HUMANITAS
RESEARCH HOSPITAL

LA CO-MUTAZIONE SF3B1–JAK2/MPL RIMODELLA LA DIFFERENZIAZIONE ERITROIDE E PROMUOVE LA DOMINANZA CLONALE INFIAMMATORIA NELL'MDS/MPN-RS-T: EVIDENZE DA ANALISI MULTI-OMICHE A SINGOLA CELLULA

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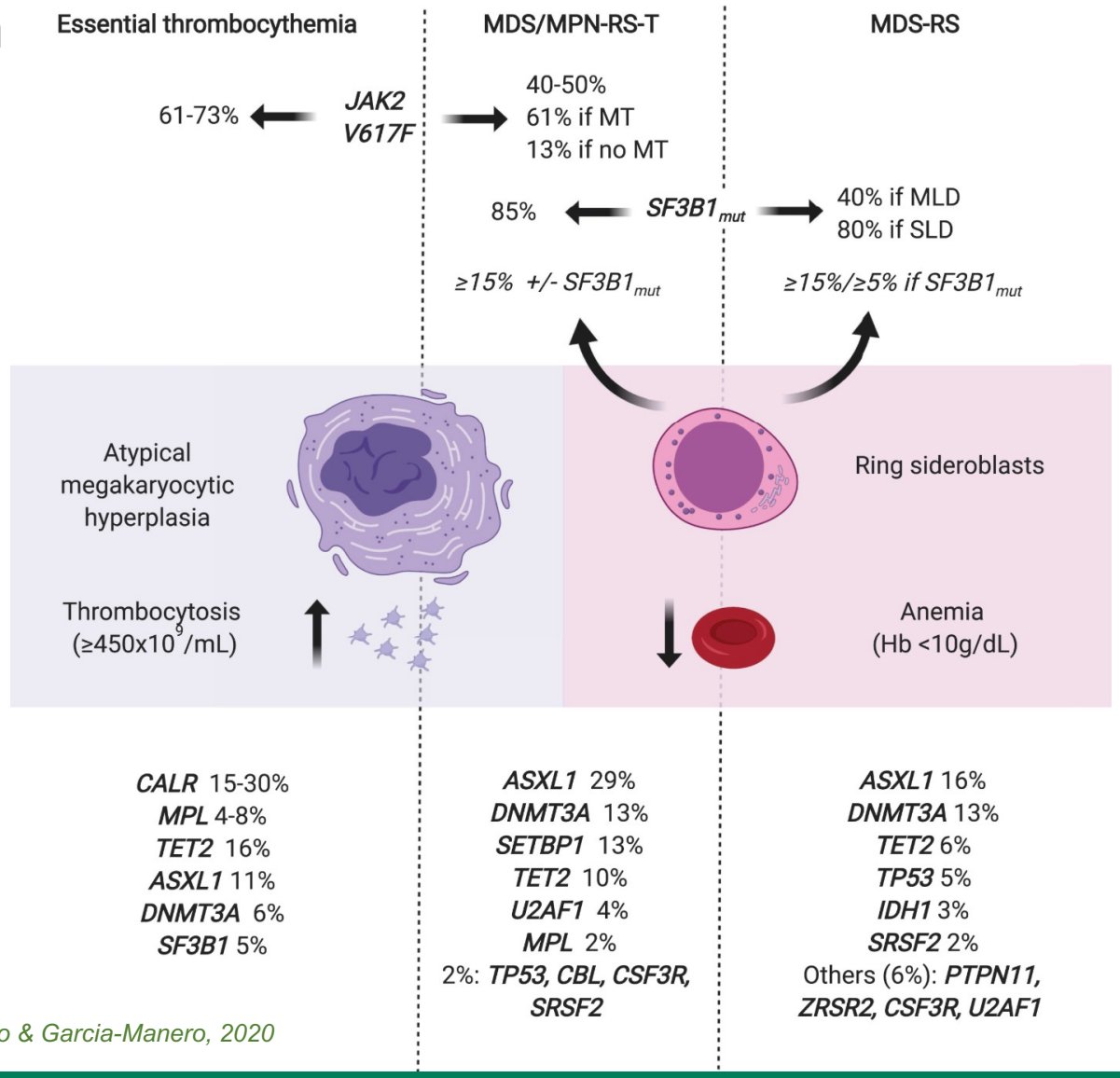
MDS/MPN-RS-T

- An ultra- rare haematologic neoplasm overlapping MDS and MPN diseases
- Anemia, bone marrow dysplasia with ring sideroblasts and persistent thrombocytosis with proliferation of large and morphologically atypical megakaryocytes
- The two “opposite” phenotypes derive from mutations in different genes:
  - splicing genes → BM dysplasia
  - genes of the JAK/STAT pathway → thrombocytosis

