REGIONALE SIES – Delegazione Piemonte

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Chromatin architecture regulates cell identity and oncogenic signaling in pediatric T-ALL

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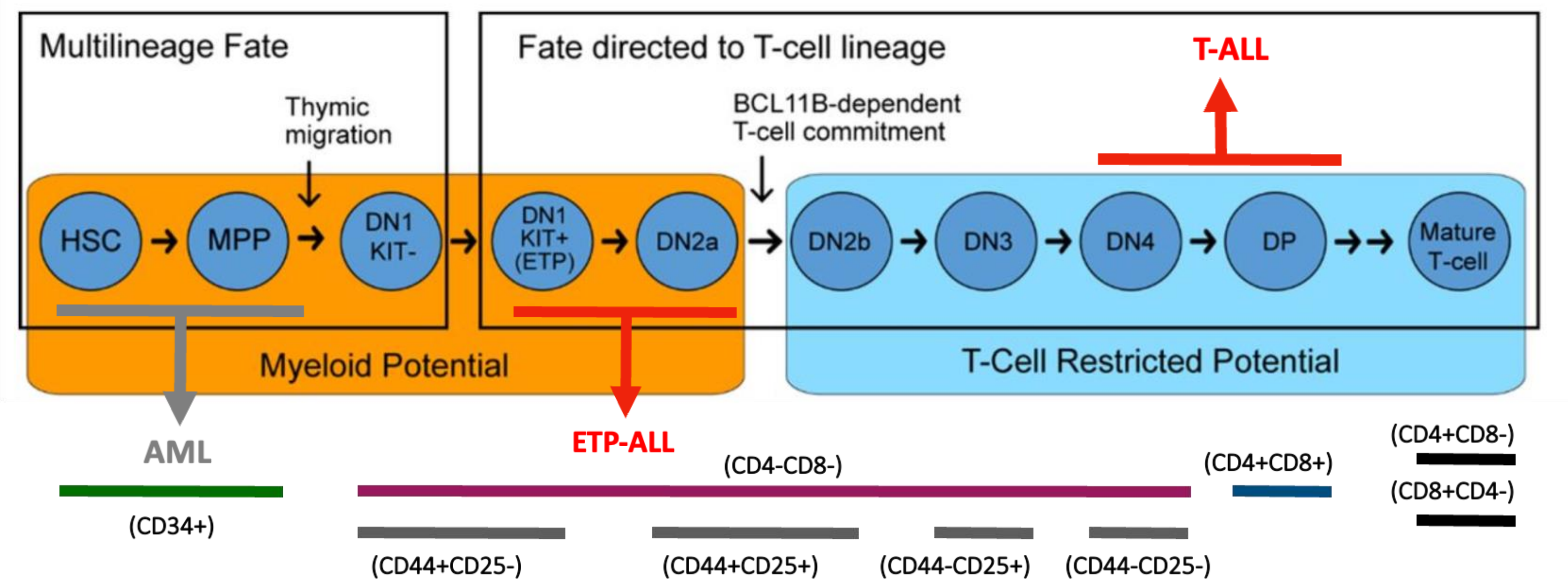
TORINO, ACCADEMIA DI MEDICINA | 4-5 GIUGNO 2026

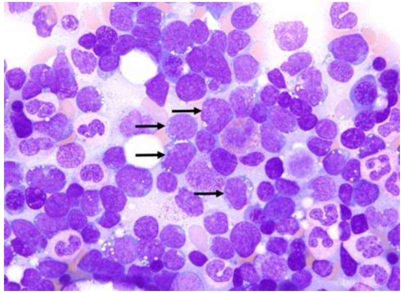
Disclosures of Francesco Boccalatte

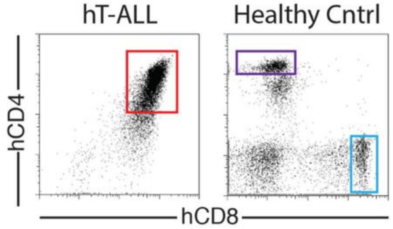
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

