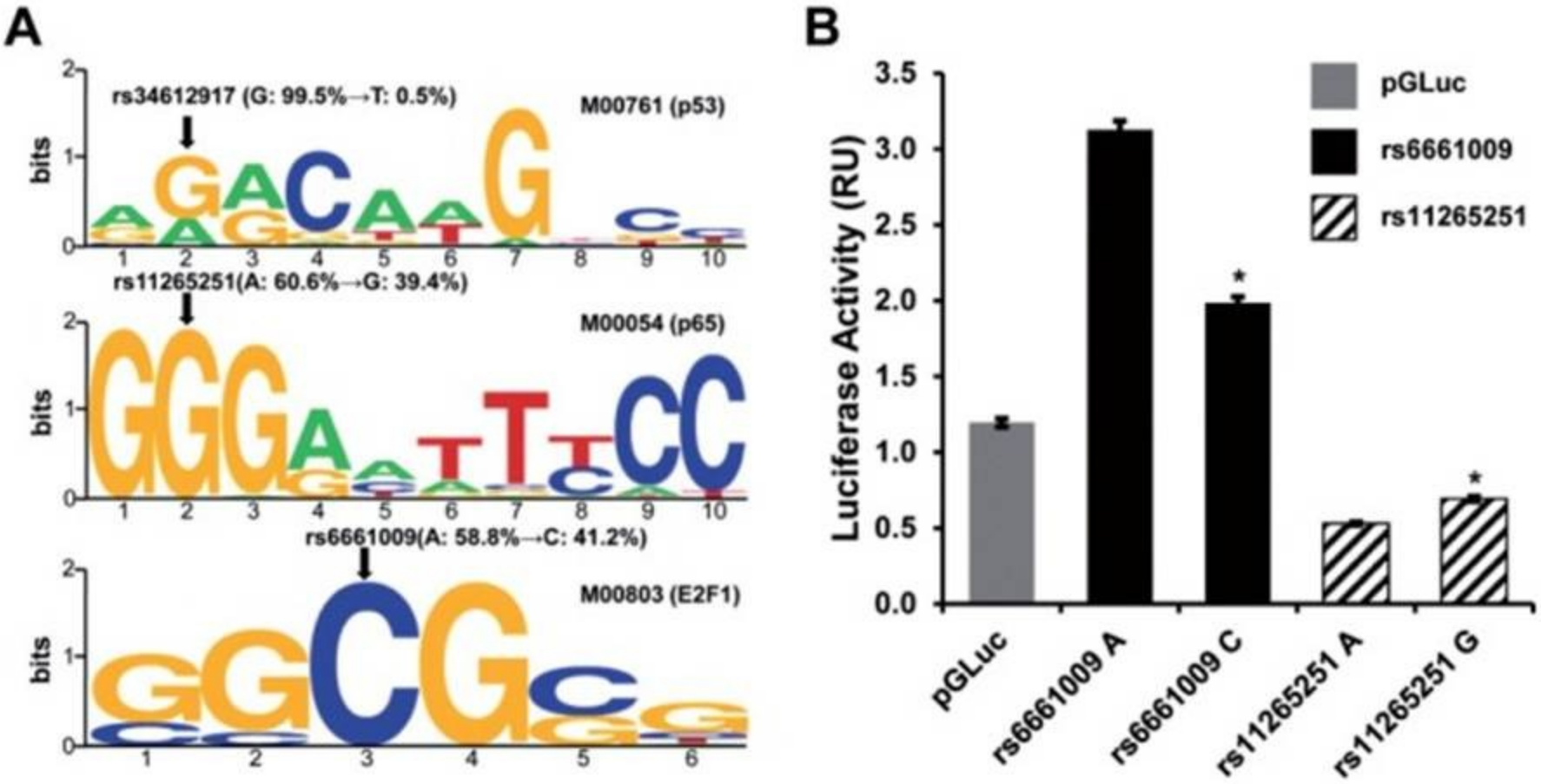

A
rs34612917 (G: 99.5%→T: 0.5%)
M00761 (p53)
bits
rs11265251(A: 60.6%→G: 39.4%)
M00054 (p65)
rs6661009(A: 58.8%→C: 41.2%)
M00803 (E2F1)
B
Luciferase Activity (RU)
pGLuc
rs6661009
rs11265251
pGLuc
rs6661009 A
rs6661009 C
rs11265251 A
rs11265251 G

- [ ] a. it is a functional assay where the SNP rs6661009 C gives a significant increase of the transcription with respect of variant A. The other SNP rs11265251G leads to a significant decrease in transcription.
- [ ] b. The single nucleotide variation in the consensus sequence does not affect the trascription factor binding and the luciferace gene reporter activity is the same.
- [x] c. the mutation in E2F1 binding site (rs6661009 A-->C) gives a significative decrease in the luciferase activity with the mutation (so gene transcription is decreased). Instead, in the mutation in p65 binding site (rs11265251A-->G) gives a signifiative increase in the luciferase activity with the mutation (so gene transcription in increased) ✓
- [ ] d. For p65 they change the second A in a G and they obtain a significative reduction in the luciferase activity. For E2F1 they change the third A in a C and they obtain a significative increase in the the luciferase activity.
- [x] e. The single nucleotide variation in the consensus sequence changes the expression of the trascription factors as showing by luciferace assay. ✗

OVERVIEW OF MOLECULAR APPROACHS TO STUDY SUPER-ENHANCERS

LUCIFERASE ACTVITY WITH VECTOR THAT CONTAINS COSTITUENT ENHANCER → ROLE OF SE IN GENE TRANSCRIPTION REGULATION

CRISPR-CAS9 SYSTEM TO DELETE COSTITUENT ENHANCER

- ROLE OF SE IN GENE EXPRESSION
- ROLE OF SE IN TRANSCRIPION FACTOR BINDING
- ROLE OF SE IN CHROMATIN REMODELING

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

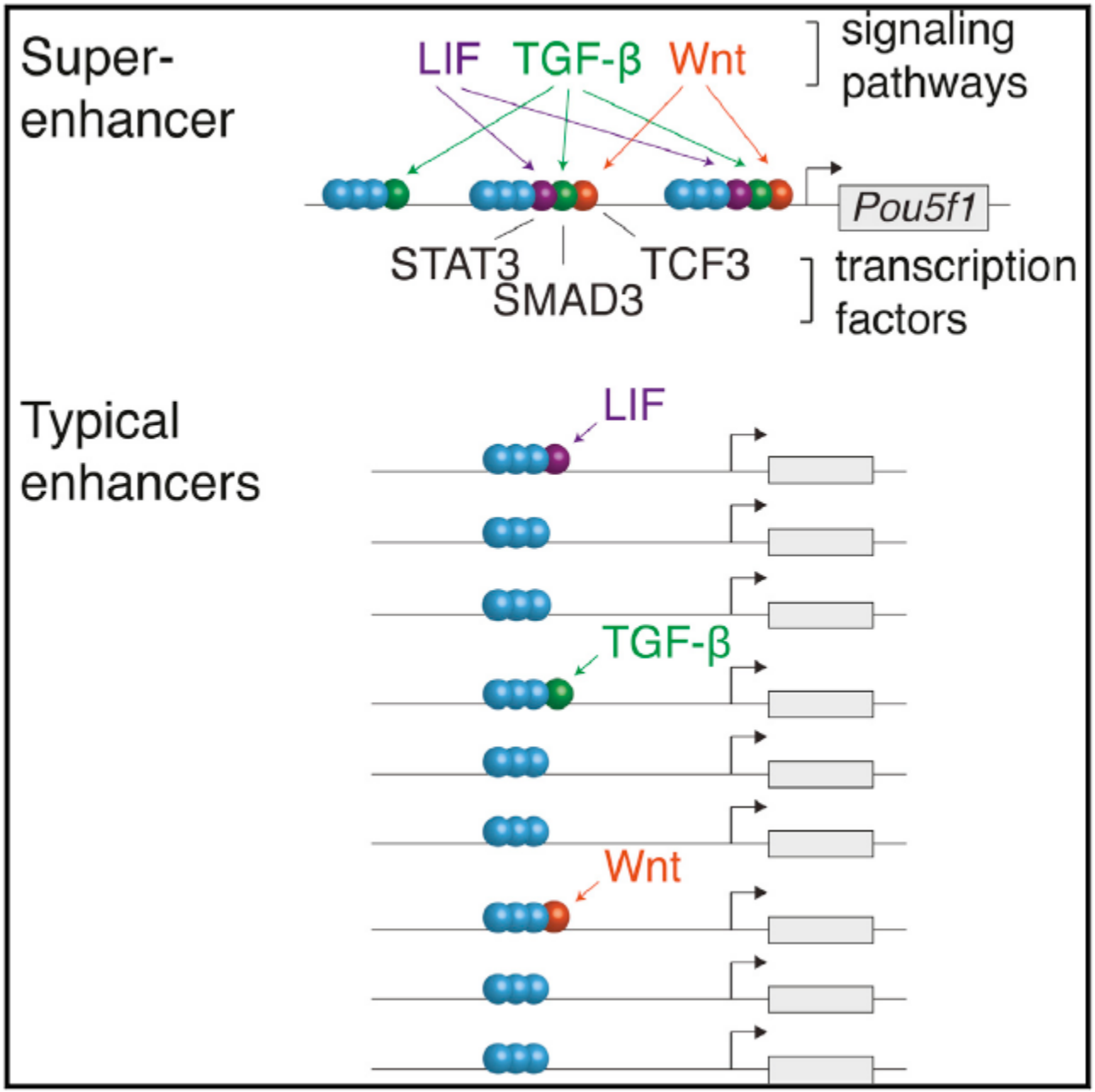
AIM

Super-enhancers (SE)
Functional costituent units

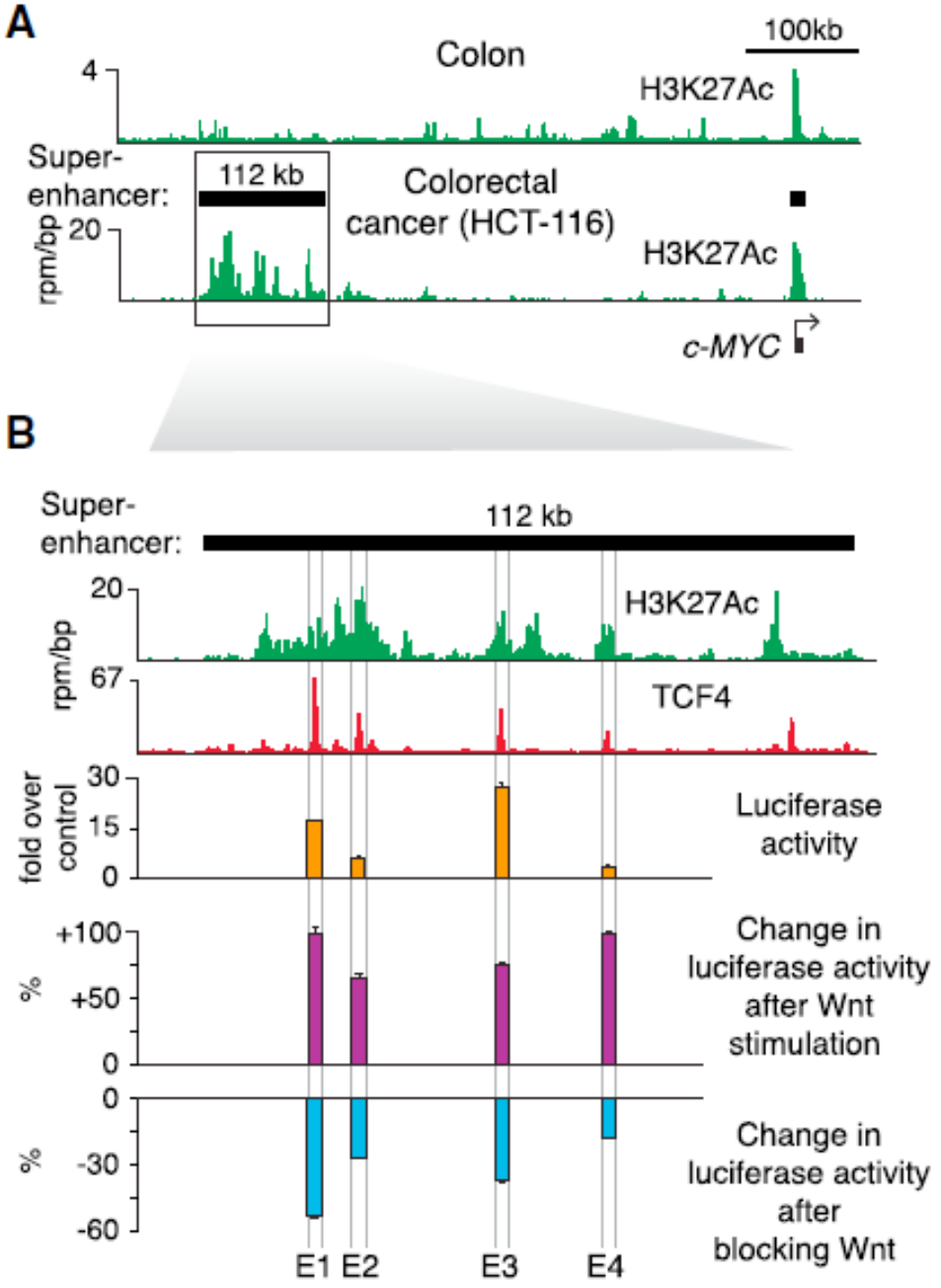
Cancer cells SE target
for oncogenic signalling

