DEFINITION

Superenhancers

are → **Cluster of enhancers**

recruit → **Transcription Factors**

with specific function → **LDTF**, **SDTF**

Are associated with → **Cell-type specific genes**

Are involved → **Cell identity**

Cell Development

Disease outcome

Such as → **Oncogenesis**, **Auto immunity**, **Neuro degerative**

WHY ARE SE IMPORTANT?

Methods to study Superenhancers:
From prediction of SE by ChIP-Seq to experimental validation

Convergence of Developmental and Oncogenic Signaling Pathways at Transcriptional Super-Enhancers

Denes Hnisz,[1,4] Jurian Schuijers,[1,4] Charles Y. Lin,[2] Abraham S. Weintraub,[1,3] Brian J. Abraham,[1] Tong Ihn Lee,[1] James E. Bradner,[2] and Richard A. Young[1,3,*]

[1]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA
[2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
[3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

SUMMARY

Super-enhancers and stretch enhancers (SEs) drive expression of genes that play prominent roles in normal and disease cells, but the functional importance of these clustered enhancer elements is poorly understood, so it is not clear why genes key to cell identity have evolved regulation by such elements. Here, we show that SEs consist of functional constituent units that concentrate multiple developmental signaling pathways at key pluripotency genes in embryonic stem cells and confer enhanced responsiveness to signaling of their associated genes. Cancer cells frequently acquire SEs at genes that promote tumorigenesis, and we show that these genes are especially sensitive to perturbation of oncogenic signaling pathways. Super-enhancers thus provide a platform for signaling pathways to regulate genes that control cell identity during development and tumorigenesis.
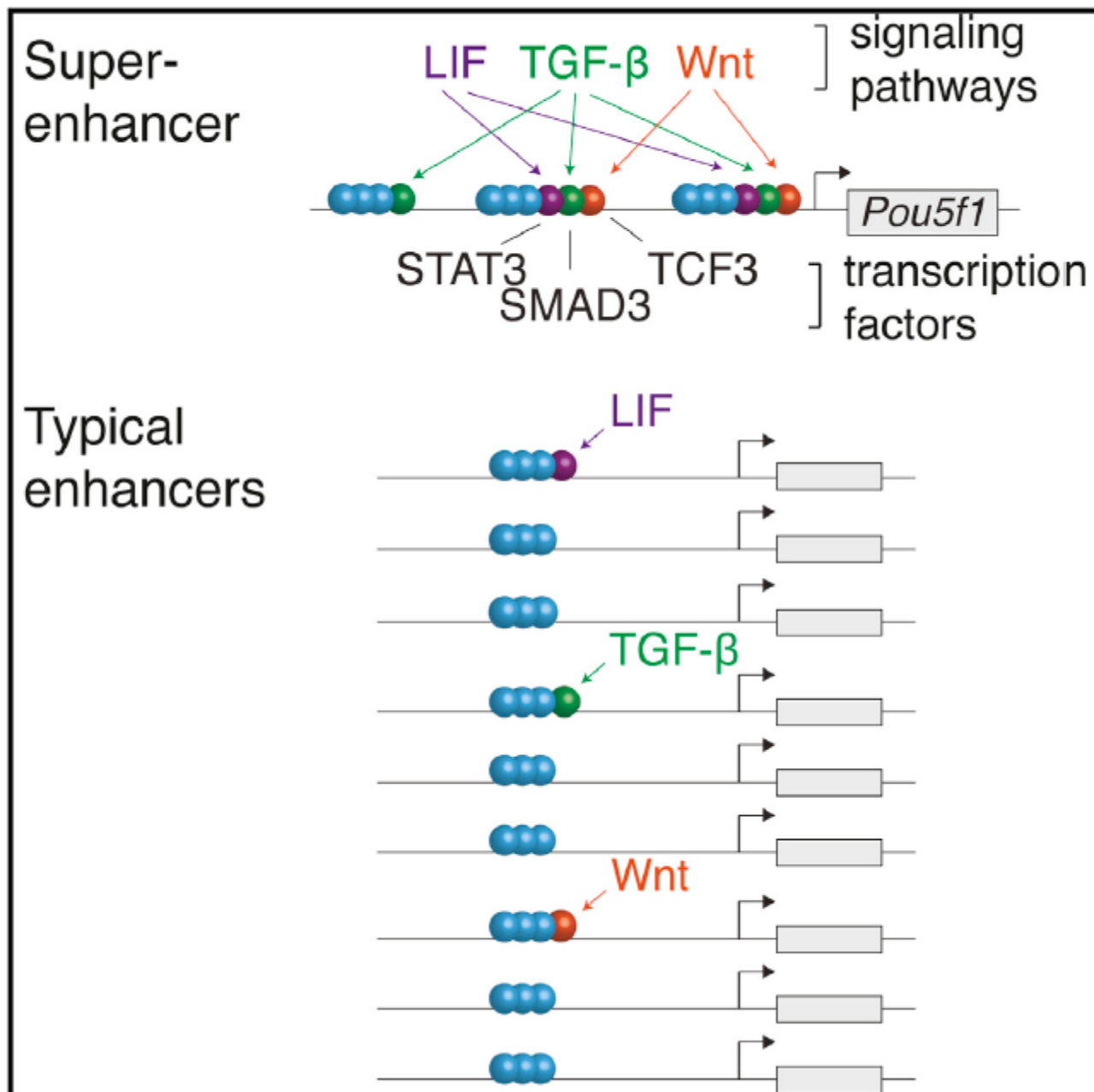
BACKGROUND

AIM

Super-enhancers (SE)
Functional costituent units

Cancer cells SE target
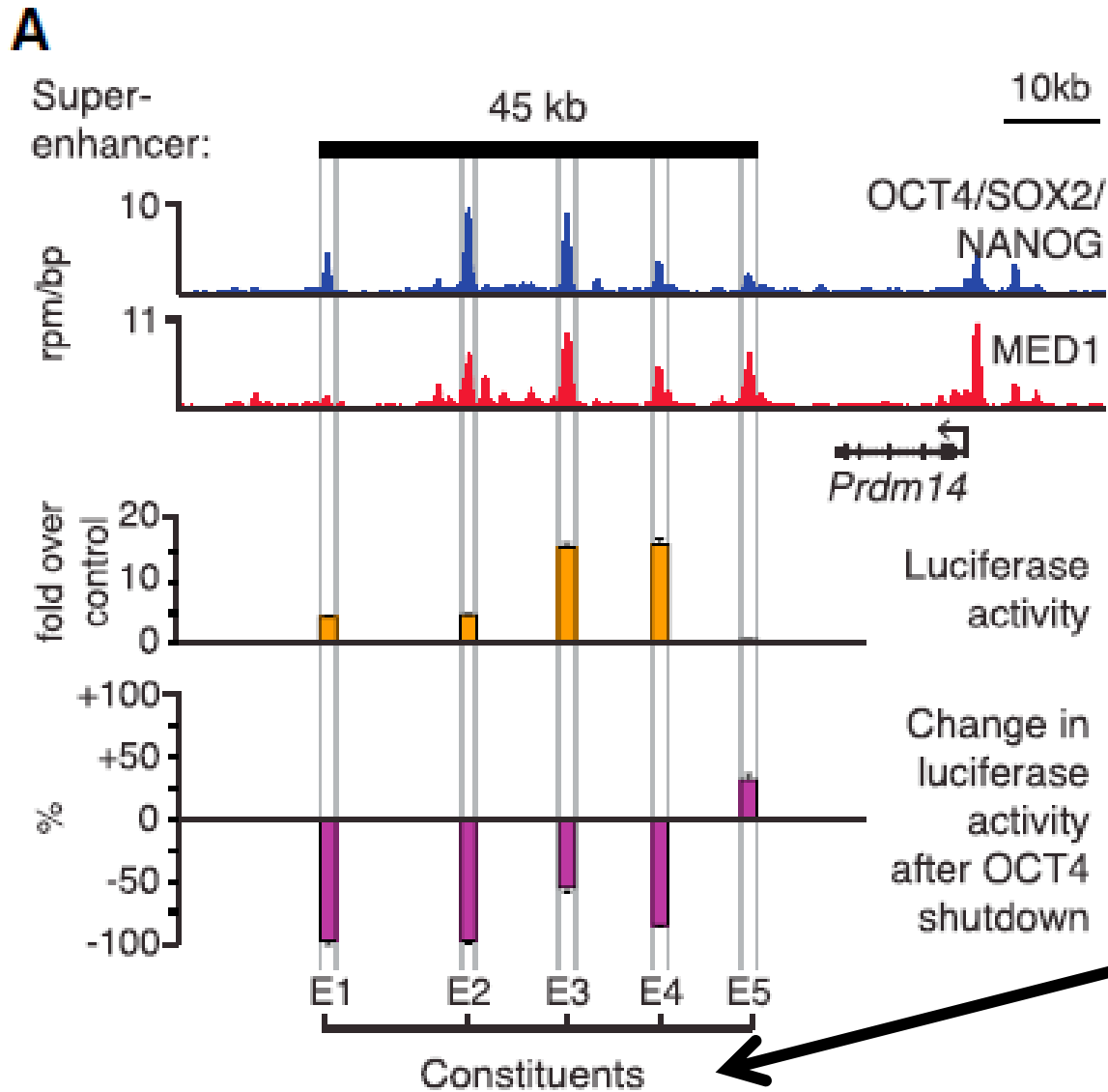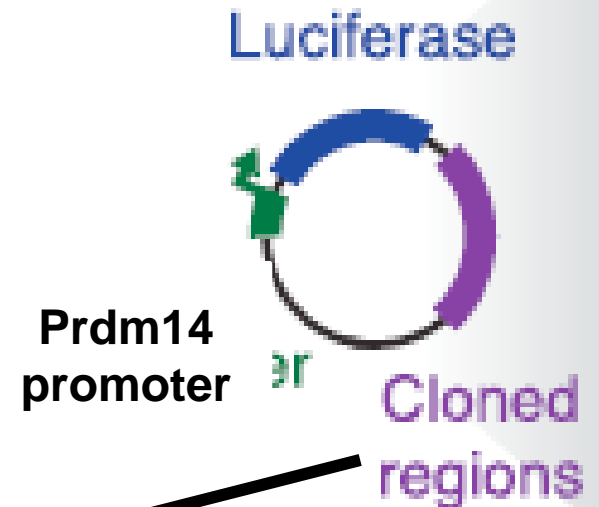**for oncogenic signalling**

CONCLUSION

Graphical Abstract

Highlights

- Super-enhancers (SEs) consist of clusters of active enhancers

- SEs are frequently bound by terminal transcription factors of signaling pathways

- SE-driven genes are especially responsive to signaling input

- SEs acquired in cancer cells are responsive to oncogenic signaling
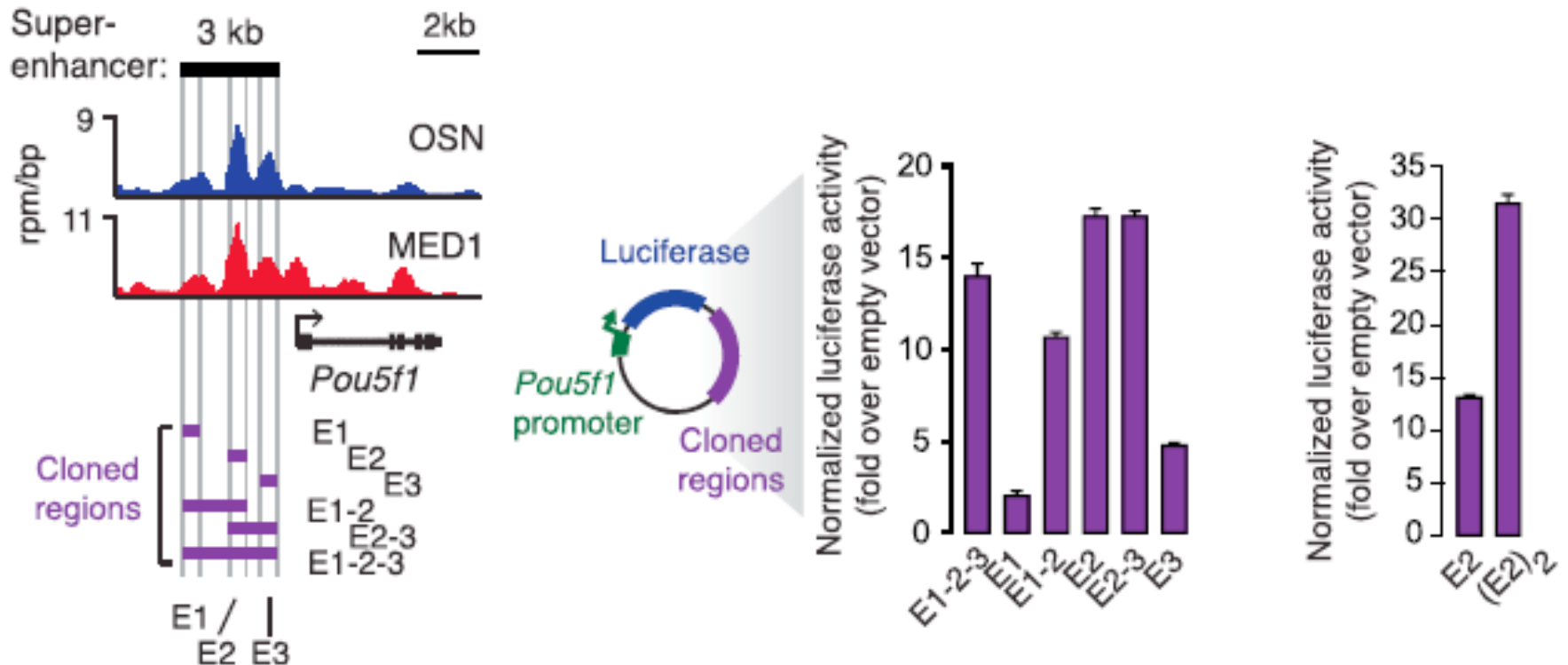
ACTIVITY OF SUPER-ENHANCER CONSTITUENTS

A

Super-enhancer:

