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Supplemental information

An interdependent network of functional enhancers regulates transcription and EZH2 loading at the INK4a/ARF locus

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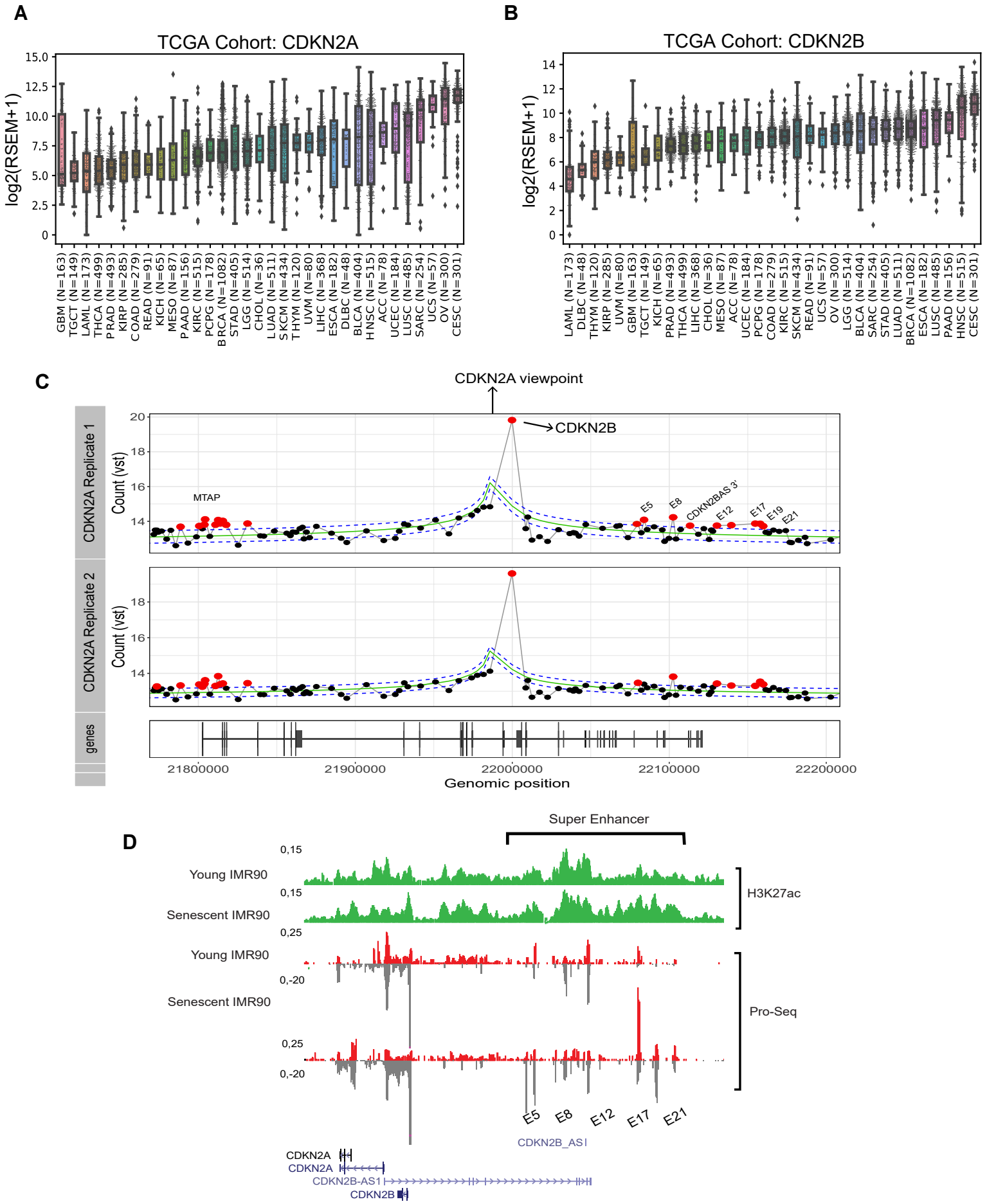
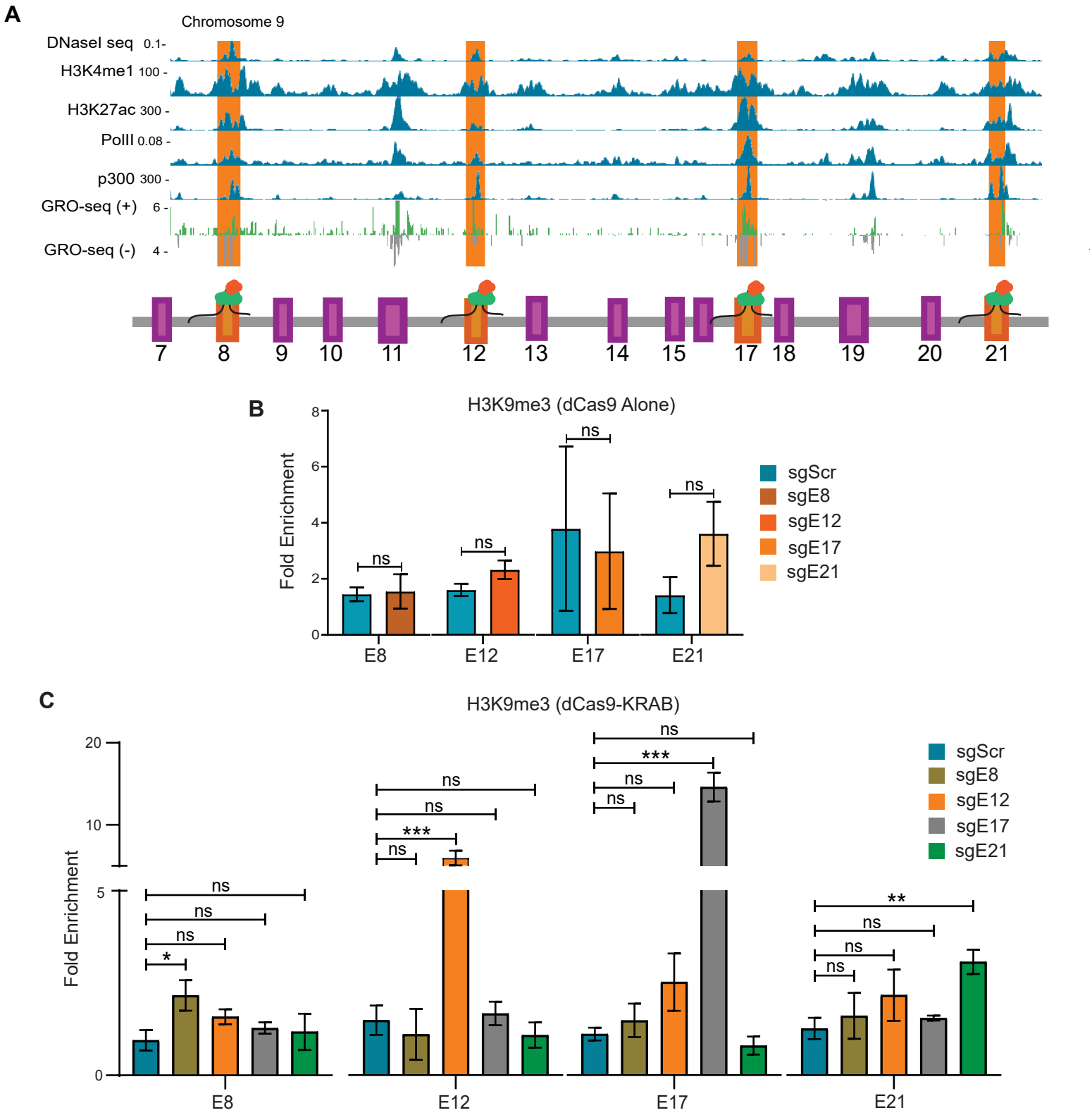
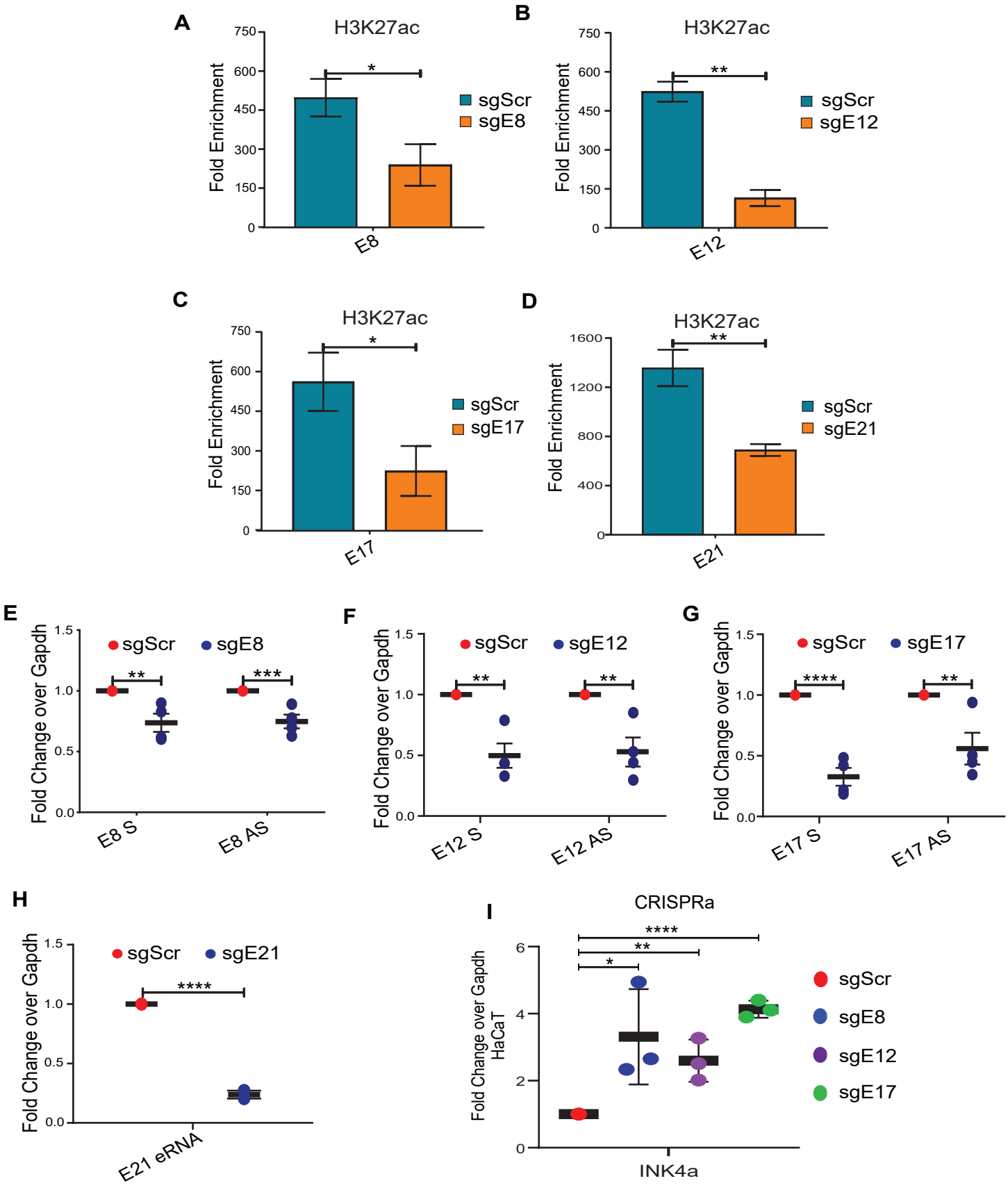


