ENHANCER

ACTIVATION

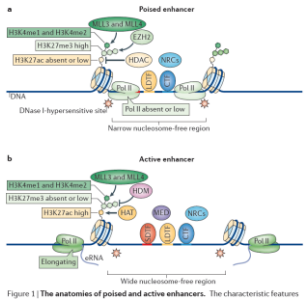
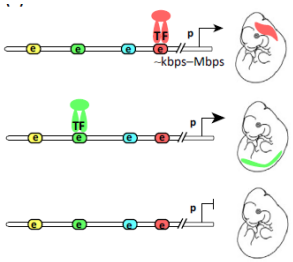


Figure 1 | **The anatomies of poised and active enhancers.** The characteristic features

CHARACTERISTICS



Enhancers in tissue/cell-specific gene expression

SELECTION

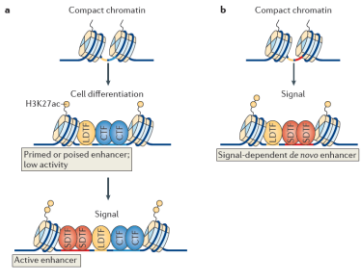
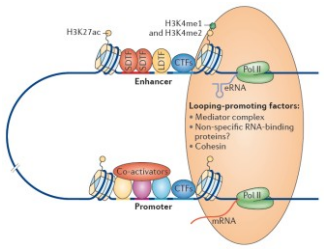


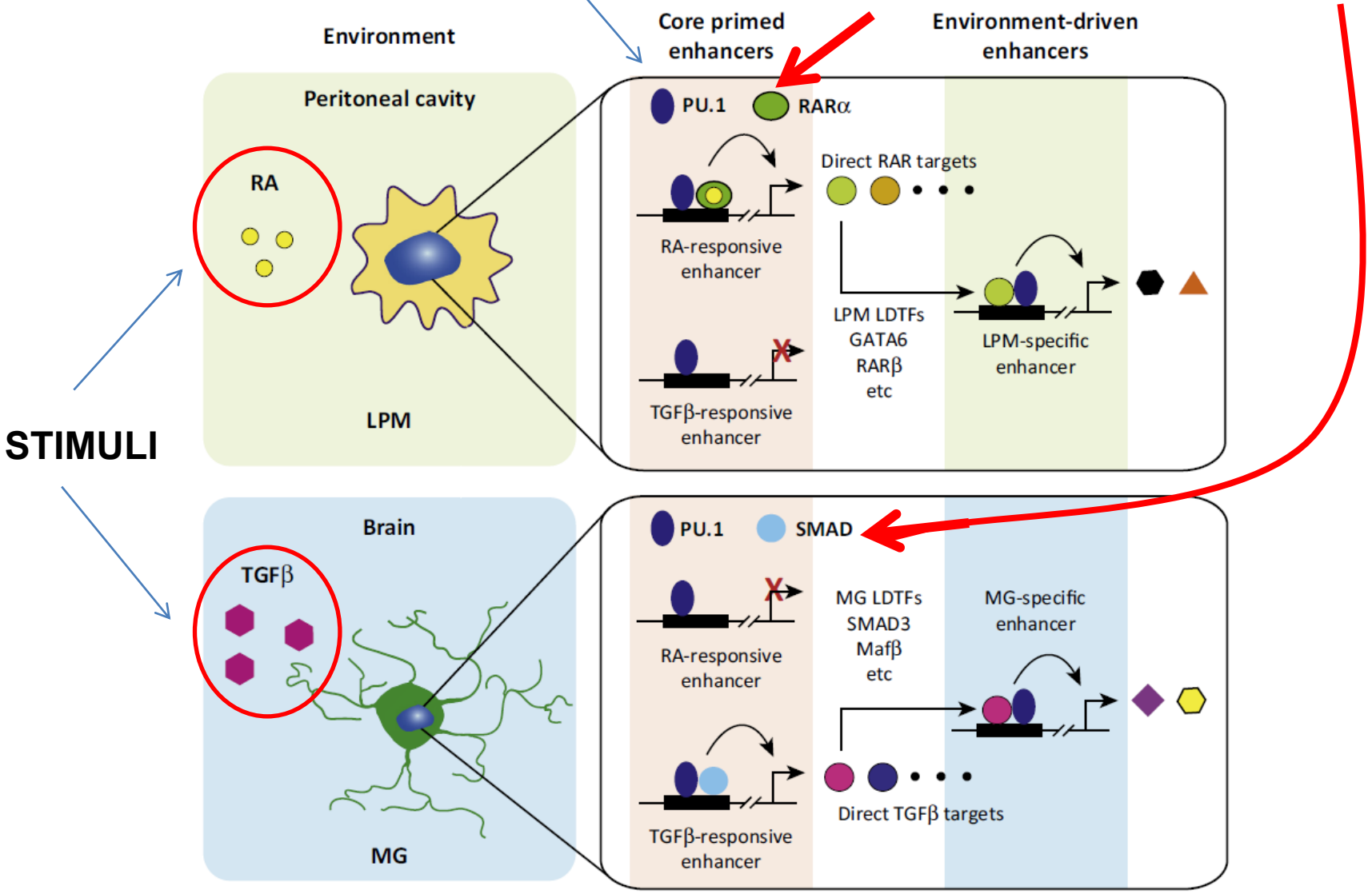
Figure 3 | **Cell type-specific enhancer selection and activation.** **a** | Collaborative

FUNCTION



LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS

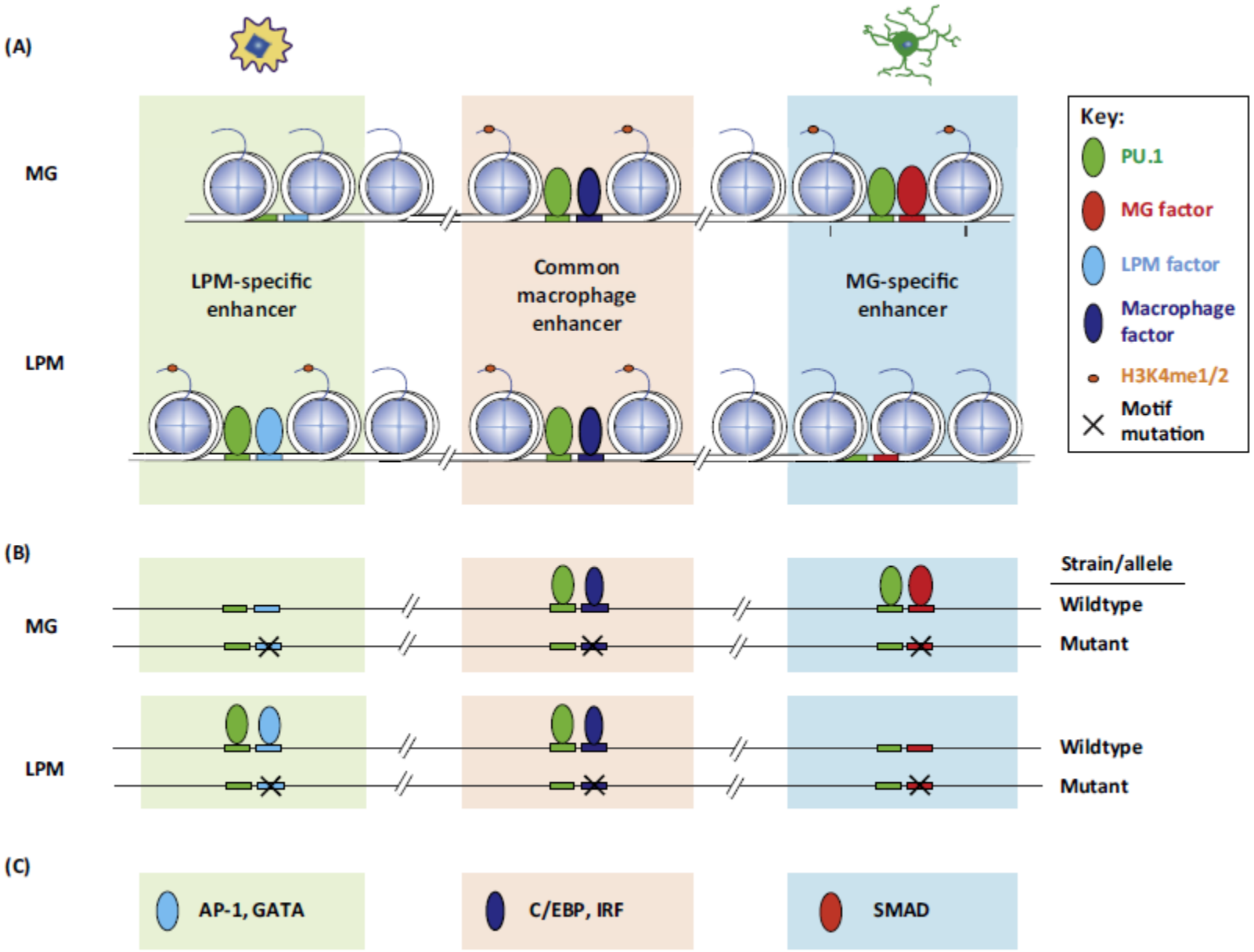
SDTF: SIGNAL –DETERMINING TRANSCRIPTION FACTORS



Romanoski et al., 2015

Trends in Immunology September 2015, Vol. 36, No. 9

Using natural genetic variation to discover regulatory networks



Romanoski et al., 2015

TRENDS in Immunology

Using natural genetic variation to discover regulatory networks

- Different macrophage populations exhibited common and specific enhancers
- Cell-type specific enhancers depend on constant environmental regulation, es tissues context
- SNPs, in proximity of LDTF, could indicate that a mutation in the recognition motif for a collaborative transcription factor

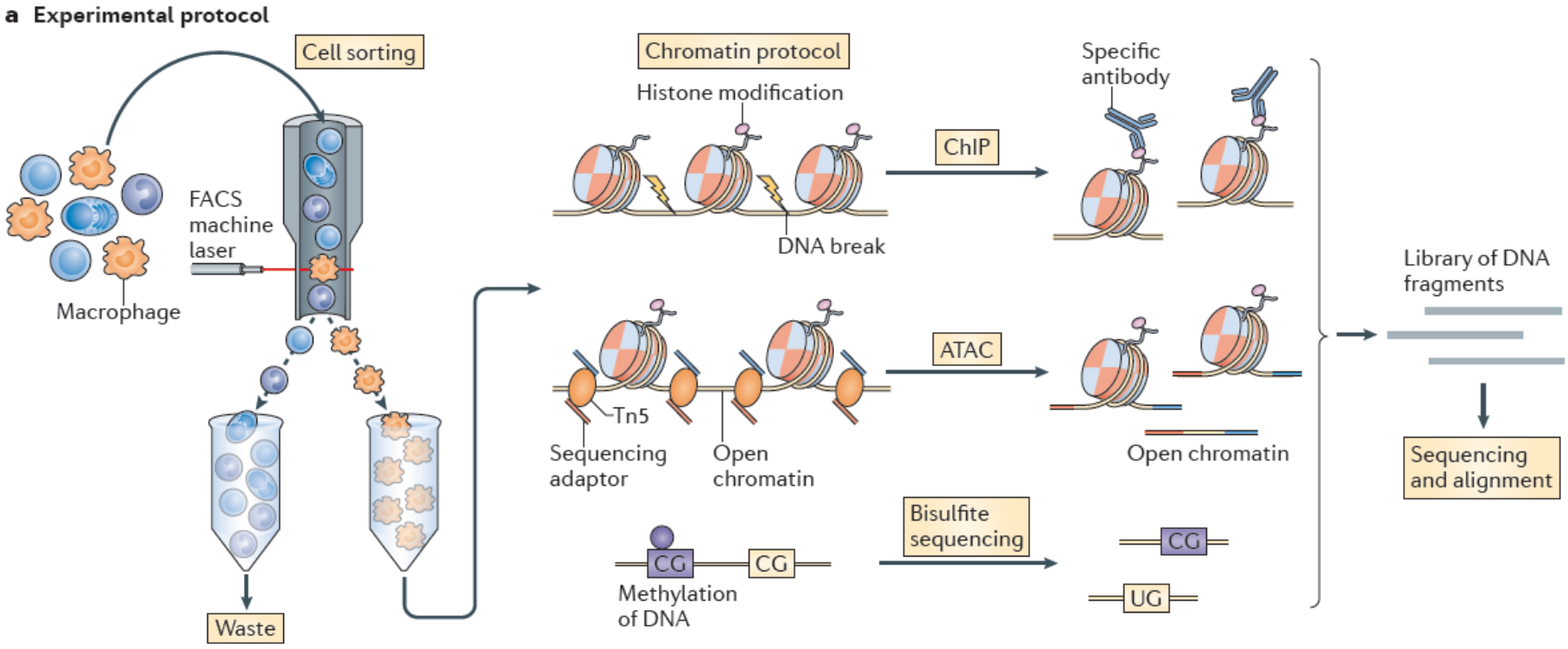
What is the techinque that you can use to identify cell-type specific enhancers?

Making the case for chromatin profiling: a new tool to investigate the immune-regulatory landscape

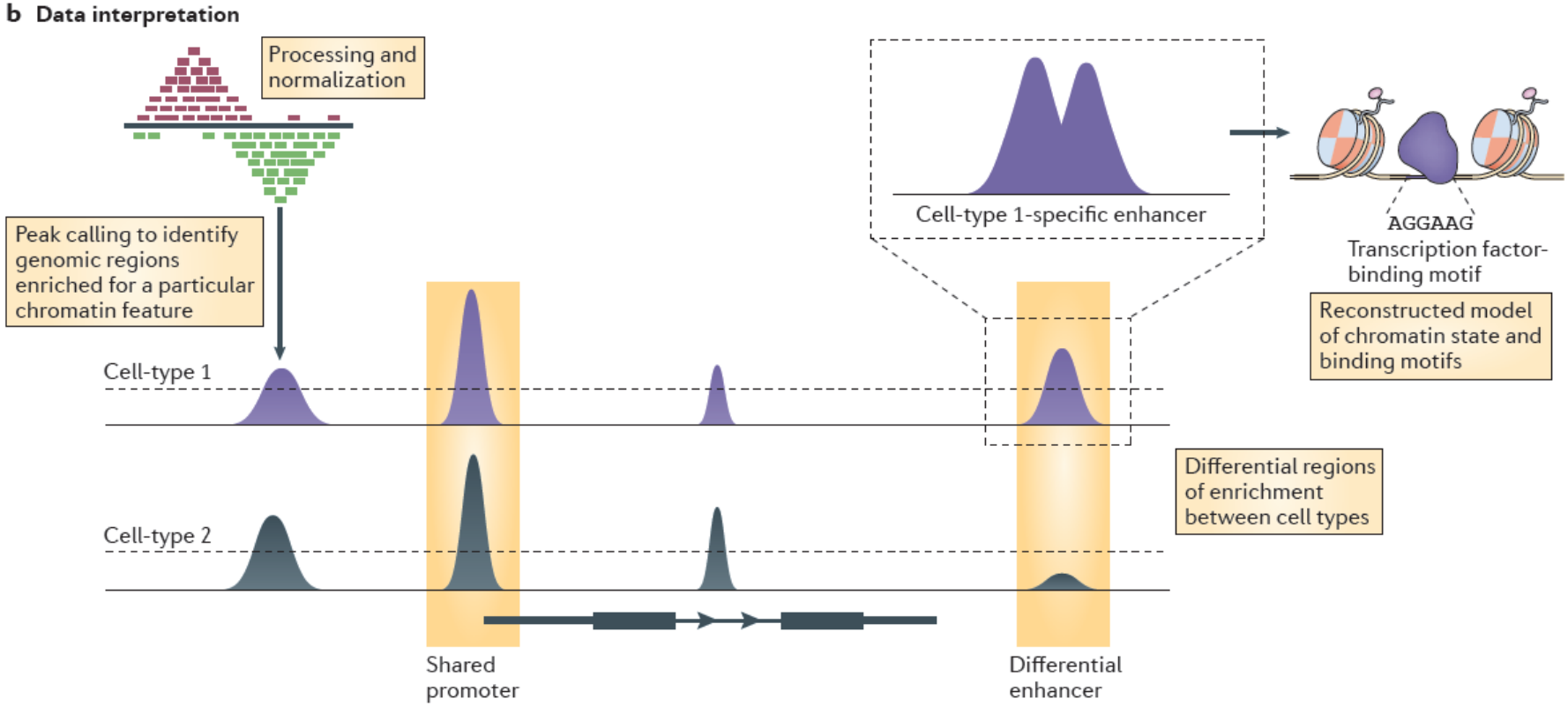
Deborah R. Winter, Steffen Jung and Ido Amit

Abstract | Recent technological advances have enabled researchers to accurately and efficiently assay the chromatin dynamics of scarce cell populations. In this Opinion article, we advocate the application of these technologies to central questions in immunology. Unlike changes to other molecular structures in the cell, chromatin features can reveal the past (developmental history), present (current activity) and future (potential response to challenges) of a given immune cell type; chromatin profiling is therefore an important new tool for studying the immune-regulatory networks of health and disease.

