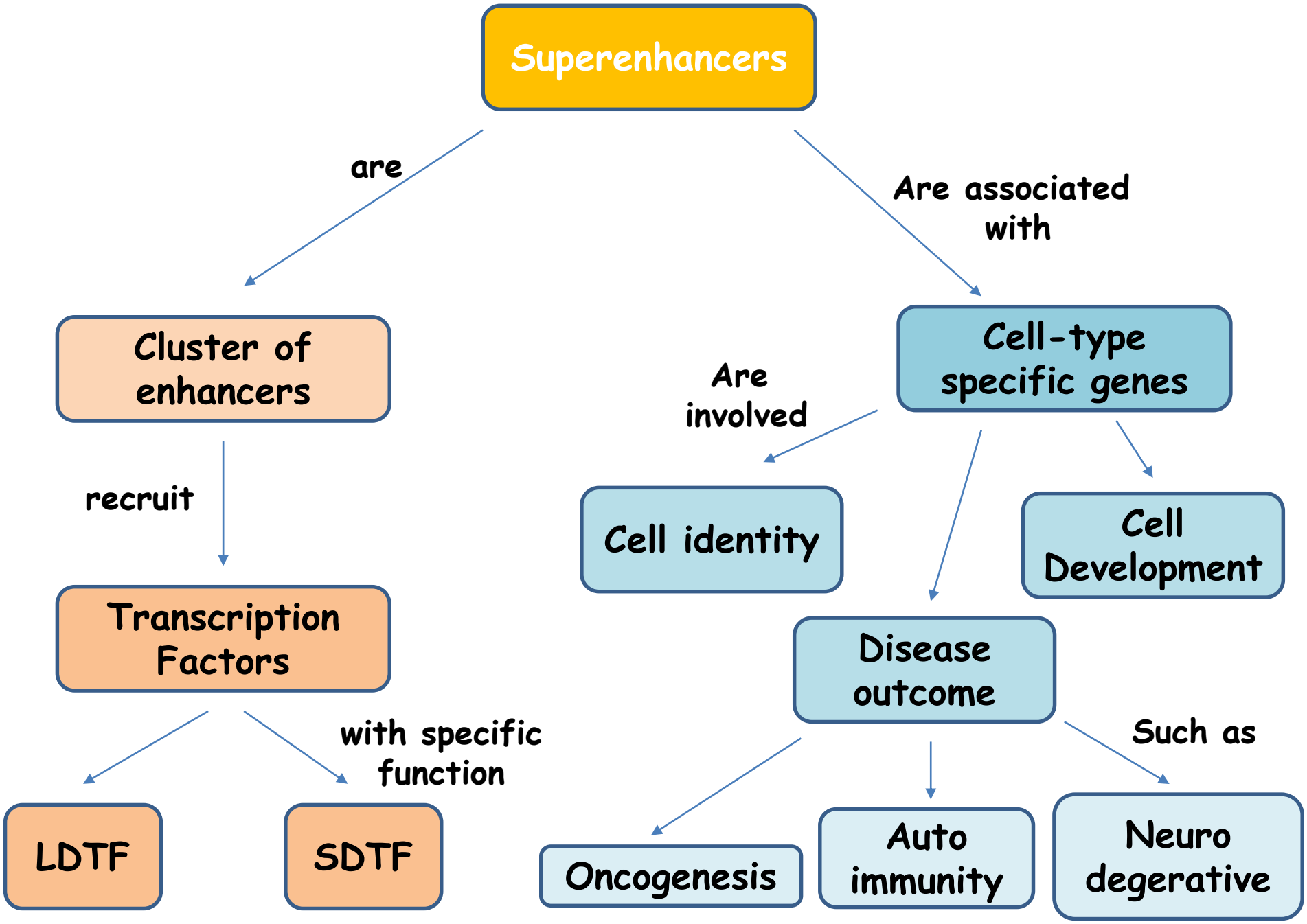
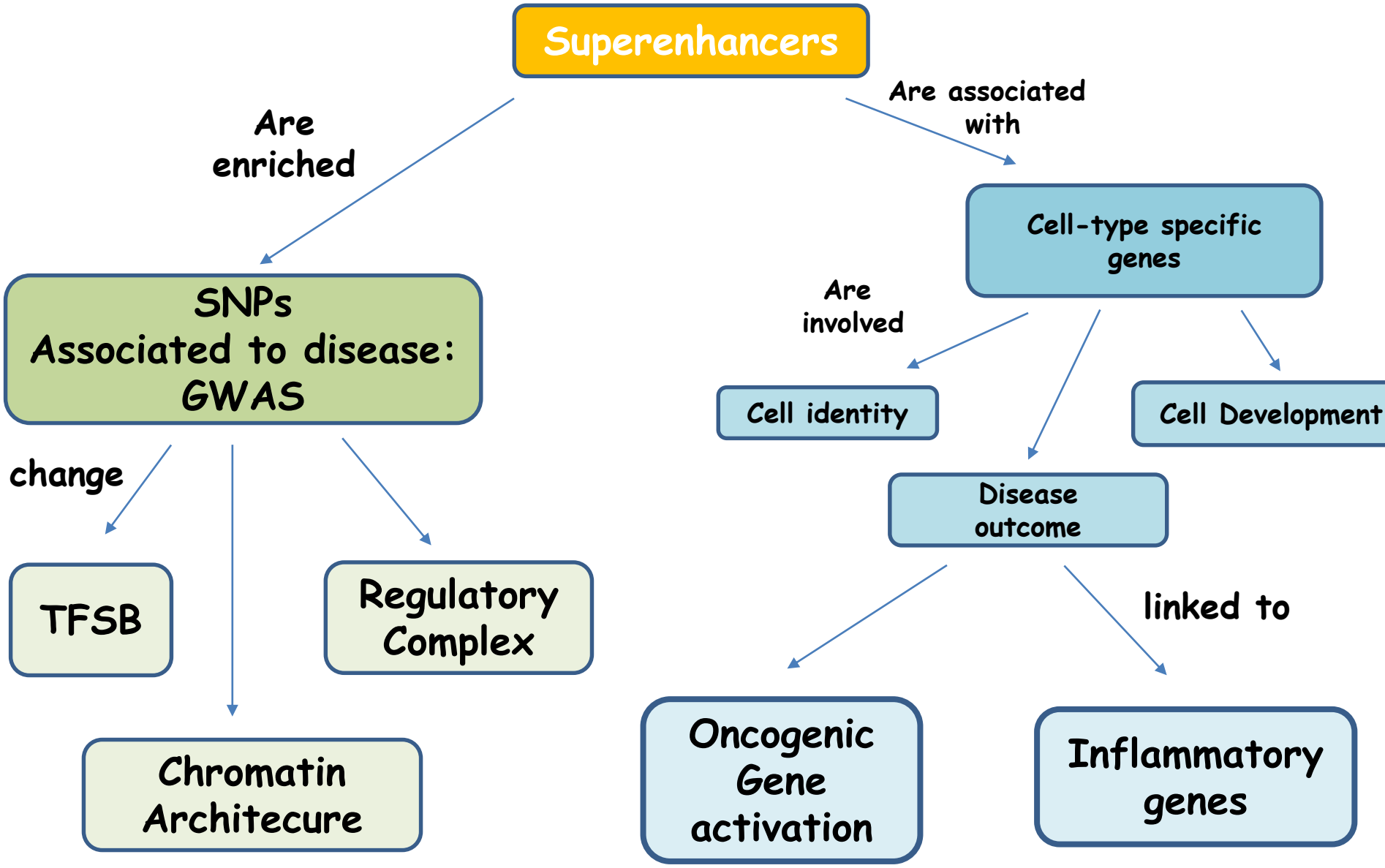
DEFINITION


Superenhancers
are
Are associated with
Cluster of enhancers
Cell-type specific genes
Are involved
recruit
Cell identity
Cell Development
Transcription Factors
Disease outcome
with specific function
Such as
LDTF
SDTF
Oncogenesis
Auto immunity
Neuro degerative

WHY ARE SE IMPORTANT?



From Superenhancers to drug discovery

RESEARCH ARTICLE

Superenhancer Analysis Defines Novel Epigenomic Subtypes of Non-APL AML, Including an RARα Dependency Targetable by SY-1425, a Potent and Selective RARα Agonist

Michael R. McKeown[1], M. Ryan Corces[2], Matthew L. Eaton[1], Chris Fiore[1], Emily Lee[1], Jeremy T. Lopez[1], Mei Wei Chen[1], Darren Smith[1], Steven M. Chan[3], Julie L. Koenig[2], Kathryn Austgen[1], Matthew G. Guenther[1], David A. Orlando[1], Jakob Lovén[1], Christian C. Fritz[1], and Ravindra Majeti[2,4]

