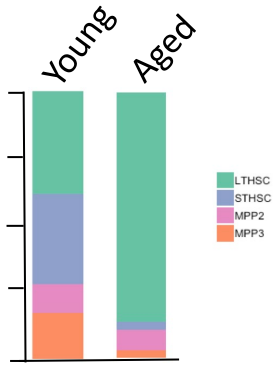
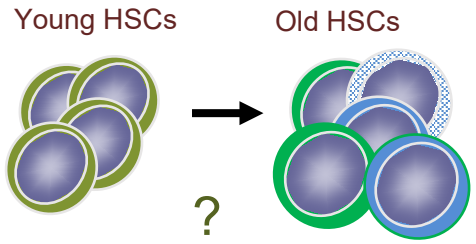




Mechanisms of HSC aging : a system biology approach





- How HSC pool evolved with age ?
- What are the intrinsic changes ?
- What are the regulators of these changes ?

Elisabeth REMY, Mathematicien CNRS, AMU

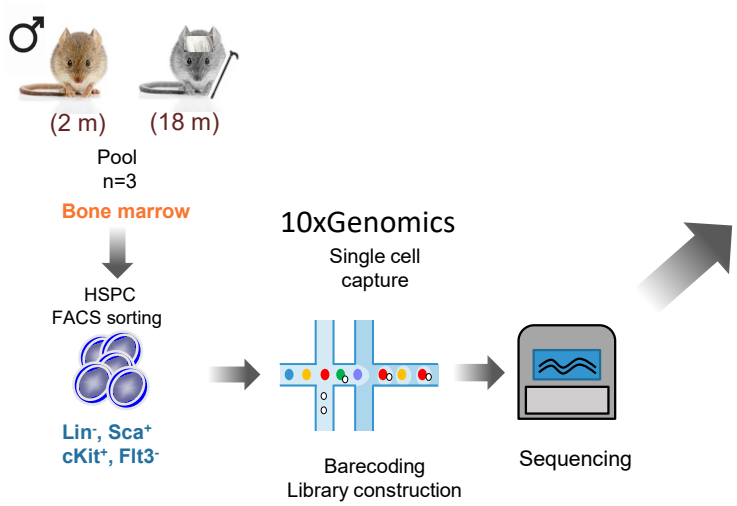
Experimental design

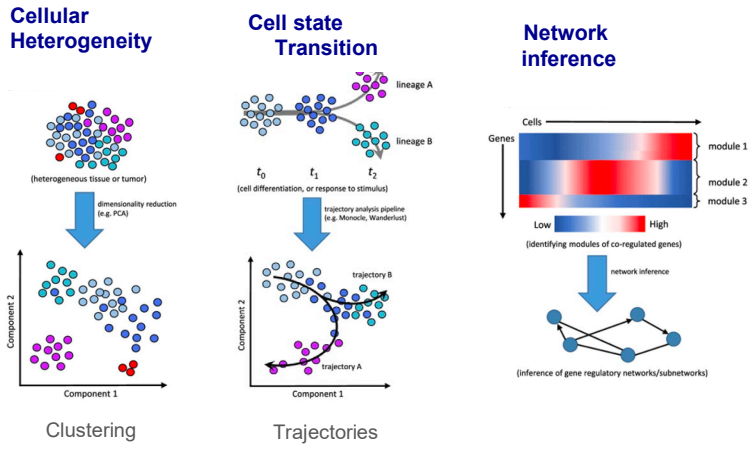
1 HSC purification

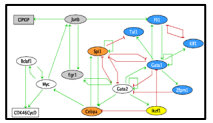
2 Single cell seq data

3 Computational analysis

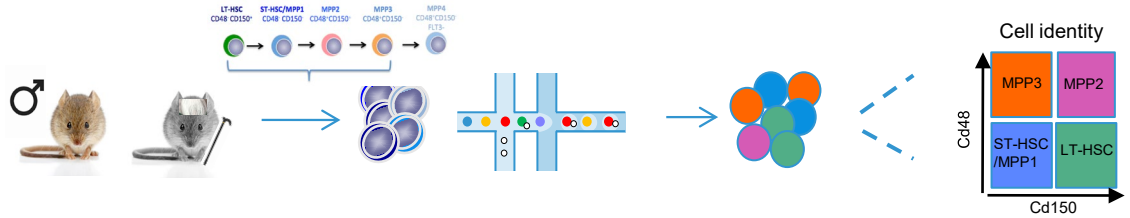
4 Mathematical modeling

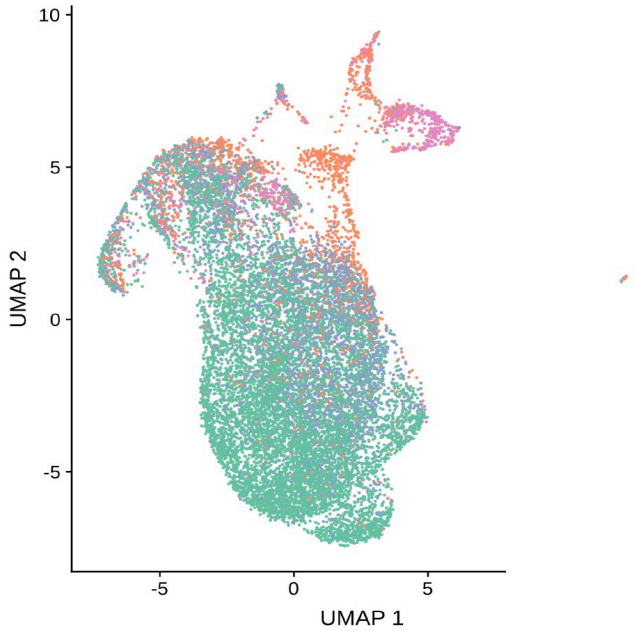




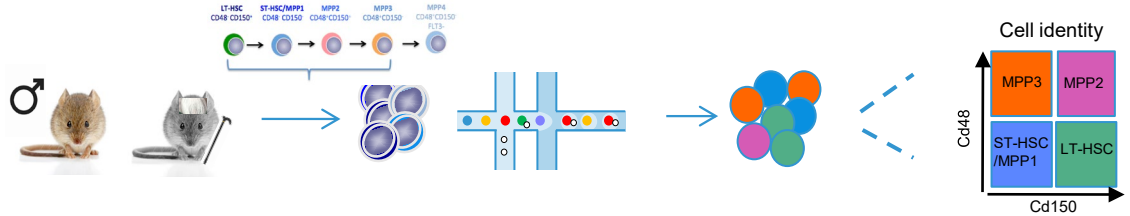


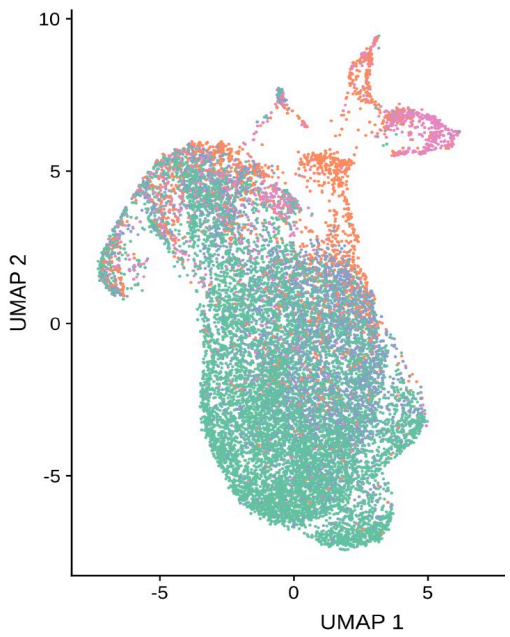
Mechanisms of HSC aging : a system biology approach