ChIP-Seq profile

45 kb

10kb

rpm/bp

10

OCT4/SOX2/NANOG

11

MED1

Prdm14

fold over control

20

10

0

Luciferase activity

+100

+50

0

-50

-100

%

Change in luciferase activity after OCT4 shutdown

E1   E2   E3   E4   E5

Constituents

Luciferase

Prdm14 promoter

Cloned regions

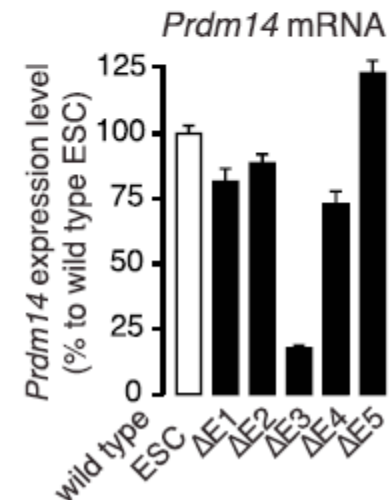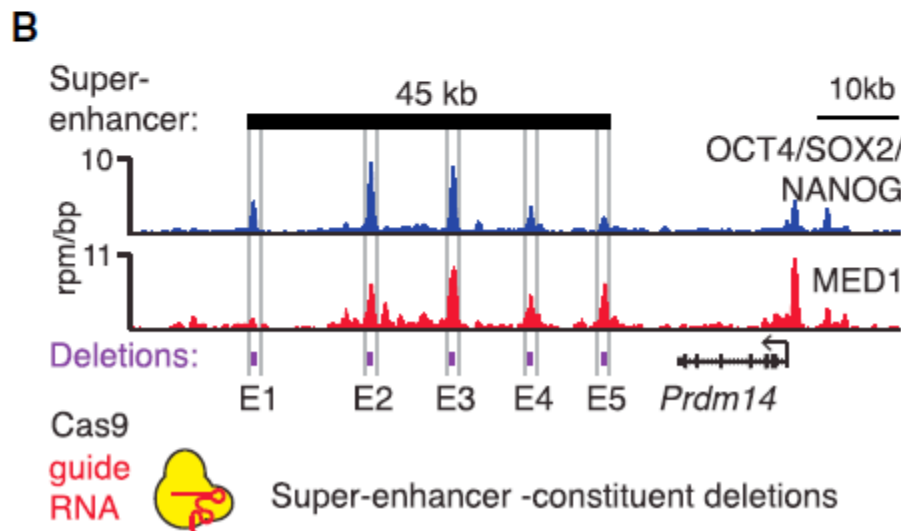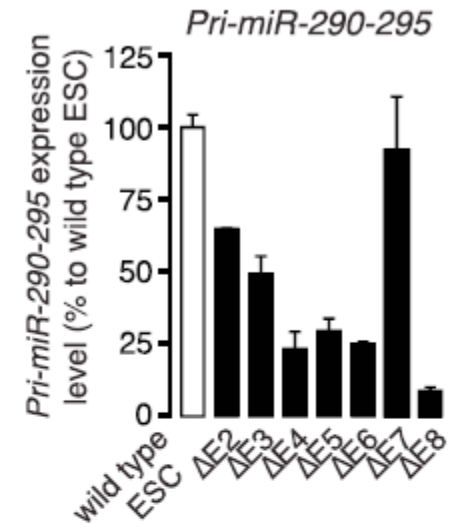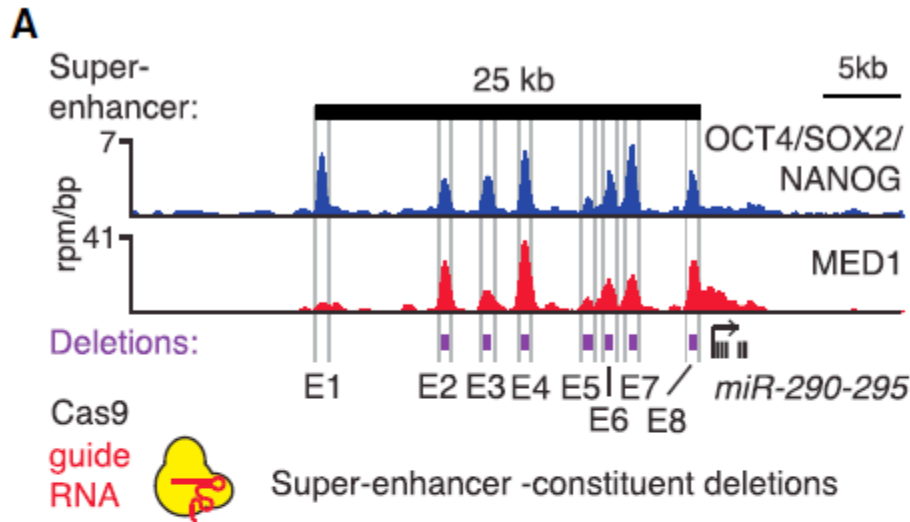# QUESTION: DO "SE CONSTITUENTS" ACT ADDITIVELY, SINERGISTICALLY OR EXERT A COMPLEX INFLUENCE?

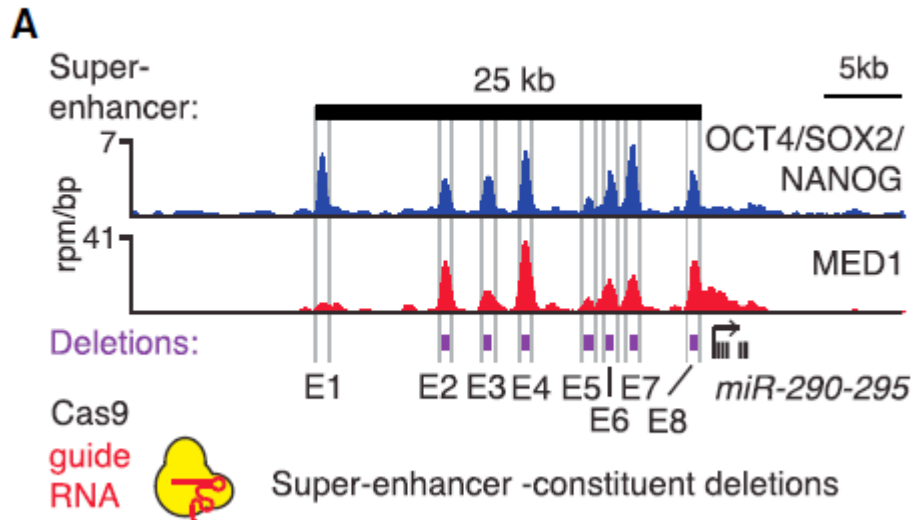## E2 has high activity, E1 and E3 influence E2 activity

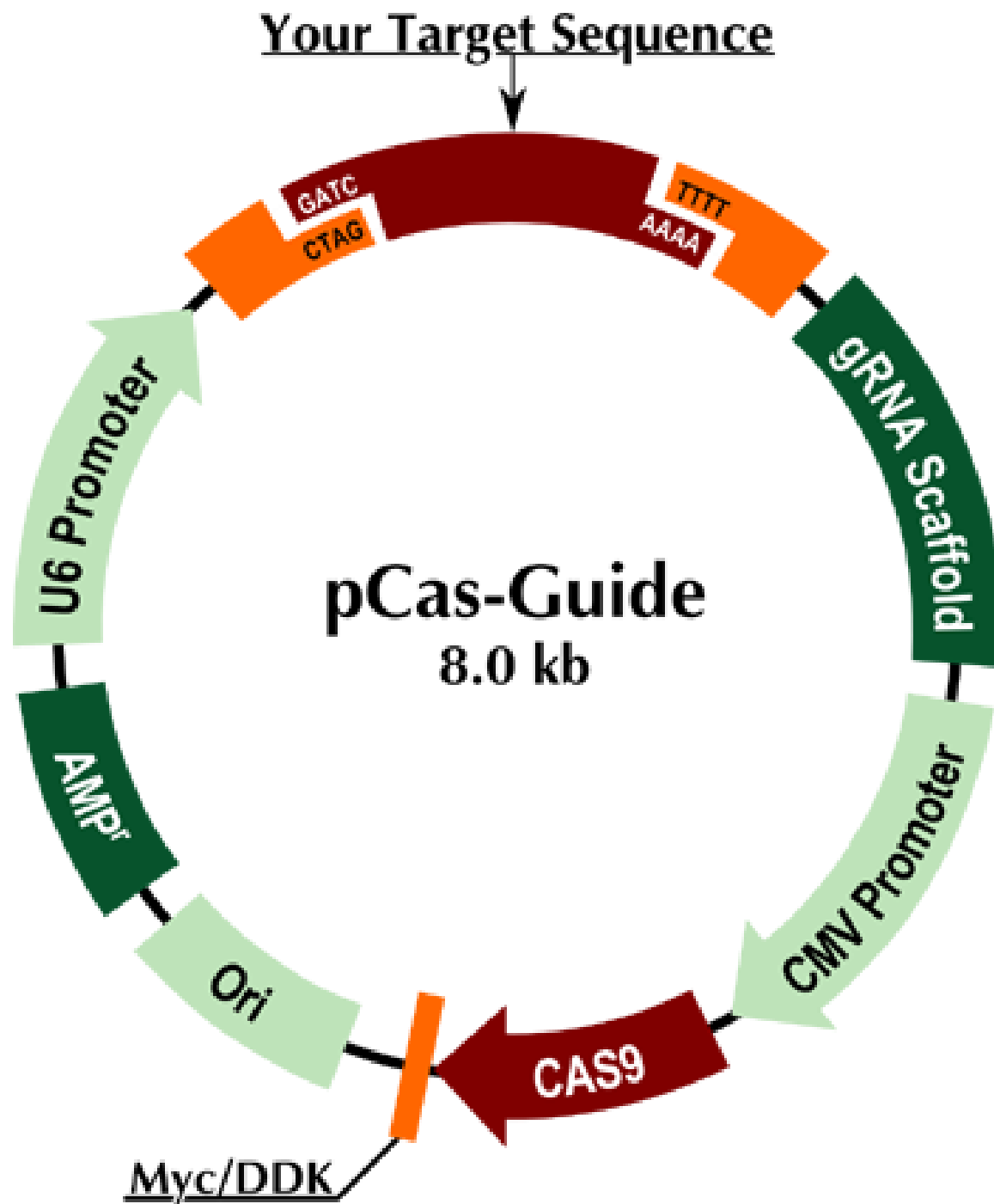# CONTRIBUTIONS OF SUPER-ENHANCER COSTITUENTS TO GENE EXPRESSION IN VIVO

QUESTION: HOW DOES SUPER-ENHANCER COSTITUENTS REGULATE GENE EXPRESSION IN VIVO?

METHOD: DELETION OF SPECIFIC GENOMIC REGIONS

TECHNIQUE: CRISPR/CAS9

ChIP-Seq DATA used to design STUDY ON COSTITUENTS ENHANCERS FUNCTION

Your Target Sequence

GATC
CTAG
TTTT
AAAA

U6 Promoter

gRNA Scaffold

pCas-Guide
8.0 kb

AMPr

CMV Promoter

Ori

CAS9

Myc/DDK

Genome editing

Genome editing was performed using CRISPR/Cas9 essentially as described (Wang et al., 2013). Briefly, target-specific oligonucleotides were cloned into a plasmid carrying a codon-optimized version of Cas9 (pX330, Addgene: 42230). The genomic sequences complementary to guide RNAs are listed in Supplemental Table 2. V6.5 murine ESCs were transfected with two plasmids expressing Cas9 and sgRNA targeting regions around 200 basepairs up- and down-stream of the center of the targeted SE-constituent (as defined by OCT4/SOX2/NANOG co-binding; see below), respectively. A plasmid expressing PGK-puroR was also co-transfected. Transfection was carried out with the X-fect reagent (Clontech) according to the manufacturer's instructions. One day after transfection, cells were re-plated on DR4 MEF feeder layers. One day after re-plating puromycin (2µg/ml) was added for three days. Subsequently, puromycin was withdrawn for three to four days. Individual colonies were picked, and genotyped by PCR, and the edited alleles were verified by Sanger sequencing. All cell lines used in subsequent experiments were homozygous deletion lines. Reference and deletion allele sequences are listed in Supplemental Table 2.