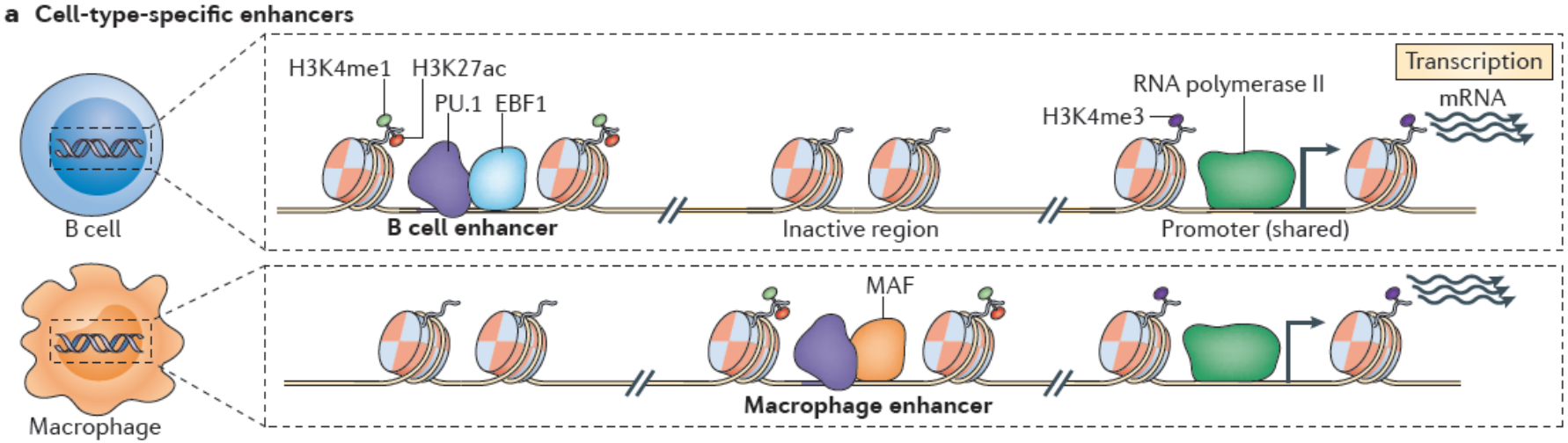
Methods for identification of genomic regulatory regions



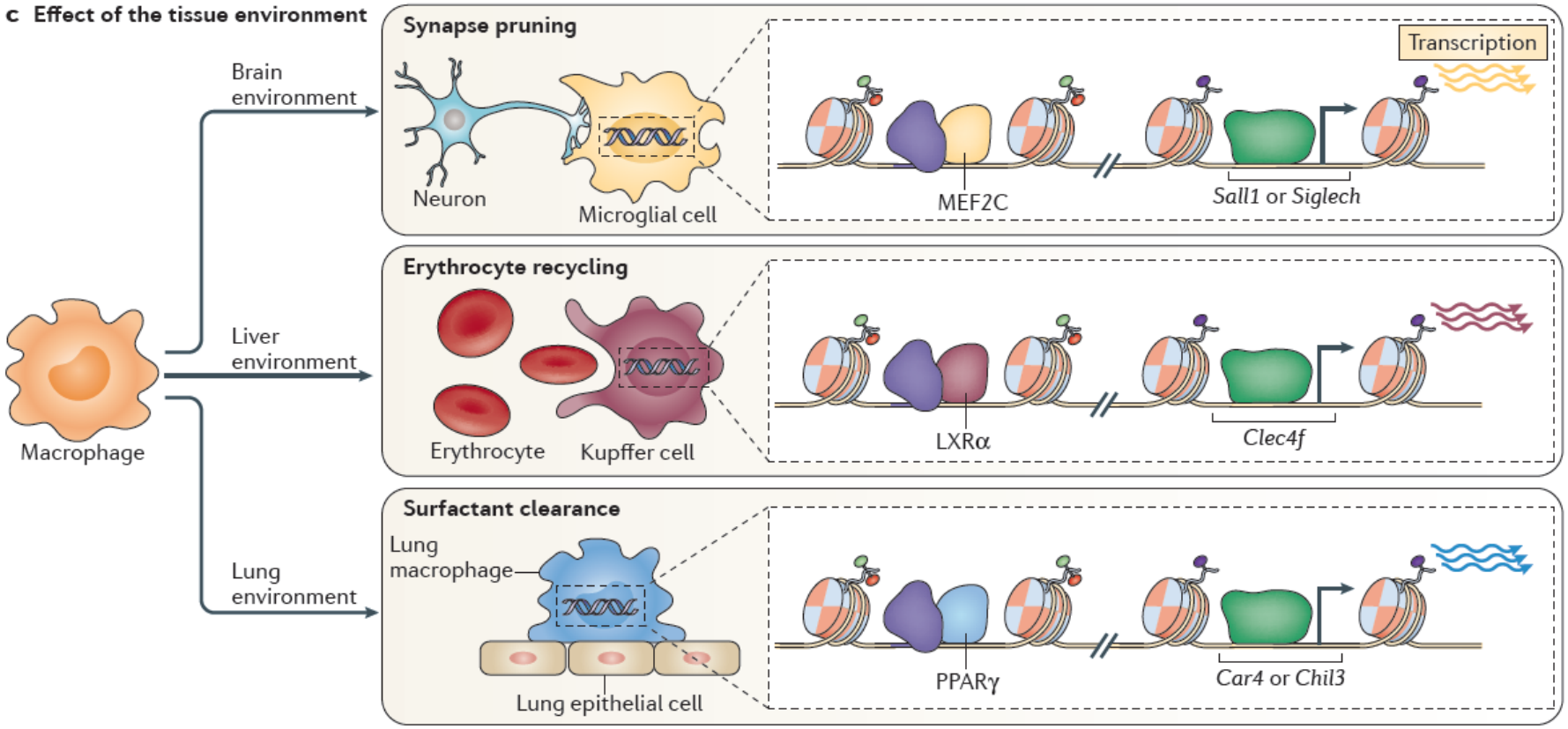
From reads to DNA elements function



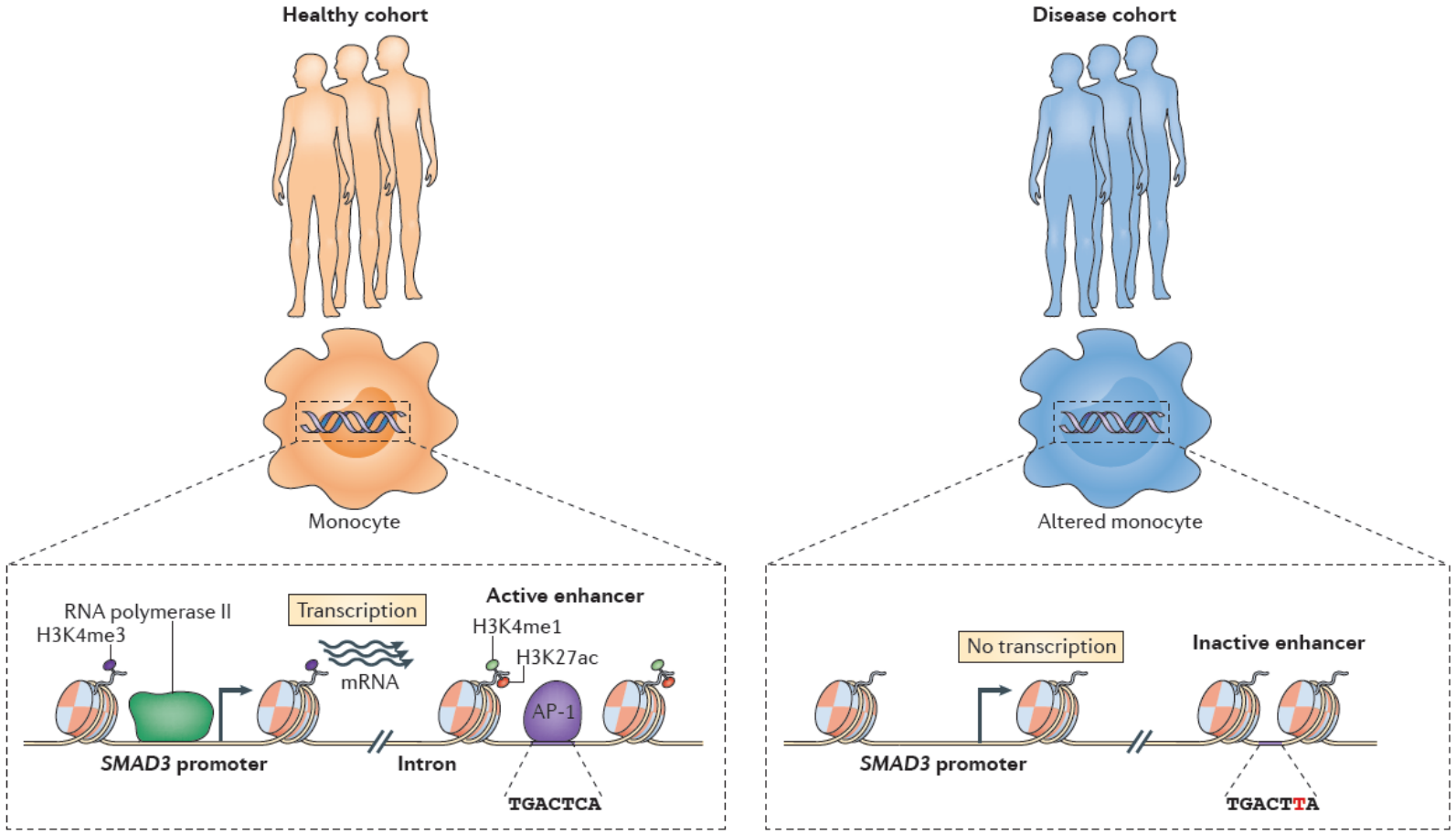
Cell-type-specific enhancers to regulate same genes



Effect of the tissue environment



Association of human chromatin data and susceptibility to immune disease

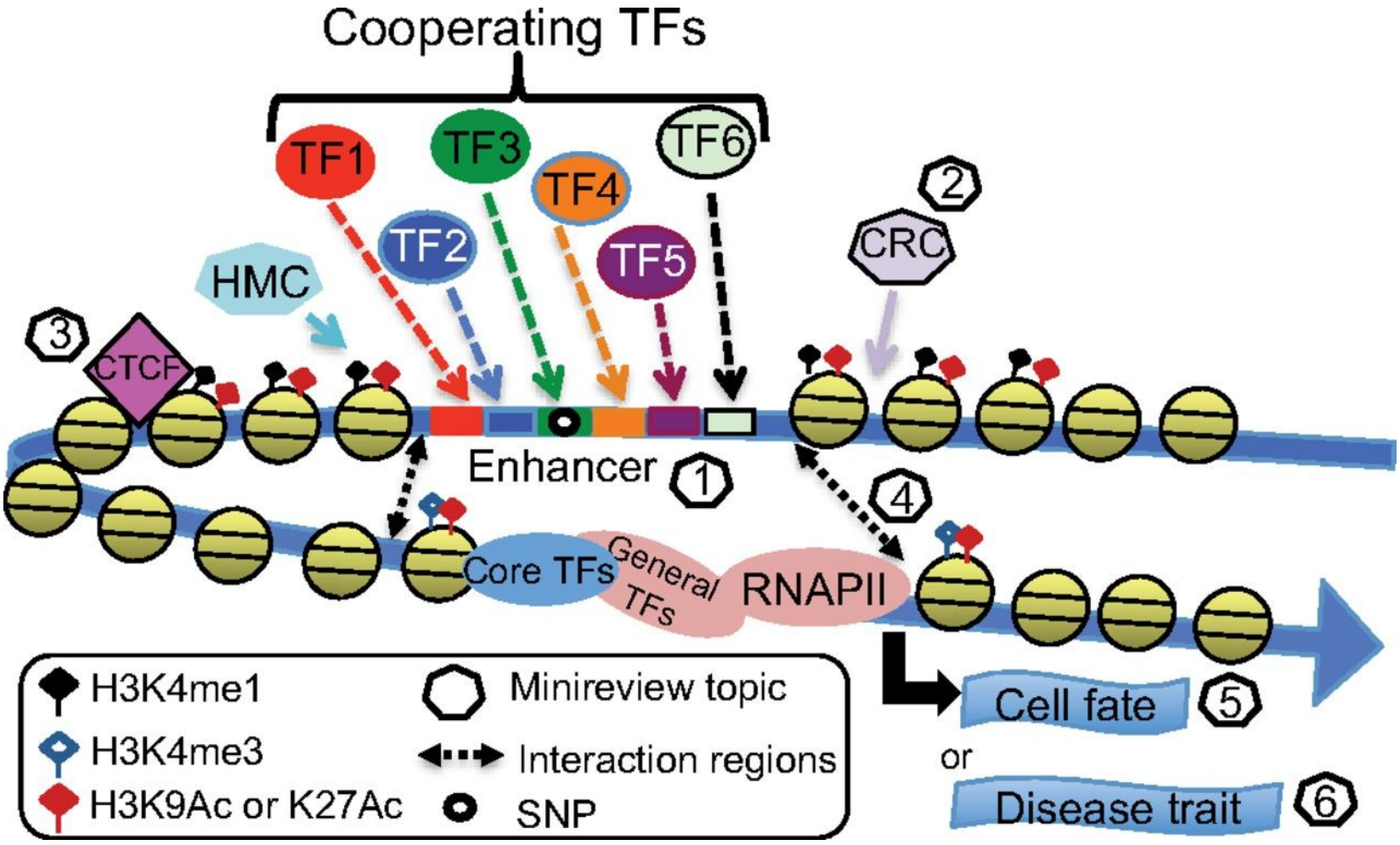


SNPs in the genomic regulatory regions **may change the TF binding to DNA and enhancer**, associated with monocytes derived from healthy donor, **become inactive** in monocyted derived from patients

SNPs in the genomic regulatory regions may affect:

- **Enhancer Activation: loss of TFs interaction or TFs recruitment.**
- **Enhancer Selection: loss or association of LTDF**
- **Alteration of timing or specific tissues activation**
- **Long range interaction between genomic regulatory regions**

Genome-wide characterizations of regulatory regions.



Peggy J. Farnham J. Biol. Chem. 2012;287:30885-30887

How are SNPs studying in genome-wide manner?

Super-Enhancers in the Control of Cell Identity and Disease

Denes Hnisz,[1,3] Brian J. Abraham,[1,3] Tong Ihn Lee,[1,3] Ashley Lau,[1,2] Violaine Saint-André,[1] Alla A. Sigova,[1] Heather A. Hoke,[1,2] 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]These authors contributed equally to this work

*Correspondence: young@wi.mit.edu

http://dx.doi.org/10.1016/j.cell.2013.09.053

SUMMARY

Super-enhancers are large clusters of transcriptional enhancers that drive expression of genes that define cell identity. Improved understanding of the roles that super-enhancers play in biology would be afforded by knowing the constellation of factors that constitute these domains and by identifying super-enhancers across the spectrum of human cell types. We describe here the population of transcription factors, cofactors, chromatin regulators, and transcription apparatus occupying super-enhancers in embryonic stem cells and evidence that super-enhancers are highly transcribed. We produce a catalog of super-enhancers in a broad range of human cell types and find that super-enhancers associate with genes that control and define the biology of these cells. Interestingly, disease-associated variation is especially enriched in the super-enhancers of disease-relevant cell types. Furthermore, we find that cancer cells generate super-enhancers at oncogenes and other genes important in tumor pathogenesis. Thus, super-enhancers play key roles in human cell identity in health and in disease.

SUMMARY

Super-enhancers are large clusters of transcriptional enhancers that drive expression of genes that define cell identity. Improved understanding of the roles that super-enhancers play in biology would be afforded by knowing the constellation of factors that constitute these domains and by identifying super-enhancers across the spectrum of human cell types. We describe here the population of transcription factors, cofactors, chromatin regulators, and transcription apparatus occupying super-enhancers in embryonic stem cells and evidence that super-enhancers are highly transcribed. We produce a catalog of super-enhancers in a broad range of human cell types and find that super-enhancers associate with genes that control and define the biology of these cells. Interestingly, disease-associated variation is especially enriched in the super-enhancers of disease-relevant cell types. Furthermore, we find that cancer cells generate super-enhancers at oncogenes and other genes important in tumor pathogenesis. Thus, super-enhancers play key roles in human cell identity in health and in disease.