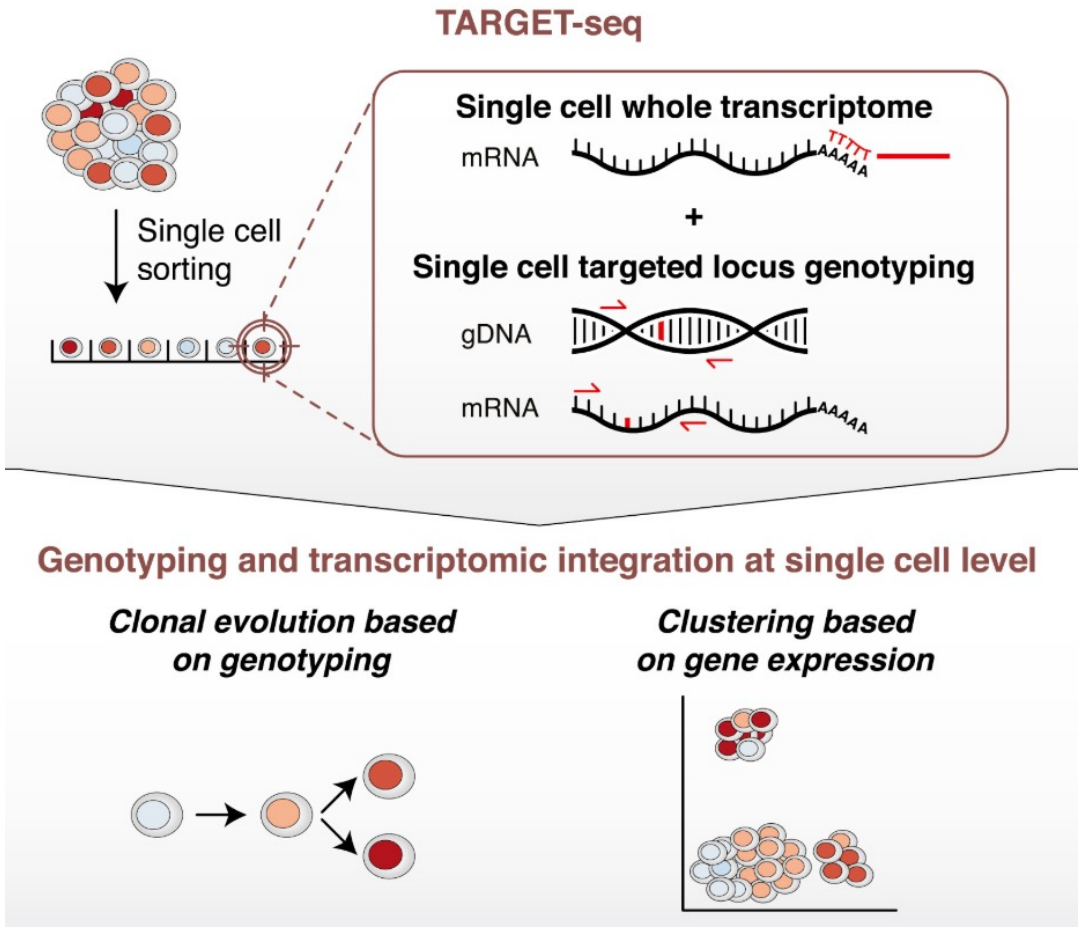


Montalban-Bravo & Garcia-Manero, 2020

Single-cell multi-omics (TARGET-seq)

TARGET-Seq (Rodriguez-Meira, 2019) allow simultaneous study of somatic mutations and RNA profile on the same single cell.

We aimed to use TARGET-Seq in MDS/MPN-RS-T patients:
- to elucidate the clonal evolution of the disease
- to correlate gene expression with the genotype of the cell
- to identify pathways that might be effective target for therapeutic intervention

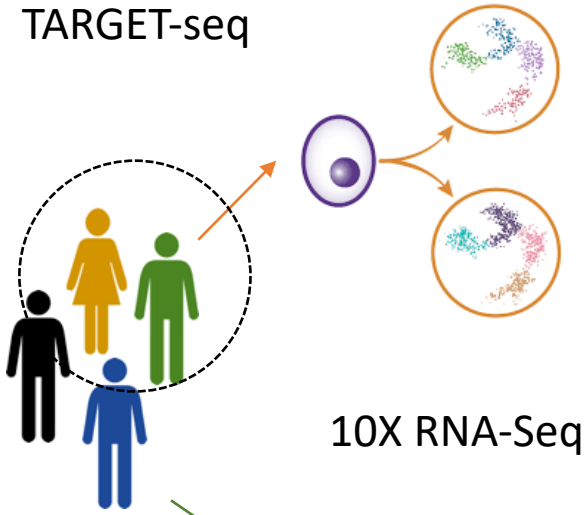


Chaligne et al., Molecular Cell, 2019

Methods

TARGET-seq

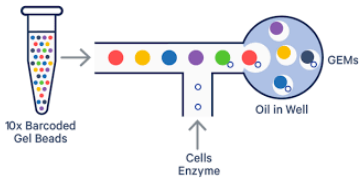
6 pts



Mutational profile

RNA profile

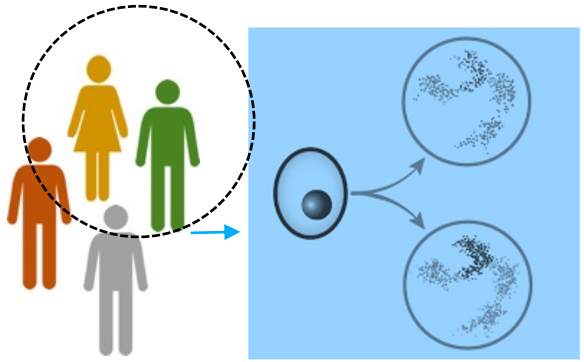
10X RNA-Seq



RNA profile

10X RNA+ATAC

4 pts



Chromatin profile

RNA profile*

- Characterization of the disease in single cell
- Pathway involvment in genotype-phenotype