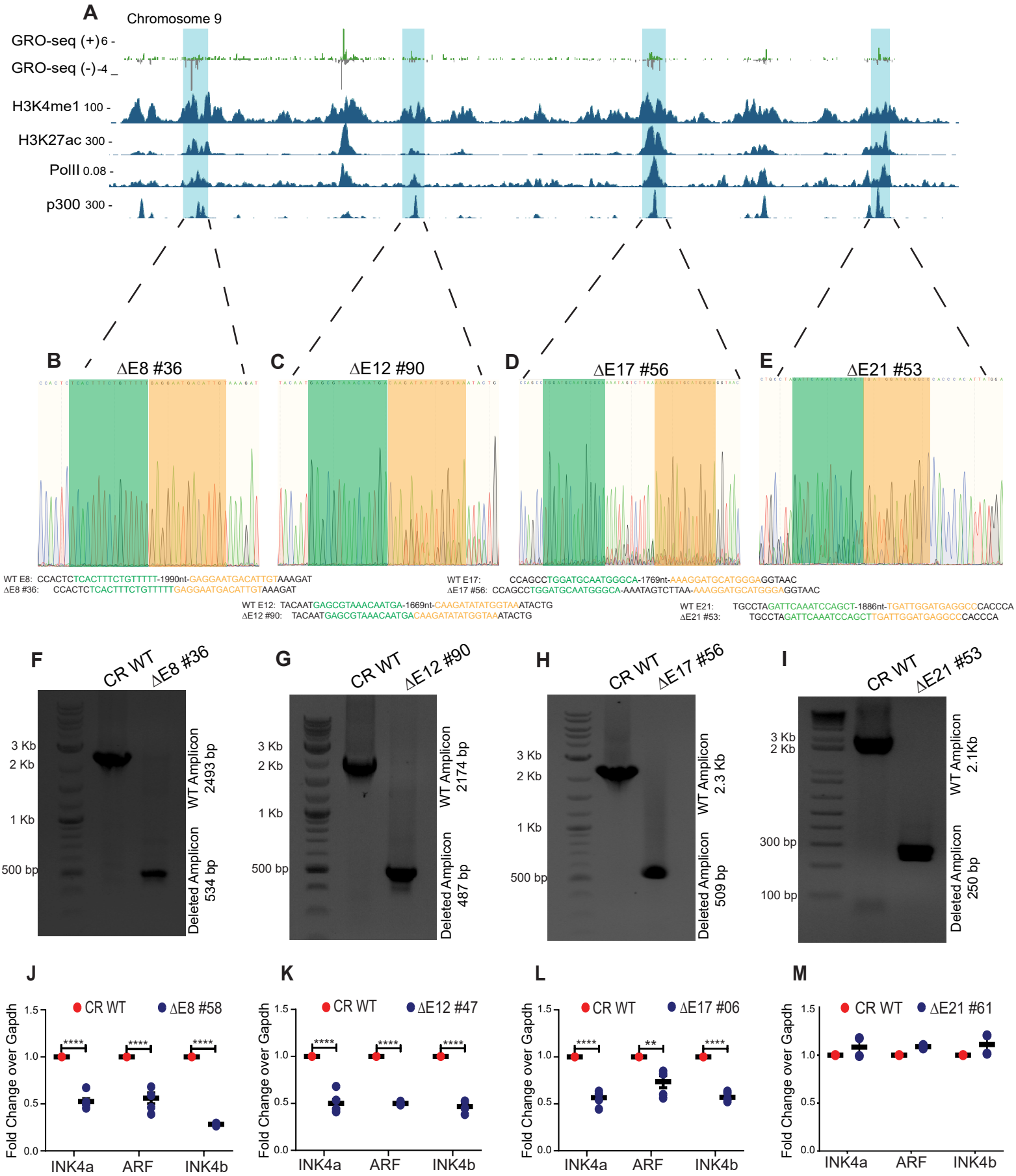
Supplementary Figure 1 (Related to Figure 1 and Figure 2). CDKN2A/2B expression is high in cervical tumors: (A) CDKN2A and (B) CDKN2B expression profiles in tumors of different origins. The expression of CDKN2A/2B is highest in tumors of cervical origin. (C) 4C plots showing the interactions from CDKN2A viewpoint, red dots represent the significant interactions. Several red dots are marked by their corresponding regulatory element. Both replicates have been individually plotted. (D) UCSC genome browser snapshots of H3K27ac, and PRO-seq in young and replicative senescent IMR90 fibroblasts. The enhancers are annotated below the track.