CONCLUSION

Graphical Abstract



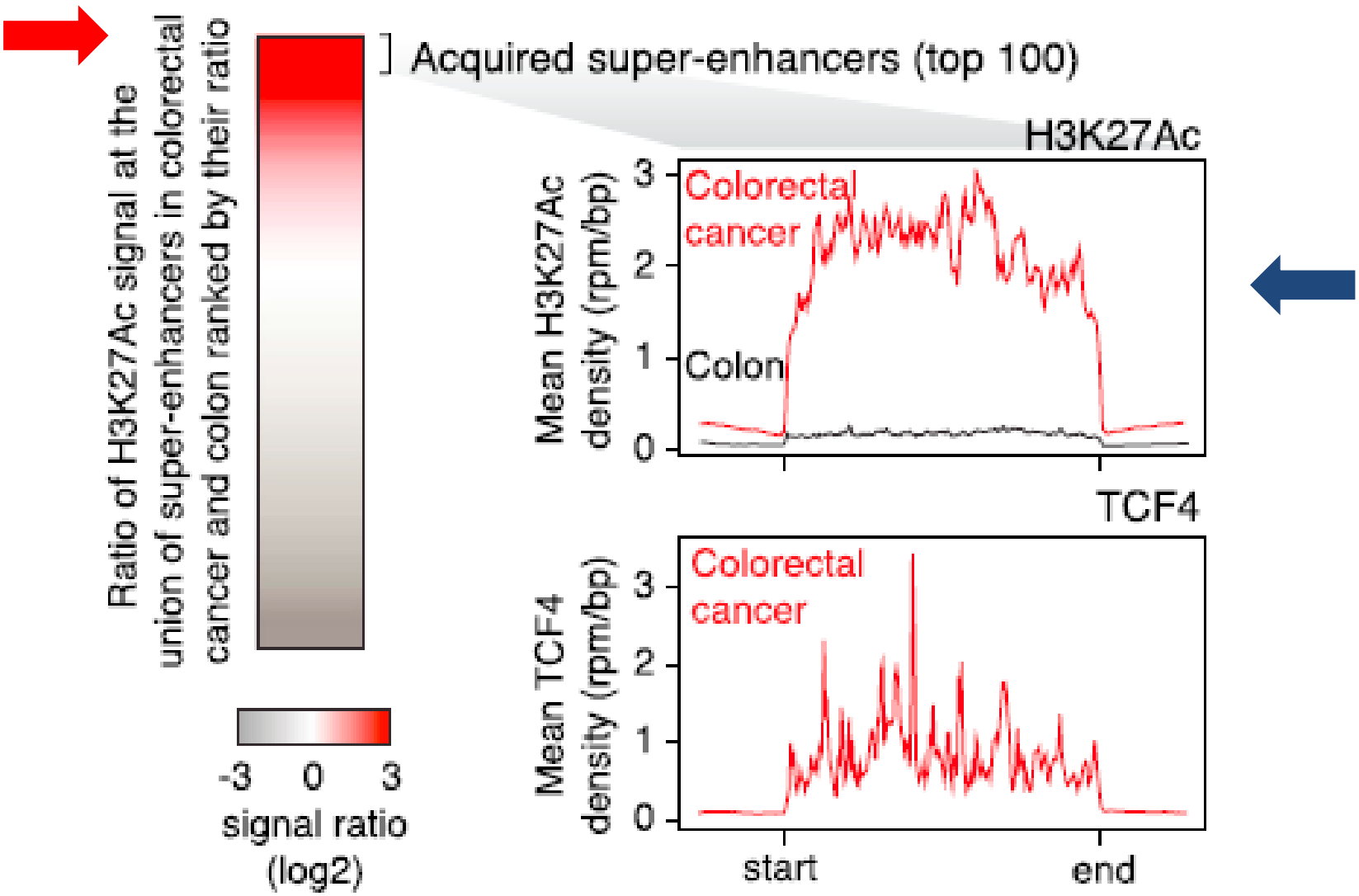
SE function on c-Myc locus



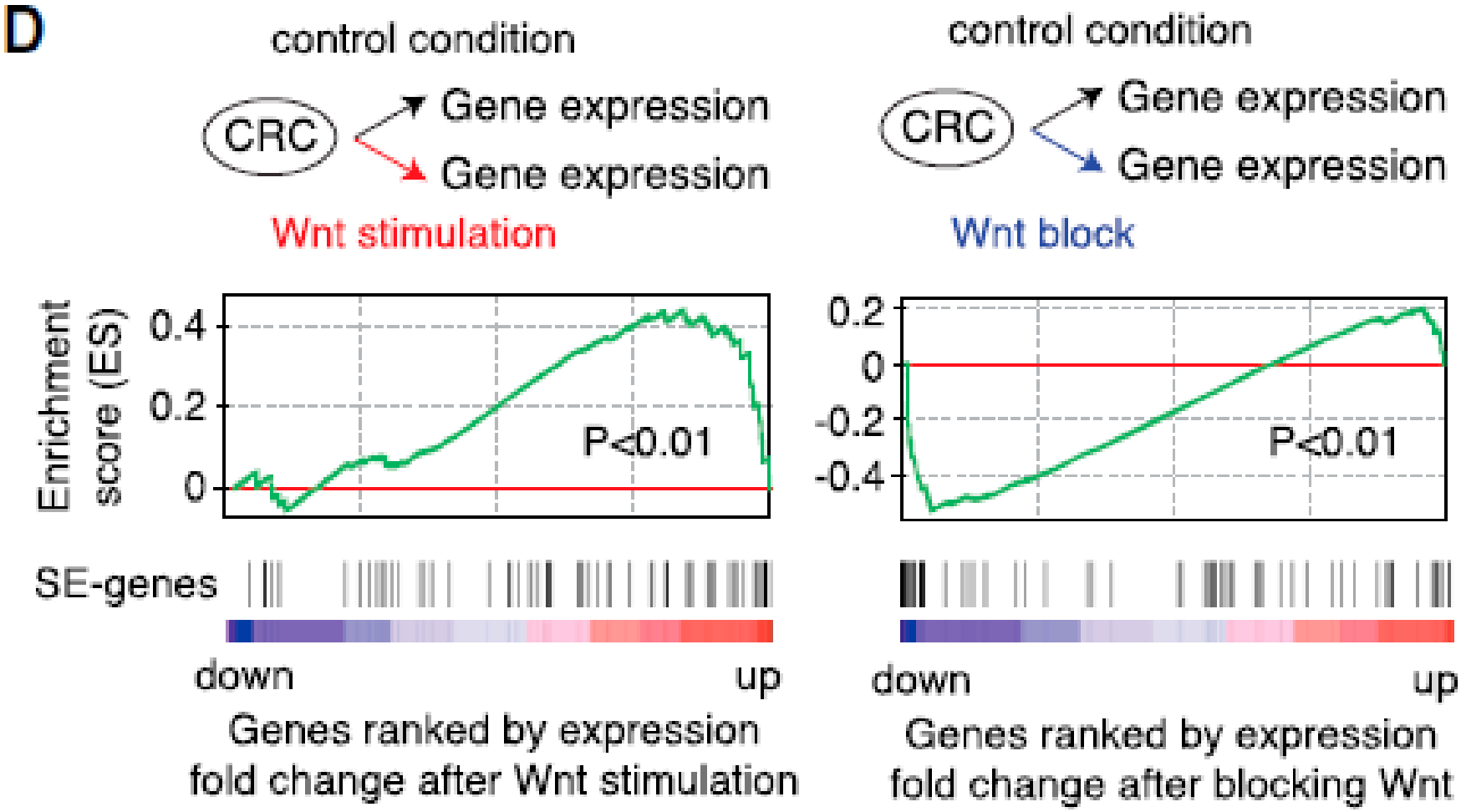
ChIP-seq binding profiles for H3K27Ac at the c-MYC locus in colon and colorectal cancer cells (HCT-116).

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.

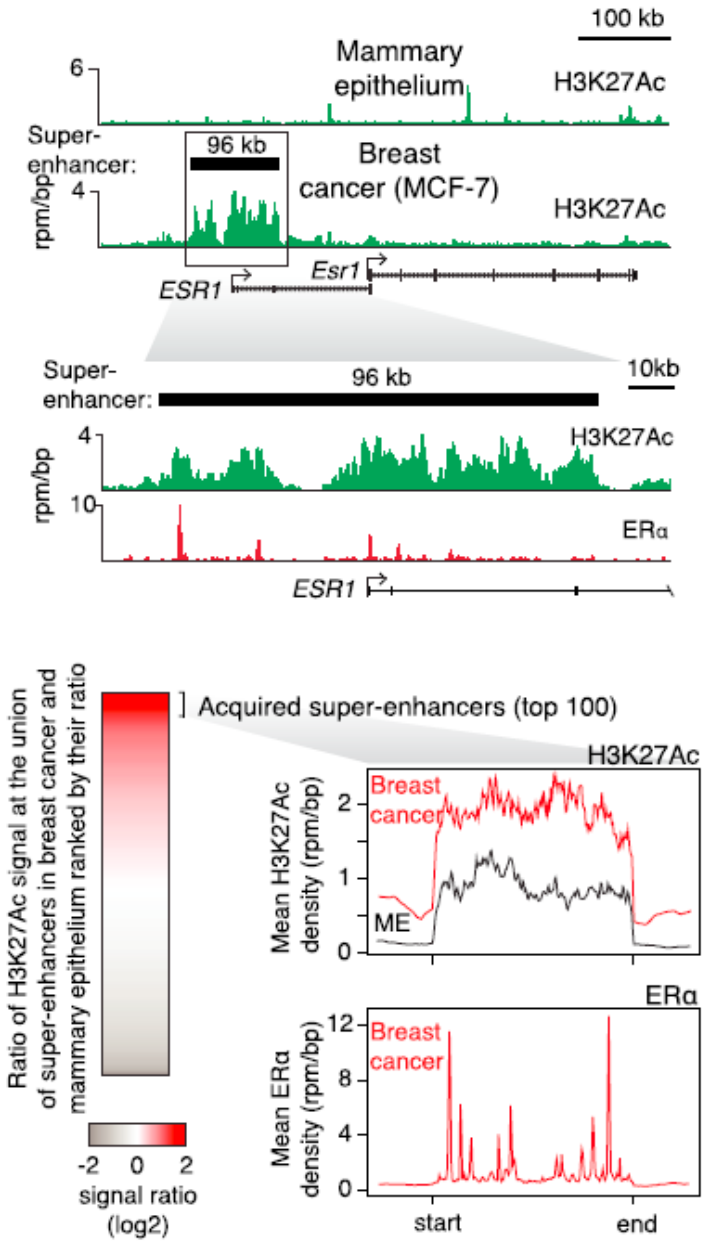
Left: ratio of H3K27Ac in CRC (HCT-116) versus normal colon tissue used densities at the union of SEs identified in the two samples. **Right:** metagene representation of H3K27Ac and TCF4 ChIP-seq densities at the regions corresponding to the top 100 acquired super-enhancers.



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-



SEs in breast cancer cell lines



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

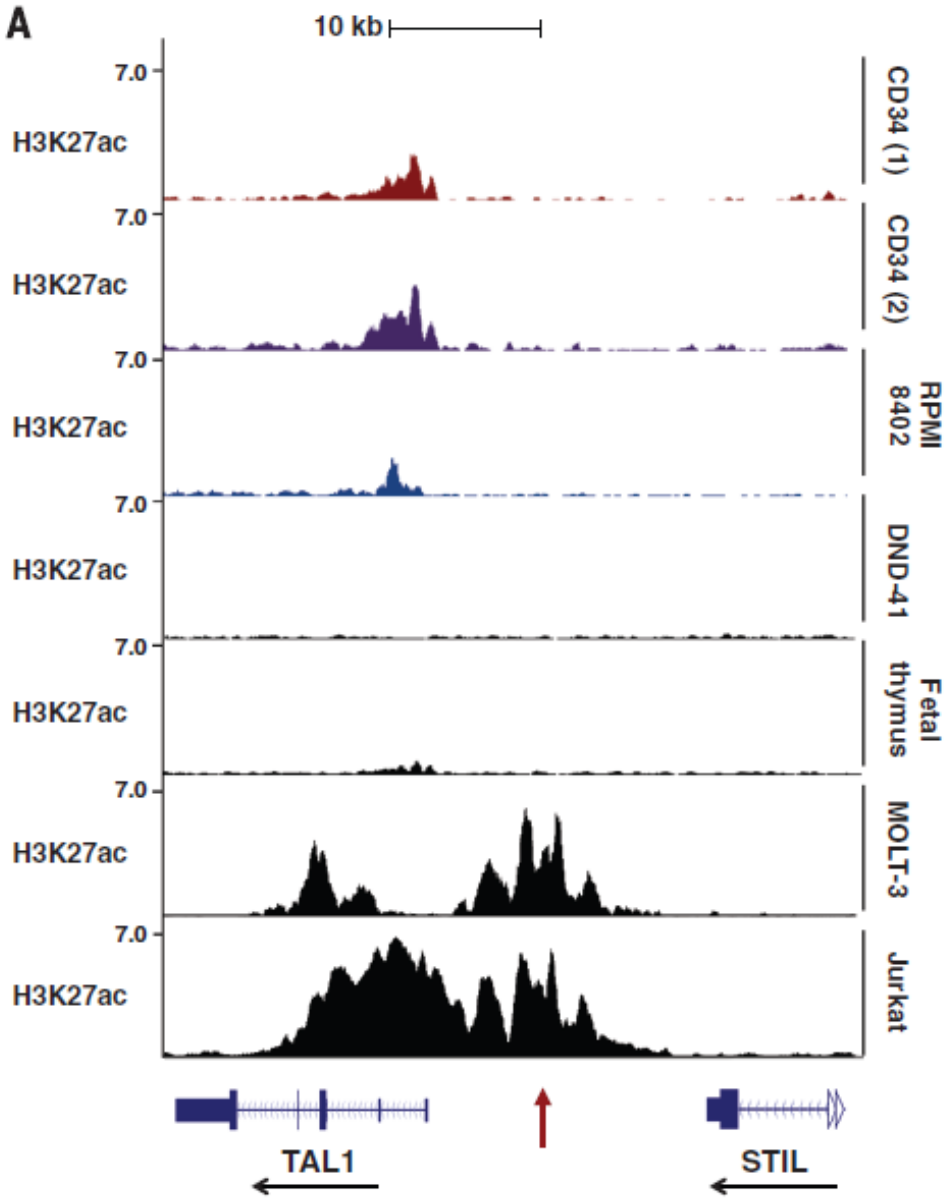
Super-enhancers (SE) upstream TAL1

MYB form Leukemogenic Transcriptional Complex

MYB binds T-ALL cells SEs

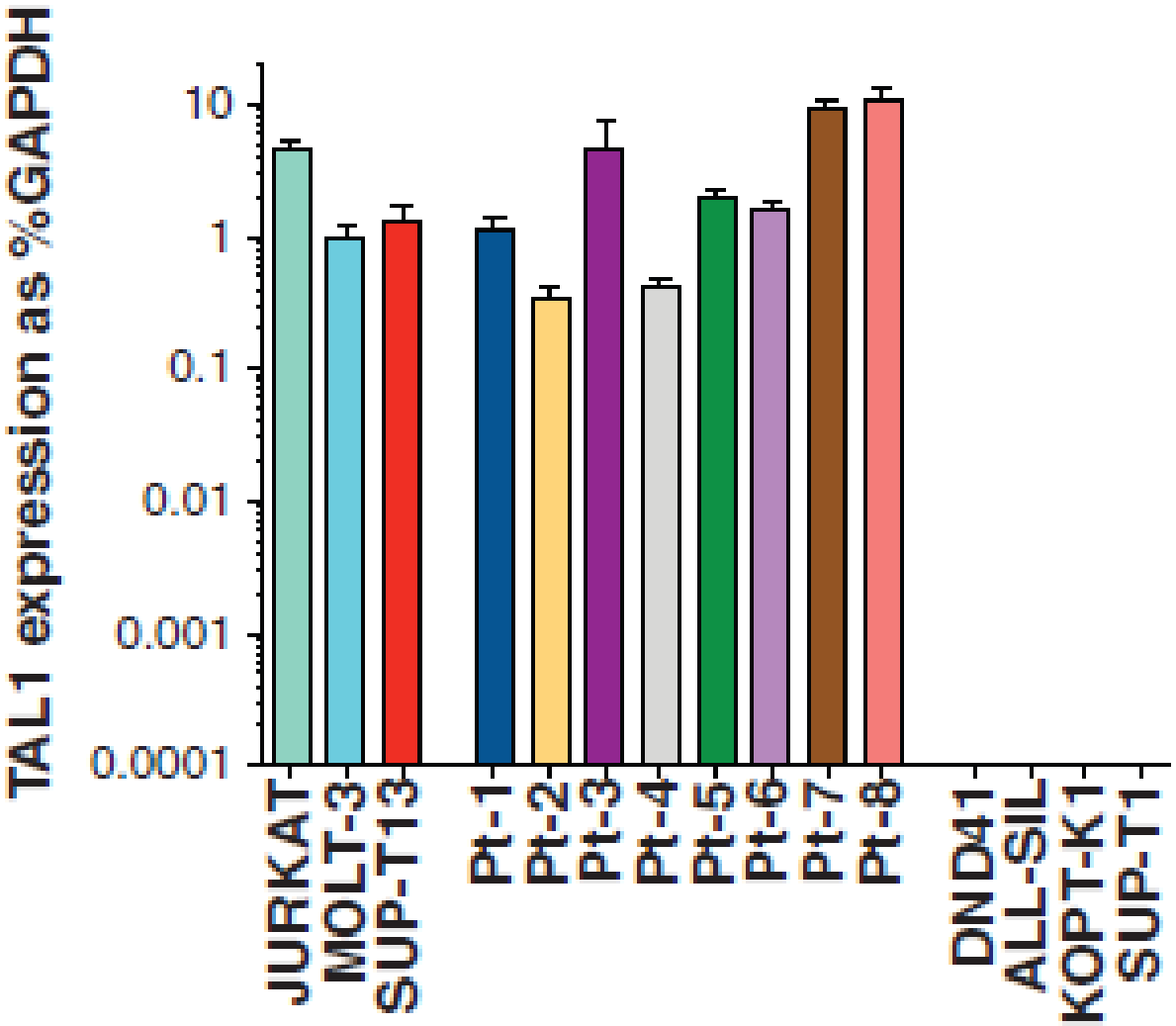
CONCLUSION

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).



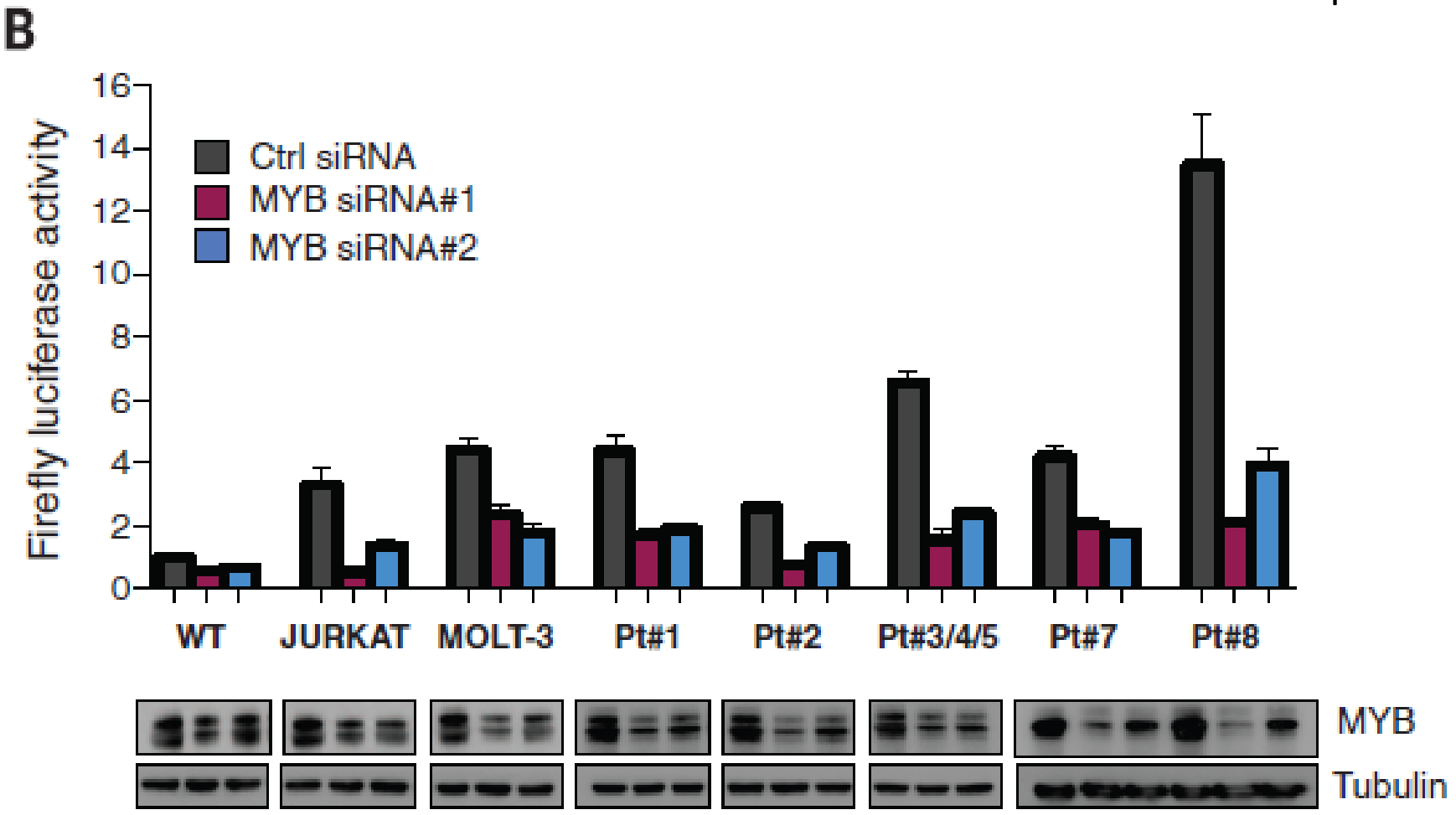
Mutations of the TAL1 enhancer activate through recruitment of MYB.