DEFINITION

AIM

1) Protein complexes

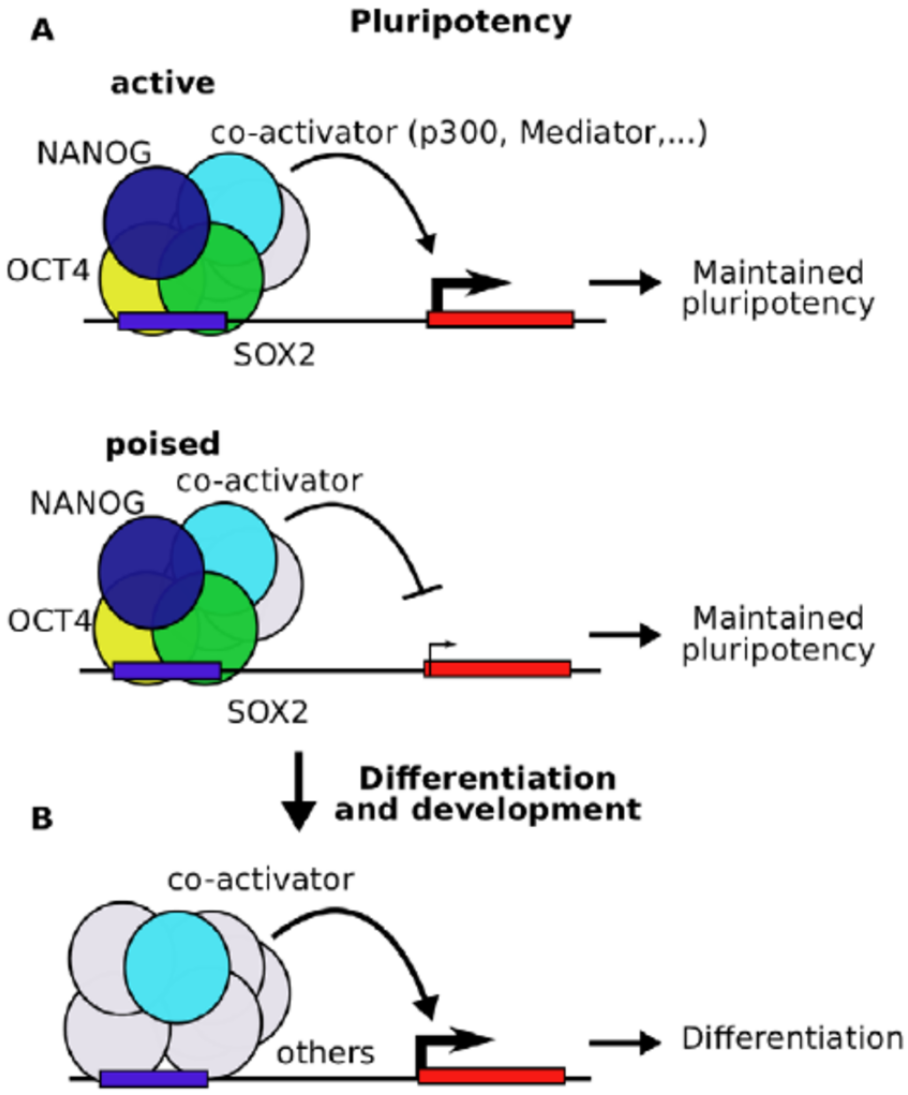
2) SE cell type-specific

3) SNPs linked to disease in SE

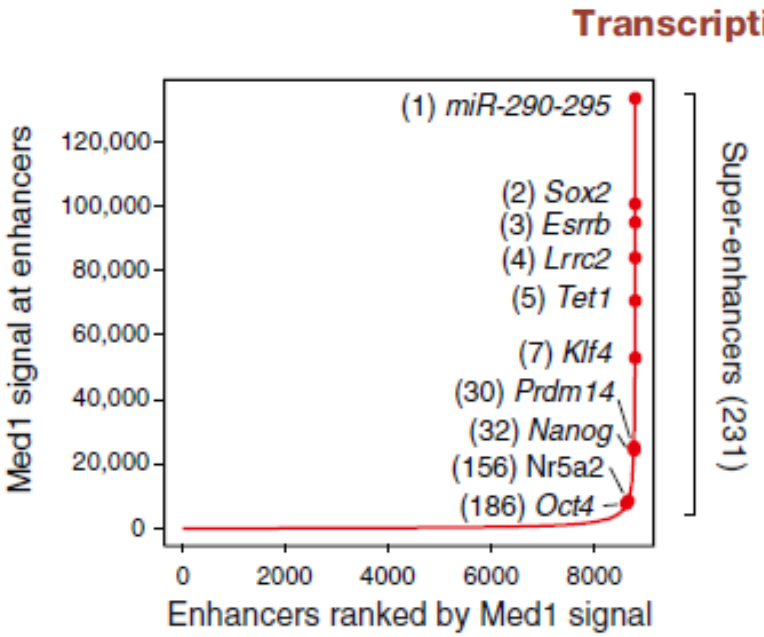
CONCLUSION

Murine Embrionic stem cells (ESC)

SOX2, Nanog, OCT4: transcription factors that bind SE controlling genes for mantained pluripotency



Mediator Coactivator Complexes and Master TFs are bound at Super-enhancers



Super-enhancers are clusters of enhancers—formed by binding of high levels of master transcription factors and Mediator coactivator—that drive high-level expression of genes encoding key regulators of cell identity (Figure 1A) (Whyte et al., 2013). Five ESC transcription factors were previously shown to occupy super-enhancers (Oct4, Sox2, Nanog, Klf4, and Esrrb) (Whyte et al., 2013), but there are many additional transcription factors that contribute to the control of ESCs (Ng and Surani, 2011; Orkin and Hochedlinger, 2011; Young, 2011). We compiled ChIP-seq data for 15 additional transcription factors in ESCs, for which high-quality ChIP-seq data were available, and investigated whether they occupy enhancers defined by Oct4, Sox2, and Nanog (OSN) co-occupancy (Whyte et al., 2013) (Table S1 avail-

(A) Distribution of Med1 ChIP-seq signal at enhancers reveals two classes of enhancers in ESCs. Enhancer regions are plotted in an increasing order based on their input-normalized Med1 ChIP-seq signal. Super-enhancers are defined as the population of enhancers above the inflection point of the curve. Example super-enhancers are highlighted along with their respective ranks and their associated genes.

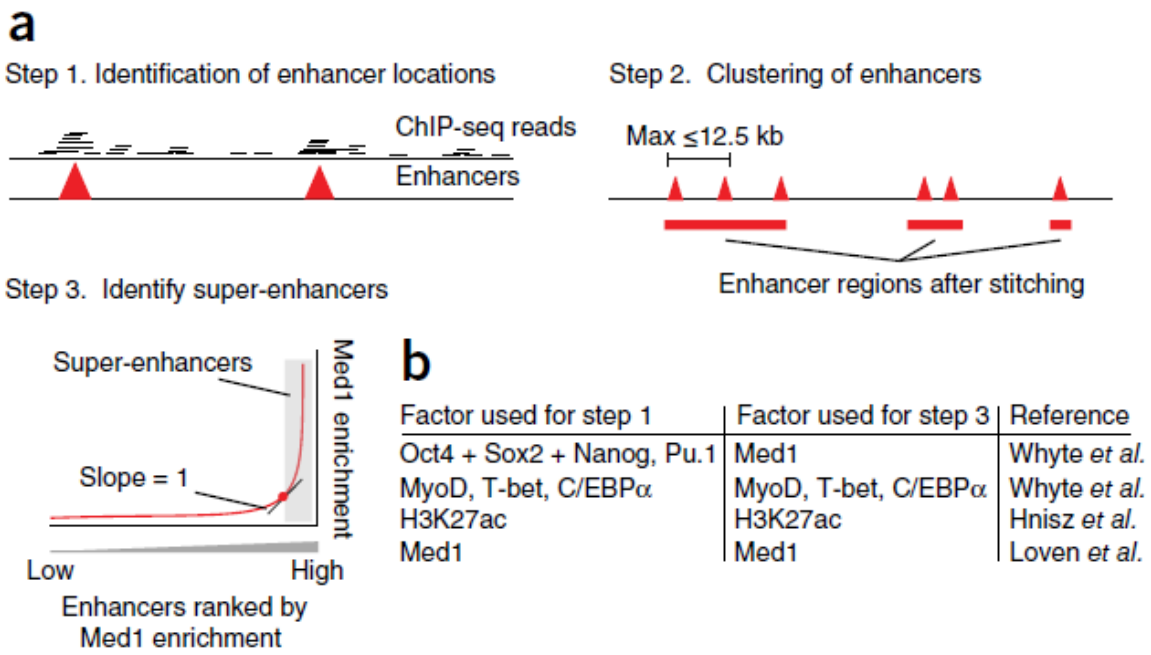
Bioinformatic analysis for the definition of SE:

- Signal in proximity of the gene

- signal extended in the genomic regions that identify SE

- increased numbers of reads into SE respect to costituent, single enhancer in the SE

- increased signal into SE respect to typical enhancer



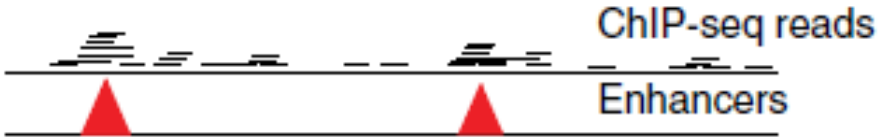
b

Factor used for step 1	Factor used for step 3	Reference
Oct4 + Sox2 + Nanog, Pu.1	Med1	Whyte *et al.*
MyoD, T-bet, C/EBPα	MyoD, T-bet, C/EBPα	Whyte *et al.*
H3K27ac	H3K27ac	Hnisz *et al.*
Med1	Med1	Loven *et al.*

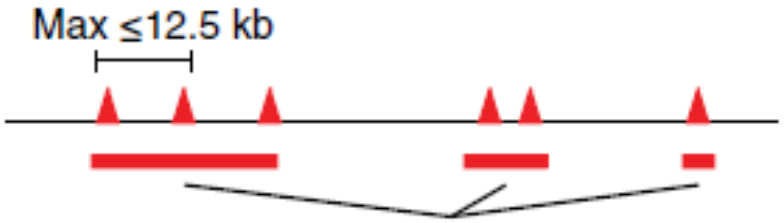
Super-enhancers.

a

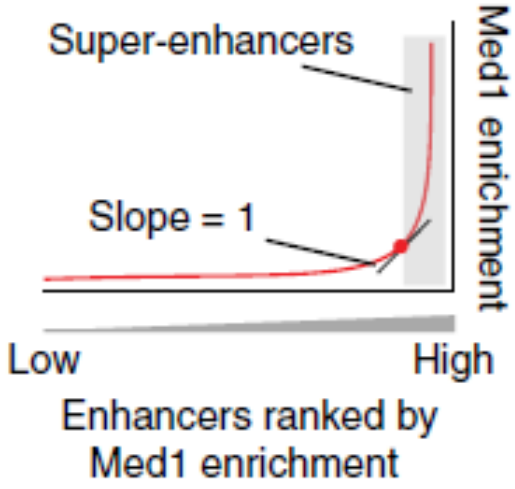
Step 1. Identification of enhancer locations



Step 2. Clustering of enhancers



Step 3. Identify super-enhancers



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MyoD, T-bet, C/EBPα	MyoD, T-bet, C/EBPα	Whyte *et al.*
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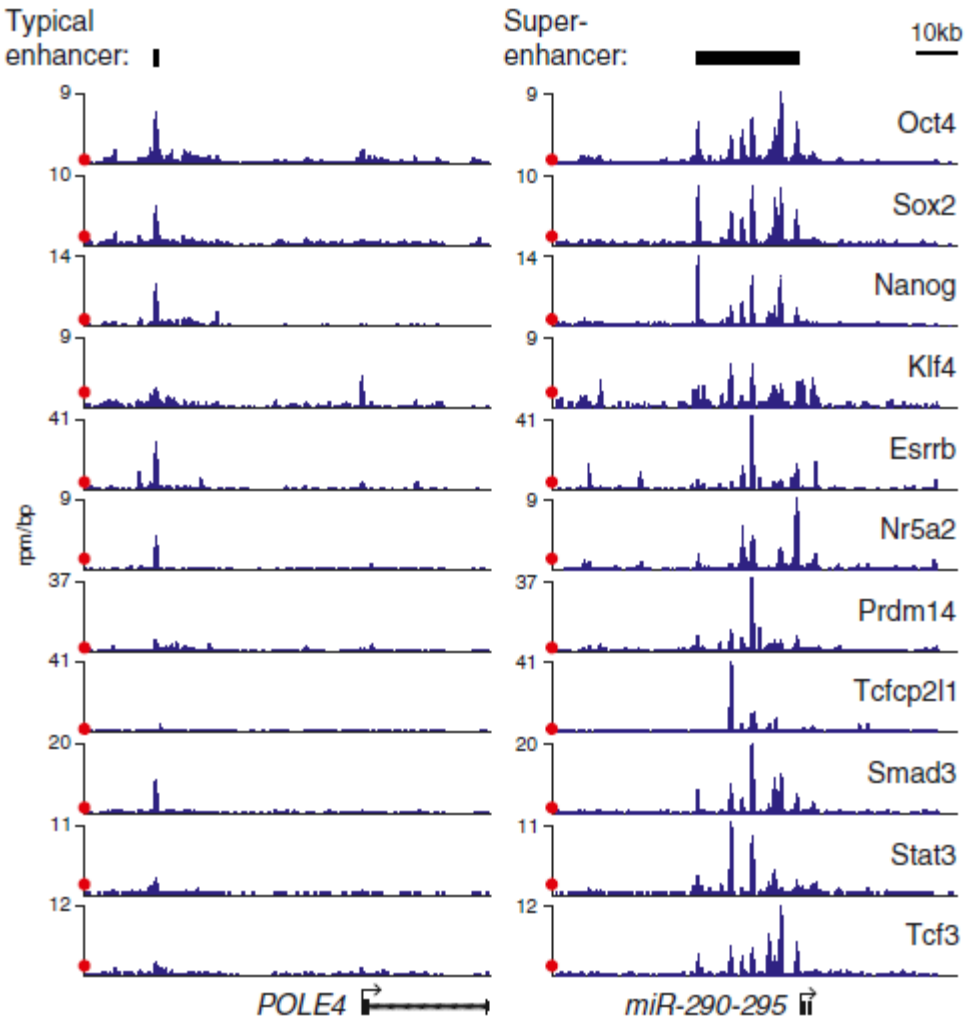
Med1	Med1	Loven *et al.*