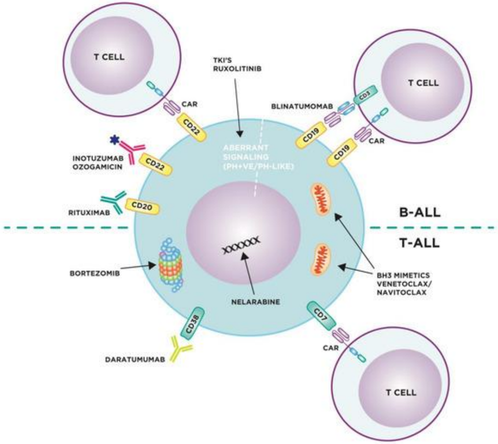
No conflicts of interest to declare

Leukemia may arise at different stages of T cell development









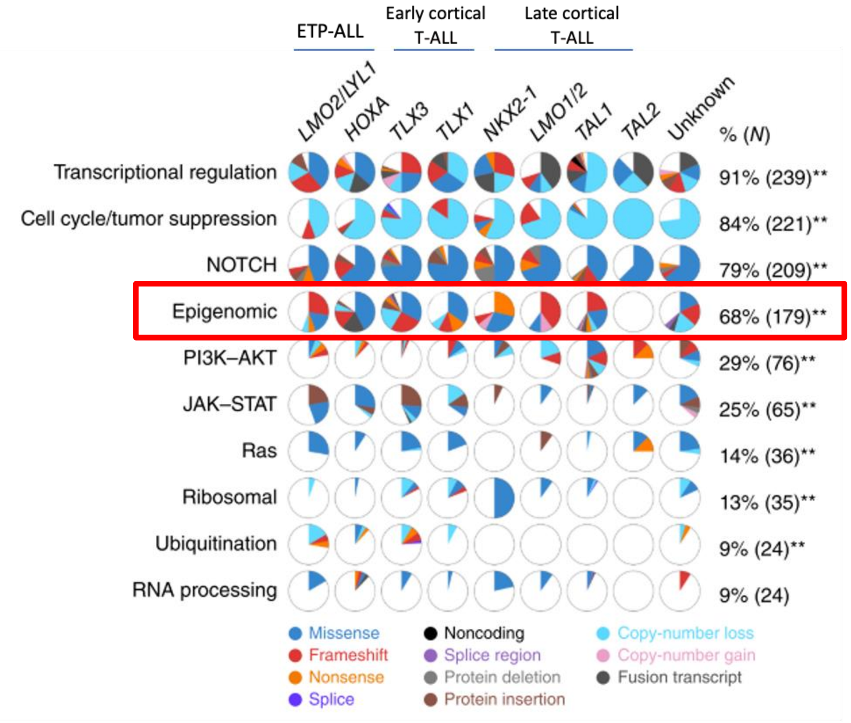
Salvaris and Fedele – J. Pers. Med 2021

Unmet clinical needs in T-ALL

- Genomic methods have limited prognostic value
- Lack of targeted therapies (*)
- High rate of relapse (especially ETP-ALL subtypes)

(*) Unlike for B-ALL, there are no FDA-approved targeted therapies or immunotherapies for T-ALL

Epigenomic alterations are recurrent across T-ALL subtypes



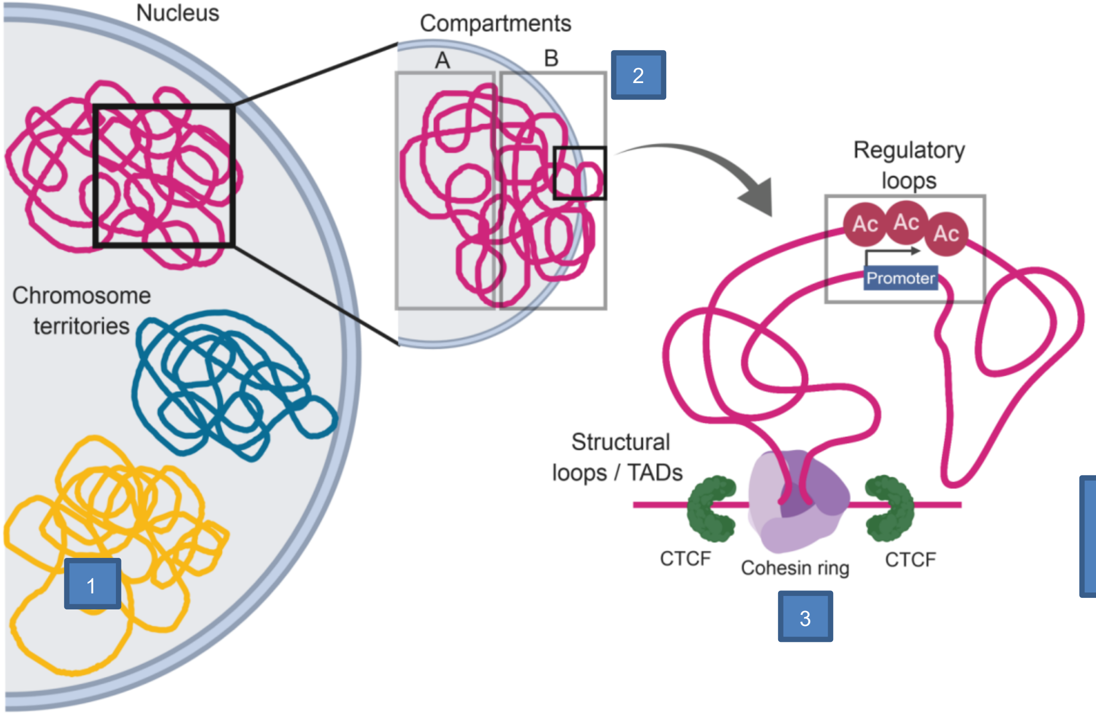
Liu et al – Nature Genetics 2017

Almost every T-ALL patient carries mutations in key epigenomic regulators:

- CTCF
- MED12
- EZH2
- TET2
- DNMT3A
- EED
- PHF6
- SUZ12
- UTX
- Various TFs…

Hierarchical chromatin organization in mammalian cells

Figure 1



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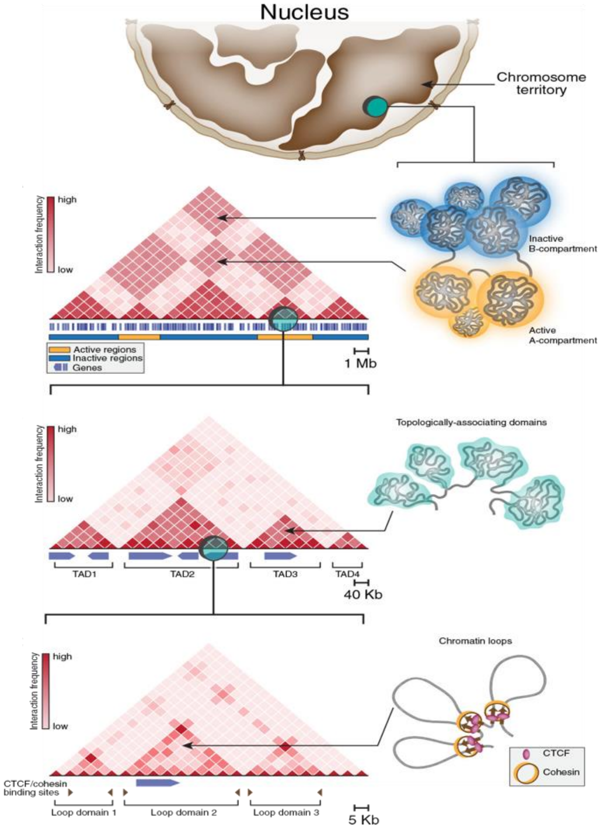
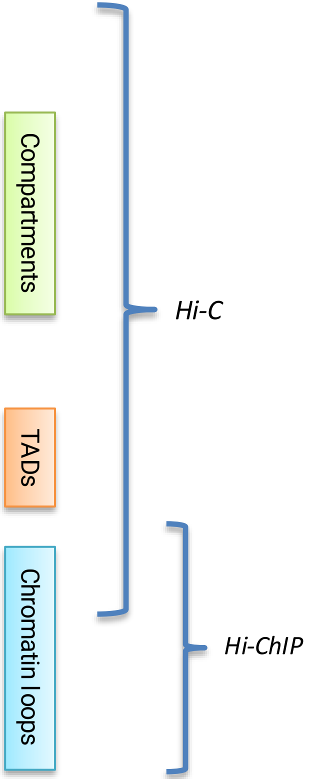
3D chromatin conformation