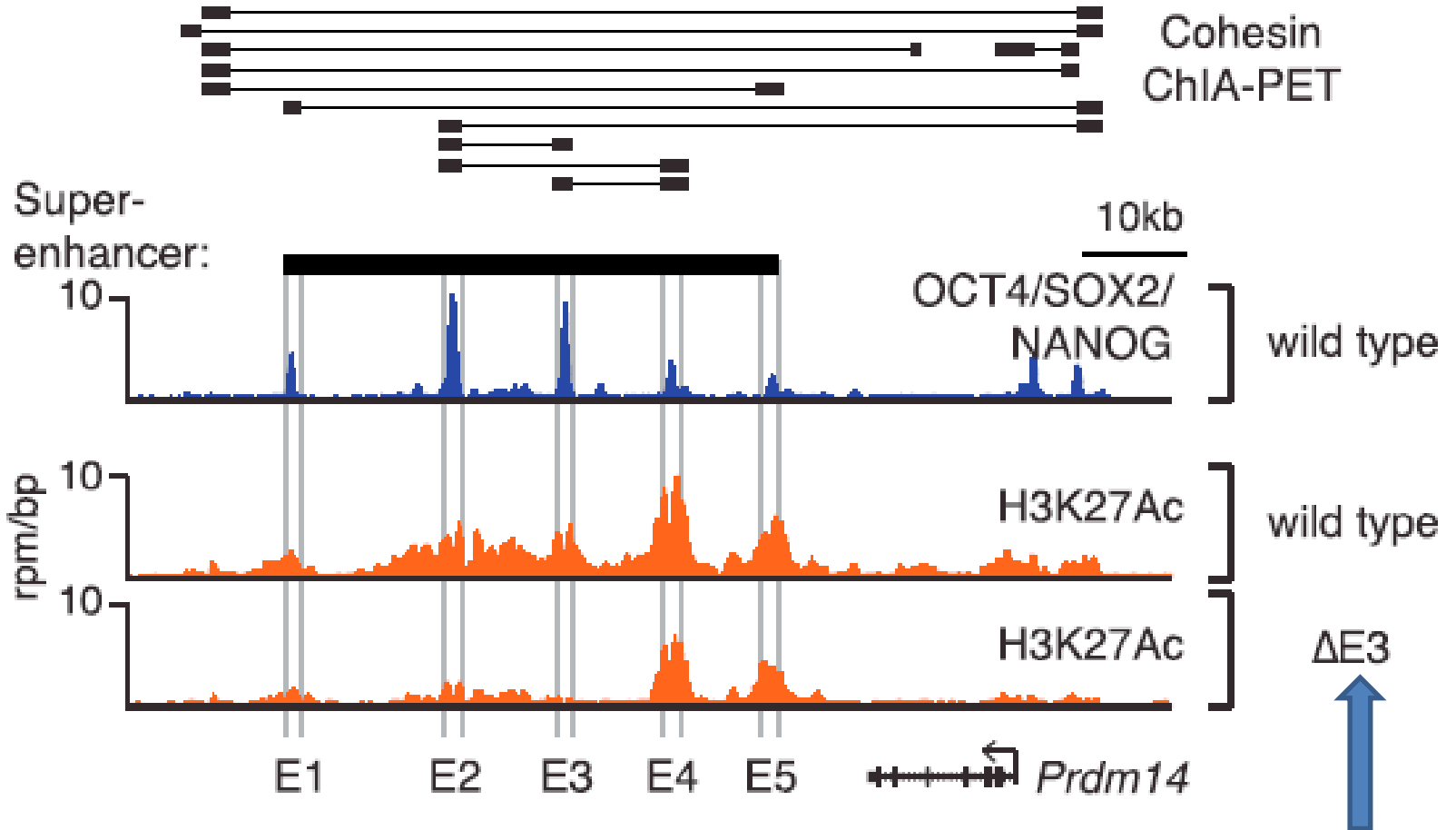# LUCIFERASE ACTIVITY AND DELETION OF SPECIFIC ENHANCER
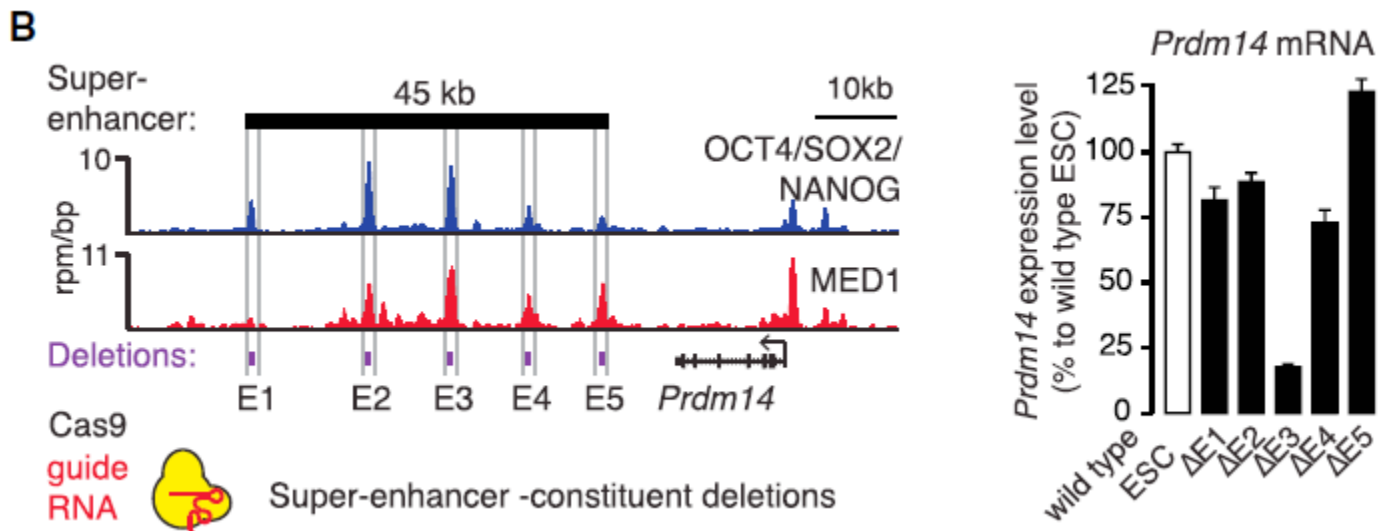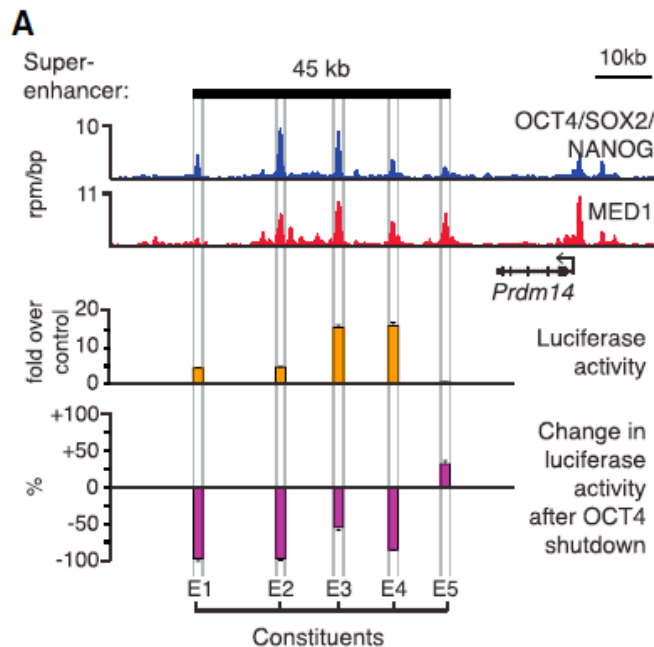
LUCIFERASE ACTIVITY AND DELETION OF SPECIFIC ENHANCER
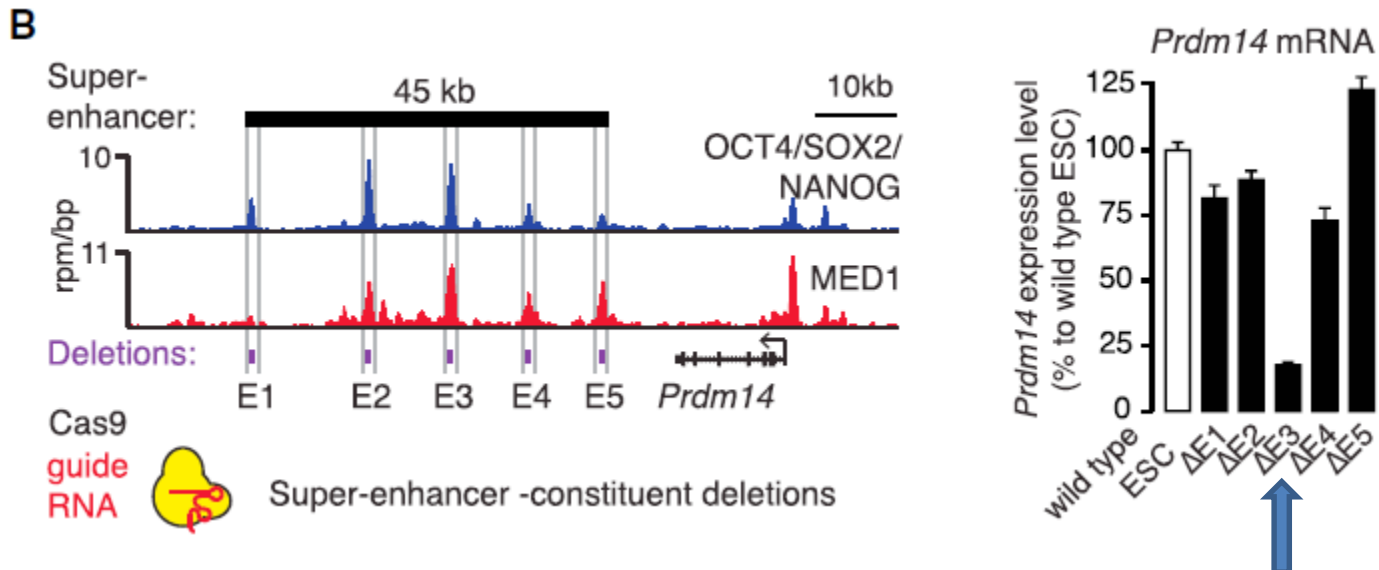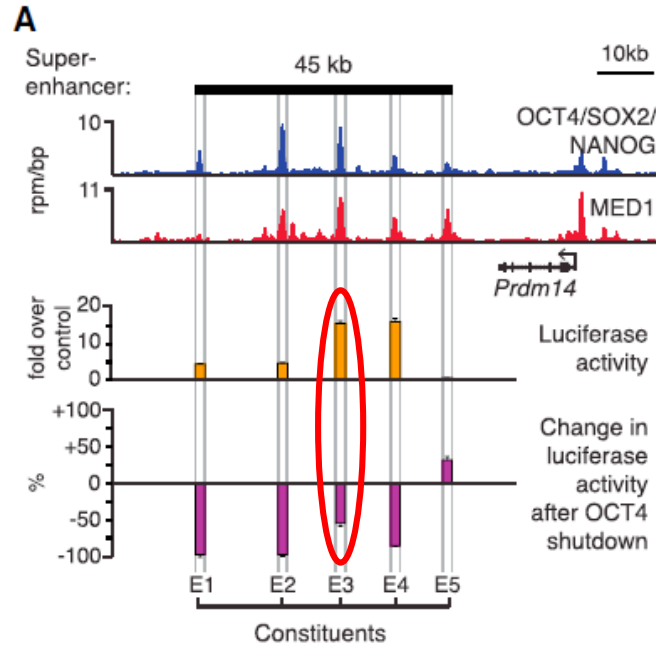
IDENTIFICATION OF INTERACTION
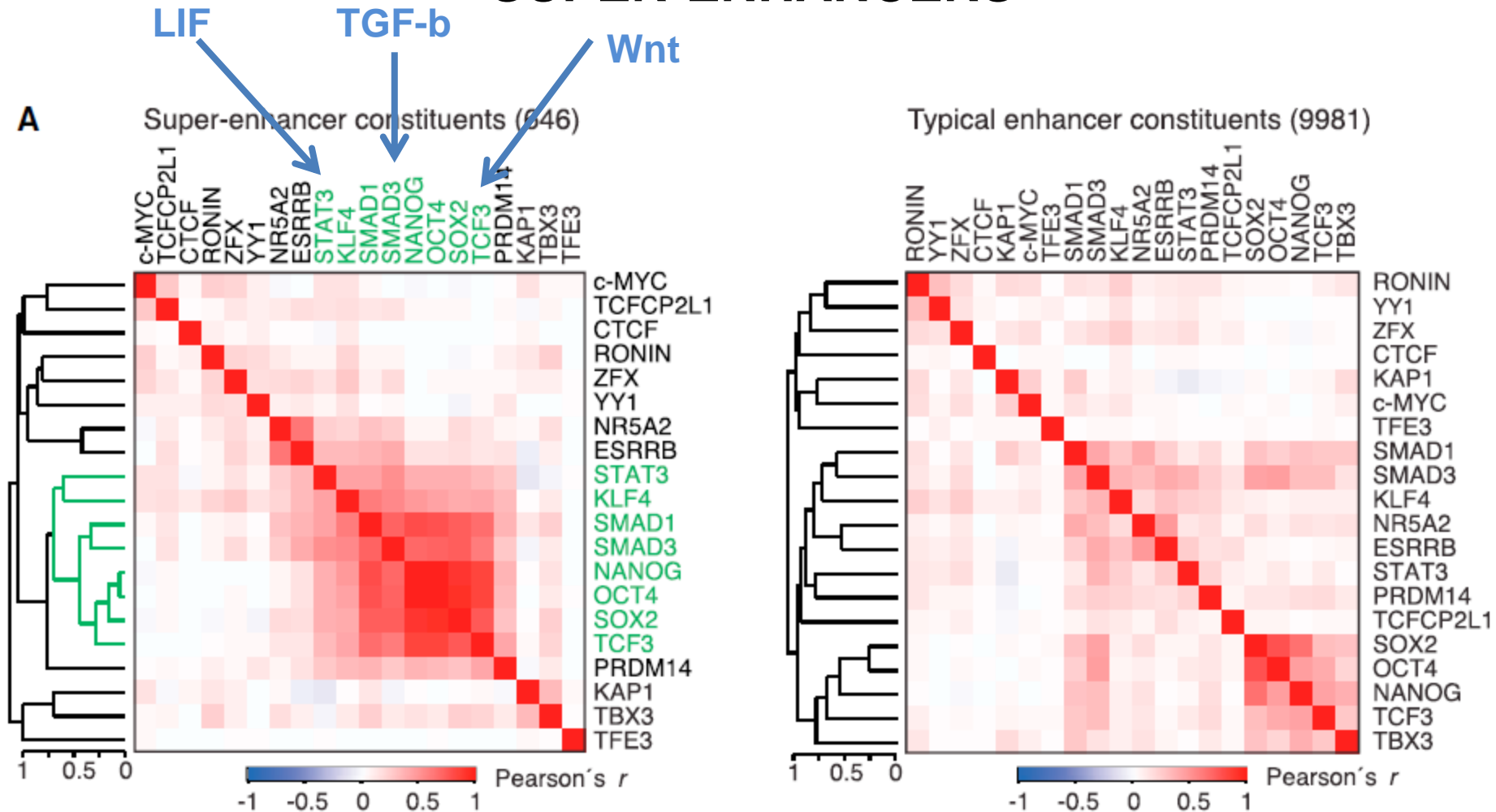# BETWEEN SPECIFIC COSTITUENTS ENHANCERS

DELETION OF E3 INDUCES EPIGENETIC MODIFICATIONS:
H3K27Ac reduction

SUPER-ENHANCERS ENRICHMENT
OF TRANSCRIPTION FACTORS PATTERN

# SPECIFIC MULTIPLE TRANSCRIPTION FACTORS BIND SUPER-ENHANCERS

Hierarchical clustering of 20 transcription factor ChIP-seq binding profiles at super-enhancer and typical enhancer constituents. A set of factors with binding profiles similar to OCT4, SOX2, and NANOG is highlighted in green.