A Myb primary motif

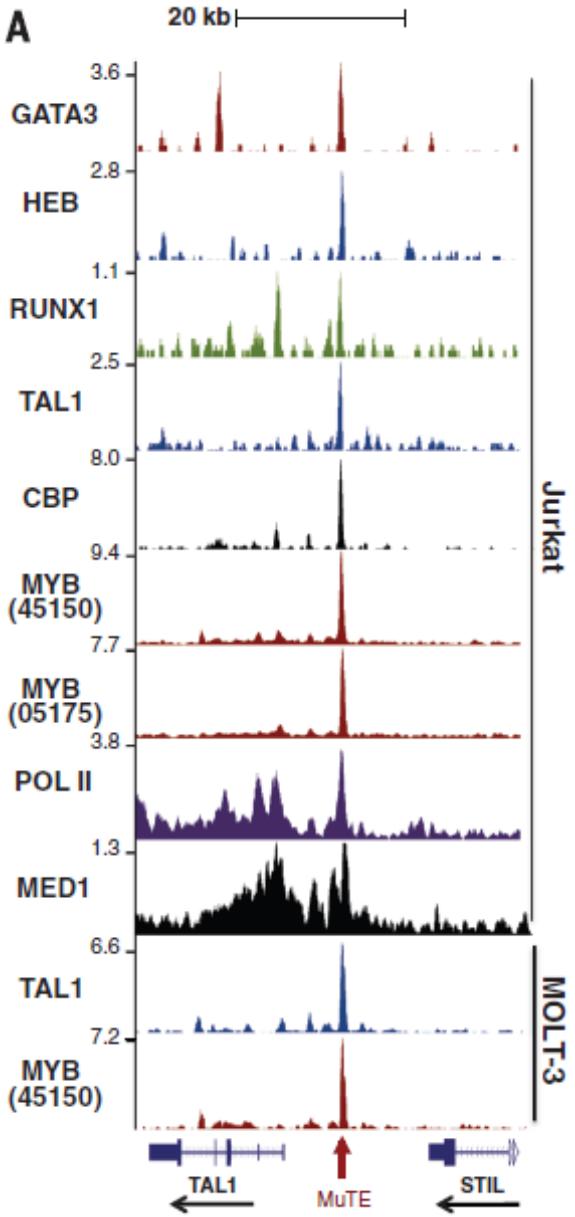
WT	GACGTA
Jurkat	[GACGGTTA]GGA[AACGGTA]
MOLT-3	GACGGTTA
Patient #1	GACCGTTTA
Patient #2	GCCGTTA
Patient #3	GACCGTTA
Patient #4	GACCGTTA
Patient #5	GACCGTTA
Patient #6	GACGGTTA
Patient #7	GACGGTTA
Patient #8	GACGGTTTA

TAL1 enhancer TRANSCRIPTION ACTIVITY USING LUCIFERASE ASSAY

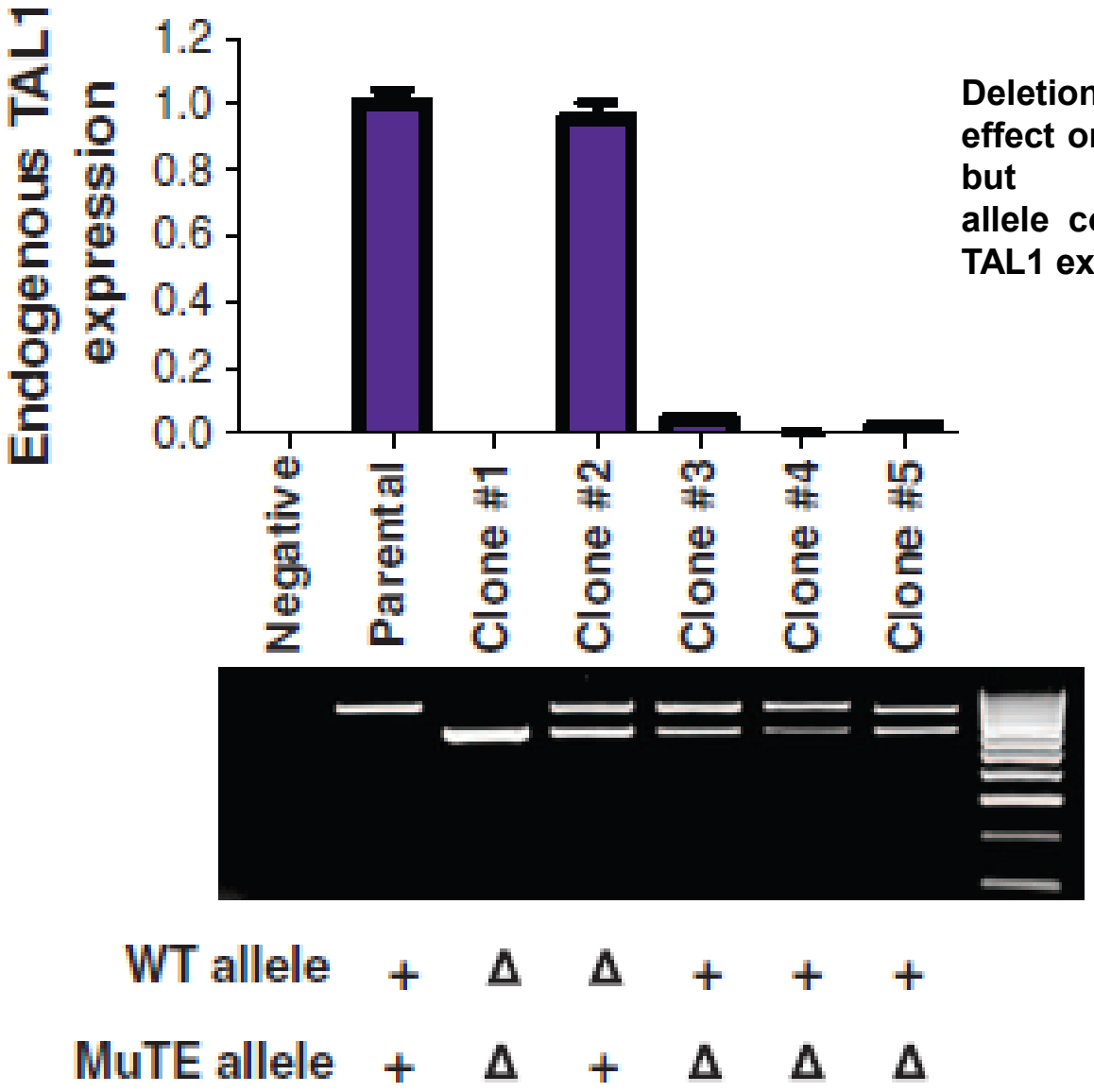
MYB binds the mutant TAL1 enhancer site and is a member of the TAL1 complex



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

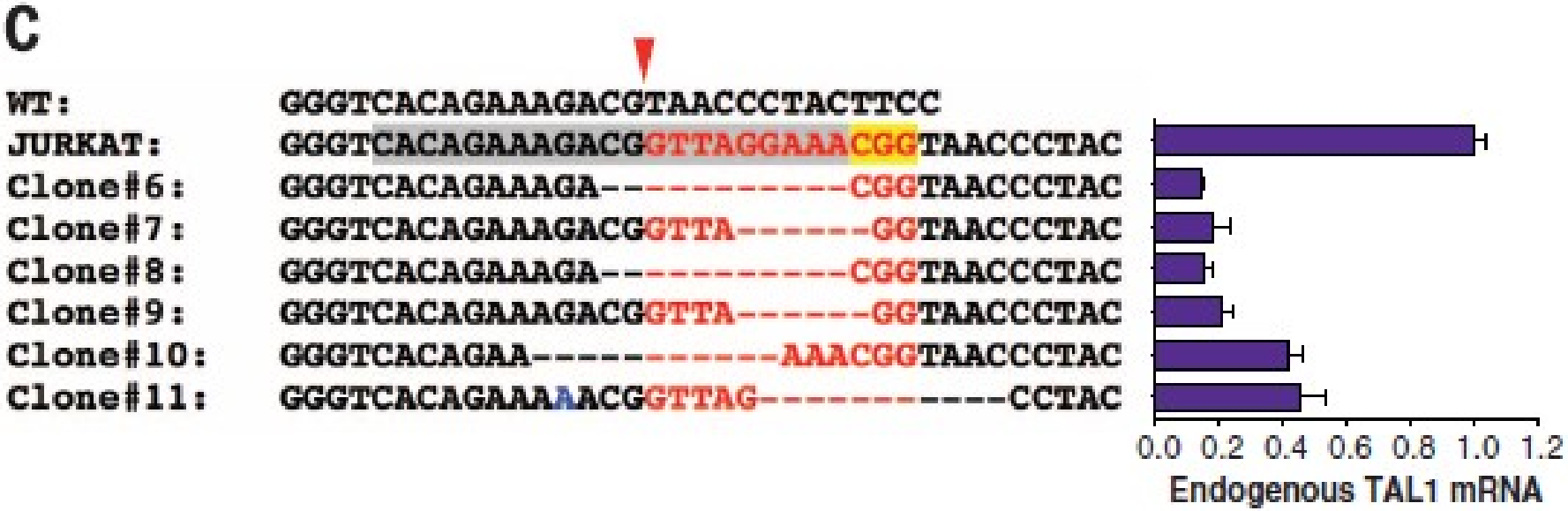


Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1

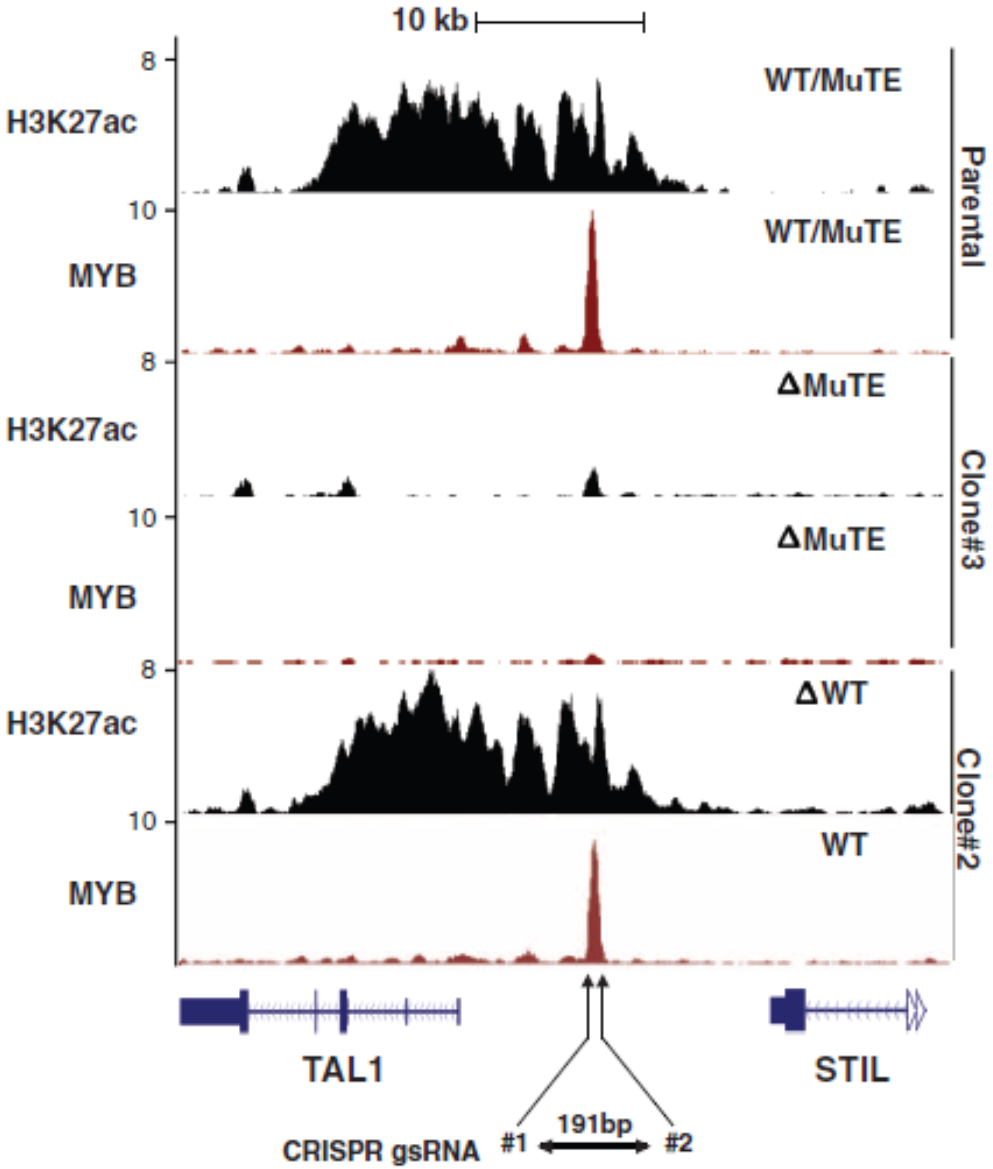


Deletion of the wild type allele had no effect on endogenous TAL1 mRNA levels, but deletion of the mutant allele completely abrogated endogenous TAL1 expression

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

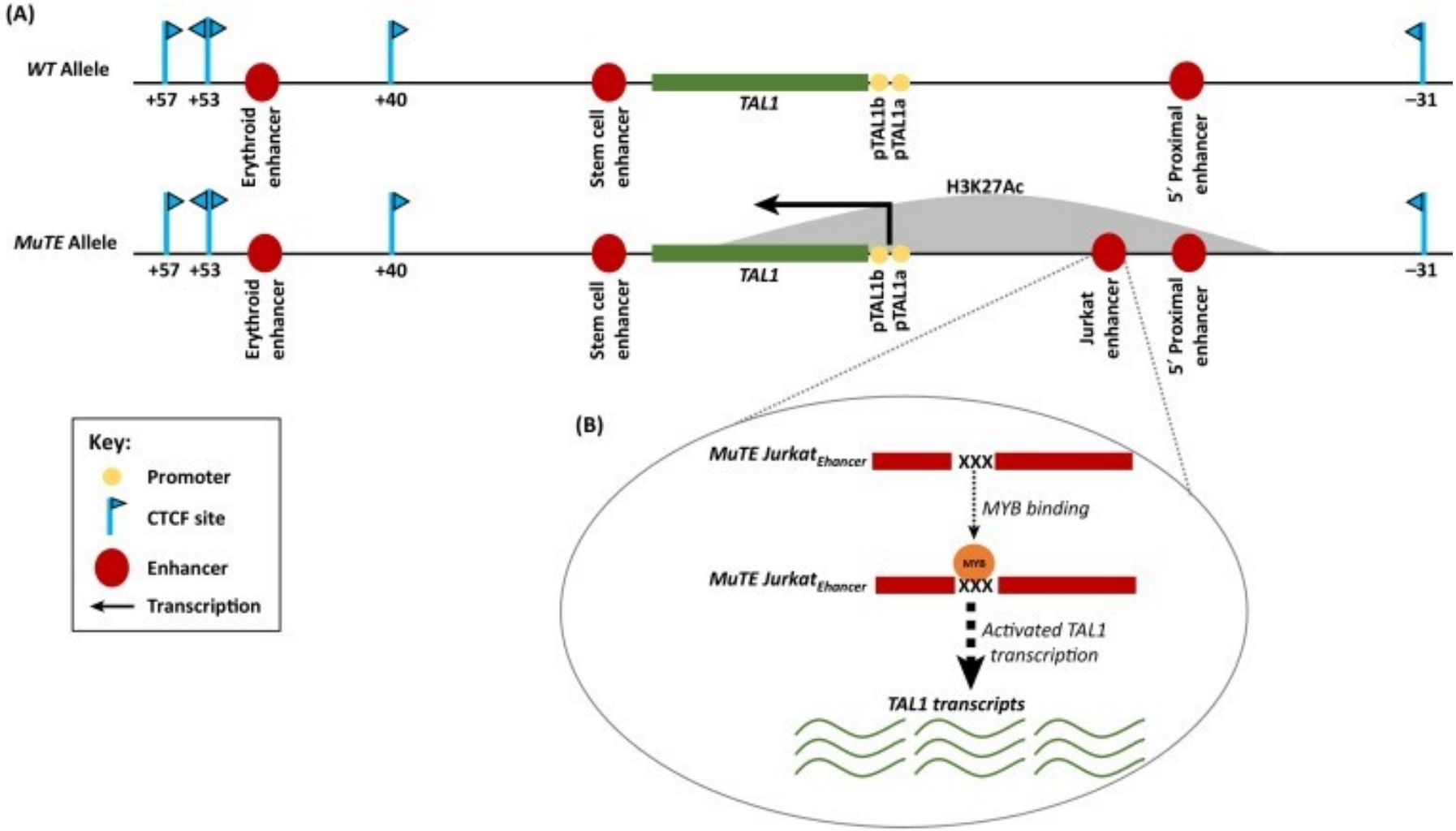


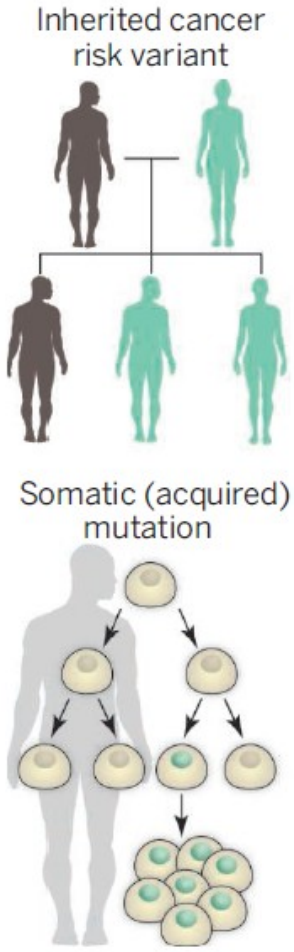
ChIP-seq tracks for H3K27ac and MYB at the STIL-TAL1 locus from selected CRISPR-Cas9 clones