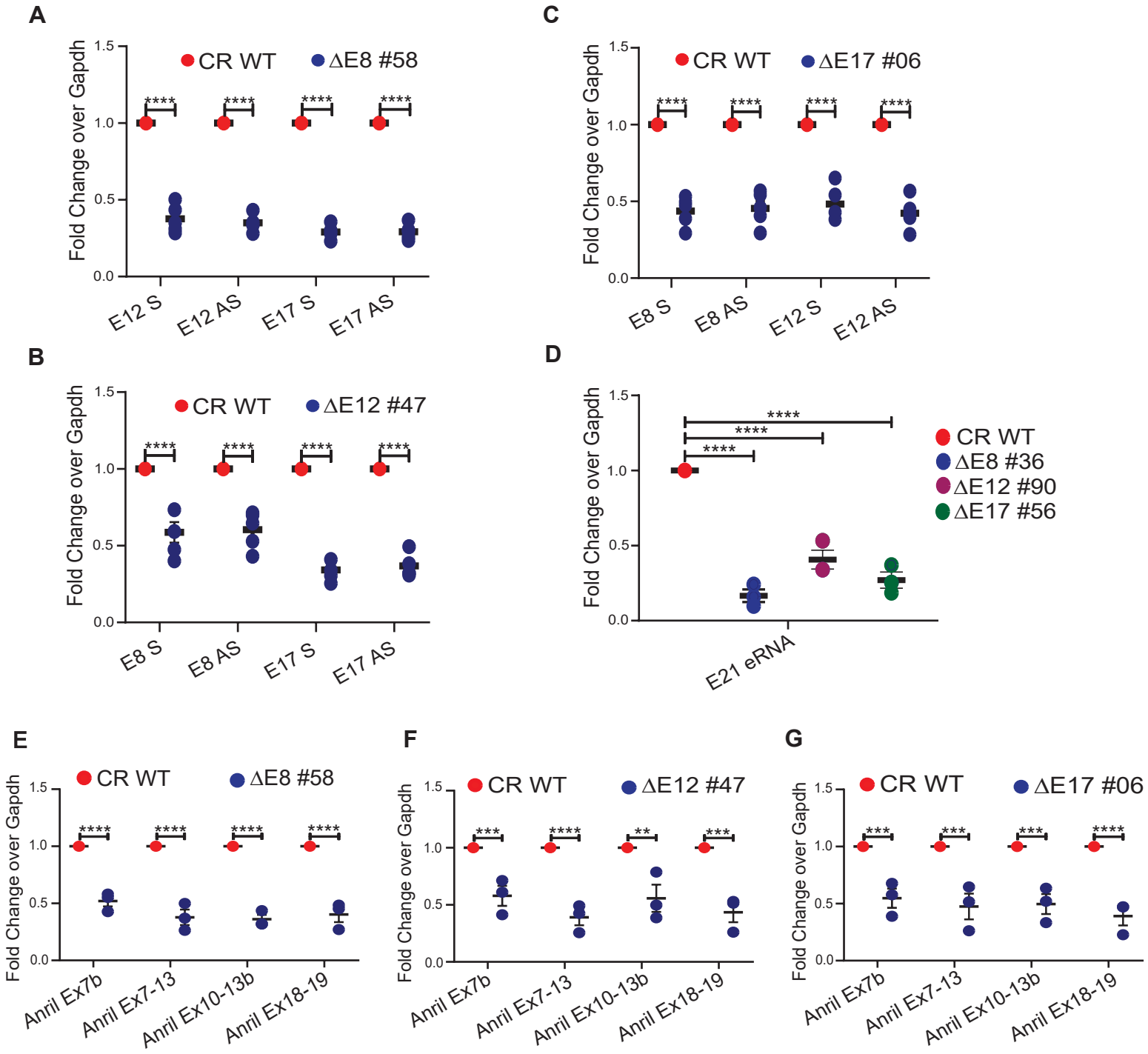
Supplementary Figure 2 (Related to Figure 3). dCas9-KRAB mediated silencing of enhancers results in enrichment of H3K9me3 only on targeted regions: (A) ChIP-seq tracks showing the active enhancer histone marks, PolII, p300 on the enhancers interacting with INK4a/ARF promoters and GRO-seq tracks showing enhancers are transcriptionally active. Highlighted regions are the enhancers that were silenced by specific guide RNAs along with constitutively expressing dCas9-KRAB. (B) H3K9me3 doesn't enrich on enhancers when targeted with dCas9 alone. (C) CRISPRi mediated via dCas9-KRAB results in enrichment of H3K9me3 on targeted regions. H3K9me3 only enriches on the targeted region and doesn't extend to other nearby enhancers. Statistical significance is determined by unpaired t-test (*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; ns p>0.05) and error bars denote SEM.



