Enhancer-hijacking in complex karyotype Acute Myeloid Leukemia

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EHA-SWG Scientific Meeting on sAML 2025 in Berlin, Spain

Disclosures

- **Consultant** for Epicelya
- **Editor-in-chief** the *International Journal of Cancer*

Transforming events in AML

Translocations leading to oncofusion genes

e.g.: t(15;17) leading to PML/RARα,

e.g.: t(8;21) leading to AML1/ETO

e.g.: translocations involving *MLL or RUNX1*

Gene mutations some occurring in clonal hematopoiesis

e.g.: *DNMT3A, IDH1/2, TET*

Inversion on chromosome 3

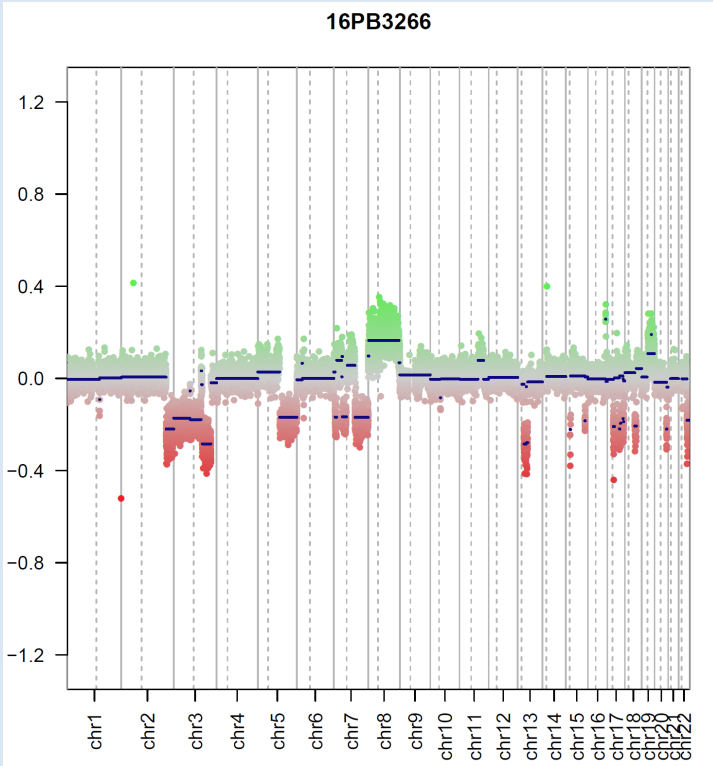
e.g.: *inv*(3)(q21q26·2) activation of *EVI1*

plus haploinsufficiency of *GATA2*

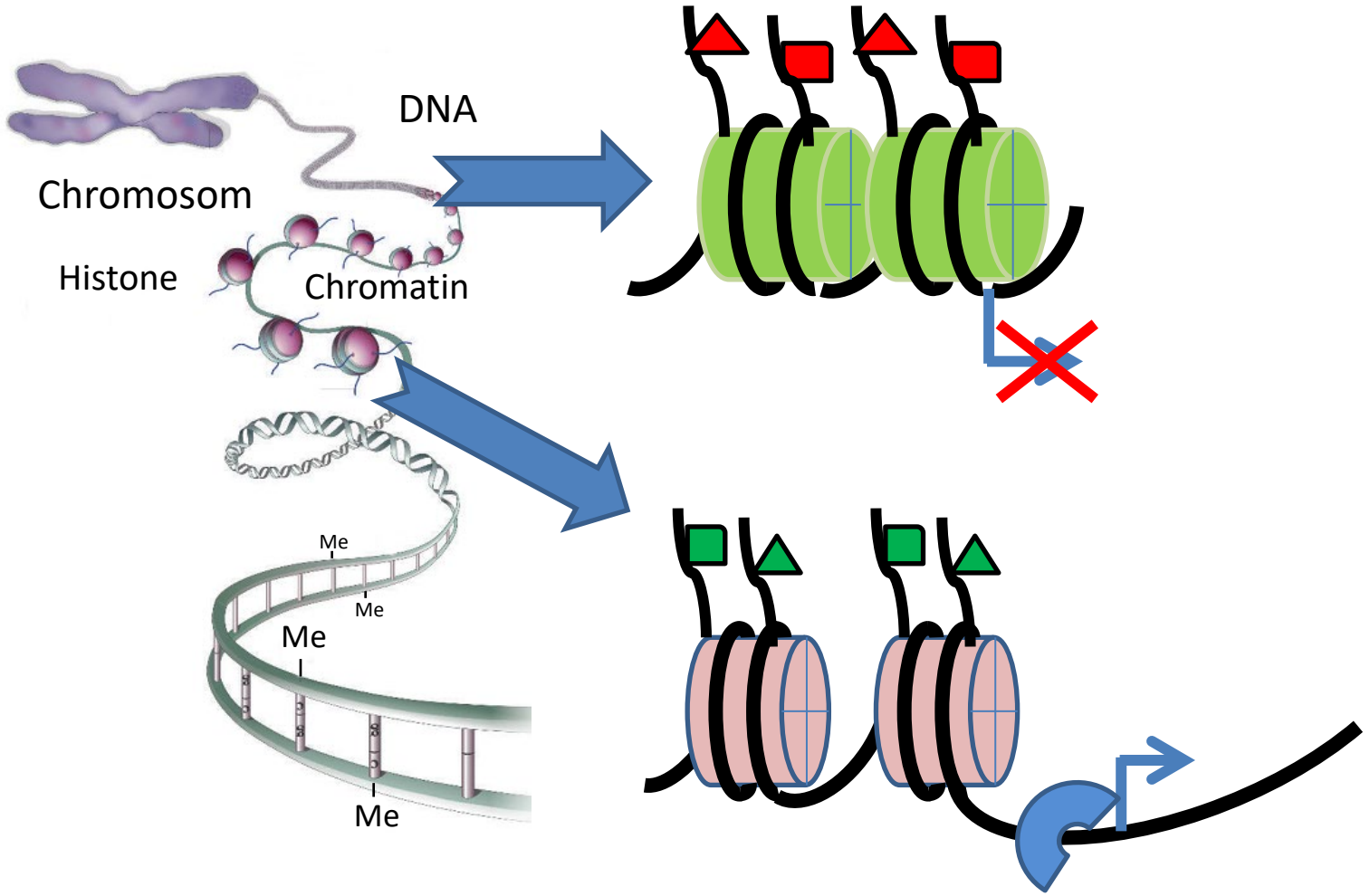
Deletions:

e.g.: chromosome 7q … unknown mechanism

e.g.: chromosome 5q … unknown mechanism



Epigenetic Modifications



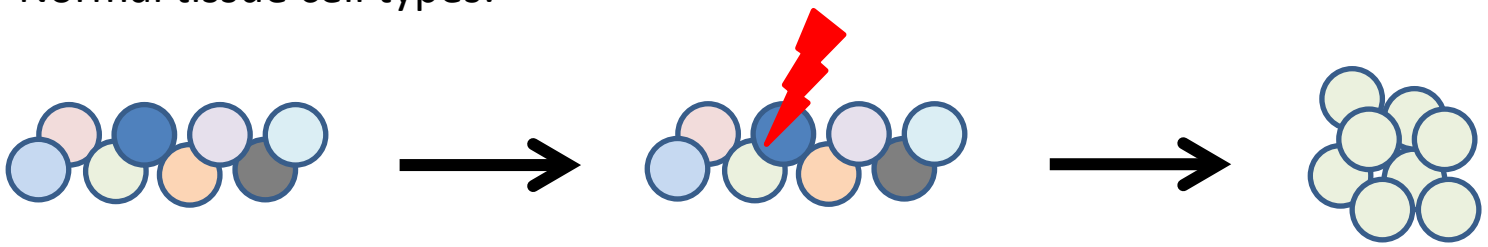
DNA Methylation

Histone Modifications

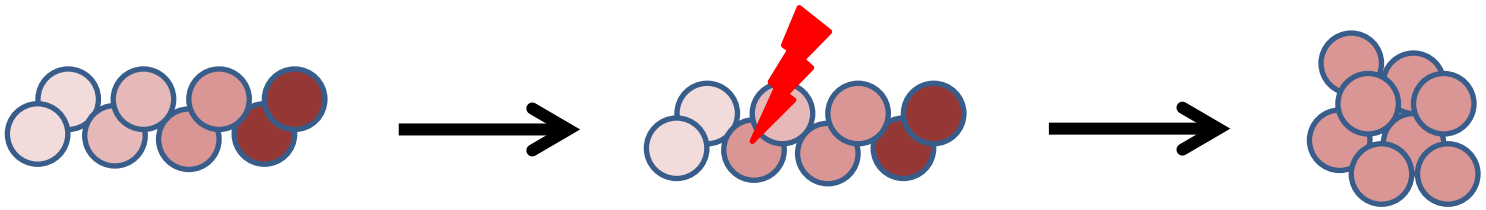
Nucleosome Position

Cancer cells inherit the Epigenome of the cell-of-origin

Normal tissue cell types:

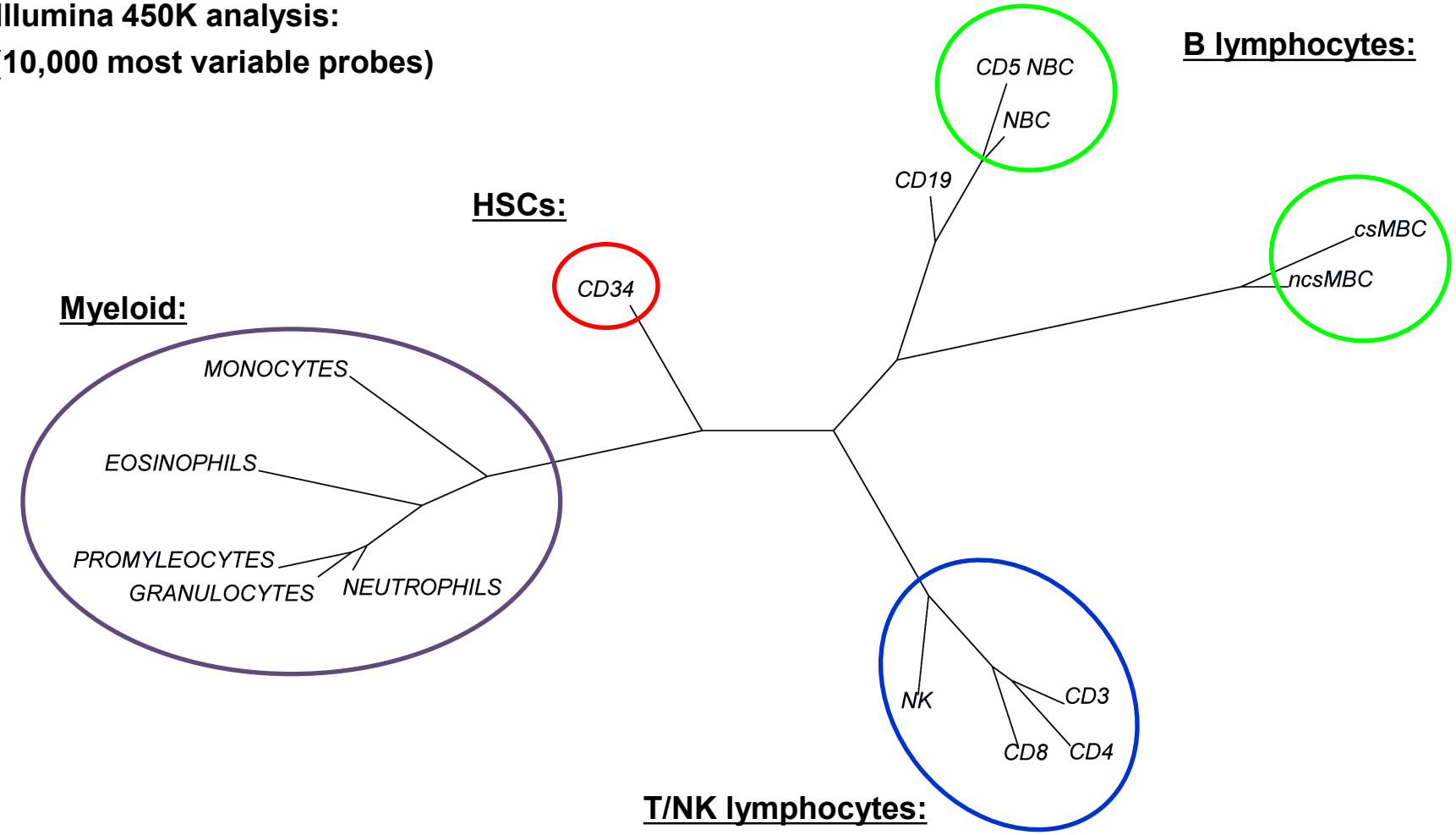


Different developmental stages, aging and microenvironment:



Phylo(epi)genetic analysis of the development of blood cell types:

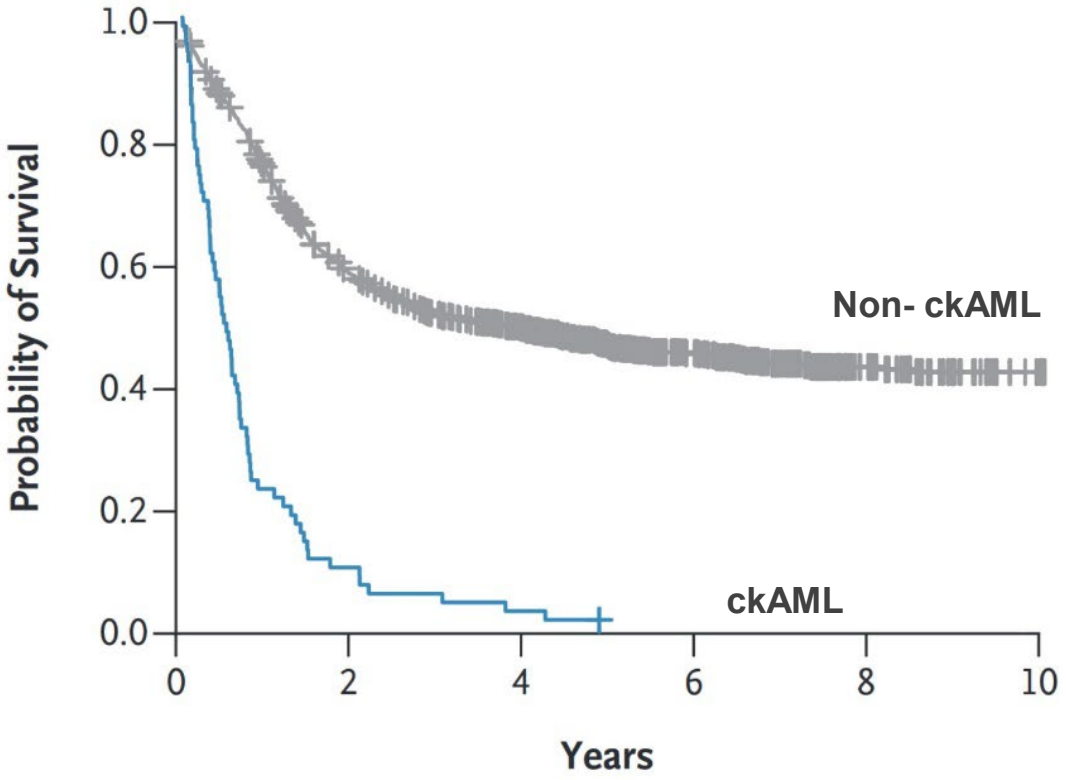
Illumina 450K analysis:
(10,000 most variable probes)



