





Lineage-specific dynamic and pre-established enhancer–promoter contacts cooperate in terminal differentiation

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- **Biological systems**

epidermal differentiation in vitro (D0;D3;D6)

undifferentiated progenitor-containing cell populations (day 0)

early (day 3)

late (day 6)

- **Data set**

Hi-C (1.36 billion reads)

Promoter capture Hi-C (1.09 billion reads)

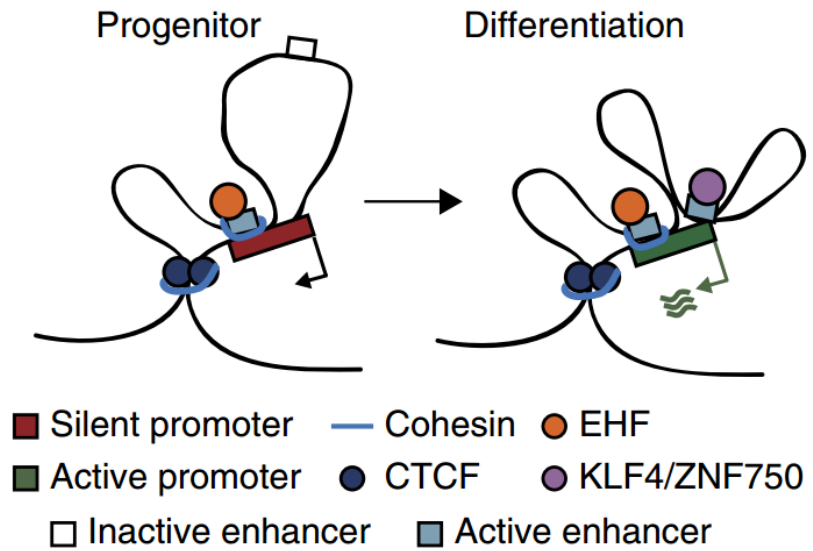
ATAC-seq

RNA-seq

ChIP-seq: KLF4 ZNF750 EHF H3K27ac

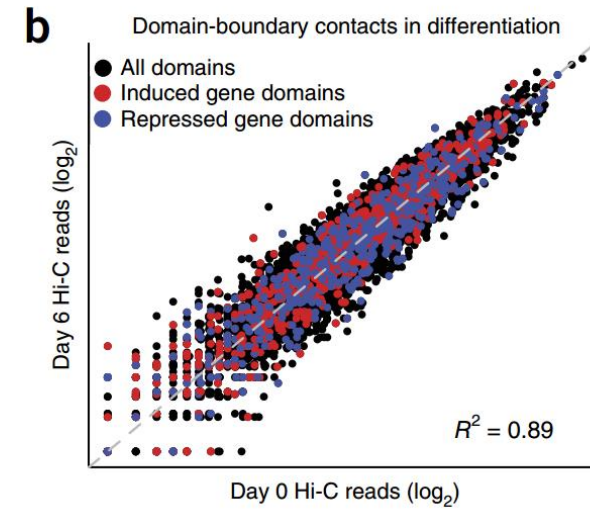
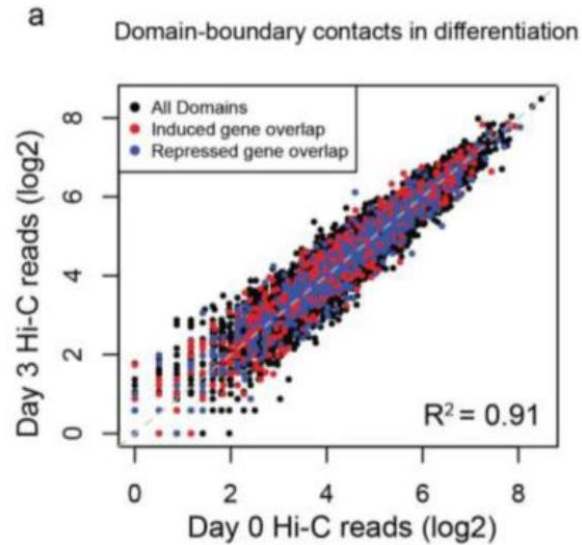
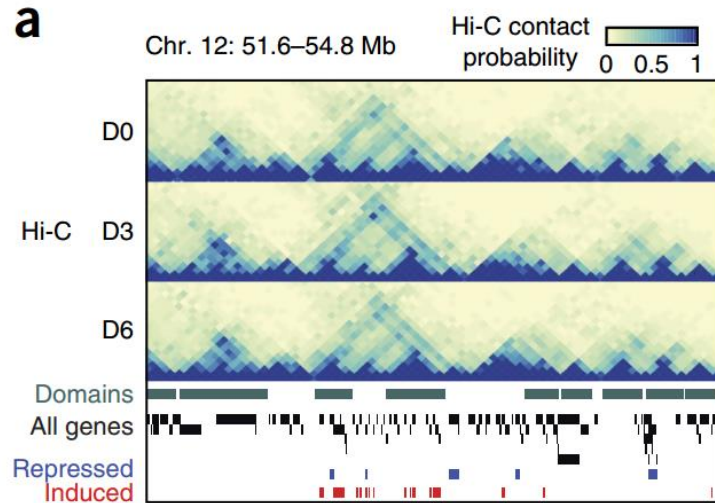
UMI-4C

model



		WT	EHFi
stable E-P	regulator	EHF (stable expressed)	
	contact	stable	not affected
	H3K27ac	stable	not affected
	differentiation-induced genes	induced	reduced
		WT	KLF4i;ZNF750i
gained E-P	regulator	KLF4;ZNF750	
	contact	gained	reduced
	H3K27ac	gained	reduced
	differentiation-induced genes	induced	reduced

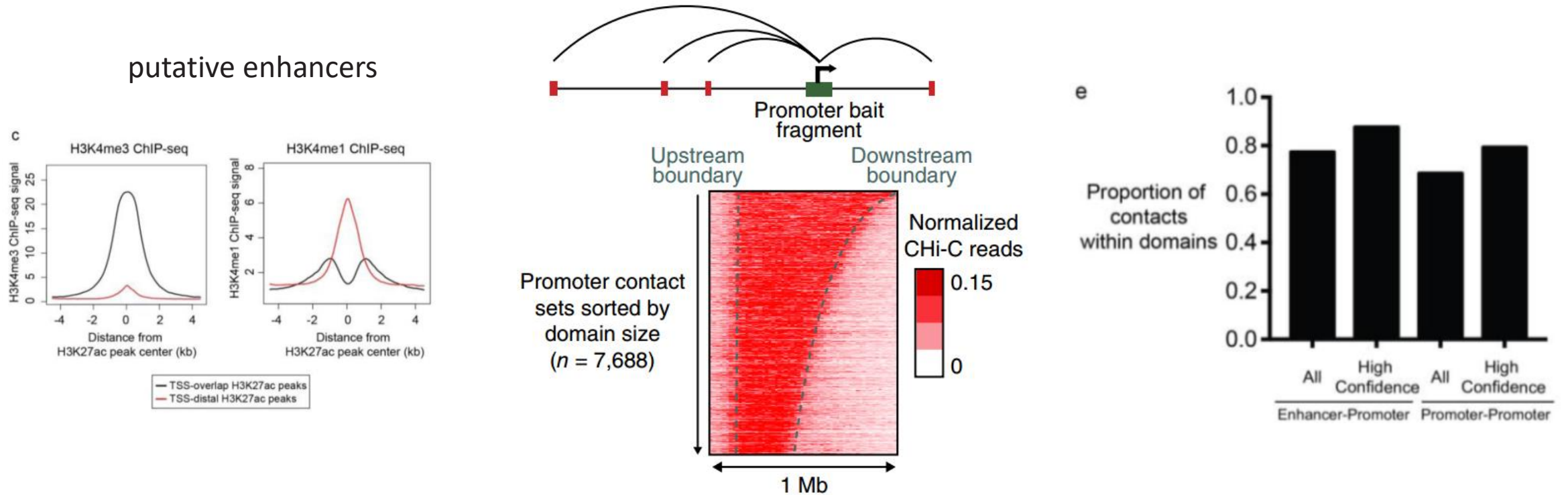
Domain boundary contacts



	All Domains	Dynamic boundary domains			
		Decreasing		Increasing	
		D3 vs. D0	D6 vs. D0	D3 vs. D0	D6 vs. D0
# of domains	4309	49	162	55	166
# induced genes	729 (100%)	12 (1.6%)	20 (2.7%)	15 (2.1%)	28 (3.8%)
# repressed genes	436 (100%)	4 (0.9%)	16 (3.7%)	5 (1.1%)	19 (4.4%)

Domain boundary contacts were stable during differentiation
Regulatory dynamics occur via intra-domain contacts

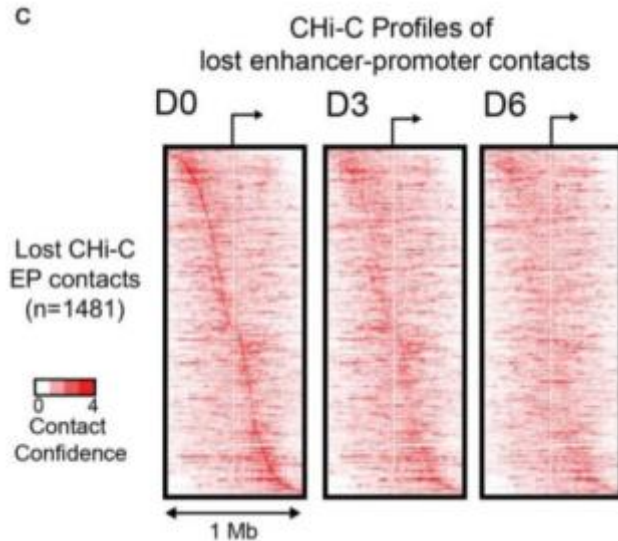
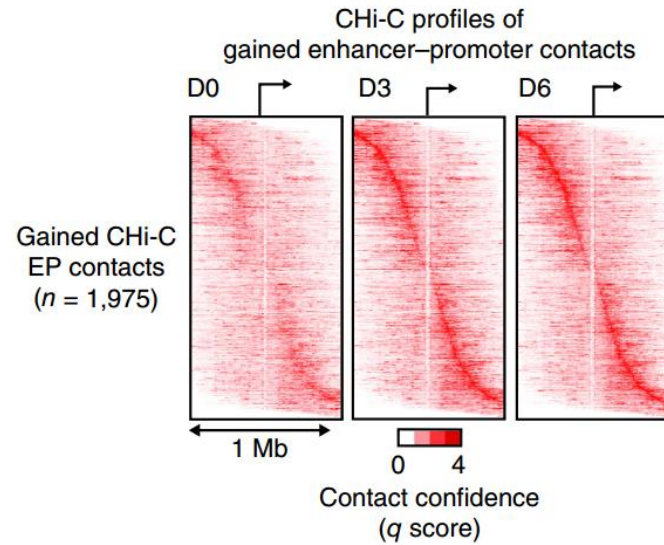
E-P contacts reside in TADs