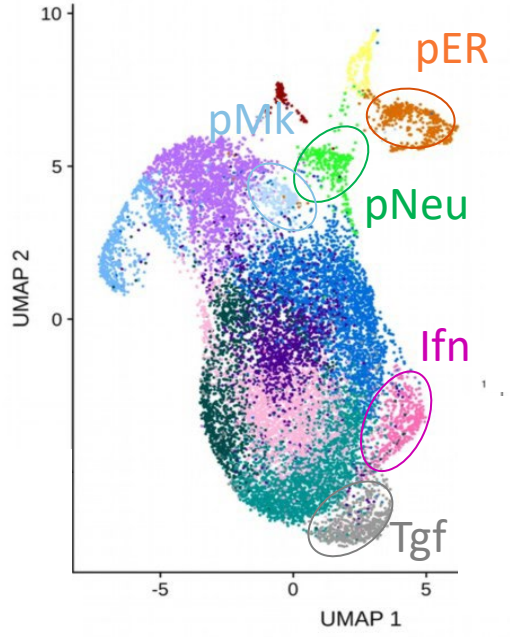




Heterogeneity of HSCs



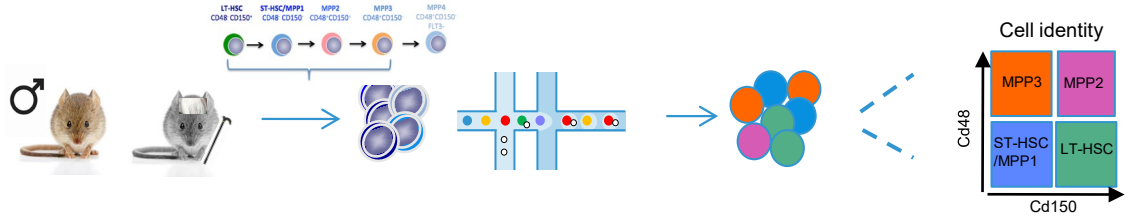


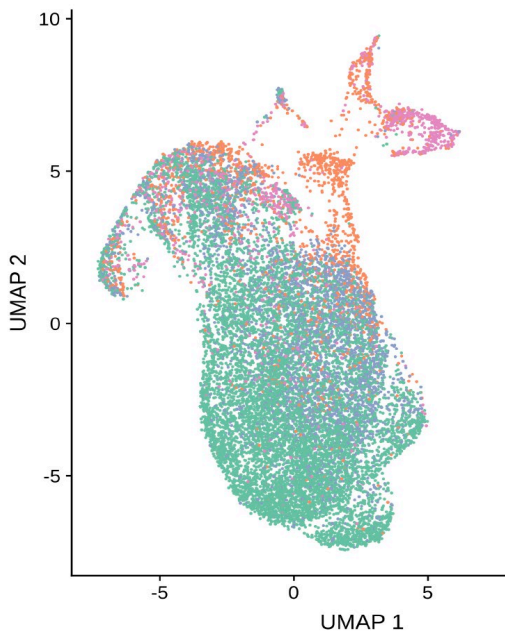


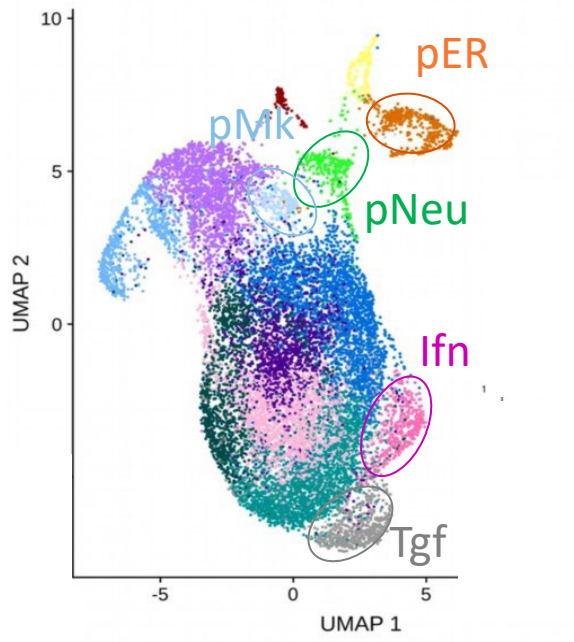
4 types

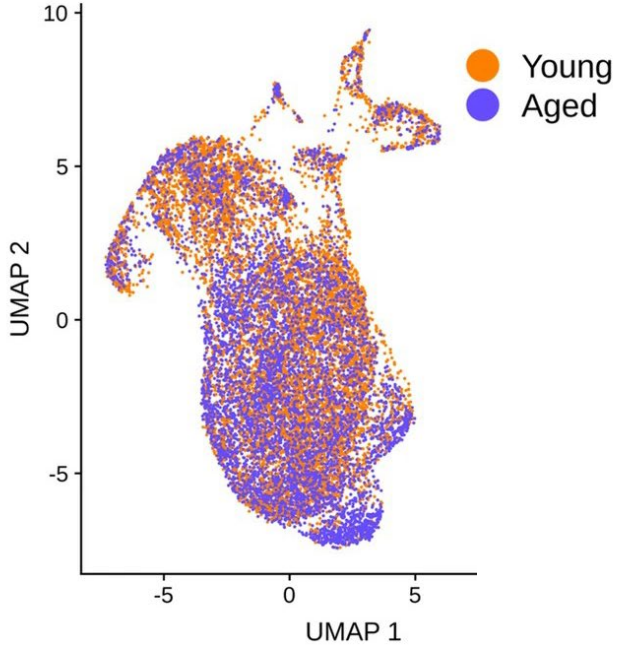
15 clusters

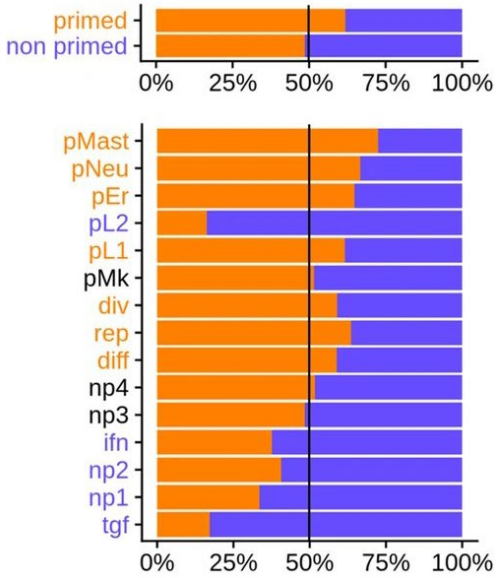
Impact of aging on HSPC clusters







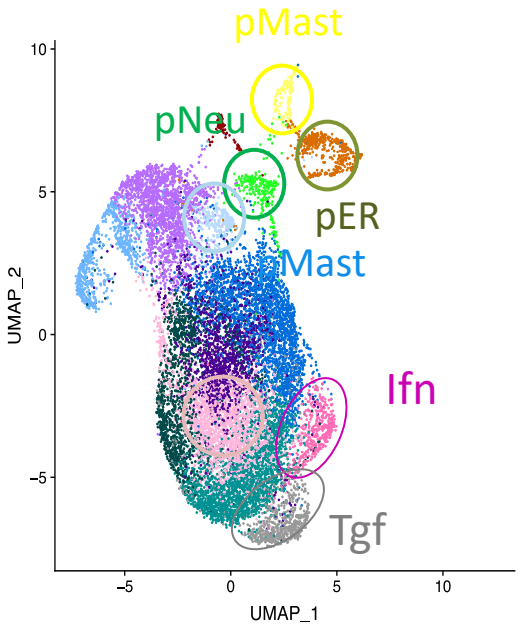


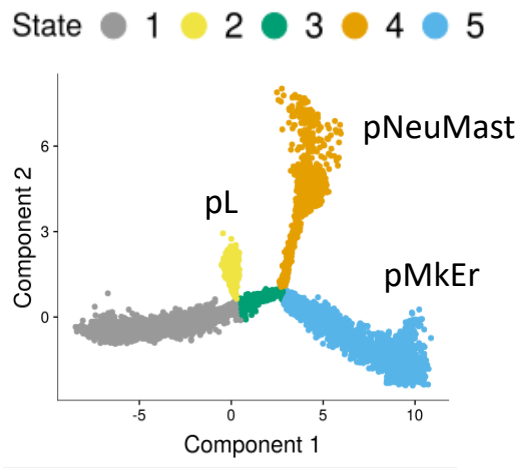


- Phenotypically defined LT-HSCs are heterogeneous and prepared to external signal.

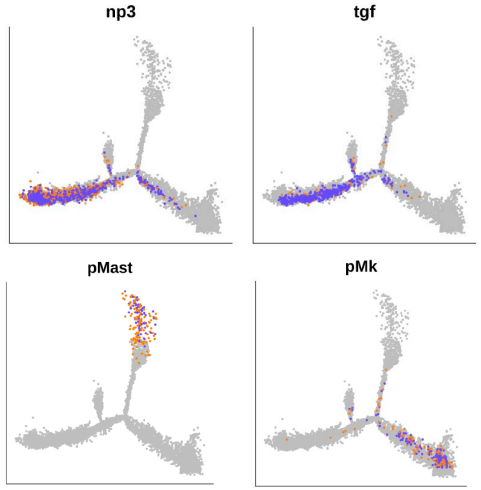
- Aging affects more cells in which signalling is already activated.

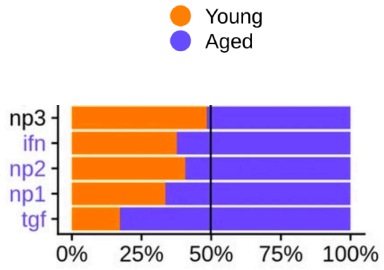
Defining the most immature HSC





Pseudotime ordering with Monocle
(Qiu et al, Nat methods 2017)



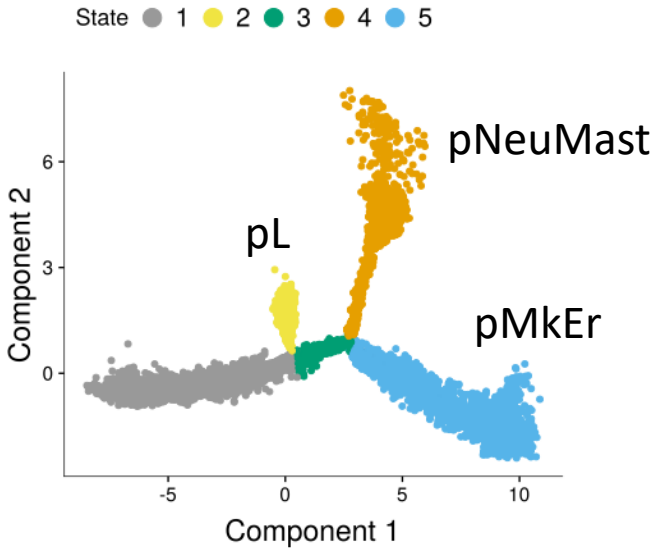


° np3 cells have the fewest gene markers

Ifitm1, Pdzk1ip1, Mllt3, Ly6a, Ltb, Sult1a1, Cd74, Meg3

° np3 cell proportion does not change upon aging

Aged HSCs are delayed concomitantly in differentiation and cell cycle progression