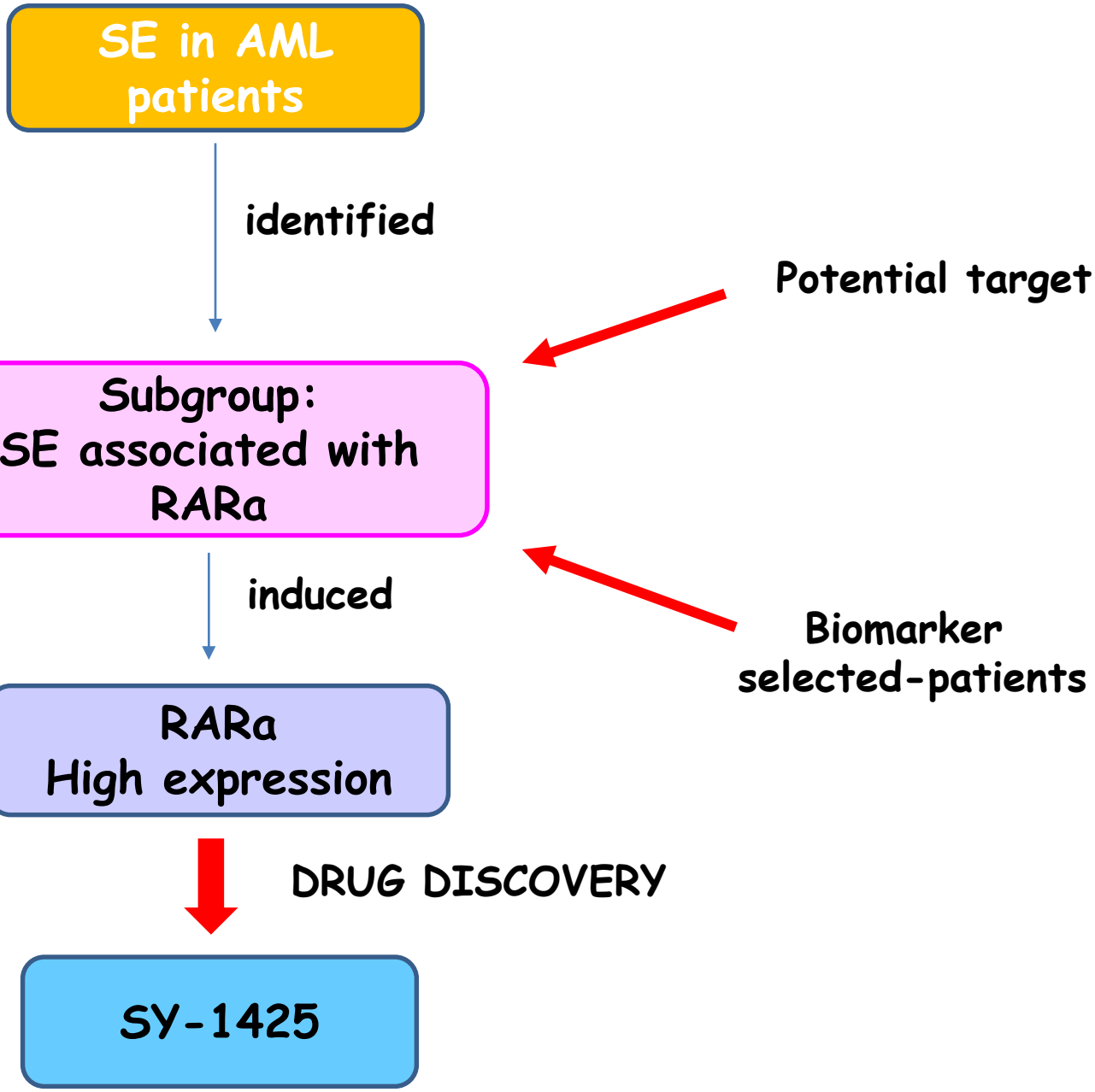
ABSTRACT

We characterized the enhancer landscape of 66 patients with acute myeloid leukemia (AML), identifying 6 novel subgroups and their associated regulatory loci. These subgroups are defined by their superenhancer (SE) maps, orthogonal to somatic mutations, and are associated with distinct leukemic cell states. Examination of transcriptional drivers for these epigenomic subtypes uncovers a subset of patients with a particularly strong SE at the retinoic acid receptor alpha (*RARA*) gene locus. The presence of a *RARA* SE and concomitant high levels of *RARA* mRNA predisposes cell lines and *ex vivo* models to exquisite sensitivity to a selective agonist of RARα, SY-1425 (tamibarotene). Furthermore, only AML patient-derived xenograft (PDX) models with high *RARA* mRNA were found to respond to SY-1425. Mechanistically, we show that the response to SY-1425 in RARA-high AML cells is similar to that of acute promyelocytic leukemia treated with retinoids, characterized by the induction of known retinoic acid response genes, increased differentiation, and loss of proliferation.

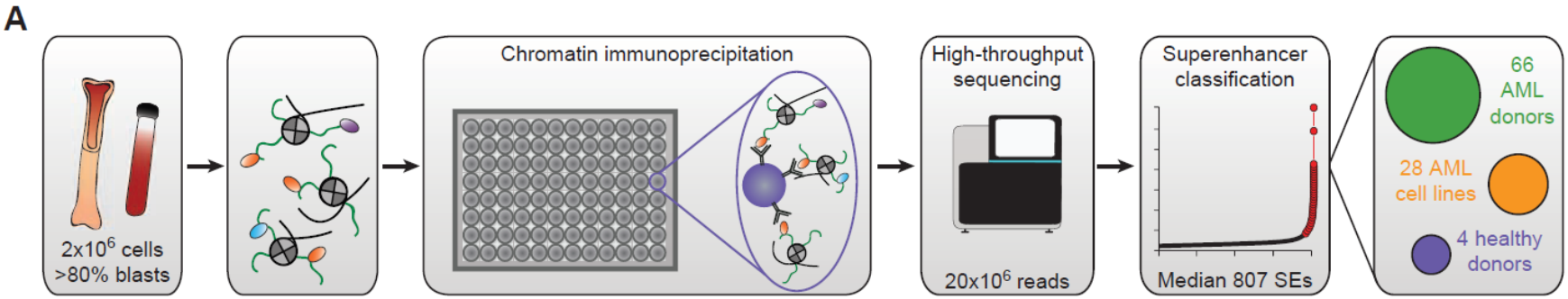
SIGNIFICANCE: We use the SE landscape of primary human AML to elucidate transcriptional circuitry and identify novel cancer vulnerabilities. A subset of patients were found to have an SE at *RARA*, which is predictive for response to SY-1425, a potent and selective RARα agonist, in preclinical models, forming the rationale for its clinical investigation in biomarker-selected patients. *Cancer Discov; 7(10); 1136–53. ©2017 AACR.*

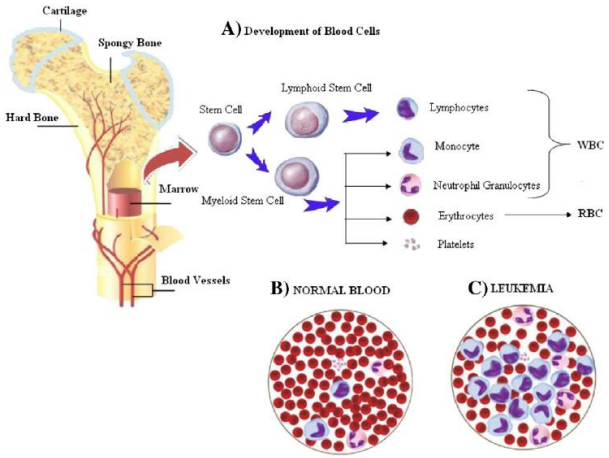
See related commentary by Wang and Aifantis, p. 1065.

Drug discovery using SE



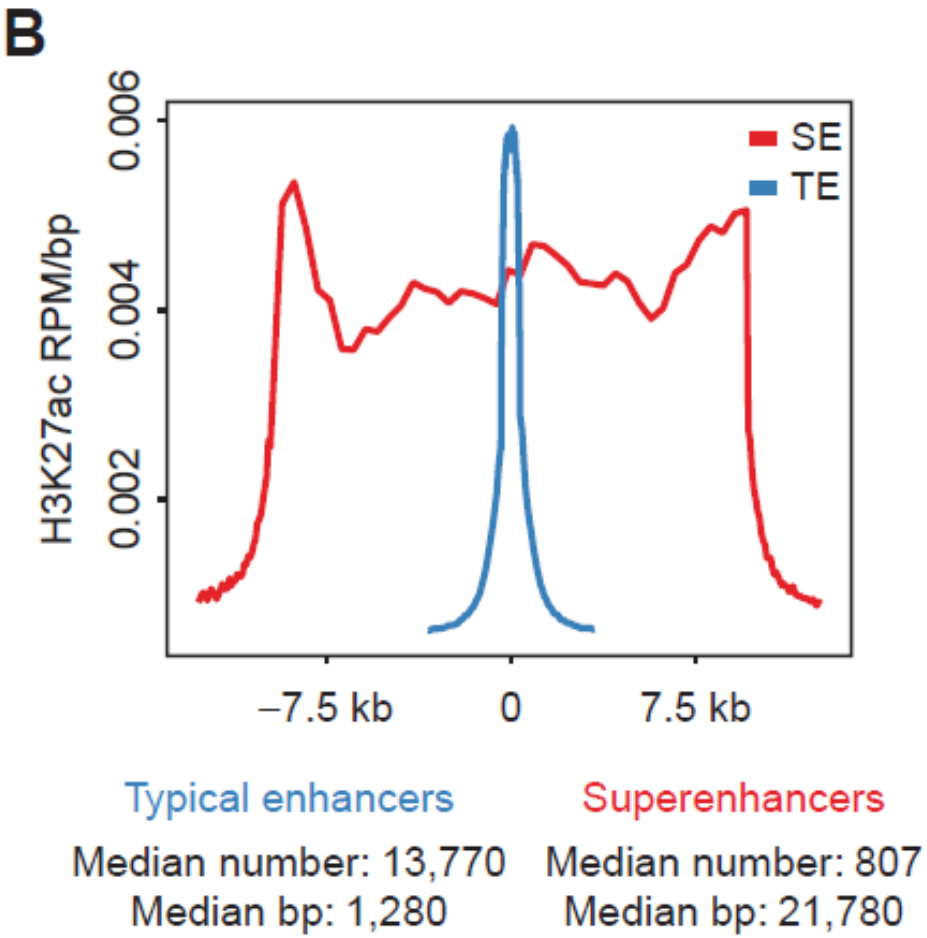
Schematic of the high-throughput chromatin immunoprecipitation pipeline used to generate SE maps in human AML and normal blood cells



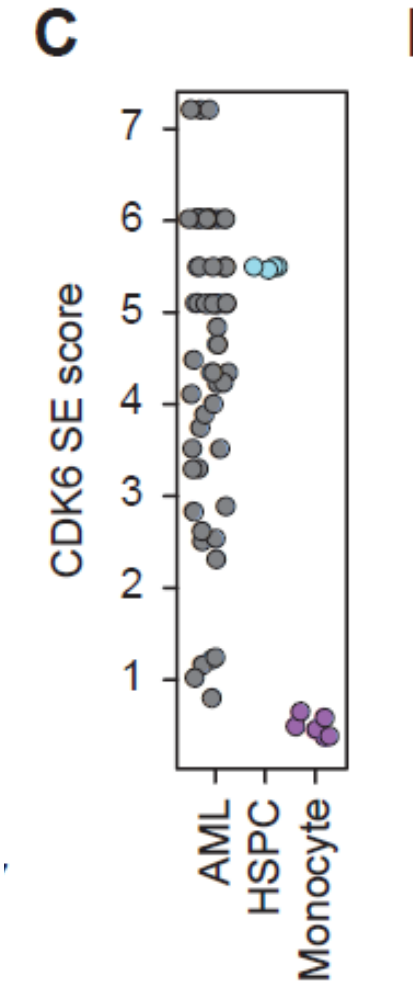


Metapeak representation of typical enhancers (TE; blue) and superenhancers (SE; red) in a single patient with AML (SU223).