enhancers. An examination of the pattern of transcription factor binding to super-enhancer constituents provided a hypothesis to resolve this conundrum (Figure 3A, Table S3). The terminal TFs of the Wnt (TCF3), TGF-β (SMAD3), and LIF (STAT3) signaling pathways, which play essential roles in transcriptional control of the stem cell state (Ng and Surani, 2011; Young, 2011), were among the TFs whose binding pattern to SE constituents was most similar to that of OCT4, SOX2, and NANOG at SE constituents (Figure 3A). Most SE constituents (75%) were occupied by at least one of these three TFs, whereas only 43% of typical enhancer constituents were bound by one of the three (Figure S3A). More importantly, 98% of super-enhancers were **1TF** bound by at least one, 86% were bound **2TFs** 46% were bound by all three signaling **3TFs**
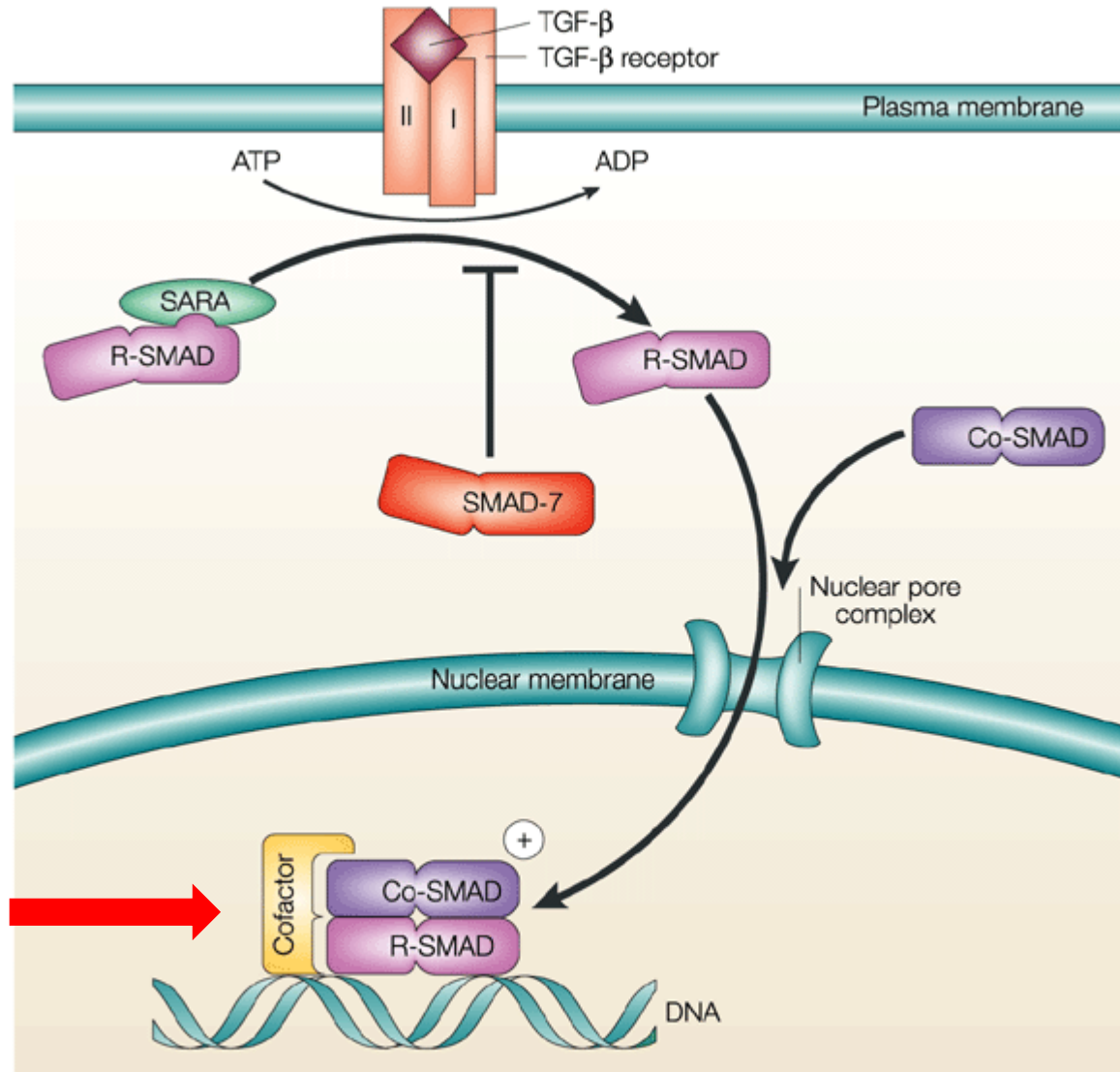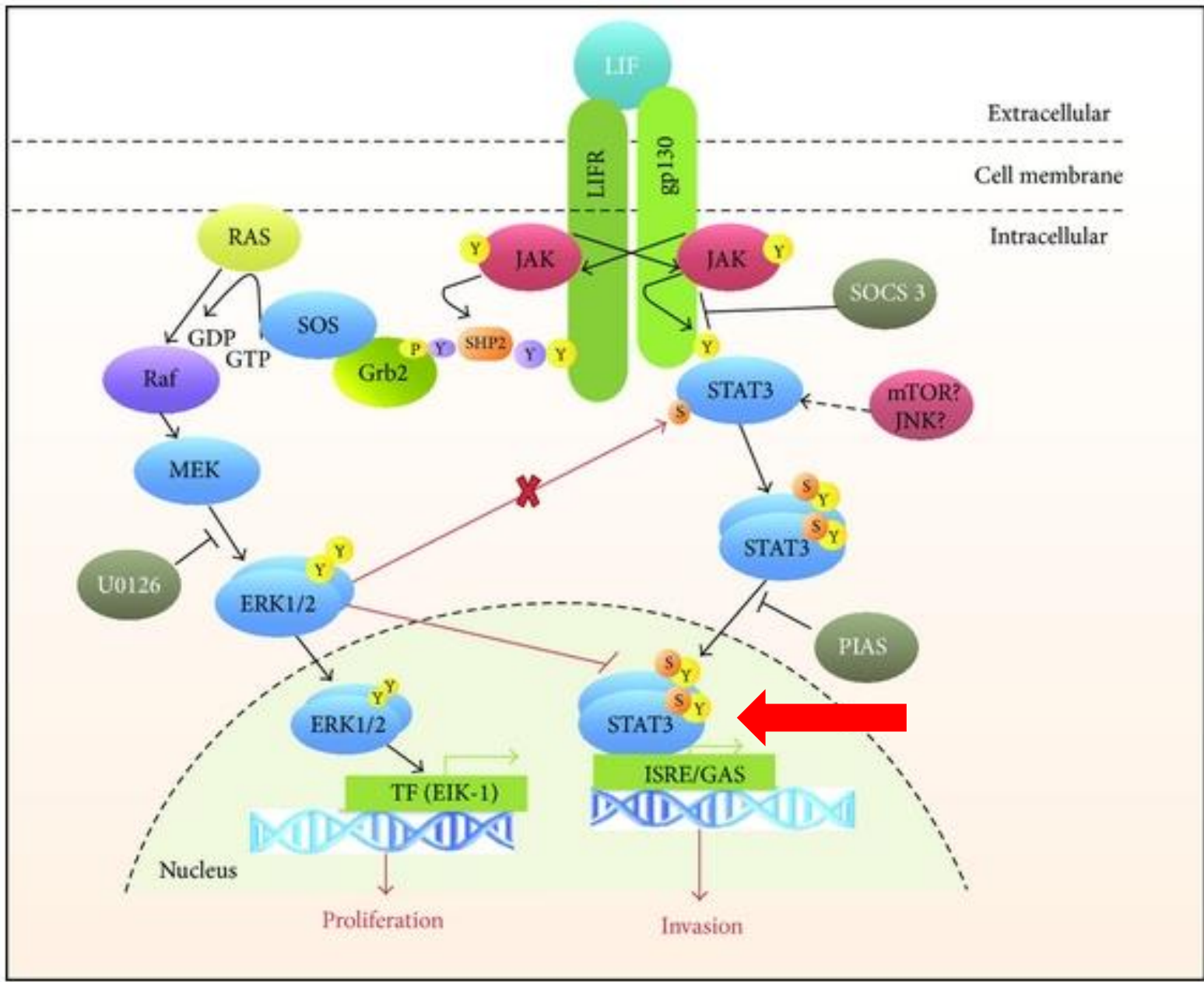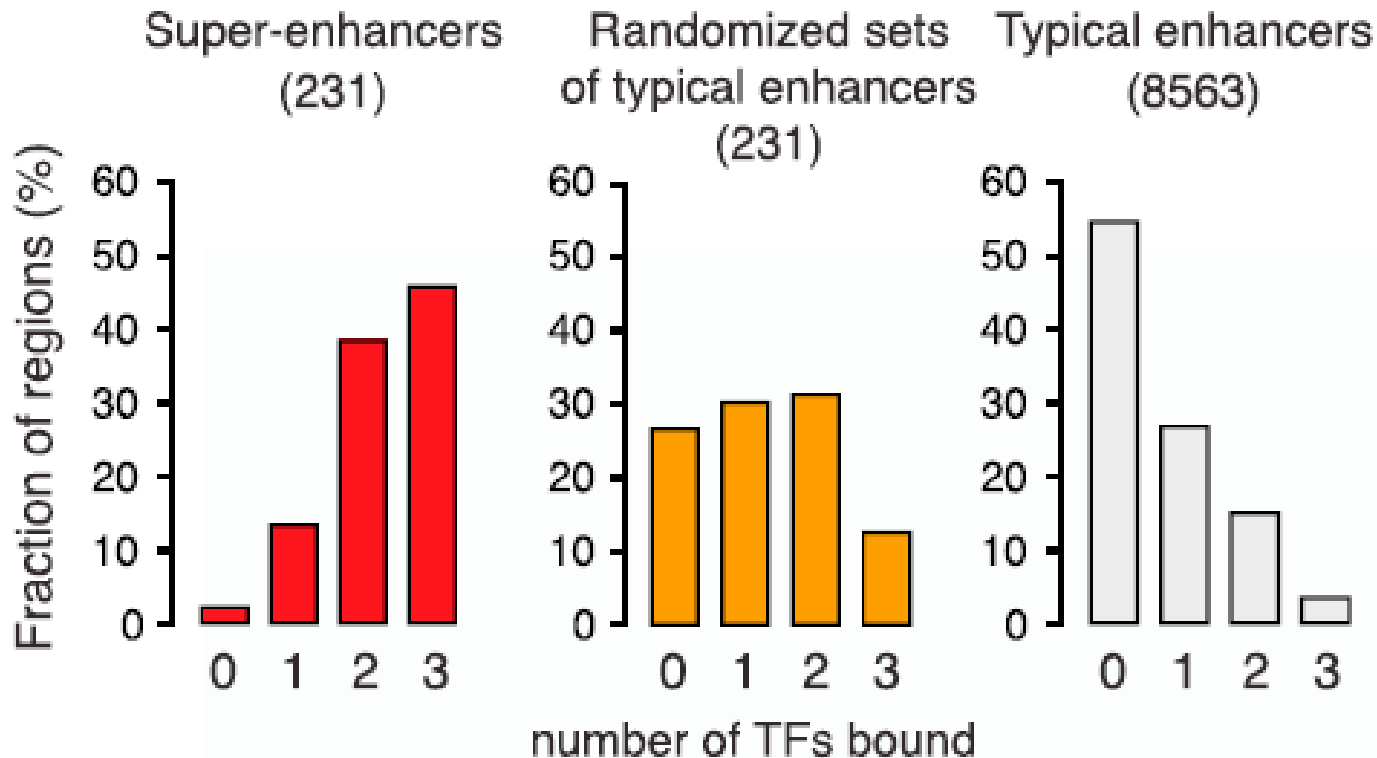
WNT PATHWAY
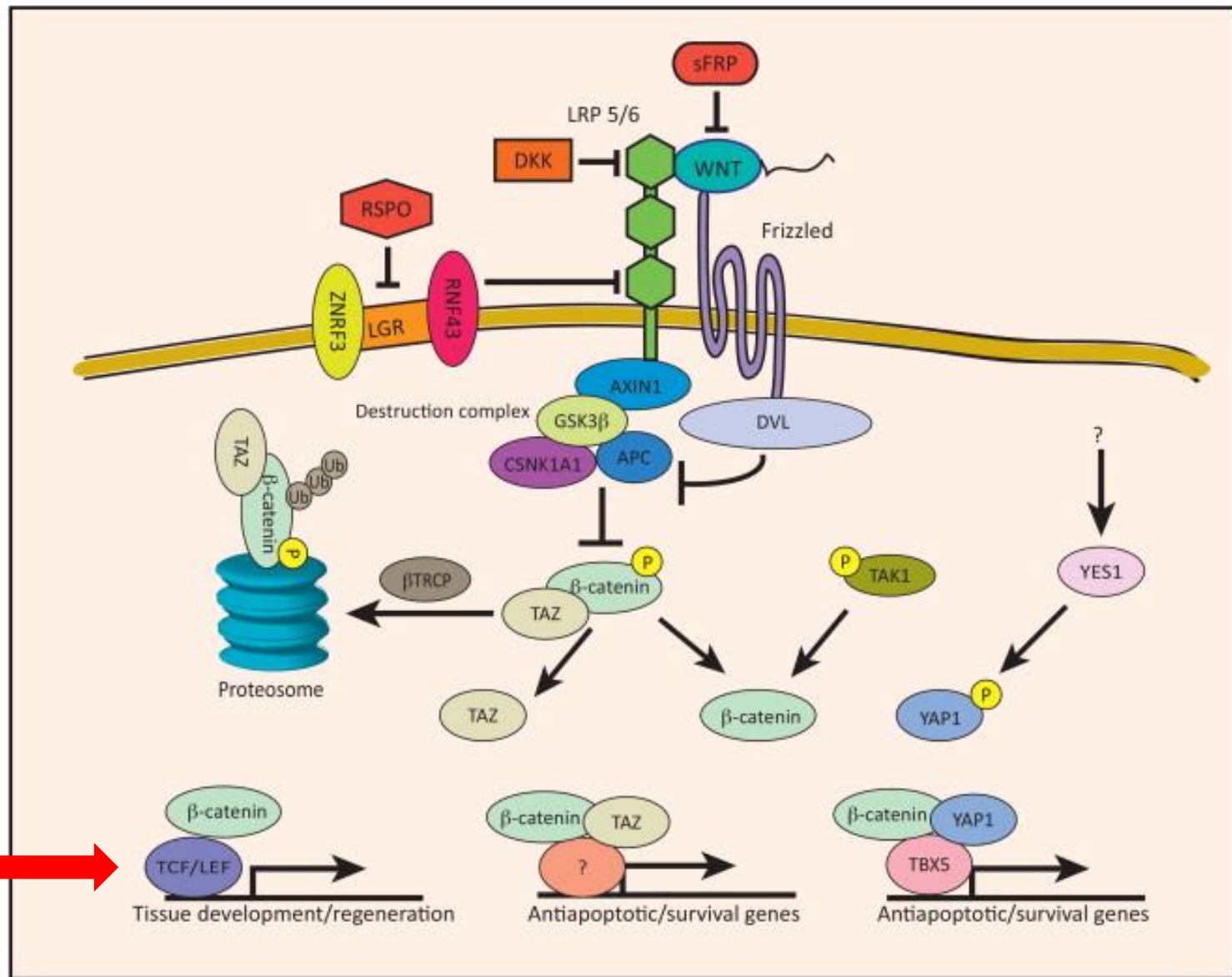
TGF beta PATHWAY

TCF3, SMAD3, STAT3, regulated by oncogenic pathways, bind constituent enhancers in SE.
No same pattern in randomized set of typical enhancers.

B

Binding motifs for TCF3, SMAD3, and STAT3 and the p values for their enrichment in super-enhancer constituent enhancers in murine and human ESCs. The motif of CTCF is not found enriched and serves as a negative control.