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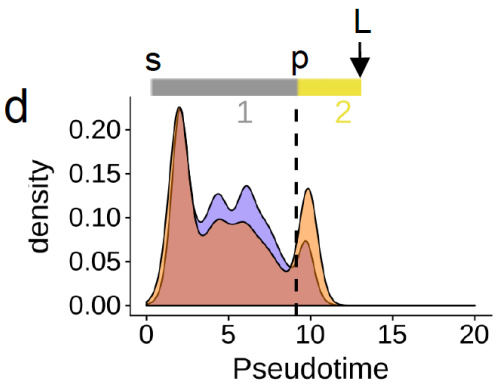
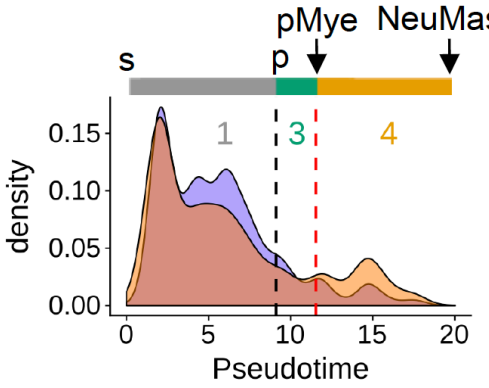
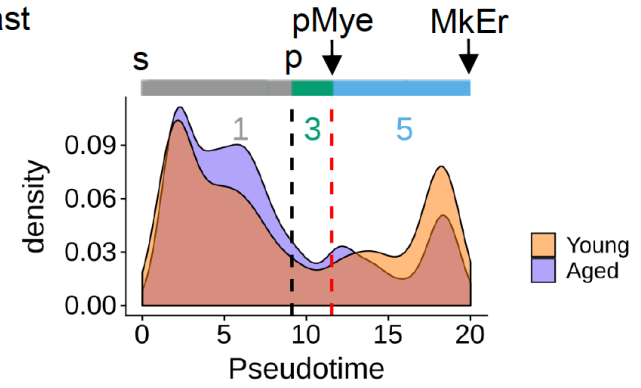
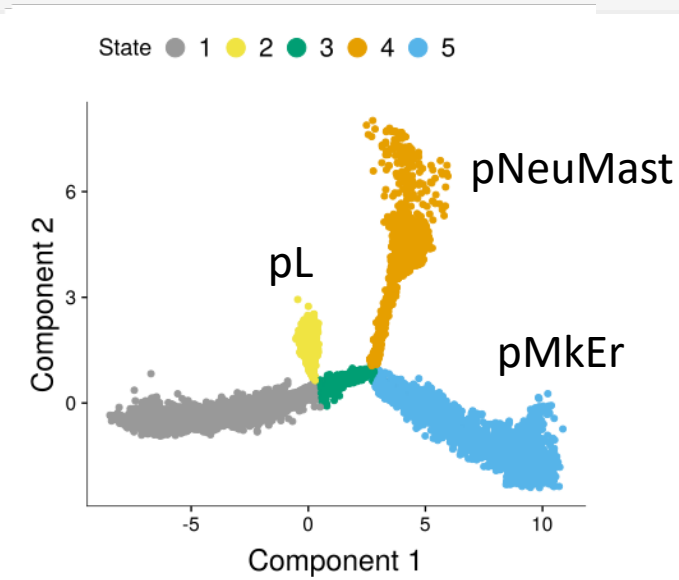
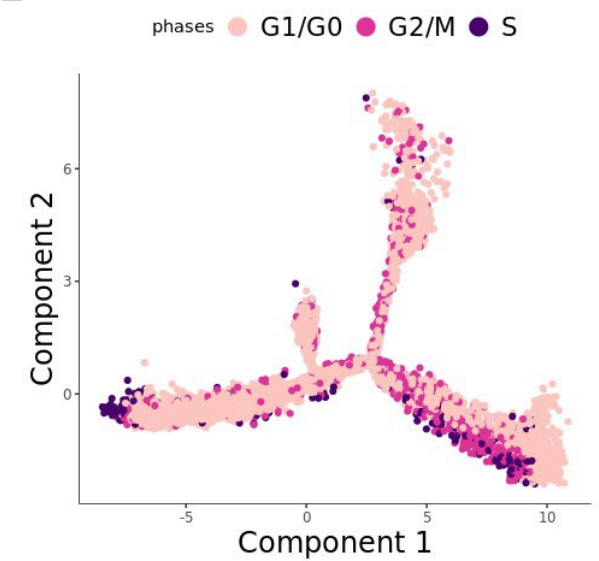


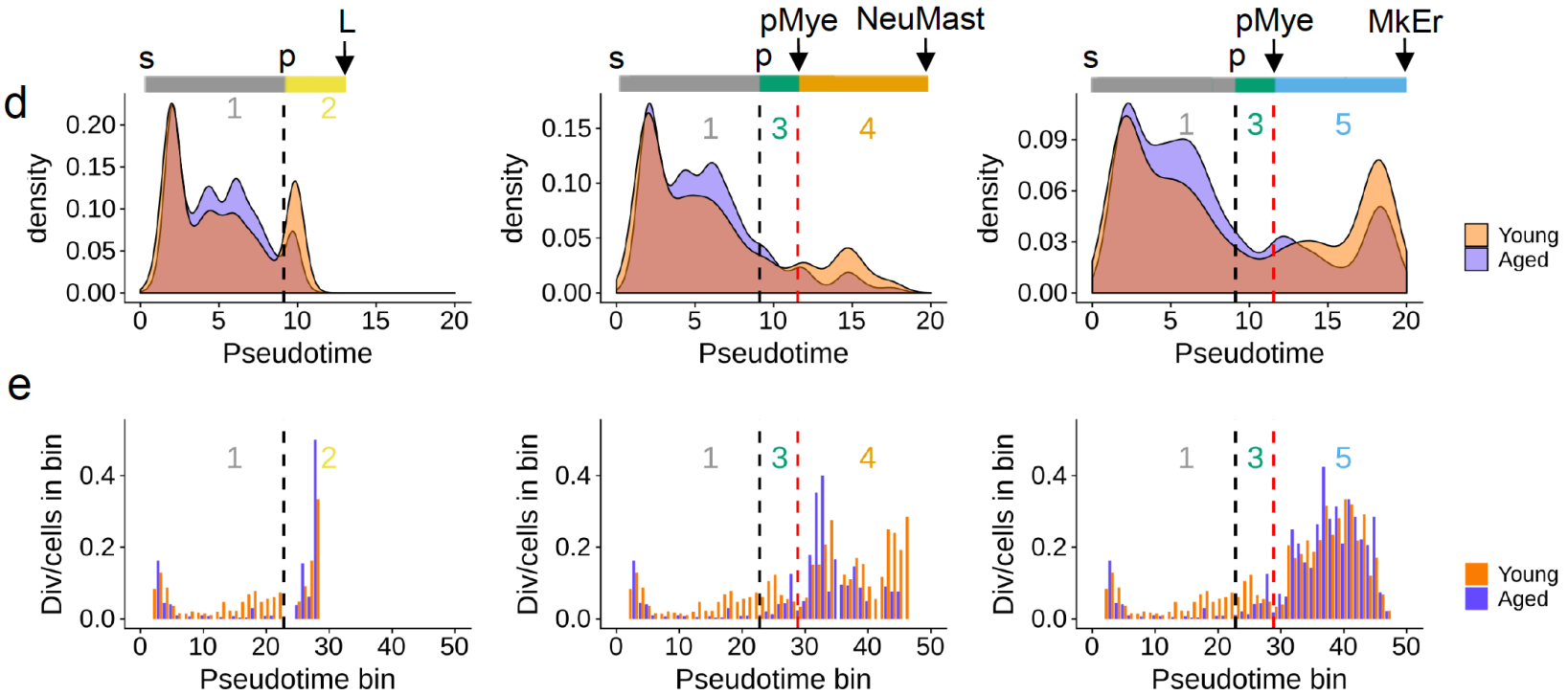




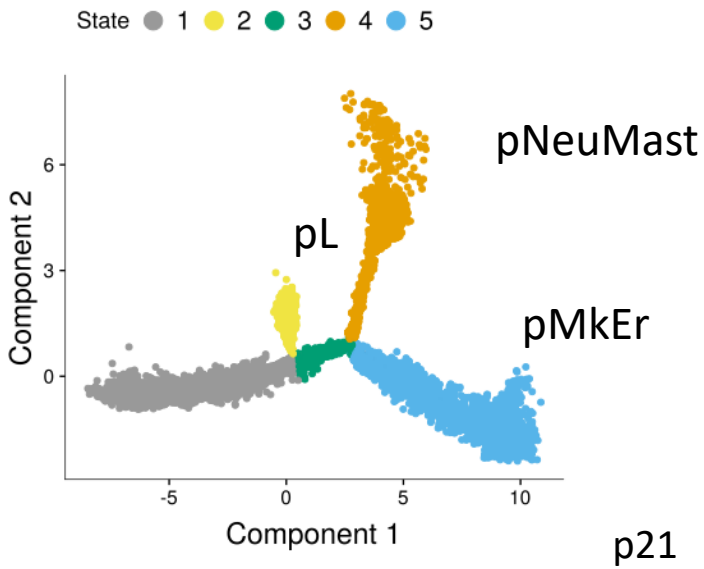
Aged HSCs are delayed concomitantly in differentiation and cell-cycle progression