Chi-C identified 207,663 enhancer–promoter contacts and 89,752 promoter–promoter contacts

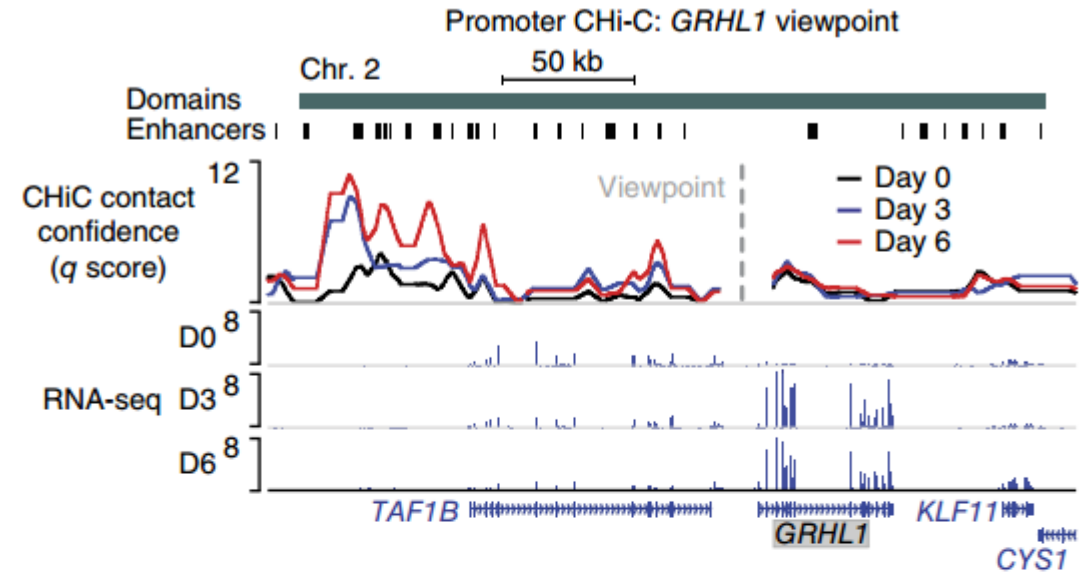
Both P-P and E-P contacts were largely restricted to single domains

Contact changes

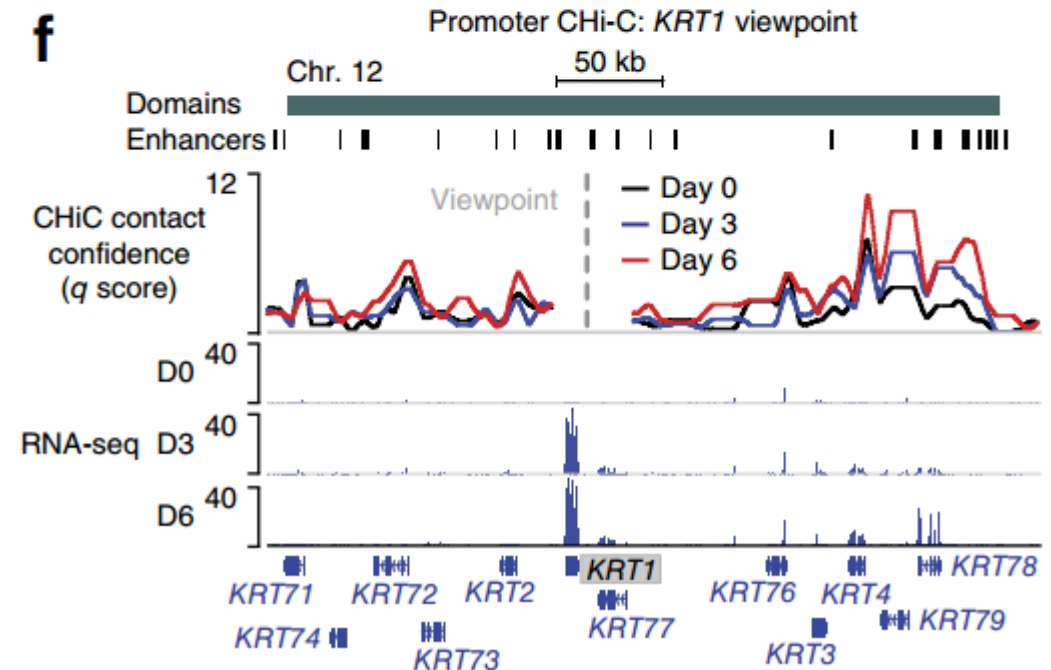


3,575 increased E-P contacts (>2-fold: 1,975)
 3,207 decreased E-P contacts (>2-fold: 1,481)

e

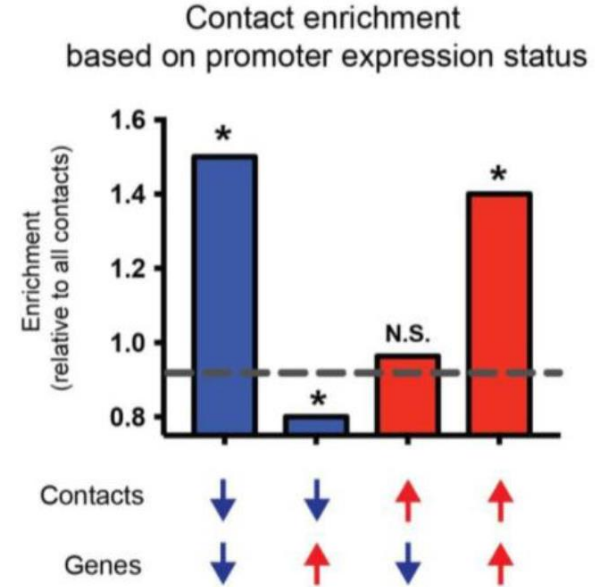


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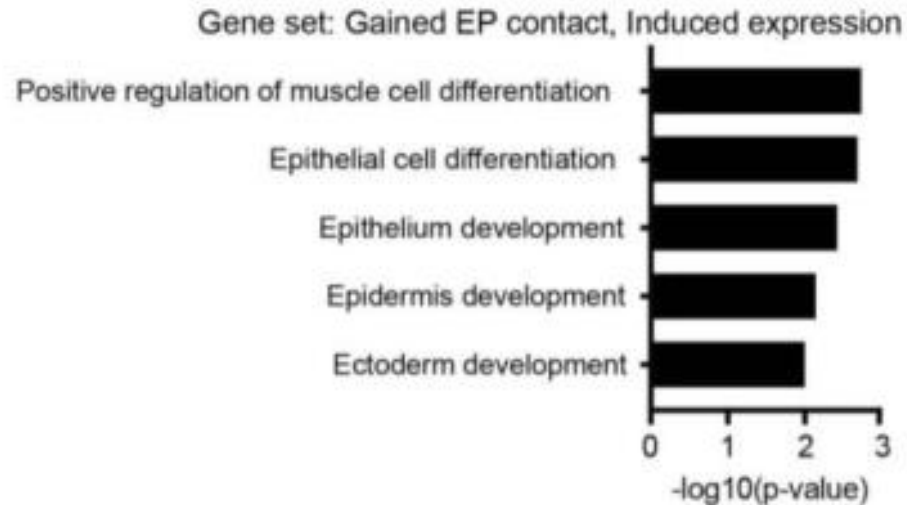
Contact changes and expression

	All Contacts	Dynamic Enhancer-Promoter contacts			
		Decreasing		Increasing	
		D3 vs. D0	D6 vs. D0	D3 vs. D0	D6 vs. D0
# of contacts	89711	905	2686	1107	2856
# induced genes	898 (100%)	40 (4.5%)	94(10.5%)	77 (8.6%)	205 (22.8%)
# repressed genes	605 (100%)	47 (7.8%)	95 (15.7%)	24 (4.0%)	75 (12.4%)

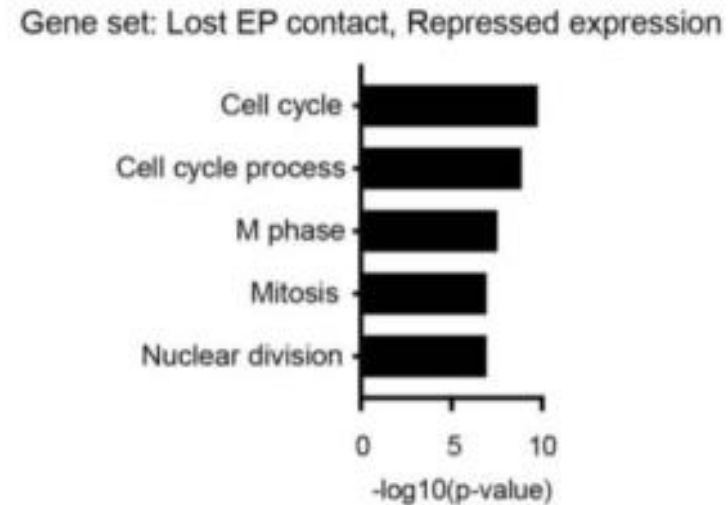


global bias

increased contacts - differentiation-induced genes,
reduced contacts - differentiation-repressed genes