RNA

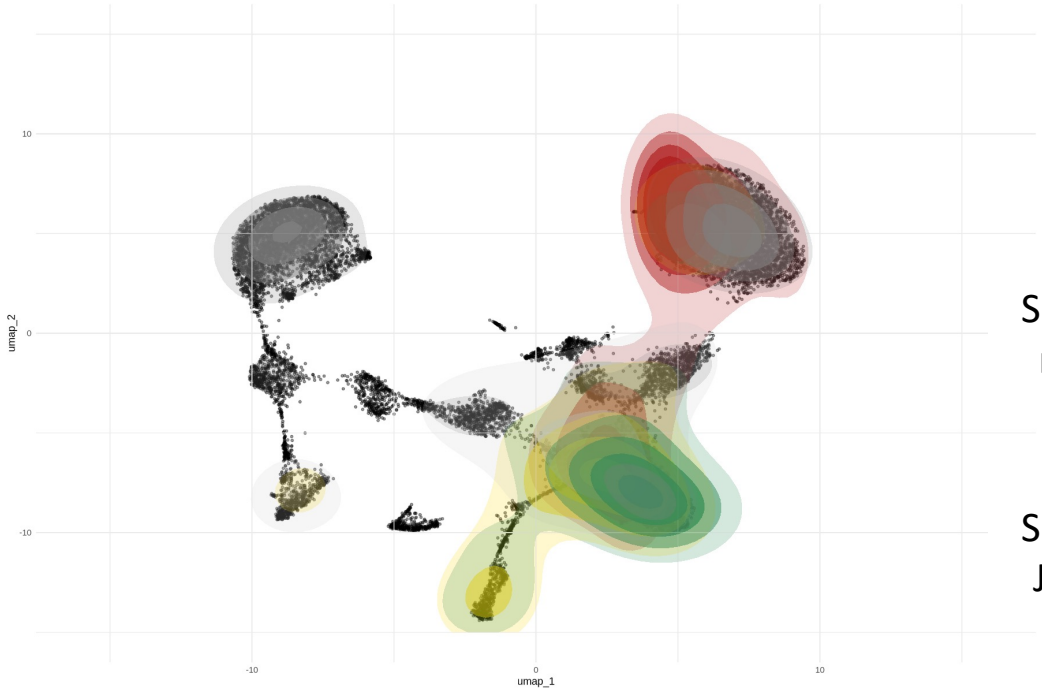
RNA

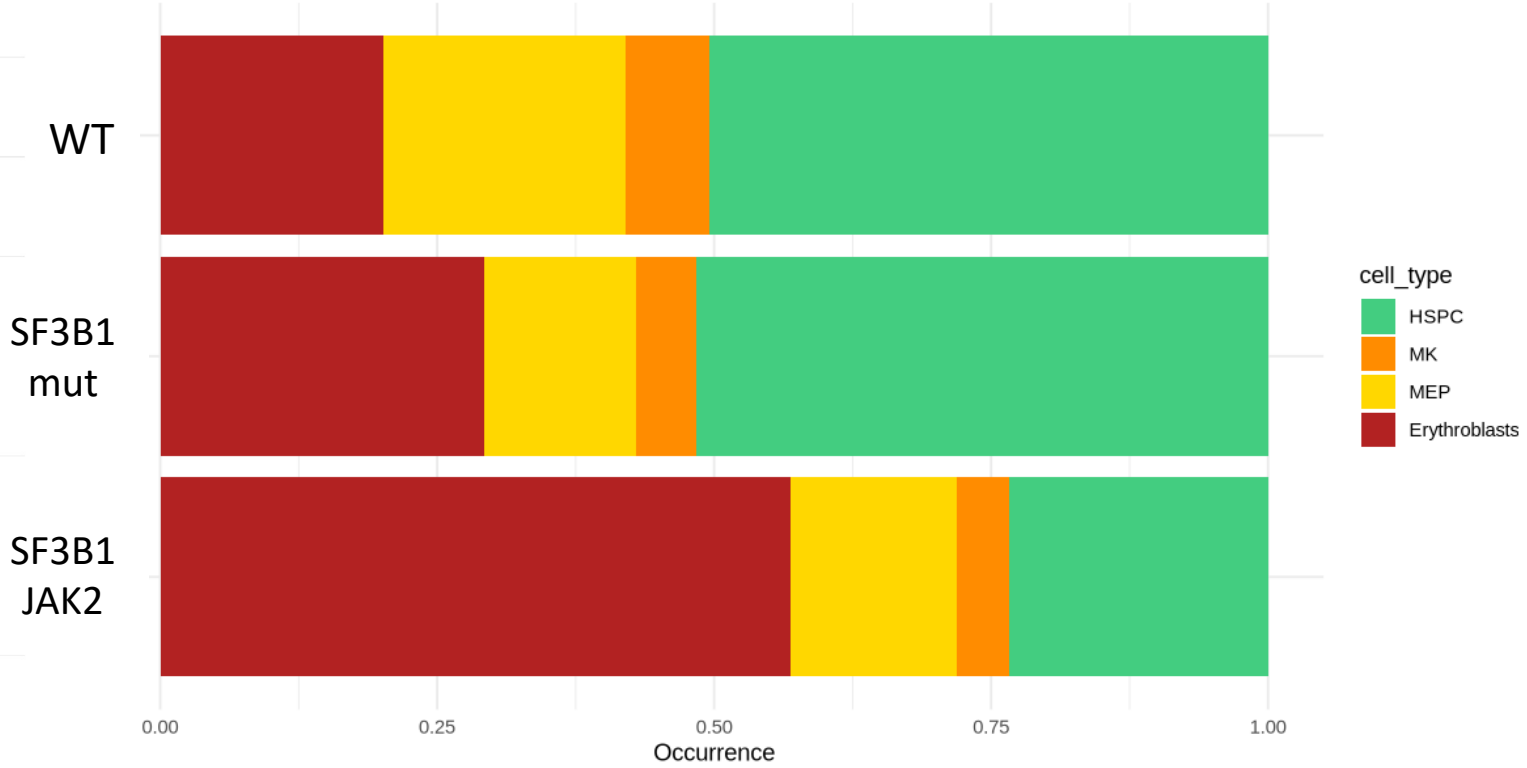
RNA

- Complexity reduction using less expensive technique
- Target for therapeutic intervention