3D genomics

Kloetgen et al. Trends in Immunology, 2019

Methods to study the 3D chromatin conformation




Compartments

TADs

Chromatin loops

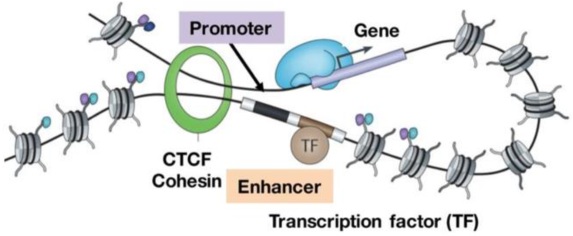
Hi-C

Hi-ChIP

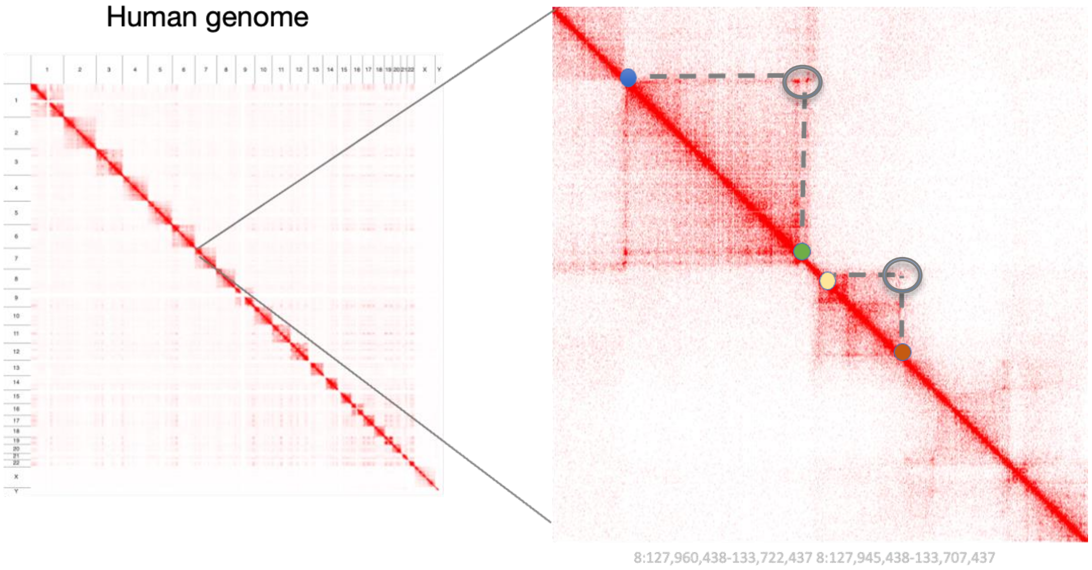
Impact of chromatin conformation on

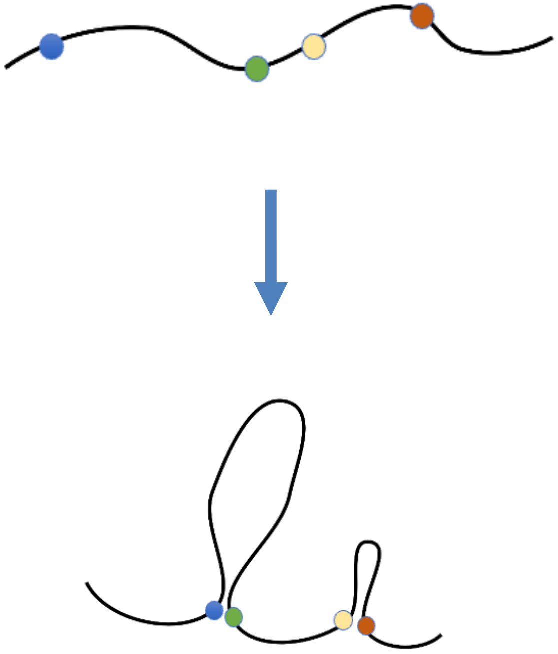
- *Transformation*
- *Cell behavior*
- *Drug resistance*

Chromatin loops regulate gene expression by bringing together (or keeping apart) genes and regulatory elements



How to read 3D chromatin conformation maps (Hi-C)