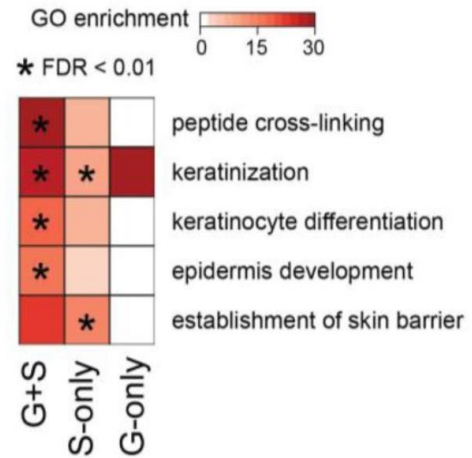
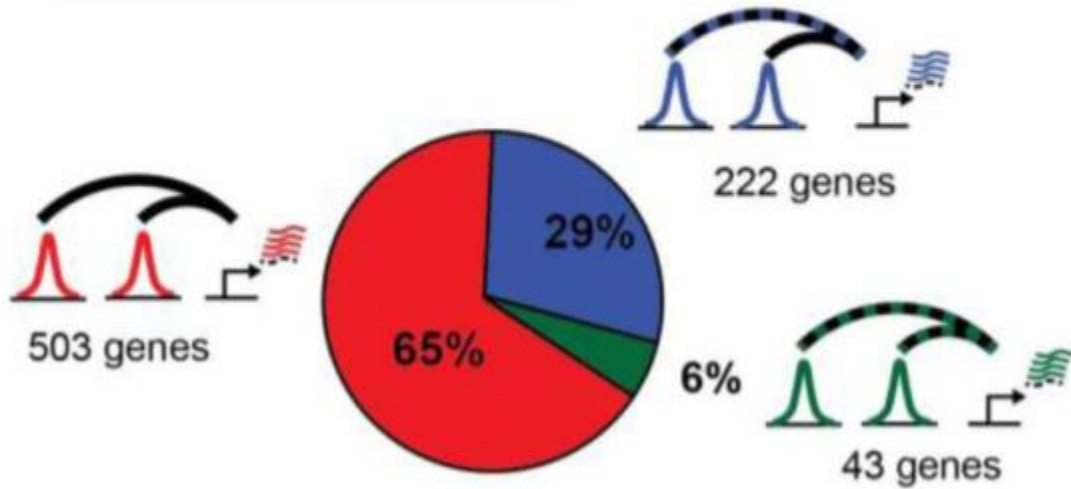
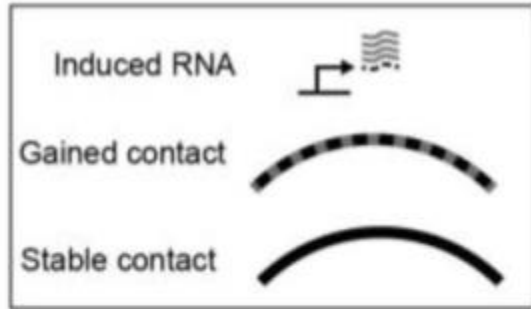


epidermal differentiation terms

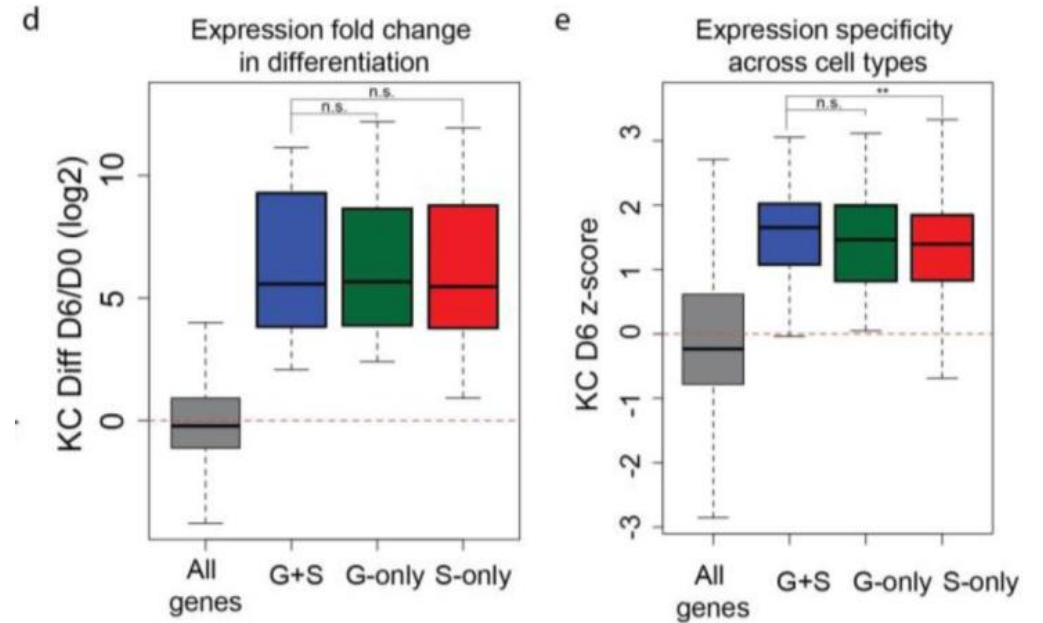


progenitor state, such as proliferation

Induced genes engage in stable E-P contacts



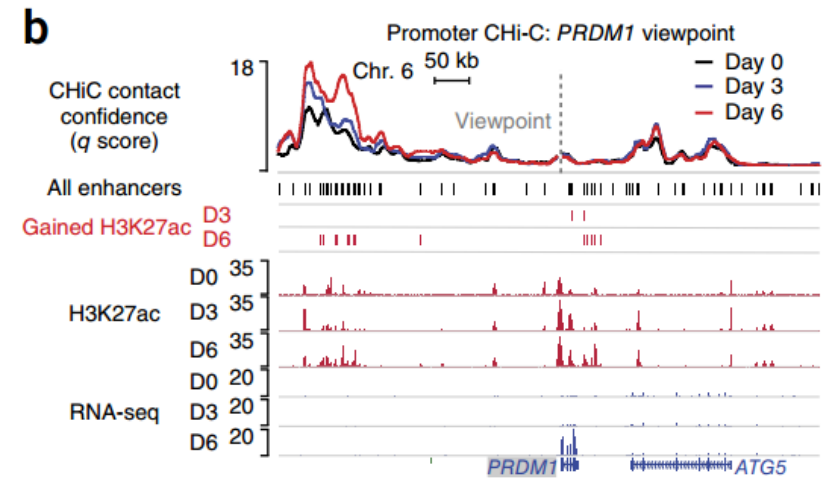
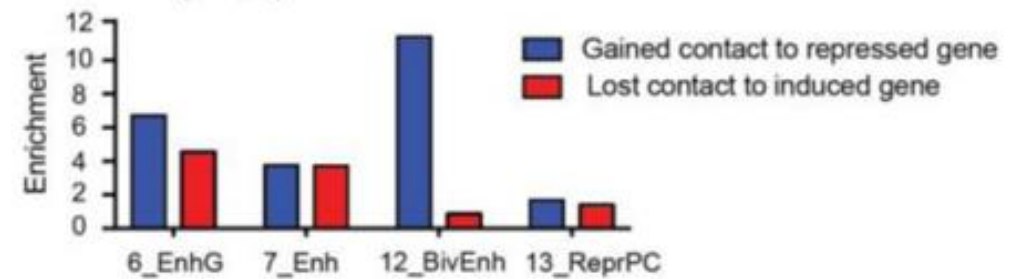
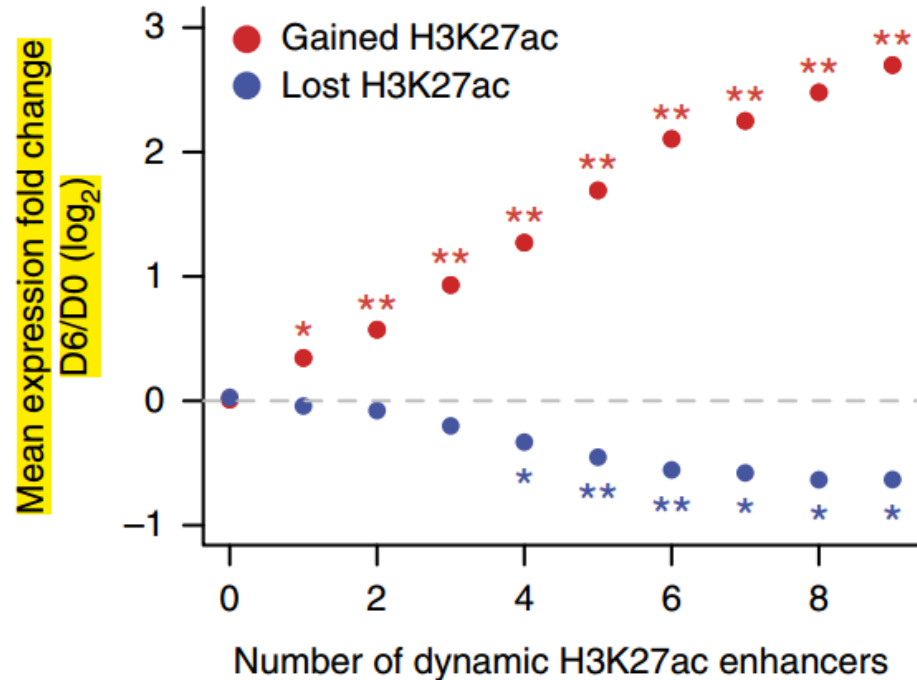
differentiation genes within each category of E-P contacts had similar GO terms



tissue-invariant structural contacts form a universal architecture that guides tissue-specific enhancer–promoter interactions ??