- Transcriptional program in genotype-phenotype

Cells Differentiation and mutational status



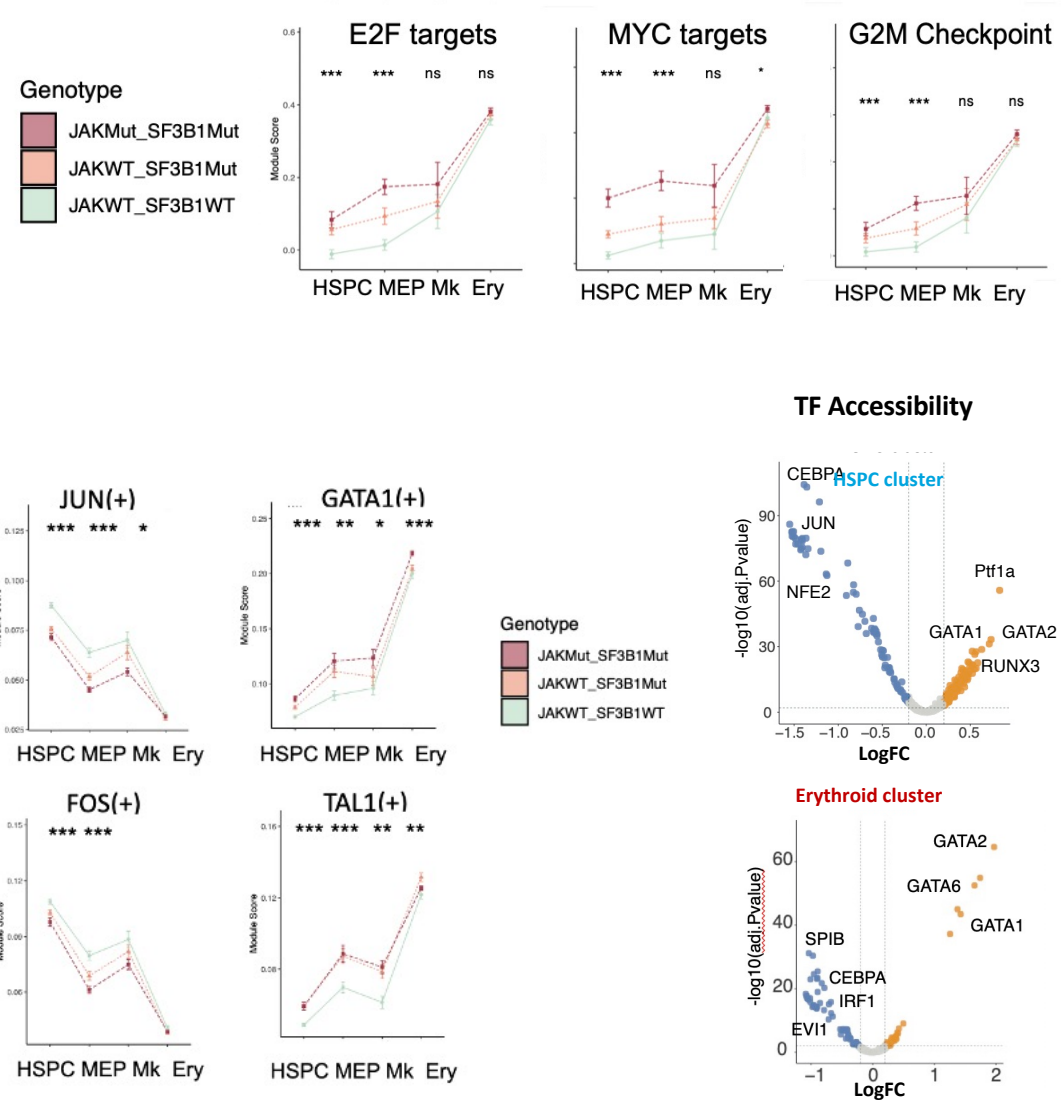


- We identified a significant **increase** in the **erythroblast fraction** attributable to the combined effect of both mutations (Fisher test, $p<0.001$).
- Double-mutant erythroblasts showed upregulation of genes involved in late erythroid differentiation, including ALAS2, SLC25A37 (mitoferrin-1), ABCB10, and FTMT, all crucial for heme synthesis and mitochondrial iron transport. This upregulation is consistent with a cellular response to iron overload and oxidative stress, a hallmark of sideroblast formation

TARGET-seq Analysis

- Erythroid commitment is driven by the **activation** of specific transcriptional and **proliferative pathways** (e.g., MYC, E2F targets) throughout the differentiation trajectory (T- test, p<0.001).

- Transcriptional regulation in double-mutated cells is characterized by **increased expression of erythroid regulators**. scATAC-seq shows significantly **higher accessibility** in their target regions, supporting an epigenetic commitment to erythroid differentiation in double-mutated cells



Any role for MYC and E2F?

HEMATOPOIESIS AND STEM CELLS | SEPTEMBER 3, 2009

c-Myc-mediated control of cell fate in megakaryocyte-erythrocyte progenitors

Yinshi Guo , Chao Niu , Peter Breslin , Minghui Tang , Shubin Zhang , Wei Wei , Ameet R. Kini , Gladell P. Paner , Serhan Alkan , Stephan W. Morris , Manuel Diaz , Patrick J. Stiff , Jiwang Zhang

ARTICLE | VOLUME 107, ISSUE 2, P247-258, OCTOBER 19, 2001

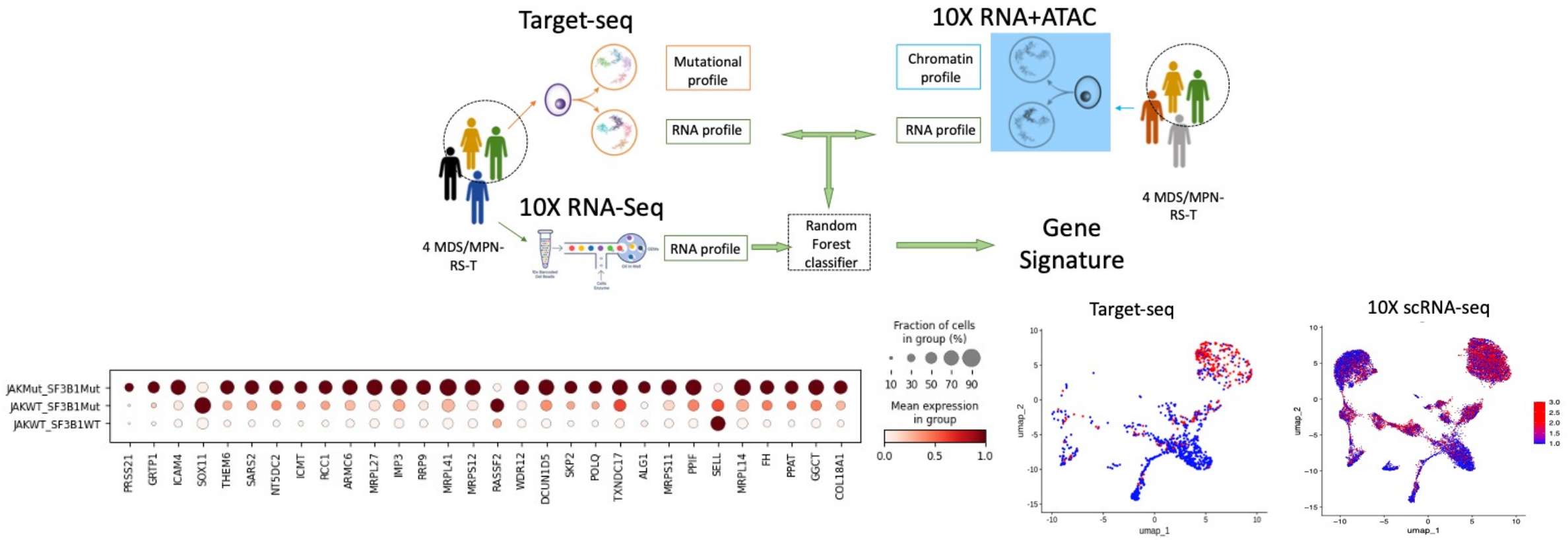
E2F Repression by C/EBPα Is Required for Adipogenesis and Granulopoiesis In Vivo