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

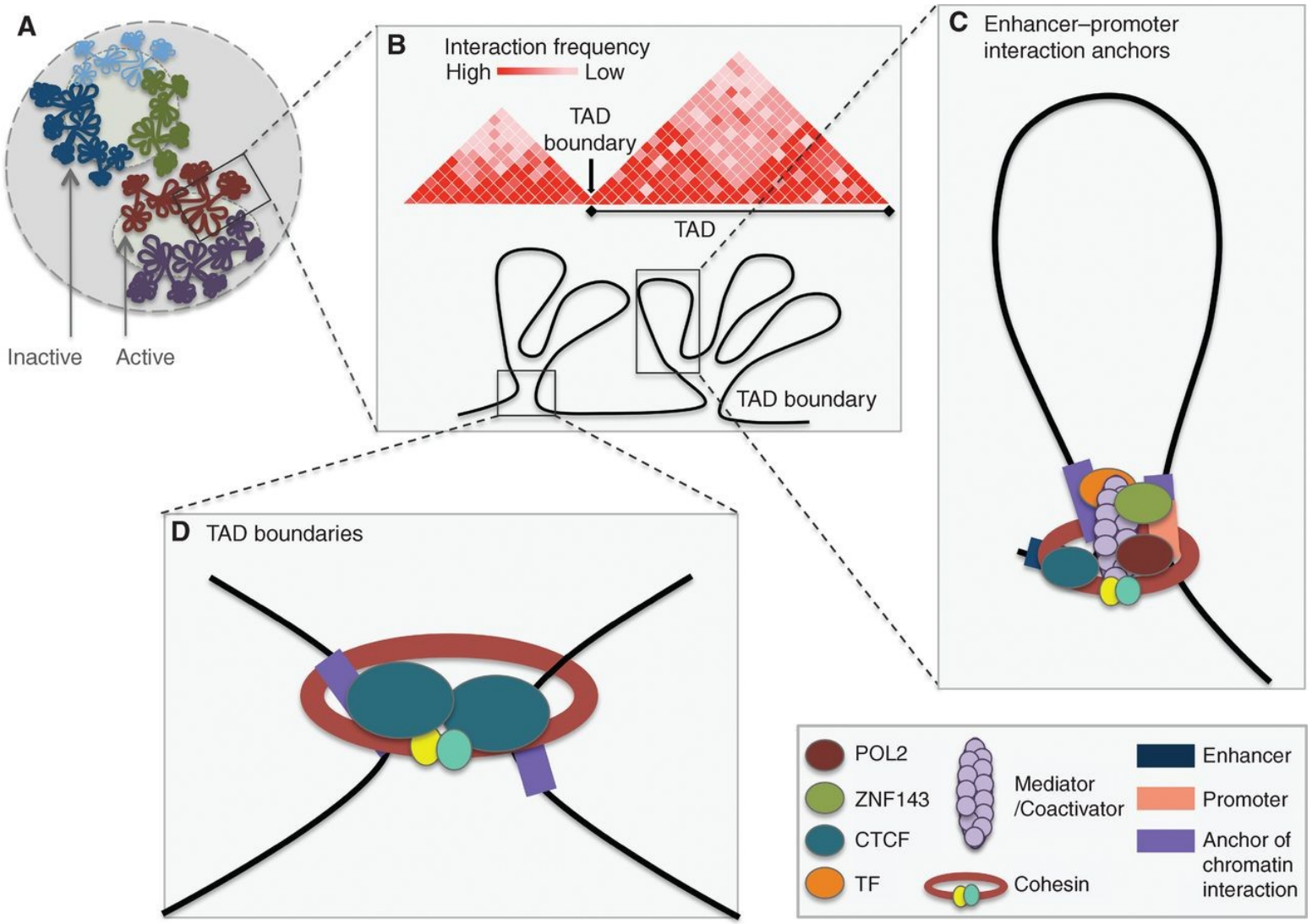




Genetics of cancer. Both inherited variants (**top**) and acquired mutations (**bottom**) can contribute to tumorigenesis.

Our findings show that **somatic mutation of noncoding intergenic elements** can lead to **binding** of master transcription factors, such as **MYB**, which in turn aberrantly initiate super-enhancers that mediate overexpression of oncogenes. This raises the possibility that **acquisition of such enhancer mutations may constitute a general mechanism of carcinogenesis** used in other types of human cancers. Mechanisms of aberrant superenhancer formation in malignancy have broad implications not only for molecular pathogenesis but also for clinical management. Drugs that target key components of the transcriptional machinery, such as BRD4 and CDK7, have recently been shown to preferentially target tumor-specific super-enhancers, which provides a novel strategy to capitalize on these abnormalities for improved cancer therapy.

GENE REGULATION:
ROLE OF LONG RANGE INTERACTIONS



Activation of proto-oncogenes by disruption of chromosome neighborhoods

Denes Hnisz,[1*] Abraham S. Weintraub,[1,2*] Daniel S. Day,[1] Anne-Laure Valton,[3] Rasmus O. Bak,[4] Charles H. Li,[1,2] Johanna Goldmann,[1] Bryan R. Lajoie,[3] Zi Peng Fan,[1,5] Alla A. Sigova,[1] Jessica Reddy,[1,2] Diego Borges-Rivera,[1,2] Tong Ihn Lee,[1] Rudolf Jaenisch,[1,2] Matthew H. Porteus,[4] Job Dekker,[3,6] Richard A. Young[1,2]†

Oncogenes are activated through well-known chromosomal alterations such as gene fusion, translocation, and focal amplification. In light of recent evidence that the control of key genes depends on chromosome structures called insulated neighborhoods, we investigated whether proto-oncogenes occur within these structures and whether oncogene activation can occur via disruption of insulated neighborhood boundaries in cancer cells. We mapped insulated neighborhoods in T cell acute lymphoblastic leukemia (T-ALL) and found that tumor cell genomes contain recurrent microdeletions that eliminate the boundary sites of insulated neighborhoods containing prominent T-ALL proto-oncogenes. Perturbation of such boundaries in nonmalignant cells was sufficient to activate proto-oncogenes. Mutations affecting chromosome neighborhood boundaries were found in many types of cancer. Thus, oncogene activation can occur via genetic alterations that disrupt insulated neighborhoods in malignant cells.

Oncogenes are activated through well-known chromosomal alterations such as gene fusion, translocation, and focal amplification. In light of recent evidence that the control of key genes depends on chromosome structures called insulated neighborhoods, we investigated whether proto-oncogenes occur within these structures and whether oncogene activation can occur via disruption of insulated neighborhood boundaries in cancer cells. We mapped insulated neighborhoods in T cell acute lymphoblastic leukemia (T-ALL) and found that tumor cell genomes contain recurrent microdeletions that eliminate the boundary sites of insulated neighborhoods containing prominent T-ALL proto-oncogenes. Perturbation of such boundaries in nonmalignant cells was sufficient to activate proto-oncogenes. Mutations affecting chromosome neighborhood boundaries were found in many types of cancer. Thus, oncogene activation can occur via genetic alterations that disrupt insulated neighborhoods in malignant cells.