GS or G genes exhibited more lineage-specific expression than S-only genes

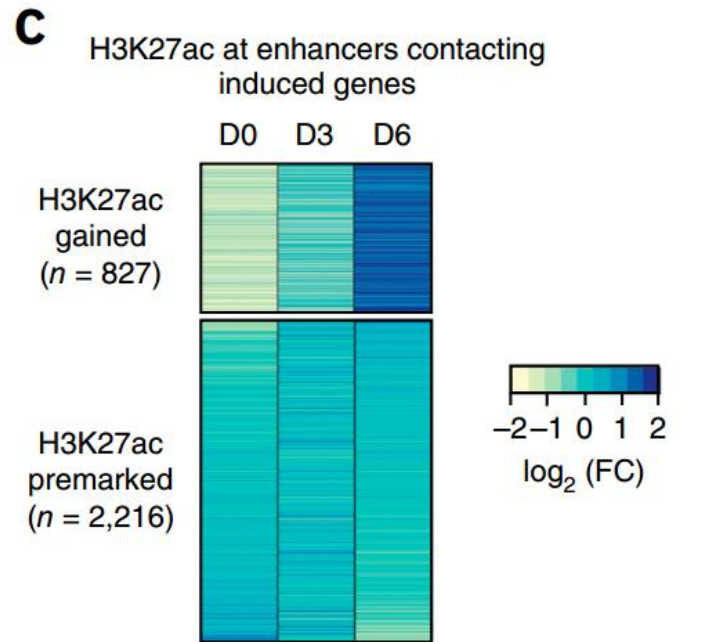
enhancer chromatin state; E-P contact; gene expression



gene induction \sim number of H3K27ac-gained enhancers

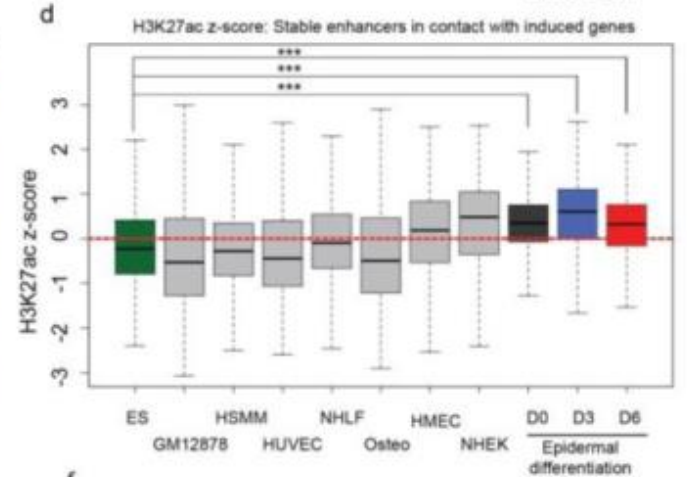
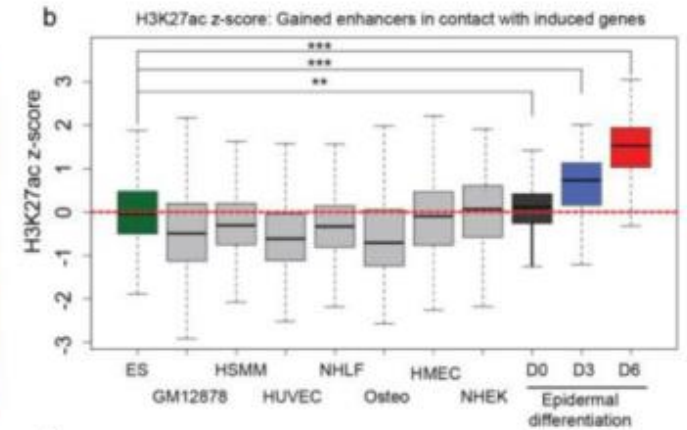
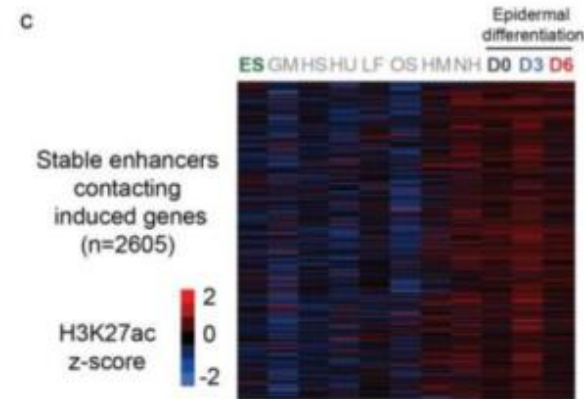
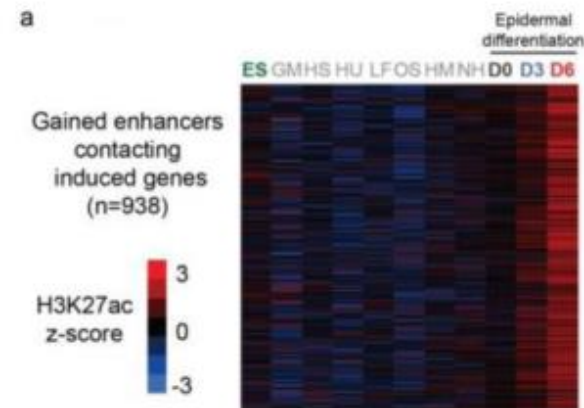
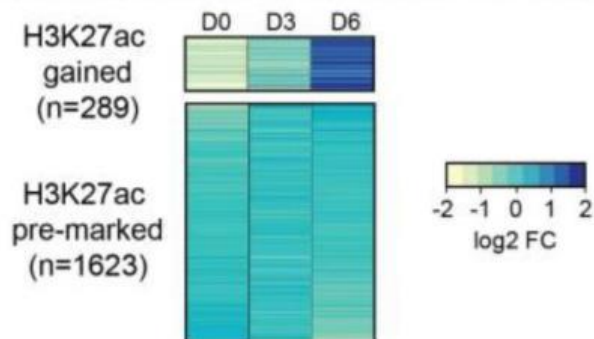
multiple classes of enhancers with distinct H3K27ac dynamics interact with a gene, not only to provide regulatory robustness but also to increase the magnitude of gene induction.

Lineage specificity of induced gene-linked enhancers



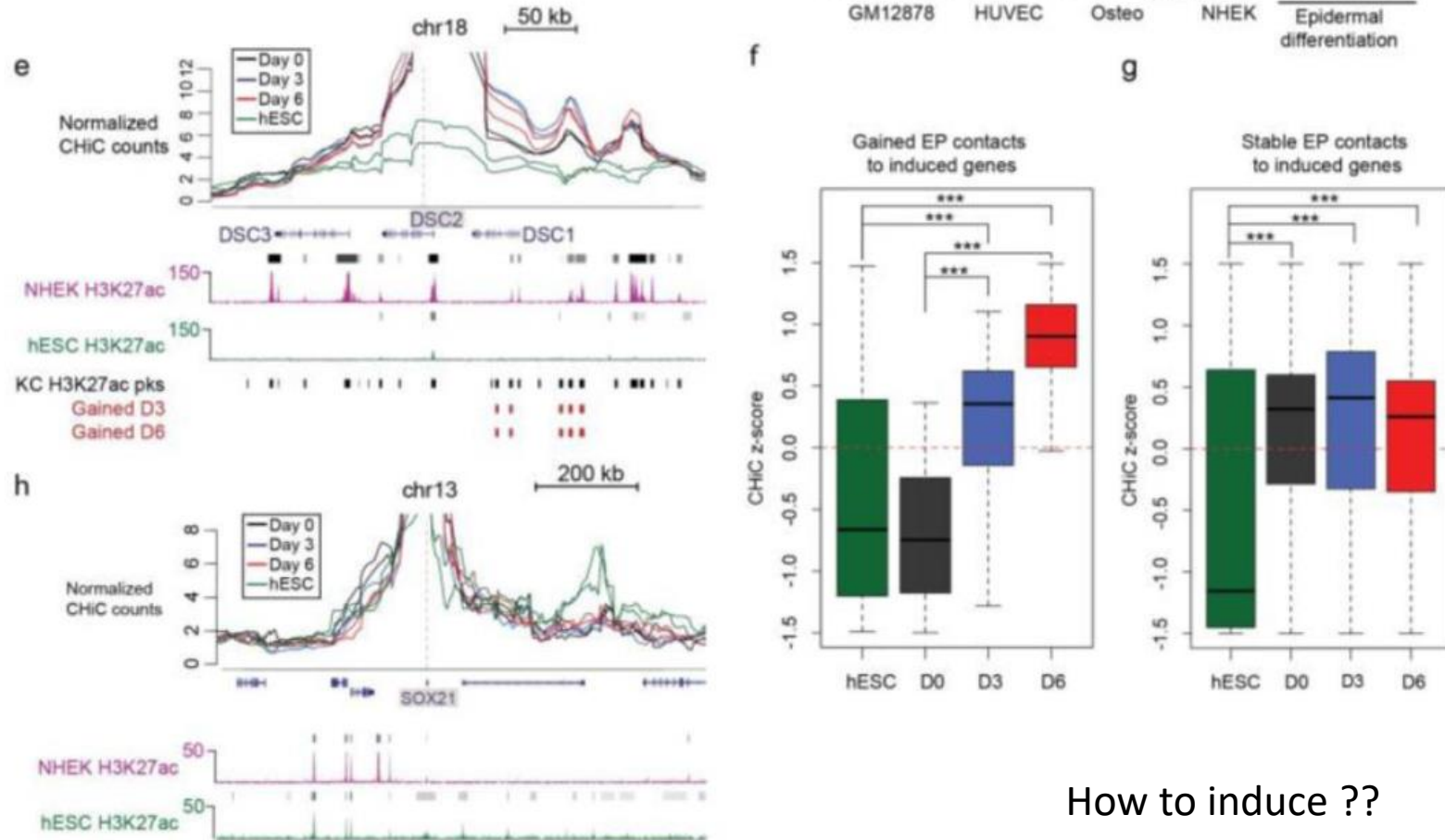
3,043 enhancers in contact with differentiation-induced genes