Bo T. Porse • Thomas Å. Pedersen • Xiufeng Xu • Bo Lindberg • Ulla M. Wewer • Lennart Friis-Hansen • Claus Nerlov • Show less

c-Myc knockout mice develop anemia, thrombocytosis, and leukopenia. Bone marrow analysis revealed a significant increase in megakaryocytopoiesis (with abnormal megakaryocyte morphology), blockage of erythrocyte differentiation, and reduction in myelolymphopoiesis

E2F repression by C/EBPα is essential for granulopoiesis. Mice harboring E2F repression-deficient C/EBPα alleles, had decreased granulocyte population in the bone marrow, paralleled by an increase in immature erythroid cells.

Data Integration



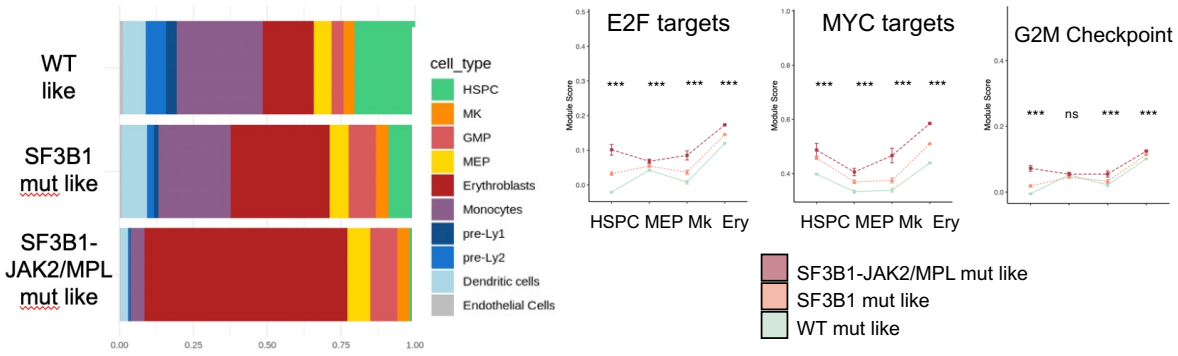
- Using a machine learning approach, we identified a **gene signature** capable of detecting **double-mutated cells** along differentiation trajectories

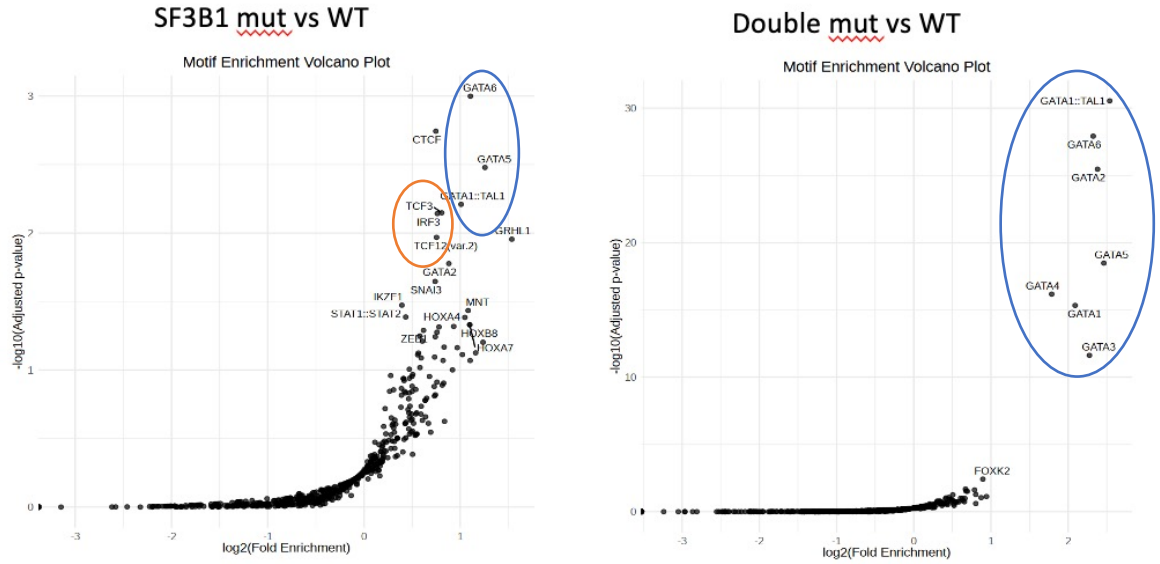
Data Integration Validation

- In non-genotyped cells, the signature module score **identified** profiles resembling **WT-like**, ***SF3B1*-mutant-like**, and ***SF3B1-JAK2/MPL*-mutant-like** cells.

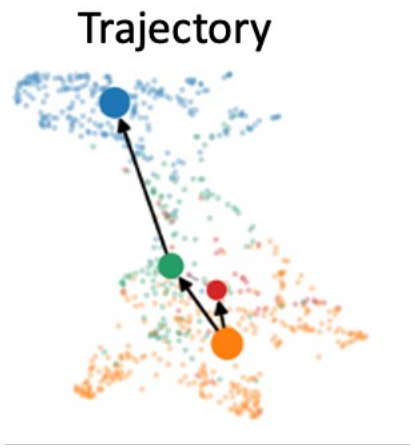
- **RNA genotyping on 10X scRNAseq** reveals increased **erythroid differentiation** and **proliferative pathway** activity, similar to patterns observed with DNA genotyping

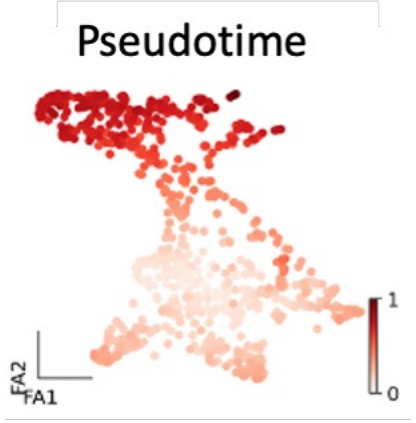
- **RNA genotyping on scATACseq.** Motif accessibility analysis with chromVAR revealed increased accessibility of **GATA family motifs** (e.g., GATA1:TAL1, GATA2, GATA4, GATA6) in double-mutated-like cells vs WT-like. SF3B1-mutated-like cells showed enriched motifs for both GATA factors and additional stem cell mantainance regulators like TCF3