Mediator Coactivator Complexes and Master TFs are bound at Super-enhancers

Chromatin Immunoprepitation Binding Profiles at target genes

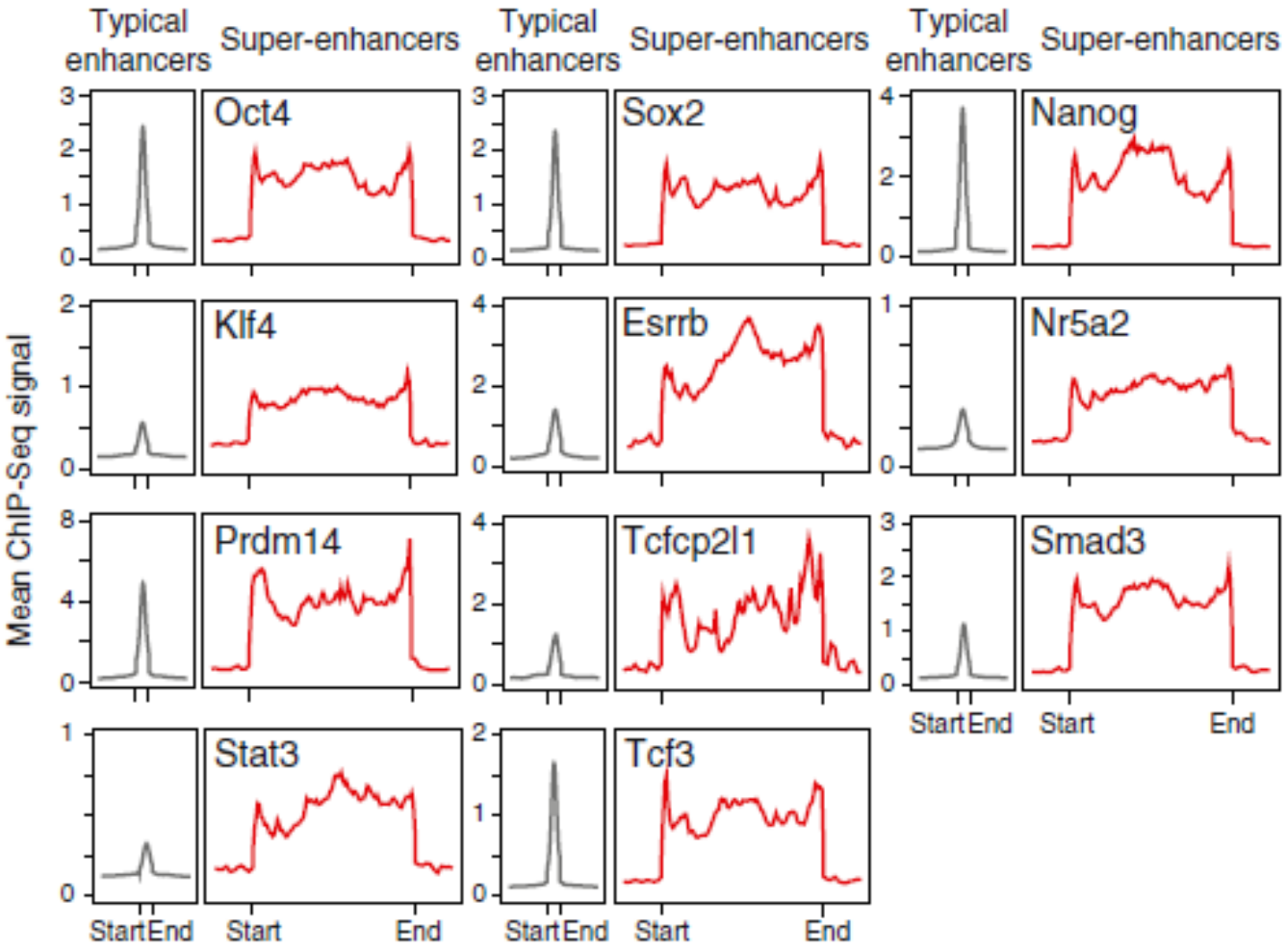


Transcription Factors

Specific Loci

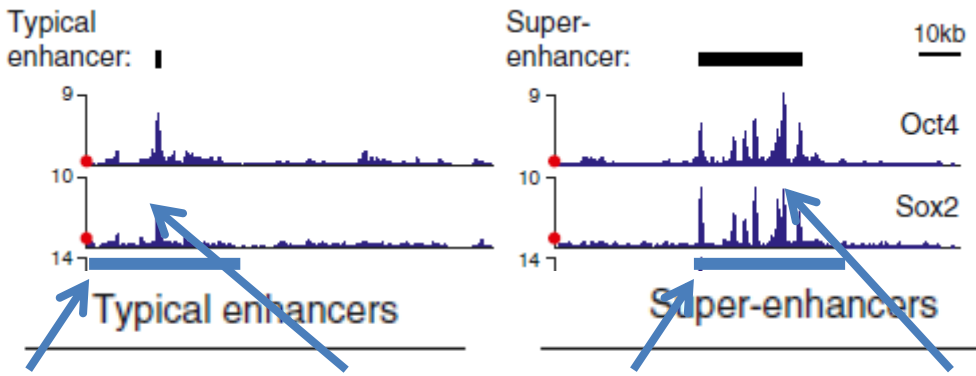
(B) ChIP-seq binding profiles for the indicated transcription factors at the *POLE4* and *miR-290–295* loci in ESCs. Red dots indicate the median enrichment of all bound regions in the respective ChIP-seq data sets and are positioned at maximum 20% of the axis height. rpm/bp, reads per million per base pair.

ChIP-seq signal across SE domains



(C) Metagene representations of the mean ChIP-seq signal for the indicated transcription factors across typical enhancers and super-enhancer domains. Metagenes are centered on the enhancer region, and the length of the enhancer reflects the difference in median lengths (703 bp for typical enhancers, 8,667 bp for super-enhancers). Additional 3 kb surrounding each enhancer region is also shown.

Reads distribution in regions and constituents (single enhancers into SE) (rpm/bp)

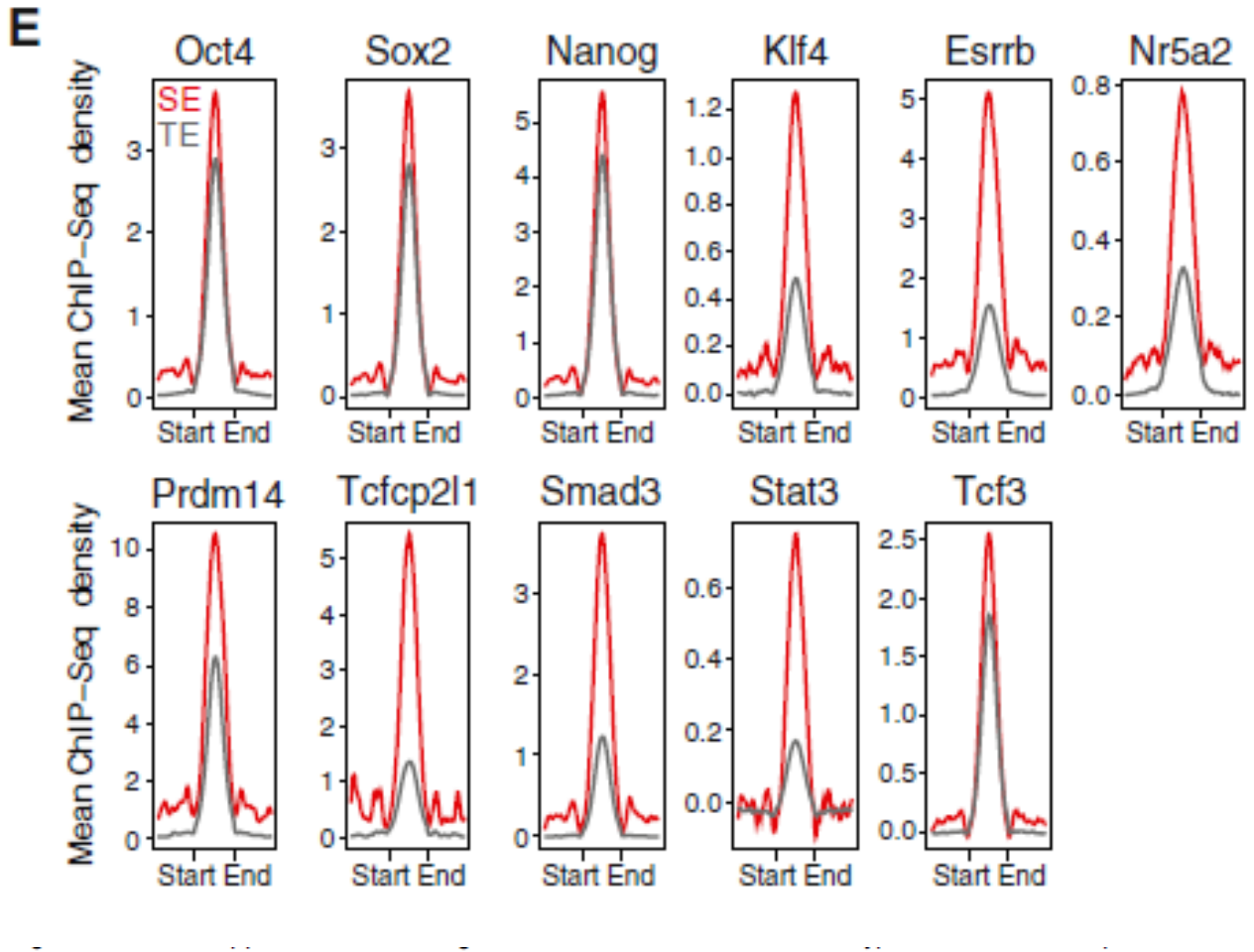


D

	Typical enhancers					Super-enhancers				
	Regions		Constituents			Regions		Constituents		
	Total signal	Density	Total signal	Density	Enhancer reads %	Total signal	Density	Total signal	Density	Enhancer reads %
Oct4:	1x	1x	1x	1x	90 %	4.5x	0.9x	1.6x	1.3x	10 %
Sox2:	1x	1x	1x	1x	90 %	4.2x	1.8x	1.7x	1.2x	10 %
Nanog:	1x	1x	1x	1x	91 %	3.9x	0.9x	1.5x	1.2x	9 %
Klf4:	1x	1x	1x	1x	85 %	8.4x	1.9x	2.9x	2.2x	15 %
Esrrb:	1x	1x	1x	1x	81 %	10.8x	2.6x	3.8x	3.0x	19 %
Nr5a2:	1x	1x	1x	1x	85 %	7.5x	1.7x	2.7x	2.1x	15 %
Prdm14:	1x	1x	1x	1x	87 %	6.2x	5.4x	2.3x	1.9x	13 %
Tcfcp2l1:	1x	1x	1x	1x	80 %	10.7x	0.9x	4.1x	3.3x	20 %
Smad3:	1x	1x	1x	1x	82 %	9.1x	2.8x	3.6x	2.8x	18 %
Stat3:	1x	1x	1x	1x	83 %	7.4x	5.8x	3.5x	2.5x	17 %
Tcf3:	1x	1x	1x	1x	90 %	4.0x	1.1x	1.7x	1.3x	10 %

(D) Fold difference values of ChIP-seq signal between typical enhancers and super-enhancers for the indicated transcription factors. Total signal indicates the mean ChIP-seq signal (total reads) at typical enhancers and super-enhancers normalized to the mean value at typical enhancers. Density indicates the mean ChIP-seq density at constituent enhancers (rpm/bp) of typical enhancers and super-enhancers normalized to the mean value at typical enhancers. Enhancer read % indicates the percentage of all reads mapped to enhancer regions that fall in the constituents of typical enhancer or super-enhancer regions.

ChIP-Seq density on TFs binding sites



(E) Metagene representations of the mean ChIP-seq density for the indicated transcription factors across the constituent enhancers within typical enhancers and super-enhancers. Each metagene is centered on enhancer constituents. Additional 2.5 kb surrounding the constituent enhancer regions is also shown.

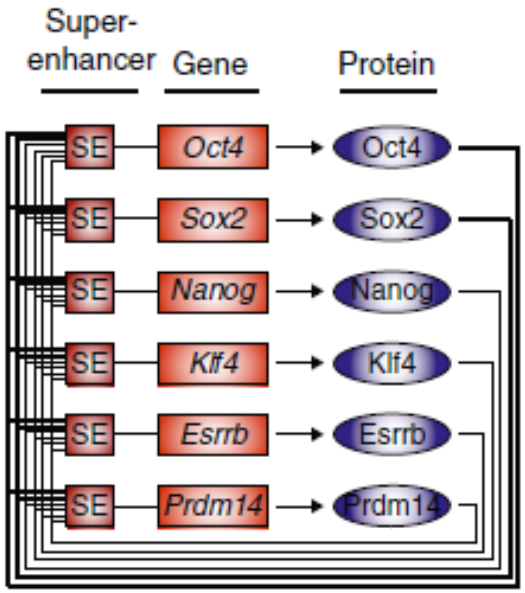
TFs motif enrichment are used to associate gene target

F

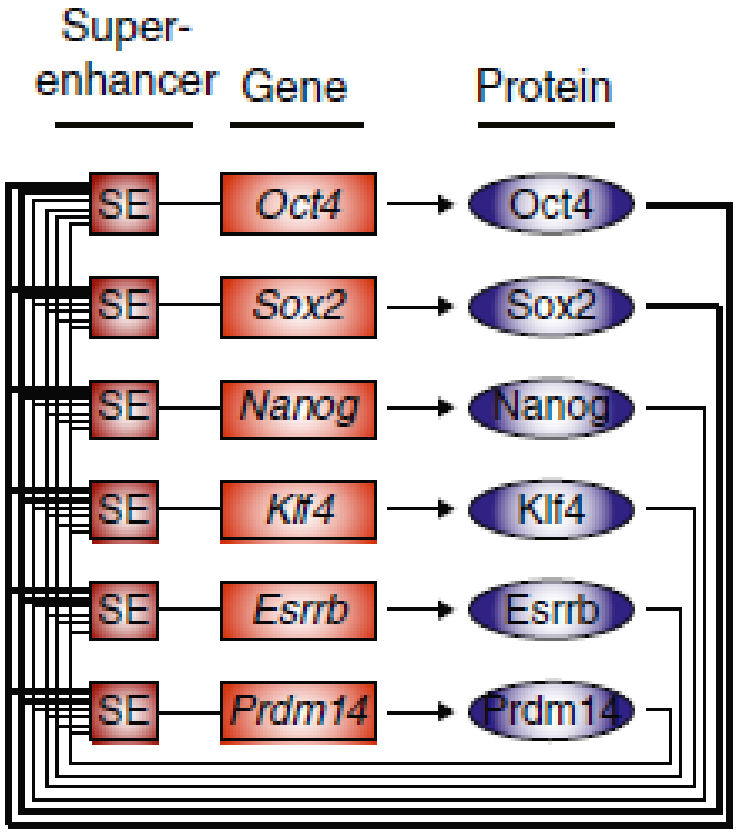
Transcription factor	Motif	P-value
Oct4		$9.19*10^{-64}$
Sox2		$3.01*10^{-67}$
Nanog		$9.46*10^{-17}$
Klf4		$4.33*10^{-6}$
Esrrb		$2.55*10^{-84}$
Nr5a2	n.a.	n.a.

Transcription factor	Motif	P-value
Prdm14	n.a.	n.a.
Tcfcp2l1		$6.83*10^{-11}$
Smad3		$9.31*10^{-11}$
Stat3		$2.90*10^{-10}$
Tcf3		$5.46*10^{-27}$

G

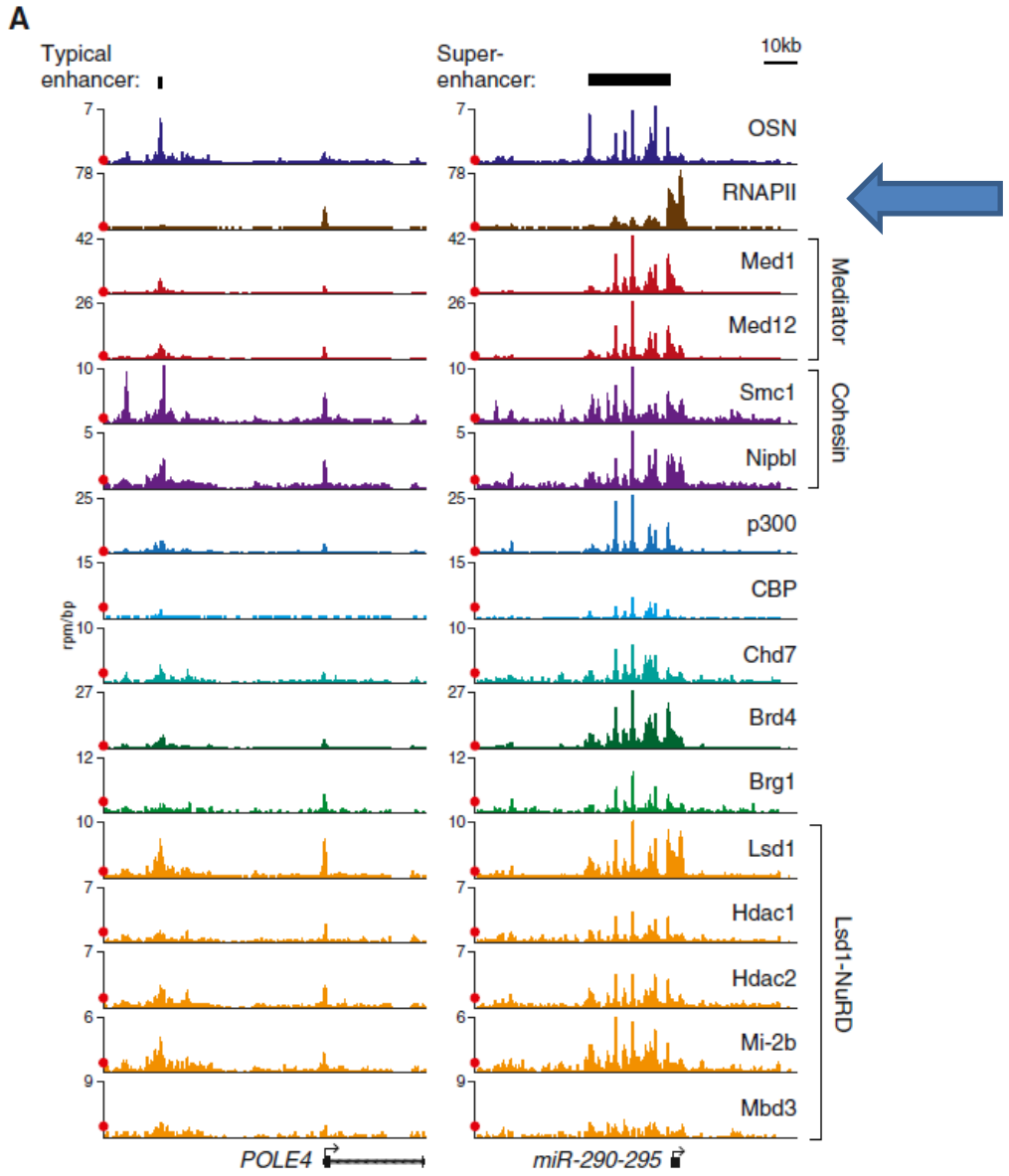


Core Transcriptional Regulatory Circuit of ESCs



(G) Revised model of the core transcriptional regulatory circuitry of ESCs. The model contains an interconnected autoregulatory loop consisting of transcription factors that meet three criteria: (1) their genes are driven by super-enhancers, (2) they co-occupy their own super-enhancers as well as those of the other transcription factor genes in the circuit, and (3) they play essential roles in regulation of ESC state and iPSC reprogramming. The layout of the circuit model was adapted from Whyte et al. (2013).