APE package in Bioconductor

Profiles downloaded from: ICGC Data Portal (http://dcc.icgc.org/web)
GEO Database (http://www.ncbi.nlm.nih.gov/geo)

Improved Outcome for Acute Myeloid Leukemia
but many patients still have a poor prognosis



modified from Papaemmanuil E *et al.* N Engl J Med, 2016

Incomplete molecular understanding of certain AML groups

But, what are we missing??

ckAML - complex karyotype AML

Dominating Concepts in Acute Myeloid Leukemia

CONCEPT 1

Genetic Loss:

Tumor suppressor↓

del(7q)
del(5q)

Classical view

CONCEPT 2

Translocation:

Oncofusion protein

PML-RARA t(15;17)
AML1-ETO t(8;21)

But:
NO tumor suppressors identified in del(5q) or del(7q)

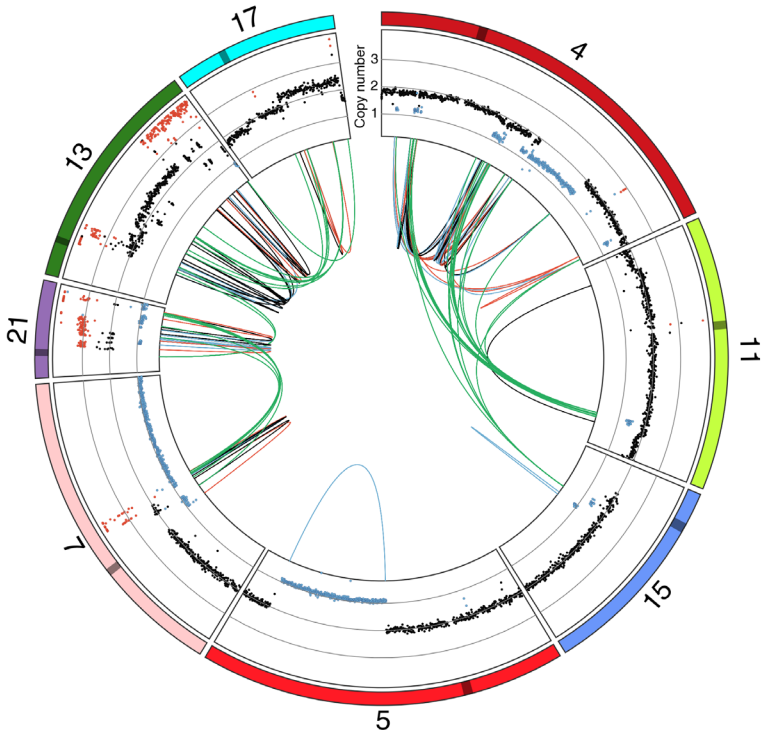
But:
several AML translocations WITHOUT oncofusion

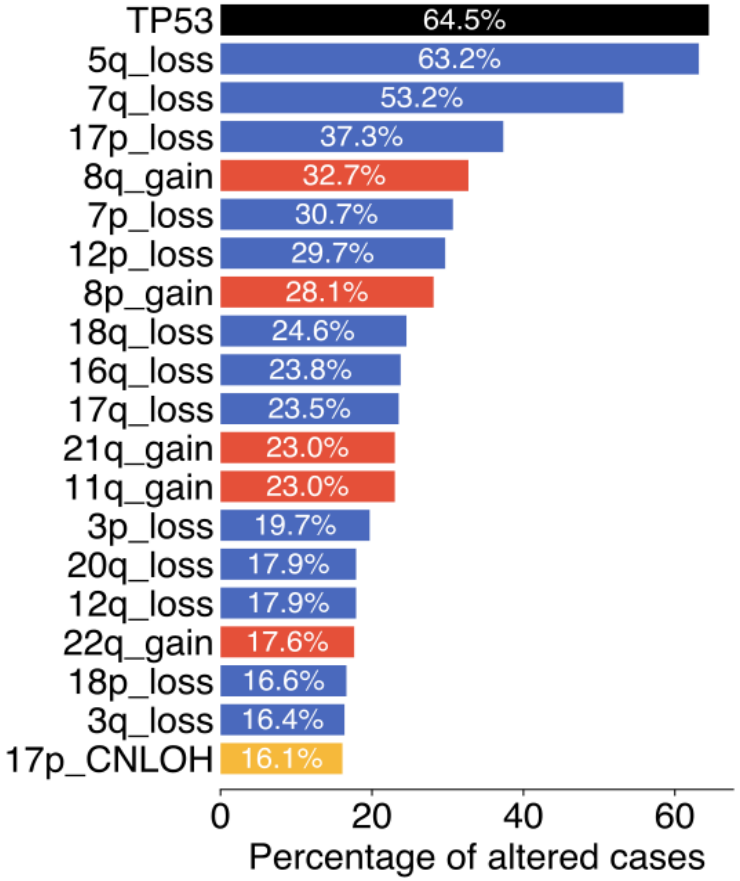
Mechanisms?

Inaba *et al.* The enigma of monosomy 7. Blood 2018

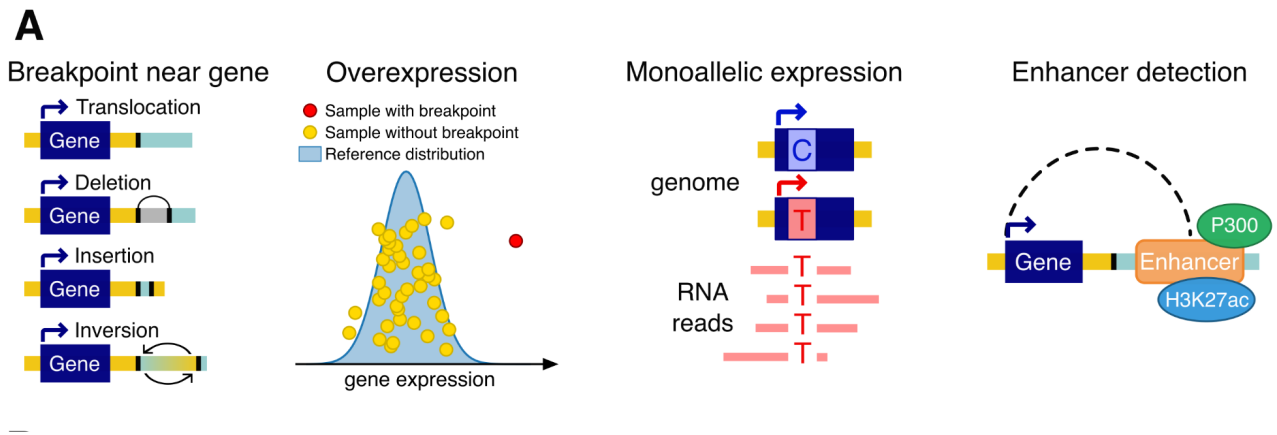
Complex karyotype AML

Cytogenetics: >3 cytogenetically visible aberrations
Molecular: often chromothripsis



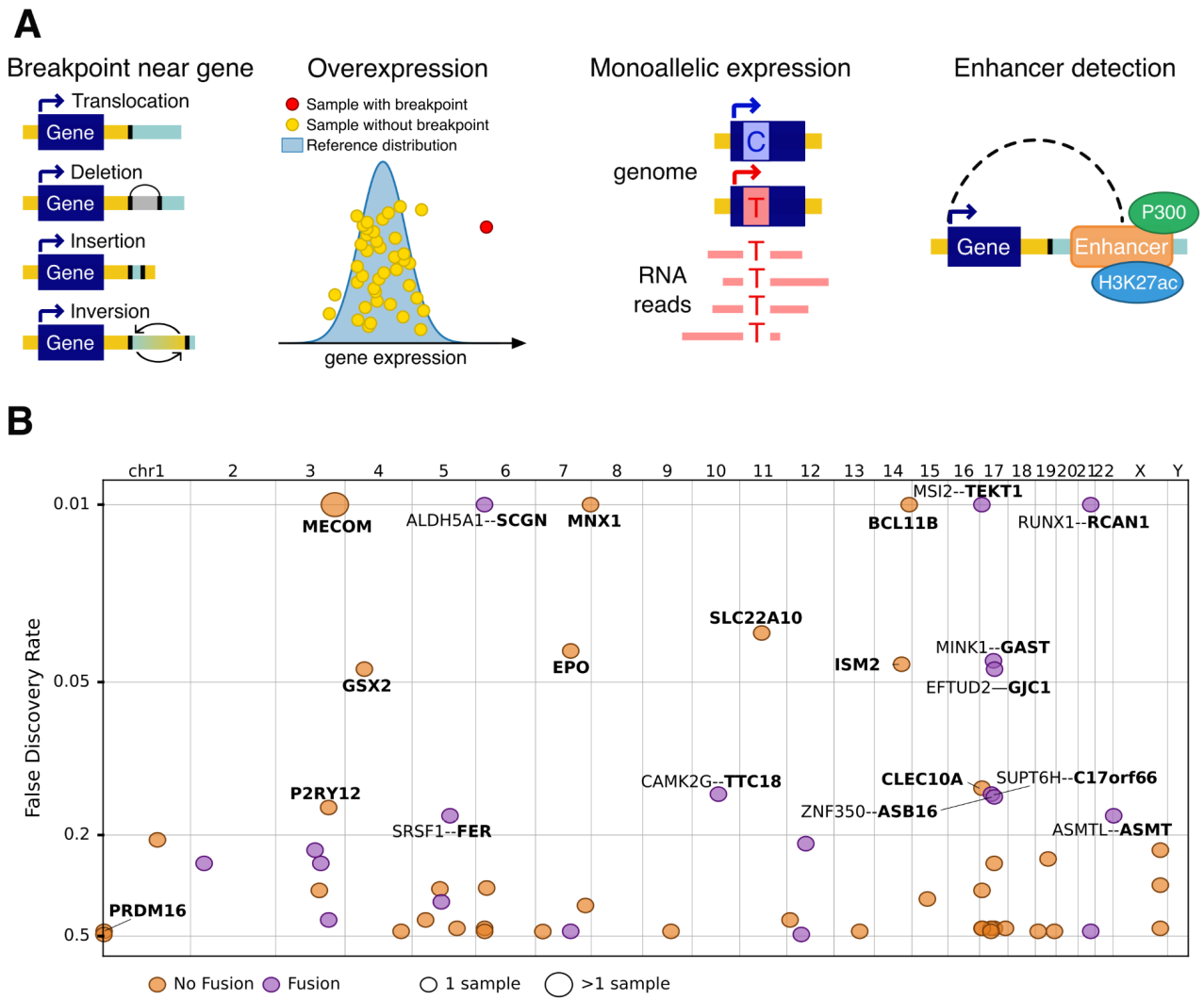


Pyjacker, a computational pipeline for the identification of enhancer-hijacking events

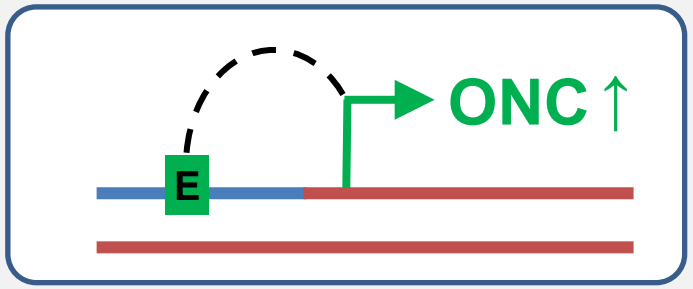


Sollier, Riedel, Toprak *et al.* Blood Cancer Discovery in press

Pyjacker, a computational pipeline for the identification of enhancer-hijacking events



Pyjacker, a computational pipeline for the identification of enhancer-hijacking events



Input: WGS + RNA-seq

1. Breakpoint detection
2. Gene upregulation
3. Monoallelic expression
4. Enhancer identification

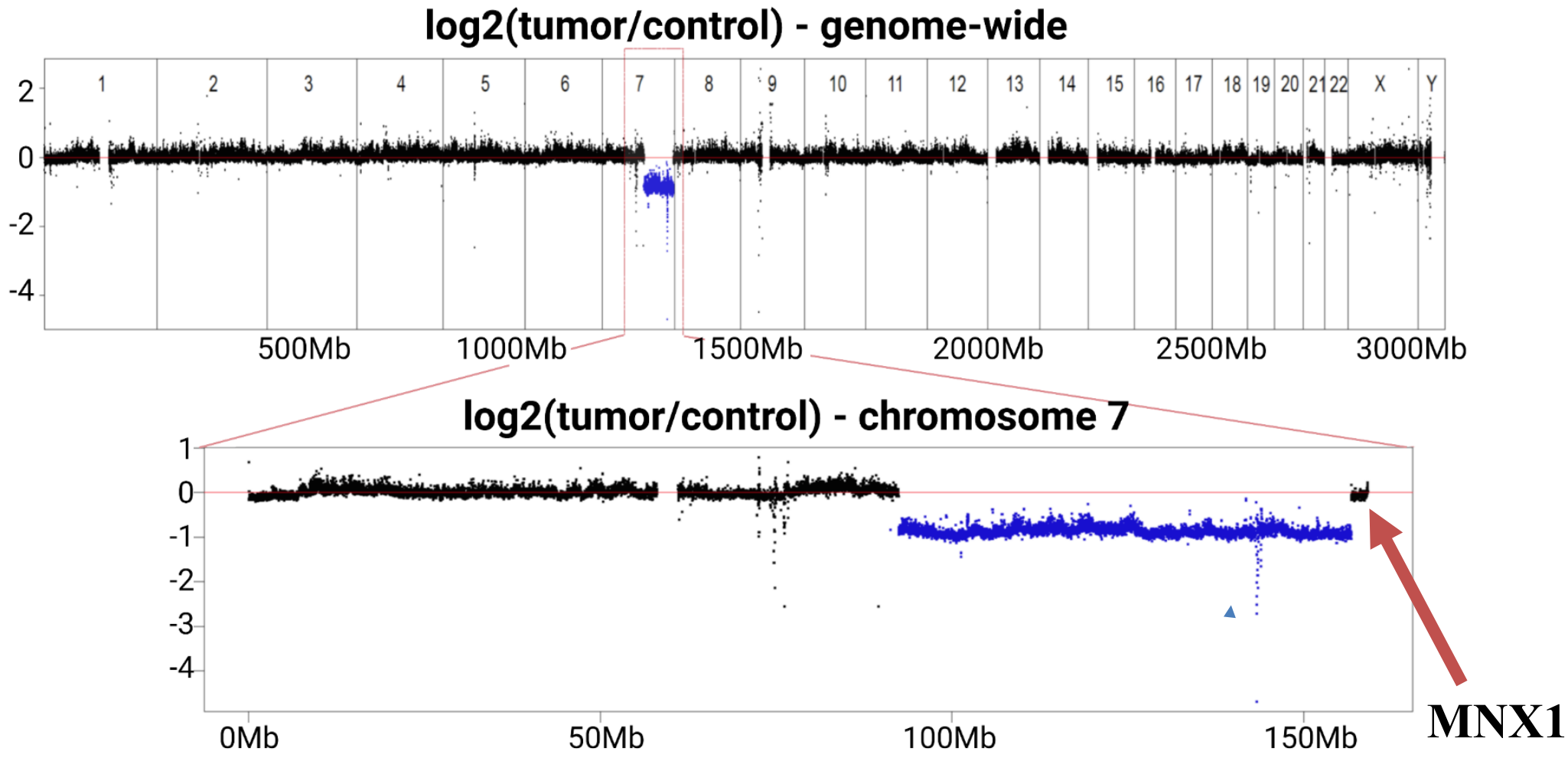
Output:

AML del(7q)
n=13

Patient	Putative oncogene	EpiR haploinsufficiency
1	EVI1 ↑	ETV6 ↓ EZH2 ↓ KMT2C ↓ KMT2E ↓
2	MNX1 ↑	EZH2 ↓ KMT2C ↓ KMT2E ↓
3	MNX1 ↑	EZH2 ↓ KMT2C ↓ KMT2E ↓
4	MNX1 ↑	EZH2 ↓ KMT2C ↓ KMT2E ↓

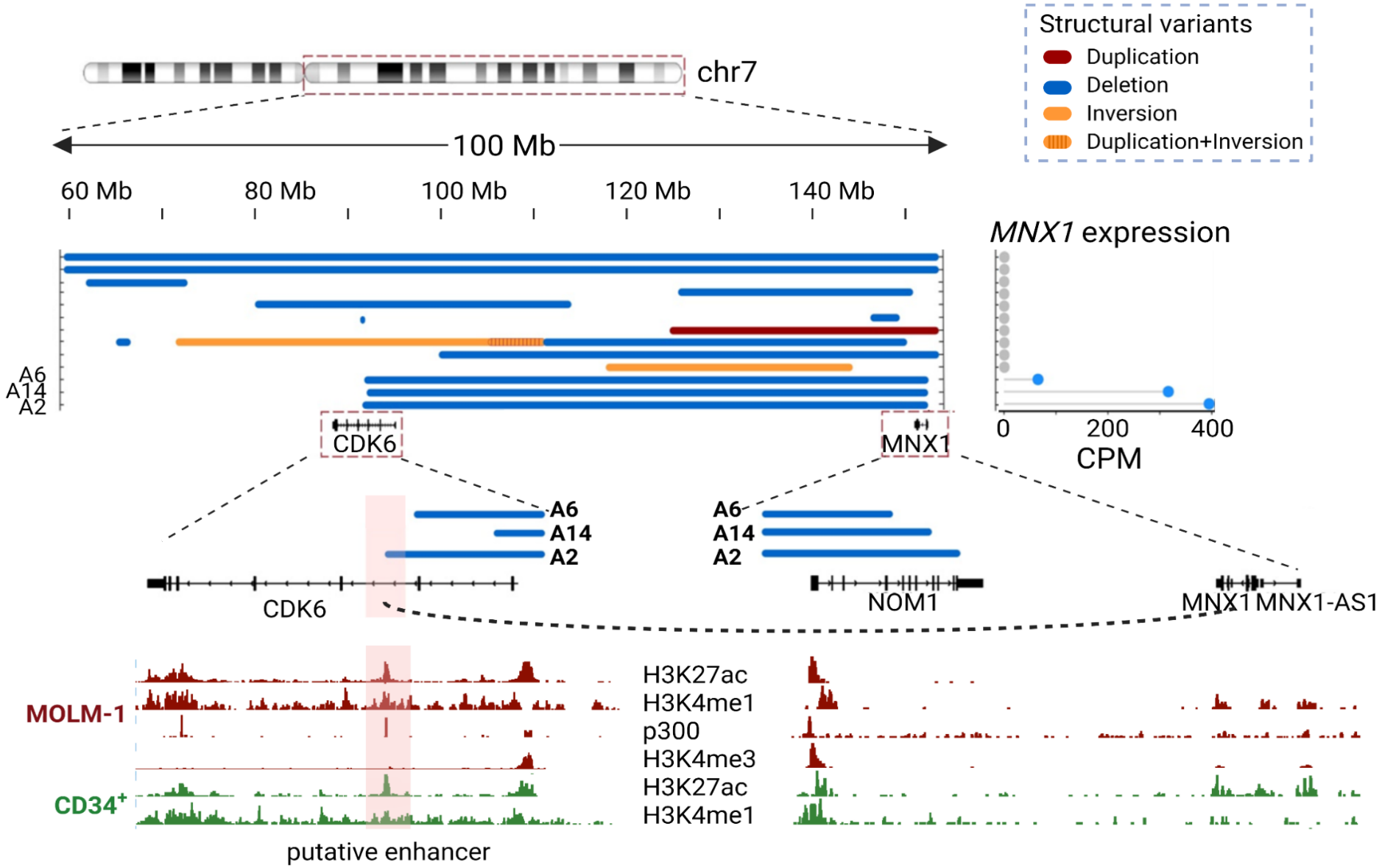
Sollier, Riedel, Toprak *et al.* Blood Cancer Discovery in press

Copy number alteration of an AML sample with del(7q)

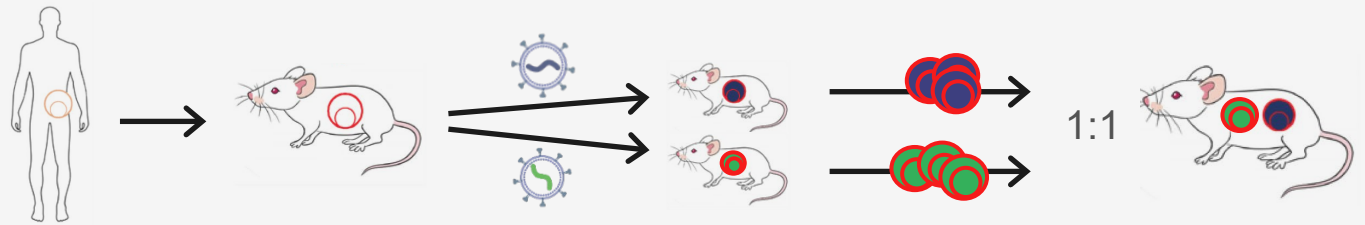


46,XY,del(7)(q21q34)[31]

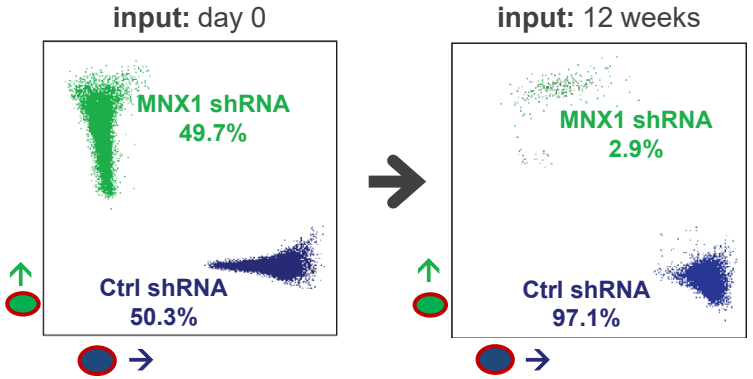
Reinterpreting Knudson's two–hit hypothesis



Oncogene Activity in PDX models



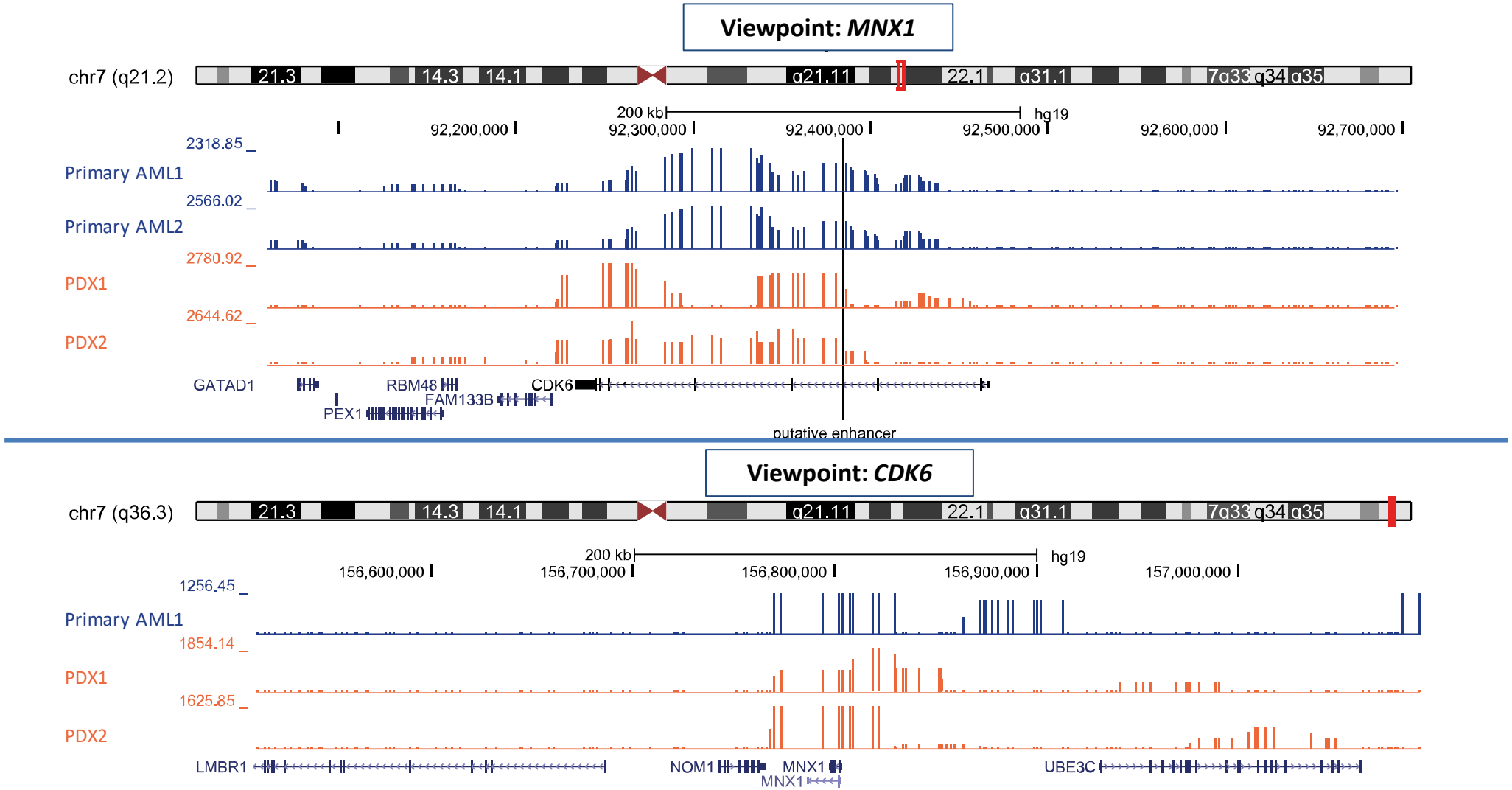
MNX1 is required for leukemic growth



Collaboration with Irmela Jeremias

Interactions between putative enhancer region and *MNX1*

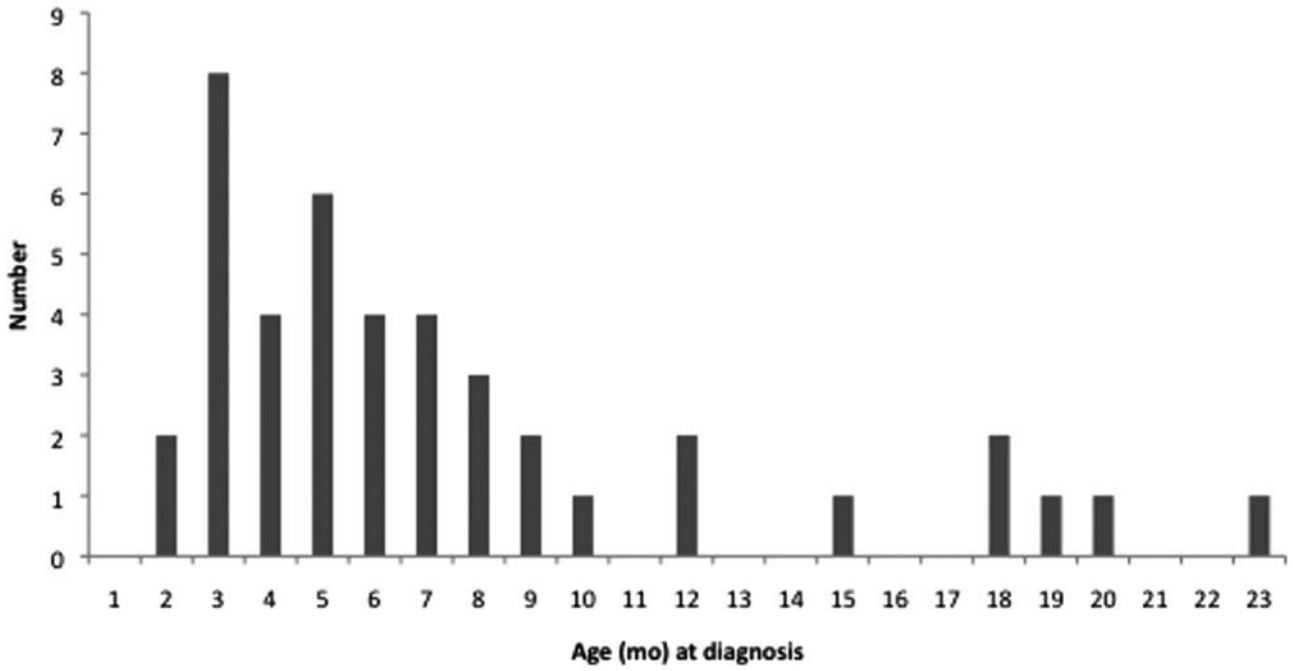
Circular chromosome conformation capture (4C)