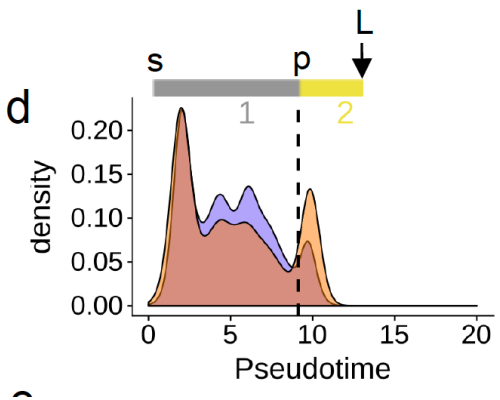


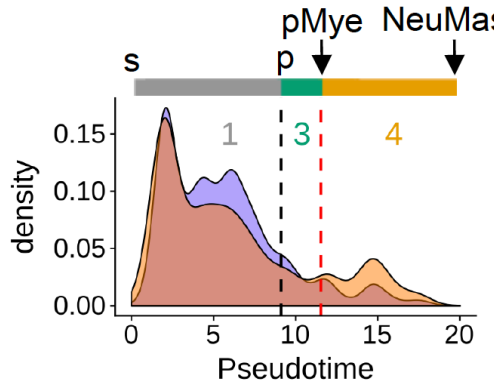


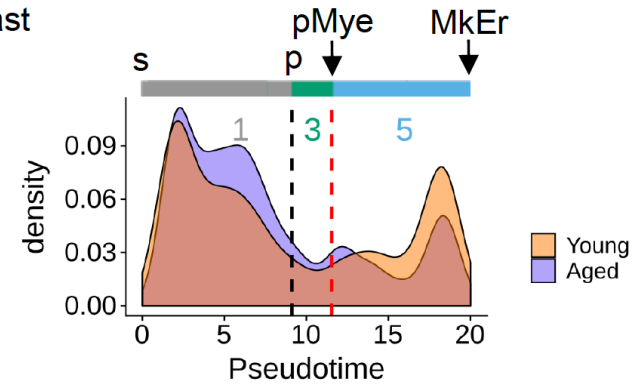


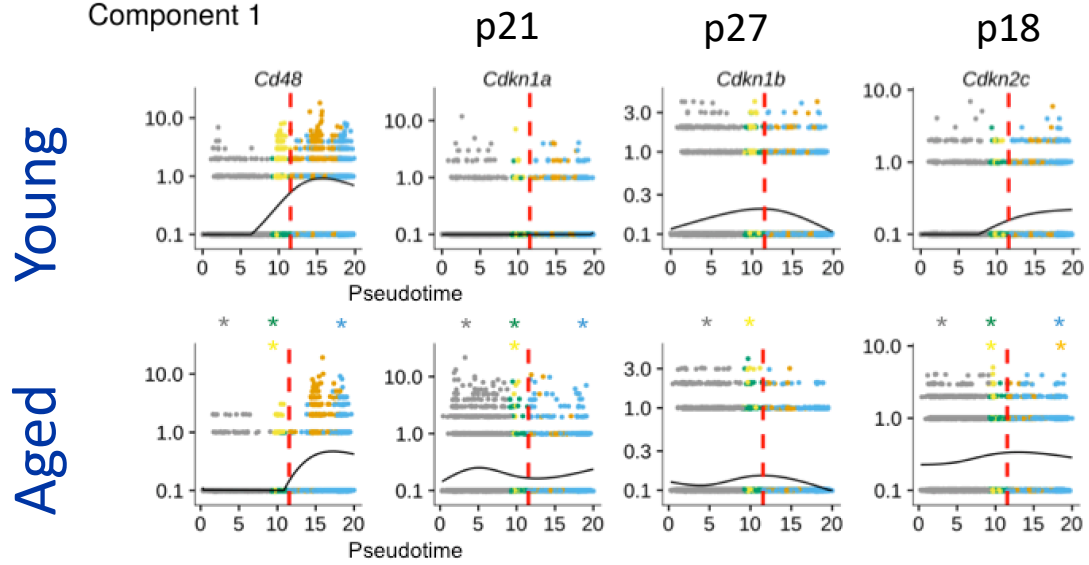
Aged HSCs are delayed concomitantly in differentiation and cell-cycle progression







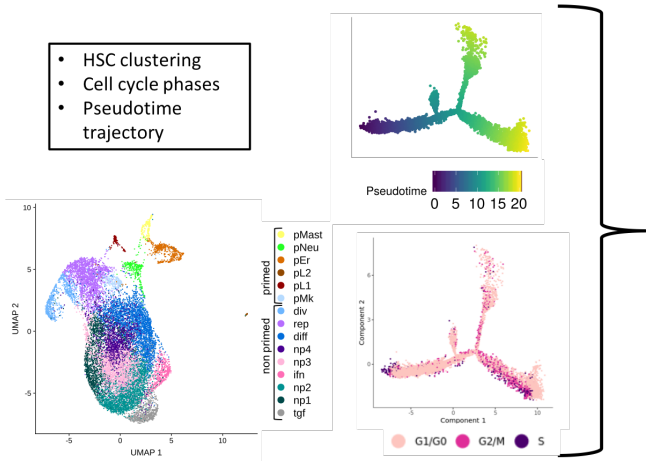


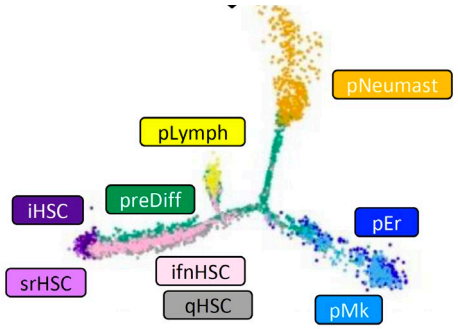


A default in cell cycle regulation in subset of HSCs that delay their commitment.