C

TF	Motif	murine ESC P-value	human ESC P-value
TCF3		5.46×10^{-27}	2.23×10^{-28}
SMAD3		9.31×10^{-11}	3.34×10^{-4}
STAT3		2.90×10^{-10}	6.26×10^{-2}
CTCF		0.45	1

Gene expression analyses

RNA-Seq RPKMs were calculated for two replicates each of murine ESCs treated with LIF for 1h (E-MTAB-1796 Arrayexpress dataset (Martello et al., 2013)) . Reads for each replicate were aligned to the mm9 reference genome using Tophat2 (Trapnell et al., 2009) version 2.0.11, using Bowtie version 2.2.1.0 and Samtools version 0.1.19.0. RPKMs per Refseq transcript were calculated from aligned reads using RPKM_count.py from RSeQC (Wang et al., 2012). Fold-changes for +/-LIF conditions were calculated by averaging RPKMs for each condition for all transcripts with the same gene name, dividing the –LIF by the +LIF average RPKM (adding one pseudocount each), and transforming by log2. Gene expression changes after blocking TGF-β signaling by the inhibitor SB431542 were downloaded from a previous study (Mullen et al., 2011). Gene expression changes after stimulation of the Wnt pathway by Wnt 3a conditioned medium were downloaded from a previous study (Cole et al., 2008).

Gene set enrichment analysis (GSEA) of gene expression changes after manipulation of the Wnt, TGF-b, and LIF pathways. "SE-genes" and "TE-genes" indicate genes associated with SEs and typical enhancers, respectively.
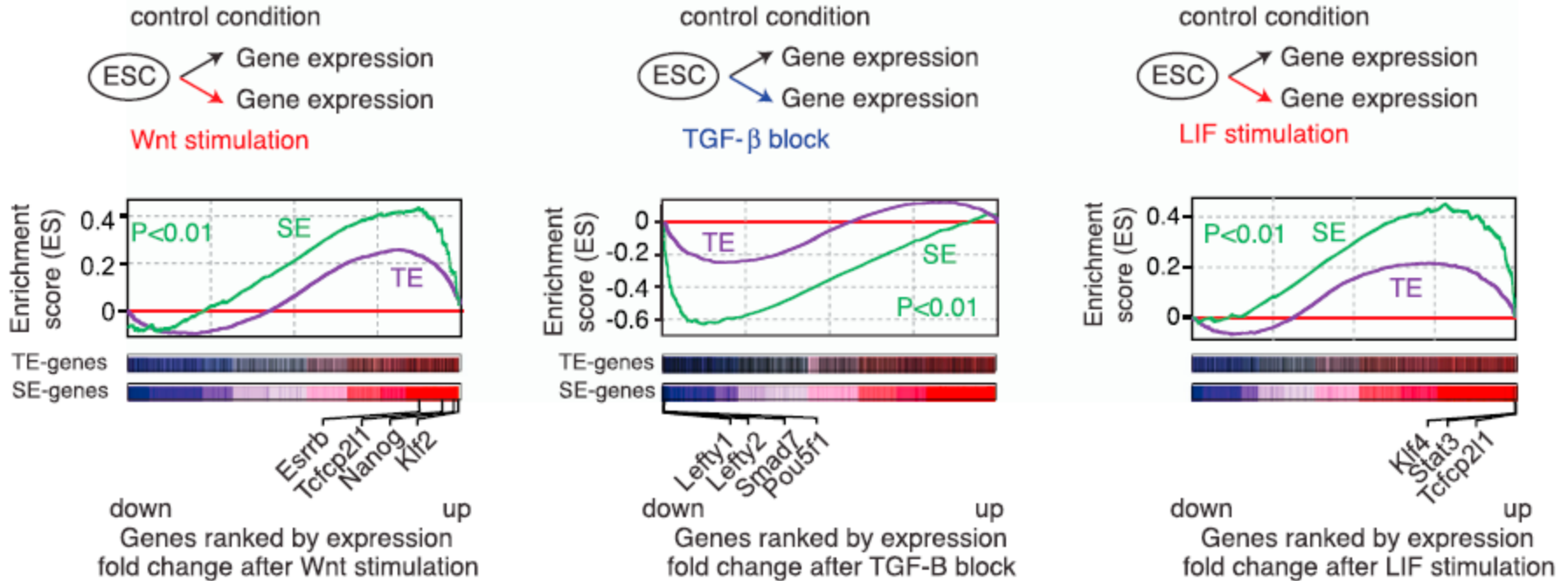
If super-enhancers confer responsiveness to the Wnt, TGF-β, and LIF pathways more frequently than typical enhancers, then stimulation or perturbation of these pathways should have a more profound effect on super-enhancer-associated genes than typical enhancer-associated genes. The results of transcriptional profiling and gene set enrichment analysis in ESCs confirm this prediction (Figure 3D); super-enhancer associated genes were found enriched among the genes whose expression exhibited the most profound changes after pathway stimulation or perturbation (Wnt: $p < 0.01$; TGF-β: $p < 0.01$; LIF: $p < 0.01$). In contrast, the enrichment for genes associated with typical enhancers was more moderate (Figure 3D). The super-enhancer-associated genes that showed a profound response to signaling included previously reported targets of these pathways that play key roles in ESC self-renewal and differentiation (Figure 3D, Figure S3G). A subset of the *Prdm14* SE constituents that are bound by signaling TFs were found to be responsive to perturbation of these signaling pathways in reporter assays (Figure S3H). These results lead us to propose that key cell identity genes have evolved a clustered enhancer structure to provide a means to respond directly to these developmentally important signaling pathways.

SE function on c-Myc locus
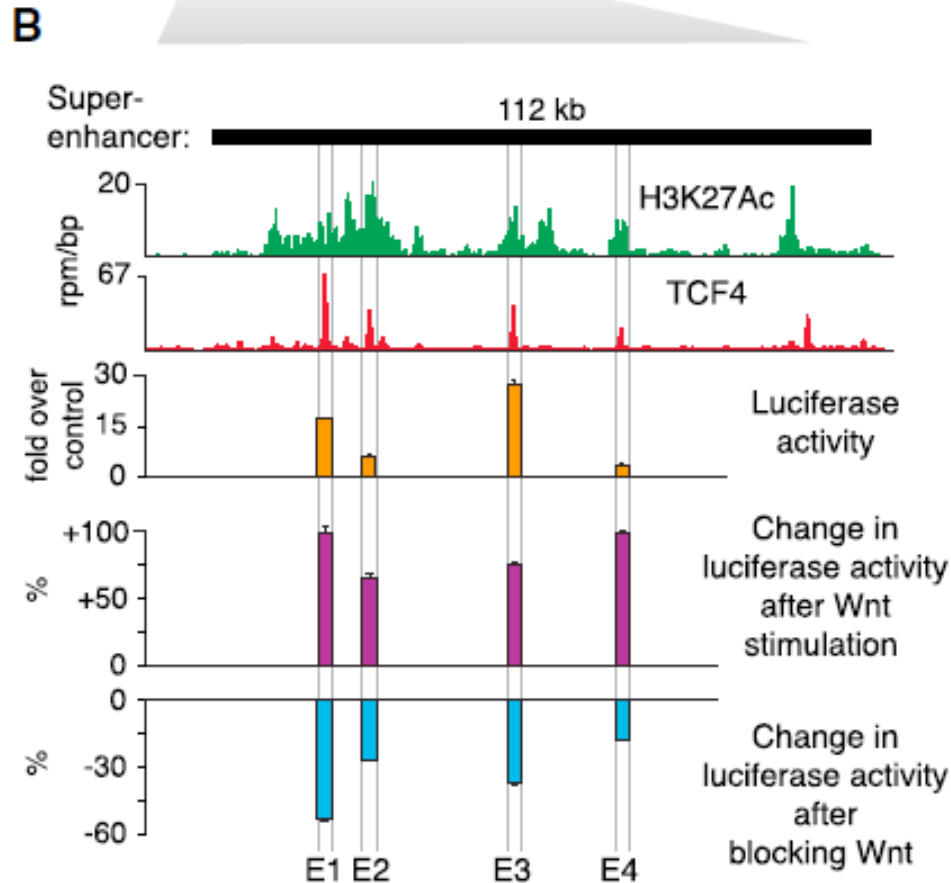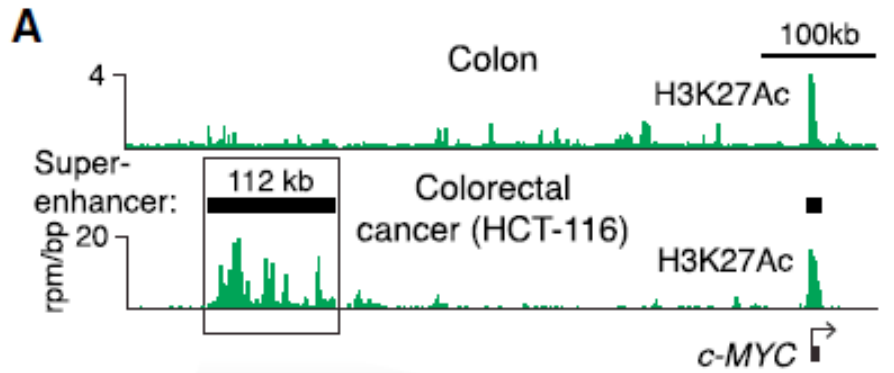
ChIP-seq binding profiles for H3K27Ac at the c-MYC locus in colon and colorectal cancer cells (HCT-116).

Wnt: V6.5 mESCs were cultured in media containing 3µM IWP-2 (STEMGENT) for 24 hours prior to transfection to suppress Wnt signaling. Cells were then transfected either in media containing 3µM IWP-2 or in media containing 50ng/µl recombinant Wnt3a (R&D). Transfected cells were incubated for 24 hours, and luciferase measurements were performed as described above.

Left: ratio of H3K27Ac in CRC (HCT-116) versus normal colon tissue used densities at the union of SEs identified in the two samples. **Right:** metagene representation of H3K27Ac and TCF4 ChIP-seq densities at the regions corresponding to the top 100 acquired super-enhancers.
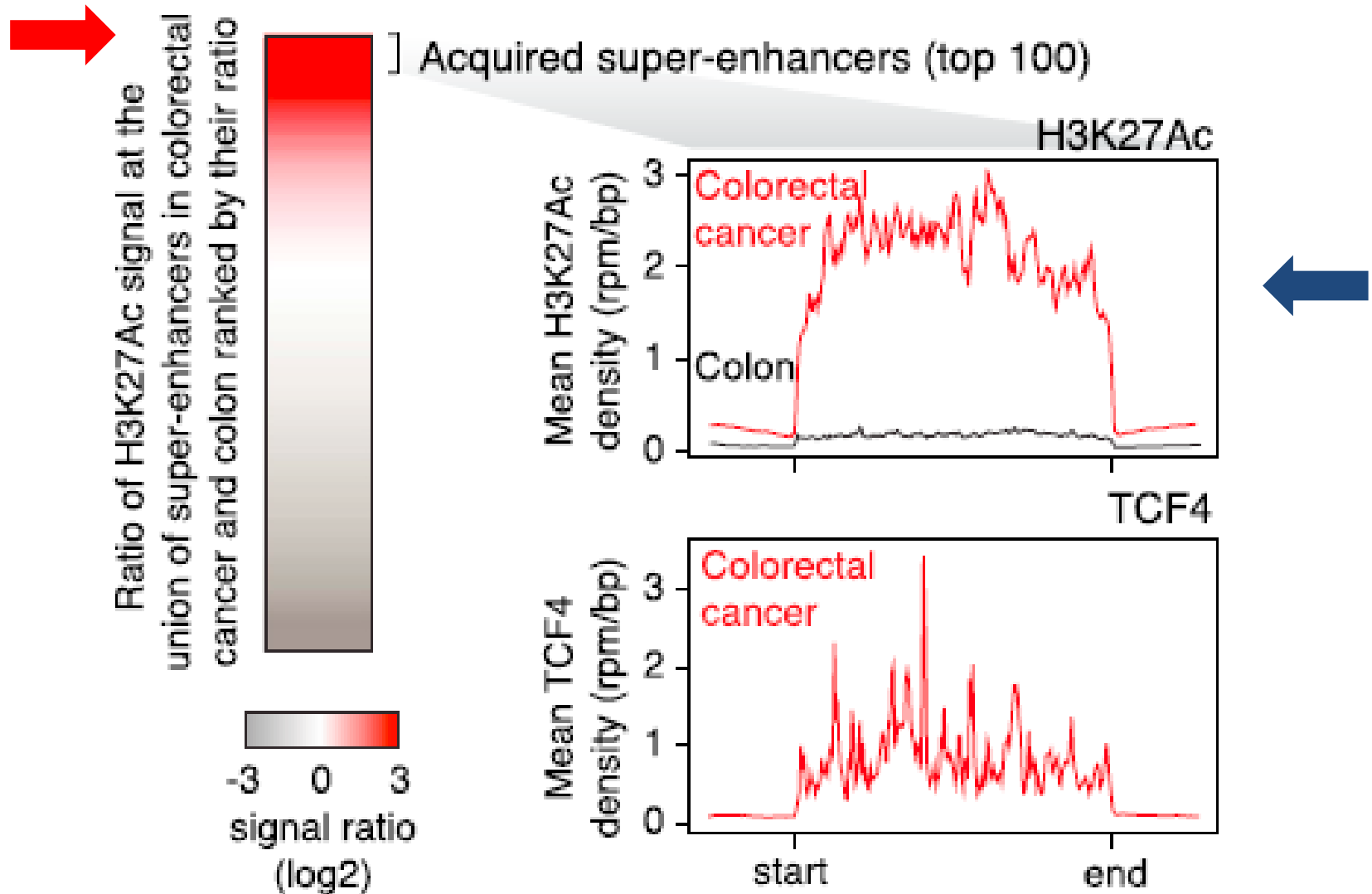
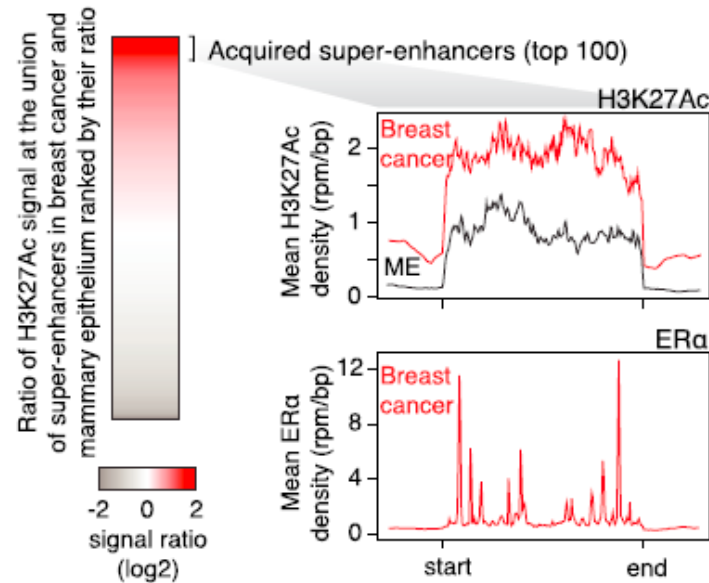ure 4C). Genes associated with these acquired super-enhancers were enriched for expression changes after stimulation or blockage of the Wnt pathway (stimulation: $p < 0.01$; blockage: $p < 0.01$), although not all super-enhancer genes showed this response (Figure 4D). These results indicate that acquired su-