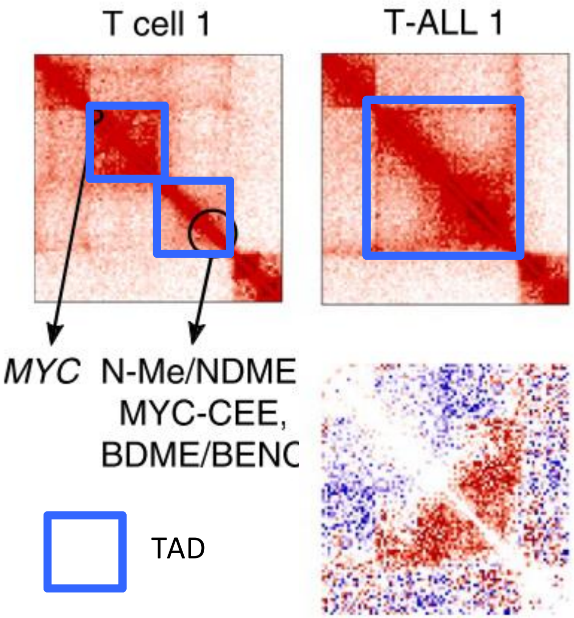




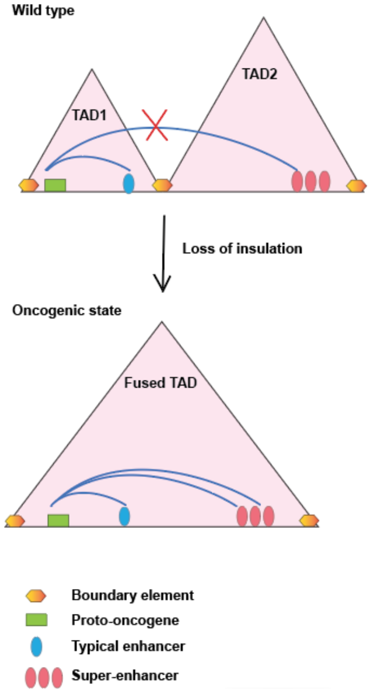
Chromatin conformation regulates MYC expression in T-ALL

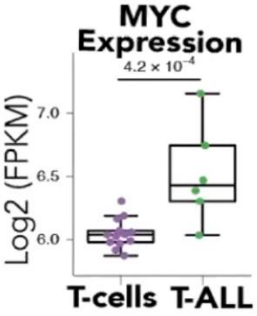
TAD fusion in leukemia

MYC locus



Super-enhancer hijacking
= long-range oncogene activation





Kloetgen, Thandapani, Ntziachristos et al – Nat Genet 2020

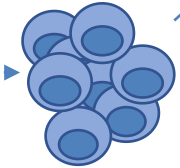
Thandapani – Pharmacol Ther 2019

Research methods

Blood sample (BM or PB)
Fresh / frozen

- *Low vs high risk*
- *Diagnosis vs relapse*

pre-processing to enrich populations of interest



~500k RNA-seq / scRNA-seq

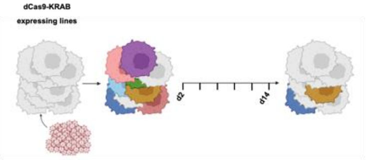
~200k DNA-seq / ONT

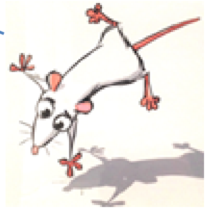
~3M HiChIP

~500k scATAC-seq

~1M (ChIP-seq)

CRISPRi screens / drug screens



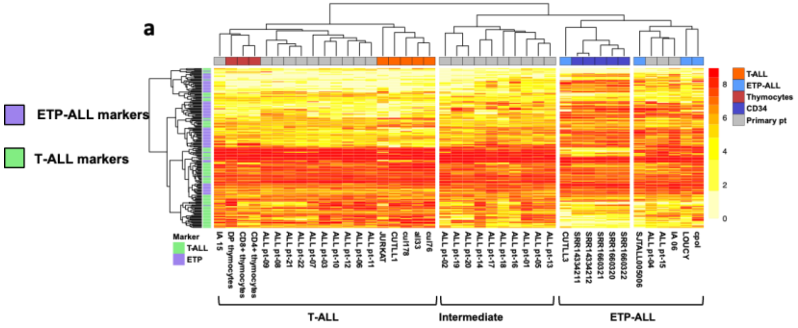


Expand blasts into NSG

Integrate with
- Clinical data (histology, morphology, flow cytometry)
- Patient history (EFS, response to therapy)
- Omics data in public repositories

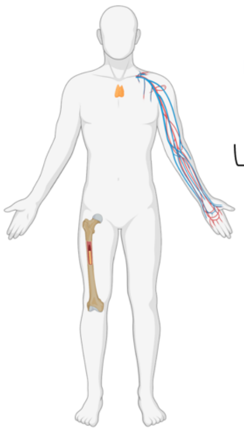
Genome-wide mapping of enhancer-promoter interactions in T-ALL

Initial profiling (RNA-seq)



Selected samples

3D chromatin conformation
(H3K27ac HiChIP)