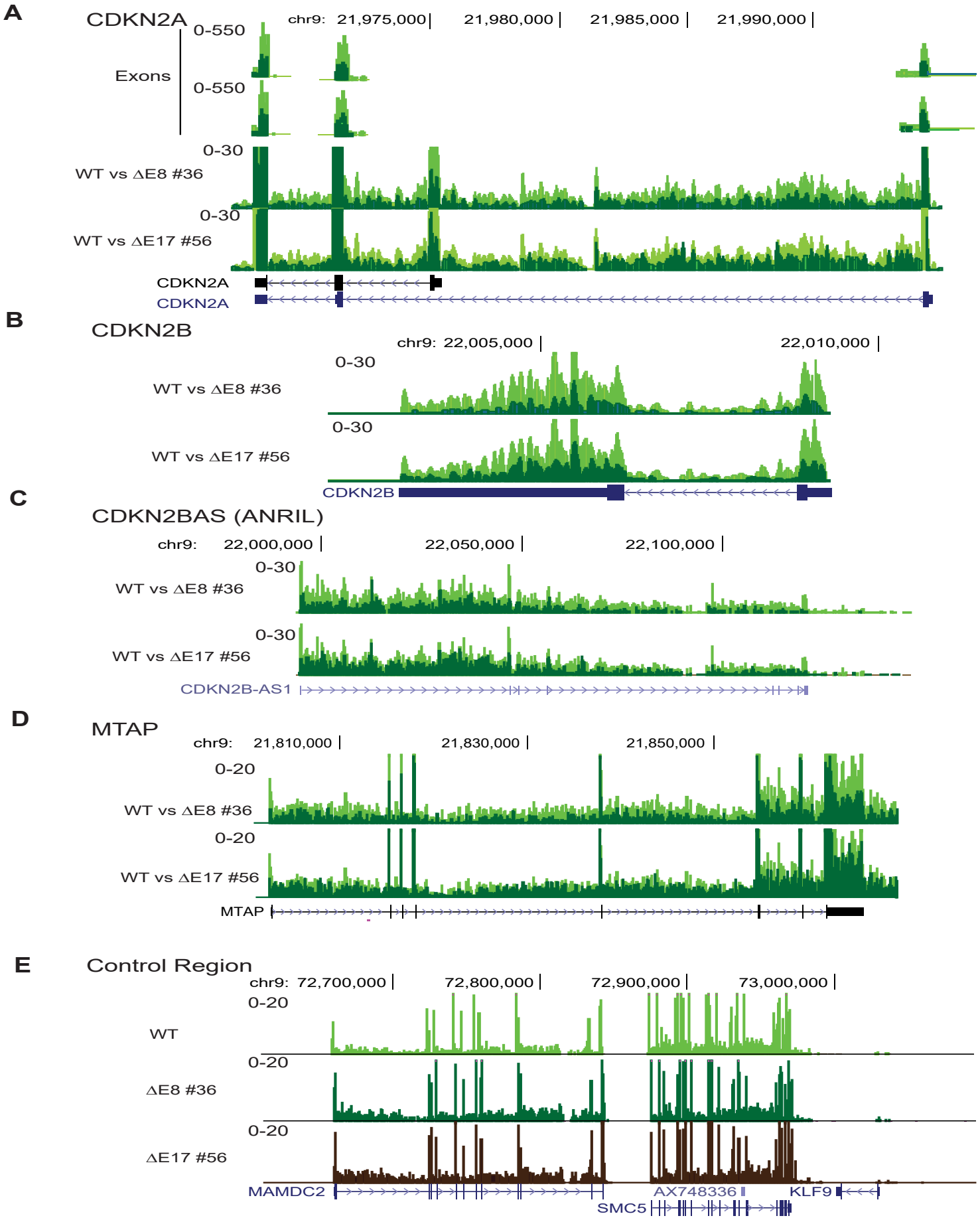
Supplementary Figure 3 (Related to Figure 3). CRISPRi mediated silencing of enhancers is confirmed by the loss of H3K27ac and eRNA: (A-D) H3K27ac enrichment goes down on the enhancers which are targeted for silencing (A) E8 enhancer, (B) E12 enhancer, (C) E17 enhancer and (D) E21 enhancer. (E-H) Enhancer silencing mediated via dCas9-KRAB system also shows loss of transcription of both sense and anti-sense eRNAs (E) E8 enhancer, (F) E12 enhancer (G) E17 enhancer and (H) E21 enhancer. (I) Enhancer activation mediated via dCas9-vpr system shows gain of transcription of INK4a gene in HaCaT cell line. Statistical significance is determined by unpaired t-test (*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; ns p>0.05) and error bars denote SEM.