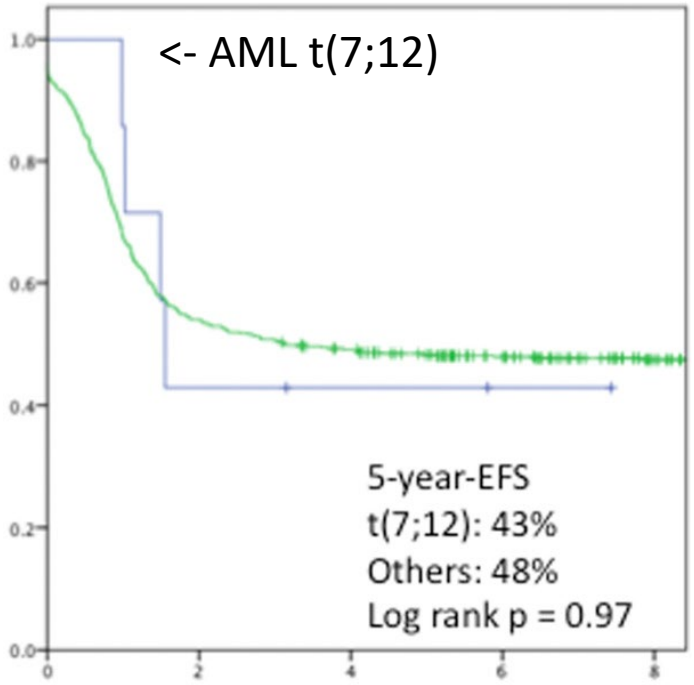


Sollier, Riedel, Toprak *et al.* Blood Cancer Discovery in press

Pediatric AML with translocation t(7;12)(q36;p13)

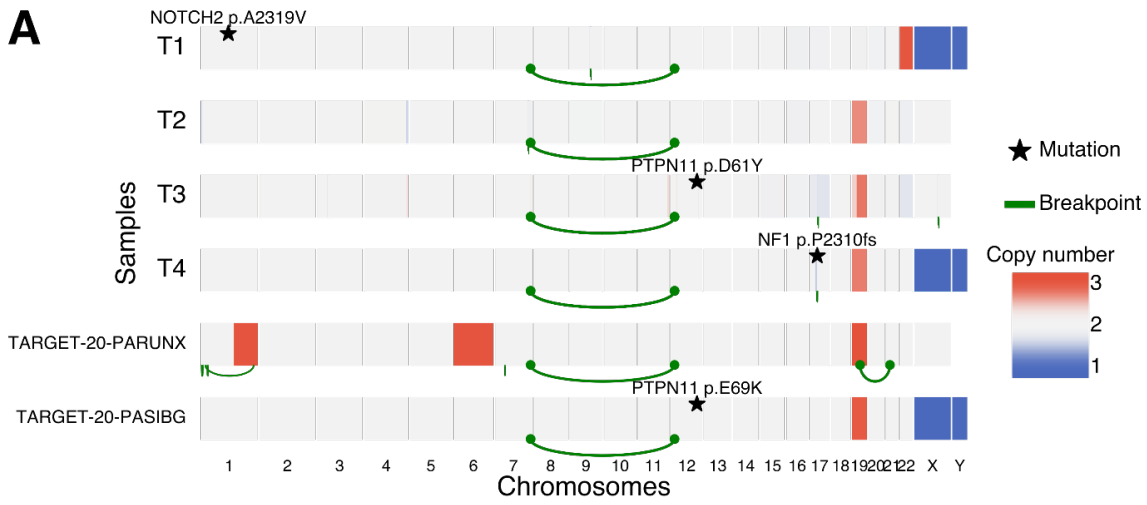
- Translocation, t(7;12) occurs in paediatric AMLs with an **age of diagnosis <12 months**
- poor outcome with a 3-year event free **survival of 0%**





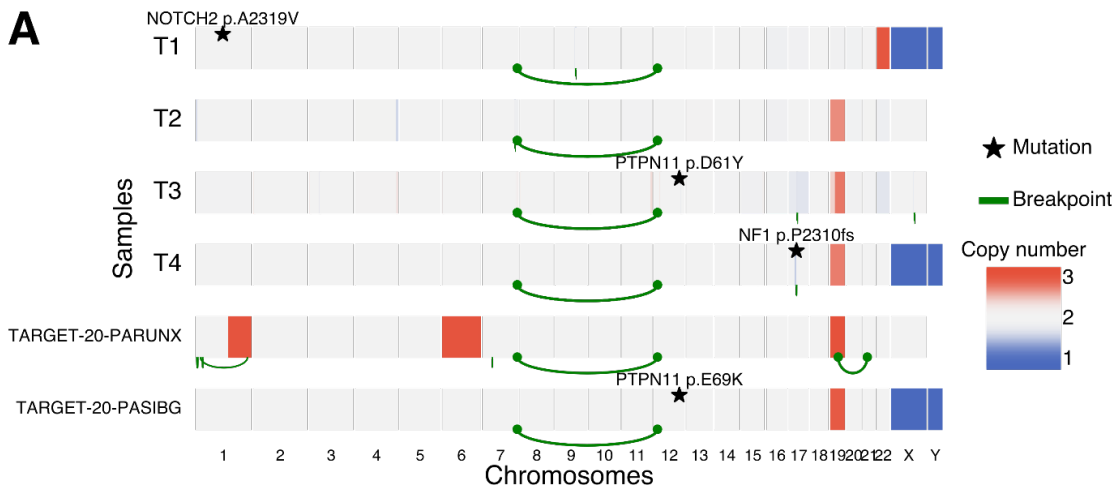
Nordic Society for Pediatric Hematology and Oncology (NOPHO-AML) Espersen et al. GCG 2018

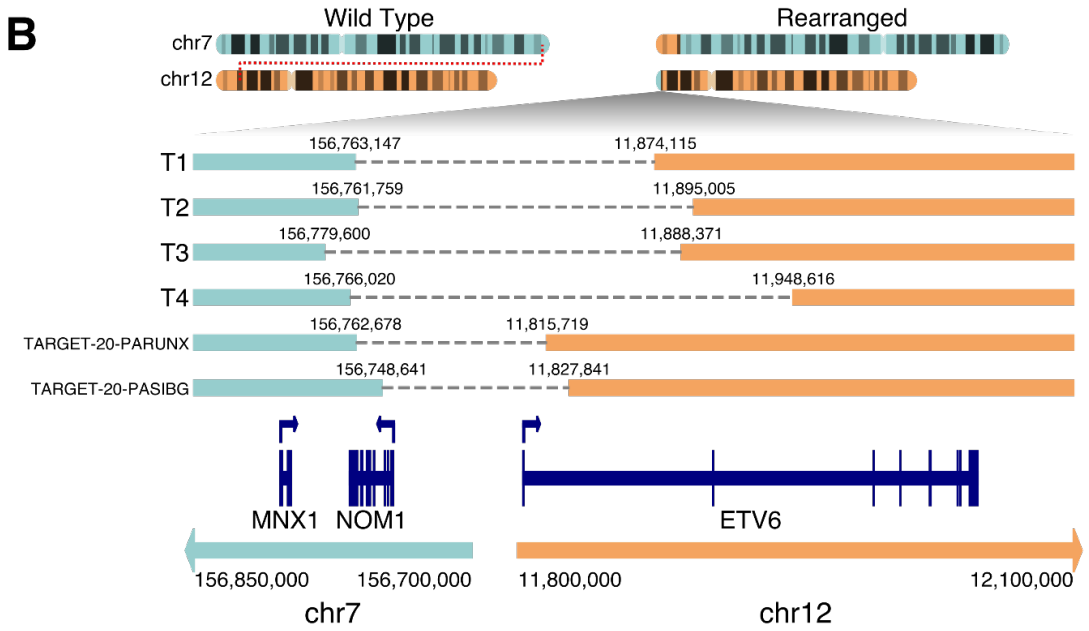
Pediatric AML with translocation t(7;12)(q36;p13)



SV and SCNA

Pediatric AML with translocation t(7;12)(q36;p13)