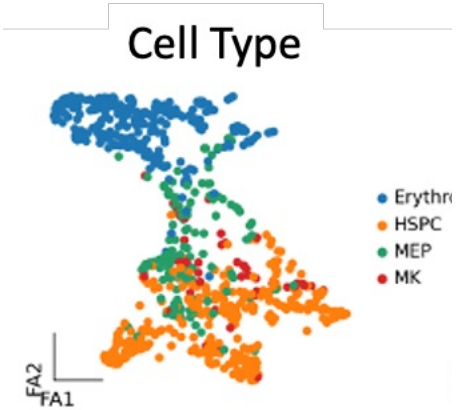


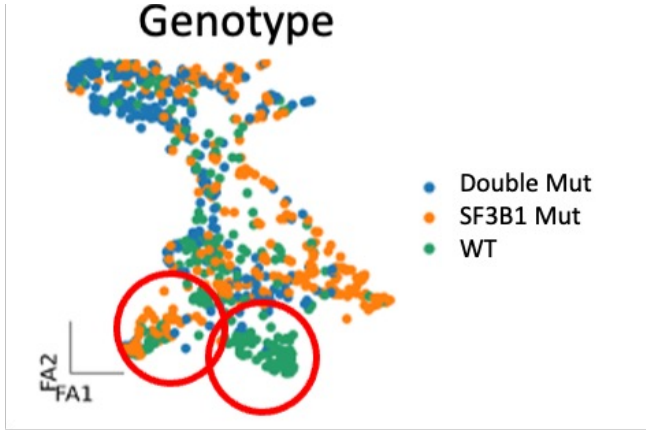


Differentiation Trajectory Analysis





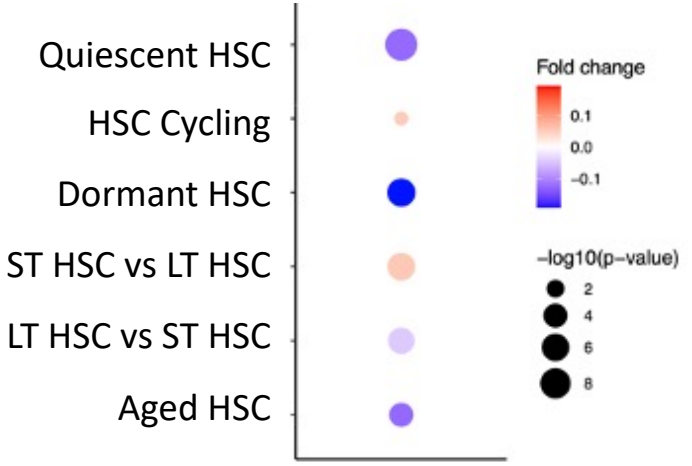




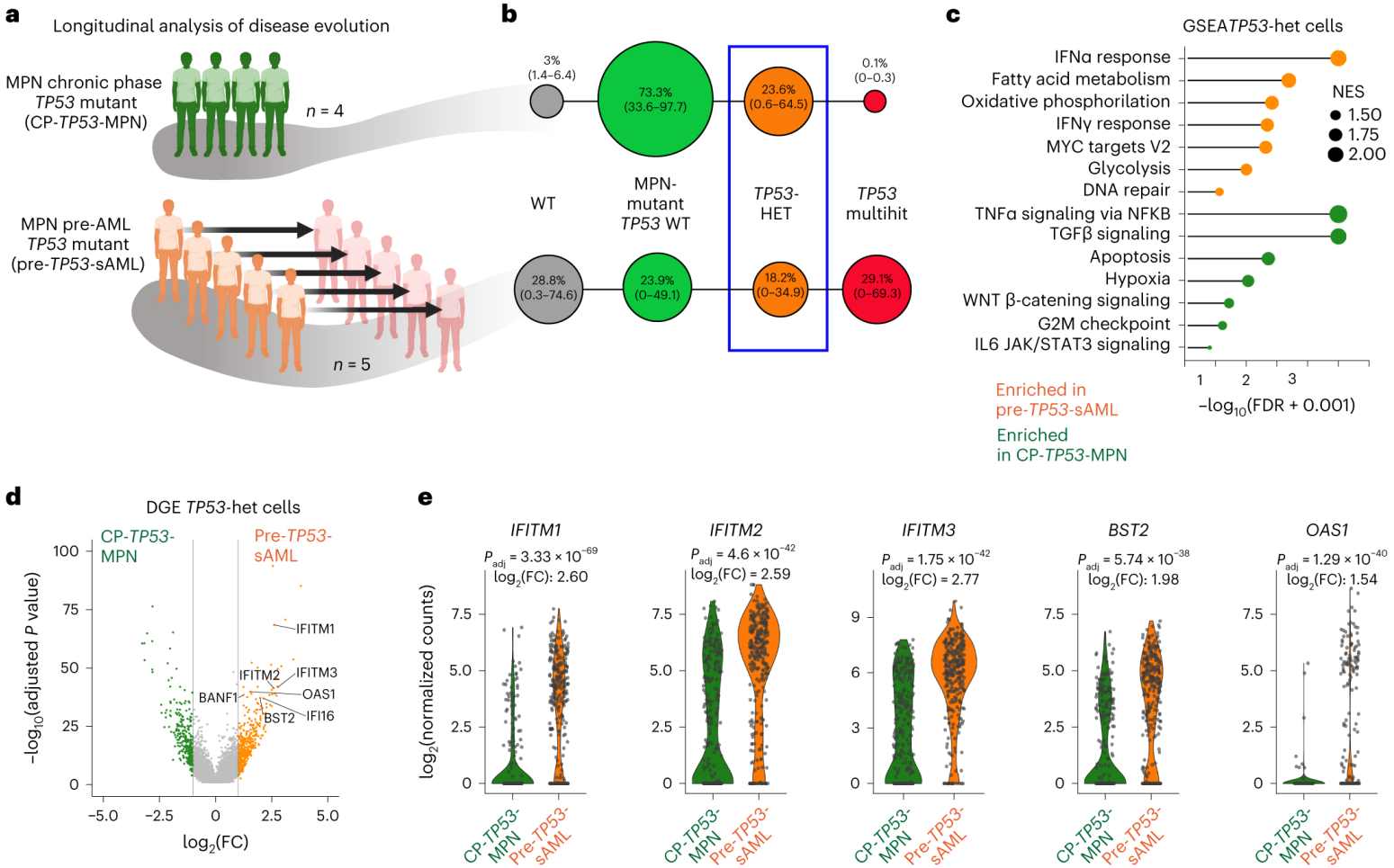
Analysis of MYC and E2F target gene activity along pseudotime revealed earlier and more pronounced activation of both pathways in the double-mutant HSC cluster, suggesting premature commitment to proliferation or differentiation compared to WT HSCs.