Super-enhancers are occupied by a large portion of the enhancer-associated RNA polymerase II

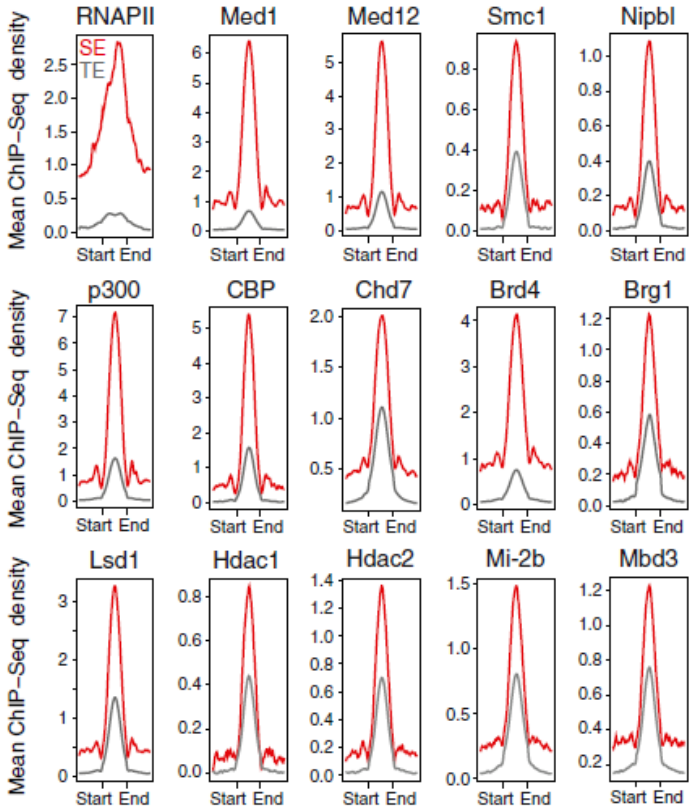


A large fraction of these enhancer cofactors are associated with super-enhancers

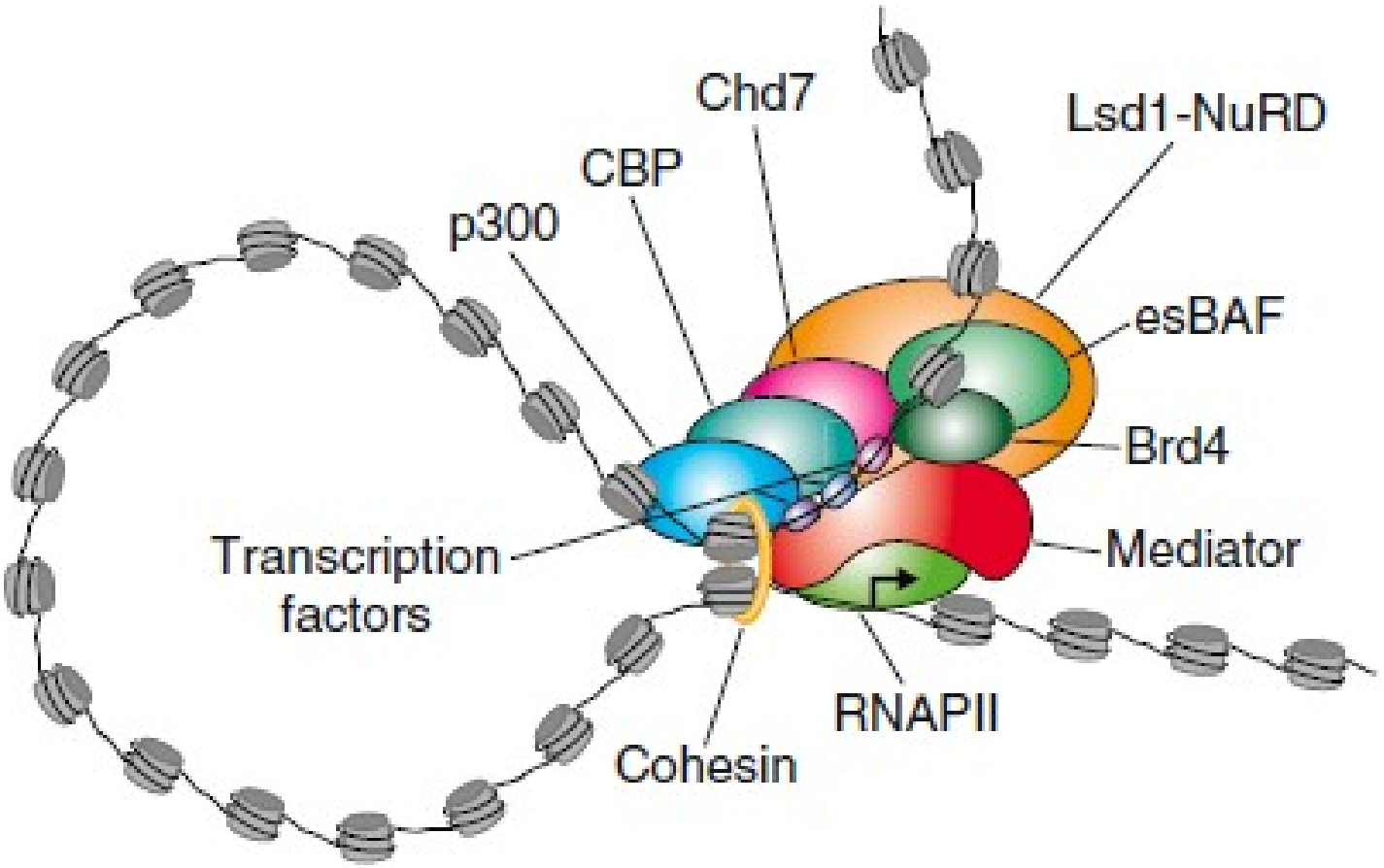
C

	Typical enhancers					Super-enhancers				
	Regions		Constituents			Regions		Constituents		
	Total signal	Density	Total signal	Density	Enhancer reads %	Total signal	Density	Total signal	Density	Enhancer reads %
RNAPII:	1x	1x	1x	1x	68 %	28.8x	8.1x	8.2x	7.1x	32 %
Med1:	1x	1x	1x	1x	64 %	28.2x	6.8x	10.3x	8.1x	36 %
Med12:	1x	1x	1x	1x	75 %	16.0x	3.5x	5.8x	4.5x	25 %
Smc1:	1x	1x	1x	1x	86 %	8.4x	2.2x	2.9x	2.3x	14 %
Nipbl:	1x	1x	1x	1x	80 %	9.4x	2.1x	3.2x	2.5x	15 %
p300:	1x	1x	1x	1x	86 %	13.3x	0.8x	2.0x	4.2x	14 %
CBP:	1x	1x	1x	1x	80 %	10.7x	2.4x	4.0x	3.1x	20 %
Chd7:	1x	1x	1x	1x	87 %	7.6x	1.5x	2.3x	1.8x	13 %
Brd4:	1x	1x	1x	1x	74 %	19.7x	4.2x	6.2x	5.0x	26 %
Brg1:	1x	1x	1x	1x	85 %	8.6x	1.5x	2.7x	2.0x	15 %
Lsd1:	1x	1x	1x	1x	85 %	9.0x	1.9x	2.9x	2.3x	15 %
Hdac1:	1x	1x	1x	1x	88 %	6.5x	1.3x	2.1x	1.6x	12 %
Hdac2:	1x	1x	1x	1x	87 %	6.9x	1.4x	2.2x	1.7x	13 %
Mi-2b:	1x	1x	1x	1x	88 %	7.4x	1.5x	2.2x	1.7x	12 %
Mbd3:	1x	1x	1x	1x	88 %	8.3x	1.5x	2.1x	1.7x	12 %
RNA:	1x	1x	1x	1x	74 %	24.3x	6.0x	5.4x	4.6x	26 %

D

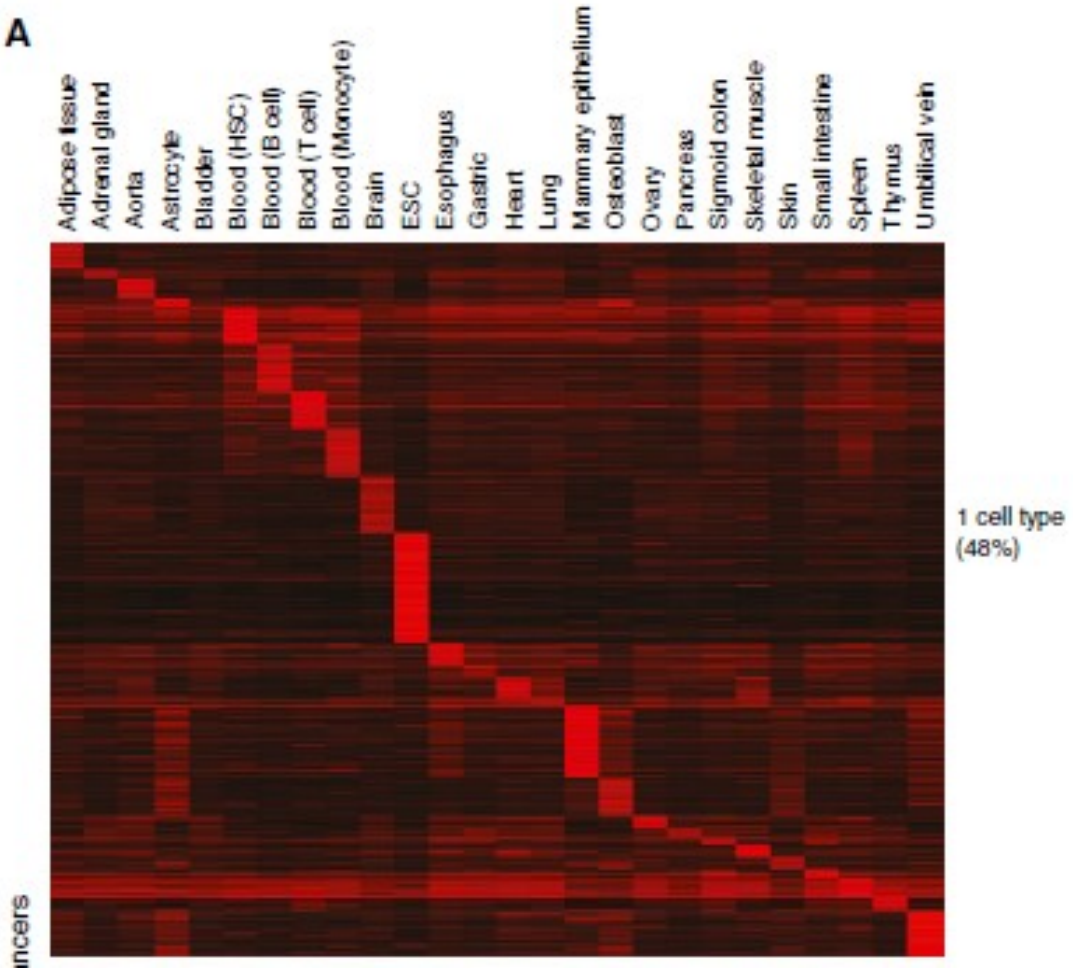


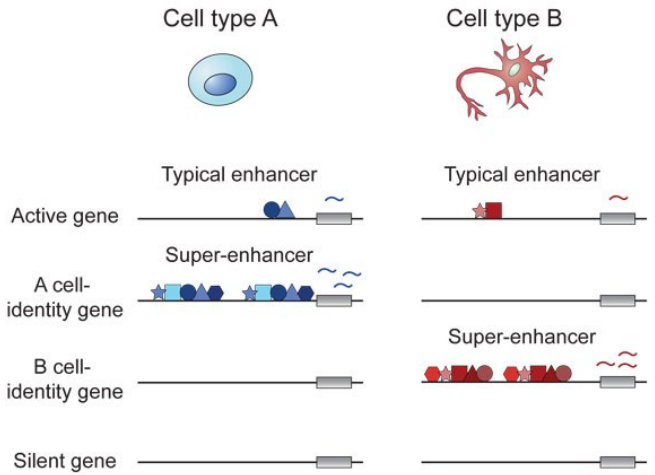
Model showing **RNAPII, transcriptional cofactors, and chromatin regulators that are found in ESC super-enhancers**. The indicated proteins are responsible for diverse enhancer-related functions, such as enhancer looping, gene activation, nucleosome remodeling, and histone modification.



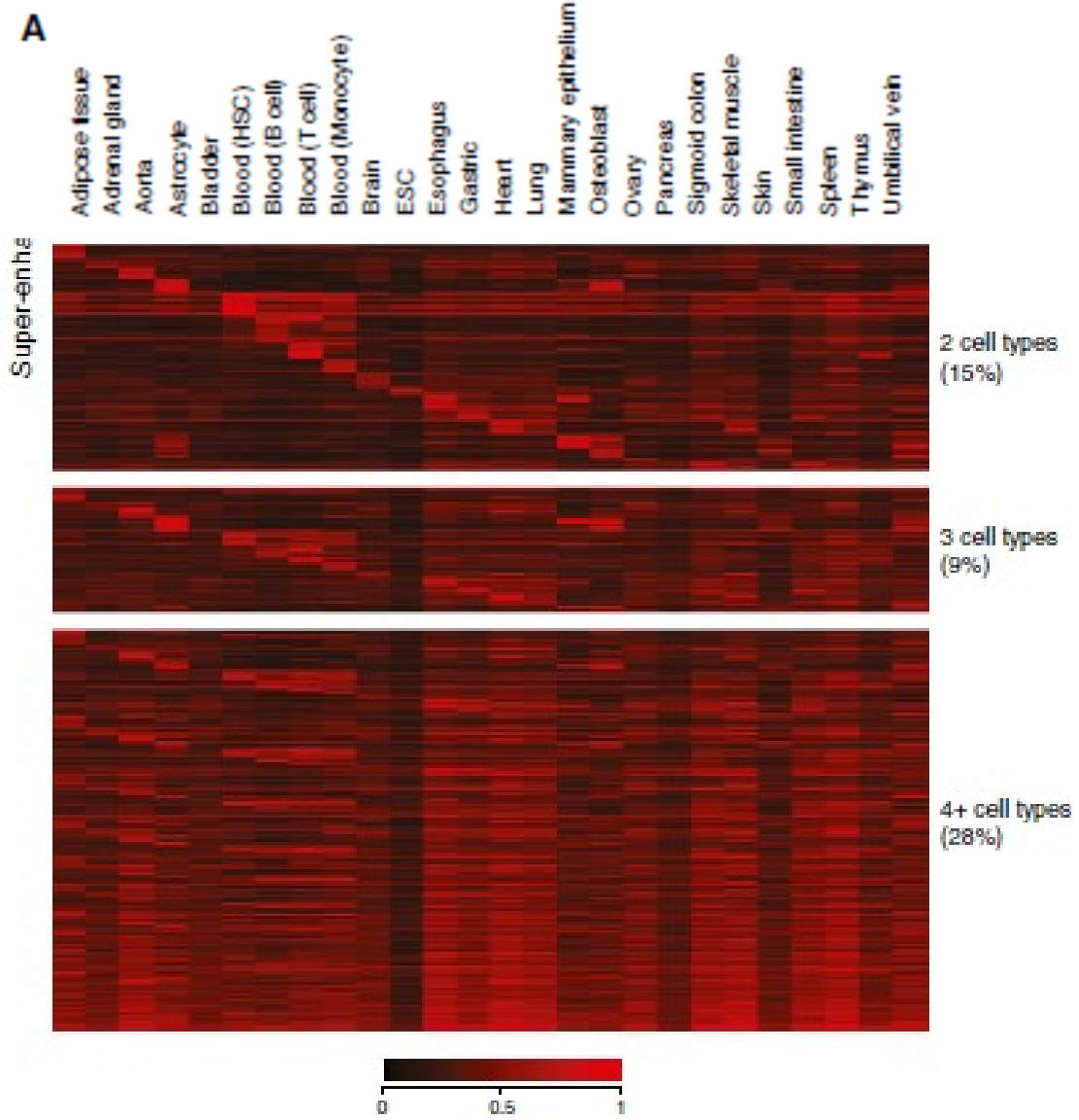
SUPERENHANCER SHARED BETWEEN SEVERAL CELL TYPES

H3K27ac ChIP-seq data are used to create a catalog of superenhancers for 86 human cell and tissue samples. A substantial portion of these super-enhancers and their associated genes are cell type specific.