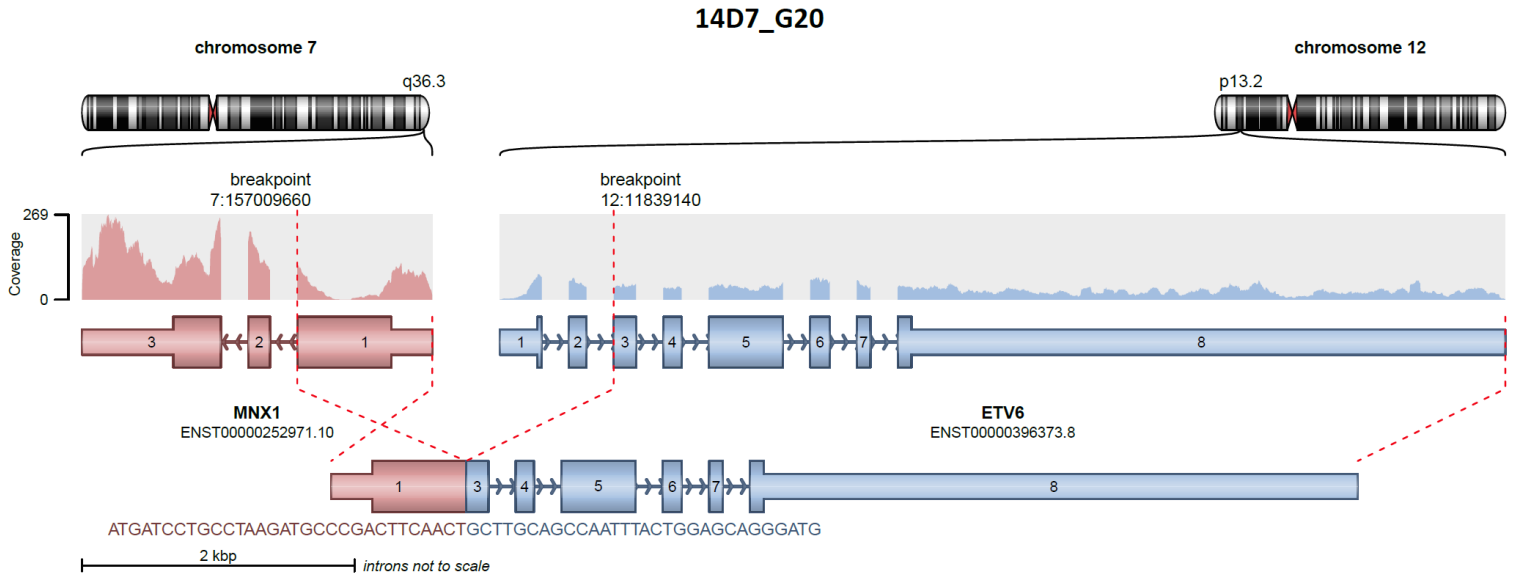


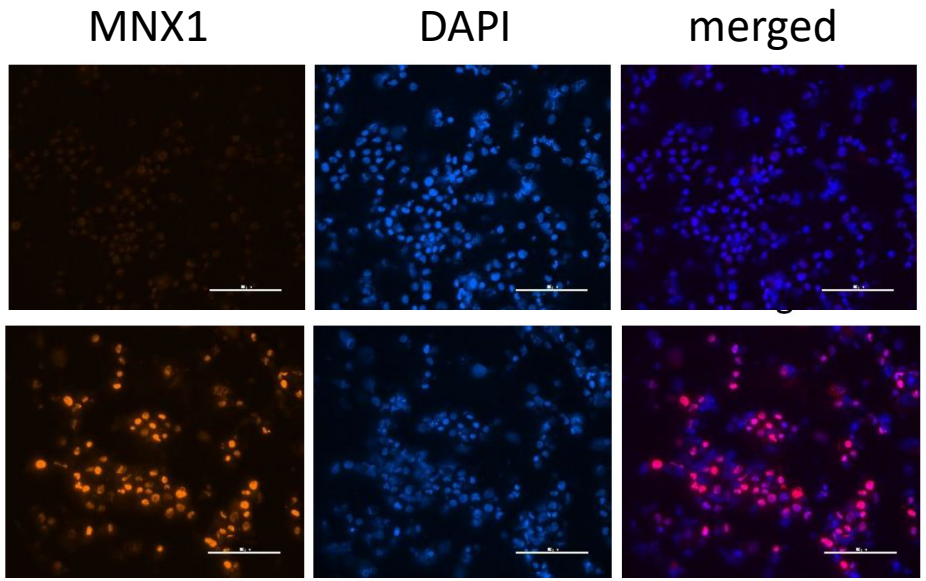


SV and SCNA

breakpoints

Differentiated iPSC cells with t(7;12) express MNX1





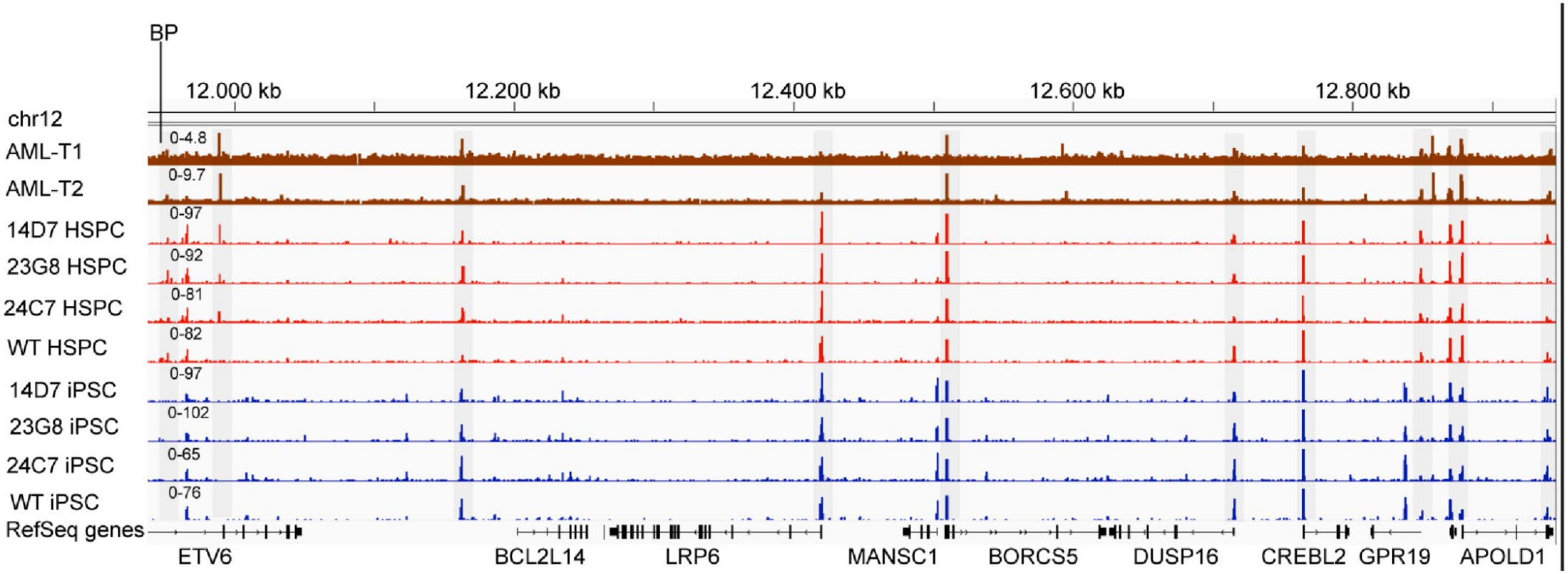
t(7;12) **iPSC** line

Differentiation

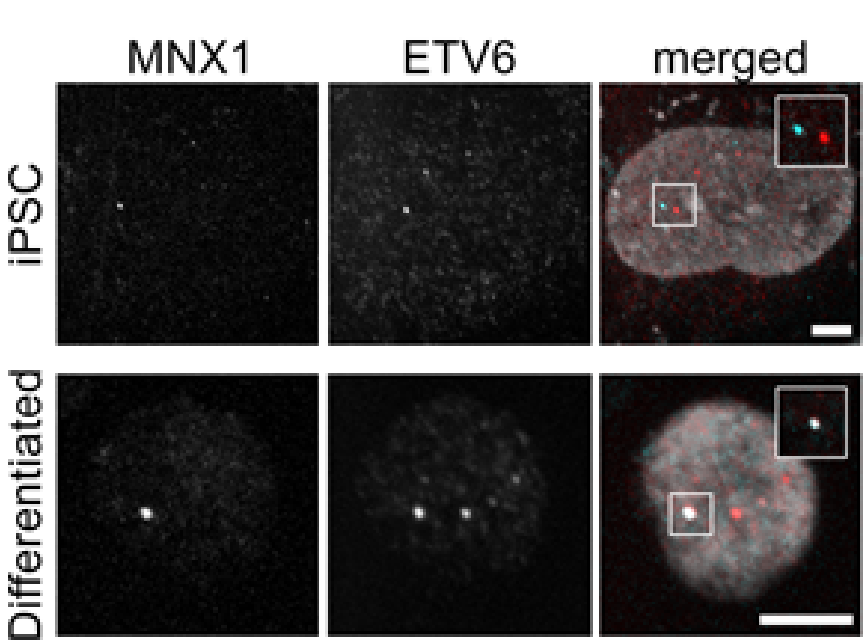
t(7;12) **HSPC** line

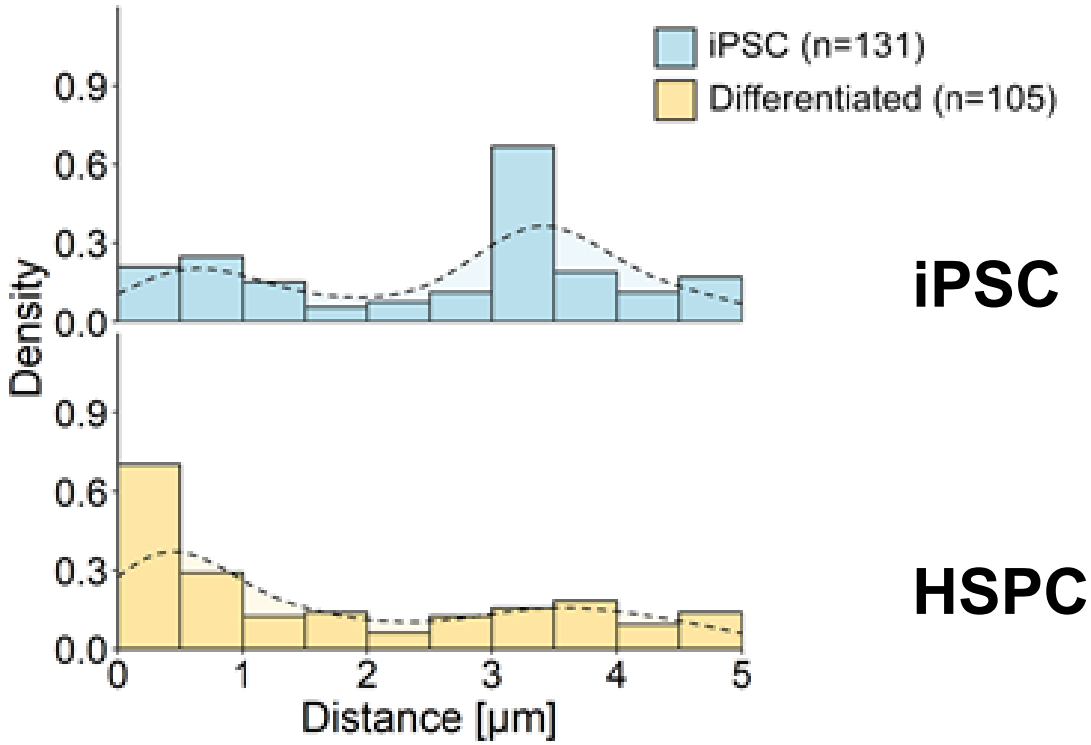
Nilsson, et al. IJC 2022

Hematopoietic enhancers mapped using ATAC-seq



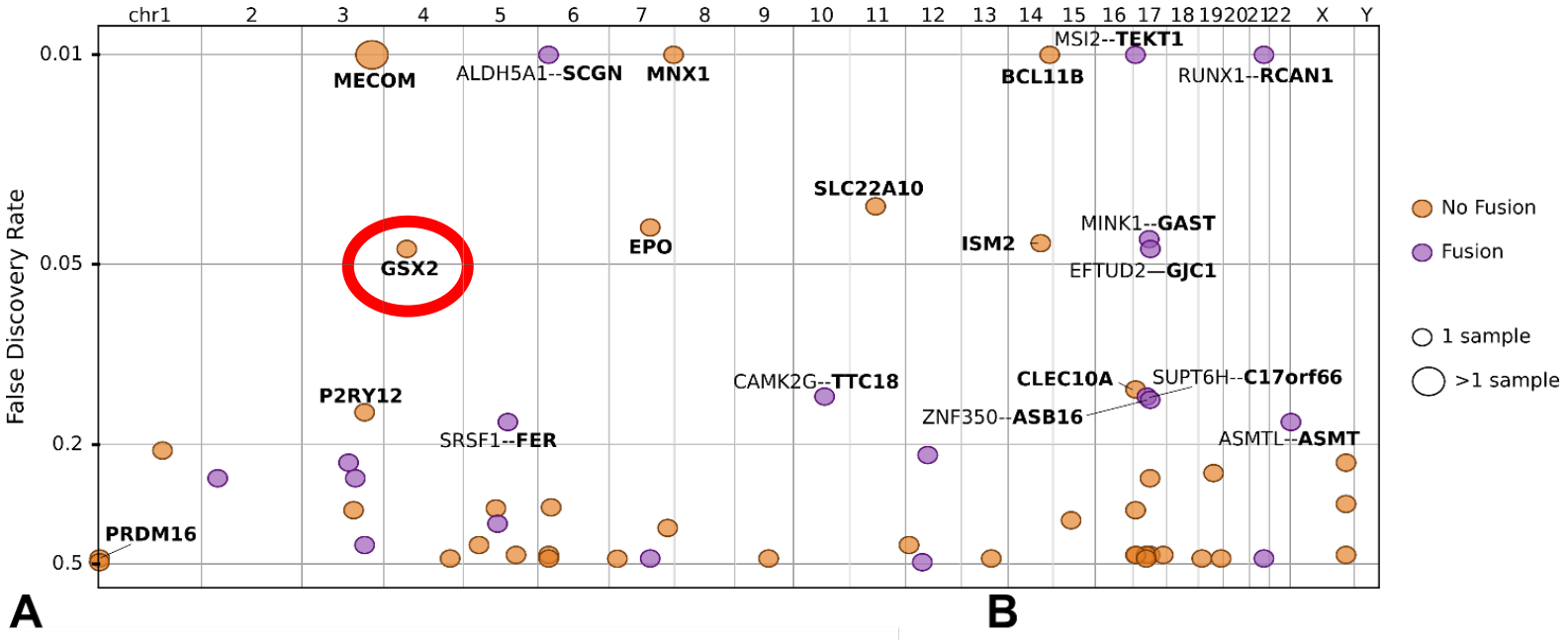
Probing the spatial proximity between MNX1 and the ETV6 enhancer

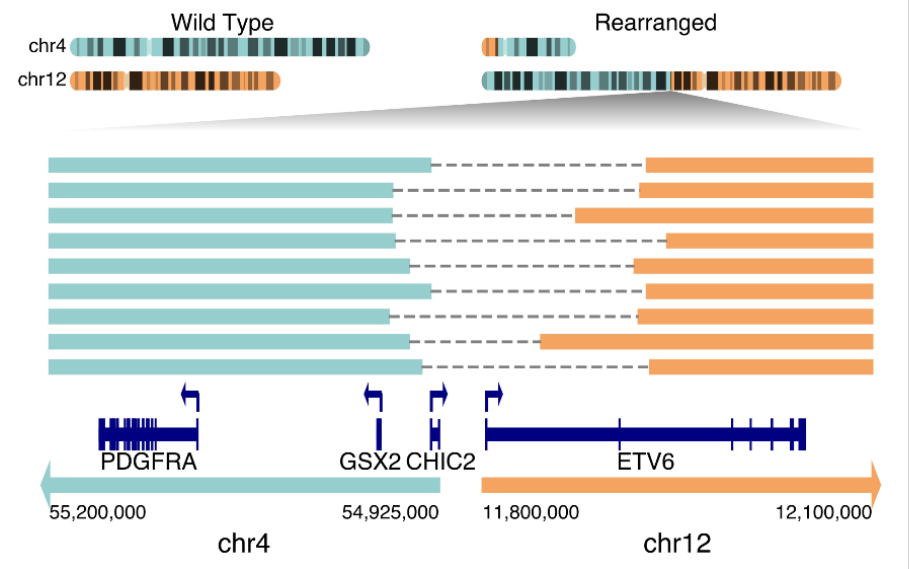


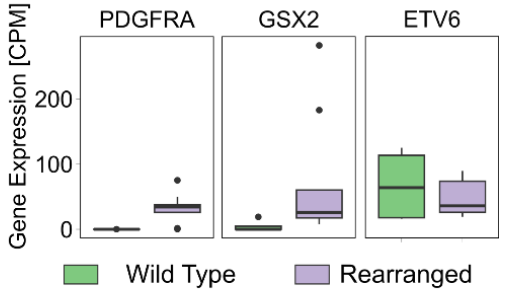


Clemens Steinek (AG Leonhard, LMU) & Anna Riedel

AML t(4;12)(q12;p13)







Enhancer Hijacking in Translocation AML

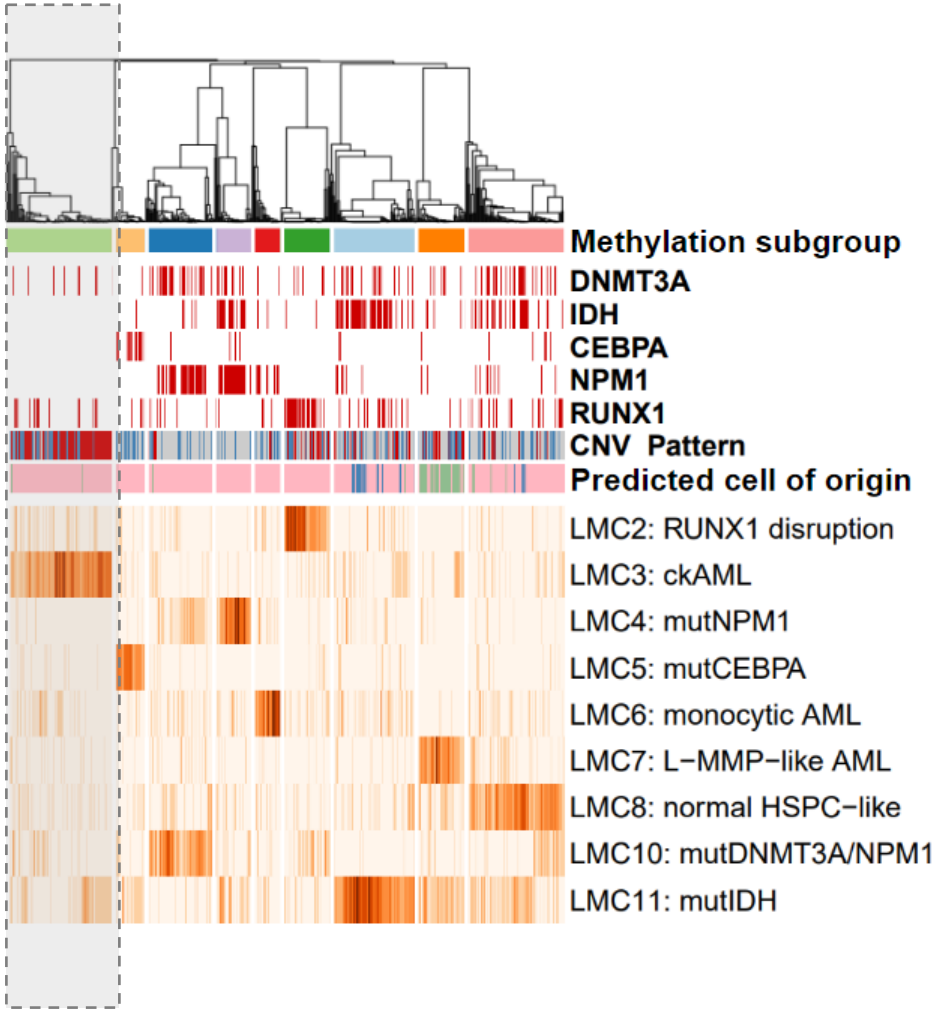
- a previously overlooked leukemogenic mechanism in AML -