H3K27ac ChIP-seq read density

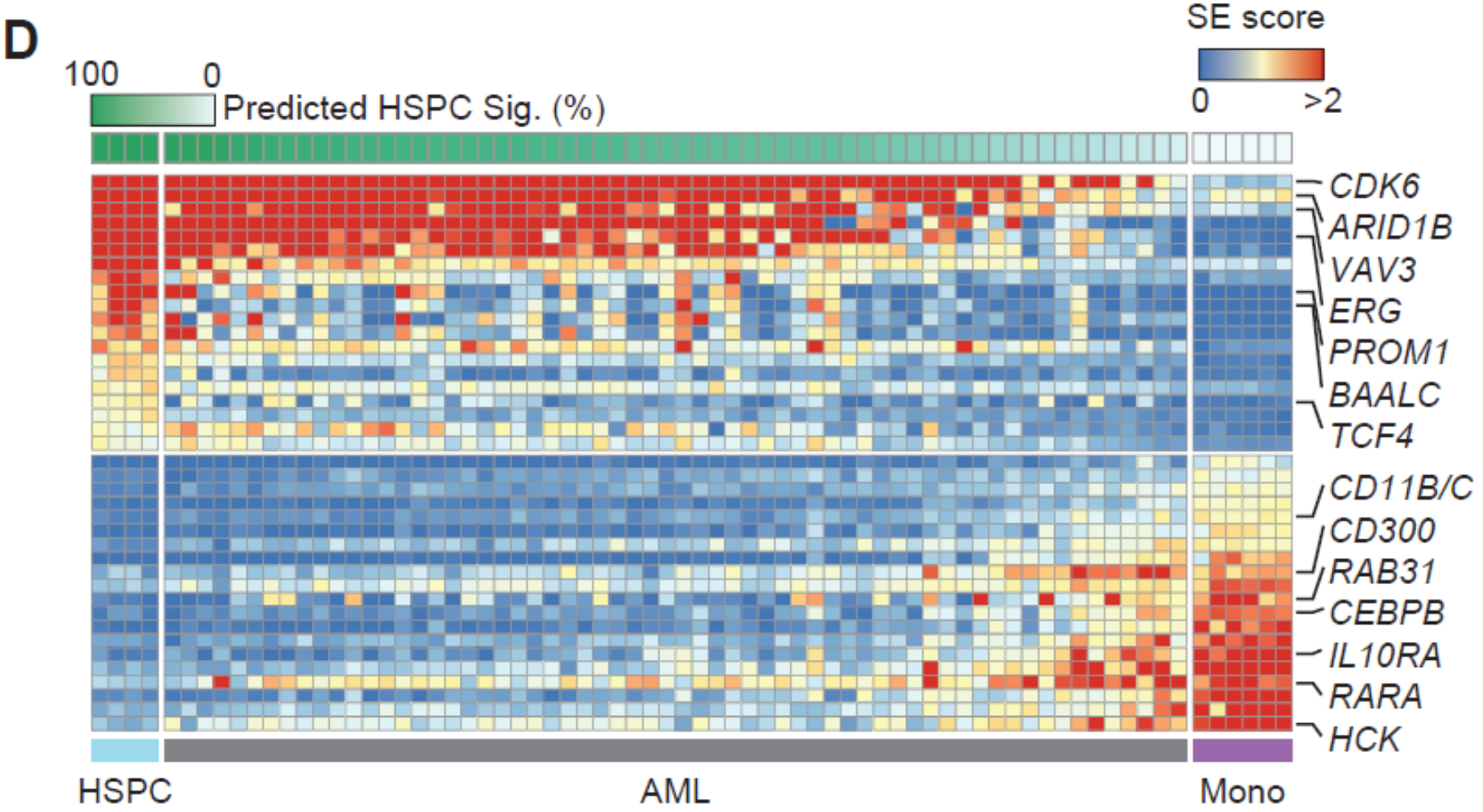


Certain key HSPC enhancers, such as the SE linked to CDK6, show strong variability across our AML cohort



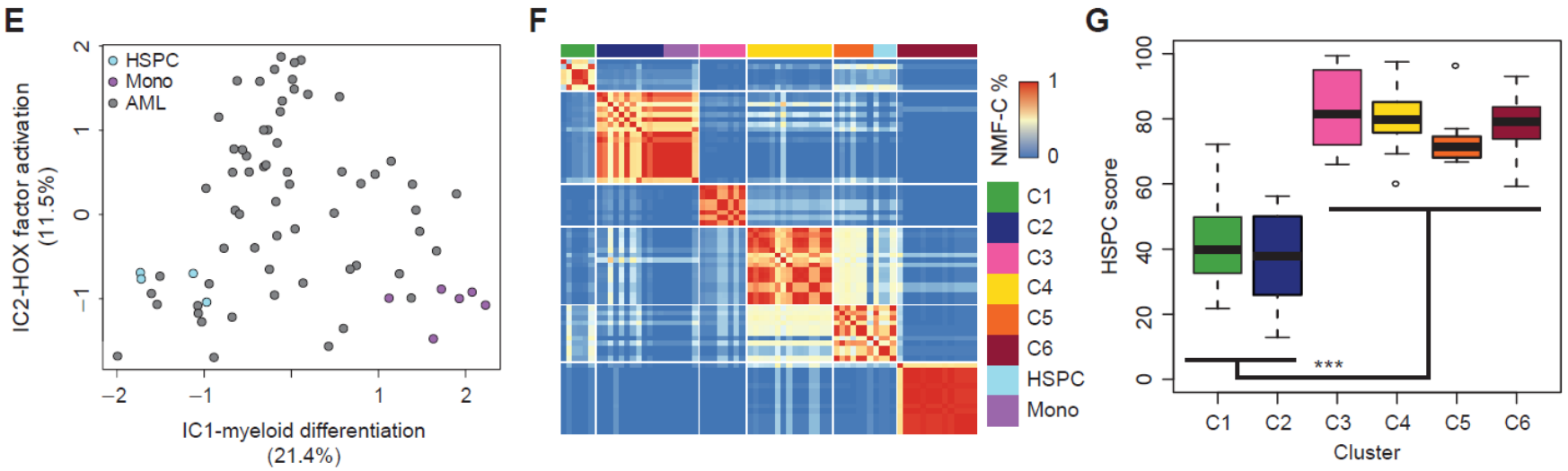
hematopoietic stem and progenitor cell (HSPC)

Certain key HSPC enhancers, such as the SE linked to CDK6, show strong variability across our AML cohort

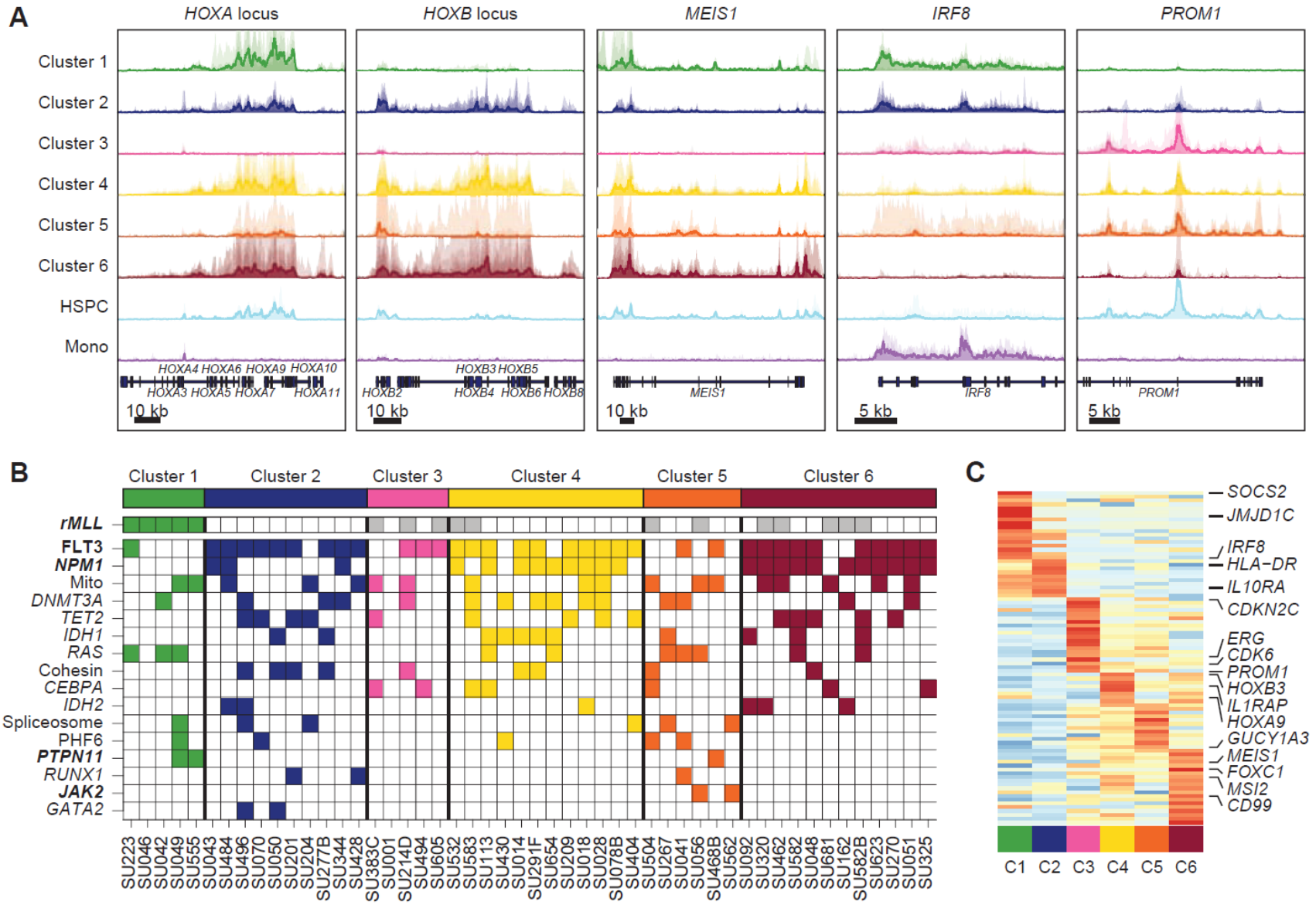


hematopoietic stem and progenitor cell (HSPC)

(top, green bar). The heat map depicts SE scores for the HSPC-associated and monocyte-associated SEs used in the classification. **E,** Independent components analysis of SE data from AML, HSPCs, and monocytes. **F,** Heat map shows the NMF consensus clustering distance (fraction of NMF iterations in which a pair of samples were grouped in the same cluster; blue, low; red, high) between each sample in this study. HSPCs (light blue) and monocytes (purple) were projected into the clustering without being allowed to contribute to the NMF. **G,** The estimated HSPC signature score (*y*-axis) for each SE-based cluster is shown (***, $P < 3 \times 10^{-8}$).

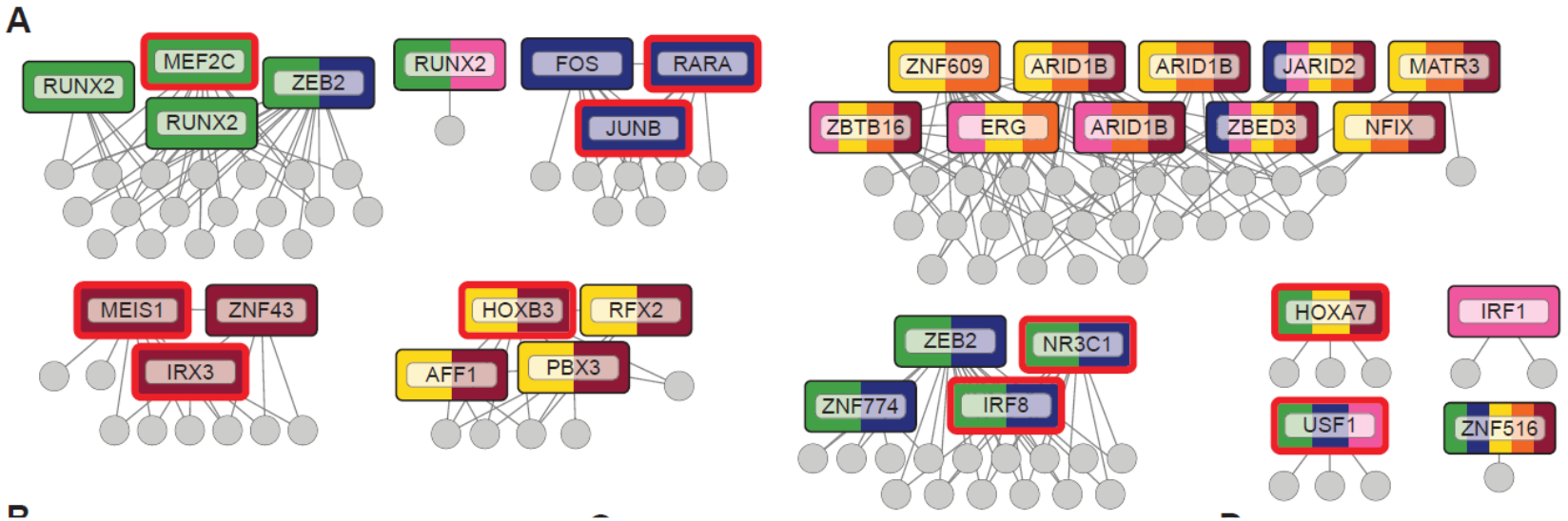


SE-derived patient subgroups associate with genotype and survival.