D

control condition

CRC — Gene expression
CRC — Gene expression

Wnt stimulation

control condition

CRC — Gene expression
CRC — Gene expression

Wnt block

P<0.01

P<0.01

SE-genes

down   up
Genes ranked by expression
fold change after Wnt stimulation

down   up
Genes ranked by expression
fold change after blocking Wnt
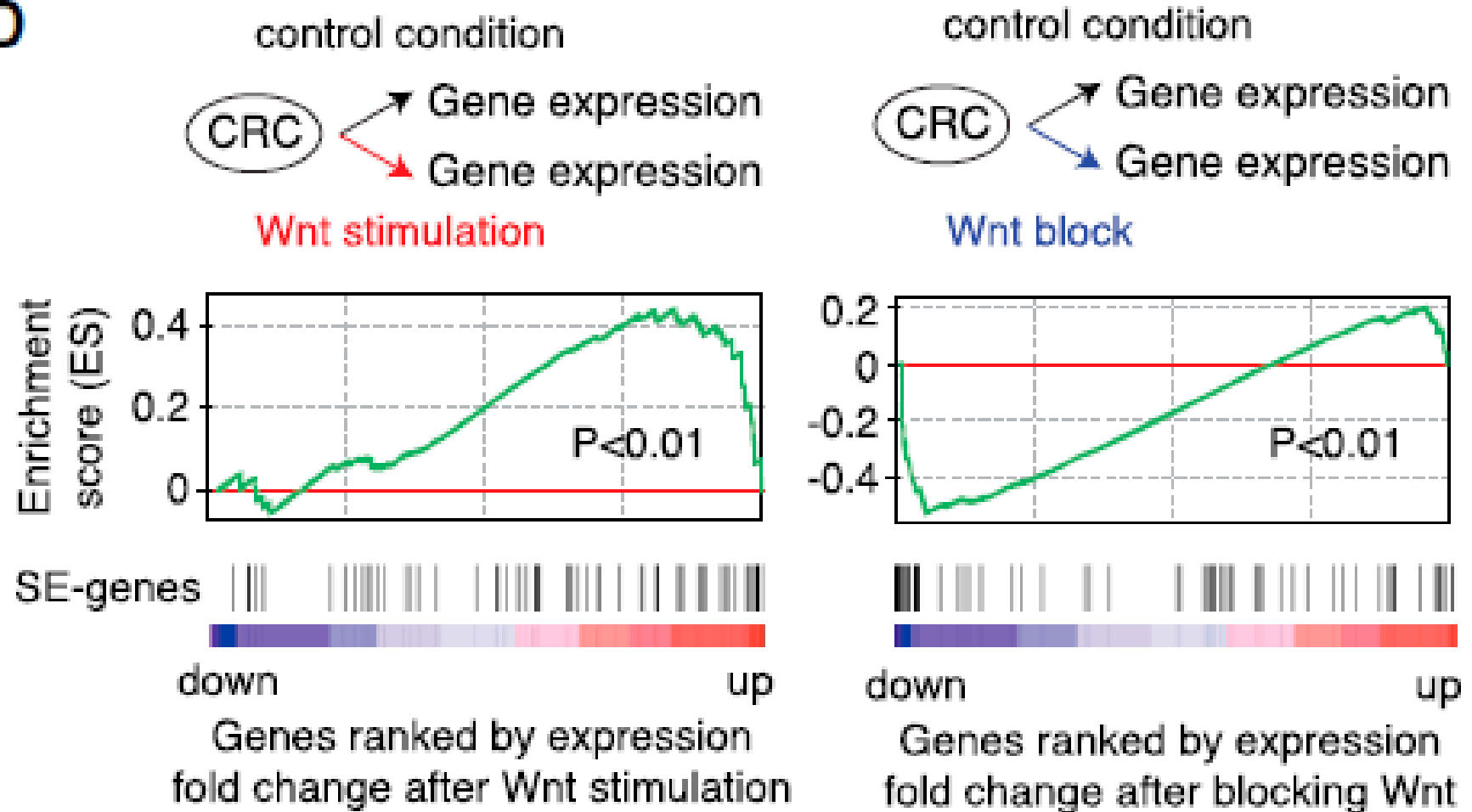
SEs in breast cancer cell lines
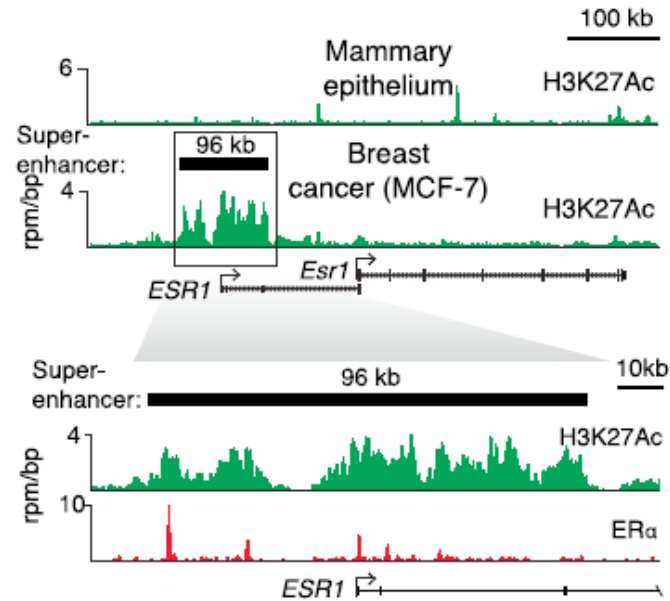
DISCUSSION

Super-enhancers control genes that play especially prominent roles in cellular physiology and disease (Brown et al., 2014; Chapuy et al., 2013; Gröschel et al., 2014; Herranz et al., 2014; Hnisz et al., 2013; Lovén et al., 2013; Mansour et al., 2014; Northcott et al., 2014; Parker et al., 2013; Siersbæk et al., 2014; Whyte et al., 2013), but there is a limited understanding of the functions of these clustered elements and, thus, why they have evolved to drive genes that play key roles in cell-type-specific biology. Our results reveal that SEs can provide a platform for signaling pathways to regulate genes that control cell identity during development and tumorigenesis.

Several lines of evidence argue that the constituent enhancers of at least some super-enhancers can act as an interdependent structural and functional unit to control their associated genes. Our results show that ESC SEs generally consist of clusters of active enhancers that have OCT4-dependent and ESC-specific functions (Figure 1) and demonstrate that optimal transcriptional activity of target genes is dependent on the presence of most of the constituent enhancers (Figure 2). Chromatin interaction data indicate that constituent enhancers physically interact within the SEs; indeed, the interactions among SE constituents in ESCs appear to be more frequent than interactions between the SE constituents and their associated gene promoters, and interactions between typical enhancers (Dowen et al., 2014). We previously noted that enhancer clusters can be gained or lost as a unit during development or oncogenesis (Hnisz et al., 2013) and have shown that large tumor SEs can collapse as a unit when depleted of the enhancer cofactor BRD4 (Lovén et al., 2013) or when a constituent is deleted (Mansour et al., 2014). In some T cell acute lymphoblastic leukemia (T-ALL) cells, a small mono-allelic insertion that creates a binding site for a master transcription factor can nucleate the formation of an oncogenic super-enhancer that involves establishment of additional transcriptional components in adjacent sites (Mansour et al., 2014). Super-enhancers produce relatively high levels of enhancer RNAs (Hnisz et al., 2013), and a recent study showed that inflammation-dependent super-enhancers form domains of coordinately regulated enhancer RNAs (Hah et al., 2015). These results, taken together, suggest that the constituent enhancers of super-enhancers can interact physically and functionally to coordinate transcriptional activity.

SEs characteristics

- OCT4 binding in ESC
- SE regulates transcription
- SE chromatin interaction
- SE role in oncogenesis
- eRNA linked to SE
- inflammation linked to SE

Our results reveal that SEs are occupied more frequently by terminal transcription factors of the Wnt, TGF-β, and LIF signaling pathways than typical enhancers in ESCs, and genes driven by SEs show a more pronounced response to the manipulation of these pathways than genes driven by typical enhancers (Figure 3). Thus, the clustered enhancer architecture of SEs may have evolved, at least in part, to provide a conduit for these signaling pathways to signal maintenance or change at genes that are key to control of cell identity. Our results also suggest that one reason that tumor cells evolve SEs at key oncogenes is to enhance the connection to oncogenic signaling pathways. The recent report of NOTCH1-driven SEs in T-ALL likely represents another example of this phenomenon (Herranz et al., 2014; Wang et al., 2014). An implication of this model is that therapies that target both oncogenic signaling pathways and super-enhancer components may be especially effective in tumor cells that have signaling and transcriptional dependencies.

Graphical Abstract
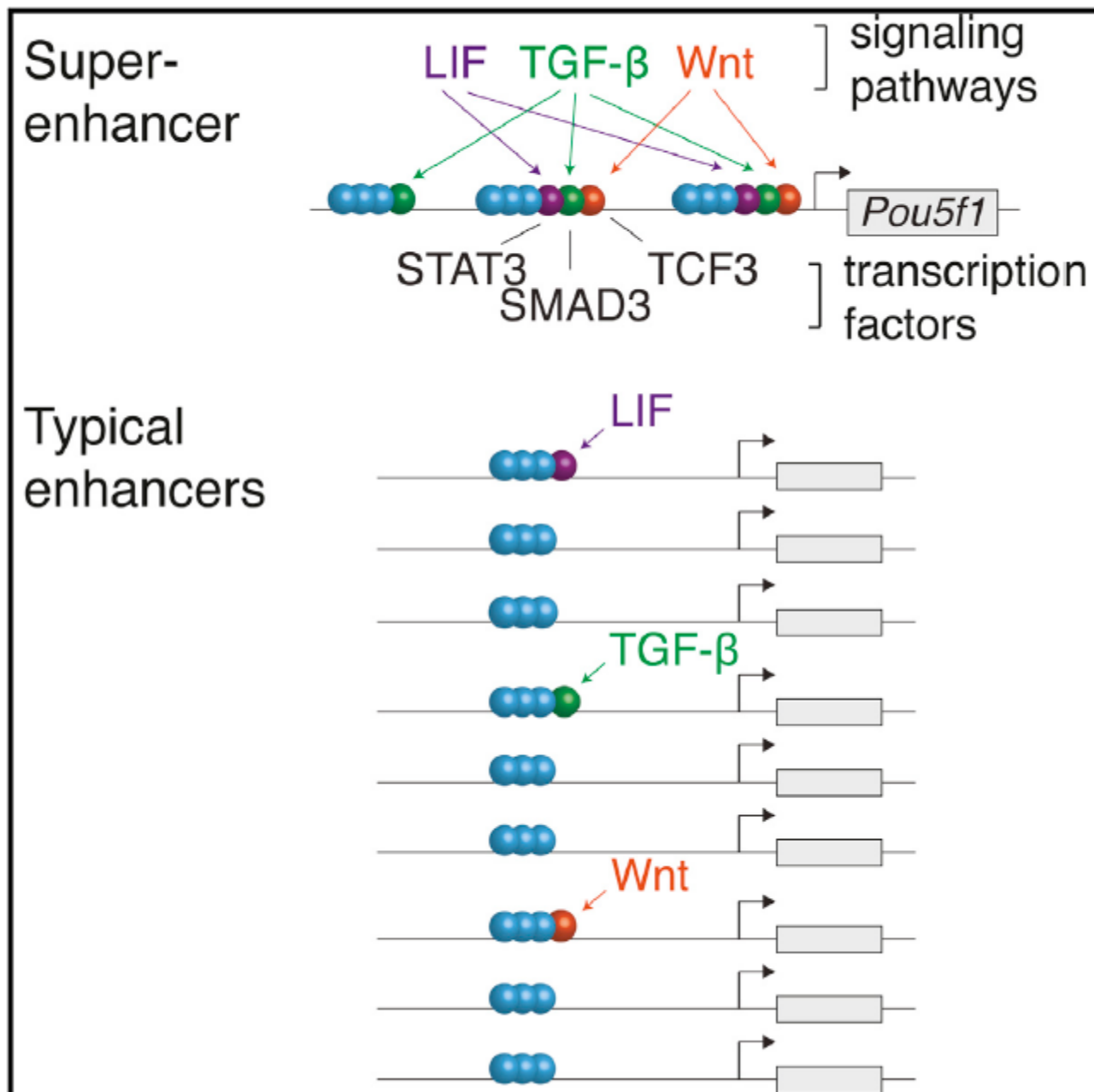
2015

2017

WE ARE A THERAPEUTICS COMPANY PASSIONATELY COMMITTED TO APPLYING OUR SCIENTIFIC LEADERSHIP IN THE EMERGING FIELD OF GENE CONTROL TO TRANSFORM THE LIVES OF PATIENTS WITH CANCER AND OTHER DISEASES.

Founded by world-leading experts in gene regulation, and led by a seasoned management team, Syros is focused on developing new medicines that control genes that cause disease. Central to our approach is a proprietary drug development platform of integrated gene control assay technologies, computational biology, chemistry, and deep biologic insights that gives us an unprecedented understanding of and control over gene regulation. Our pioneering research and drug development capabilities provide us with the distinctive ability to identify new, important disease and patient-targeted gene control medicines.