H3K27ac at promoters in contact with induced genes



stable enhancers and H3K27ac-gained enhancers were specifically marked by H3K27ac in keratinocytes

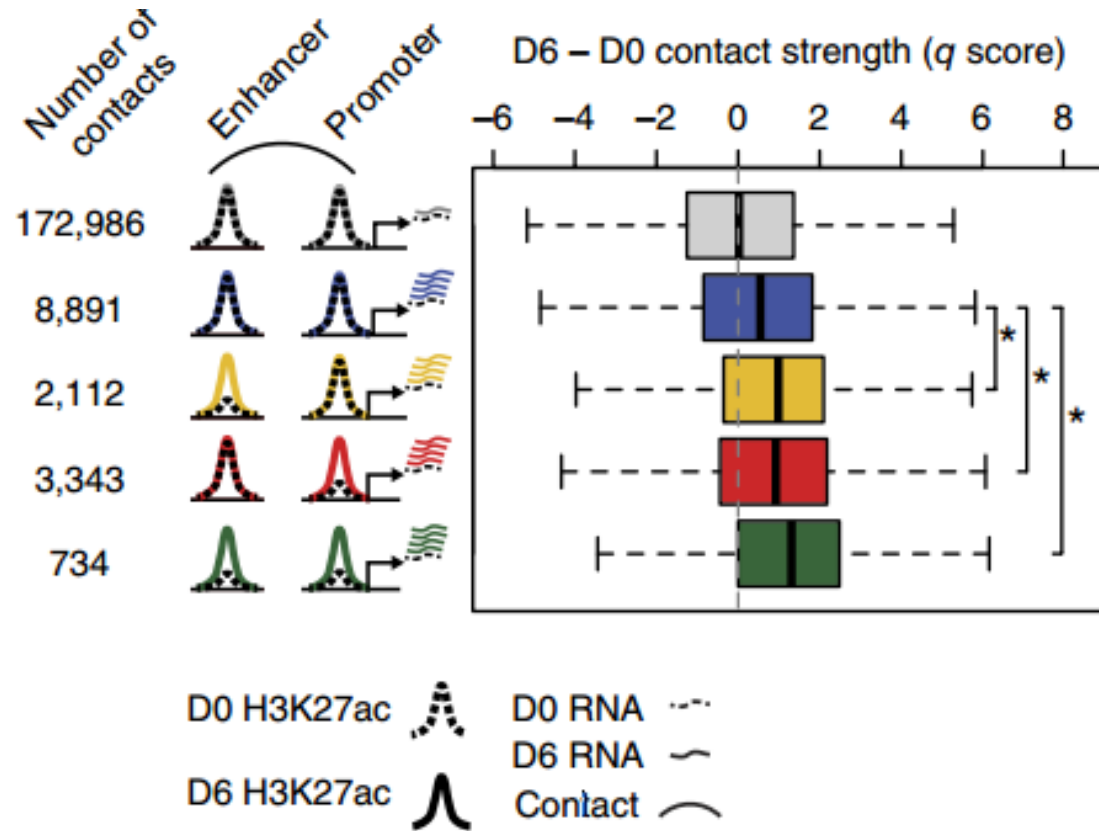
Some somatic E–P contacts is established after pluripotency but before induction of terminal differentiation genes



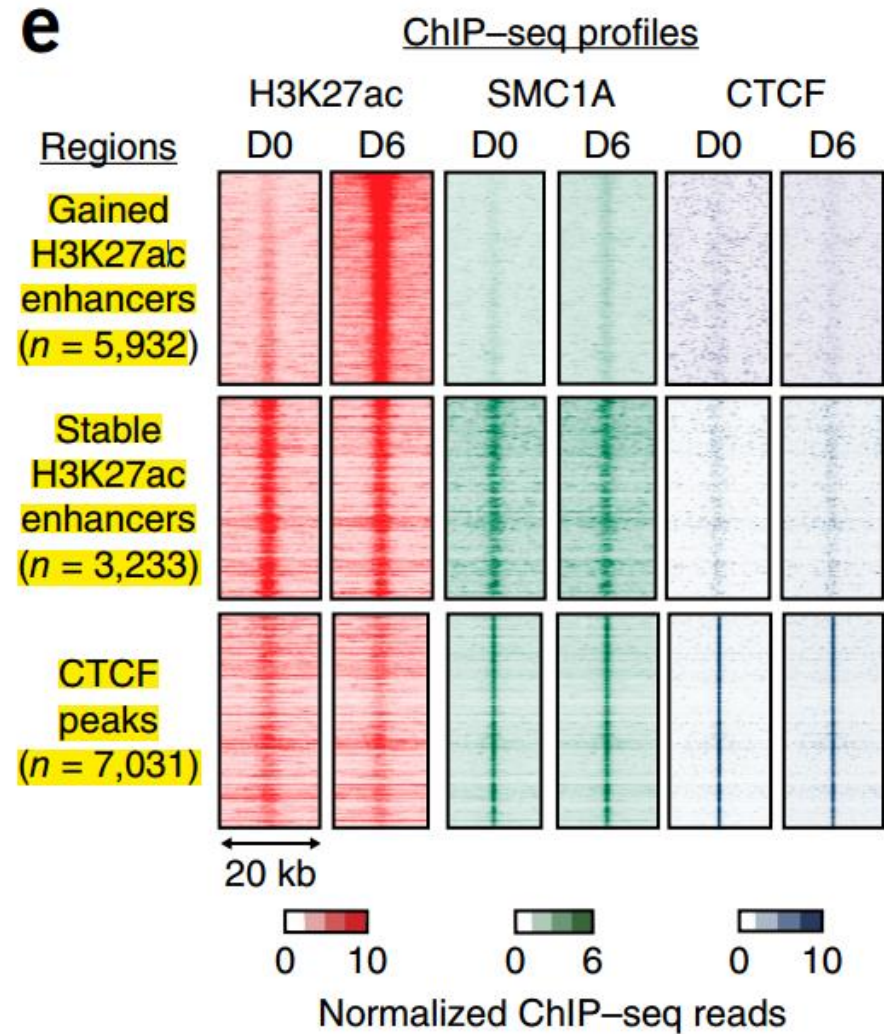
How to induce ??

both gained and pre-established enhancer–promoter contacts associated with differentiation genes showed significant reduction in signal in hESCs relative to keratinocytes, unlike the tissue-invariant contacts described in the mouse Hox loci.

chromatin state activation and contact dynamics

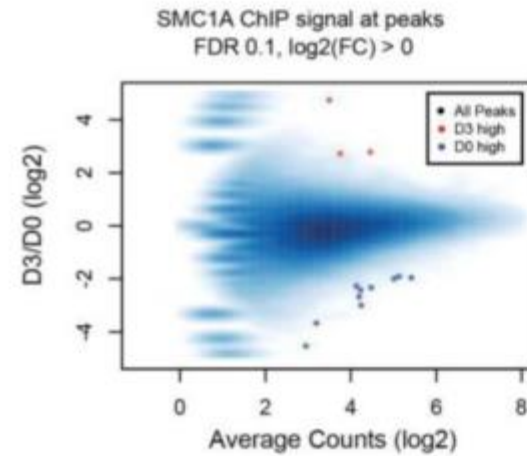
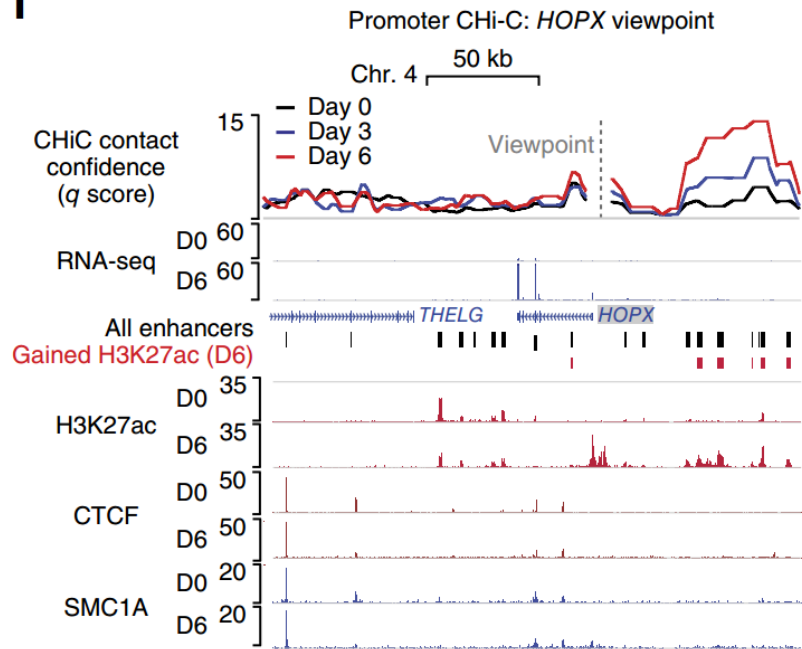


gain of H3K27ac at an enhancer or promoter was associated with significant increases in contact strength

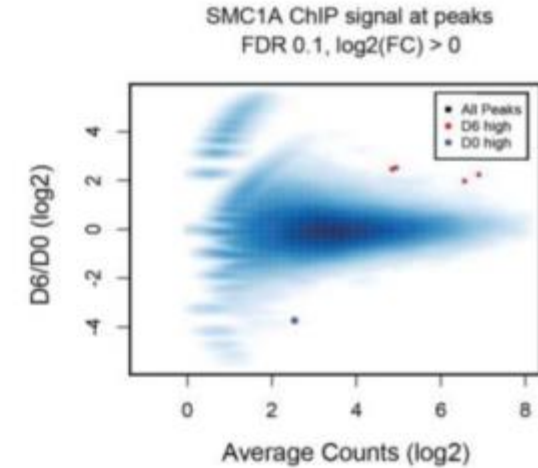


chromatin state activation and contact dynamics

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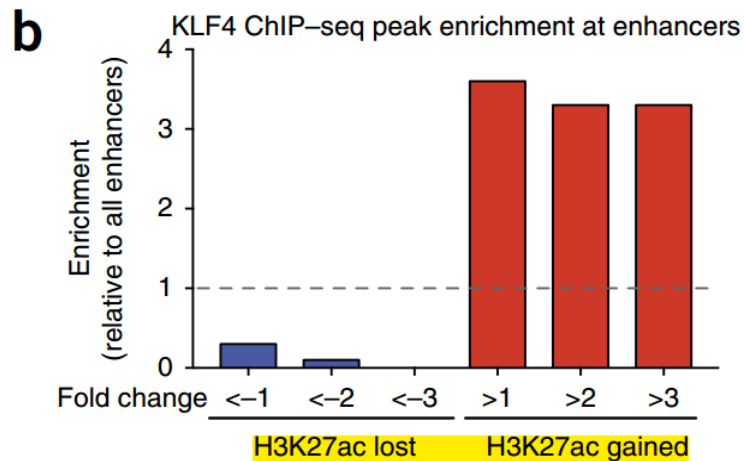
Pre-established E-P contacts are associated with premarked H3K27ac and constitutive cohesin binding at enhancers

E-P contacts acquired in differentiation are associated with enhancers that gain H3K27ac and lack cohesin.