Synthesis of a HSC boolean model with Bonesis

- HSC clustering
- Cell cycle phases
- Pseudotime trajectory



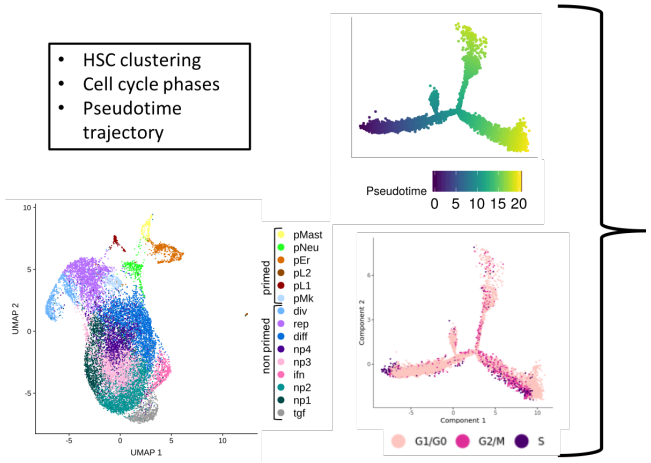


initial – intermediate - fixed

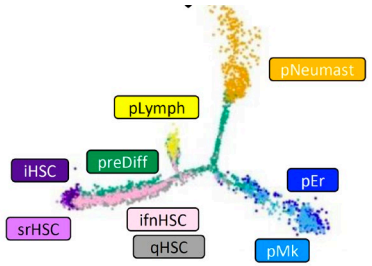
Activities per state

Synthesis of a HSC boolean model with Bonesis

- HSC clustering
- Cell cycle phases
- Pseudotime trajectory

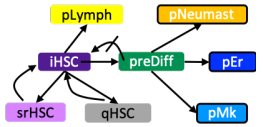


Activities per state

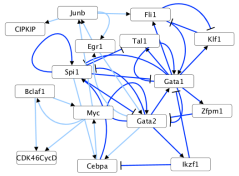


initial – intermediate - fixed

Linking partial observations to configurations
Dynamic constraints

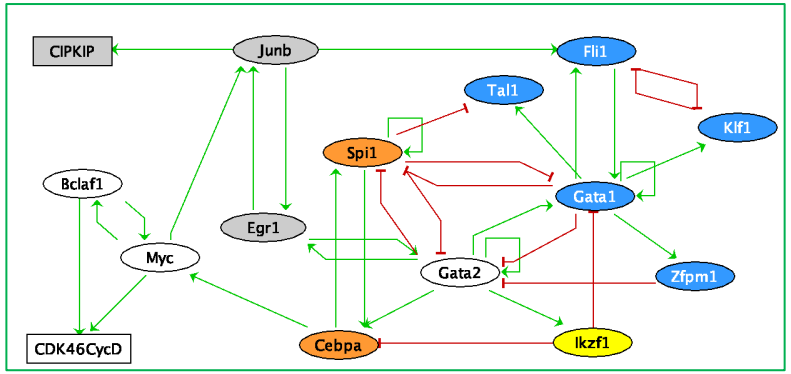


- 15 composants
- 60 interactions
- $>10^{22}$ modèles possibles



Adding constrained mutant behaviors

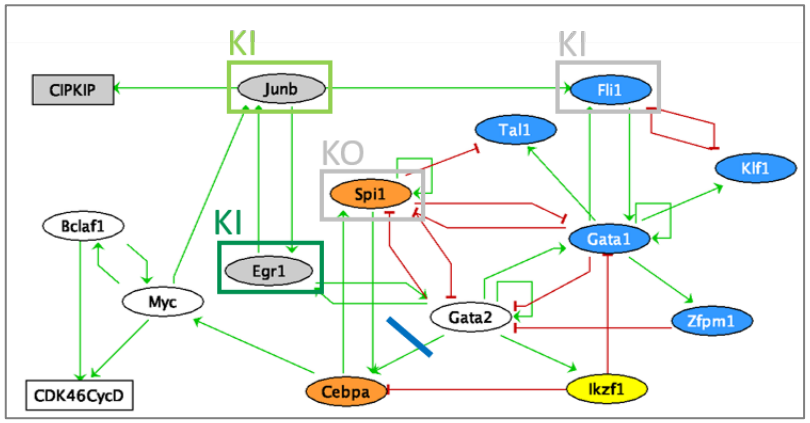
Final inference



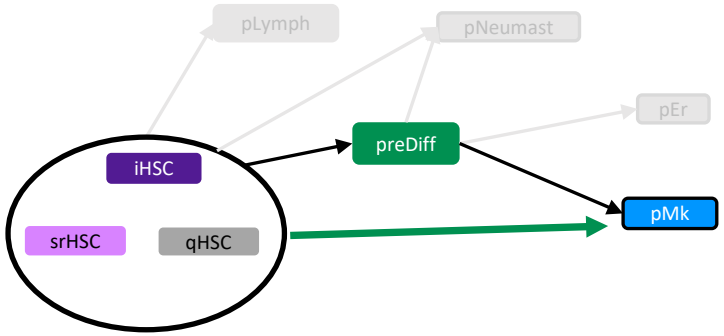
Final solution

Importance of Egr1 and Junb activation in HSC aging

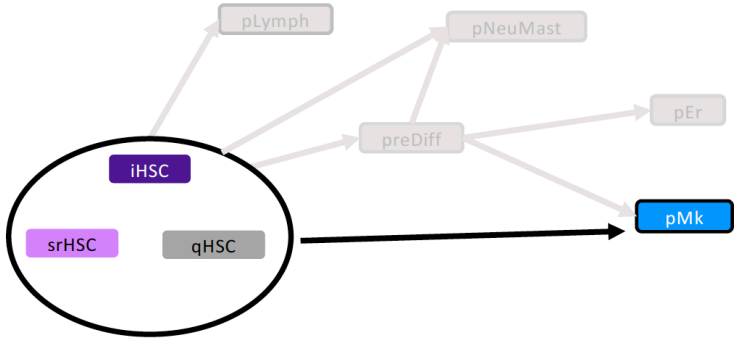
Perturbations



Egr1 KI



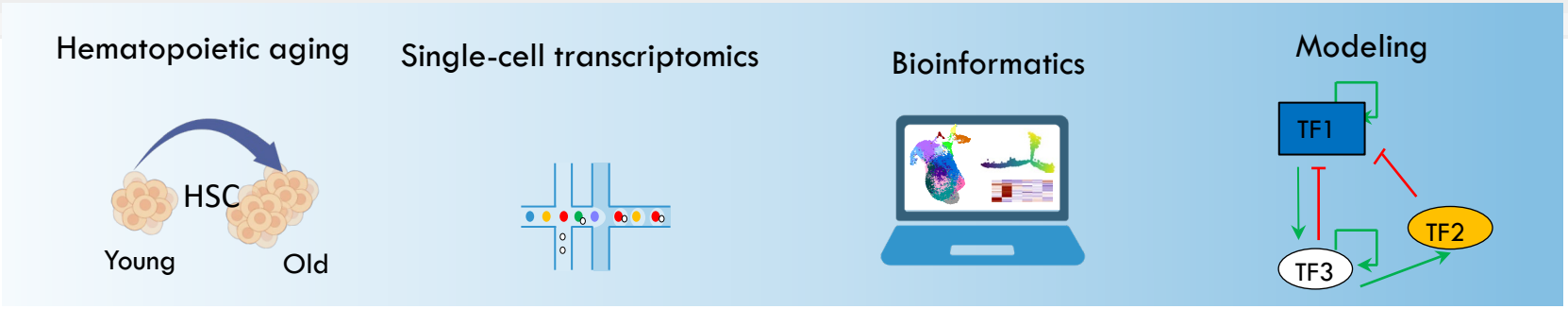
Junb KI

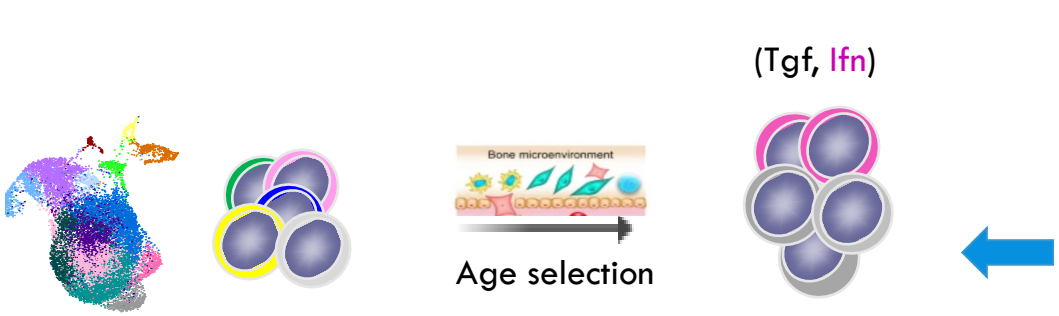


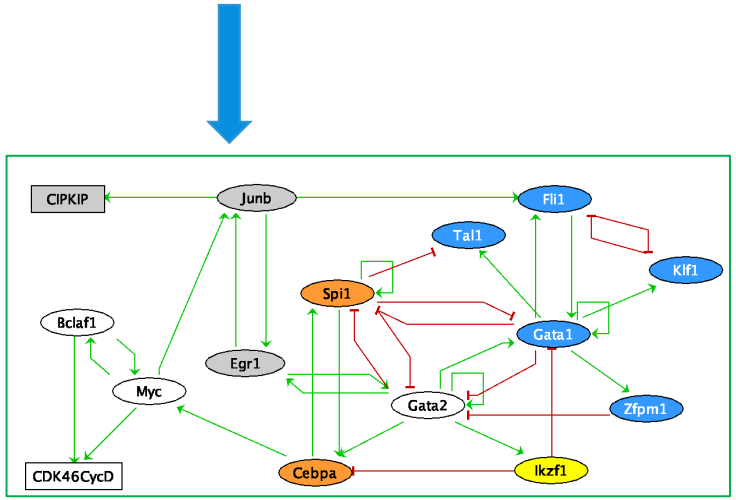
- *Egr1*, *Junb,*
- TGF-beta signalisation

(Hérault, Comp Structural Biol J., 2023)

Mechanisms of HSC aging







EGR → KLf6 → P21

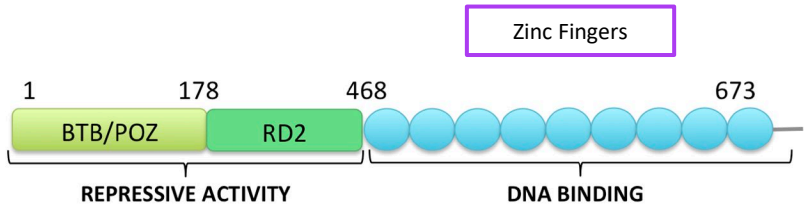
- **We resolved HSC heterogeneity and identified HSCs that accumulate during aging**

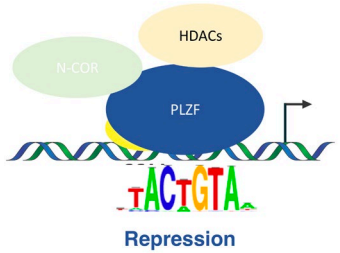
(Hérault, BMC biology, 2021)

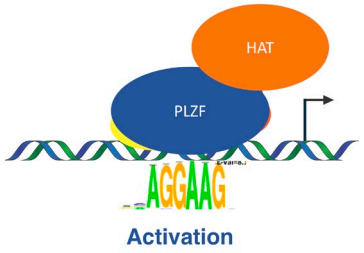
- **A regulatory network promoting aged HSC quiescence**

(Hérault, Comp Structural Biol J., 2023)

PLZF







Global Regulation of Hematopoiesis

Myeloid differentiation

Lymphocytic development

Megakaryocytic development

Musculoskeletal development

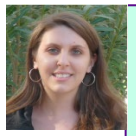
Spermatogenesis

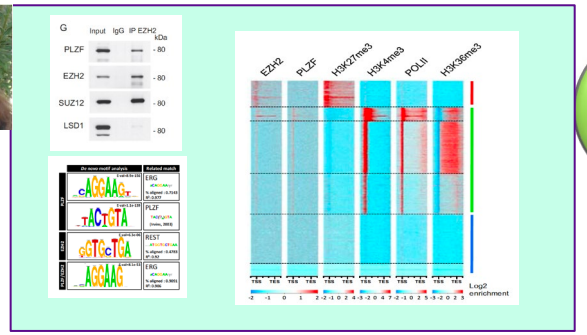
-balances cell differentiation and cell proliferation

-targets cell-cycle and immunity genes

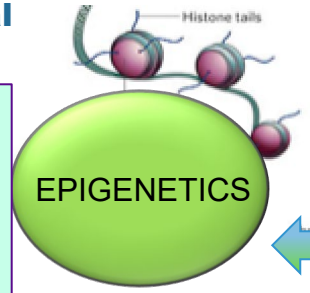
PLZF: a link between HSC aging and epigenetic

PLZF and EZH2: a non canonical relationship

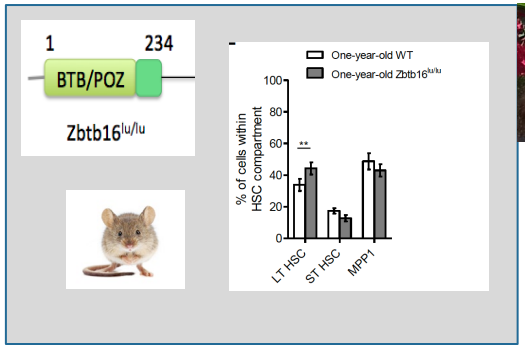




Koubi, NAR, 2018



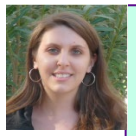
PLZF restrains HSC aging

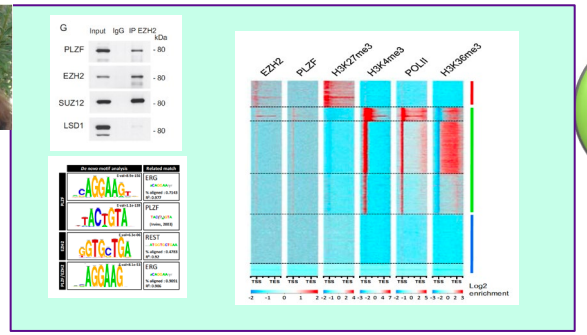


Vincent-Fabert, Blood, 2016

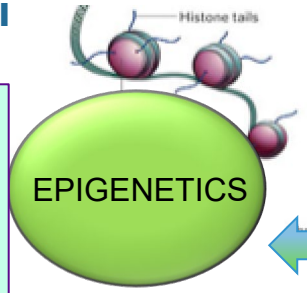
PLZF: a link between HSC aging and epigenetic