The erythroid transcription factor GATA1 also showed earlier expression in double-mutated cells, consistent with accelerated erythroid specification.

HSC Signatures: Double Mut vs WT



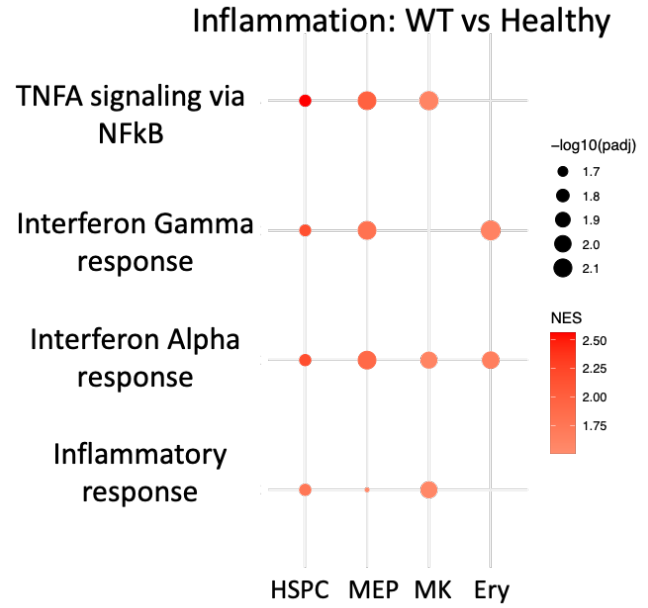
Inflammatory pathways are upregulated in TP53-mutant HSPCs before transformation



Alba Rodriguez-Meira 2023

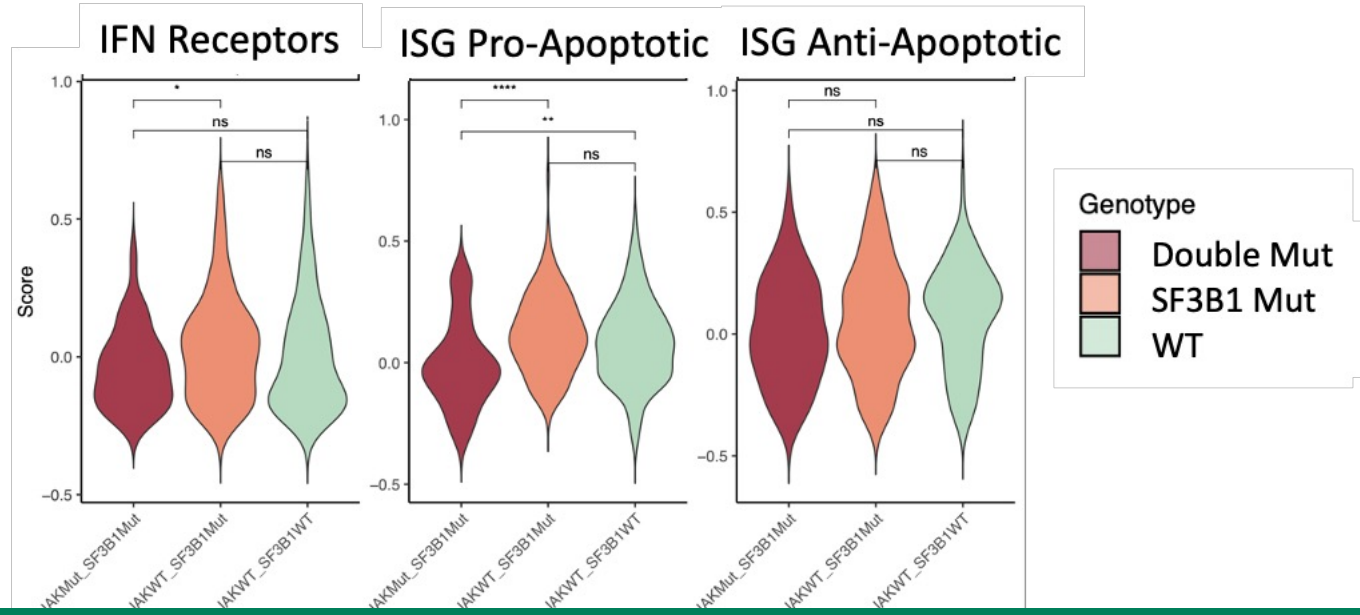
Inflammation Response in HSC

- **WT HSPCs** enriched for **Inflammatory signaling**: IFN-α, IFN-γ, TNF-α.