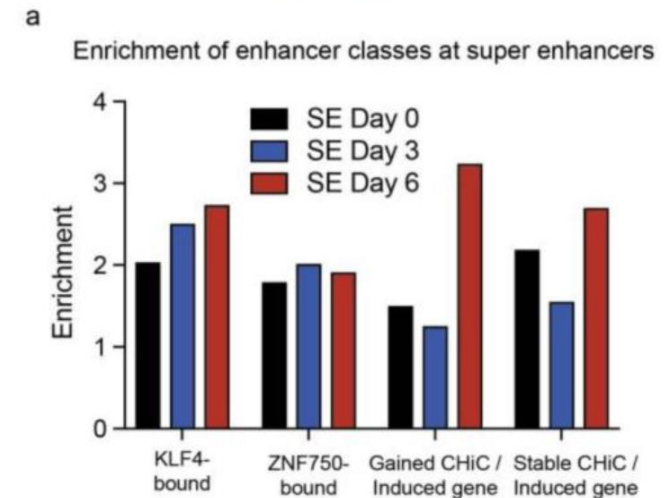
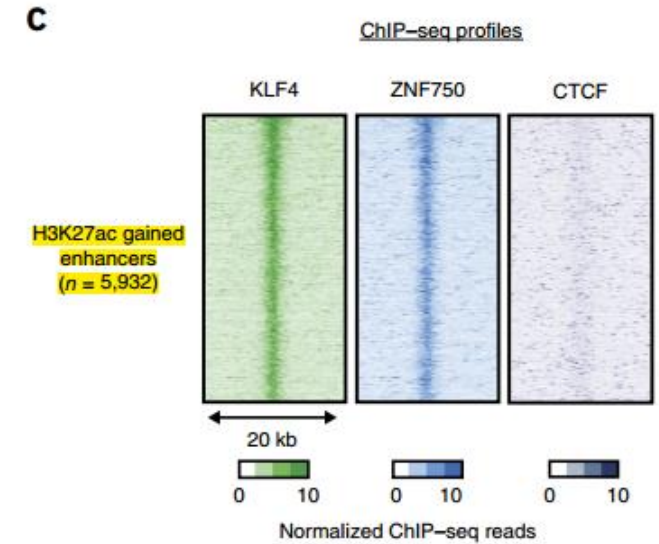
Gained enhancers and TFs

TF motif enrichment in gained enhancers

Motif family	% sites	<i>P</i> value	Motif
CEBP	27.20	1×10^{-32}	
KLF	21.61	1×10^{-9}	
LHX	25.41	1×10^{-4}	
T-box	30.48	1×10^{-4}	

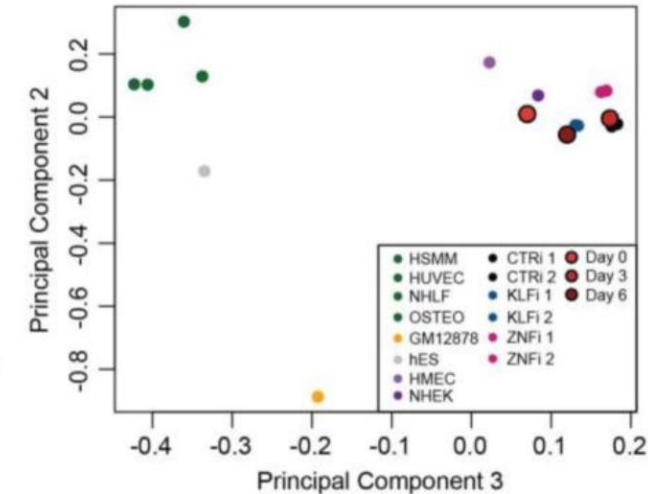
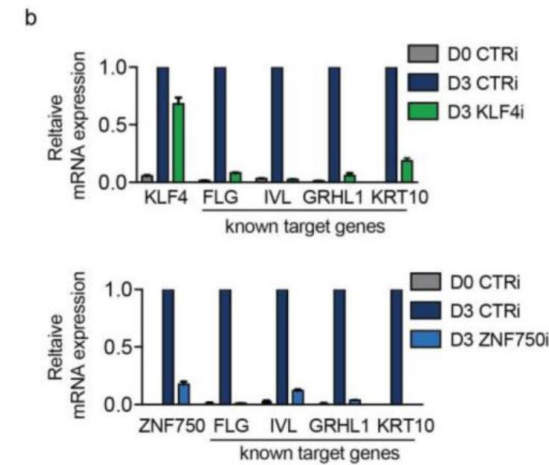
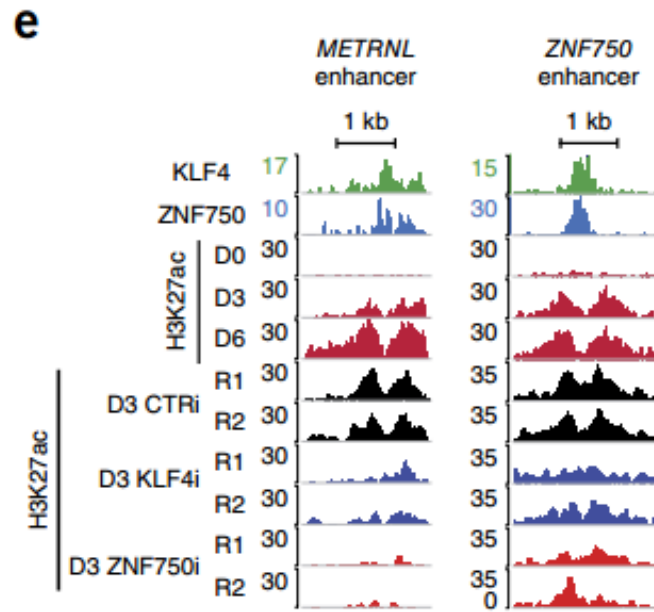
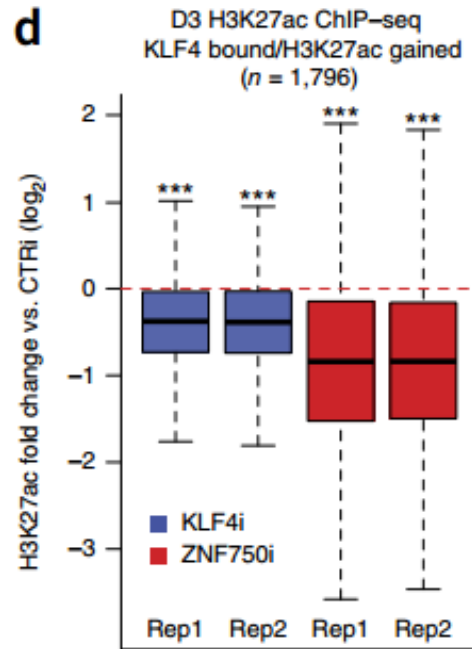


KLF4 binding was enriched at enhancers that acquired H3K27ac and depleted at enhancers that lost H3K27ac