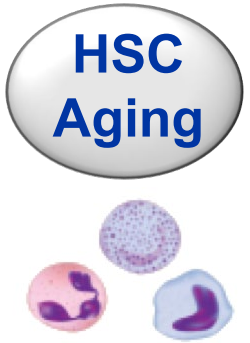
PLZF and EZH2: a non canonical relationship



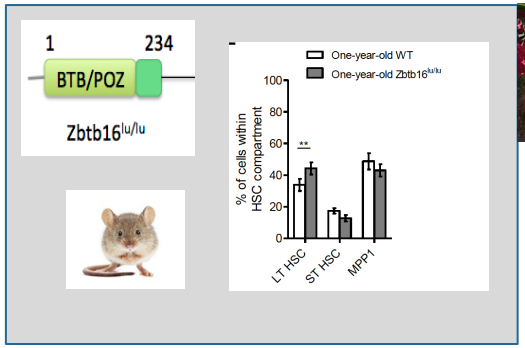


Koubi, NAR, 2018

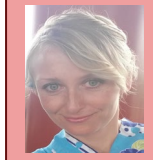


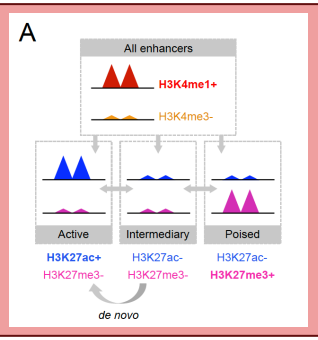


PLZF restrains HSC aging



Vincent-Fabert, Blood, 2016

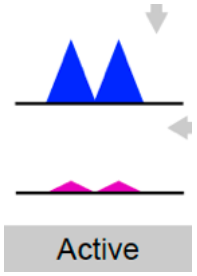




Poplineau, NAR, 2019

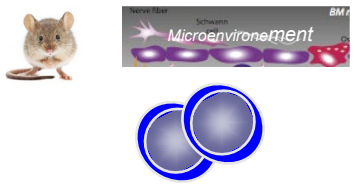
PLZF is involved in HSC aging

Gain in H3K27ac



At inflammatory genes

Zbtb16$^{lu/lu}$



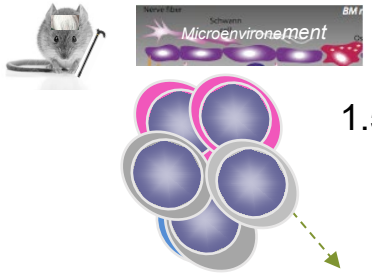
Aging

Inflammatory
Metabolism

differentiation

A more pronounced aging phenotype

Zbtb16$^{lu/lu}$



1.5 y-old zbtb16^{lu} HSCs
Aging++++

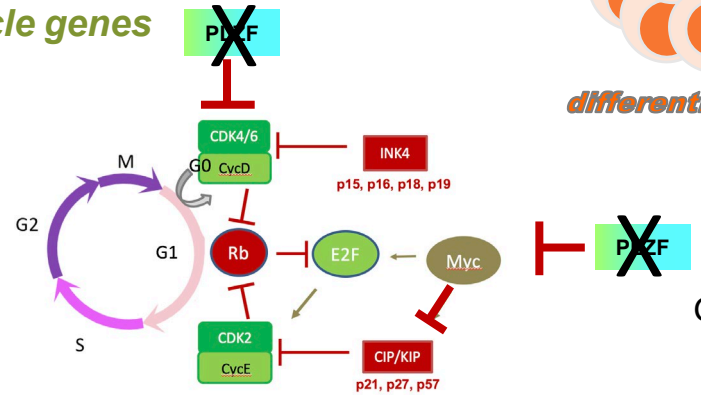
Altered differentiation

Loss of regenerative potential

Myeloid bias

HSC exhaustion

At cell-cycle genes



G1-S phase transition

Activity of PLZF in HSC aging ?

PLZF mutation increases myeloid disease frequency

PLZFmutant

1 234

BTB/POZ

$Zbtb16^{lu/lu}$

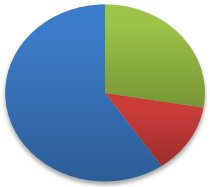
No spontaneous tumor

X

***Cdkn2a*$^{-/-}$**

Tumor model

(Krimpenfort, Nature, 2007)

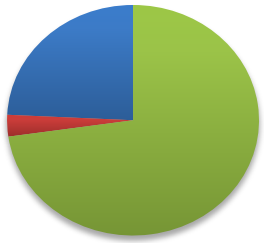


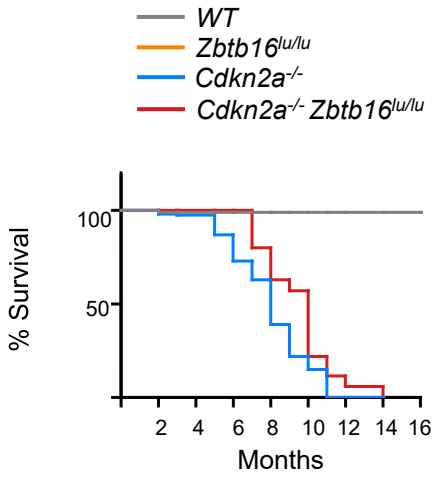
Solid tumor

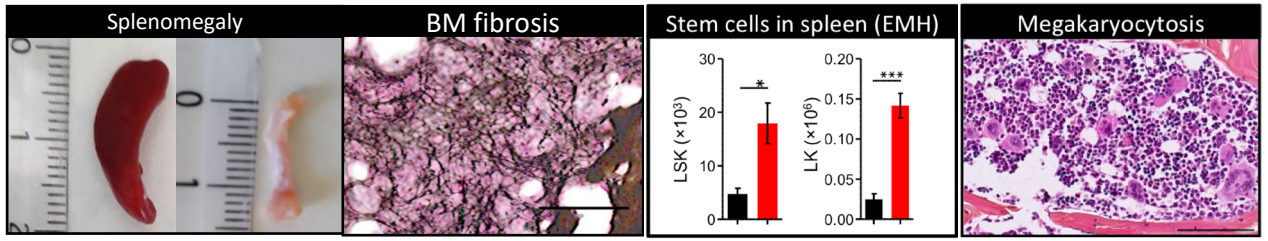
Leukemia & lymphoma

Splenomegaly

***Cdkn2a*$^{-/-}$ *Zbtb16*$^{lu/lu}$**



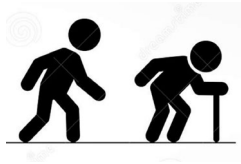




Primary Myelofibrosis (PMF)

(Haboub , in preparation)

PLZF mutation increases myeloid disease frequency



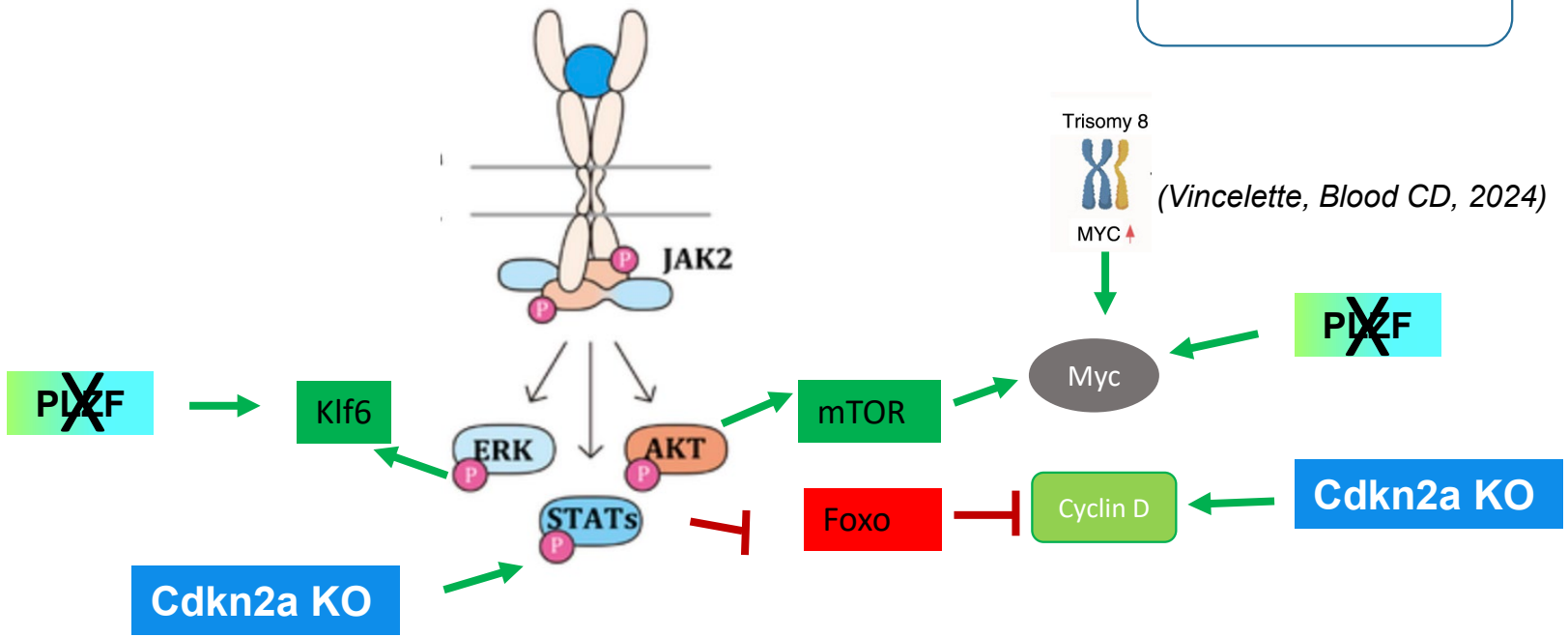
- Myelofibrosis is a **clonal hematologic malignancy** and secondary(non-clonal) **hyperproliferation of fibroblasts** with increased collagen synthesis .
- Myelofibrosis is affecting aged patients. Peak incidence of PMF is between **50 and 70 yr.**
- 90% of the patients have a mutation in JAK/STAT signaling pathway. **JAK2V617F**, **MPL** **and** **CALR**