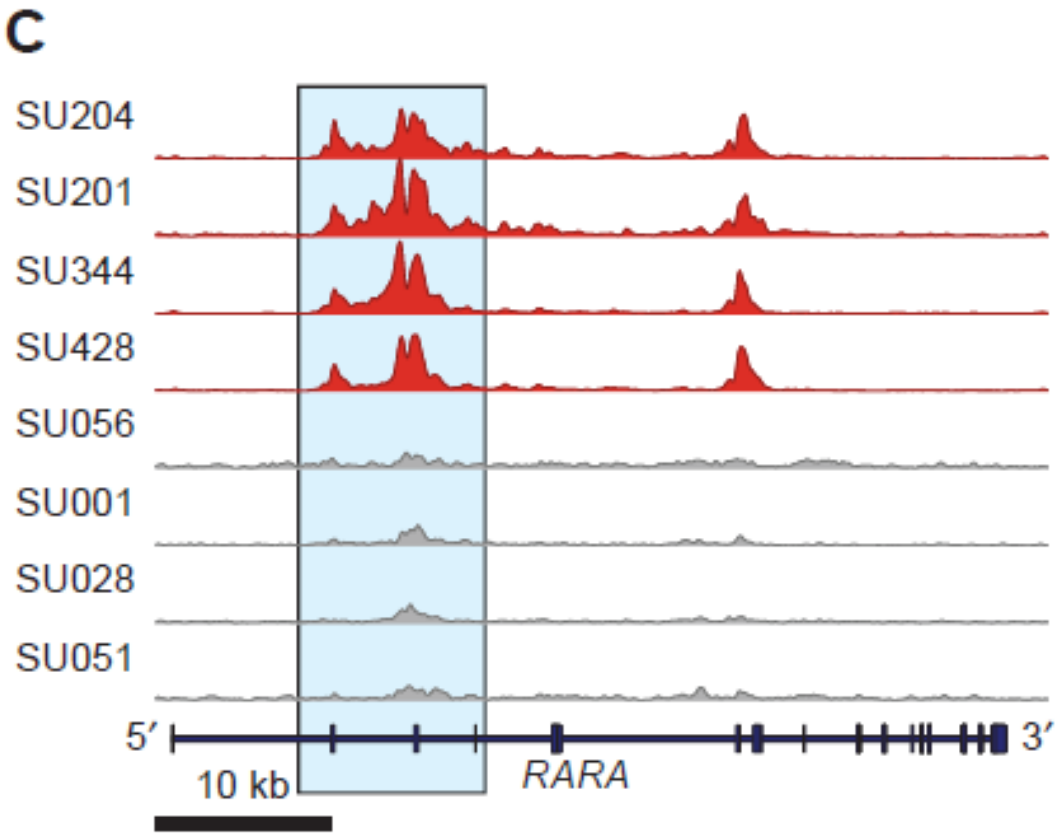
A subset of patients with AML have a superenhancer at the *RARA* locus

Mutual information network of the SE score between clusterspecific SEs.

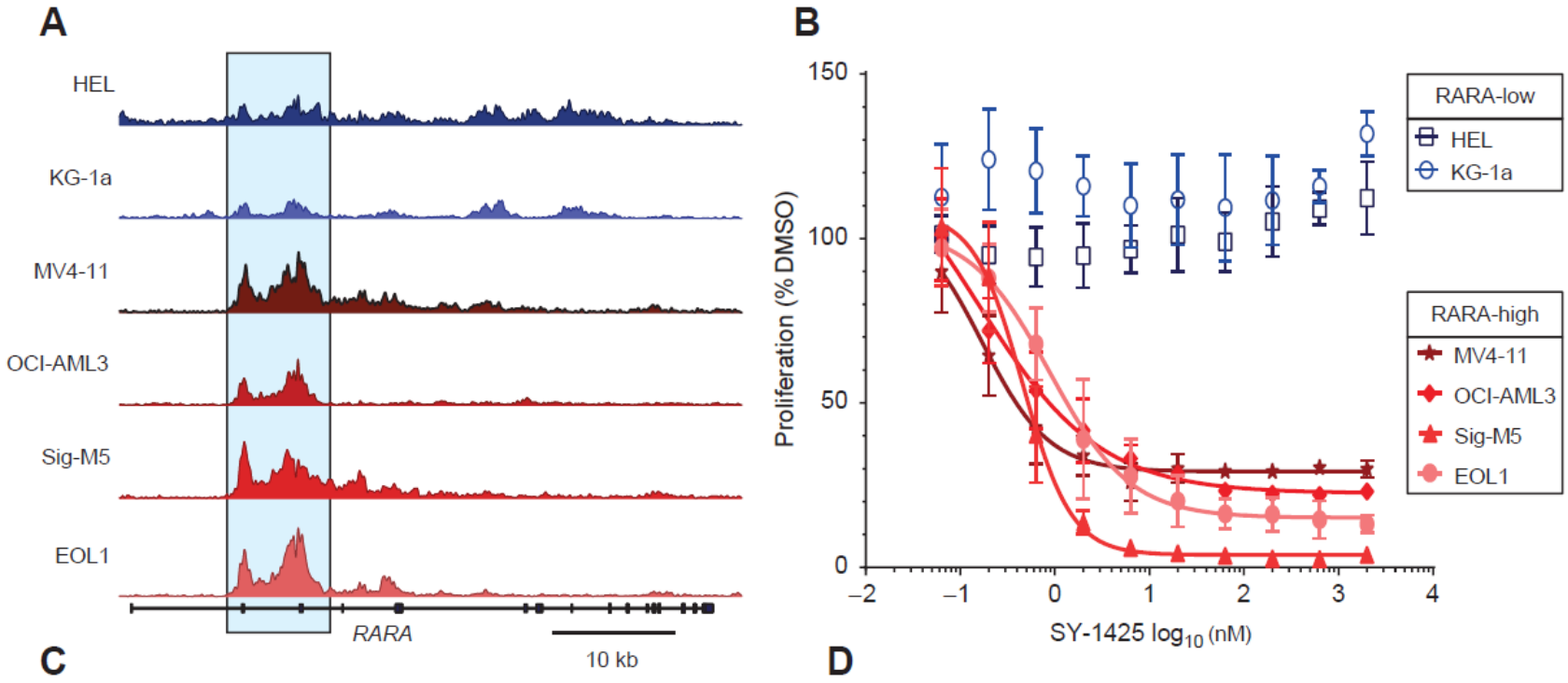


A subset of patients with AML have a superenhancer at the *RARA* locus

H3K27ac ChIP-seq tracks at chr17:38,464,514– 38,515,430 with large SEs shown in red and non-SE tracks shown in gray



The presence of a *RARA* SE correlates with sensitivity to SY-1425 in non-APL AML cell lines and PDX models

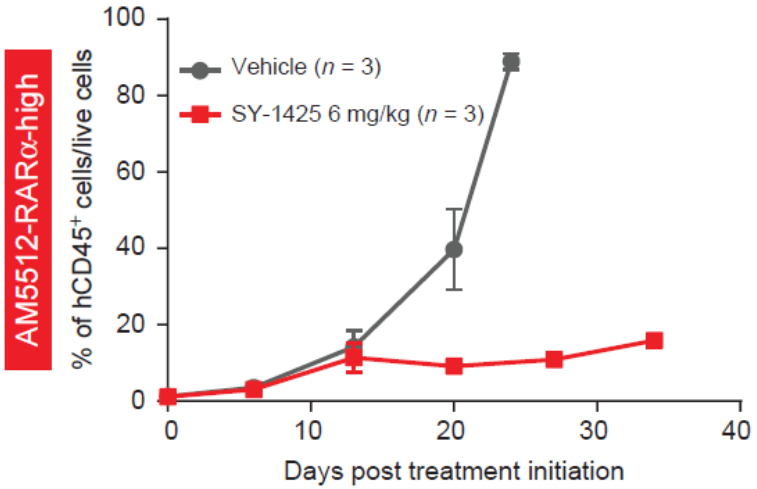


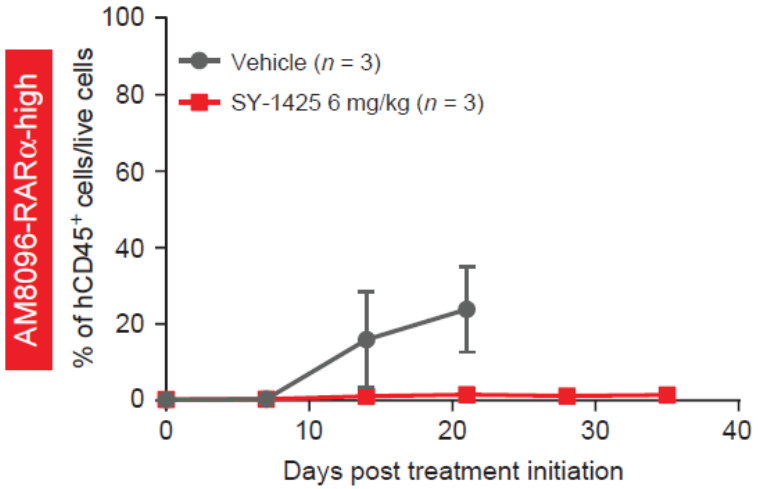
C- Effect of SY-1425 in the AM5512 non-APL RARA-high PDX model

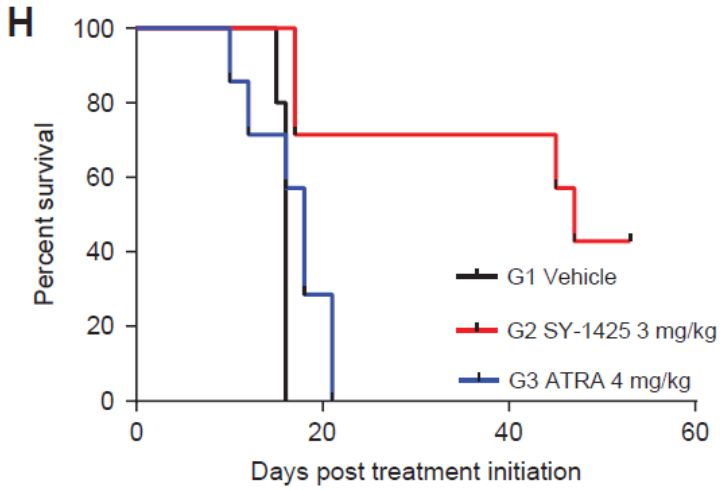
D- Effect on percent human CD45 positive in peripheral blood of SY-1425 in the AM8096 non-APL RARA-high PDX model.

Survival plot for study in **G**.