BACKGROUND

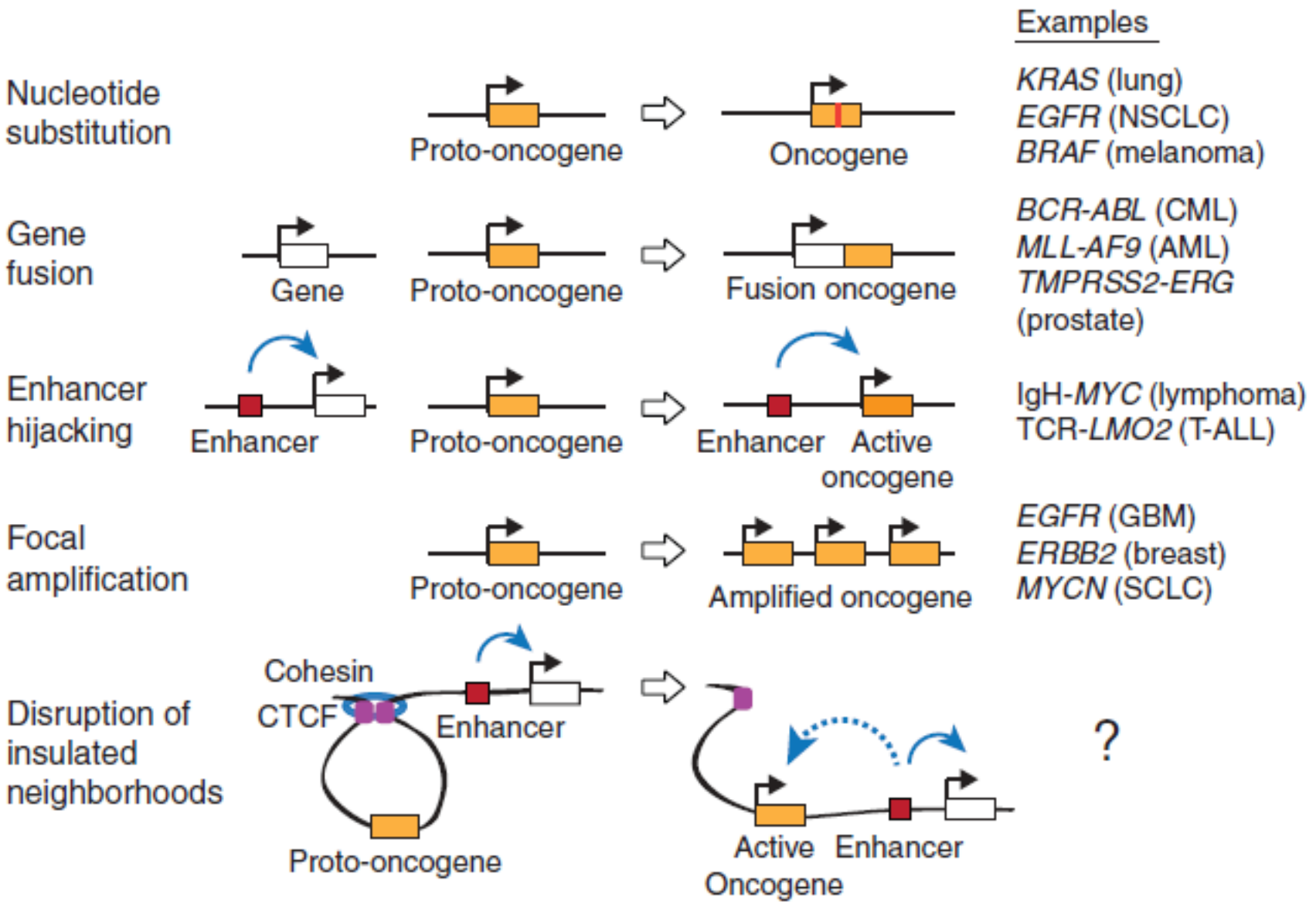
Focus

Maps of boundaries and Mutations

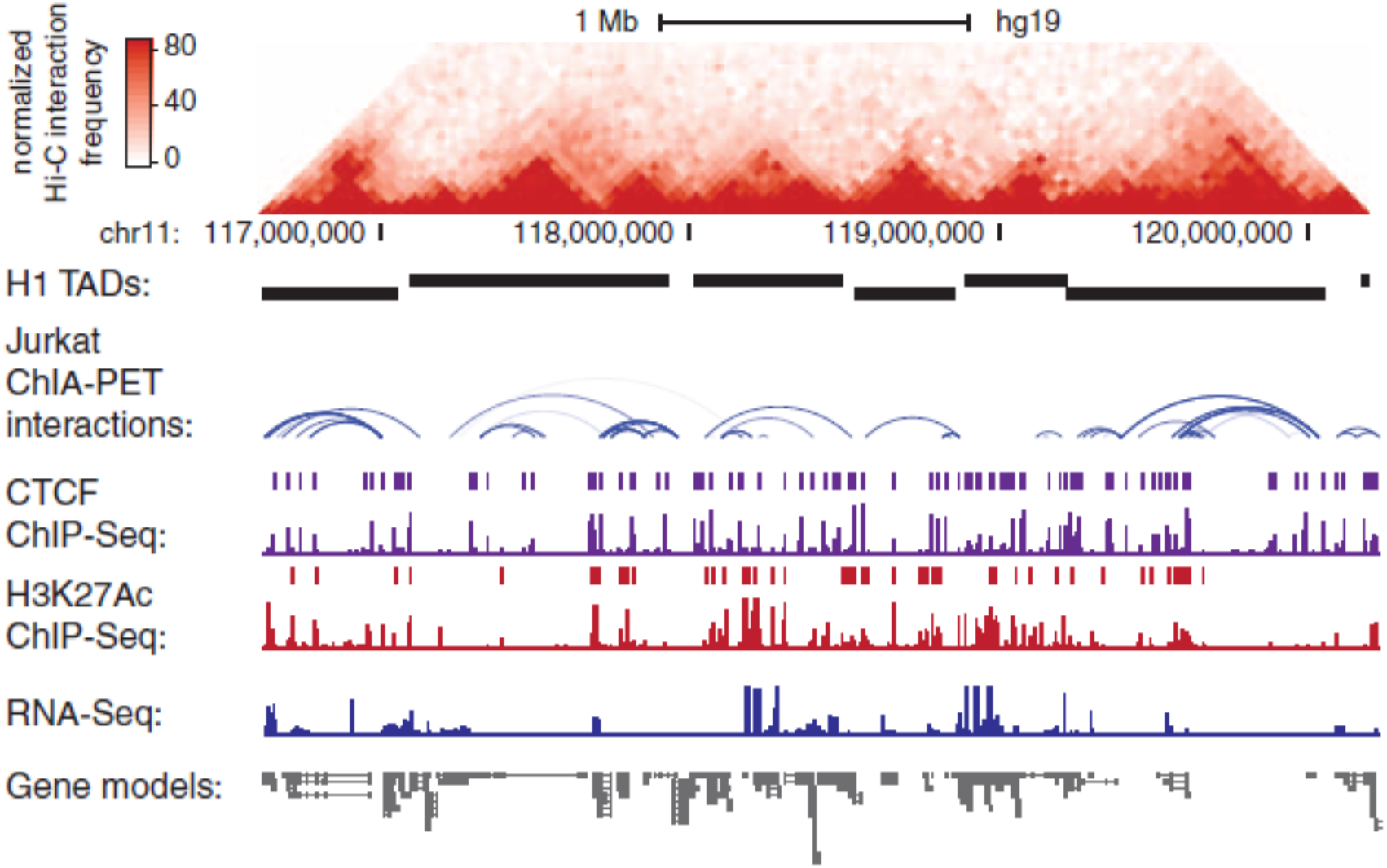
Mute in boundaries Are in Cancer

CONCLUSION

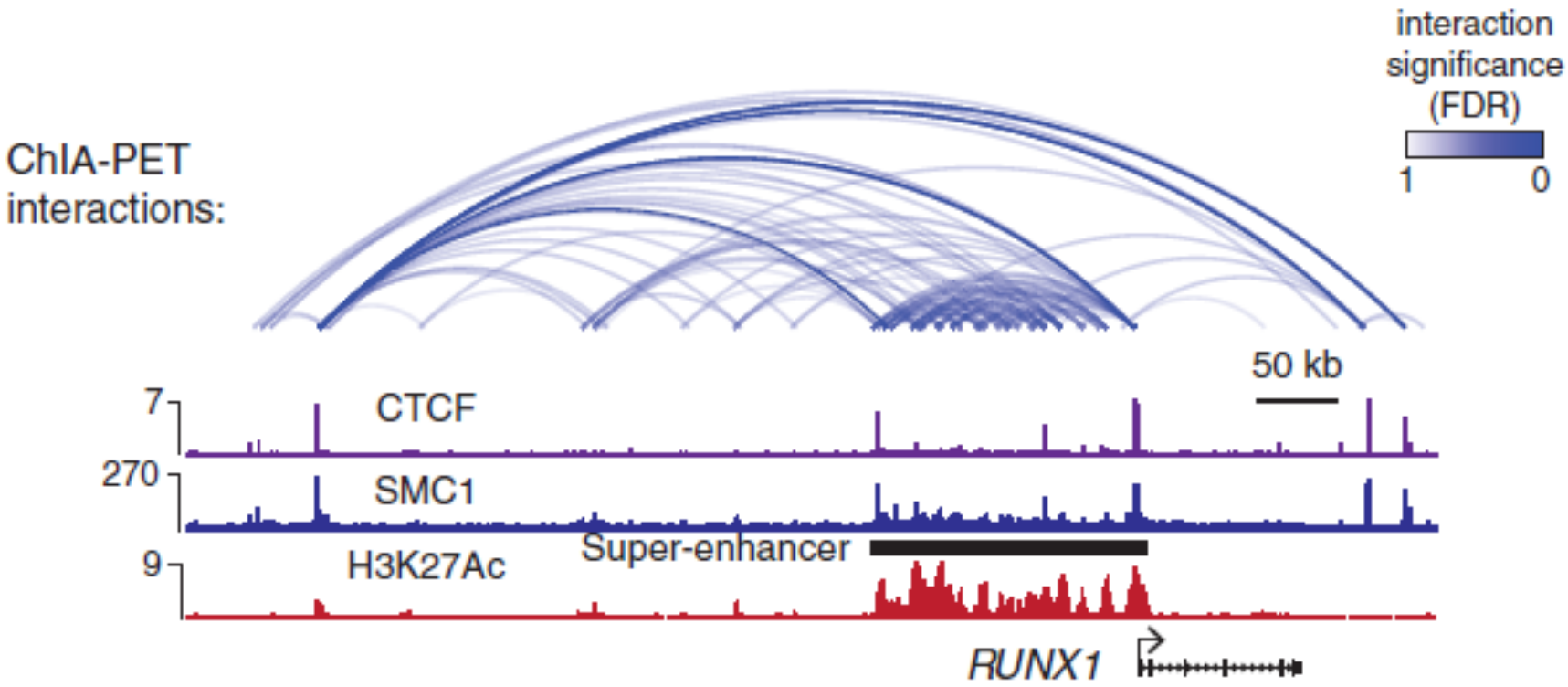
Mutations in oncogene activation

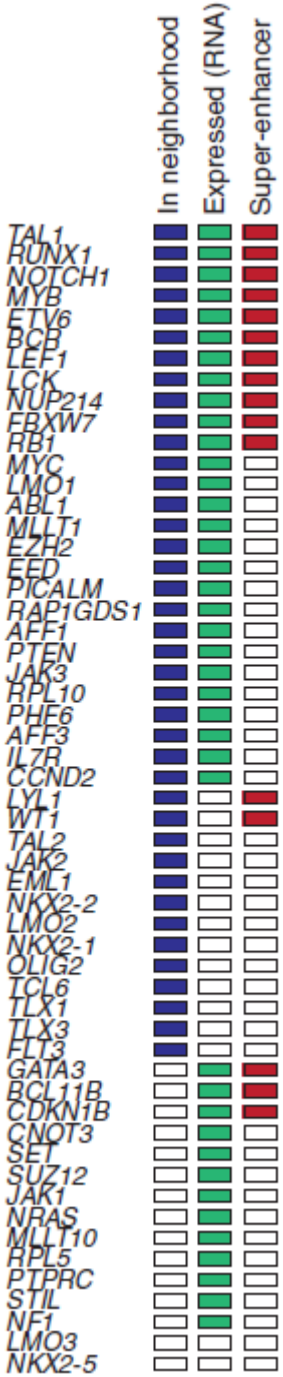


Map of the three-dimensional (3D) regulatory landscape of a tumor cell genome



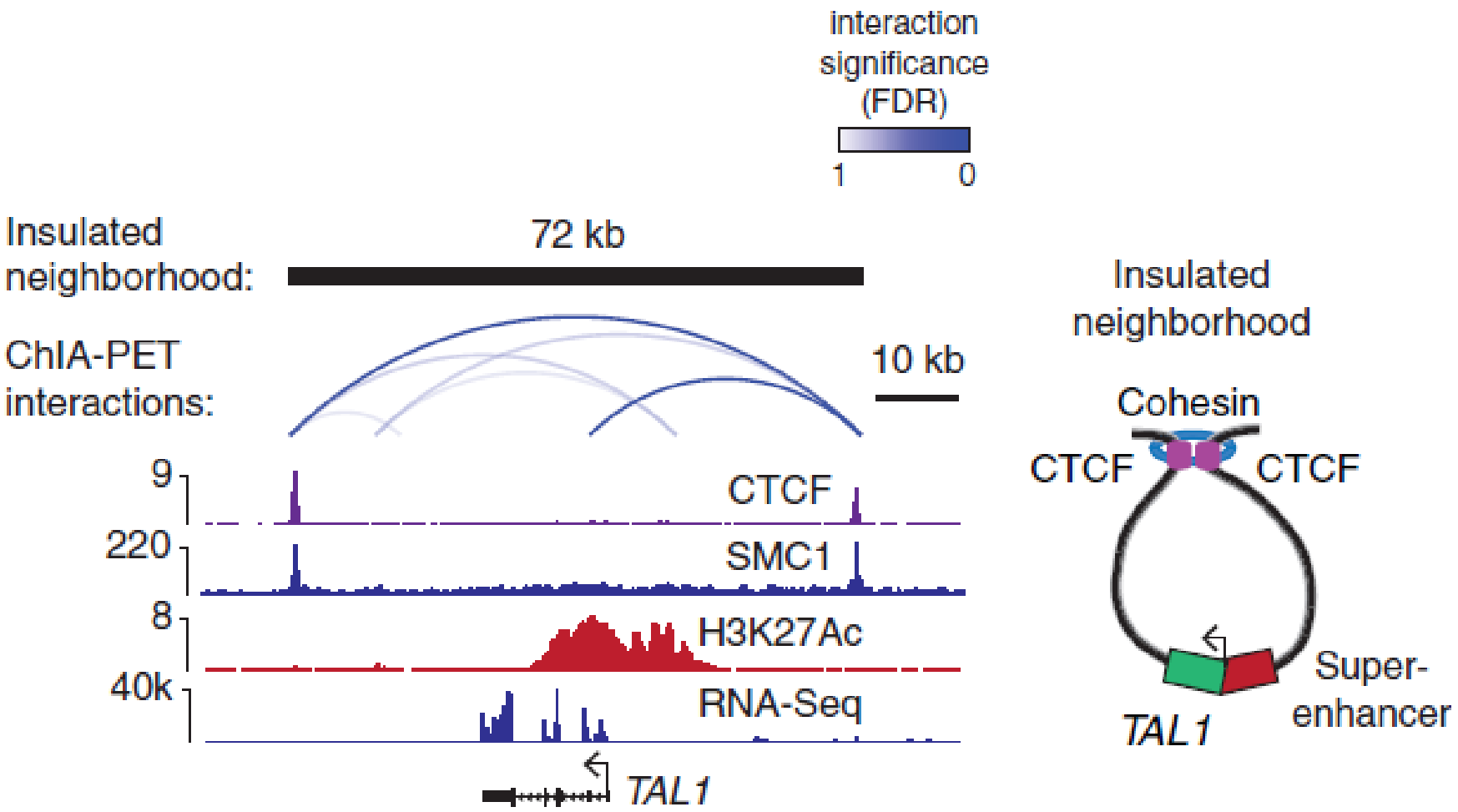
Insulated neighborhoods:
Genomic regulatory unit





Genes involved in tumorigeneis are associated with Insulated neighborhoods

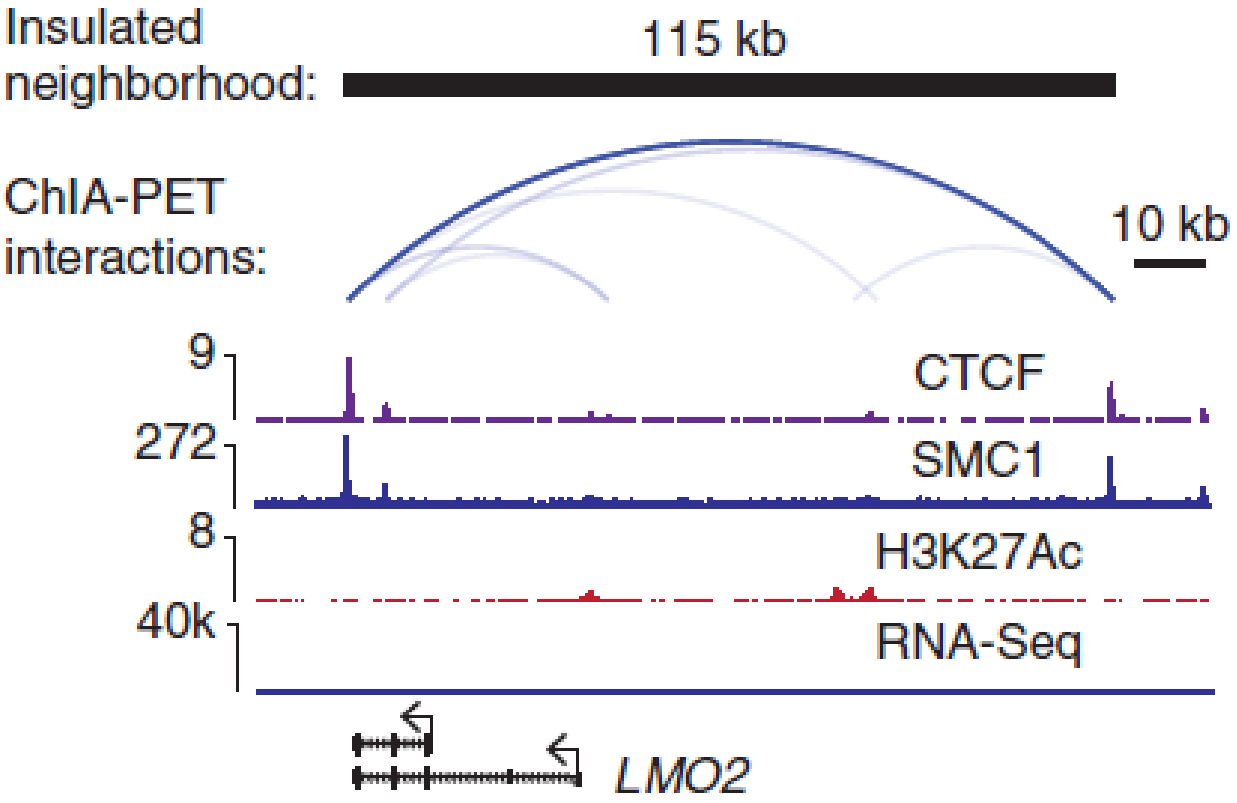
TAL1 and active super-enhancer are located within insulated neighborhood

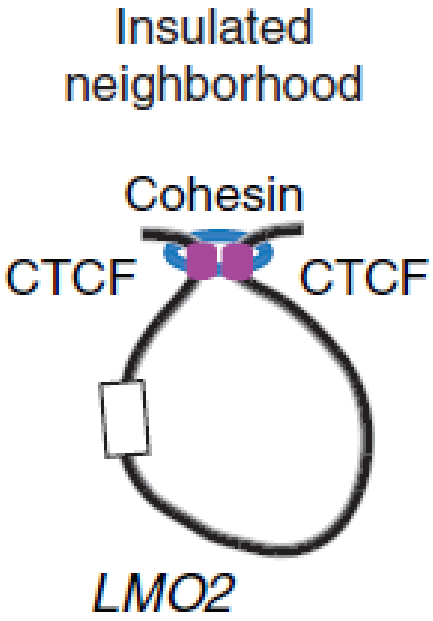


cohesin (SMC1)

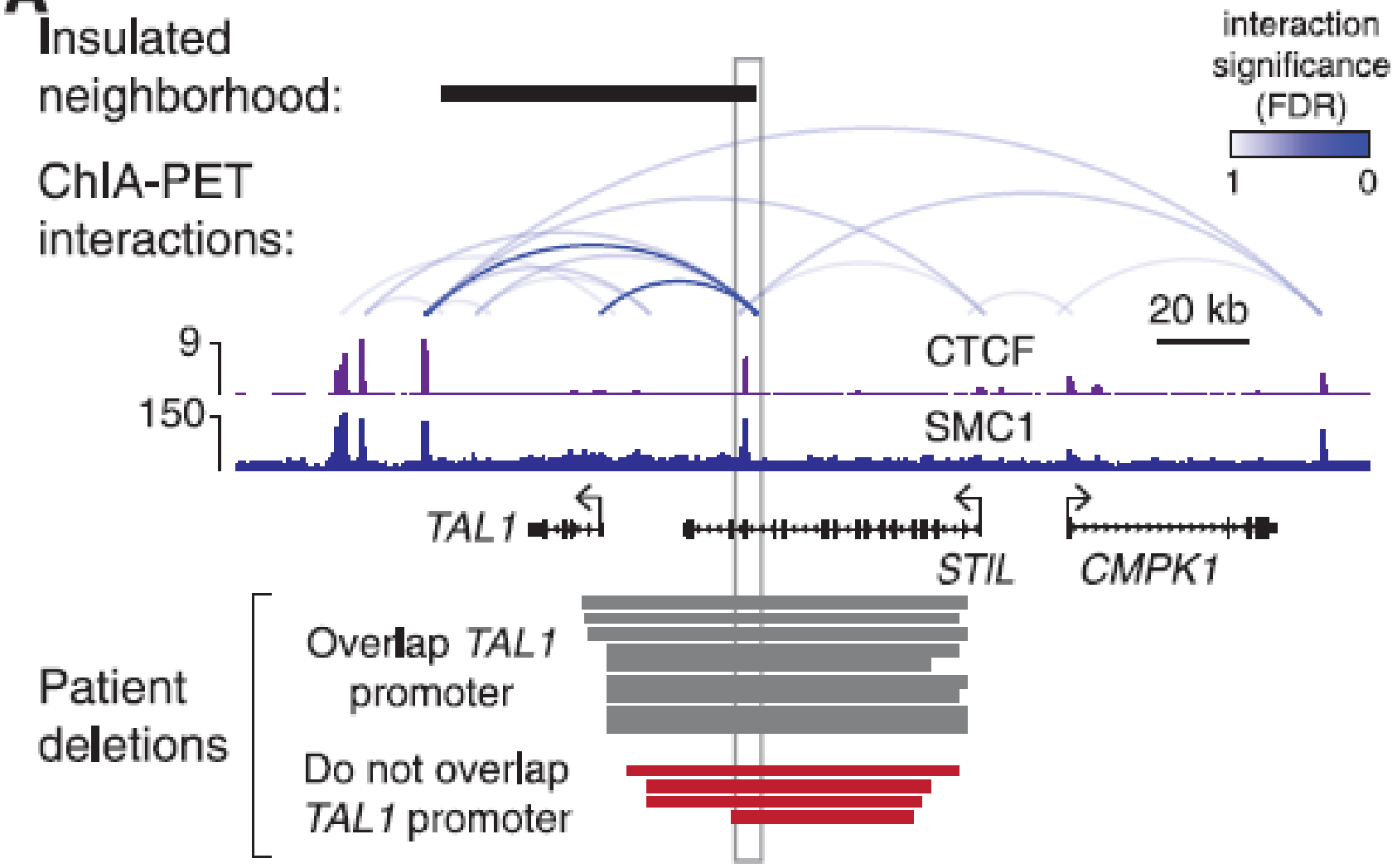
T cell acute lymphoblastic leukemia (T-ALL)
Jurkat cell line

LMO2 are in the silence region and are located within insulated neighborhood

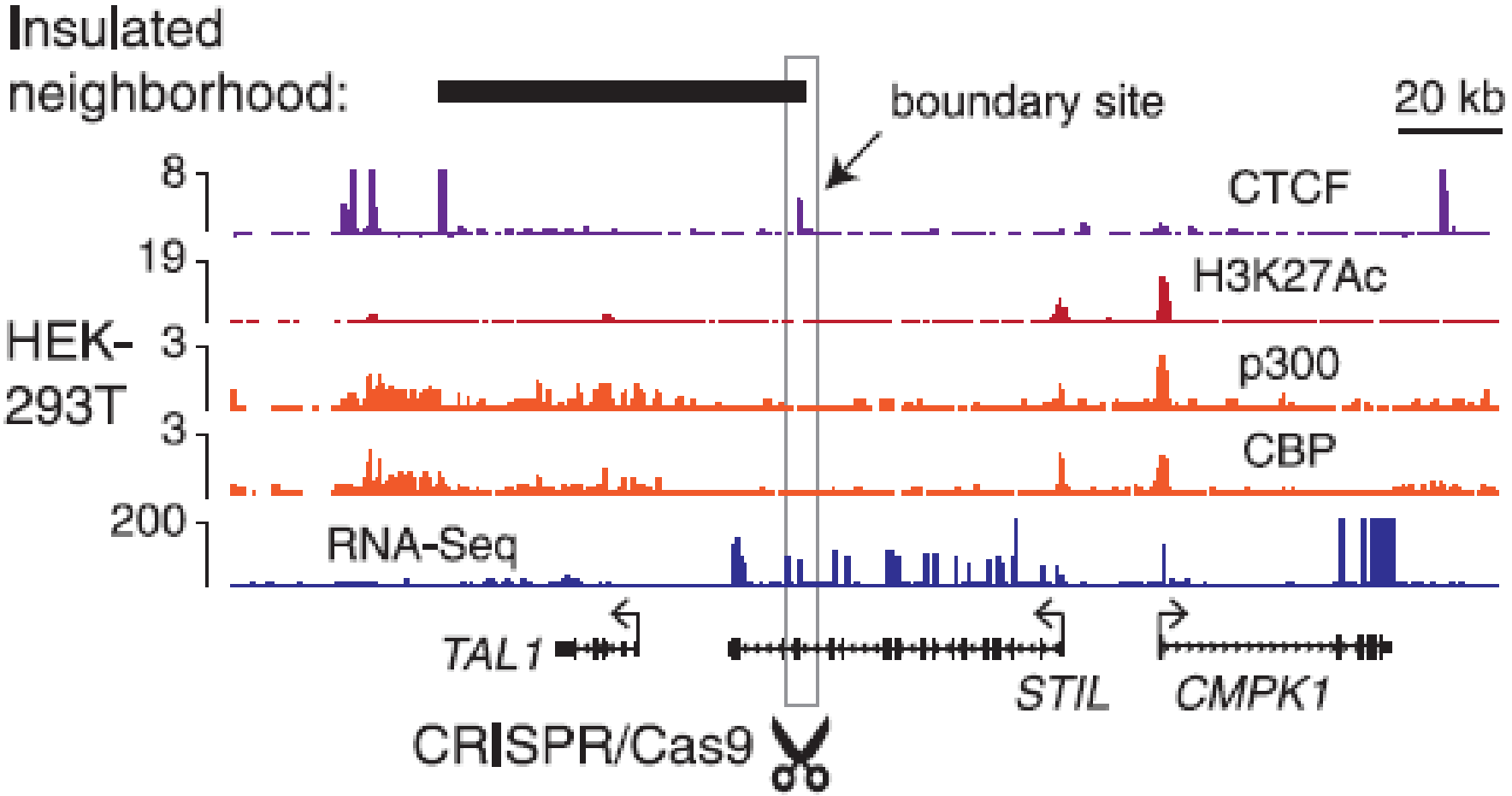




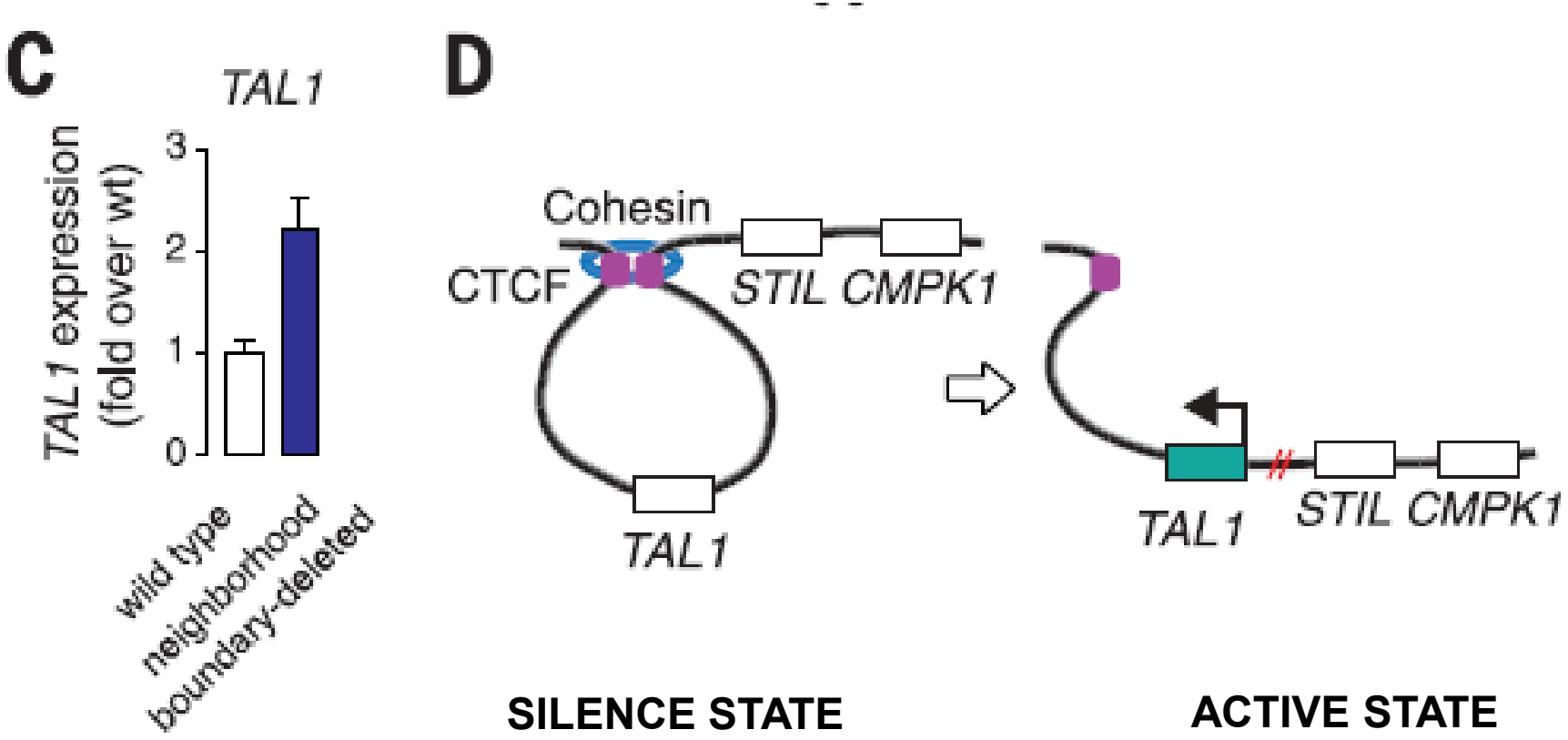
Disruption of insulated neighborhood boundaries is linked to proto-oncogene activation: TAL1 can be activated in many patients with deletions