Working together to transform patients' lives through the creation of novel gene control medicines

OUR PLATFORM

Syros is solely focused on gene control. Building on the seminal discoveries of our scientific founders, we have developed what we believe is the first proprietary platform designed to systematically and efficiently analyze non-coding regions of the genome to identify alterations in gene expression programs.
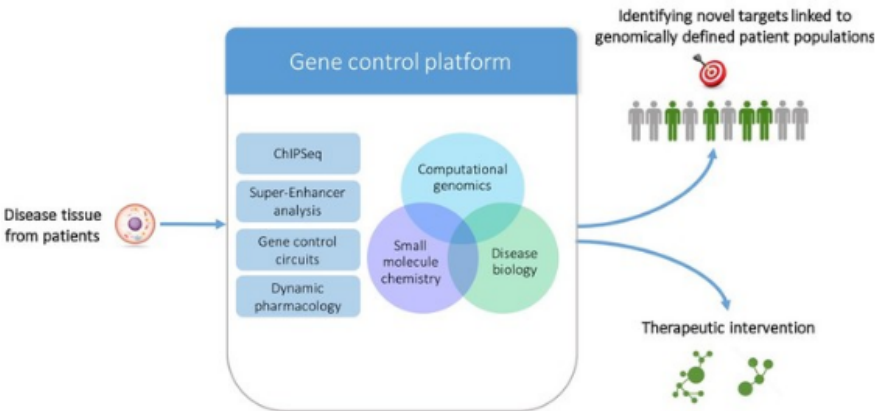
By doing so, we believe our gene control platform will allow us to:

- Identify a wide array of potential new drug targets across a range of diseases
- Provide a new lens for diagnosing and segmenting patients, including those with complex, multi-factorial diseases that have eluded segmentation with other genomic-based approaches
- Advance a new wave of medicines with the potential to influence multiple drivers of disease through a single target, making them less susceptible to drug resistance and providing patients with a more profound and durable benefit than many of today's targeted therapies.

Learn more about our platform

DRUGS DISCOVERY FROM SYROS PHARMACEUTYCAL

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
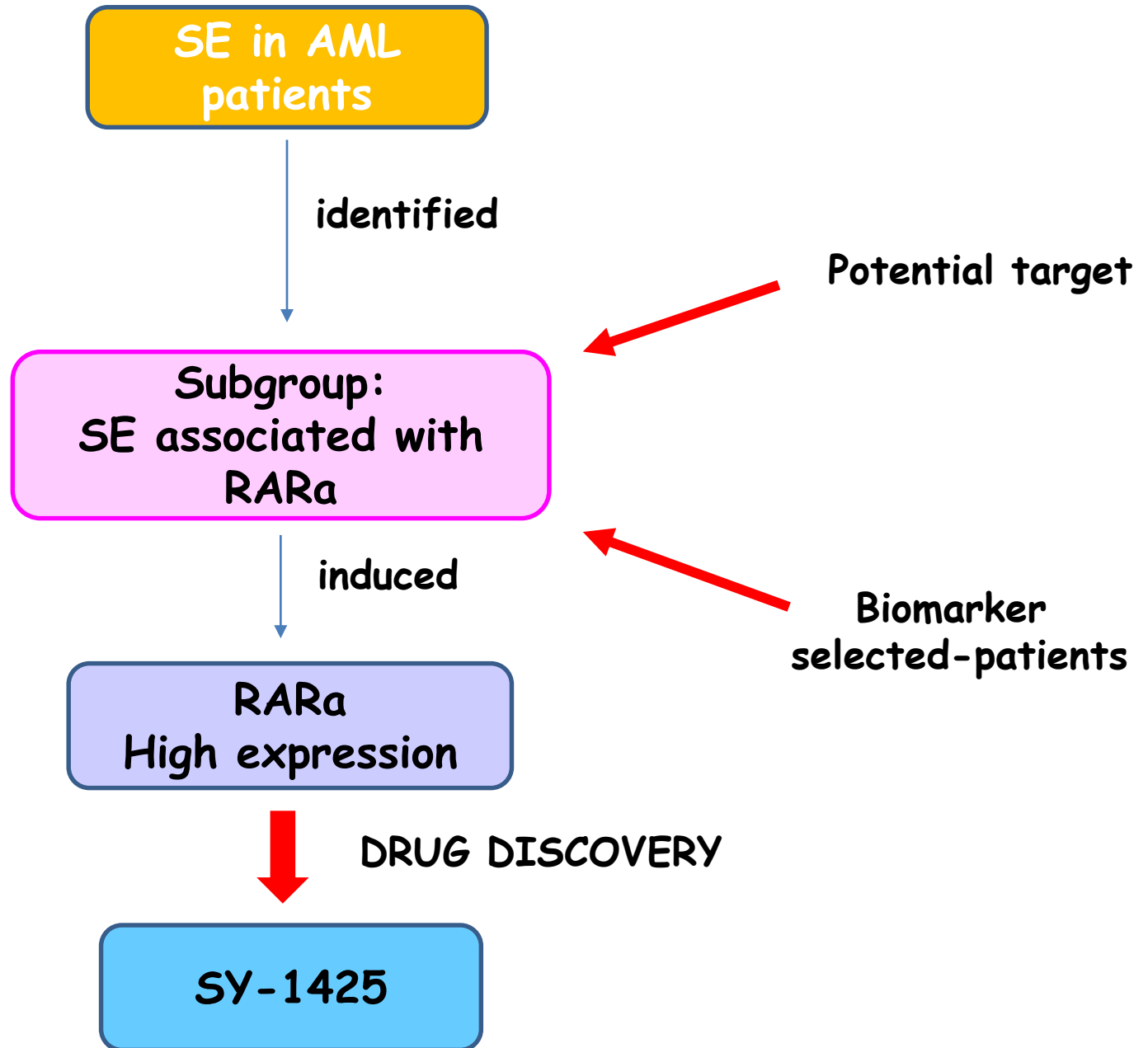
ONCOGENIC SUPER-ENHANCERS linked to SNPs IN TUMOR PROGRESSION

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.
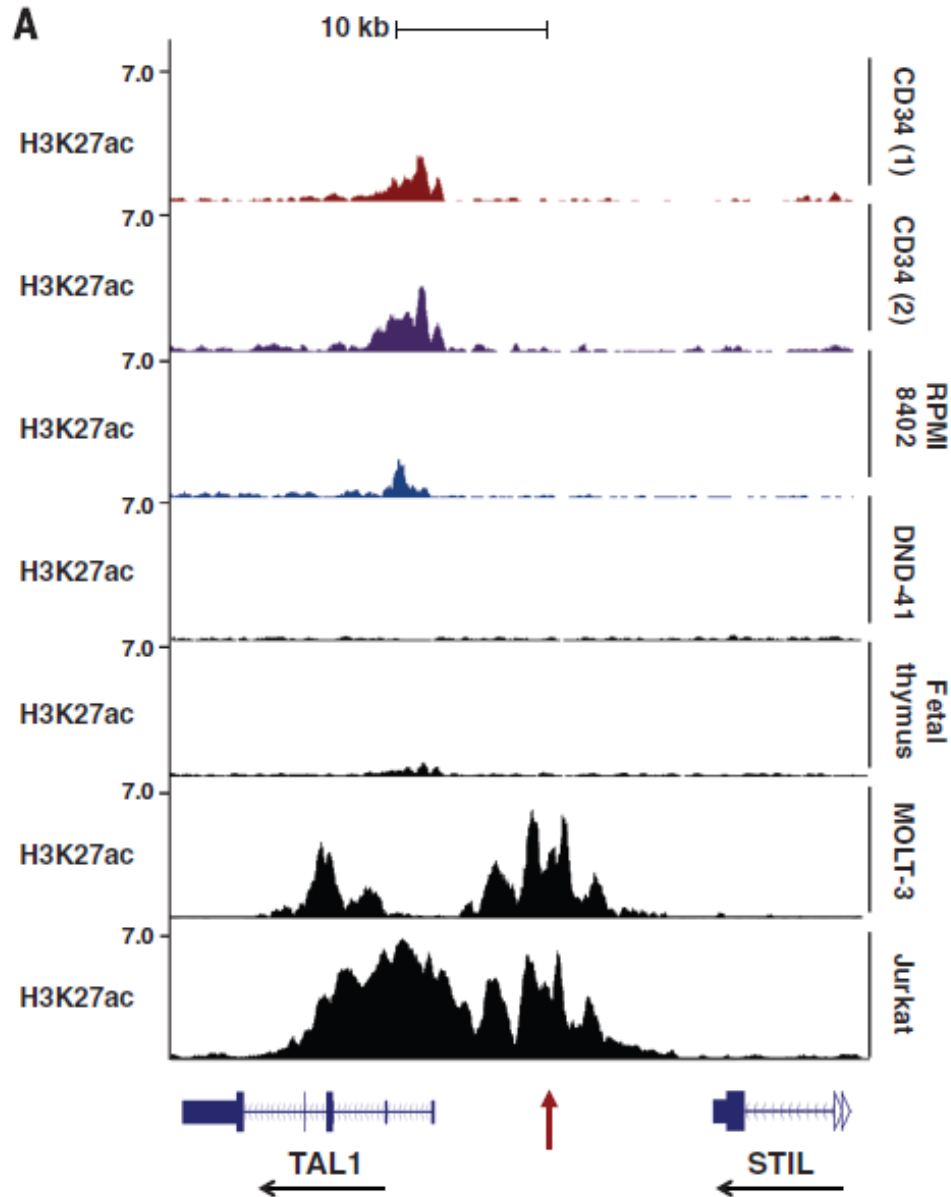
BACKGROUND

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.

hg19:	47,704,983	47,704,954

```
WT          GGGTCACAGAAAGACGTAACCCTACTTCCT
Jurkat      GGGTCACAGAAAGACGGTTAGGAAACGGTAACCCTACTT
MOLT-3      GGGTCACAGAAAGACGGTTAACCCTACTT
Patient #1  GGGTCACAGAAAGACCGTTTAACCCTACTT
Patient #2  GGGTCACAGAAAGACGCCGTTAACAGACGGTAAACTACTT
Patient #3  GGGTCACAGAAAGACCGTTAACCCTACTT
Patient #4  GGGTCACAGAAAGACCGTTAACCCTACTT
Patient #5  GGGTCACAGAAAGACCGTTAACCCTACTT
Patient #6  GGGTCACAGAAAGACGGTTAACCCTACTT
Patient #7  GGGTCACAGAAAGACGGTTACCAGTTTGAAACCCTACTT
Patient #8  GGGTCACAGAAAGACGGTTTAACCCTACTTCCTGG
```

TAL1 mRNA expression as determined by quantitative polymerase chain reaction (PCR) and expressed as percentage of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
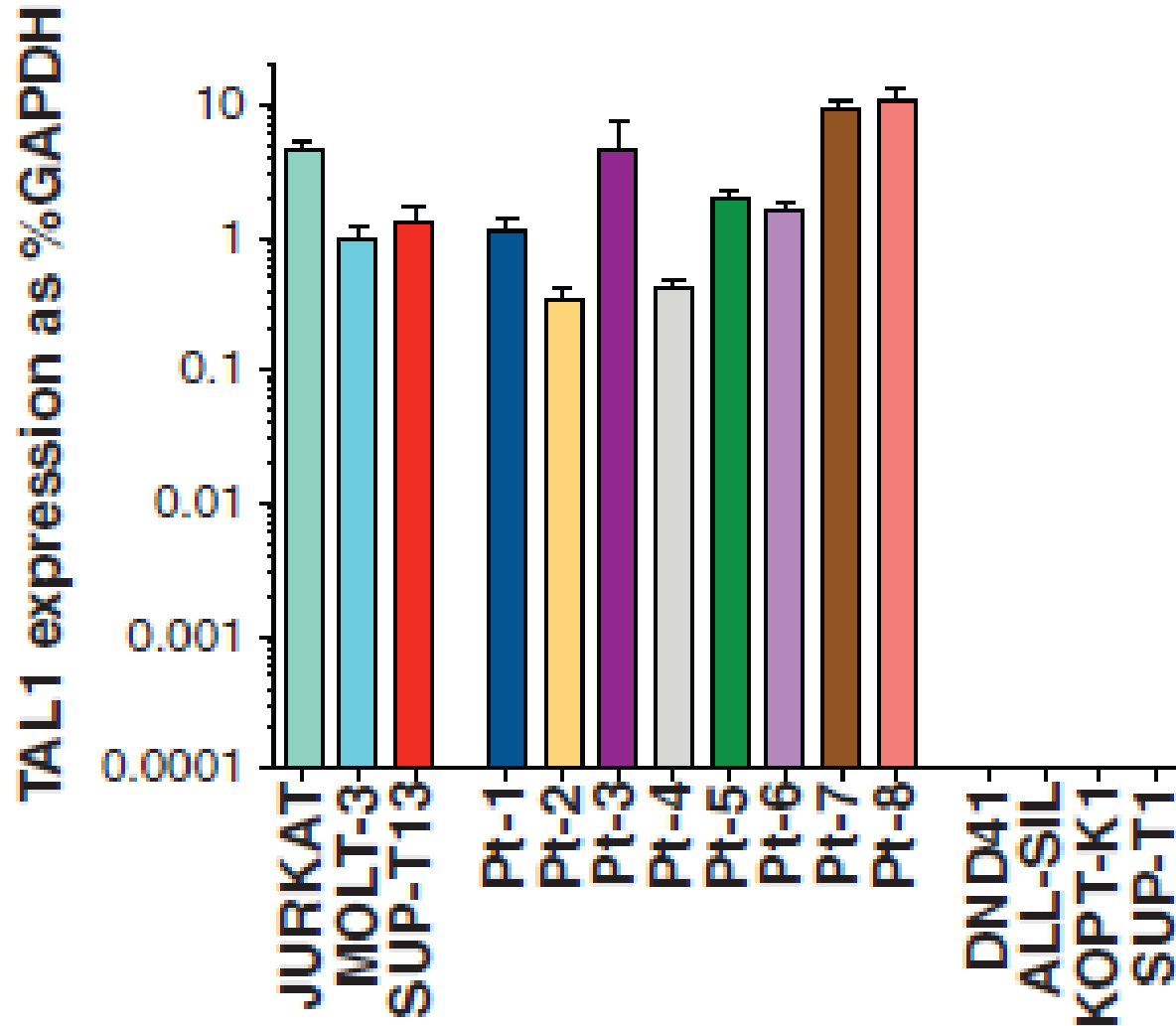
Mutations in TAL1 SE show consensus sequence for MYB, transcription factor.