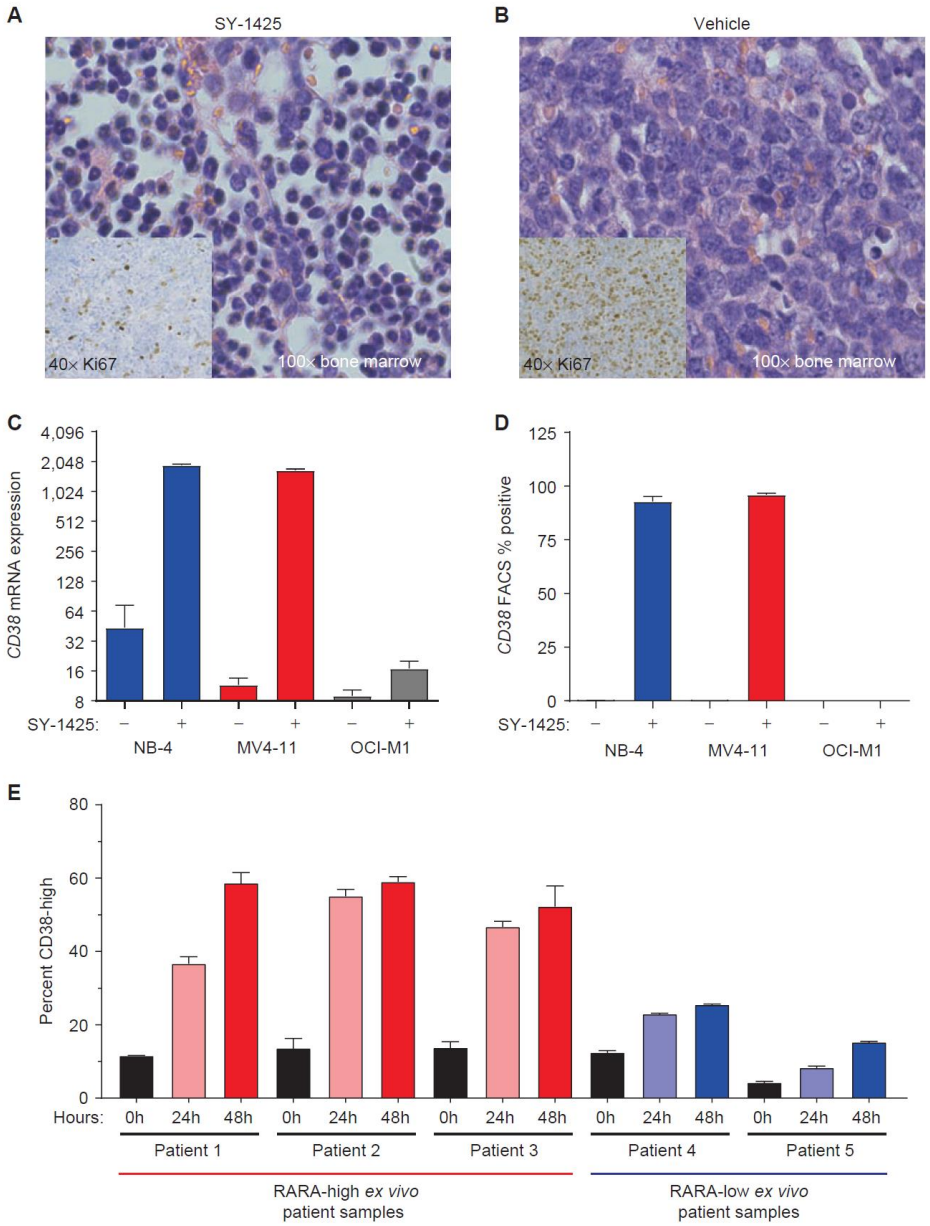
PDX models (AM5512 and AM8096) where the tumor cells contained high levels of *RARA* mRNA



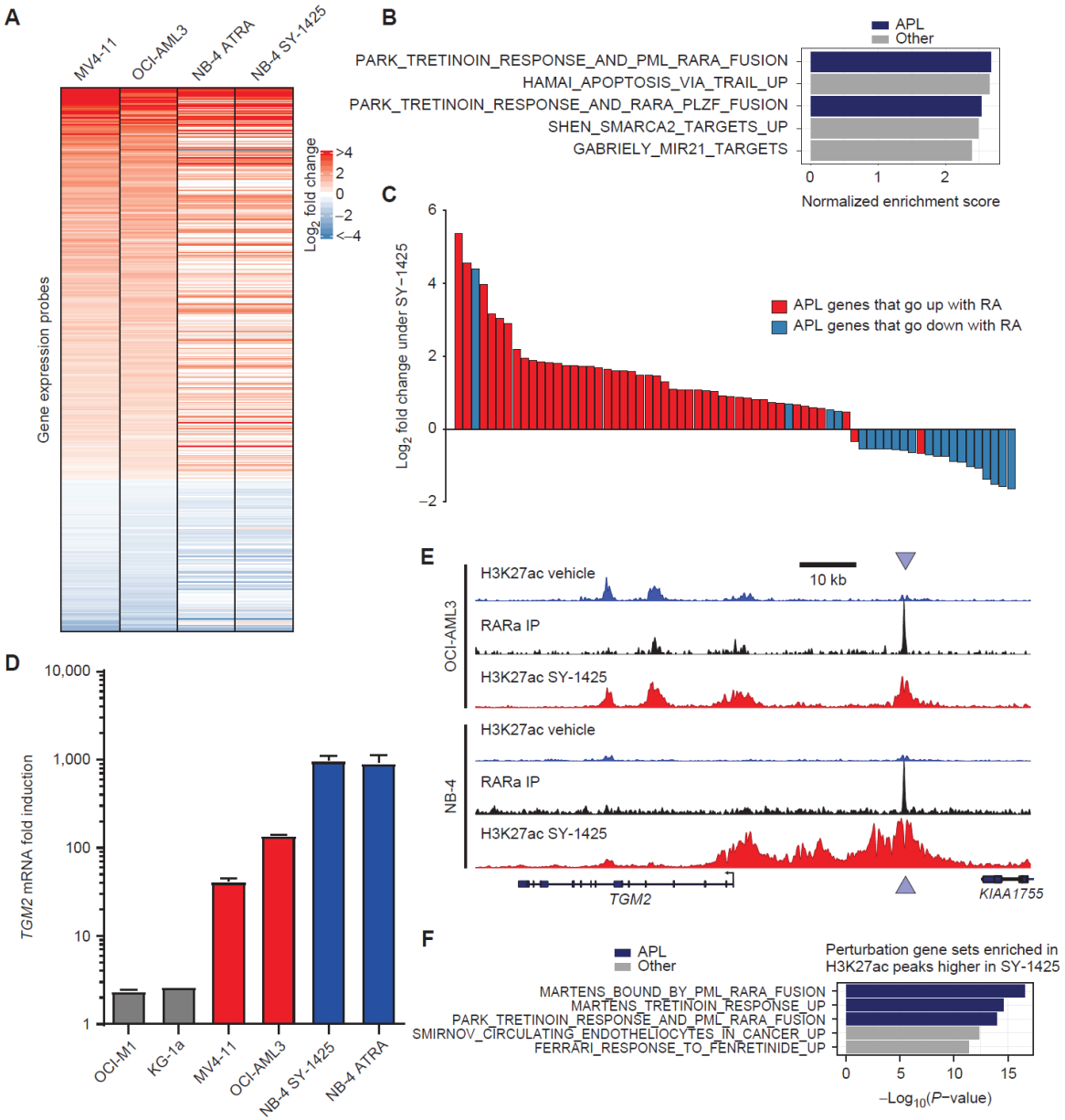




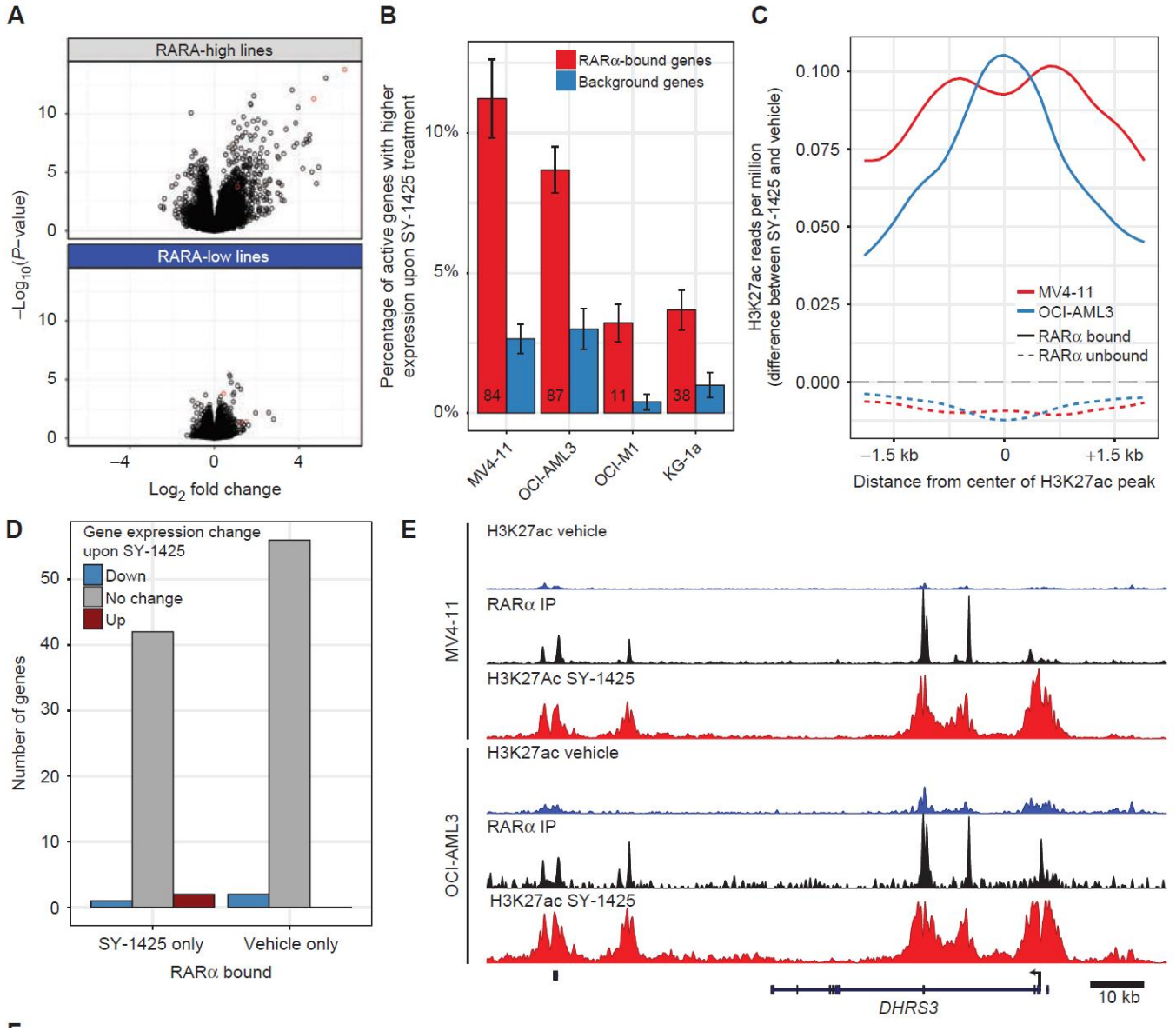
SY-1425 induces maturation in RARA-high AML