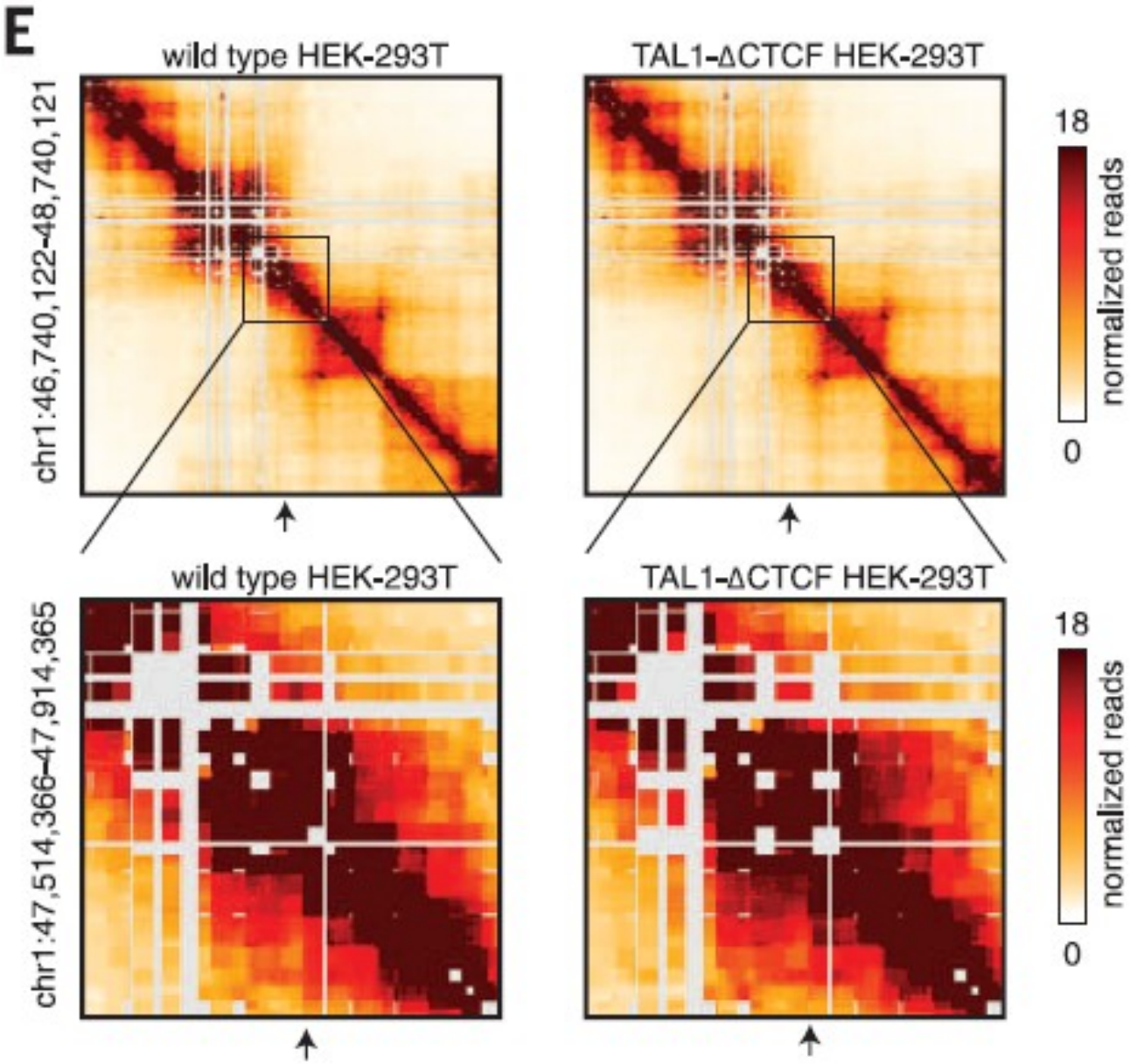
TAL1 is silent in HEK293T cells
CTCF signals define the



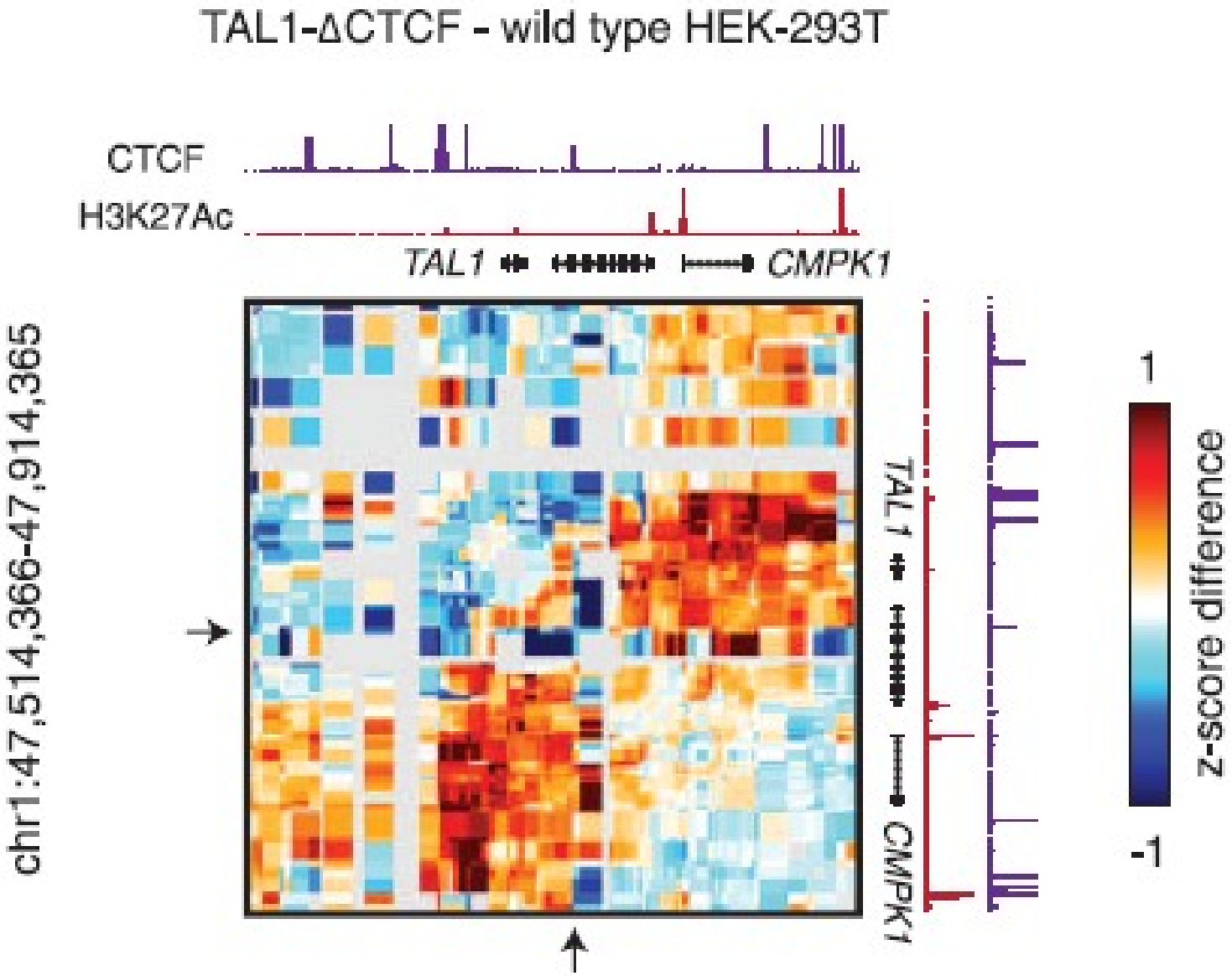
TAL1 is expressed in HEK293T using CRISPR-Cas9 system that deletes the neighborhood boundaries



Long range interaction in the boundaries are showed by 5C assay. Disruption of interactions.



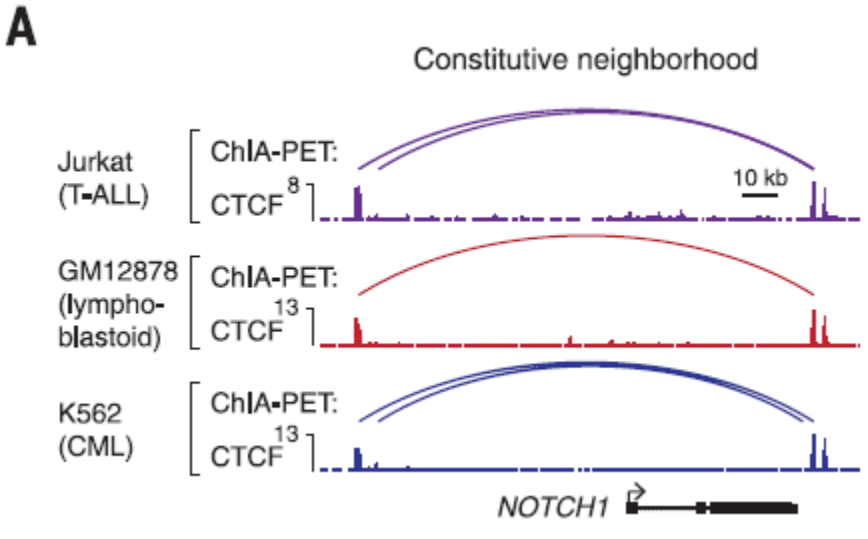
Long range interaction in the boundaries are showed by 5C assay. Disruption of interactions.

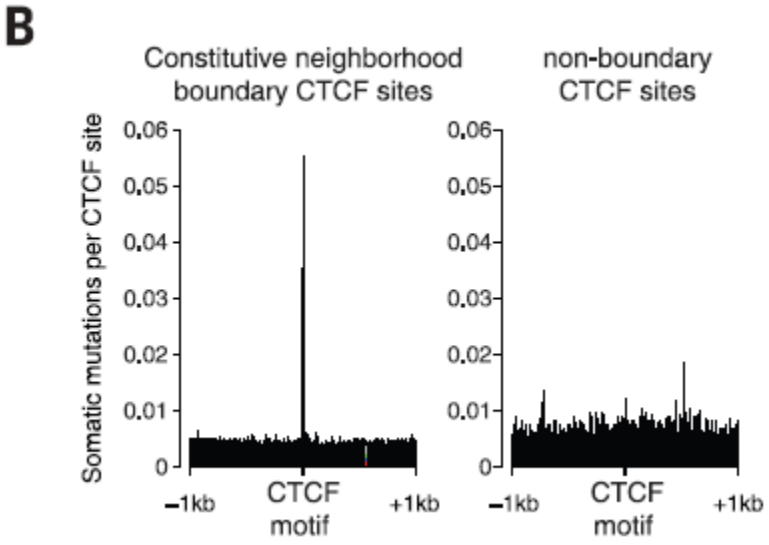


Somatic mutations of neighborhood boundaries

occur in many cancers.

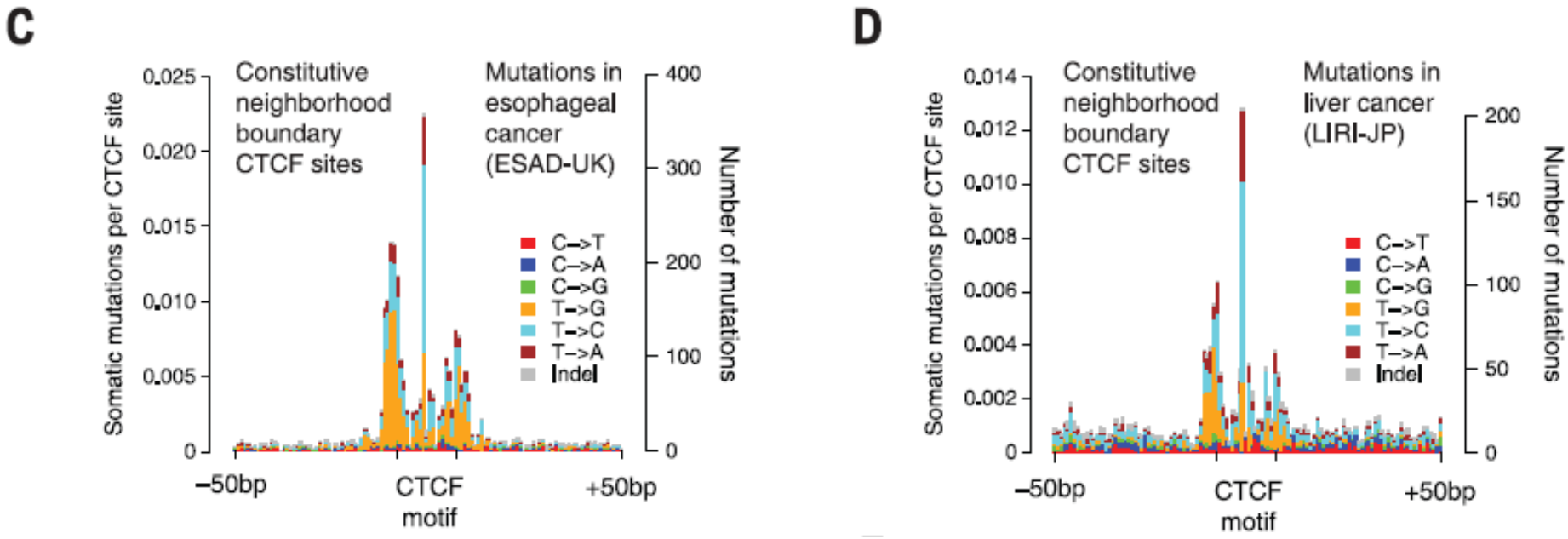
COSTITUTIVE NEIGHBORHOOD at the NOTCH1 locus ARE SIMILAR in different cell lines



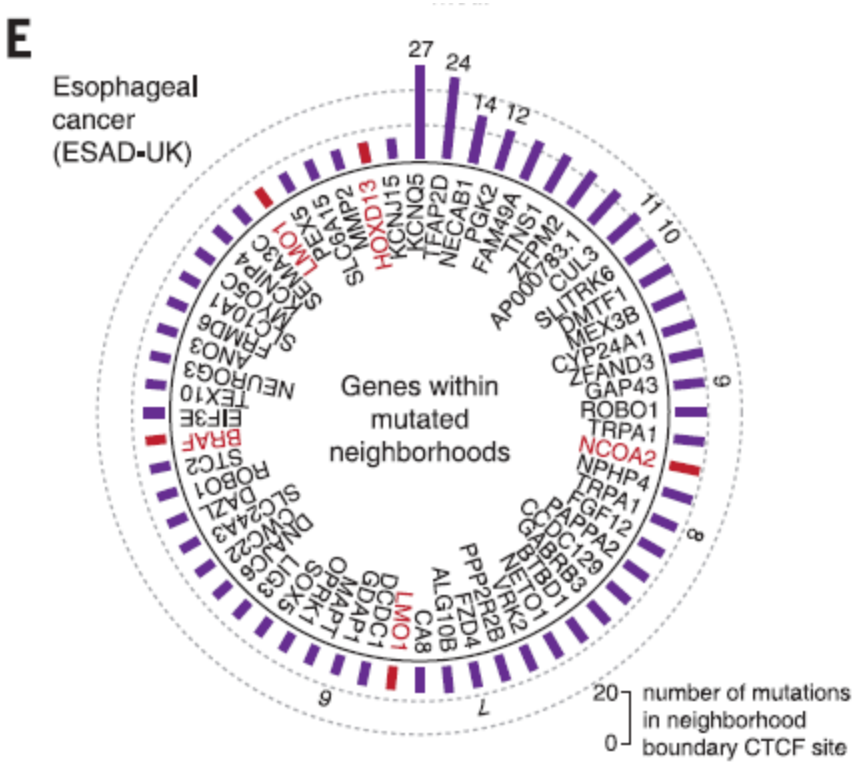


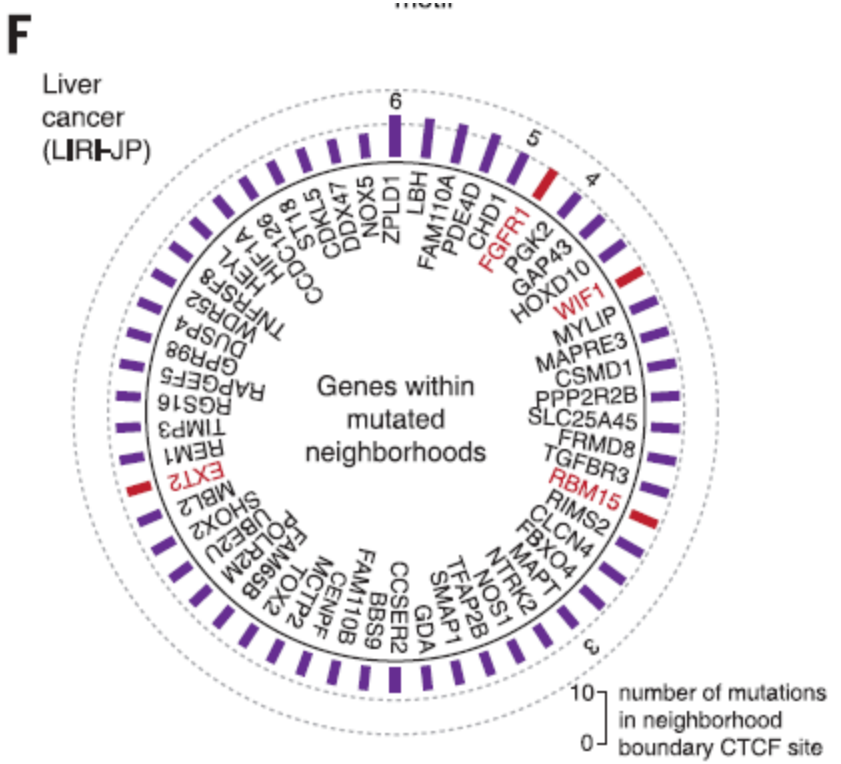
Somantic mutations are enriched in COSTITUTIVE NEIGHBORHOOD