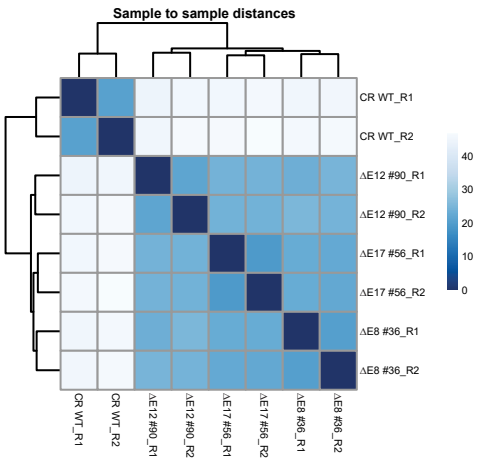
Supplementary Figure 4 (Related to Figure 3). Confirmation of homozygous enhancer deletions and expression of INK4a/ARF genes in heterozygous deletion lines: (A) ChIP-seq tracks showing H3K4me1, H3K27ac, PolII, p300 and GRO-seq signal at super enhancer region. The deleted enhancer regions are highlighted in light blue. (B), (C), (D) and (E) shows the sanger-seq chromatogram of E8, E12, E17 and E21 enhancer deletion junctions respectively. (F), (G), (H) and (I) Deletion of enhancers was confirmed by Surveyor assay. (J), (K), (L) and (M) Plots show expression levels of INK4a, ARF and INK4b genes in heterozygous set of deletion clones. Statistical significance is determined by unpaired t-test (*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; ns p>0.05) and error bars denote SEM.