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
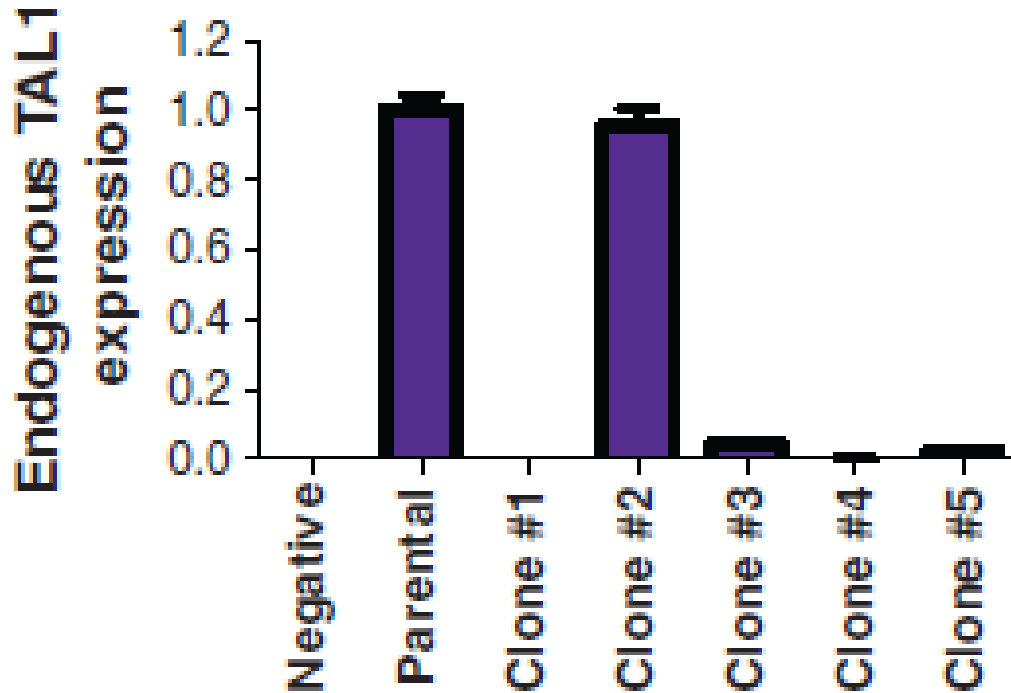MYB binds the mutant TAL1 enhancer site and is a member of the TAL1 complex
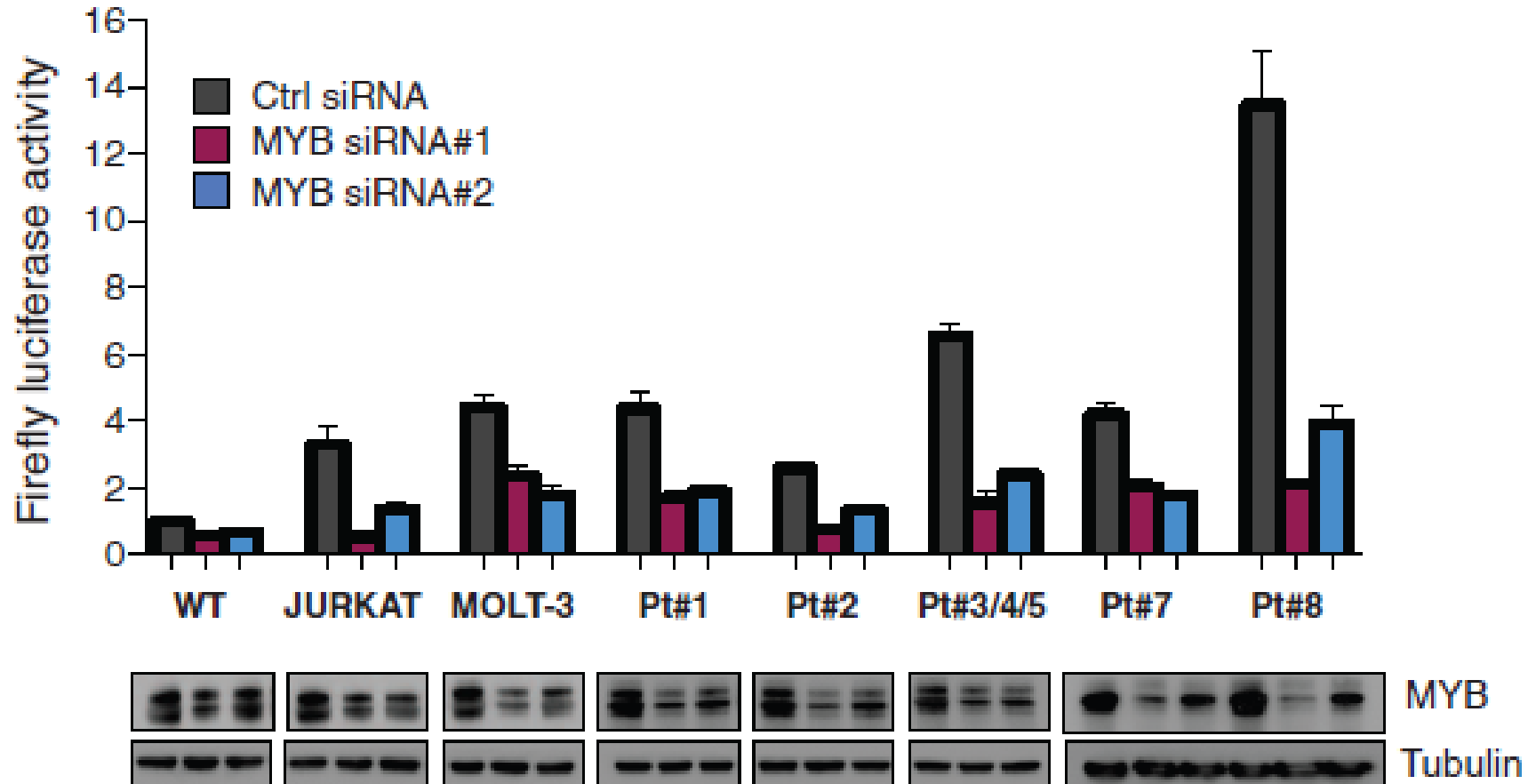
MYB binds the mutant TAL1 enhancer (MuTE) site and is a member of the TAL1 complex
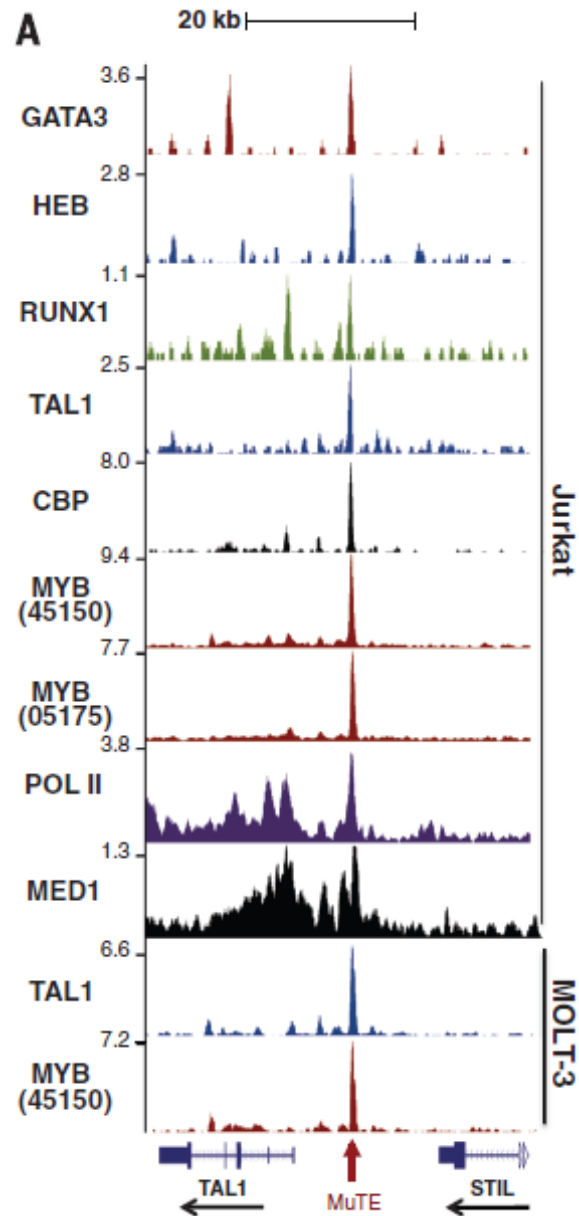
Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1
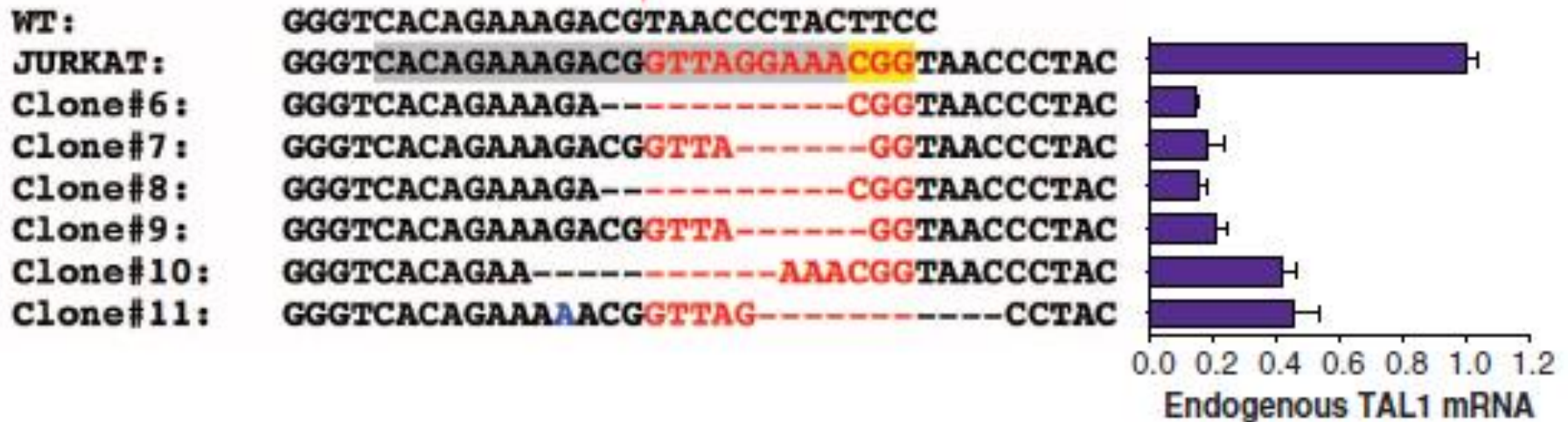

Deletion of the wild type allele had no effect on endogenous TAL1 mRNA levels, but deletion of the mutant allele completely abrogated endogenous TAL1 expression
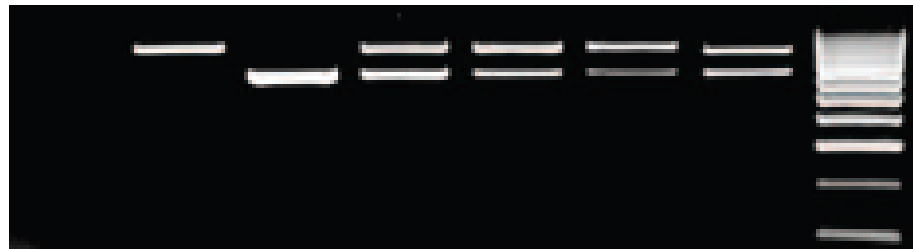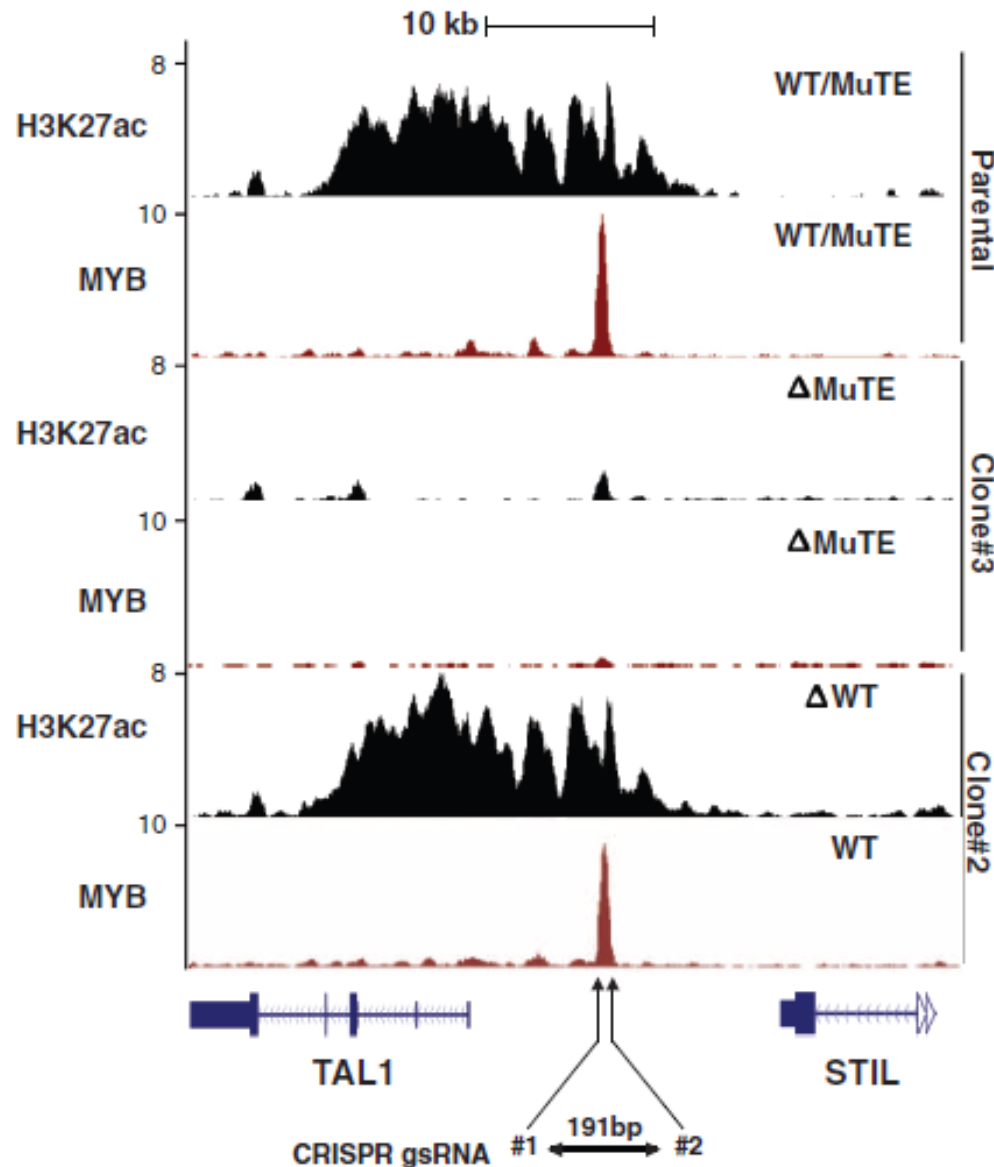
Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1

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