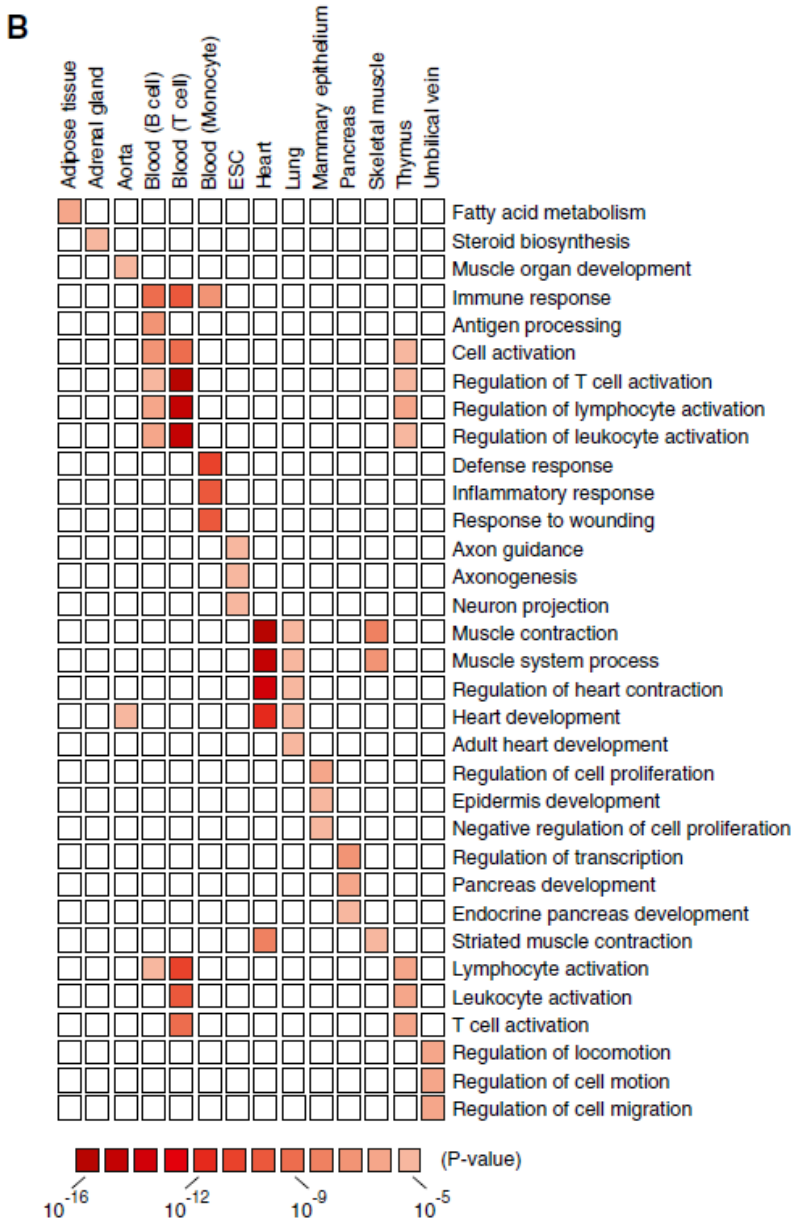




SUPERENHANCER SHARED BETWEEN SEVERAL CELL TYPES



GENE ASSOCIATED TO SUPERENHANCER IN SEVERAL CELL TYPES: GENE ONTOLOGY



Characterization of superenhancer-associated genes by **Gene Ontology analysis** revealed that they are linked to **biological processes that largely define the identities** of the respective cell and tissue types.

MASTER TRANSCRIPTION FACTORS IN SIX CELL TYPES are regulated by SE

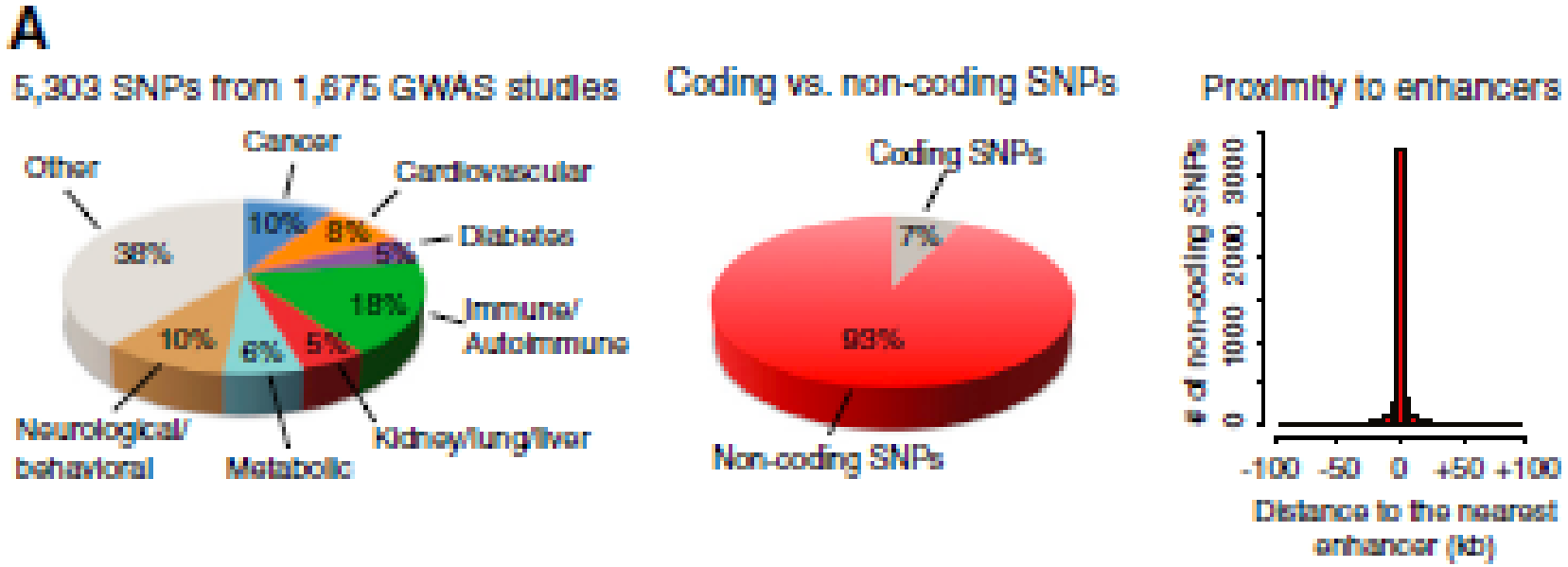
The transcriptional regulatory circuitry is formed with transcription factors that control cell states, therefore acting as Master Transcription Factor. For each cell types are found master TFs regulated by SE.

C

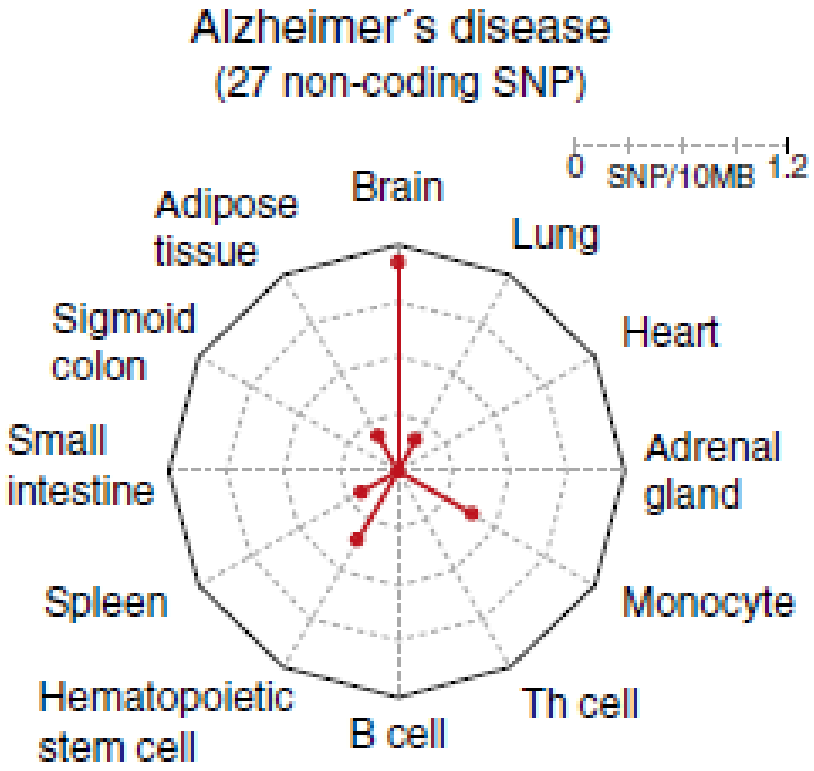
Brain	Heart	Skeletal muscle	Lung	Adipose tissue	B cell
NKX2-2	TBX20	MYOD1	NFIB	PPARG	IKZF3
OLIG1	TBX5	PITX2	TBX5	CEBPB	PAX5
BRN2	MEF2A	SIX1	CEBPA	CEBPD	BACH2
SOX10	NKX2-5	TEAD4	TBX2	CREB1	OCT2
SOX2	GATA4		TBX3		IKZF1
					IRF8

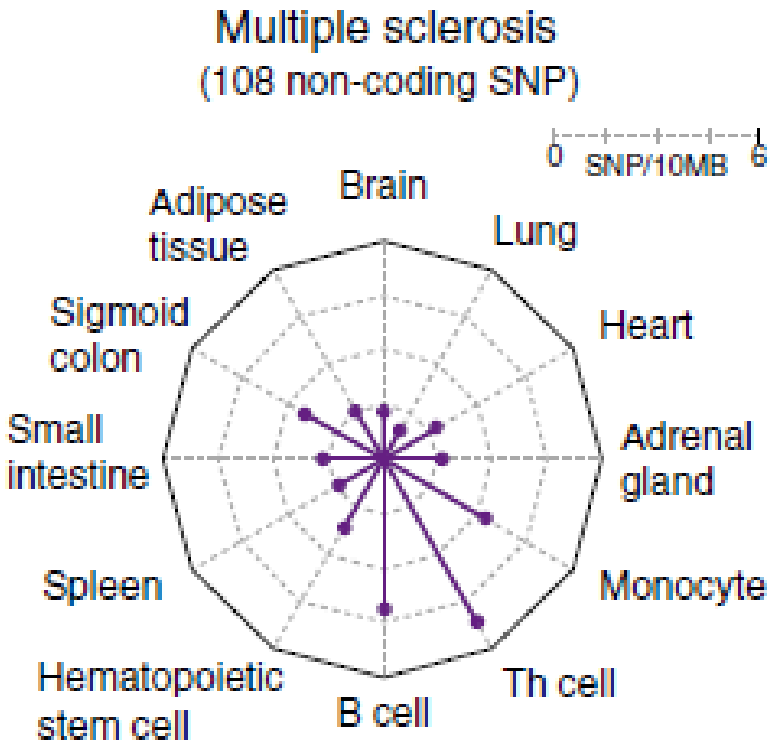
(C) Candidate master transcription factors identified in six cell types. All of these transcription factors were previously demonstrated to play key roles in the biology of the respective cell type or facilitate reprogramming to the respective cell type.

SINGLE NUCLEOTIDE MUTATIONS LINKED TO DISEASE (GWAS) ASSOCIATED TO SUPERENHANCERS



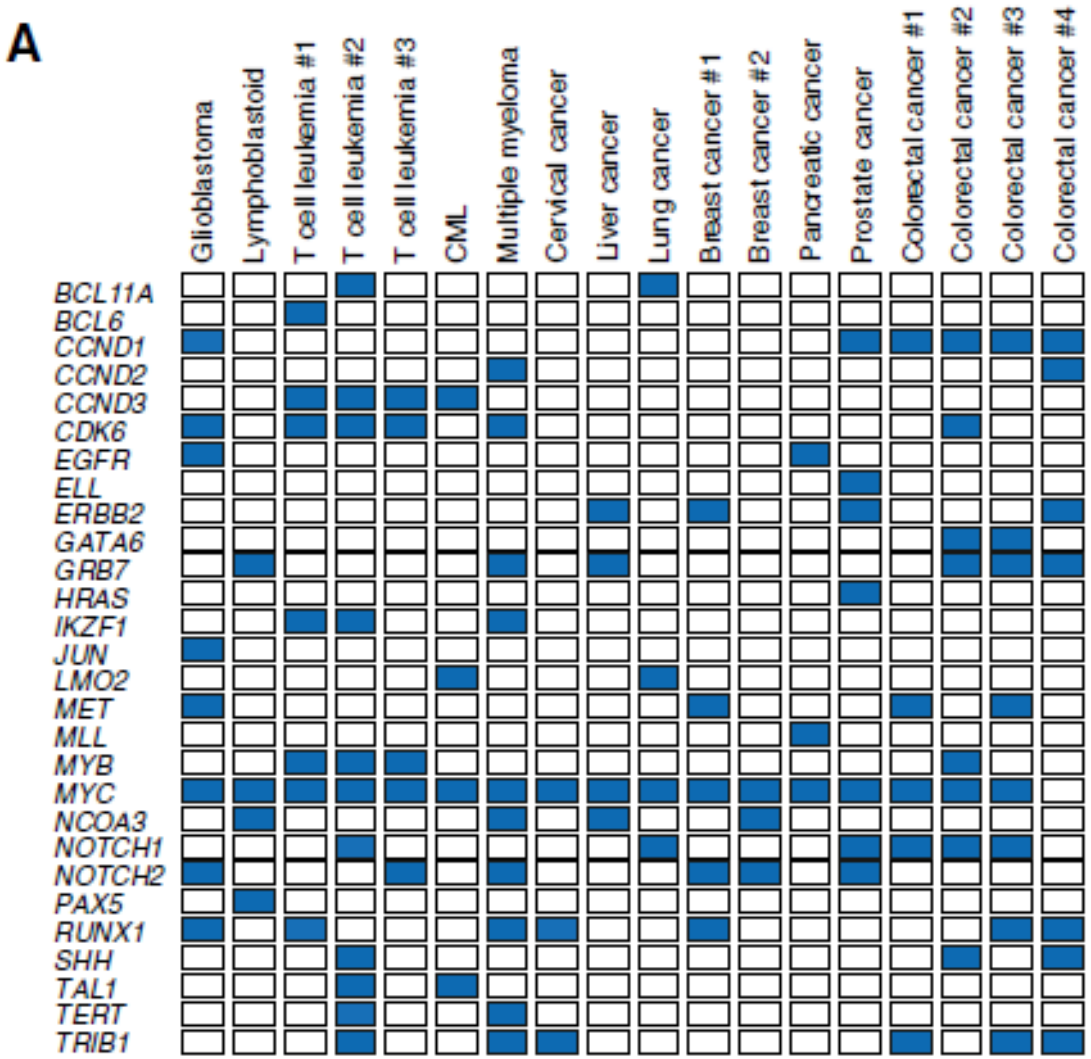
SINGLE NUCLEOTIDE MUTATIONS LINKED TO DISEASE (GWAS) ASSOCIATED TO SUPERENHANCERS