Human primary samples

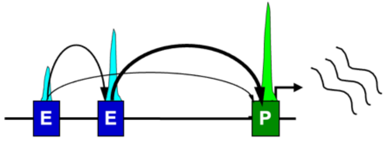
BM-derived CD34+ cells

ETP-ALL

DP thymocytes

T-ALL

H3K27ac HiChIP

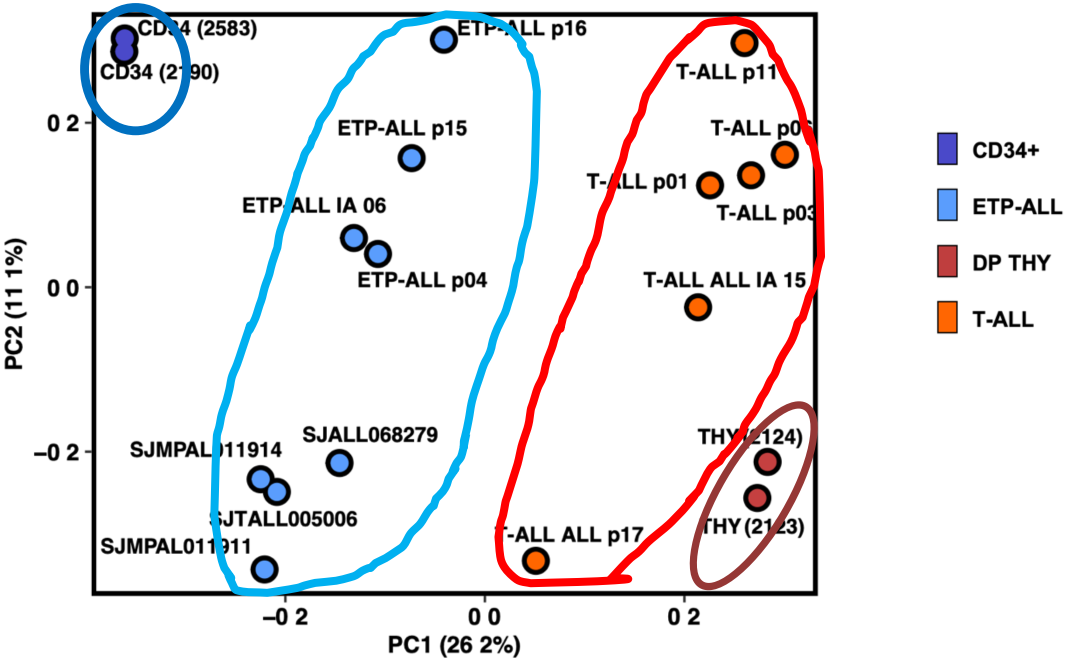


Interactivity score (IA)
(sum of counts of an anchor towards other regions)

Hub score (H)
(number of connections of an anchor towards other regions)

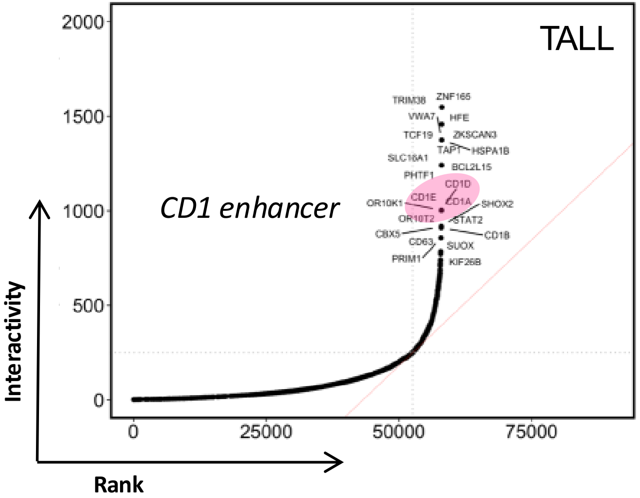
Correlate with Gene expression levels

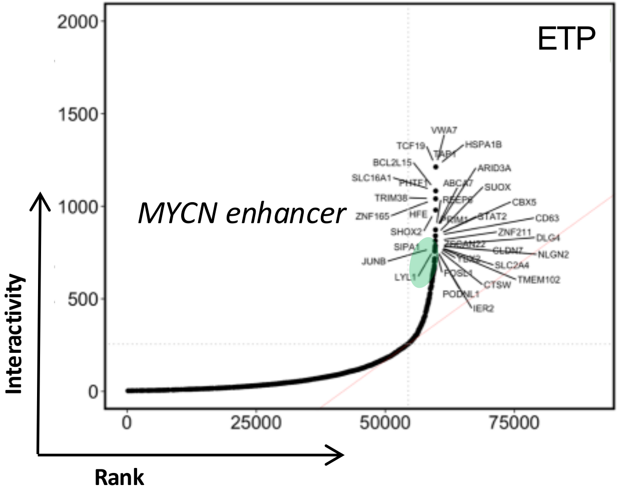
Cell types (and risk groups) are stratified by 3D chromatin interactivity



Gambi, Boccalatte*, Rodriguez* et al – Molecular Cell 2025*

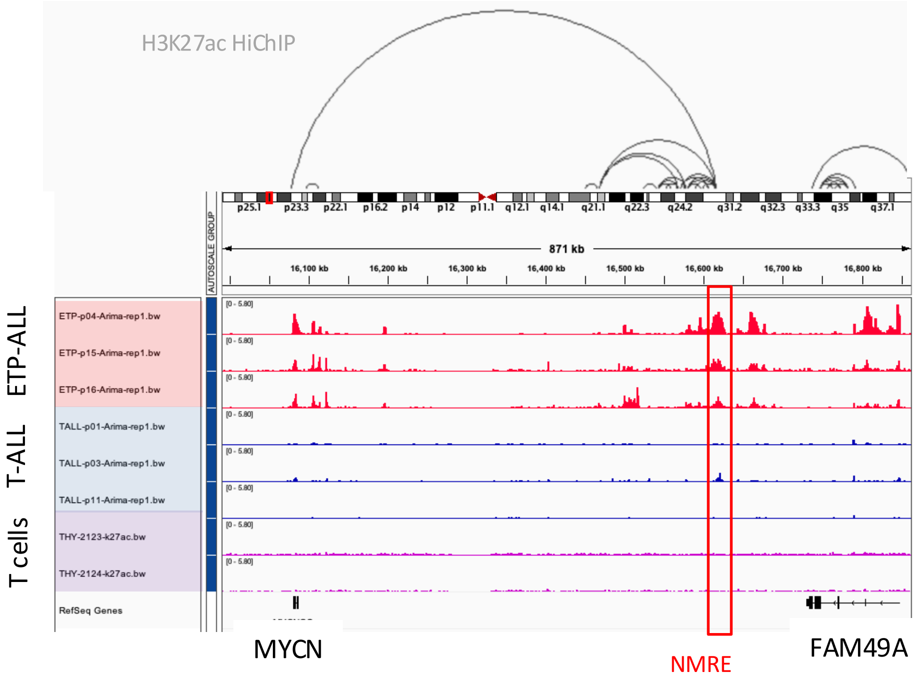
Highly connected chromatin hubs reveal known and novel targets