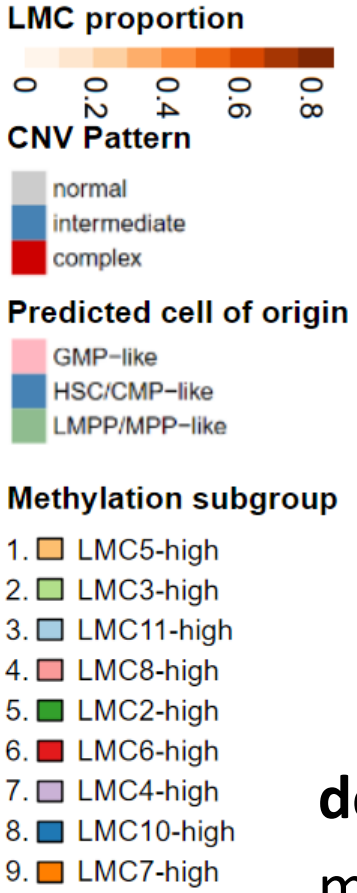
Translocations in AML leading to enhancer-hijacking and EpiR haploinsufficiency

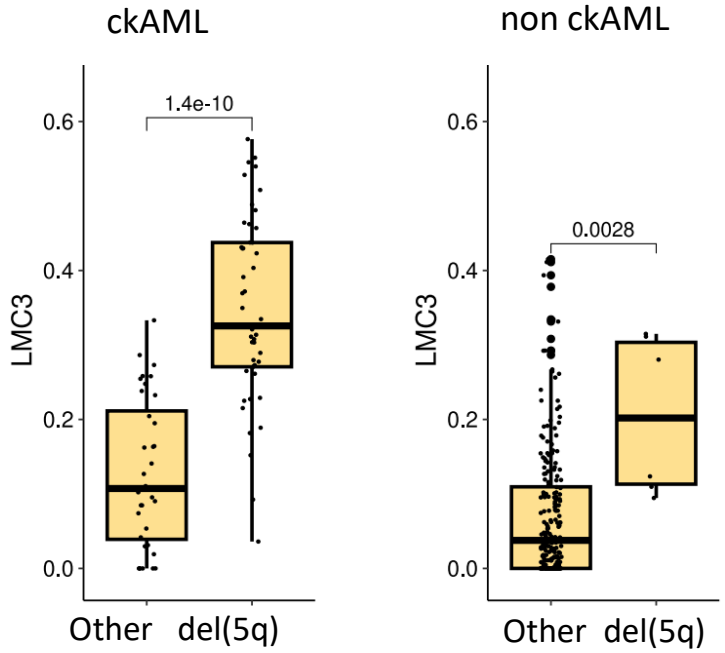
Translocation	*Oncogene*	*Haploinsufficient EpiR*	*Reference*
t(1;2)	PRDM16	ZFP36L2	List et al. *Br J Haematol*. 2024
t(1;3)	PRDM16	GATA2	Rørvik et al. 2024 *Annals of Hematology 2024*
t(4;12)	GSX2	ETV6	Everatt, Plass et al. unpublished
t(6;7)	MNX1	MYB	Weichenhan et al. *Leukemia* 2023
t(7;12)	MNX1	ETV6	Weichenhan et al. *Blood Advances* 2024

Del(5q) AML patients share a distinct DNA hypermethylation signature

DNA methylomes of 480 AML patients (median age 77 years)

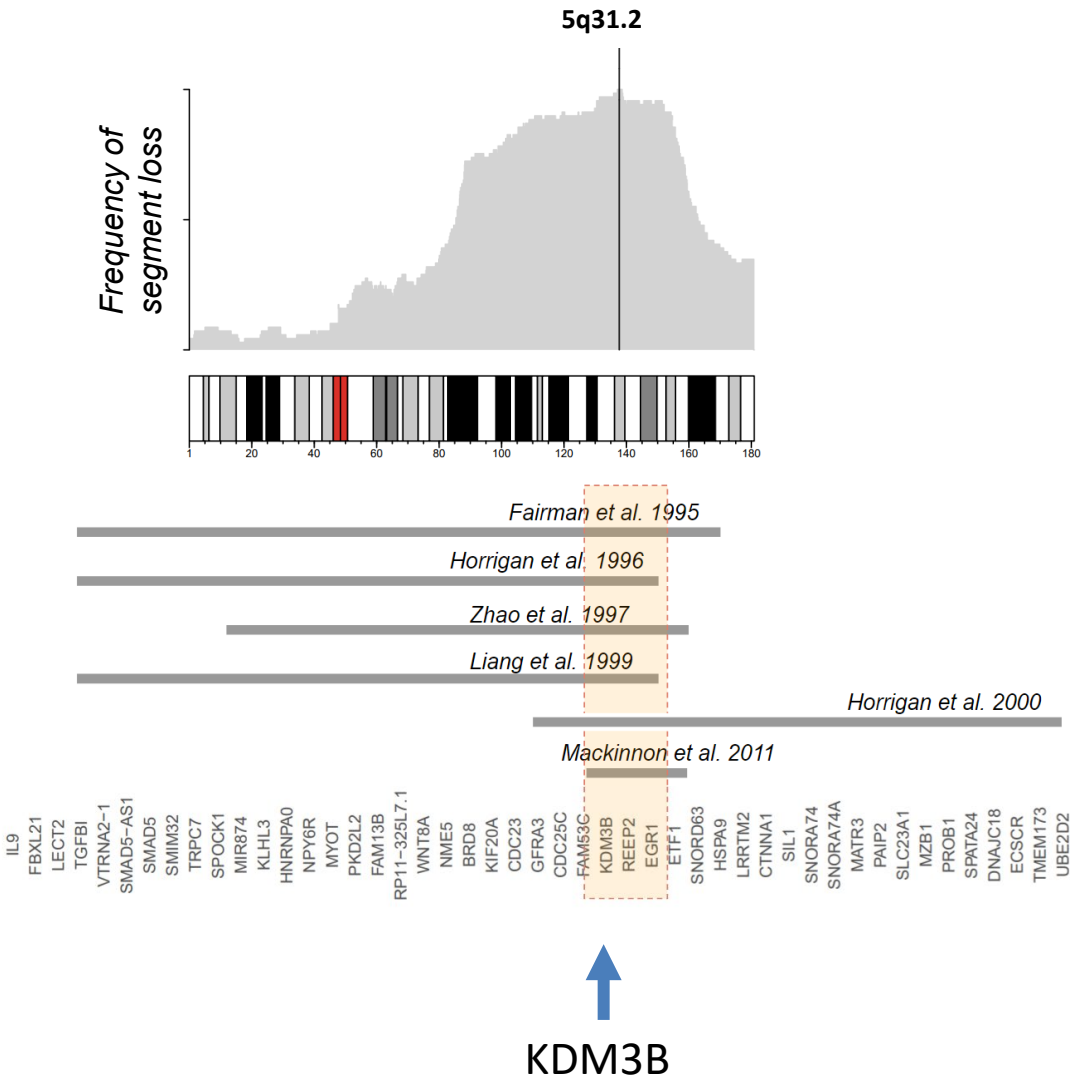






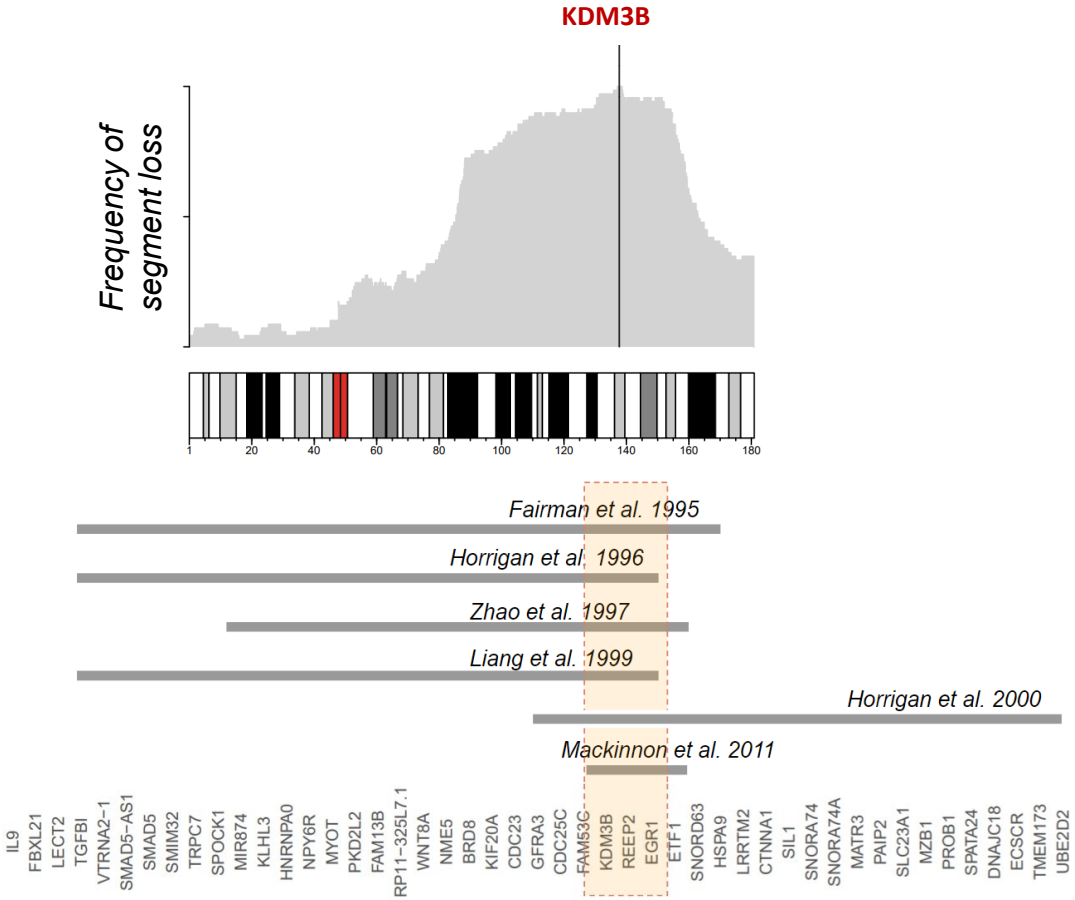
del(5q) AML may be driven by epigenetic mechanisms previously unrecognised

Reconsidering del(5q) AML from an epigenetic perspective



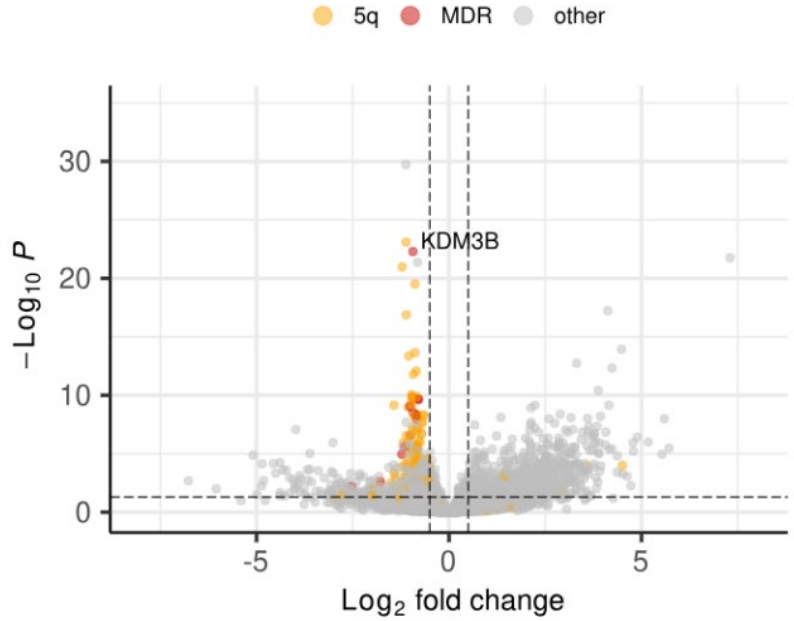
- **Most common copy number alteration** in AML
- Highly aggressive subgroup
- **Minimally deleted region** (MDR) localised to **5q31.2**
- No known **tumor suppressor** genes in the MDR

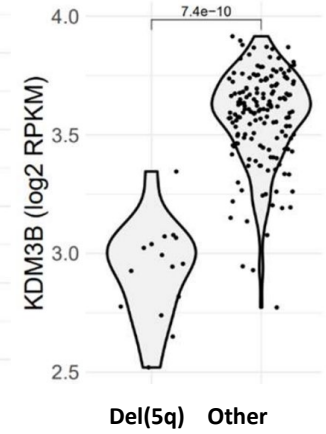
Reconsidering del(5q) AML from an epigenetic perspective



KDM3B is at the peak of the minimally deleted region

The **H3K9me1/2 demethylase KDM3B** is a likely **haploinsufficiency** candidate in del(5q) AML