KLF4- and ZNF750- bound enhancers also frequently overlapped super-enhancers

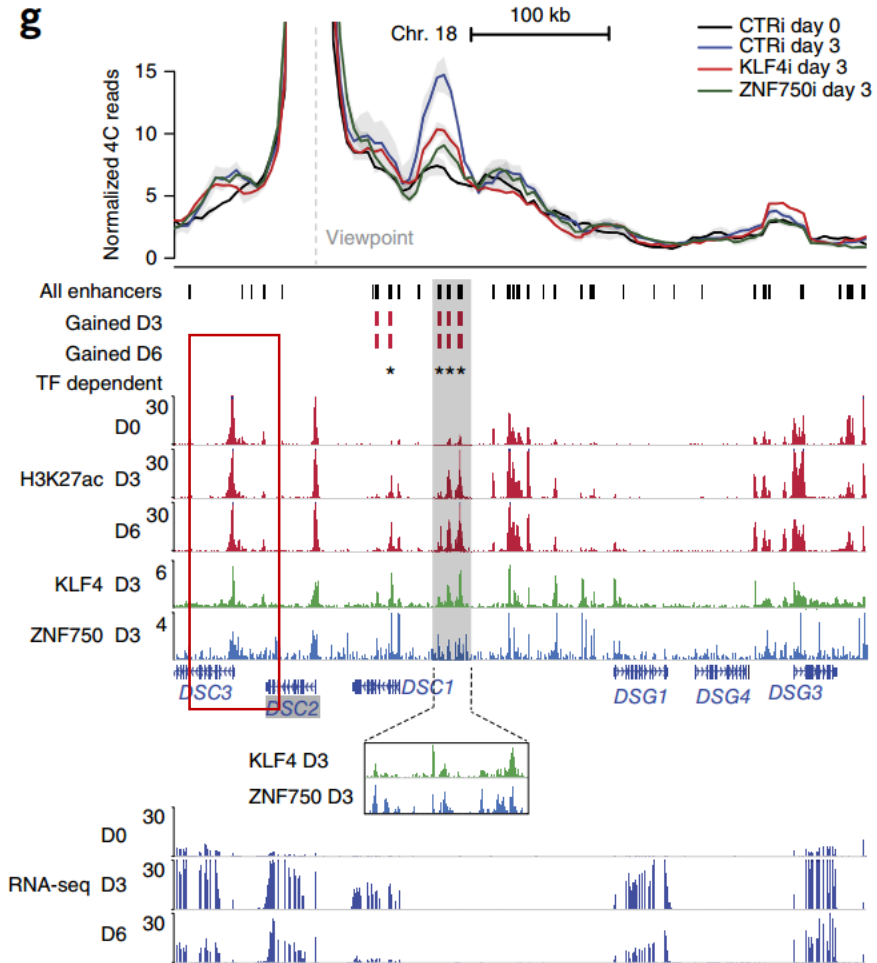
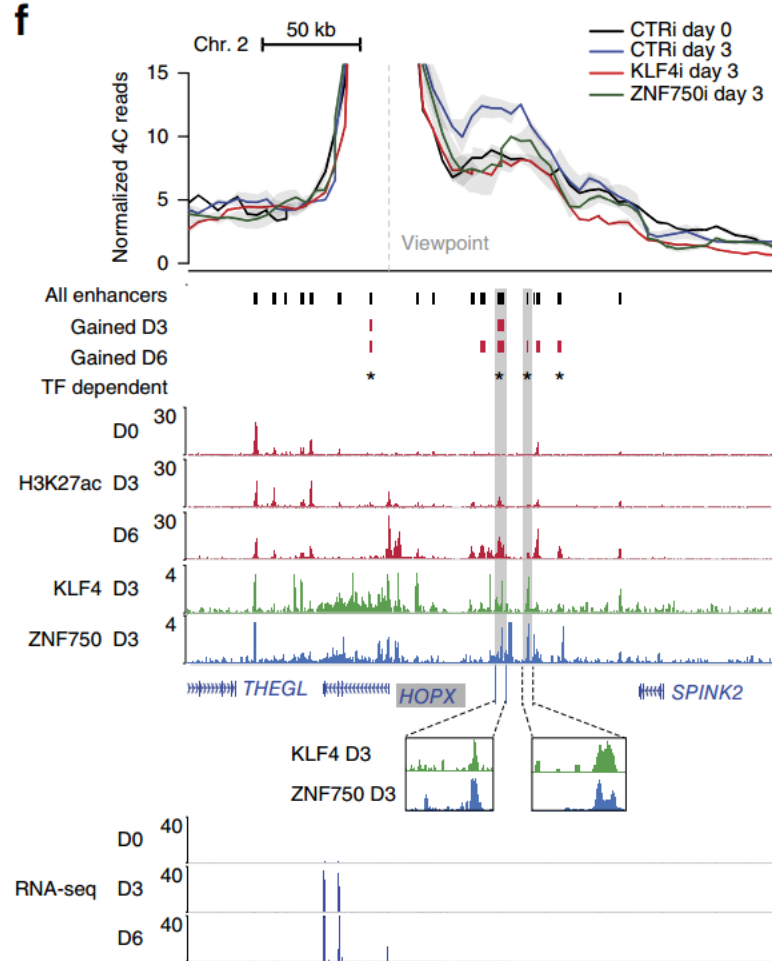
KLF4/ZNF750 contribute to enhancer activation and expression



Depletion of either factor impaired acquisition of H3K27ac at regions bound by these factors

transcription factor depletion altered epidermal differentiation but did not alter epidermal identity

KLF4/ZNF750 influence E-P contacts



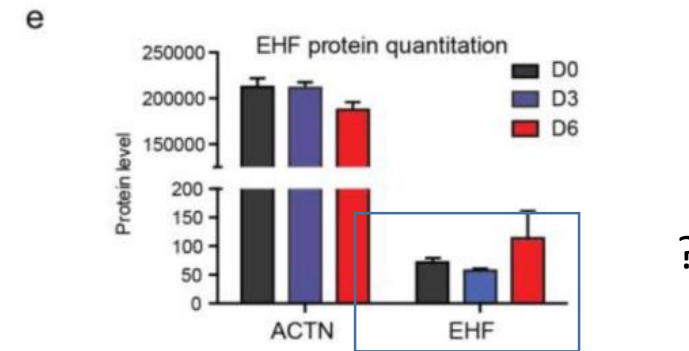
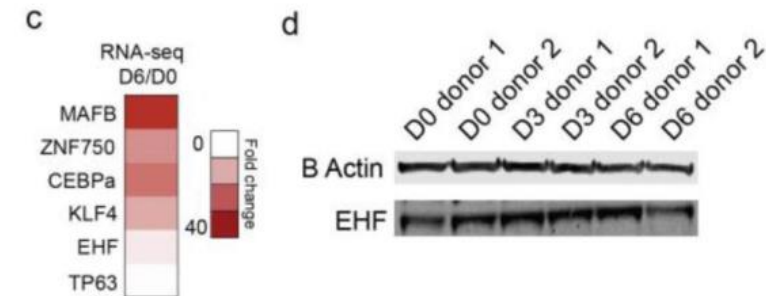
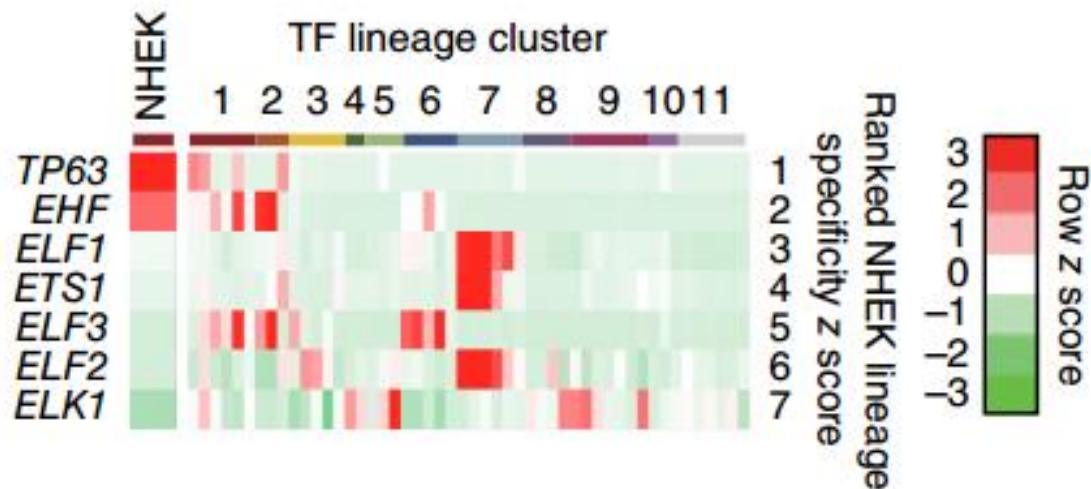
Depleting KLF4 or ZNF750 decreased contact strength. function at a subset of enhancers targeting induced genes

Stable enhancers and TFs

TF motif enrichment in premarked enhancers

Motif family	% sites	P value	Motif
ETS	26.96	1×10^{-243}	
MYB	35.59	1×10^{-76}	
E2F	21.87	1×10^{-68}	
PRDM1	11.76	1×10^{-50}	

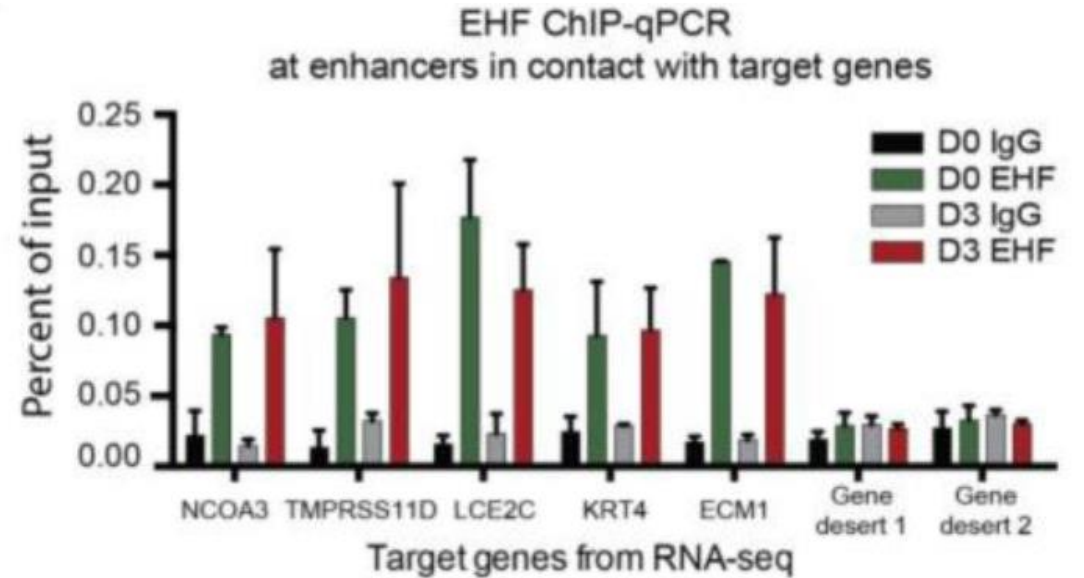
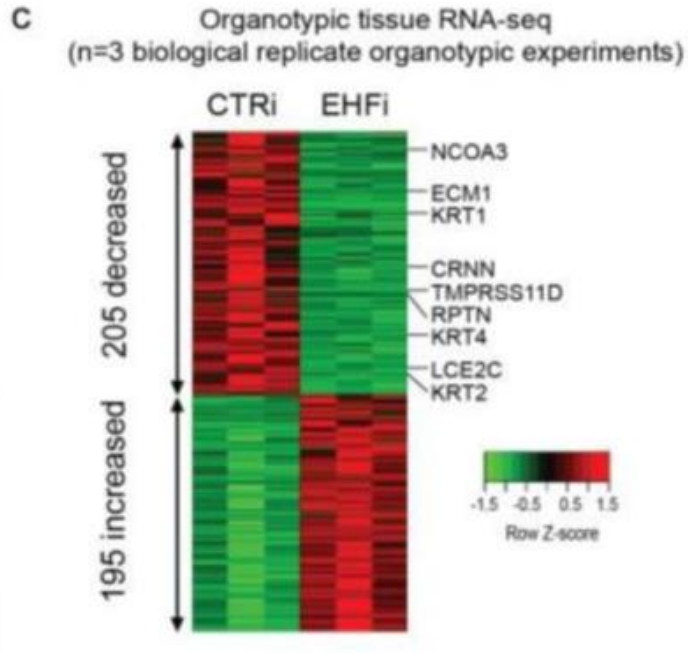
Why not these factors