Gambi, Boccalatte*, Rodriguez* et al – Molecular Cell 2025*

An ETP-selective hub is detected at the MYCN locus



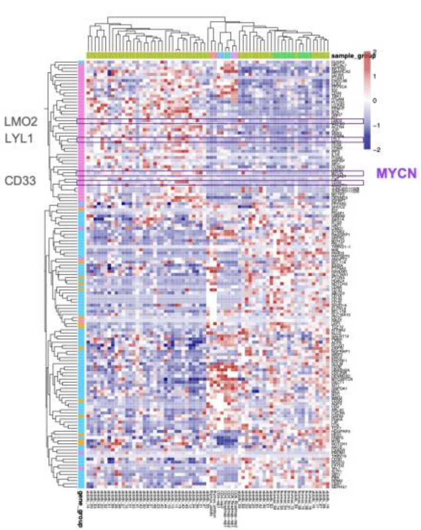
- Unannotated ncRNA
- Enhancer features
- Looping on MYCN

Does this long-range chromatin interaction correlates with an increased MYCN expression in ETP-ALL?

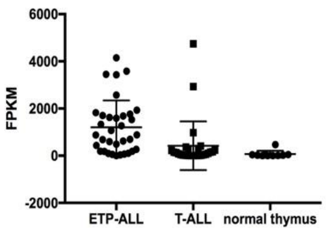
Wang, Boccalatte* et al – JEM 2025*

MYCN is robustly expressed in ETP-ALL (and correlates with NMRE expression)