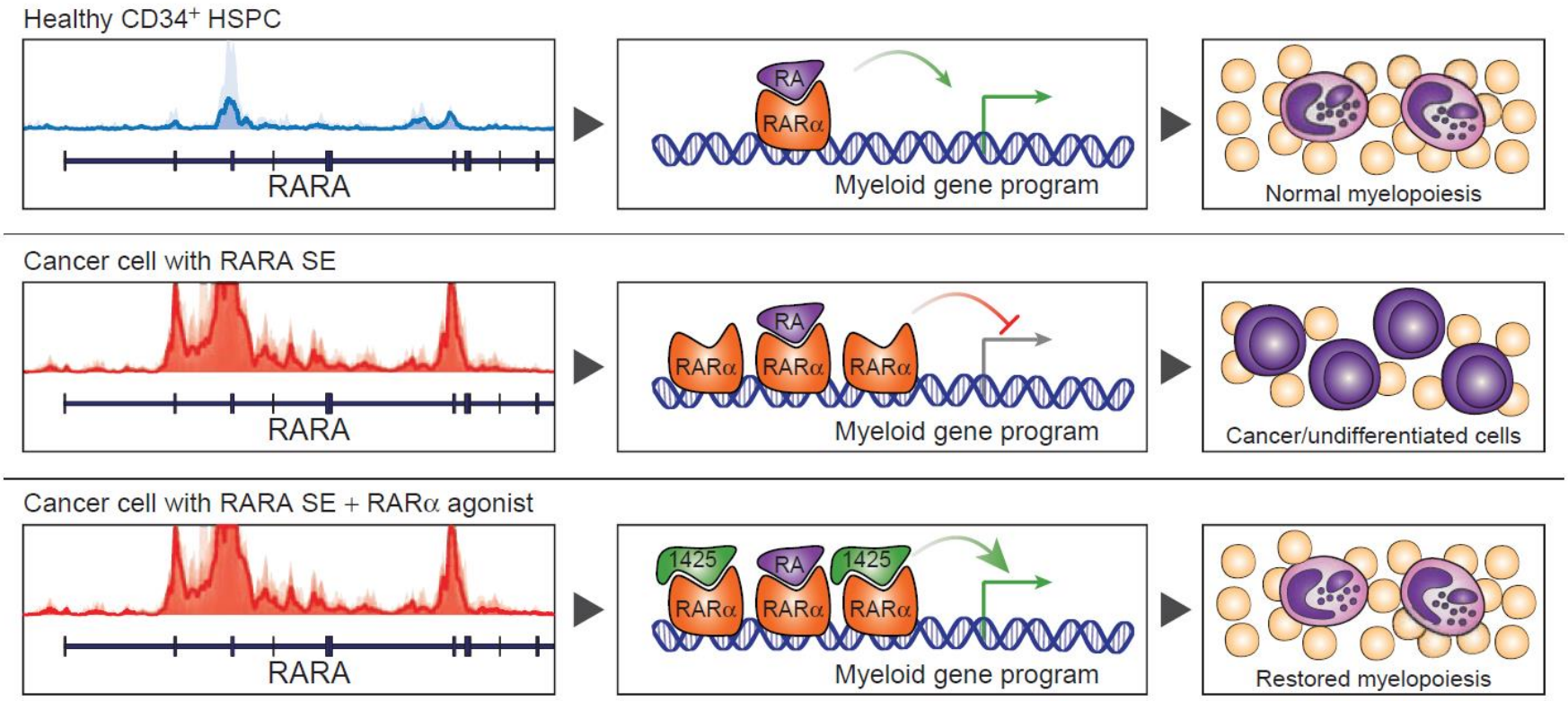


SY-1425 shows similar response in RARA-high AML cell lines to APL.



SY-1425 induces transcriptional and epigenetic alterations.




Healthy CD34+ HSPC
RARA
RA
RARα
Myeloid gene program
Normal myelopoiesis
Cancer cell with RARA SE
RARA
RA
RARα
RARα
RARα
Myeloid gene program
Cancer/undifferentiated cells
Cancer cell with RARA SE + RARα agonist
RARA
1425
RA
1425
RARα
RARα
RARα
Myeloid gene program
Restored myelopoiesis

ONCOGENIC SUPER-ENHANCERS IN TUMOR PROGRESSION

ONCOGENE REGULATION

An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element

Marc R. Mansour,[1,2] Brian J. Abraham,[3*] Lars Anders,[3*] Alla Berezovskaya,[1] Alejandro Gutierrez,[1,4] Adam D. Durbin,[1] Julia Etchin,[1] Lee Lawton,[3] Stephen E. Sallan,[1,4] Lewis B. Silverman,[1,4] Mignon L. Loh,[5] Stephen P. Hunger,[6] Takaomi Sanda,[7] Richard A. Young,[3,8†] A. Thomas Look[1,4†]

In certain human cancers, the expression of critical oncogenes is driven from large regulatory elements, called super-enhancers, that recruit much of the cell's transcriptional apparatus and are defined by extensive acetylation of histone H3 lysine 27 (H3K27ac). In a subset of T-cell acute lymphoblastic leukemia (T-ALL) cases, we found that heterozygous somatic mutations are acquired that introduce binding motifs for the MYB transcription factor in a precise noncoding site, which creates a super-enhancer upstream of the *TAL1* oncogene. MYB binds to this new site and recruits its H3K27 acetylase–binding partner CBP, as well as core components of a major leukemogenic transcriptional complex that contains RUNX1, GATA-3, and TAL1 itself. Additionally, most endogenous super-enhancers found in T-ALL cells are occupied by MYB and CBP, which suggests a general role for MYB in super-enhancer initiation. Thus, this study identifies a genetic mechanism responsible for the generation of oncogenic super-enhancers in malignant cells.

BACKGROUND

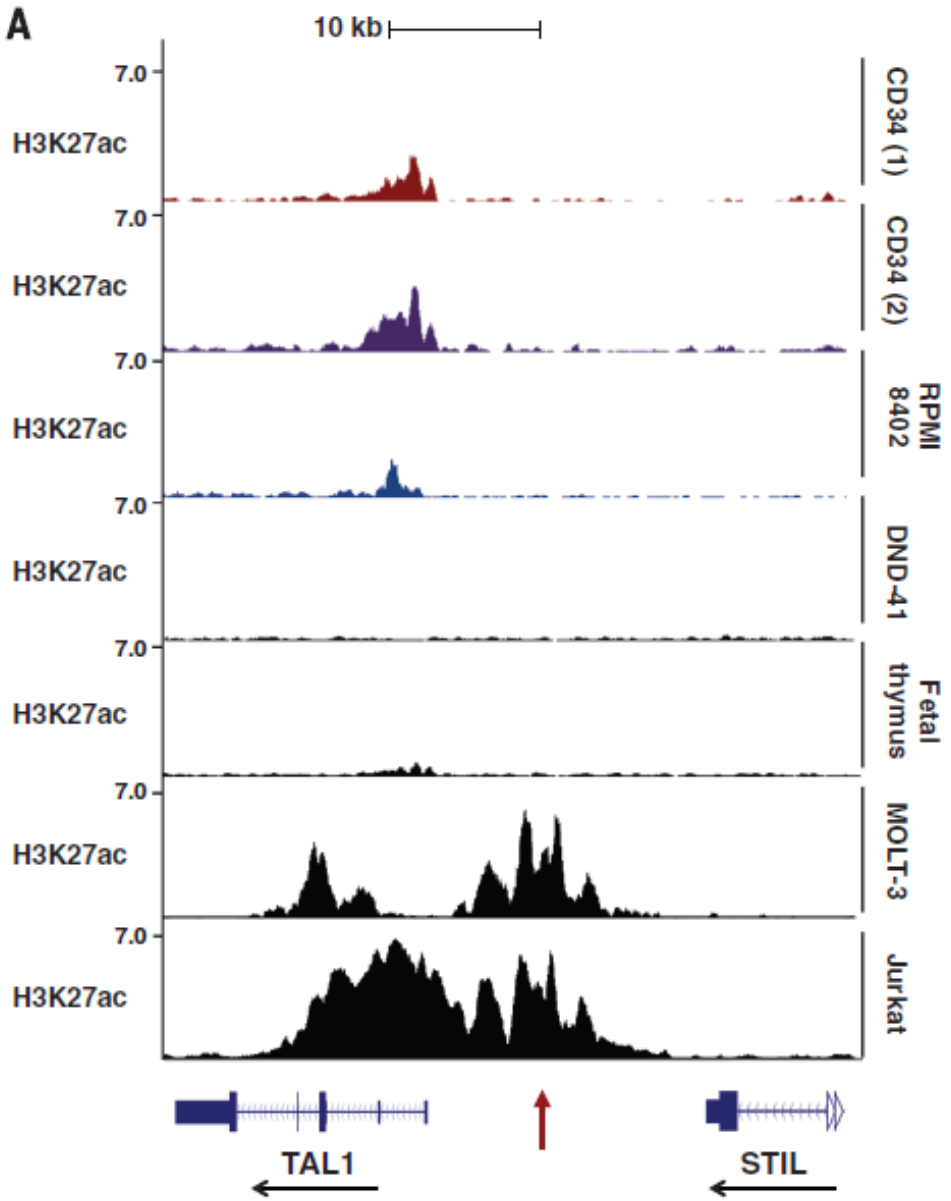
Super-enhancers (SE) upstream TAL1

MYB form Leukemogenic Transcriptional Complex

MYB binds T-ALL cells SEs

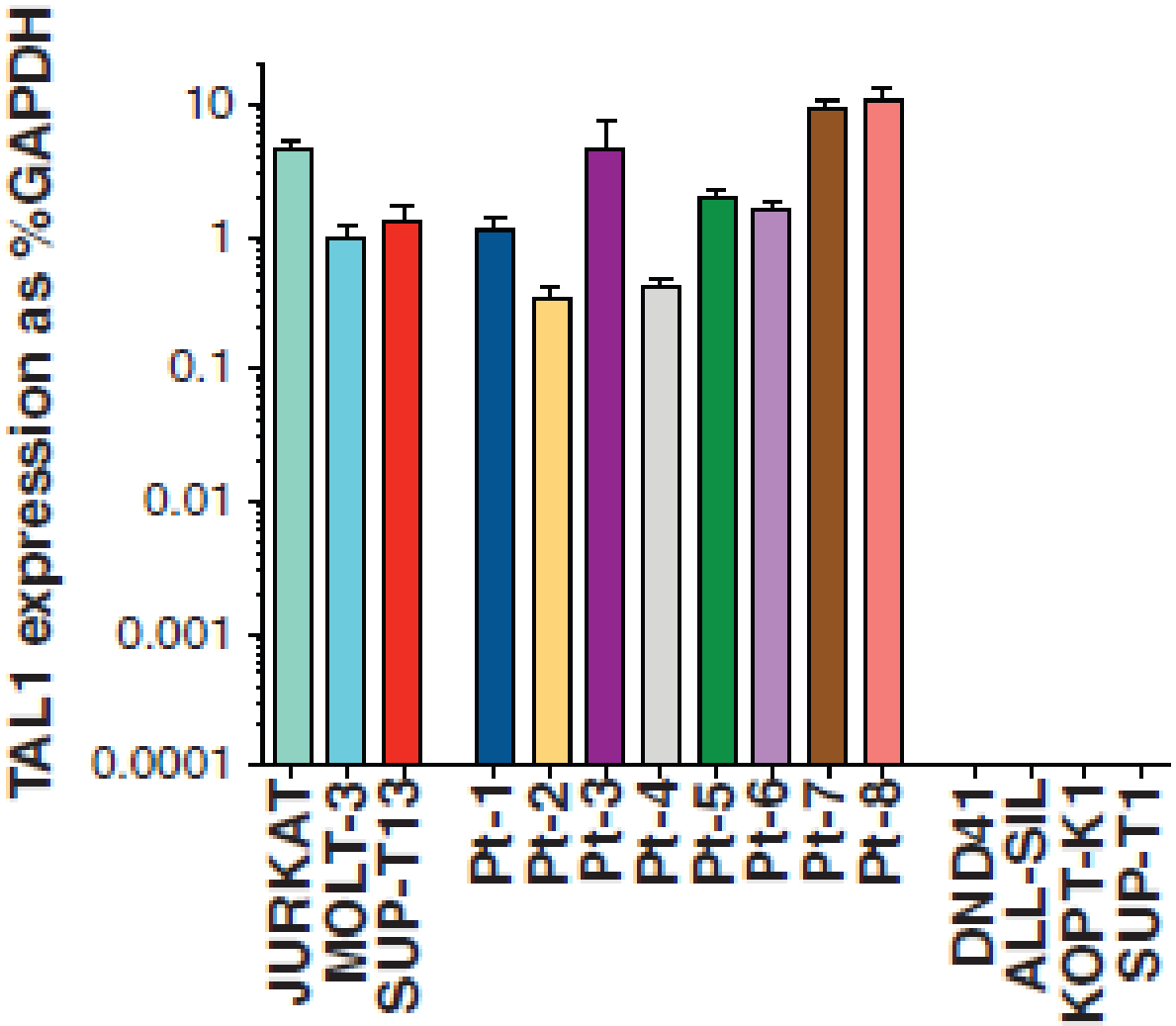
CONCLUSION

ChIP-Seq profile for H3K27ac (active enhancer mark) in different cell lines



Sequence alignments of the –7.5 kb site showing wild-type (WT) sequences in **black** and inserted sequences in **red** for Jurkat and MOLT-3 T-ALL cell lines and eight pediatric T-ALL patients. hg19, human genome build 19.