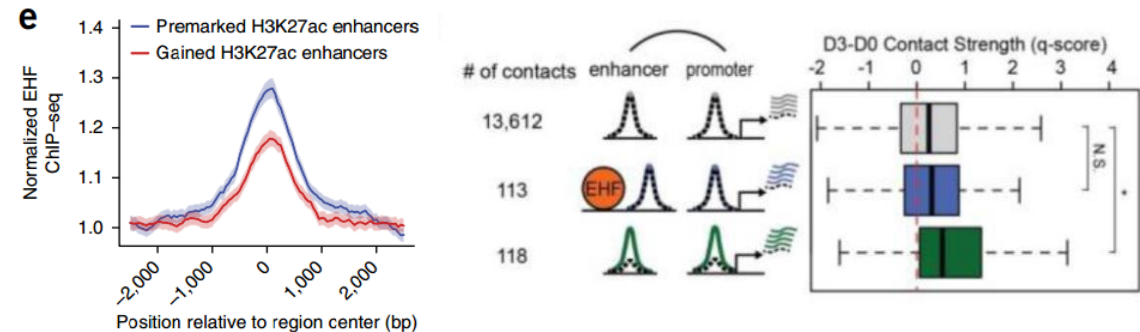
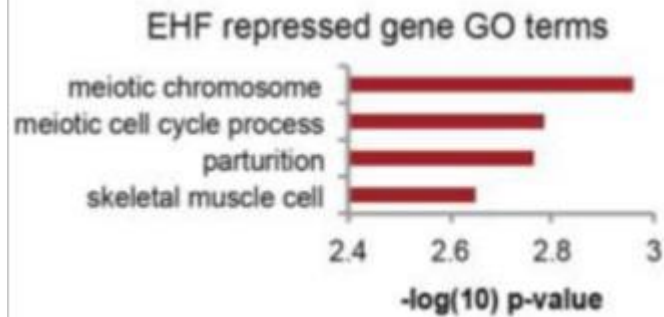
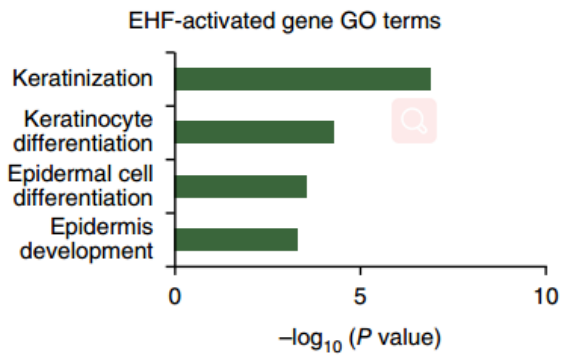
EHF expression was largely stable during differentiation

The EHF ETS-family transcription factor showed the most lineage-specific expression in stratified epithelia

EHFi



EHF binding at induced genes remained stable

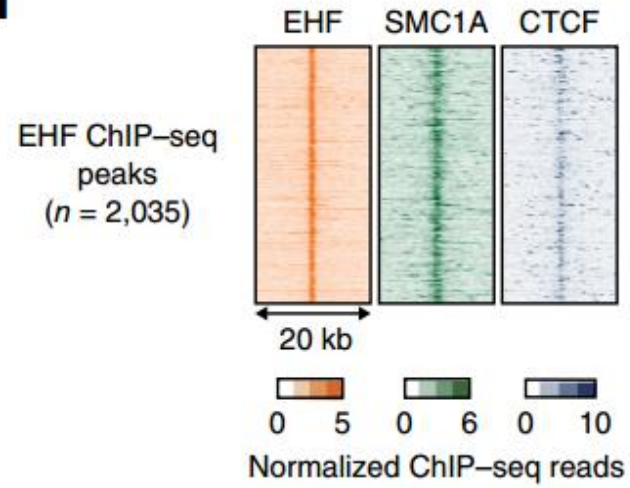


EHF is required both for induction of differentiation-related genes and for repression of ectopic gene expression.

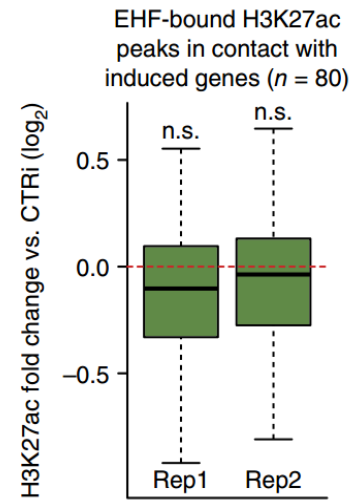
EHF-bound, H3K27ac-premarked enhancers generally engaged in stable contacts with differentiation-associated genes

EHF*i*

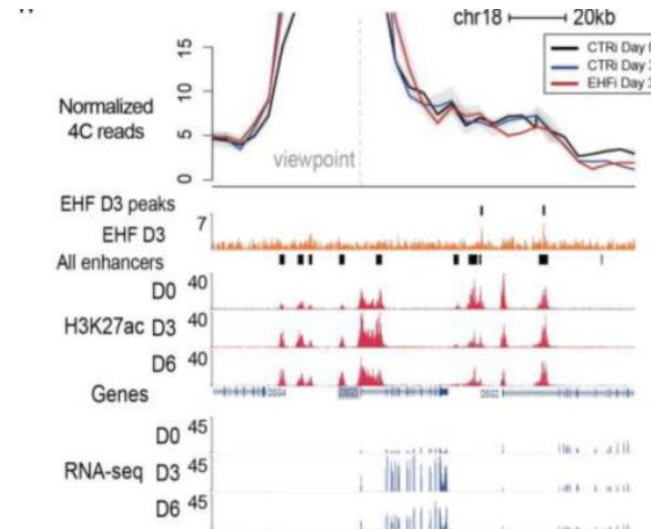
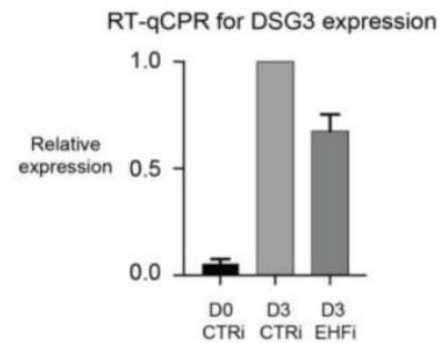
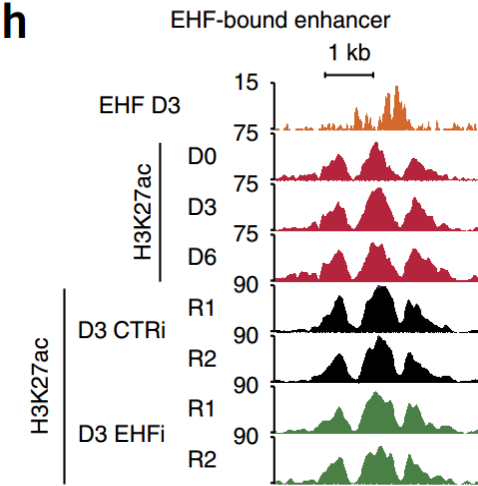
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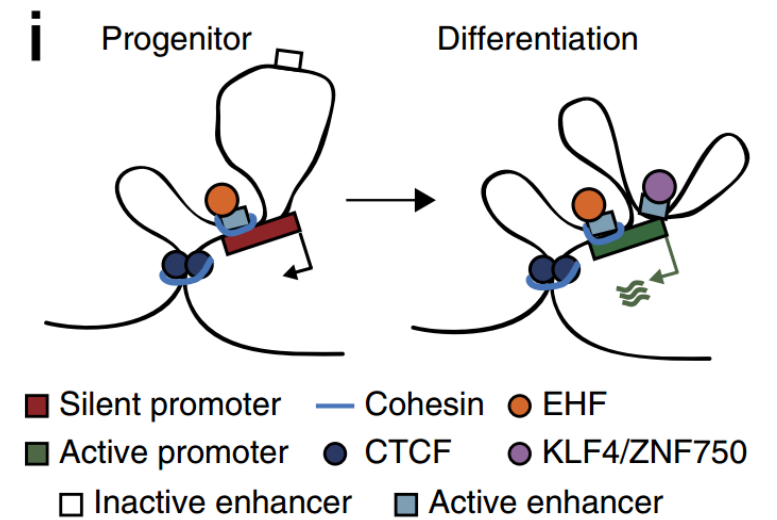
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EHF regulates gene expression in a manner distinct from KLF4 and ZNF750

Summary

		WT	EHFi
stable E-P	regulator	EHF (stable expressed)	
	contact	stable	not affected
	H3K27ac	stable	not affected
	differentiation-induced genes	induced	reduced
	cohesin colocalized	Yes	
		WT	KLF4i;ZNF750i
gained E-P	regulator	KLF4;ZNF750 (induced)	
	contact	gained	reduced
	H3K27ac	gained	reduced
	differentiation-induced genes	induced	reduced
	cohesin colocalized	Yes	



Progenitor cells partially pre-establish a regulatory apparatus that is fully engaged in terminal differentiation.

comments

- the differentiation-repressed genes ? (contact loss)
- CTCF-mediated loop changes, relation with gene expression? relation with TF-mediated E-P interaction?
- Casual relation of looping and expression changes? (editing some enhancer region)

THANK YOU