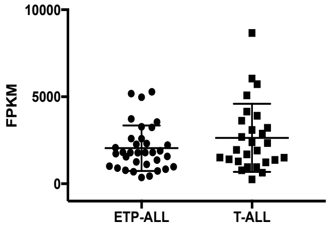
RNA-Seq



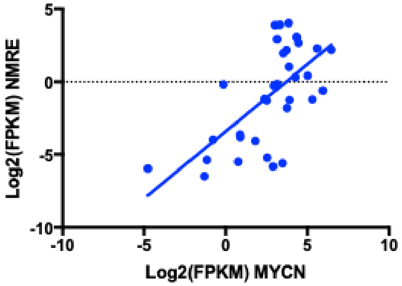
N-MYC



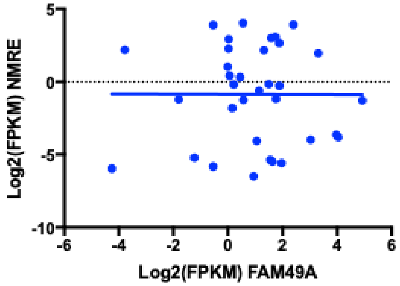
c-MYC

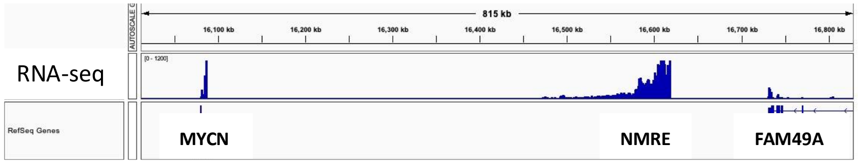


MYCN-NMRE correl in ETP-ALL



FAM49a-NMRE correl in ETP-ALL

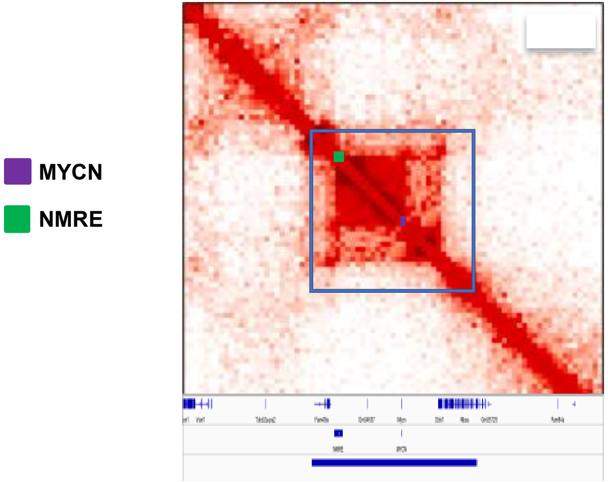




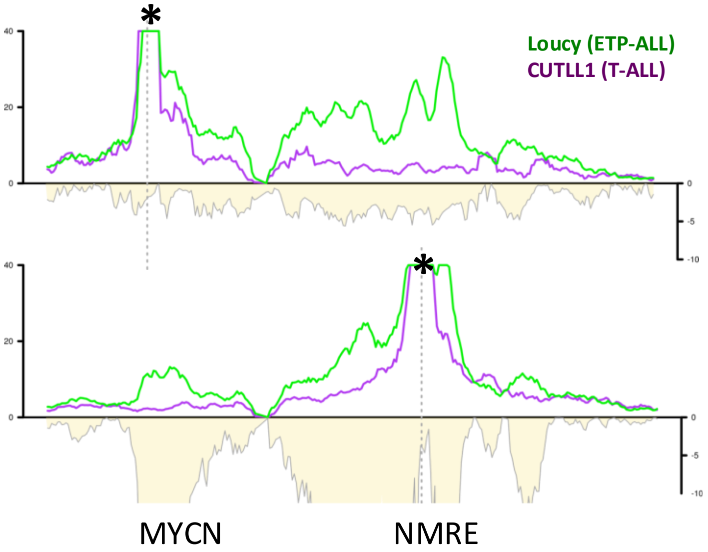
Wang, Boccalatte* et al – JEM 2025*

MYCN and its enhancer (NMRE) form a chromatin loop in ETP-ALL

Hi-C



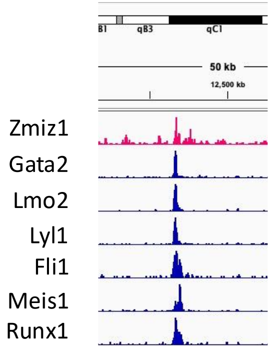
4-C sequencing

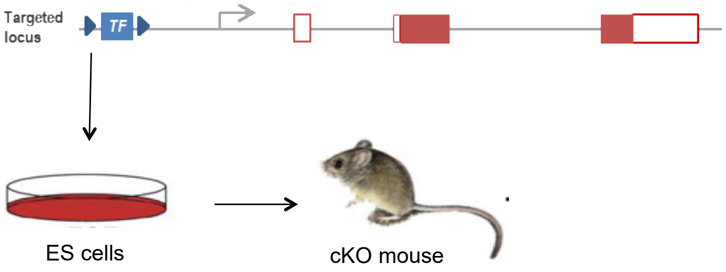


Wang, Boccalatte* et al – JEM 2025*

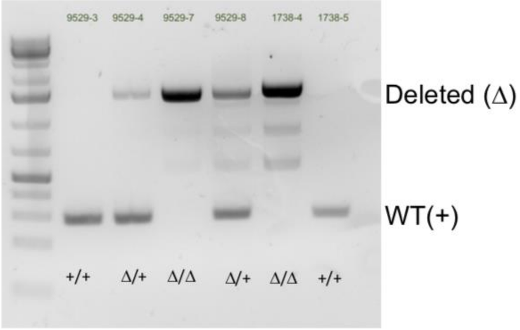
Nmre is a TF binding hotspot and it regulates Mycn expression in vivo

NMRE is a hotspot for binding of many transcription factors involved in leukemia

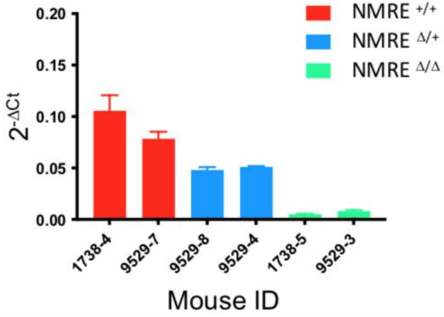




Peripheral blood cell DNA



Mycn expression in HSC



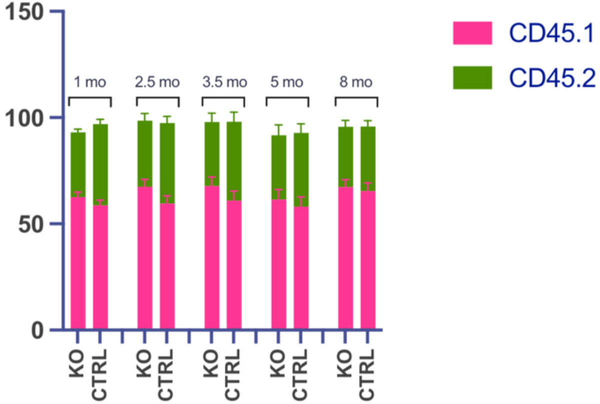
Wang, Boccalatte* et al – JEM 2025*

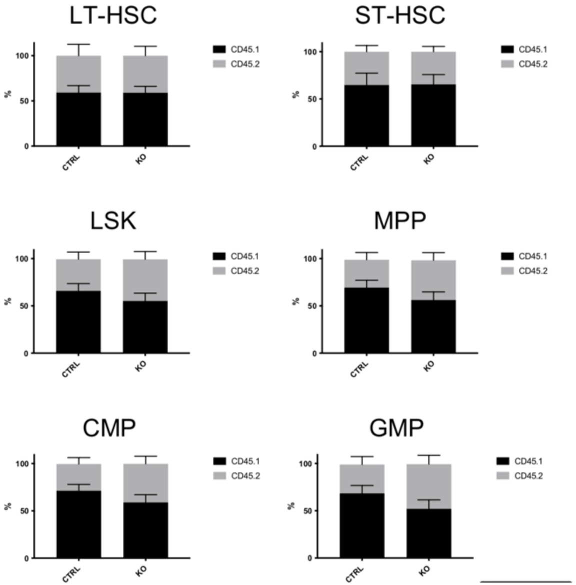
Nmre is dispensable in normal hematopoiesis