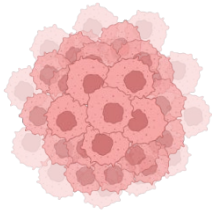




EpiR Haploinsufficiency Increases Epigenomic Heterogeneity

a novel concept proposed for tumorigenesis

normal cells

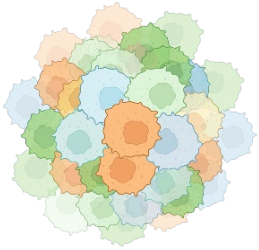


Enzymatic activity

100%

KDM3B ↓

tumor cells



50%

ONC

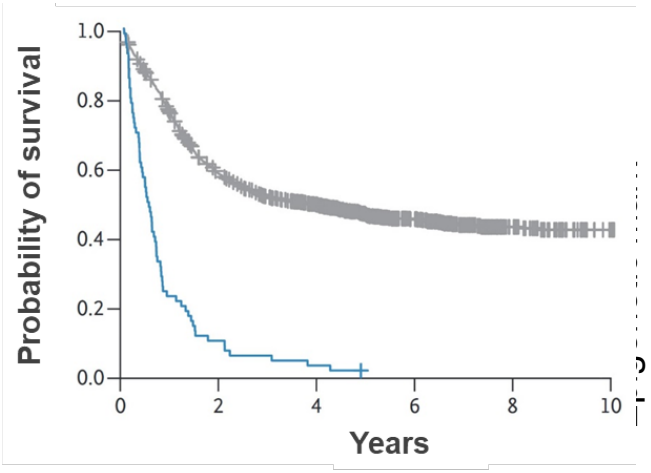
accelerated tumorigenesis

&

non-genetic therapy resistance

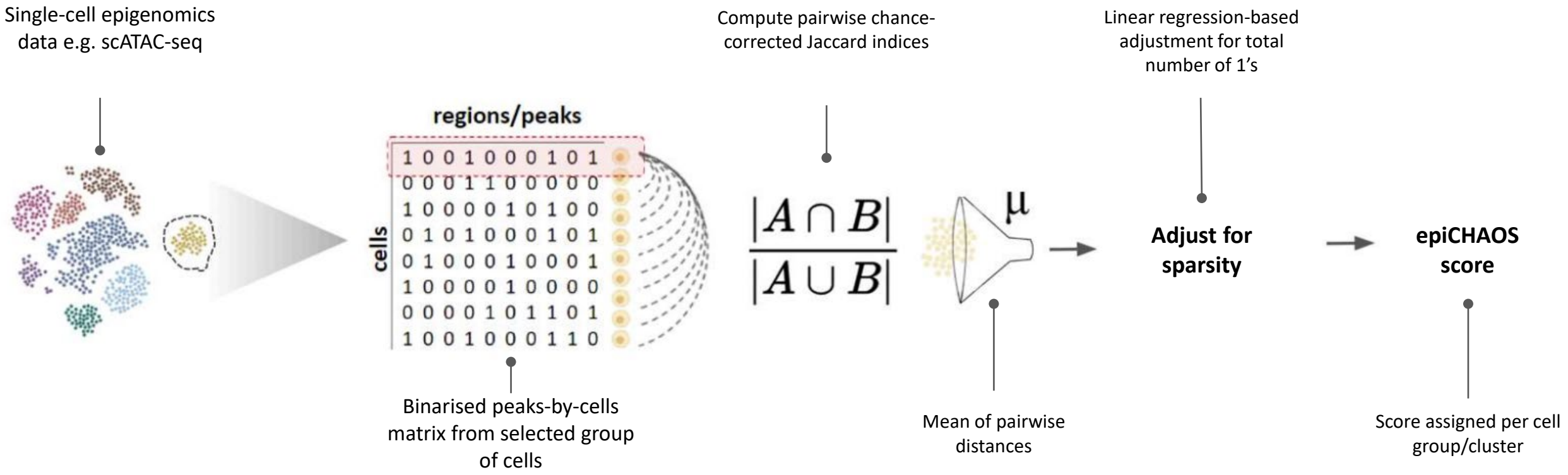
Low Epigenomic Heterogeneity

Single-cell Epigenomic Heterogeneity ↑↑



Epigenetic clone selection

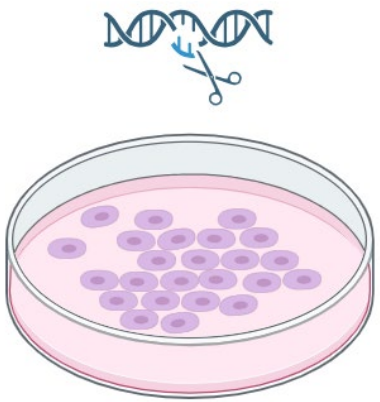
EpiCHAOS quantifies epigenetic heterogeneity in single-cell epigenomic data



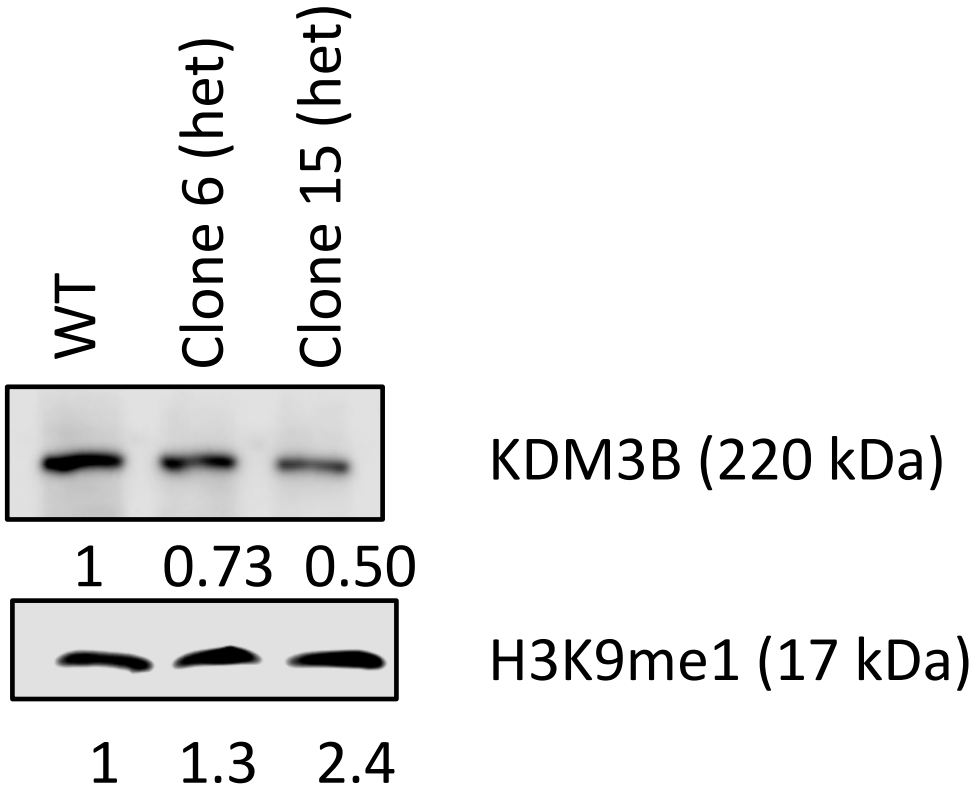
EpiCHAOS: Epigenetic/Chromatin Heterogenety Assessment of Single cells

Kelly et al., 2024 ***Genome Biology***

Does haploinsufficiency of KDM3B give rise to epigenetic heterogeneity?



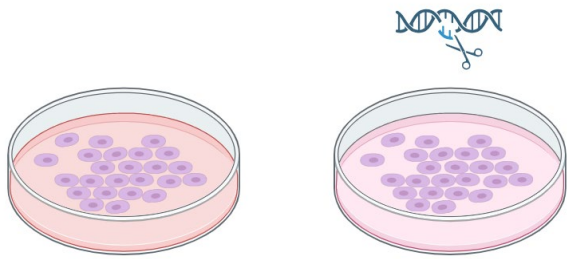
OCI-AML3



Heterozygous and homozygous disruption of KDM3B via CRISPR cas9 in OCI-AML3

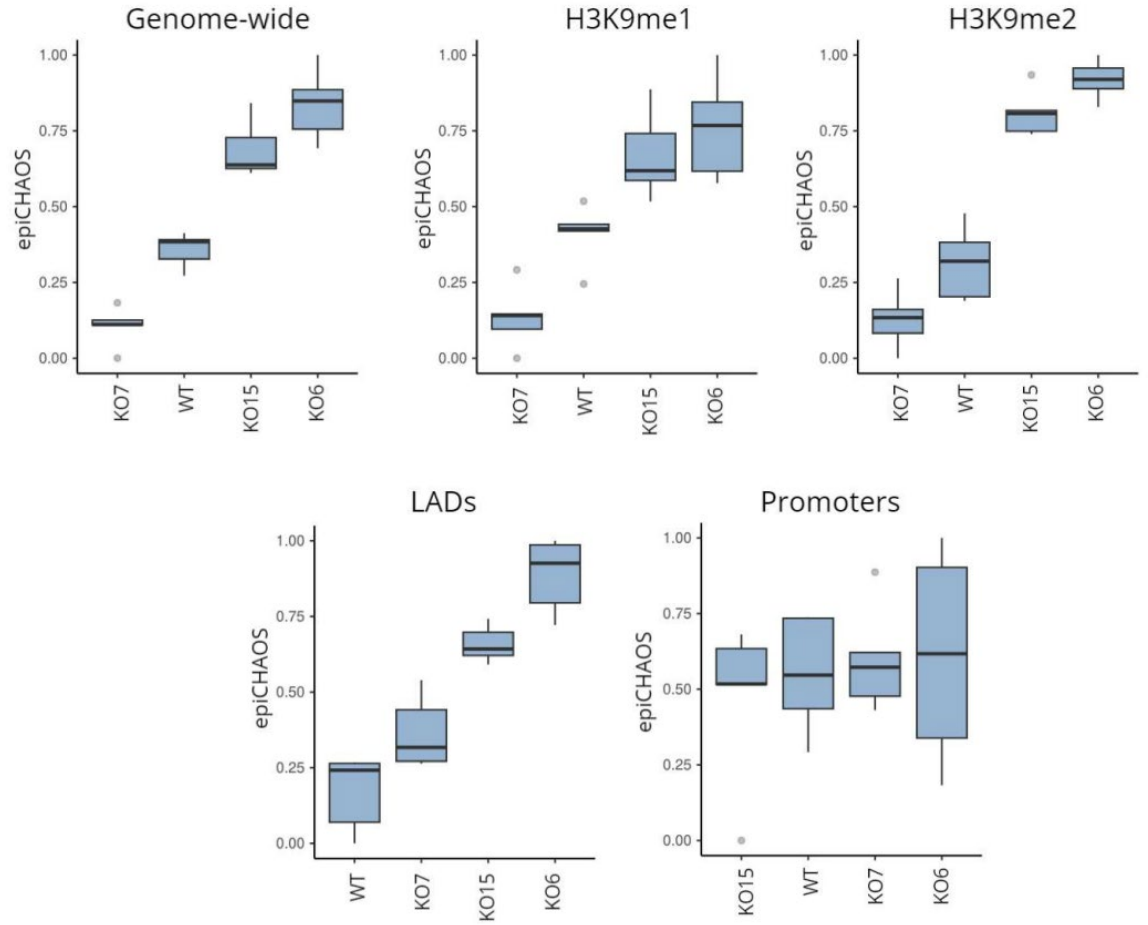
- Models haploinsuffiency in 5q- ckAML

EpiCHAOS detects increased epigenetic heterogeneity in KDM3B-/+ cells

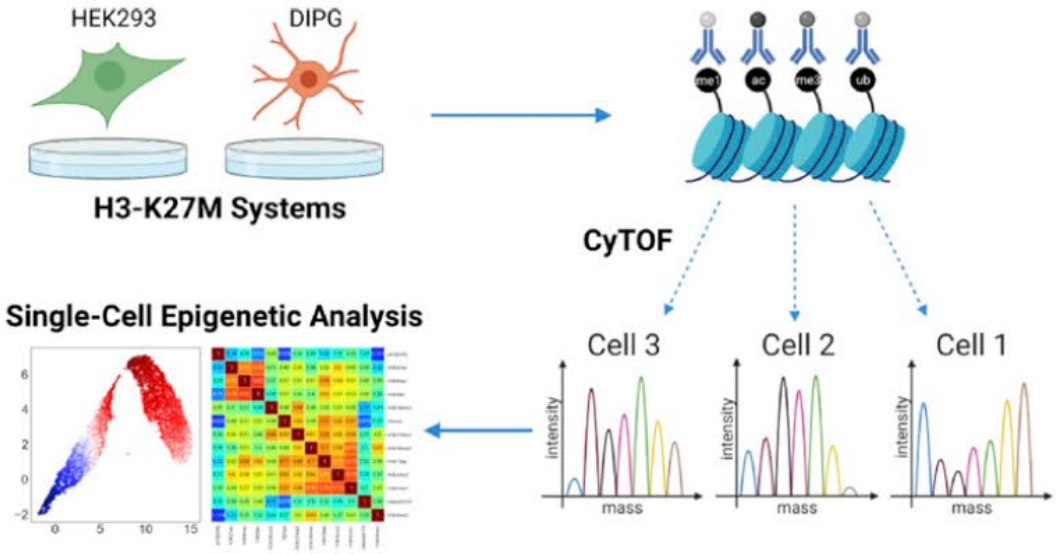


Single-cell epigenomic profiling with scATAC-seq

epiCHAOS: a metric to quantify epigenetic heterogeneity in single-cell epigenomic data



Epigenetic-focused cytometry by time-of-flight (CyTOF)



Mutant Histone

H3-K27M

Histones

H1
H1.0
H3.3
H3
H4

Cell Cycle

pH3[S28]
Ki-67
IdU
CyclinB1
pRb

Chromatin Regulators

BMI1
SIRT1
EZH2

Cancer Stemness & Oncogenesis

CD24
CD44
c-Myc
SOX2

Histone Modifications

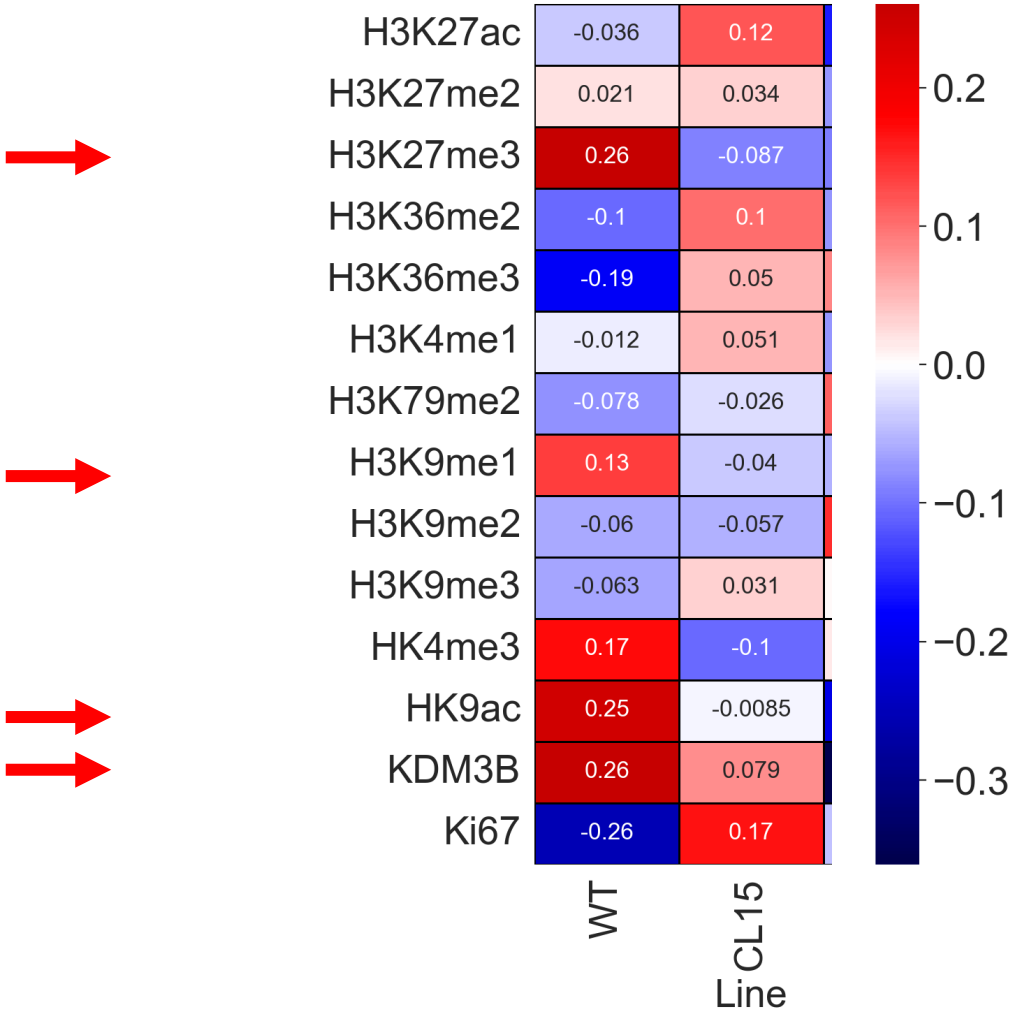
H3K27me3
H3K27ac
H3K9me3
H3K4me3
H3K27me2
H3K36me3
H3K36me2
H3K9ac
H4K16ac
H3K4me1
H3K79me2
H4K20me3
H3K64ac
γH2A.X
H2AK119ub
Cleaved H3
H3K9me2