Somantic mutations at COSTITUTIVE NEIGHBORHOOD boundary CTCF sites In esophageal and liver cancers

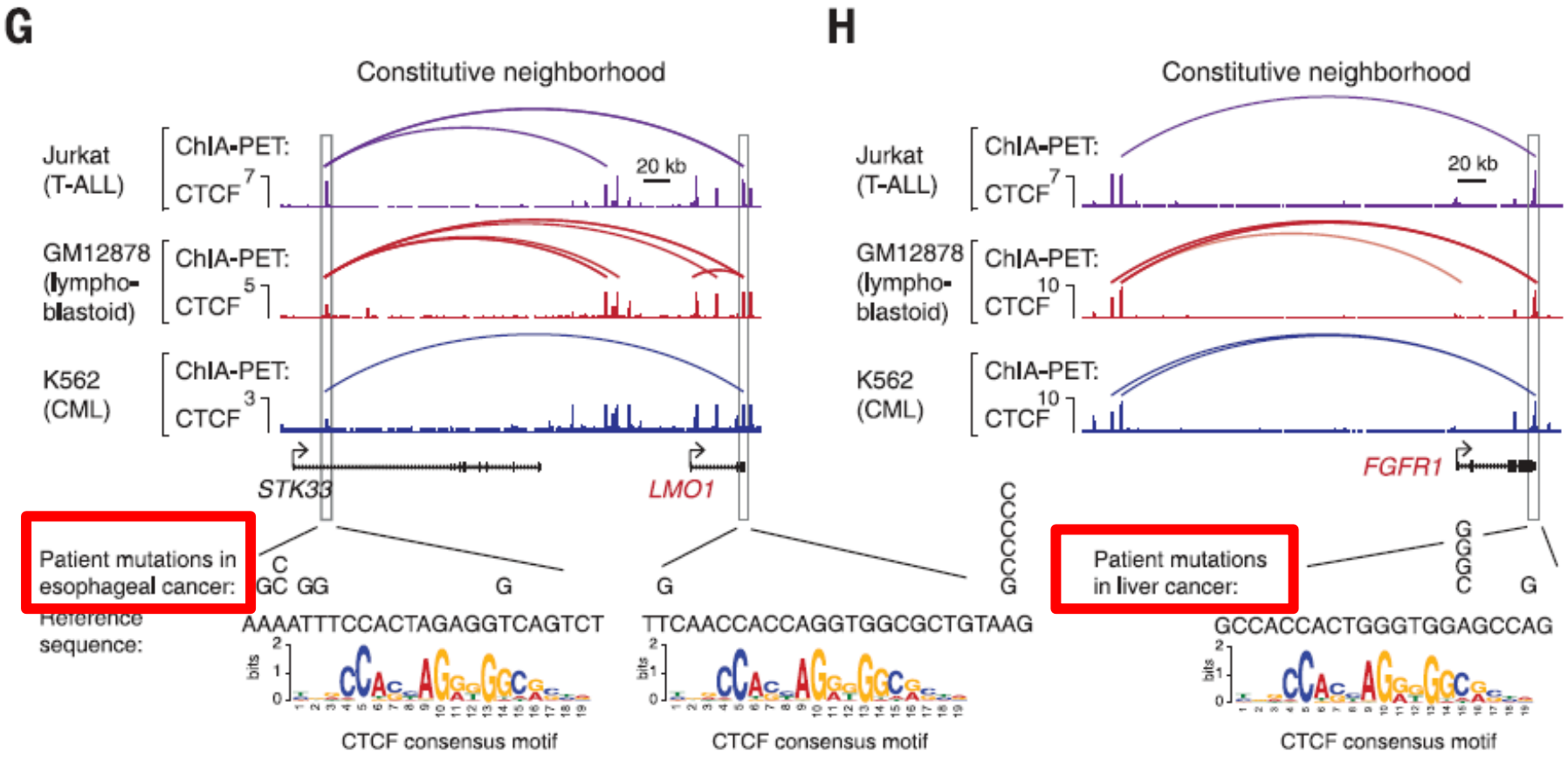


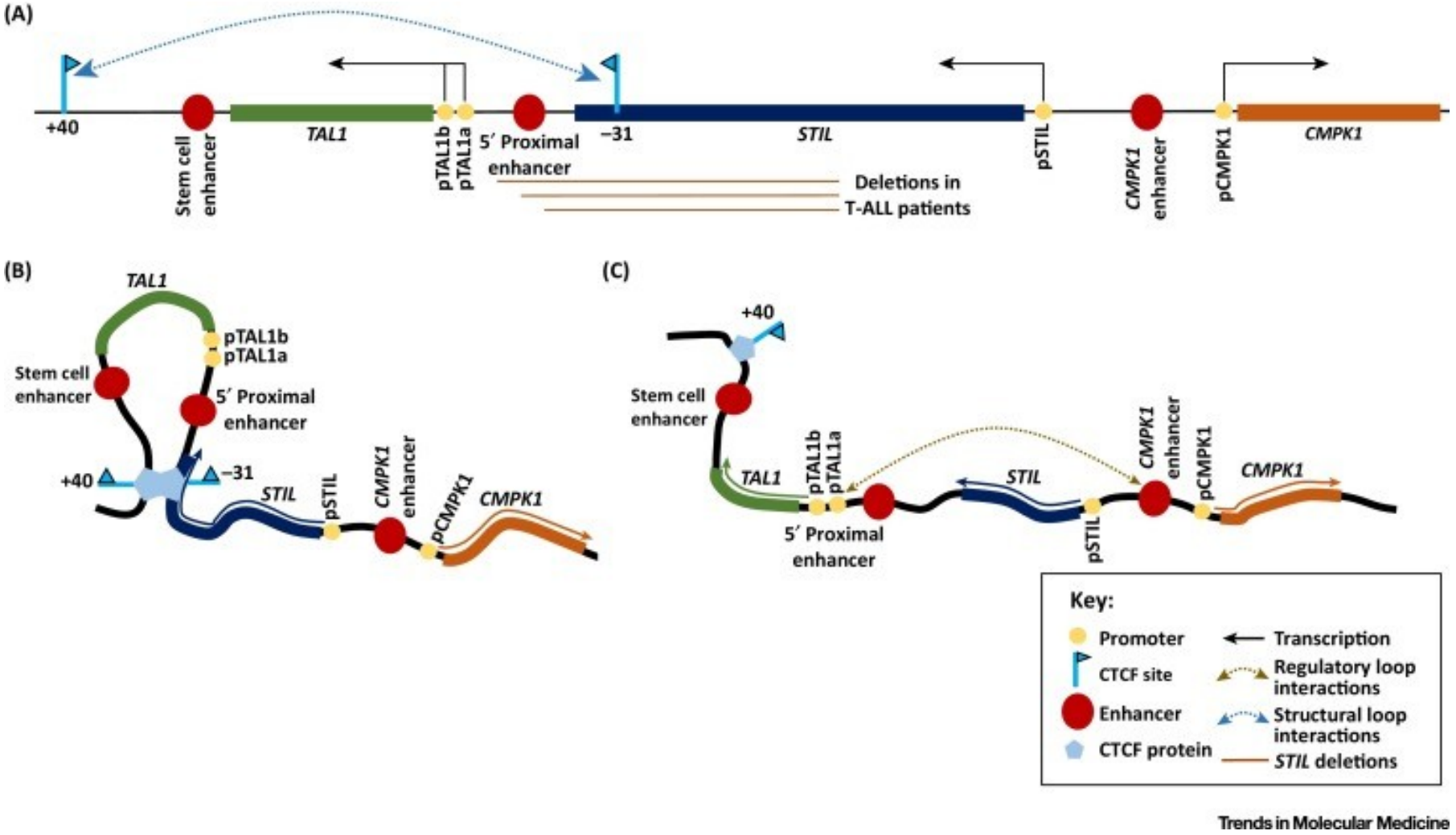
Genes in the COSTITUTIVE NEIGHBORHOOD whose boundary is recurrently mutated in cancers





Mutations in the boundery sites
In cancers



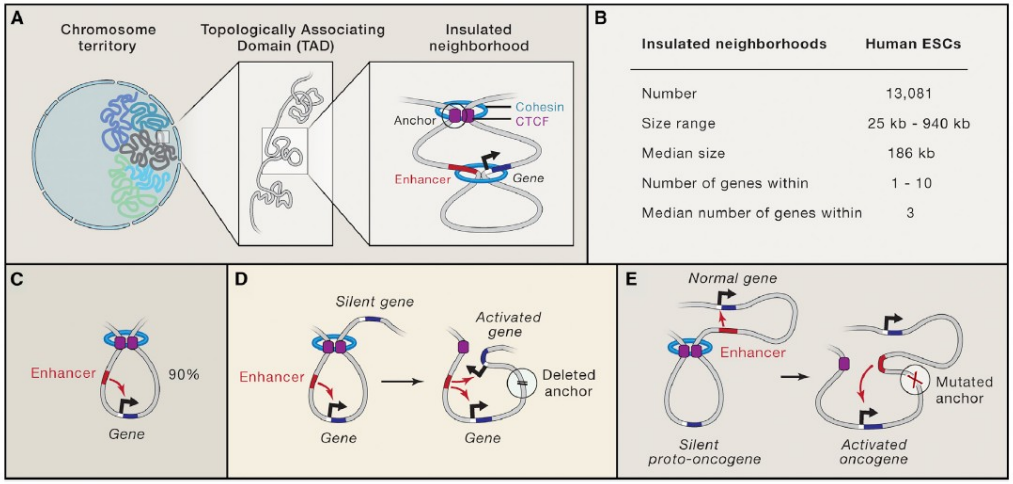


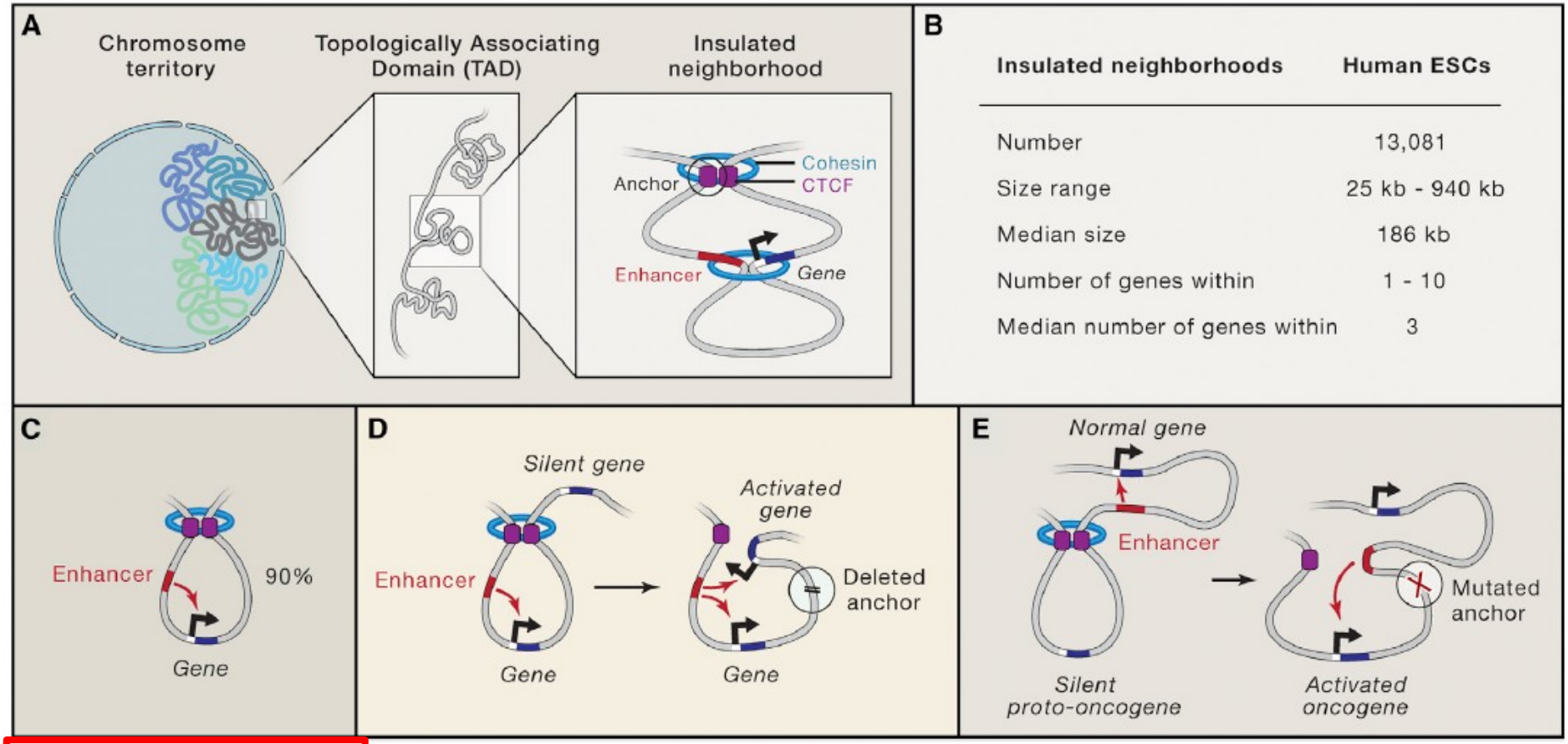
Trends in Molecular Medicine

Insulated Neighborhoods: Structural and Functional Units of Mammalian Gene Control

Denes Hnisz,[1,3,*] Daniel S. Day,[1,3,*] and Richard A. Young[1,2,*]
[1]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA
[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]Co-first author
*Correspondence: hnisz@wi.mit.edu (D.H.), dsday@wi.mit.edu (D.S.D.), young@wi.mit.edu (R.A.Y.)
http://dx.doi.org/10.1016/j.cell.2016.10.024

Understanding how transcriptional enhancers control over 20,000 protein-coding genes to maintain cell-type-specific gene expression programs in all human cells is a fundamental challenge in regulatory biology. Recent studies suggest that gene regulatory elements and their target genes generally occur within insulated neighborhoods, which are chromosomal loop structures formed by the interaction of two DNA sites bound by the CTCF protein and occupied by the cohesin complex. Here, we review evidence that insulated neighborhoods provide for specific enhancer-gene interactions, are essential for both normal gene activation and repression, form a chromosome scaffold that is largely preserved throughout development, and are perturbed by genetic and epigenetic factors in disease. Insulated neighborhoods are a powerful paradigm for gene control that provides new insights into development and disease.