WBM mix 1:1
CD45.2 Nmre-KO
CD45.1 WT

WBM mix 1:1
CD45.2 Nmre-WT
CD45.1 WT

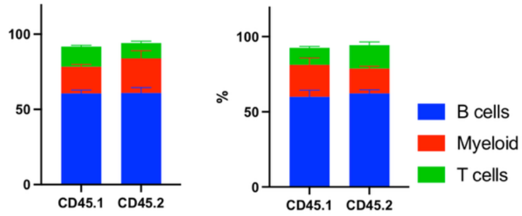
Chimerism on peripheral blood





CD45.1: WT
CD45.2: NMRE$^{\Delta/\Delta}$

CD45.1: WT
CD45.2: NMRE$^{+/+}$

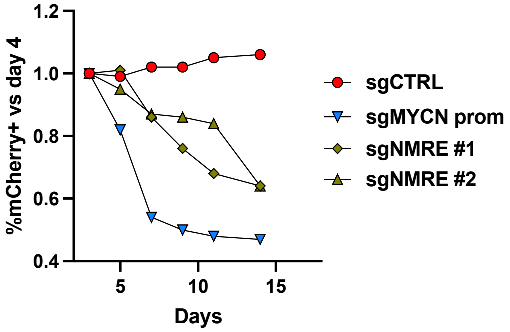


Wang, Boccalatte* et al – JEM 2025*

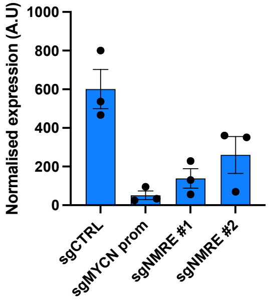
Nmre is selectively required for ETP-ALL survival

ETP-ALL

CUTLL3

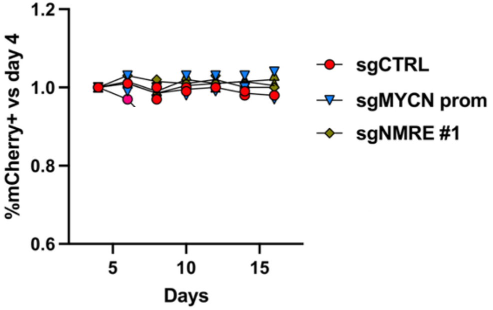


MYCN

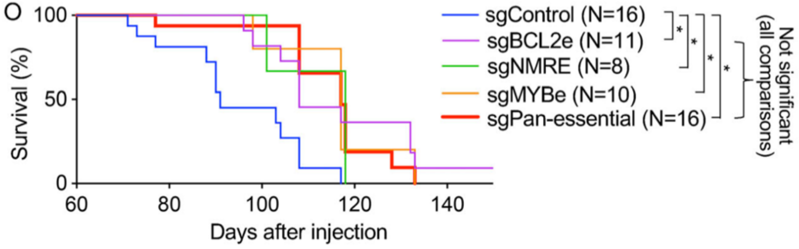


T-ALL

CUTLL1



NMRE targeting in ETP-ALL *in vivo (survival increase)*



Wang, Boccalatte* et al – JEM 2025*

Conclusions

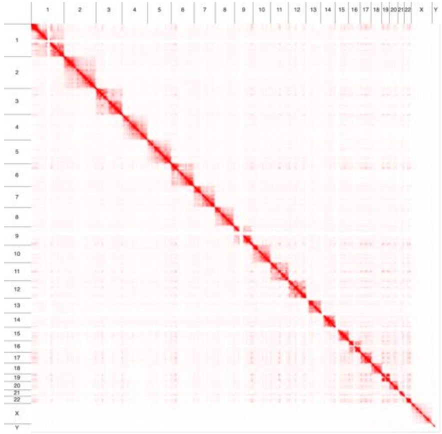
- Chromatin conformation can be efficiently captured genome-wide in acute leukemia (and virtually in every tumor)
- Chromatin interactivity provides an accurate patient stratification based on their epigenome
- Chromatin conformation capture reveals long-range interactions between enhancers and proto-oncogenes
- 3D chromatin profiling of ETP-ALL reveals a novel enhancer (NMRE) regulating MYCN expression
- Loss of the NMRE impairs ETP-ALL proliferation, while sparing normal hematopoiesis

Future plans :

- *Study of additional targets organized in highly interconnected neighborhoods (multi-hub complexes)*
- *Study of dynamic 3D chromatin hubs rewiring upon drug resistance*
- *Identify actionable chromatin loops and use them as therapeutic targets*

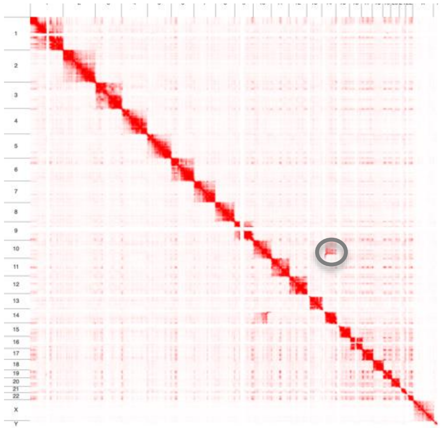
Additional insight
Chromatin conformation detects and characterizes Structural Variations

Normal karyotype



Intra-chromosomal Hi-C signal

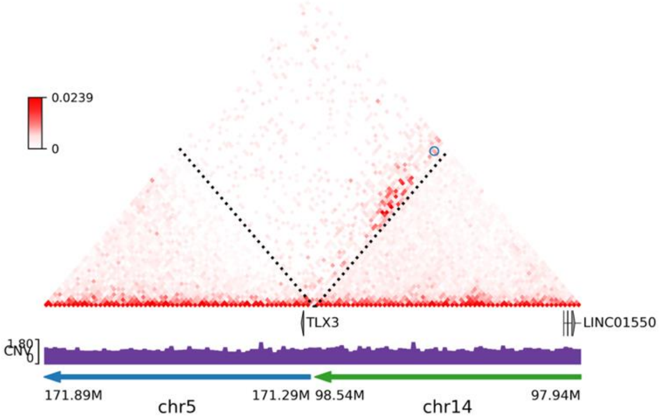
Abnormal karyotype



Inter-chromosomal Hi-C signal reveals translocation event

Hi-C has better sensitivity and resolution than standard procedures (Karyotyping or FISH)

Patient ID: PAVMUJ - Dx



We can detect the formation of new chromatin loops connecting oncogenes and regulatory elements on different chromosomes

Unpublished data

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AIRC

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