Glioma Lineage

CXCR4
GFAP
DLL3
MBP
PDGFRa

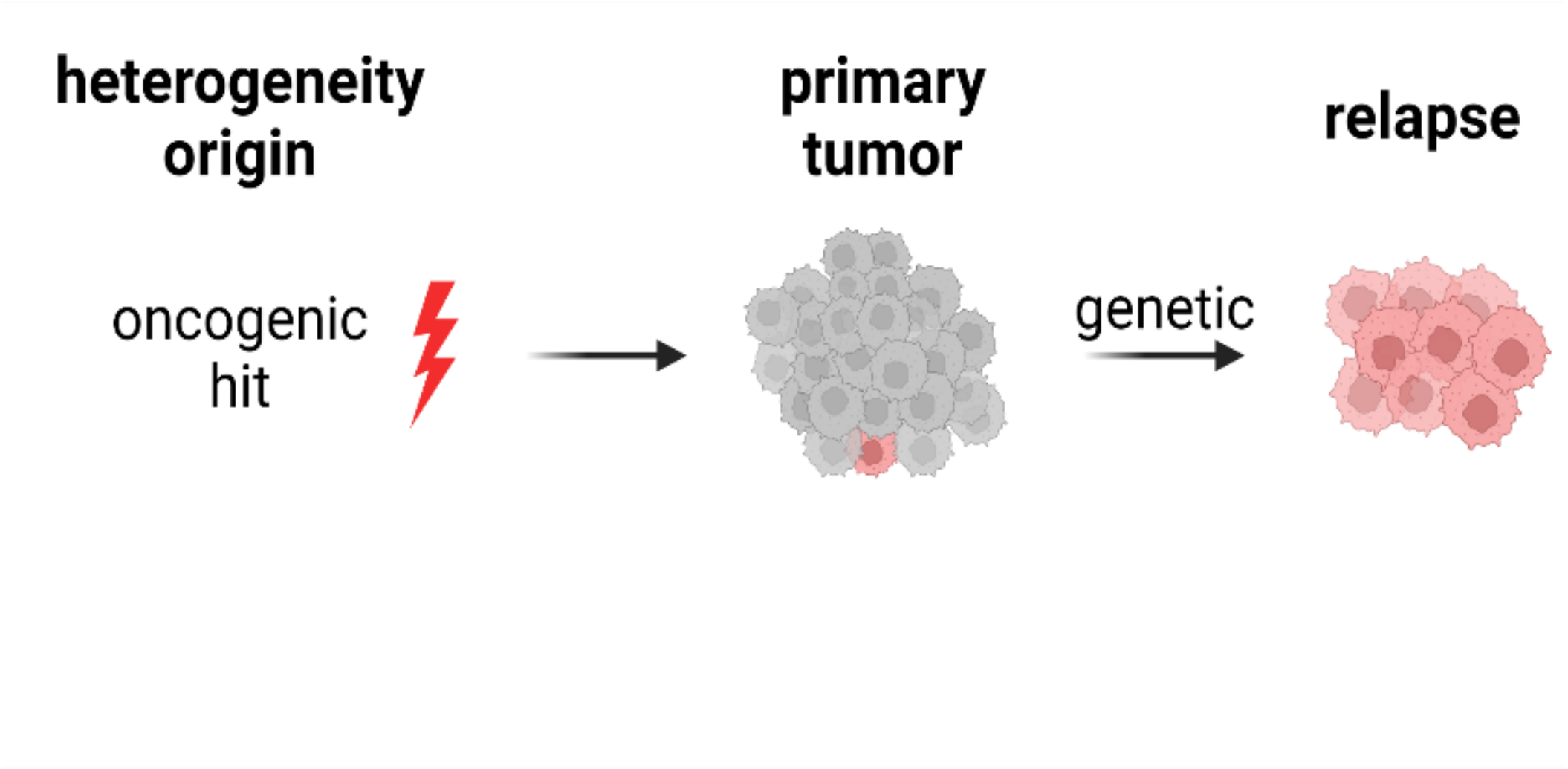
Harpaz N et al Mol Cell 2022

Preliminary CyTOF analysis of KDM3B WT and heterozygous OCI-AML clones

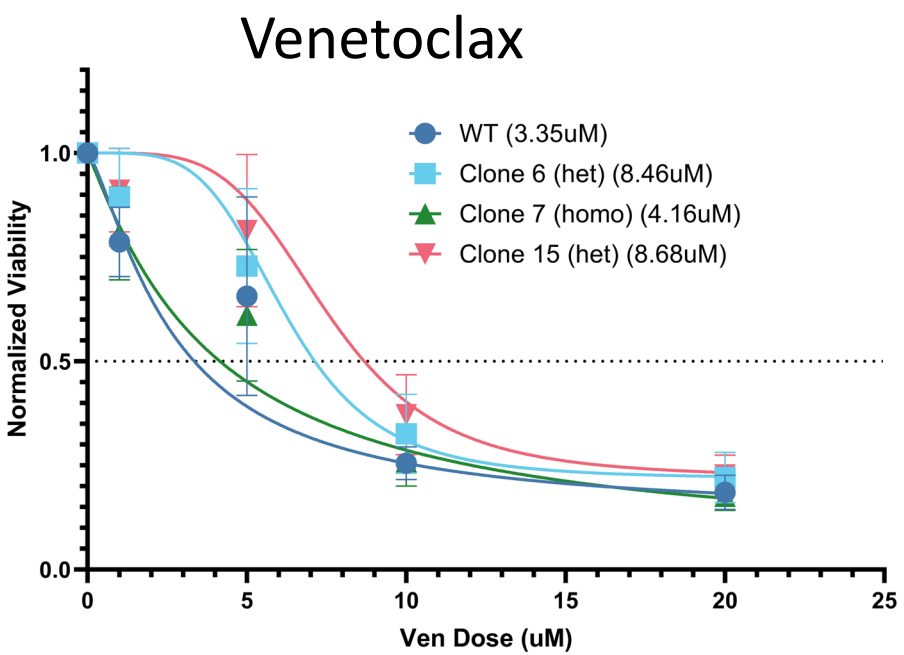


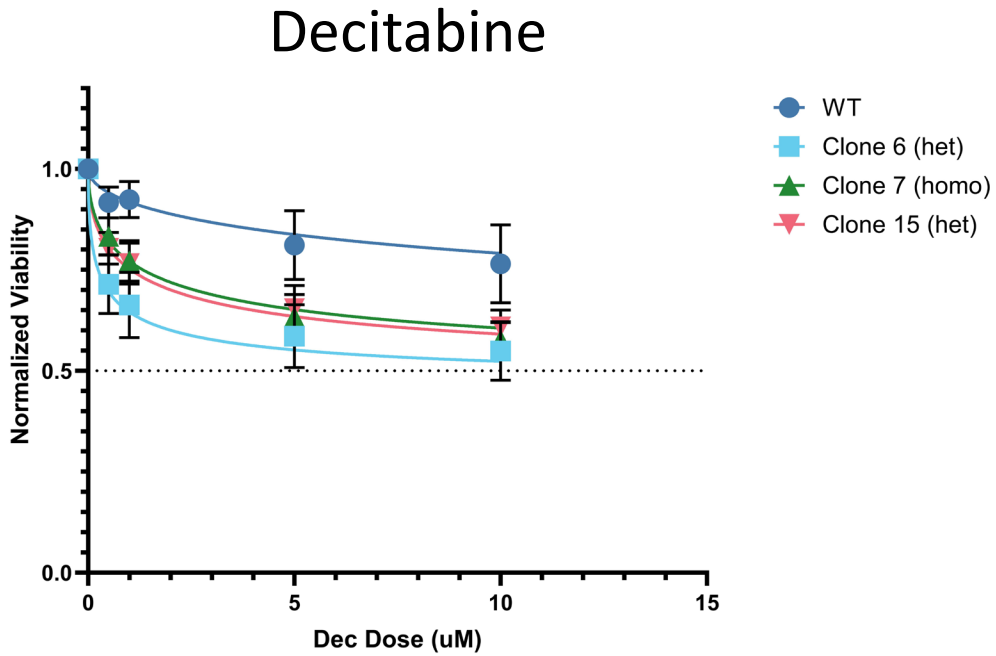
Efrat Shema unpublished

Tumor heterogeneity (genetic or epigenetic) as the foundation of therapy resistance



KDM3B haploinsufficiency increases therapy resistance against venetoclax





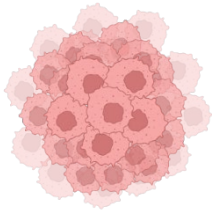
Increased resistance to venetoclax and reduced resistance to decitabine

Venetoclax – BCL2 inhibitor
Decitabine – hypomethylating agent

EpiR Haploinsufficiency Increases Epigenomic Heterogeneity

a novel concept proposed for tumorigenesis

normal cells

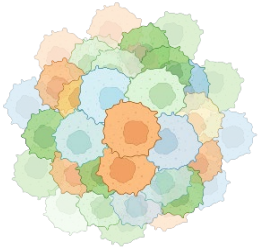


Enzymatic activity

100%

EpiR↓

tumor cells



50%

ONC

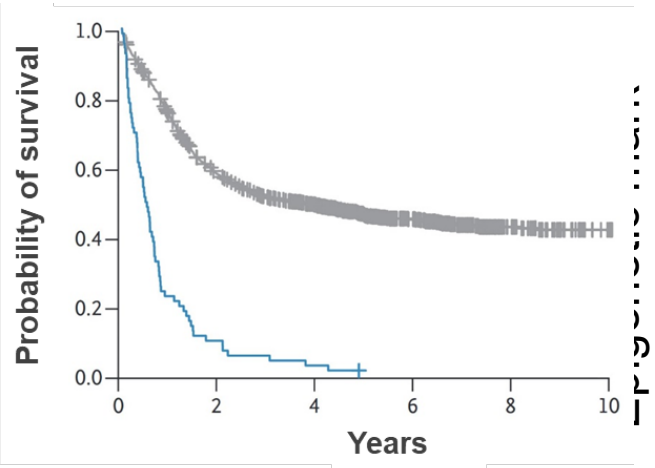
accelerated tumorigenesis

&

non-genetic therapy resistance

Low Epigenomic Heterogeneity

Single-cell Epigenomic Heterogeneity ↑↑



Epigenetic clone selection

What leads to tumor single-cell epigenetic heterogeneity?

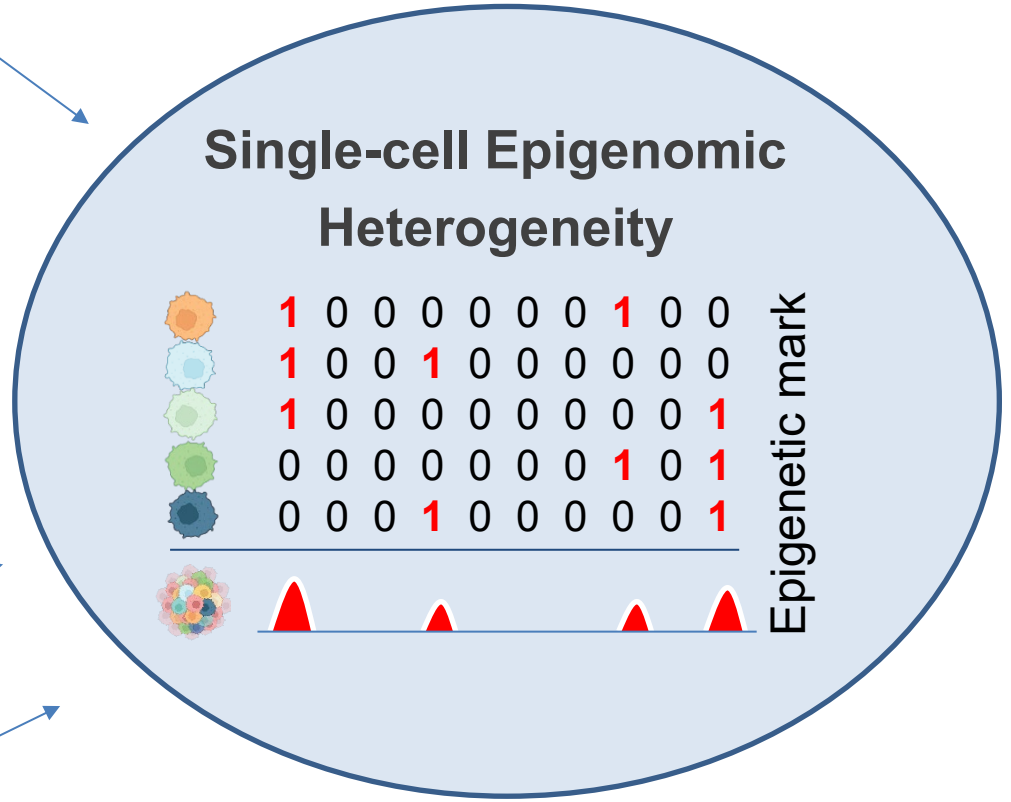
AML
KDM3B$^{+/-}$ del(5q) AML
EZH2$^{+/-}$ del(7q) AML
KMT2C$^{+/-}$ del(7q) AML
KMT2E$^{+/-}$ del(7q) AML

Breast cancer
KDM5A
amp/overexpressed

CLL
SETD2 $^{+/-}$

Lung cancer
LOY
Loss of KDM5D
loss f UTY

Oligodendroglioma
1p/19q codeletion
KDM1A $^{+/-}$



Division of Cancer Epigenomics

We are hiring!

- PhD students
- Postdocs
- **experimentalist** and
- **computational epigenomics**

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