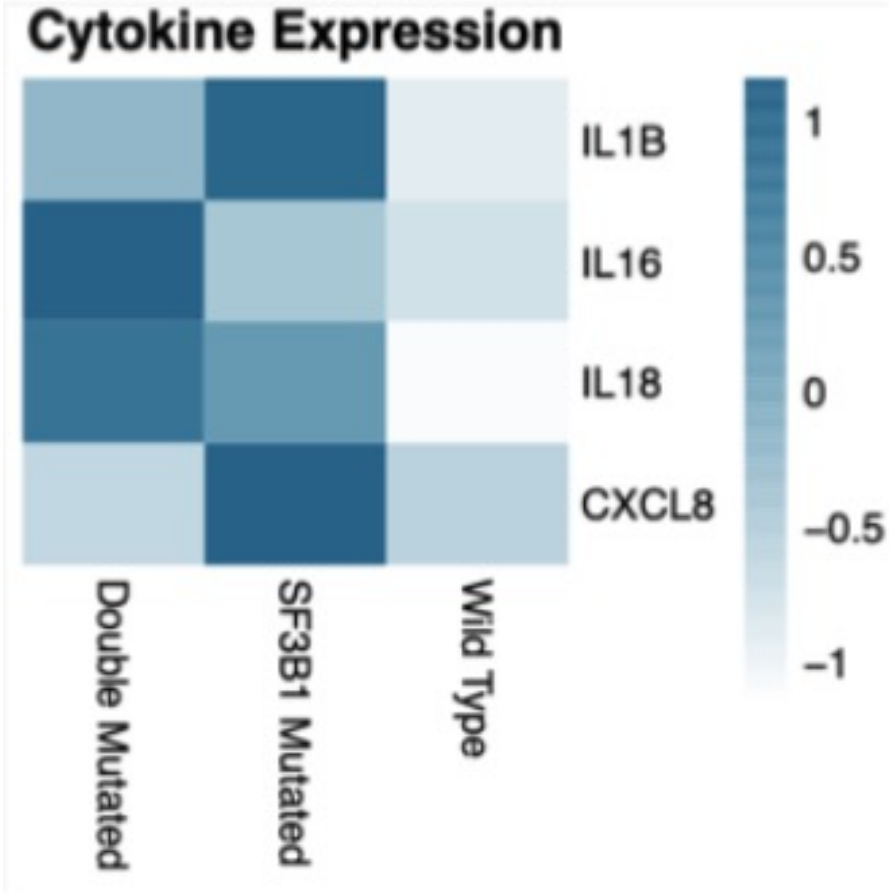
- Mutated HSC show **low response** to **IFN** with **low acrivation** of Interferon-related **pro-apoptotic** pathways

- WT transcriptional profile aligns with **aged HSCs**: Inflammation, reduced regenerative capacity.



Inflammation in Microenvironment

SF3B1-mutated cells show upregulation of inflammatory cytokines: IL1B, CXCL8, IL16, IL18



Conclusions

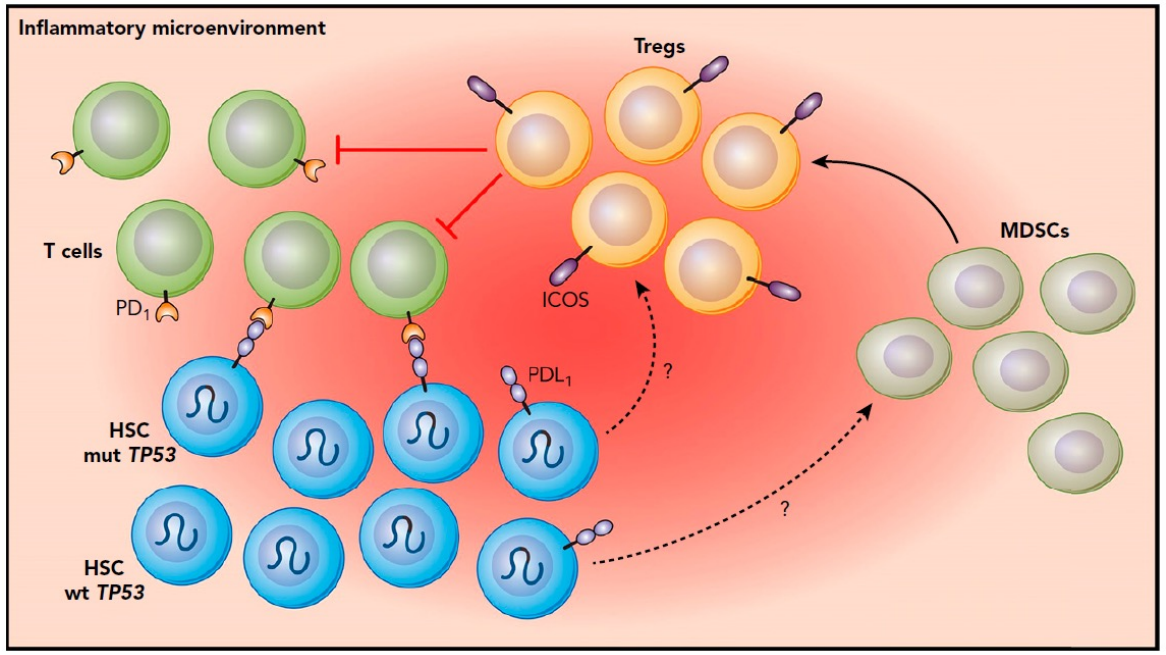
- **Single-cell multi-omics** reveals a clonal hierarchy in MDS/MPN-RS-T, with **SF3B1 as founder** and **JAK2/MPL as secondary proliferative mutations**.
- **Co-mutated HSPCs** show transcriptional/epigenetic bias toward **erythroid and megakaryocytic fates**, with activation of **proliferative pathways (MYC – E2F)**.
- A **30-gene RNA signature** distinguishes co-mutated cells by features of **dysplastic erythropoiesis**, **iron metabolism**, and **megakaryocytic priming**.
- **scATAC-seq** confirms increased **GATA motif accessibility**, supporting **erythroid bias** seen in transcriptomics.
- **WT HSPCs** display **inflammatory signaling and quiescence**, contrasting with **immune-resistant mutant clones**, suggesting **inflammation confers selective advantage**.
- Overall, **inflammation and genotype-driven erythroid skewing** co-drive the MDS/MPN-RS-T phenotype.

c-MYC

- we identified MYC target activation as a defining feature of double-mutant clones in MDS/MPN-RS-T.

- MYC overexpression is known to couple inflammation with immune evasion in hematological neoplasms: it represses class II major histocompatibility complex (MHC-II) expression and downregulates inflammatory transcriptional programs while sustaining MYC-driven transcriptional activity, consistent with our observations.

Mutant TP53 MDS and sAML have altered regulation of select checkpoint molecules.



- **Immune checkpoint overexpression (PD-L1) at the stem cell level, which is mediated by dysregulation of the mir-34a/MYC circuit**
- Reduced numbers of cytotoxic T cells
- Expansion of myeloid-derived suppressor cells (MDSCs)
- Expansion of ICOShigh/PD-1neg regulatory T cells (Tregs).

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