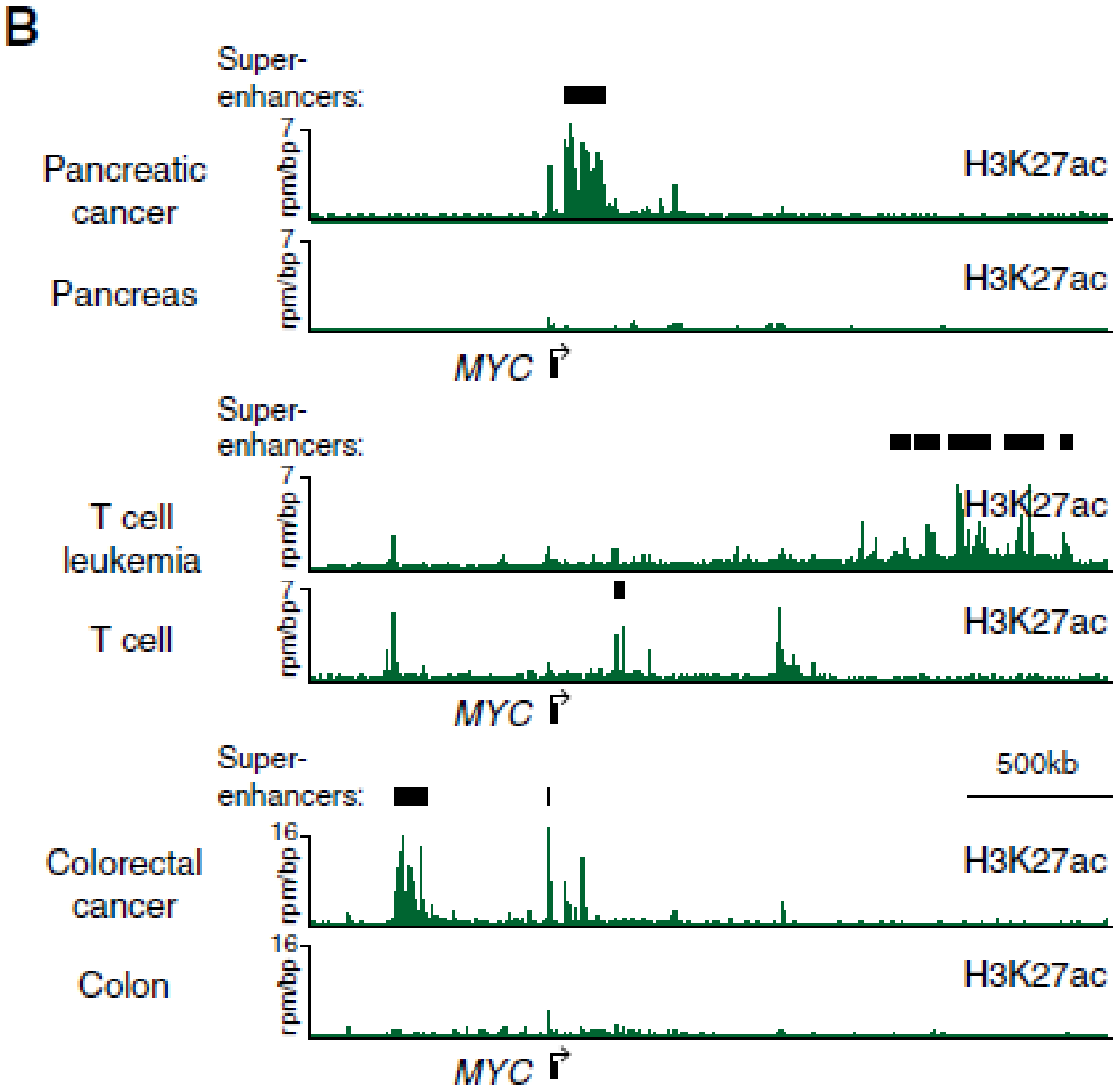


Super-enhancers in Cancer

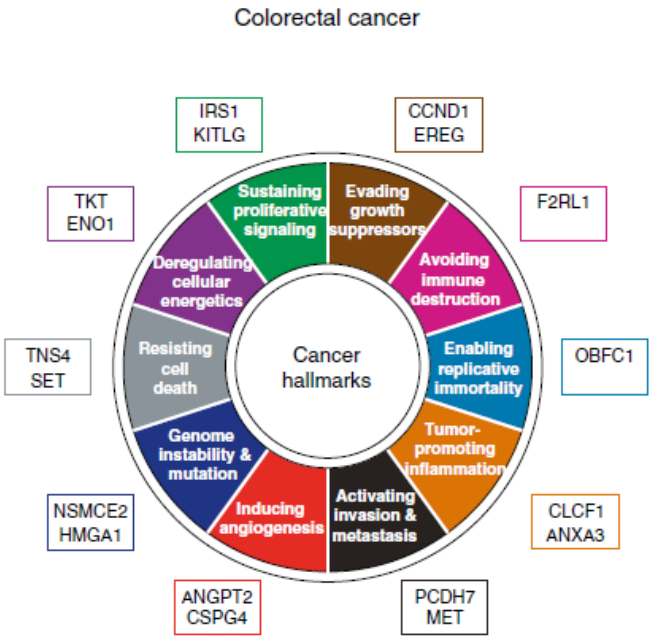
Genes associated with SE and involved in cancer progression

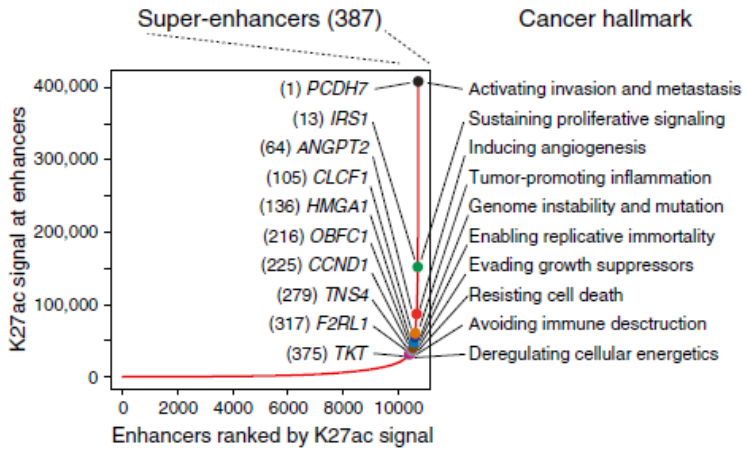


The super-enhancers formed in the MYC locus were tumor type specific



Super-enhancers are associated with genes that act as hallmarks in colonrectal cancer