Zbtb16 is a coding gene for PLZF which regulates the hematopoiesis And limit HSC cycling

Cdkn2a is a tumor supressor

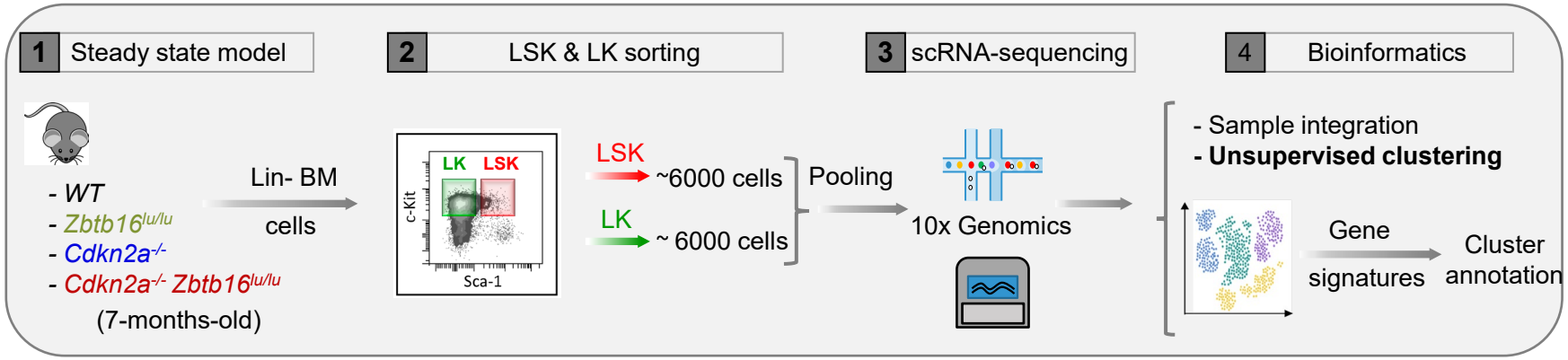
Primary Myelofibrosis (PMF)

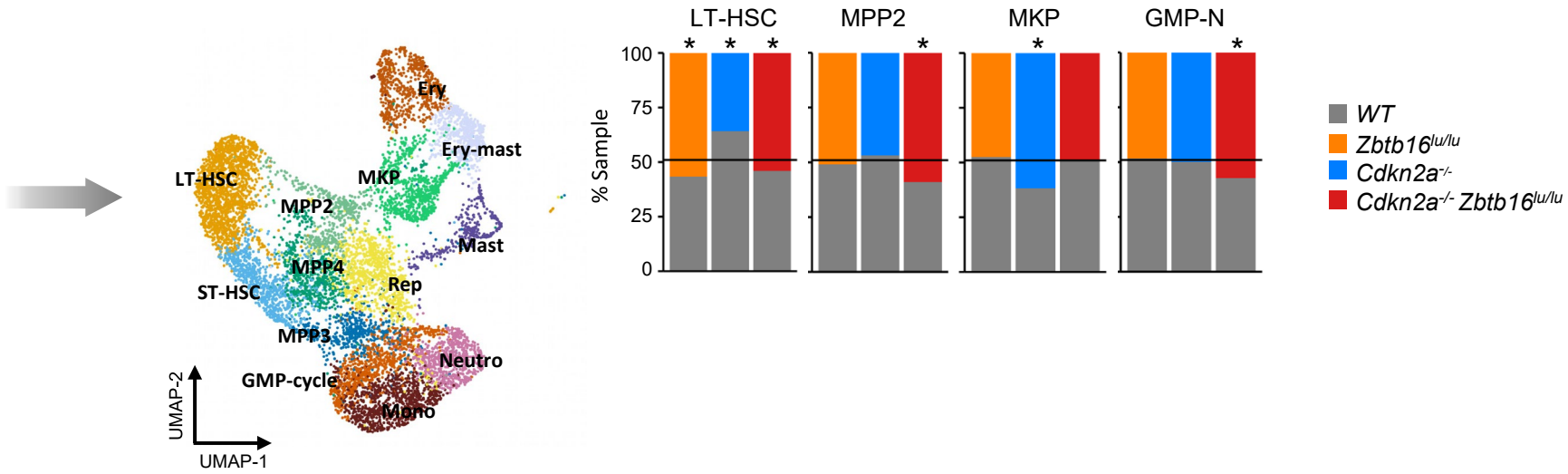
JAK2V617F, **MPL** **and** **CALR**



(Vincelette, Blood CD, 2024)

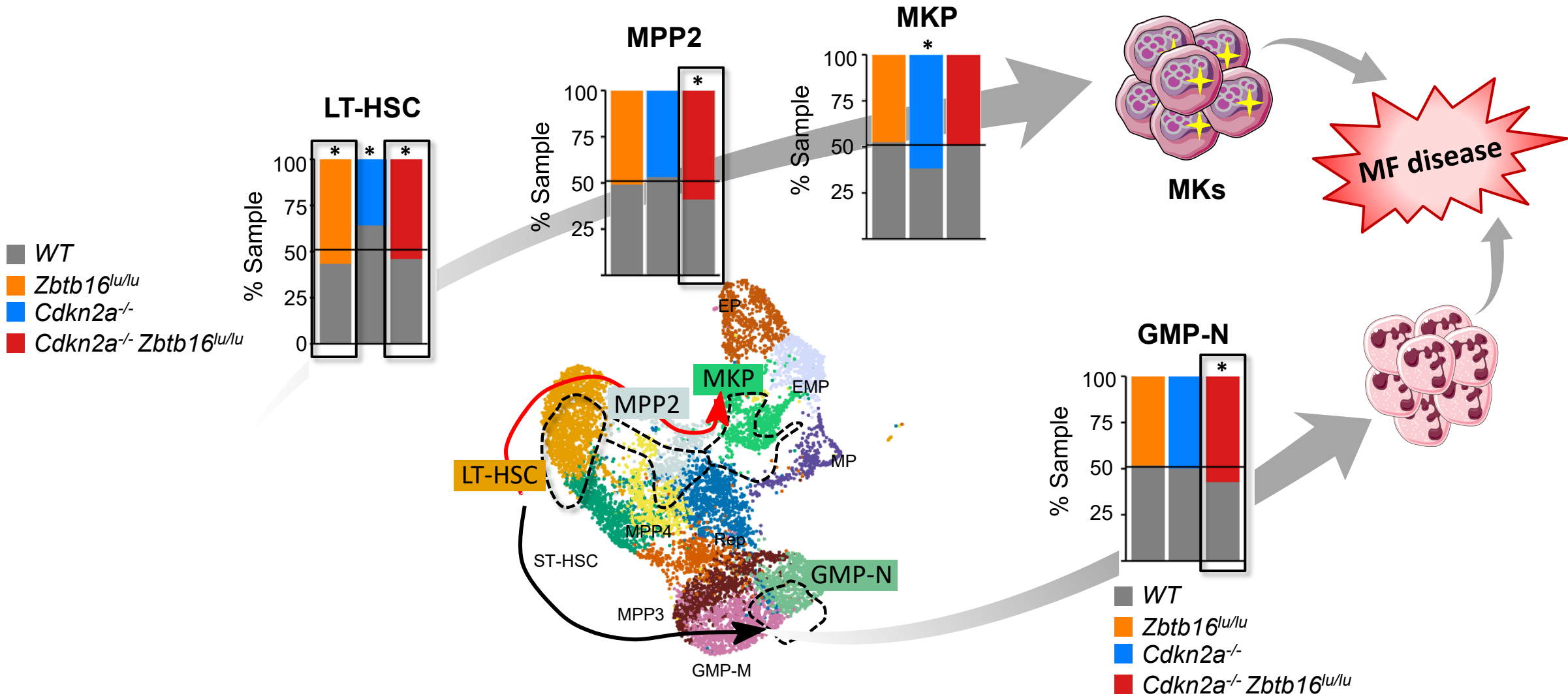
PLZF mutation increases myeloid disease frequency



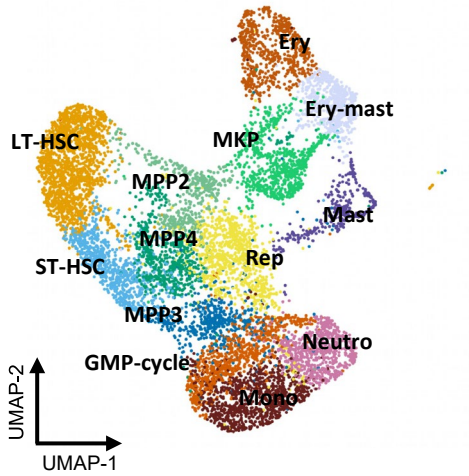


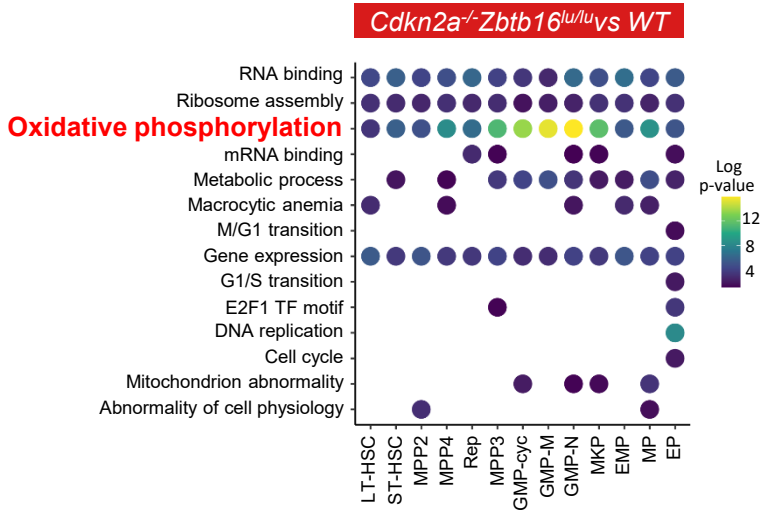
(Loreen Haboub , in preparation)