TAL1 mRNA expression as determined by quantitative polymerase chain reaction (PCR) and expressed as percentage of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).



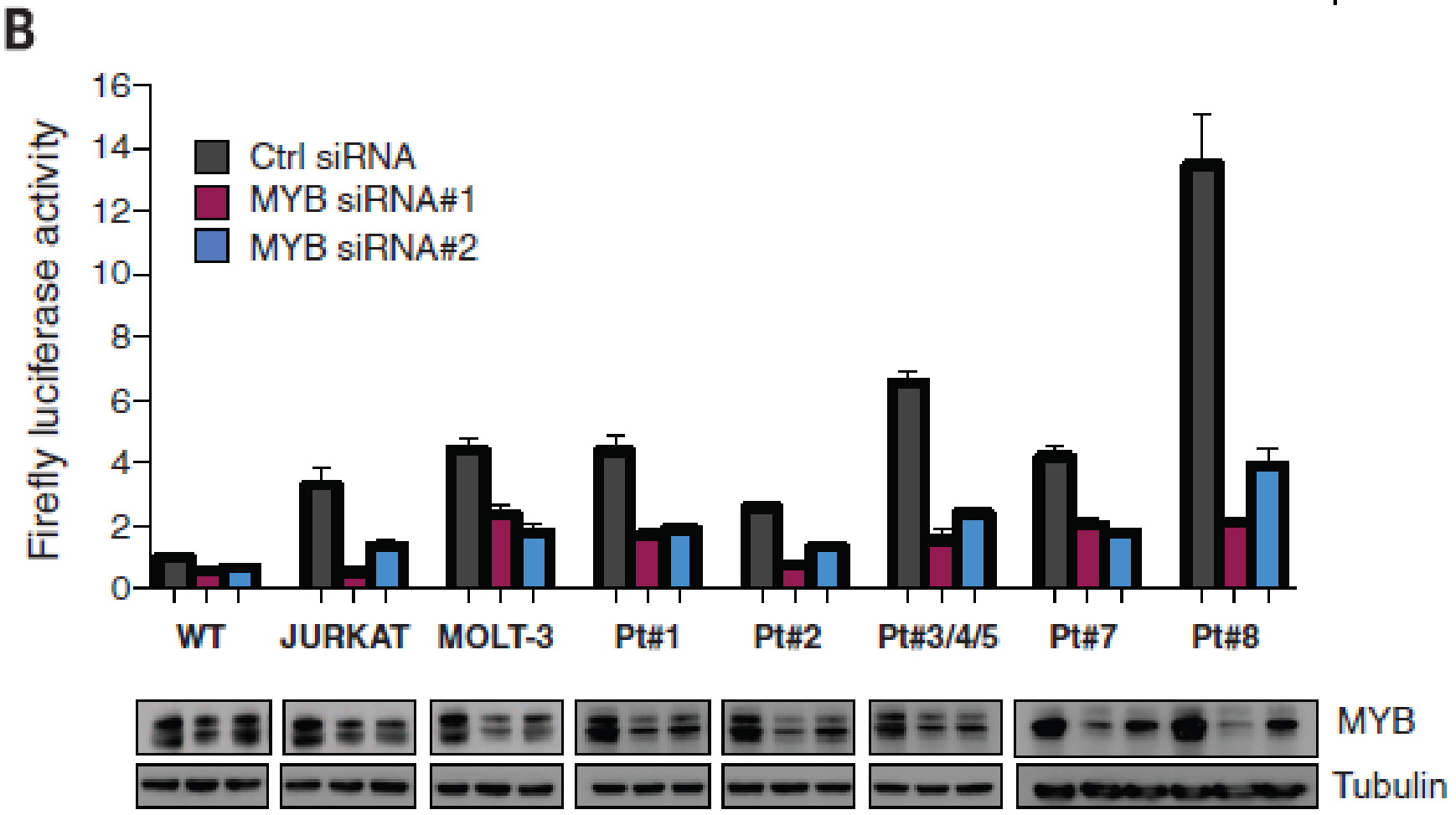
Mutations of the TAL1 enhancer activate through recruitment of MYB.

A Myb primary motif

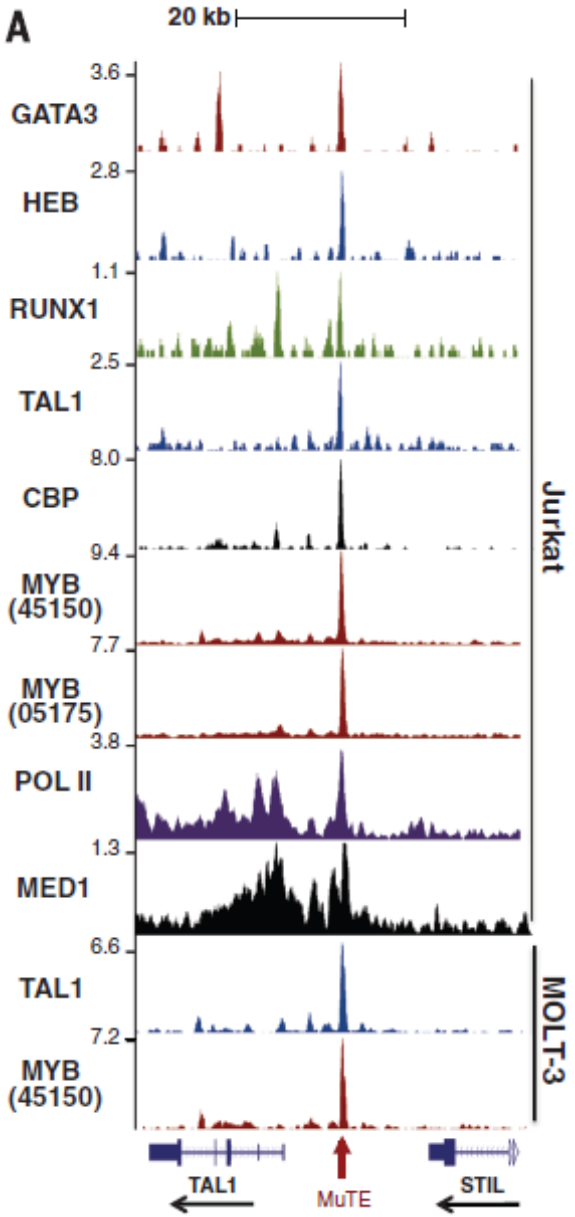
WT	GACGTA
Jurkat	[GACGGTTA]GGA[AACGGTA]
MOLT-3	GACGGTTA
Patient #1	GACCGTTTA
Patient #2	GCCGTTA
Patient #3	GACCGTTA
Patient #4	GACCGTTA
Patient #5	GACCGTTA
Patient #6	GACGGTTA
Patient #7	GACGGTTA
Patient #8	GACGGTTTA

TAL1 enhancer TRANSCRIPTION ACTIVITY USING LUCIFERASE ASSAY

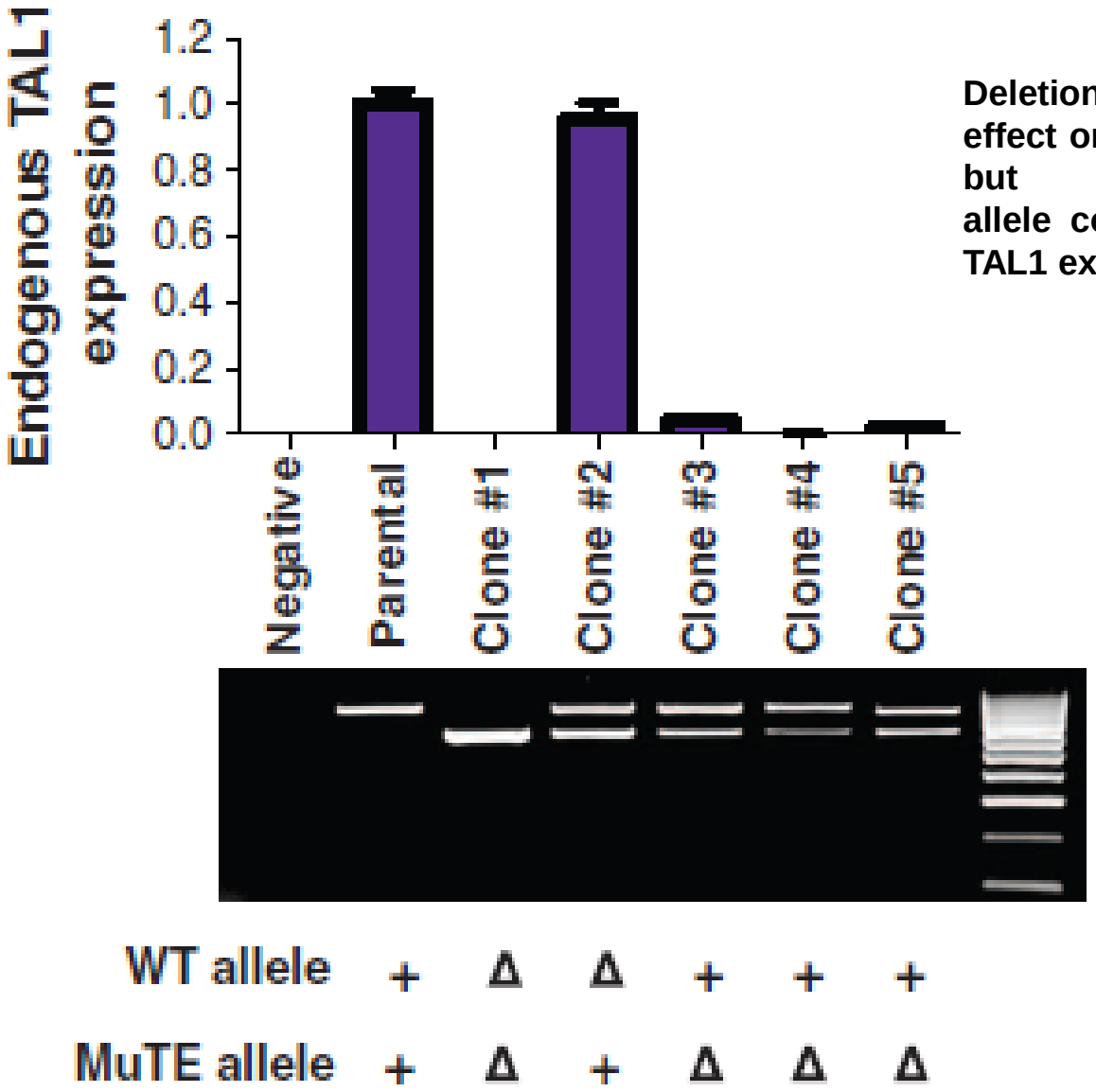
MYB binds the mutant TAL1 enhancer site and is a member of the TAL1 complex