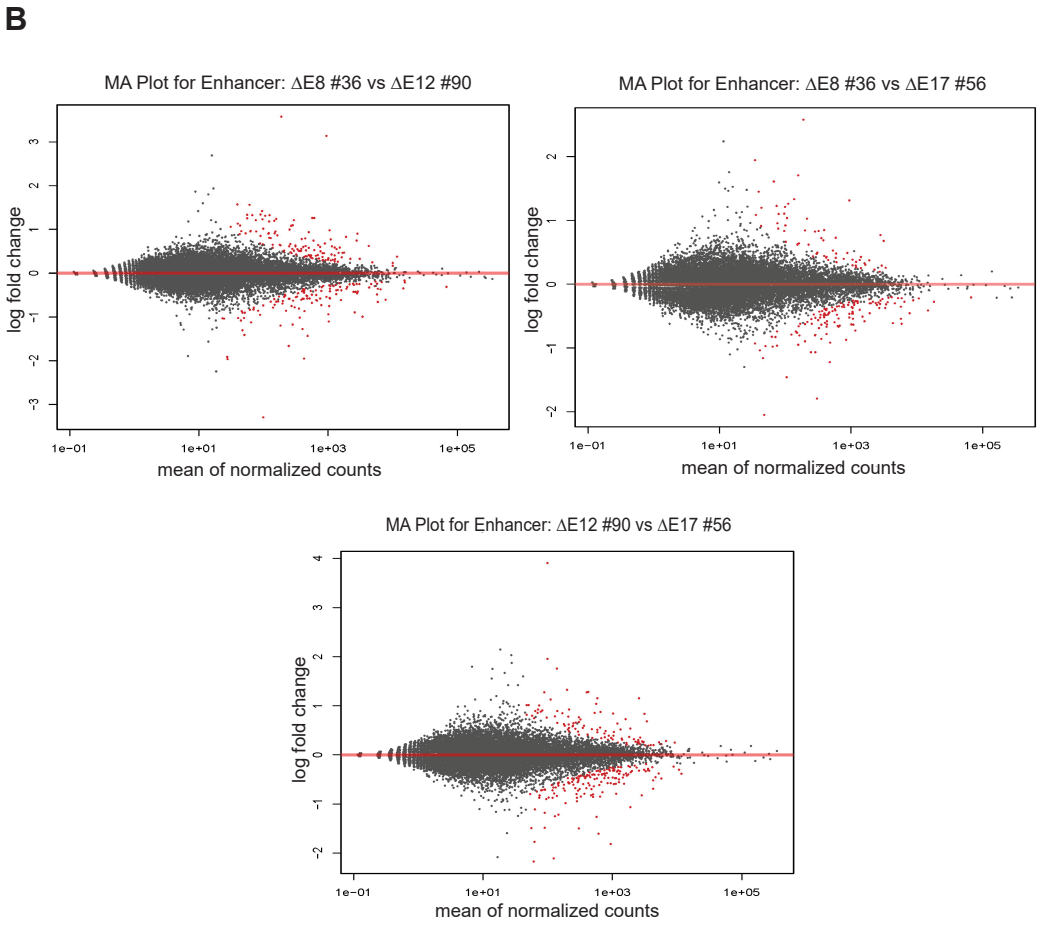


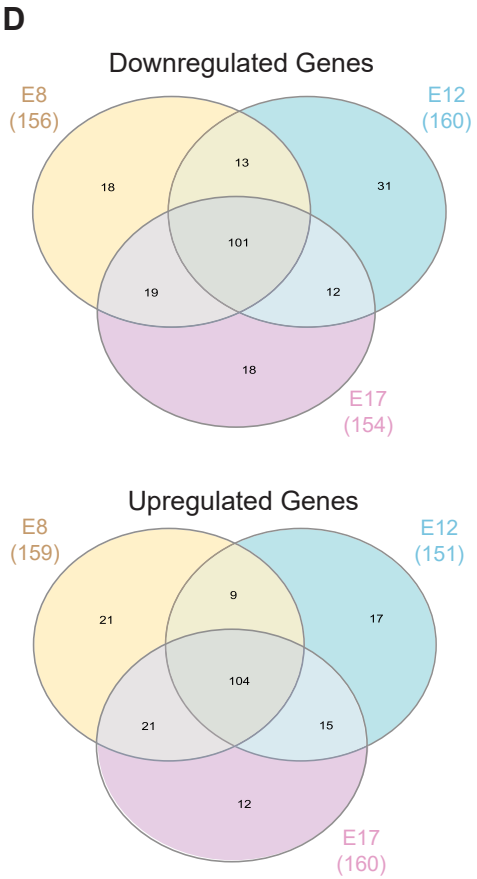
Supplementary Figure 5 (Related to Figure 4 and Figure 5). Promoter interacting enhancers are inter-dependent on each other for their transcriptional activity: (A) E12 and E17 eRNA expression in cells lines carrying heterozygous deletion of E8. (B) E8 and E17 eRNA expression in cell lines carrying heterozygous deletion of E12. (C) E8 and E12 eRNA expression carrying heterozygous deletion of E17. (D) E21 eRNA expression in cell lines carrying homozygous deletion of promoter interacting enhancers. (E-G) Expression of different ANRIL isoforms in cells carrying heterozygous deletions of (E) E8, (F) E12 and (G) E17. Statistical significance is determined by unpaired t-test (*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; ns p>0.05) and error bars denote SEM.