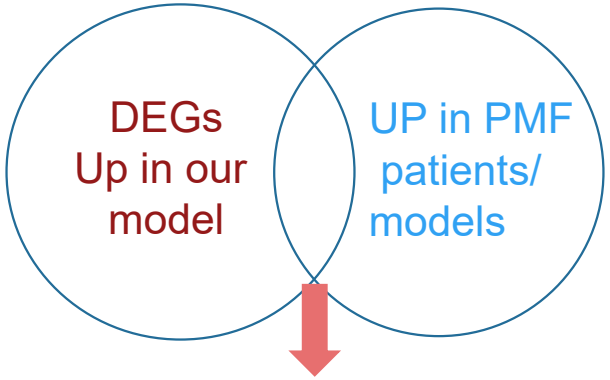
LT-HSC, MPP2 and GMP-N are populations that drive the MF pathogenesis in the *Cdkn2a*$^{-/-}$ *Zbtb16*$^{lu/lu}$ mouse model



Cdkn2a $^{-/-}$ *Zbtb16*$^{lu/lu}$ increases MPP2 and GMP compartments







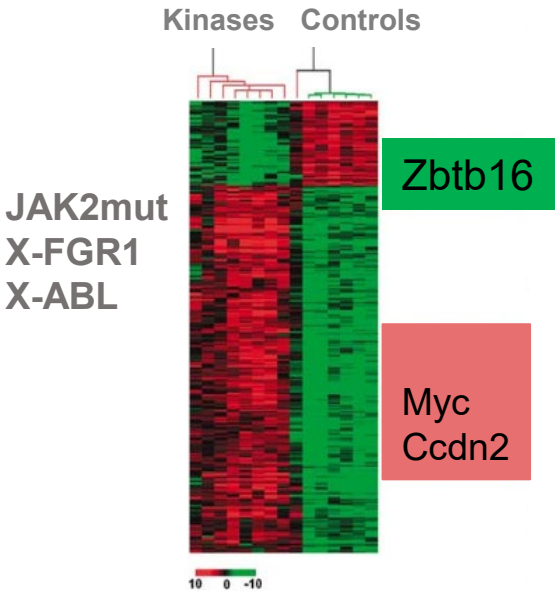
Potential interesting targets

(Cdk2, Myc, Mycn, Ezh2….)

(Loreen Haboub , in preparation)

PLZF/*ZBTB16* in human myeloproliferation

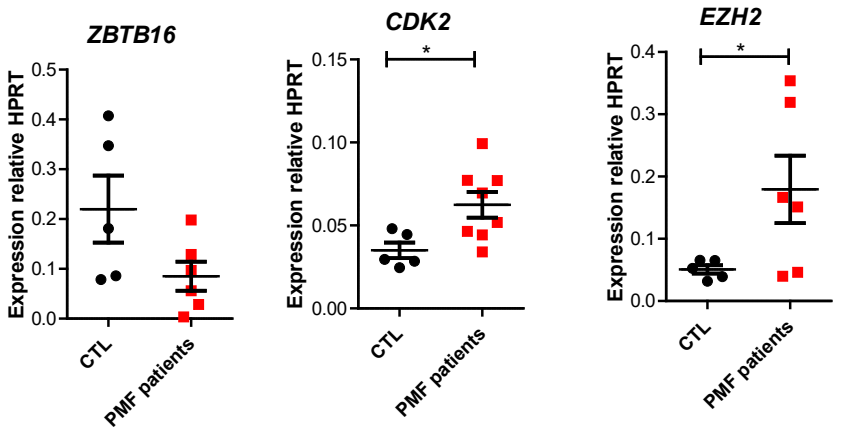
Oncogenic kinases of myeloproliferative disorders induce both protein synthesis and G1 activators.
Delaval, B, ..Wachenker, ***Birnbaum, D***
Leukemia (2006) 20, 1885–1888. doi:10.1038/sj.leu.2404361.

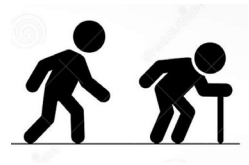


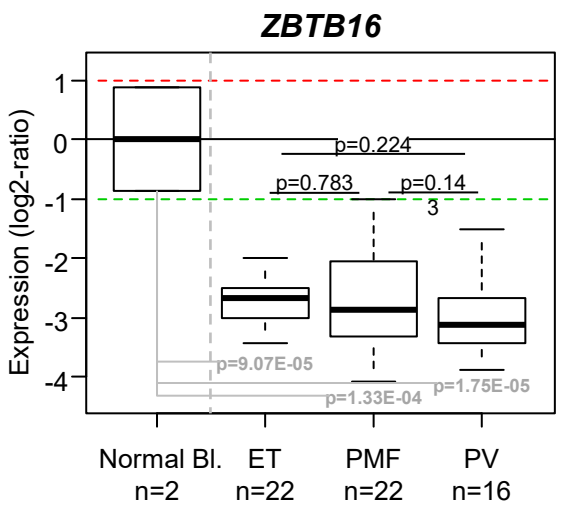
➢↗ CDK2 et EZH2 □ cycle cellulaire
➢ Corrélation avec une ↘ de ZBTB16

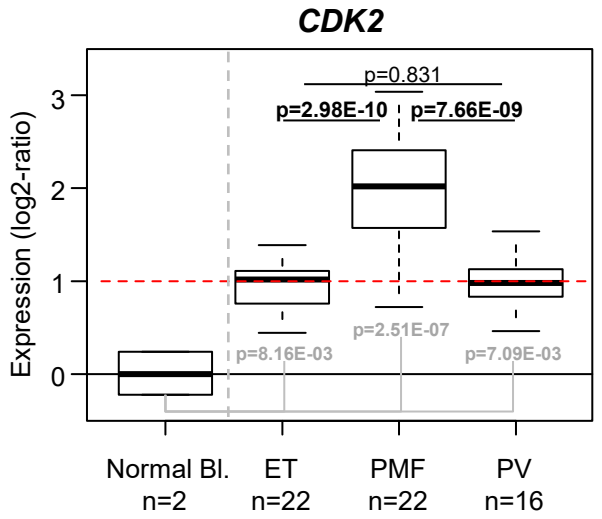
IPC patient data

Loreen Haboub
Maeva Rodies
Anne Murati









Take home message

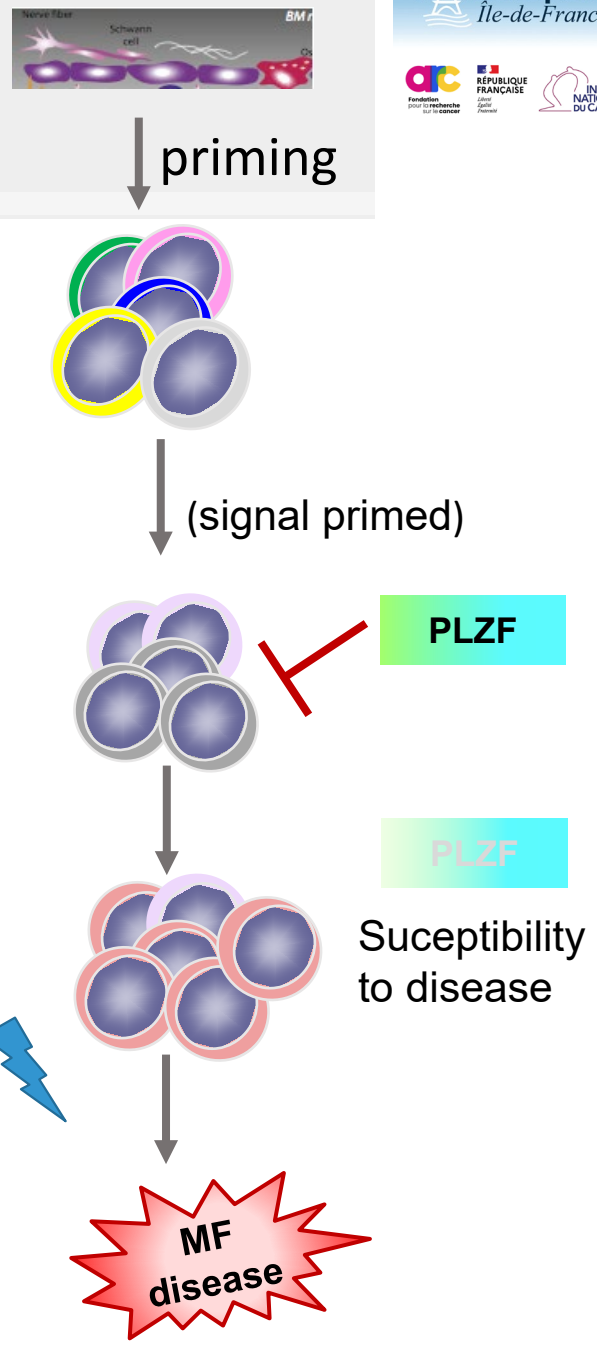
-Phenotypically defined LT-HSCs are heterogeneous and prepared to external signals.

-Aging affects more cells in which signalling is already activated and induced quiescence.

- PLZF limits aged-related increase of chromatin accessibility

-PLZF inactivation leads to aging and succeptibility to myeloproliferation
-PLZF is down regulated in PMF patients

PLZF a key epigenetic factor in human HSC aging and myeloproliferation ?



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Animal facillity

Cytometry

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