MYB binds the mutant TAL1 enhancer (MuTE) site and is a member of the TAL1 complex

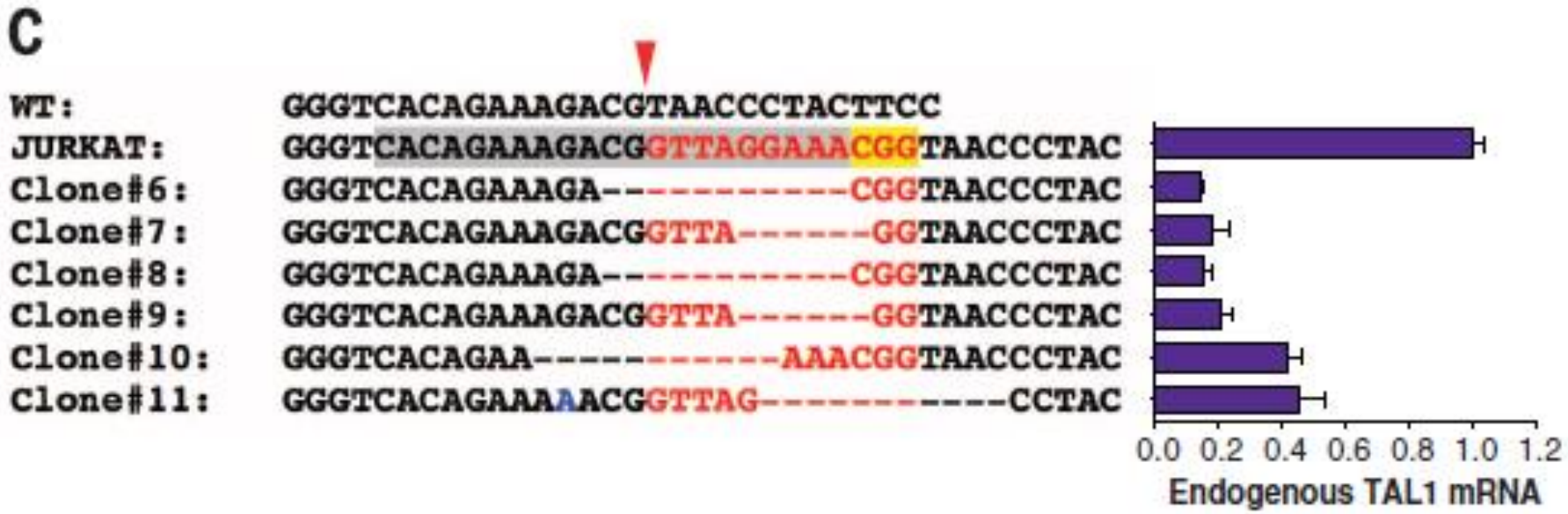


Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1

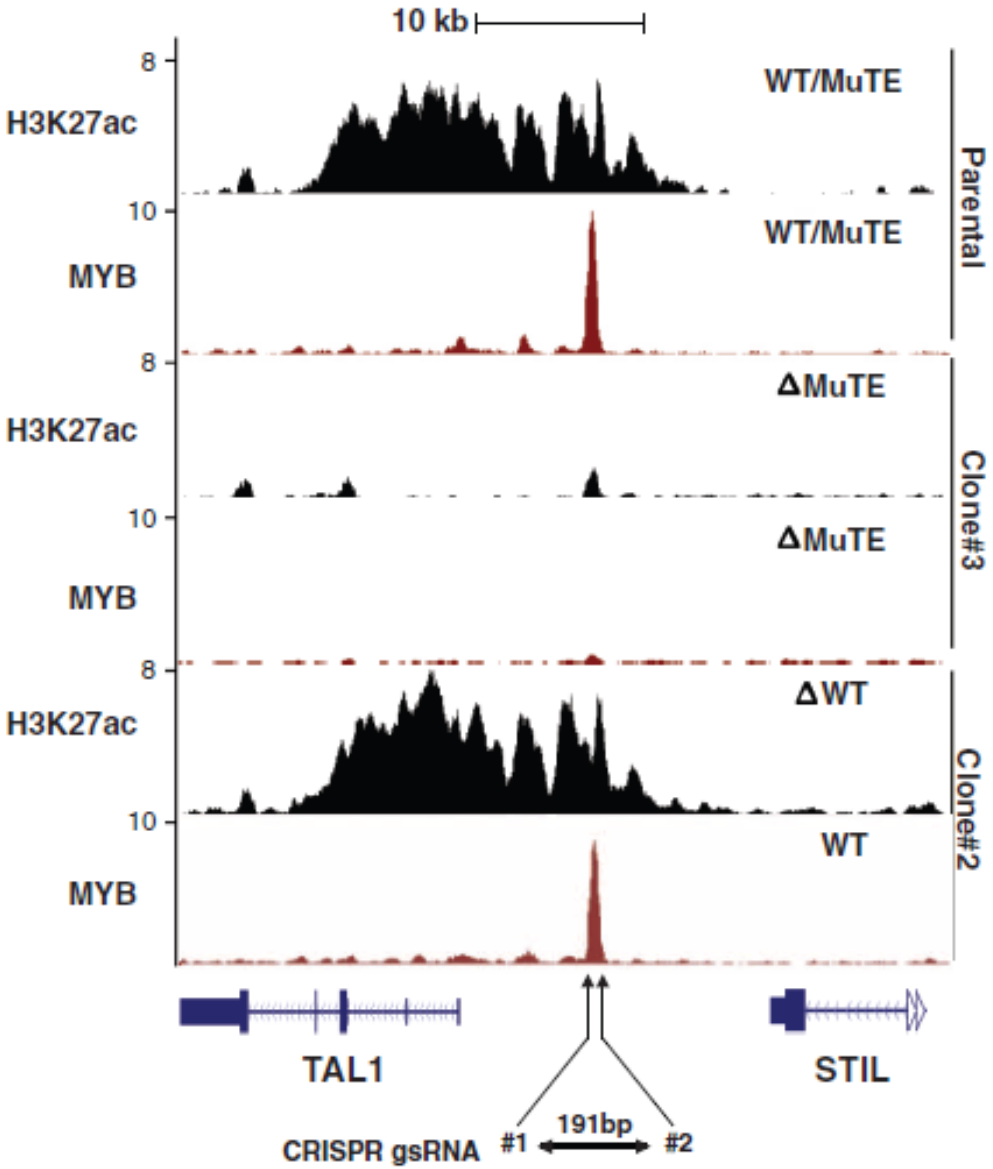


Deletion of the wild type allele had no effect on endogenous TAL1 mRNA levels, but deletion of the mutant allele completely abrogated endogenous TAL1 expression

Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1

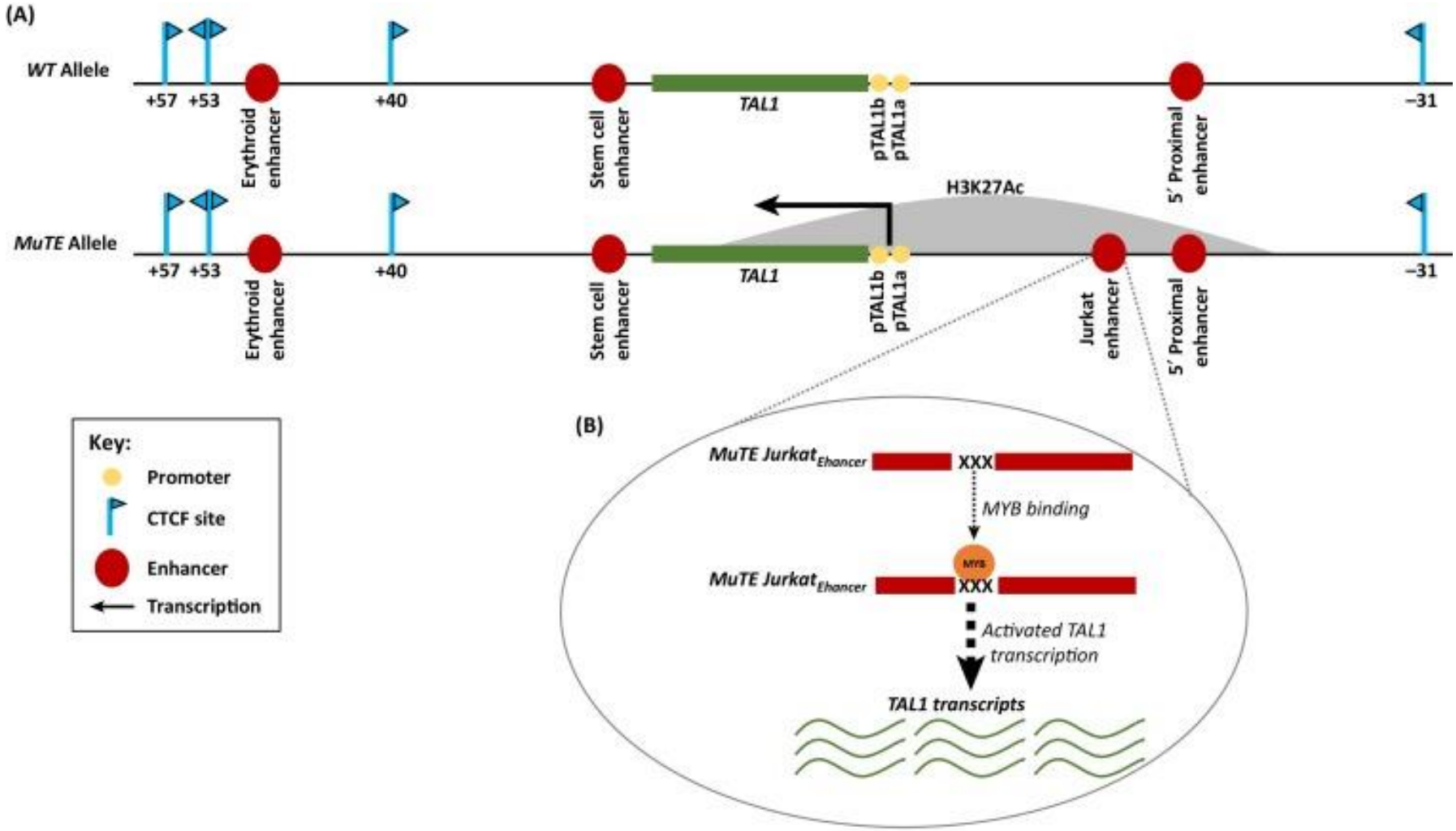


ChIP-seq tracks for H3K27ac and MYB at the STIL-TAL1 locus from selected CRISPR-Cas9 clones



Deletion of the wild type allele had no effect on H3K27ac signal and MYB binding, but deletion of the mutant allele completely abrogated H3K27ac signal and MYB binding

An Acquired Super-Enhancer Activates Monoallelic *TAL1* Transcription in T-ALL (T cell acute lymphoblastic leukemia) Cells



Trends in Molecular Medicine