IDENTIFICATION AND CHARCTERIZATION

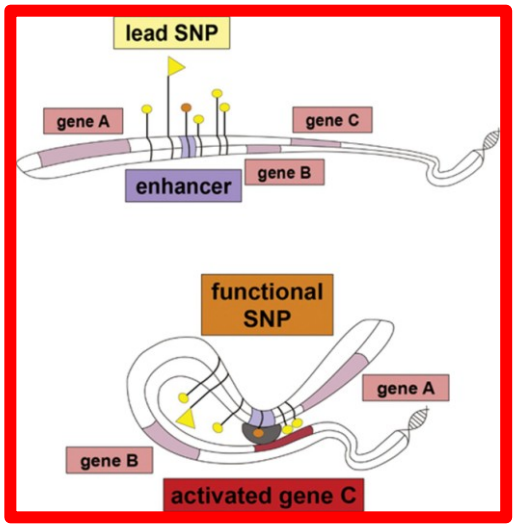
MOLECULAR MECHANISMS

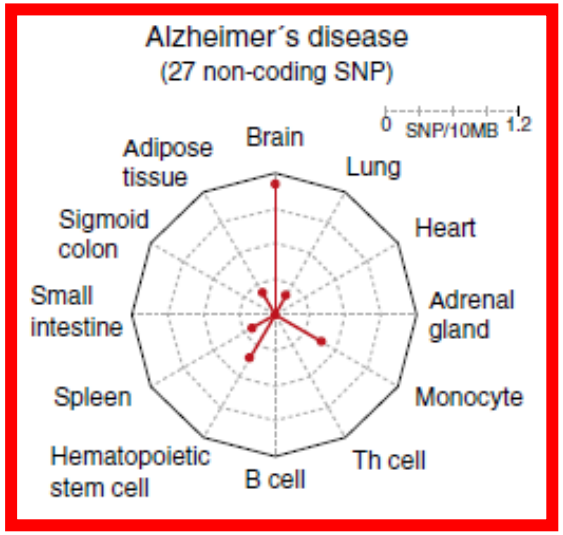
GENOMIC REGULATORY REGIONS

GENE EXPRESSION REGULATION

CELL IDENTITY

BIOLOGICAL FUNCTIONS





TO UNDERSTAND DISEASES

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

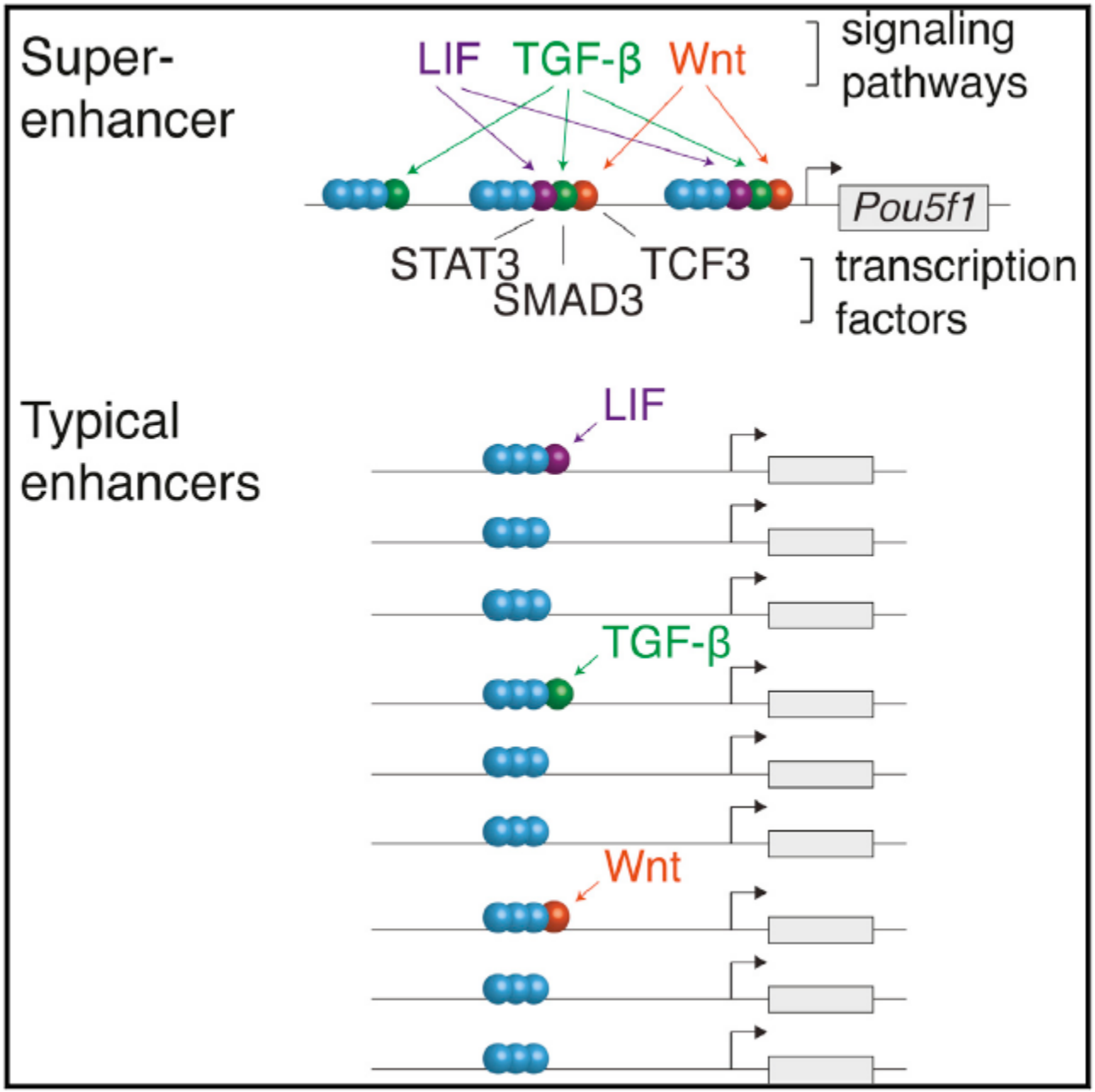
AIM

Super-enhancers (SE)
Functional costituent units

Cancer cells SE target
for oncogenic signalling

CONCLUSION

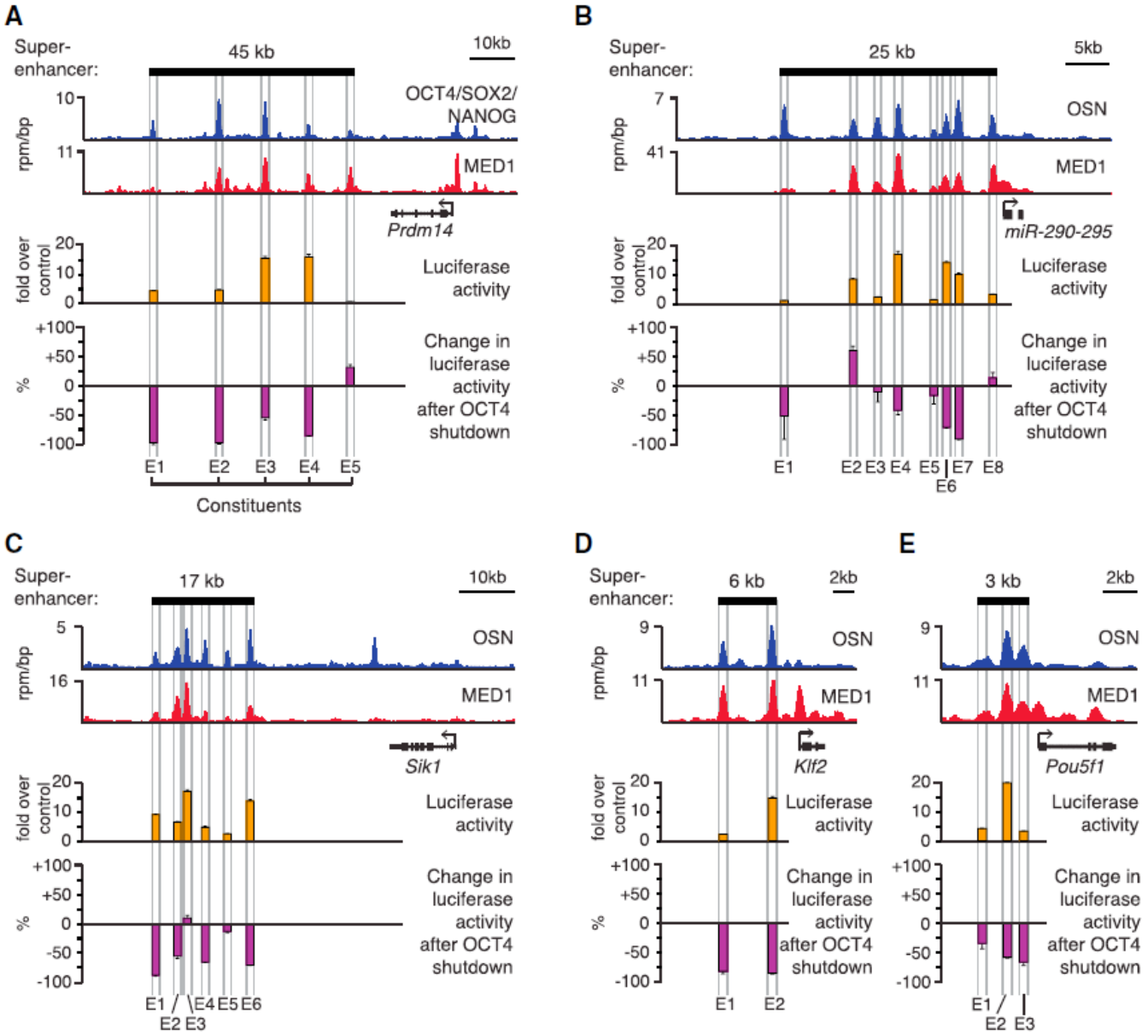
Graphical Abstract



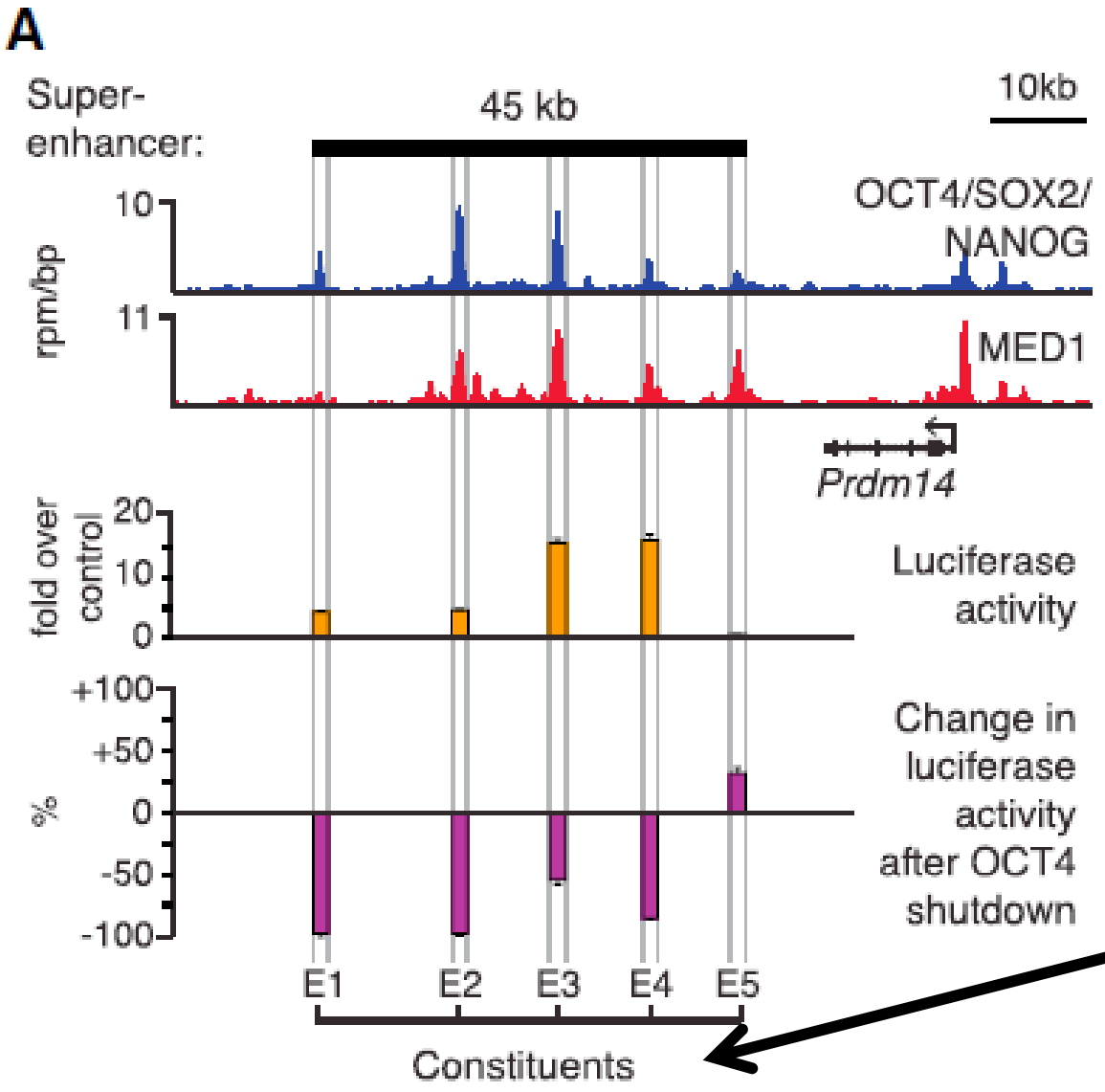
Highlights

- 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

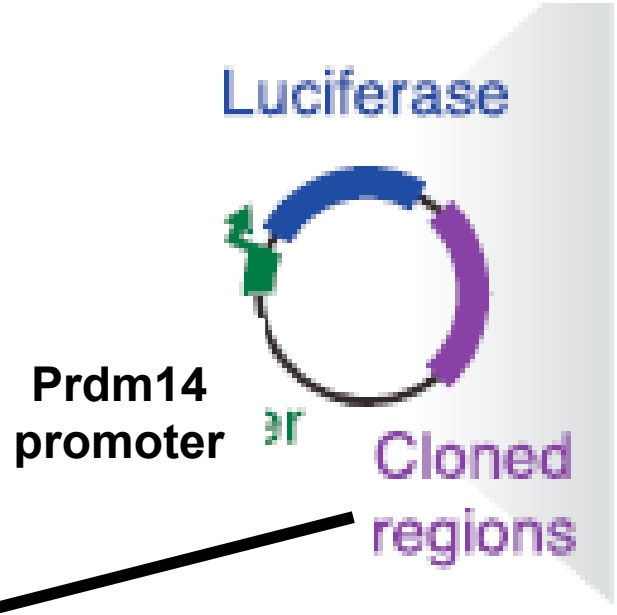
ACTIVITY OF SUPER-ENHANCER CONSTITUENTS IN SEVERAL GENOMIC LOCI



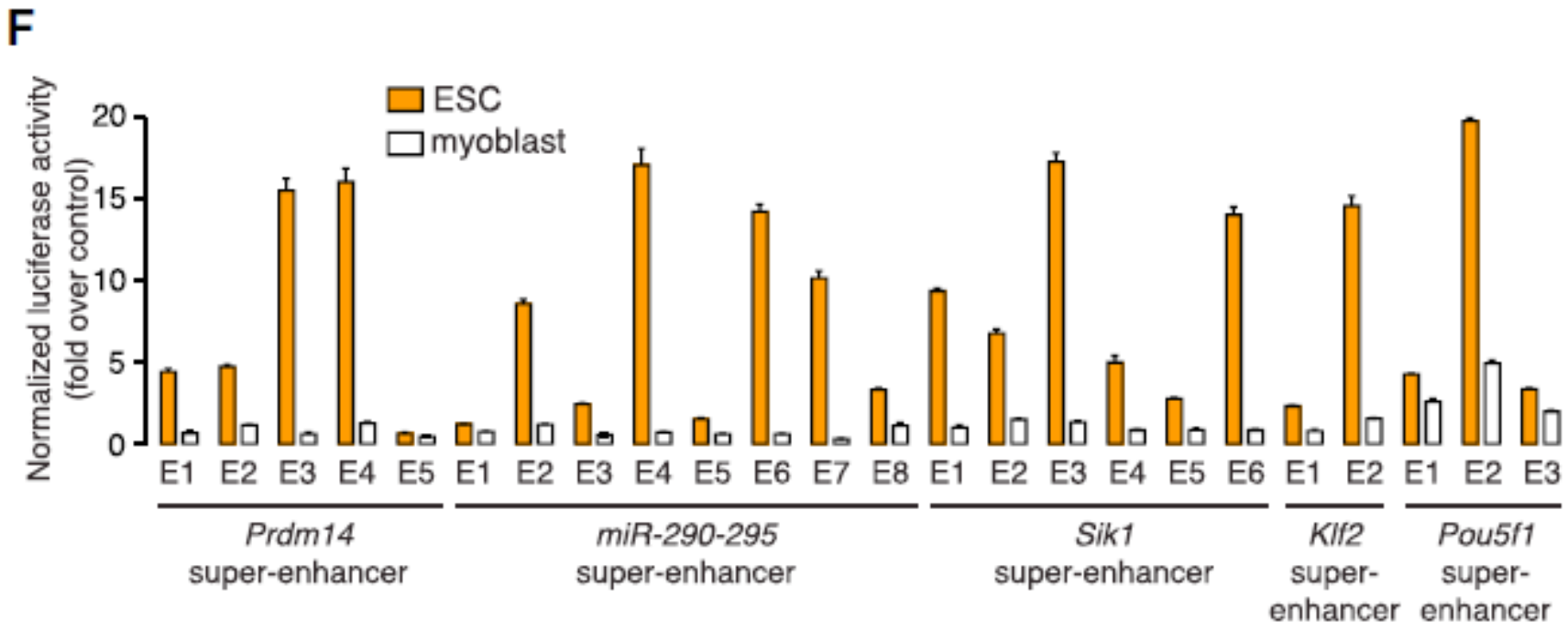
ACTIVITY OF SUPER-ENHANCER CONSTITUENTS



ChIP-Seq profile

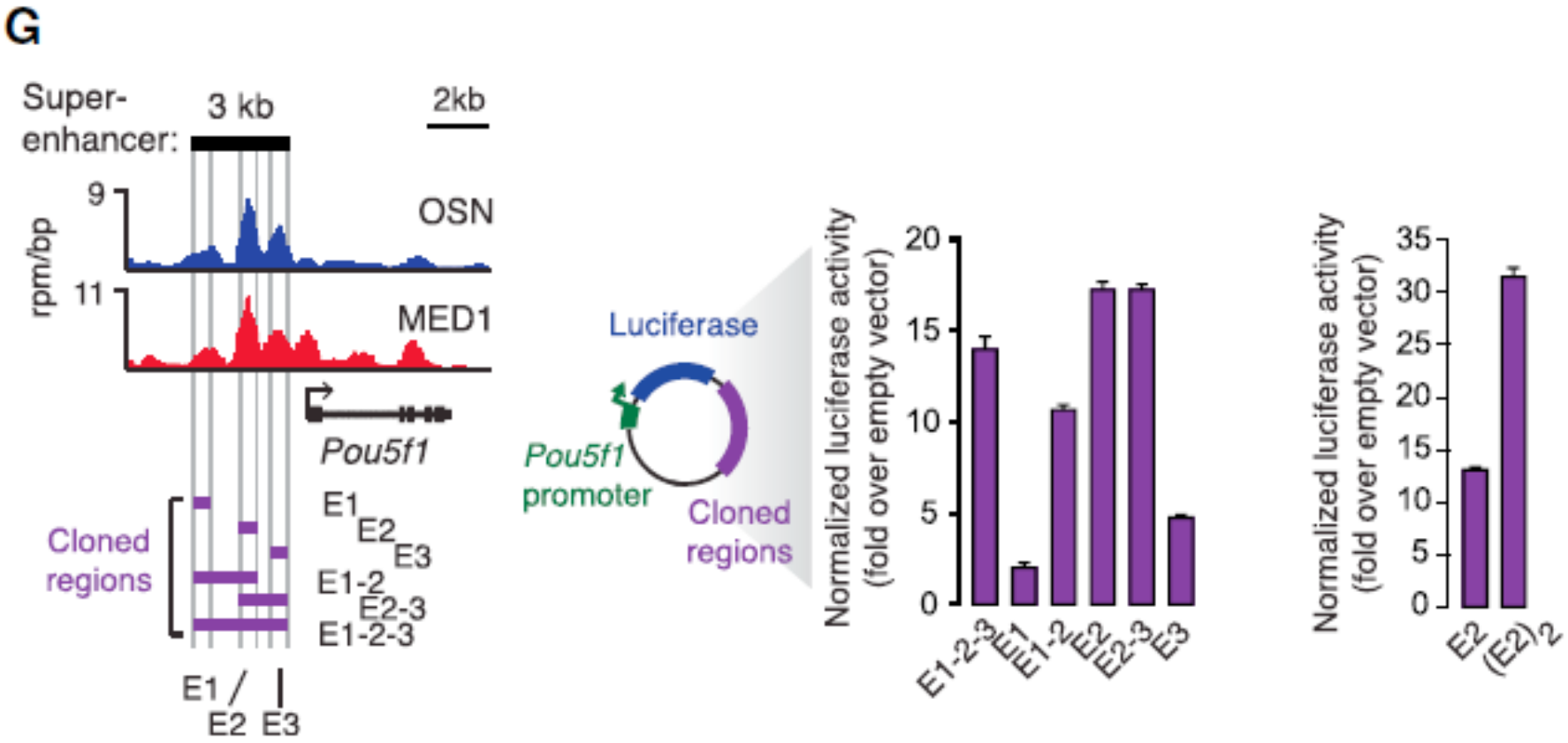


ENHANCER ACTIVITY OF SE CONSTITUENTS IS SPECIFIC FOR ESC, COMPARED TO MYOBLAST

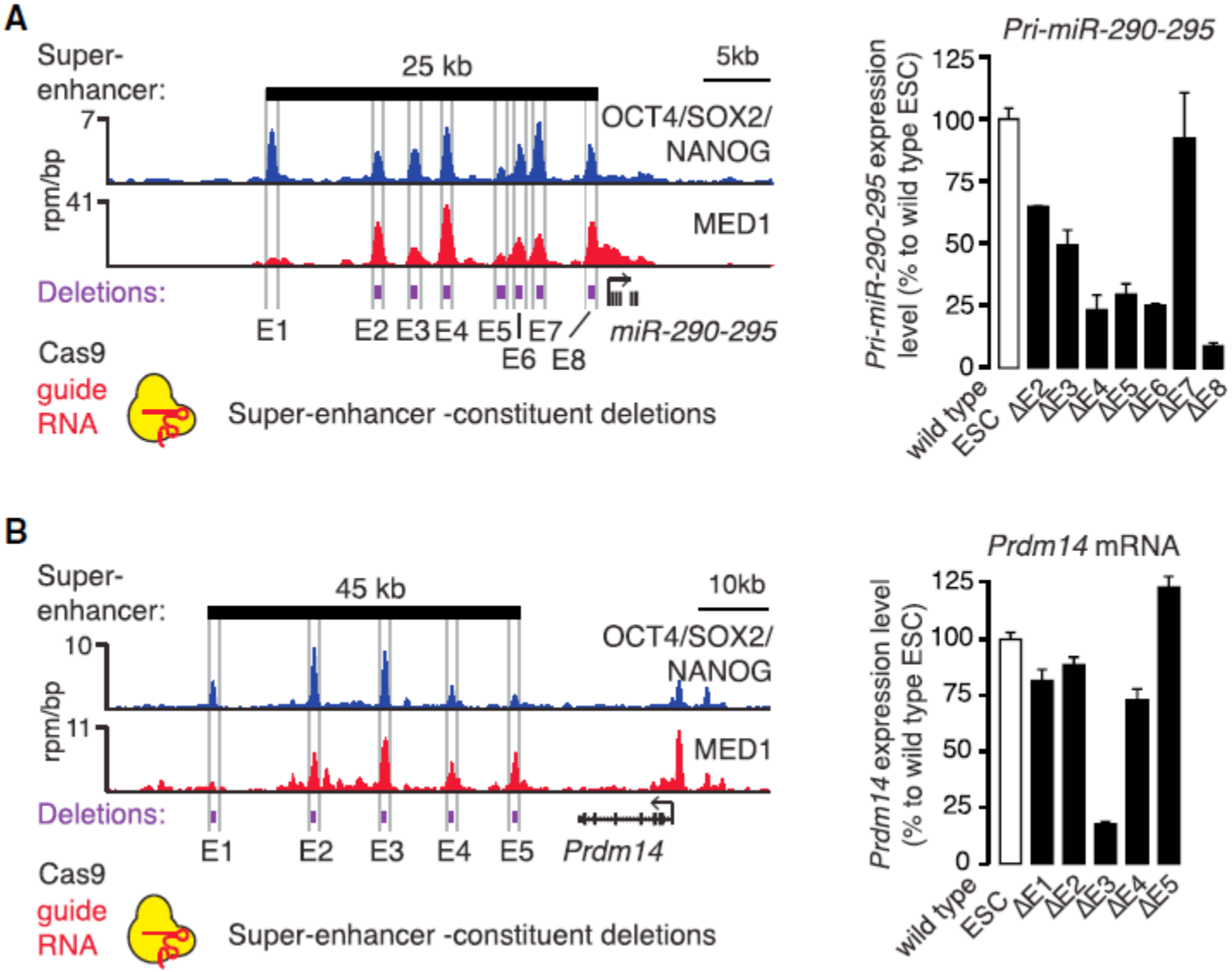


QUESTION: DO "SE CONSTITUENTS" ACT ADDITIVELY, SINERGISTICALLY OR EXERT A COMPLEX INFLUENCE?

E2 has high activity, E1 and E3 influence E2 activity



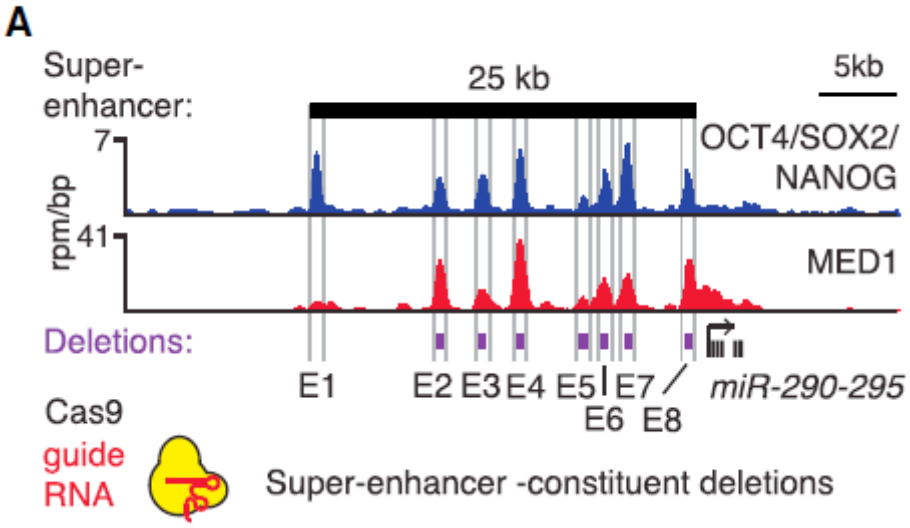
CONTRIBUTIONS OF SUPER-ENHANCER COSTITUENTS TO GENE EXPRESSION IN VIVO



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