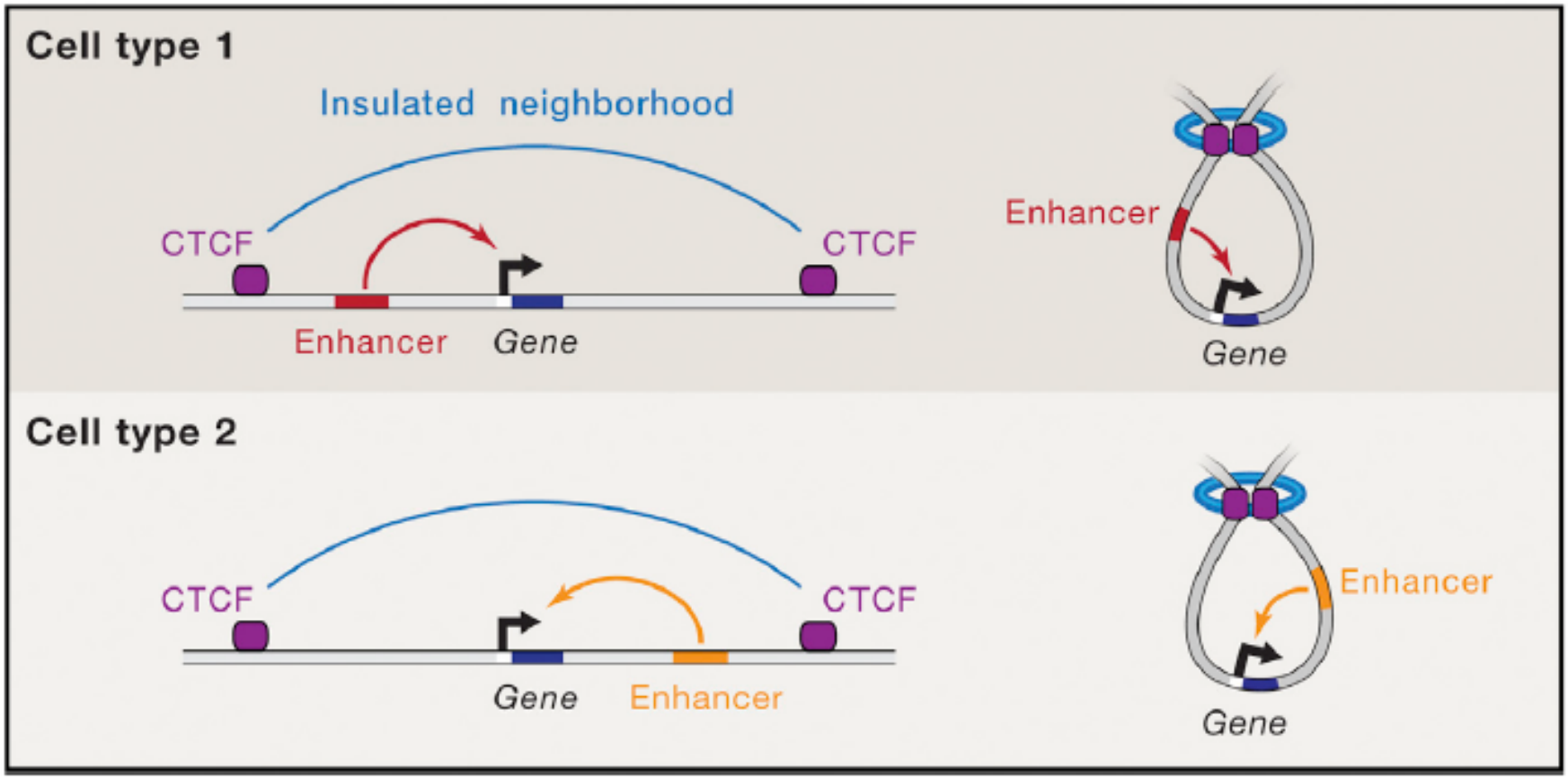
90% of enhancer-gene interactions occur **within insulated neighborhoods** in human ESCs

deletion of insulated neighborhood anchors leads to gene misregulation

mutations of insulated neighborhood anchors in tumor cells lead to oncogene activation

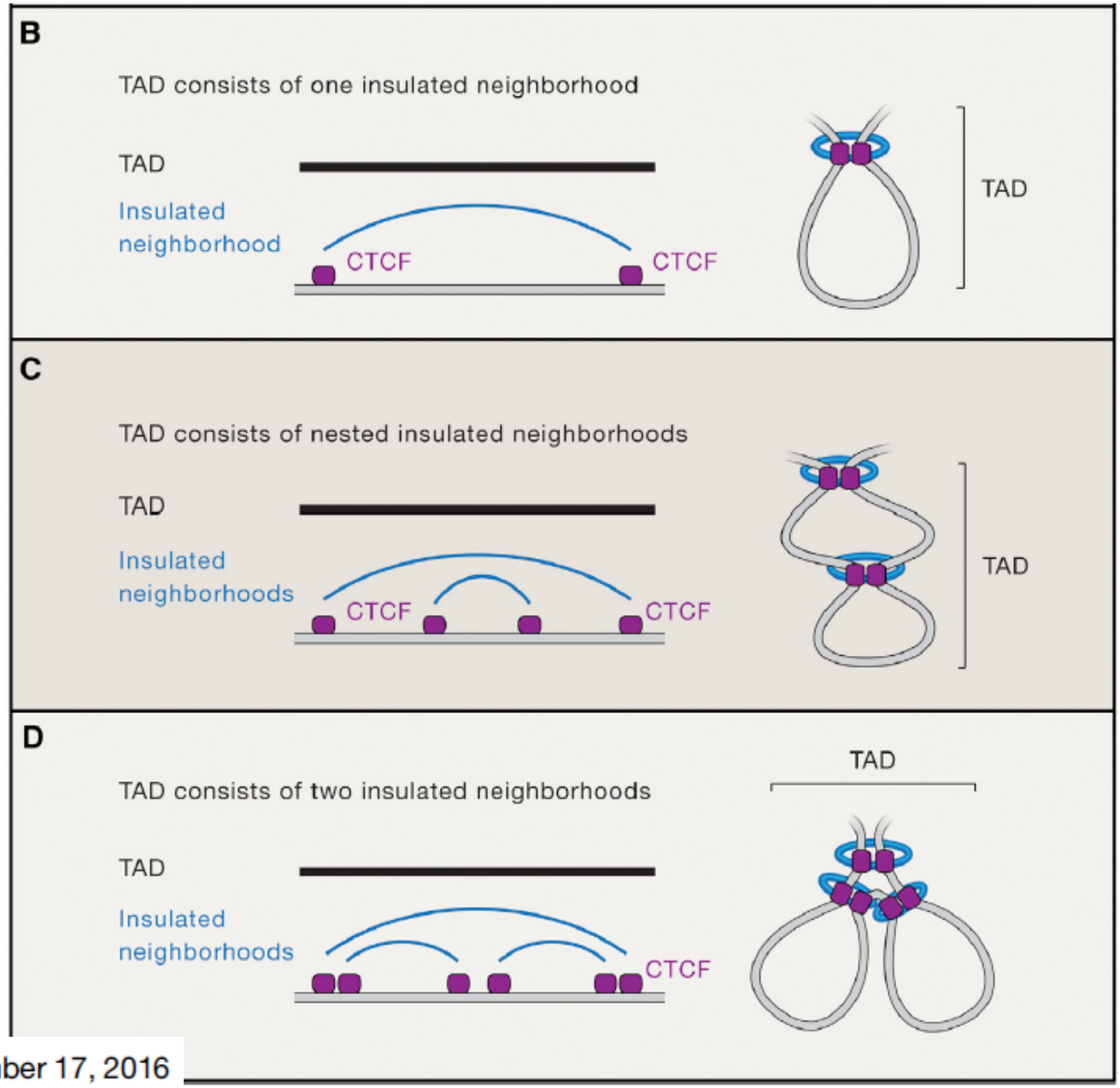
Cell *167*, November 17, 2016

Cell-type specific enhancers –gene interactions occur within the boundaries



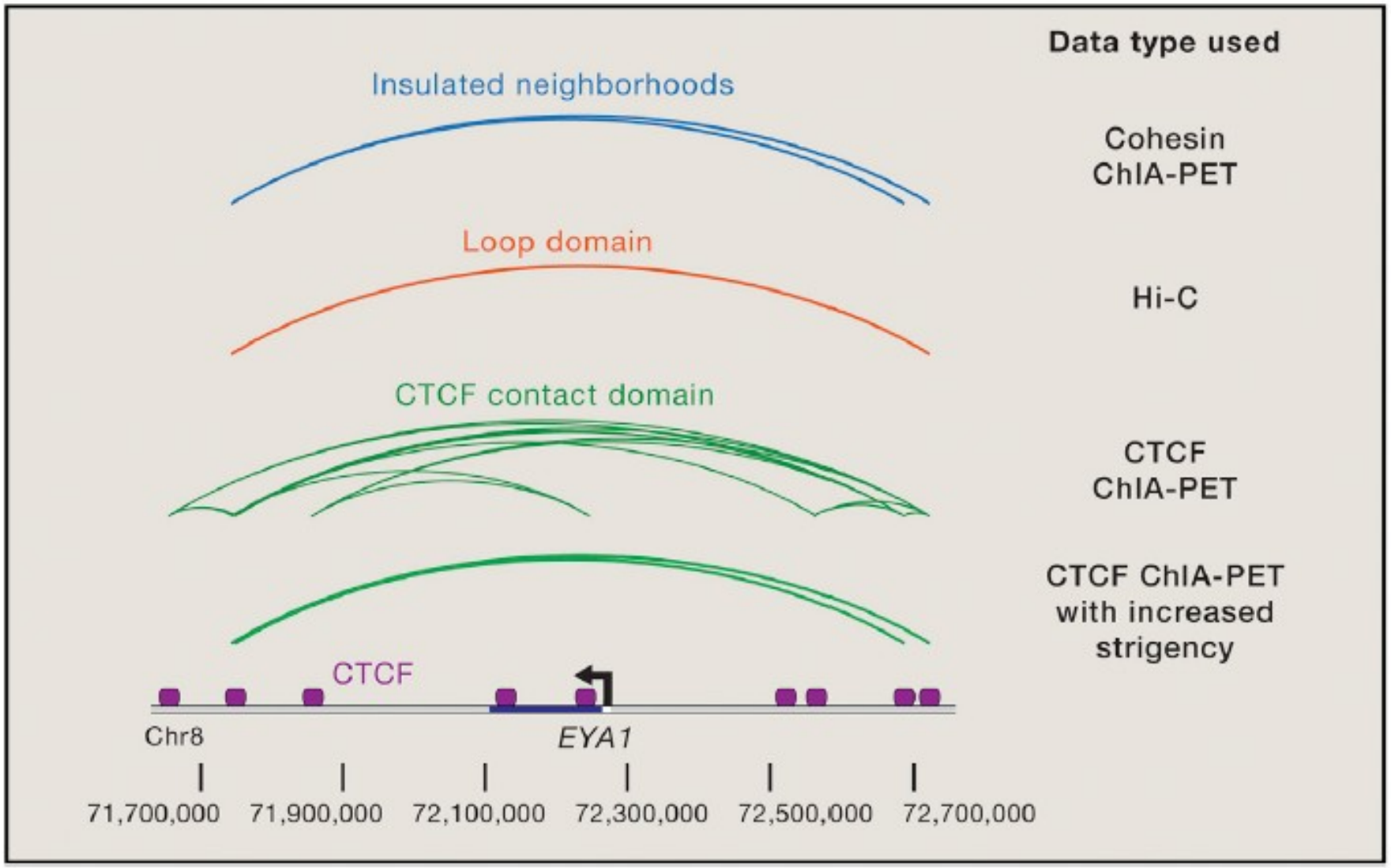
Cell *167*, November 17, 2016

Insulated neighborhoods are a major structuring component of TADs.



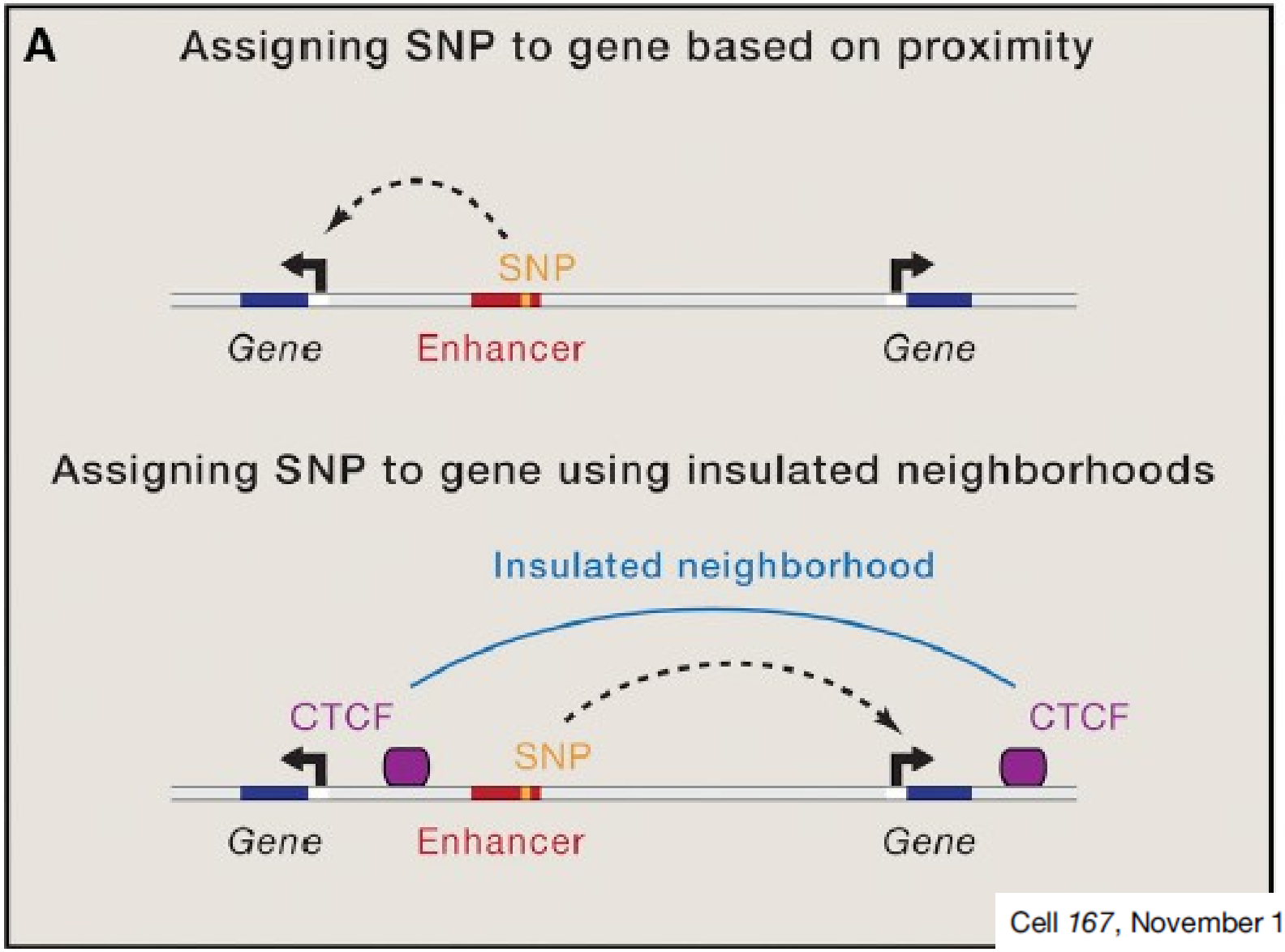
Cell *167*, November 17, 2016

Comparation between several techniques for identification of long range interaction



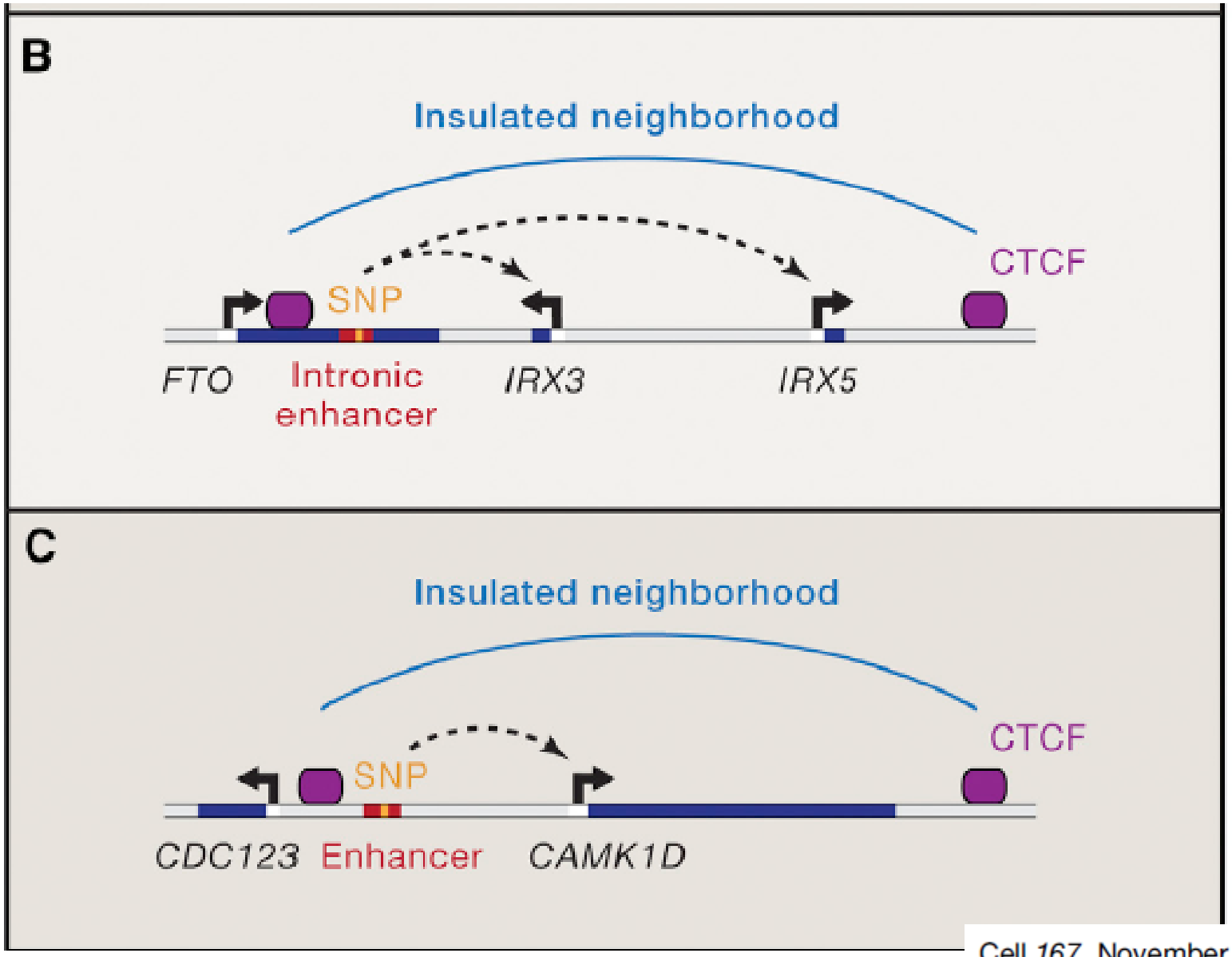
Cell *167*, November 17, 2016

How SNPs affect long range interactions of the chromatin



Cell *167*, November 17, 2016

How SNPs affect long range interactions of the chromatin



Cell *167*, November 17, 2016

Insulated neighborhoods:

are structural and functional units of gene control

are used during development to control the diverse cell identities that contribute to complex animals

form the mechanistic basis of higher-order chromosome structures, such as topologically associating domains (TADs)

genetic and epigenetic perturbations of neighborhood boundaries contribute to disease.