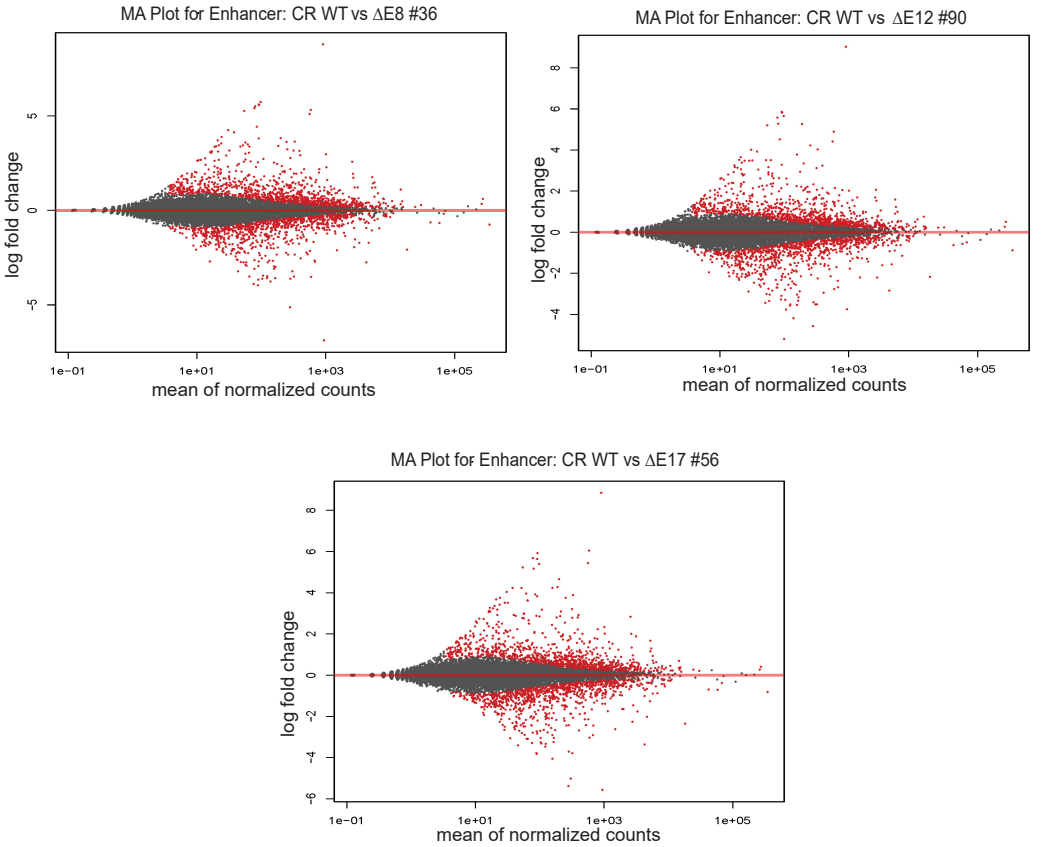


Supplementary Figure 6 (Related to Figure 7). Expression of INK4a/ARF genes is perturbed upon enhancer deletions: (A-D) UCSC browser shots of RNA-seq showing (A) signal from exons and entire gene body of CDKN2A, (B) CDKN2B, (C) CDKN2BAS, (D) MTAP expression in E8 and E17 enhancer deletion lines. (E) Control genes outside INK4a/ARF TAD showing no change in expression upon E8 and E17 deletion.









Supplementary Figure 7 (Related to Figure 7). Alterations in genome-wide transcription is similar upon any enhancer deletion: (A) Positional matrix positions together the replicates of RNA-seq upon an enhancer deletion or WT cells and (B) MA plots comparing the RNA-seq data from E8 with E12, E8 with E17, E12 with E17. (C) MA plots comparing RNA-seq from WT cells with E8, or E12, or E17 deletions. (D) Venn diagram showing the number of common, unique, down regulated genes (upper diagram) and upregulated genes (lower diagram) upon enhancer deletions.

Table S1 (Related to Figure 2, 3, 4 and 5): List of oligos used

Oligos used for qRT-PCRs:

Oligo Name	Forward Oligo (5' – 3')	Reverse Oligo (5' – 3')
p14	AACATGGTGCGCAGGTTCT	CACCAGCGTGTCCAGGAAG
p15	CGTTAAGTTTACGGCCAACG	CCATCATCATGACCTGGATCG
p16	ATGGAGCCTTCGGCTGACT	CACCAGCGTGTCCAGGAAG
Gapdh	CGCTCTCTGCTCCTCCTGTT	CCATGGTGTCTGAGCGATGT
Anril Ex7b	AGAATTCTTGATTCTTTGCTTTCC	TCCCTAGTTTTGAGGACTAAGCTACT
Anril Ex7-13	GAACTCCCGACCTCGTGATTCGC	CTTCGTAGGAAATTCCTAGCTCCGTAATC
Anril Ex10-13b	CTGTGGCCACCTTGGAGA	TGGCTTCCATAGCACCAACT
Anril Ex18-19	AATGAGGCTGAGAGCATGGGAGATAC	GAGATATAGGTTCCAGTCCTGGTTCTG
E8 S eRNA	CAGCCAACCCCCTGTATTGT	TGCTGGCTGAGTTGCAATAAC
E8 AS eRNA	CAATACAGGGGGTTGGCTGT	TGCTGCCCAATCAGAAGATG
E12 S eRNA	ATGTCAGGGCCAGAAGTCGT	AAGGTCACAGCCCTGAAGGA
E12 AS eRNA	CACCCTGACTTGTCCCACAG	CAGCAAACCACAATCCCACA
E17 S eRNA	GGGTTTACATCCCCAAAGCA	TGAACAGGGAGCAGGAGTGA
E17 AS eRNA	TGGGGTGACTCAGACCTTCA	GACGAGGAGGCGTTGAAAAC
E21 eRNA	TCACTGTGAGCAGGAAACGT	CACAGACACTTAGGCACACACAT

Oligos used for ChIP qPCRs:

Oligo Name	Forward Oligo (5' – 3')	Reverse Oligo (5' – 3')
p14TSS	ACCCAGGATATTCGGGACTCACTGAC	CGTCTCTAGCCCAGGCTAGGAGG
p15TSS	CCGTCGTCCTTCTGCGGCTTG	AGTGAGGACTCCGCGACGCGT
p16TSS	TCGCCAGGAGGAGGTCTGTGATTAC	CAGGTGGGTAGAGGGTCTGCAGC
E8 ChIP	GCTGAGGCAAGGGGACATACCAAACAC	GCTCACAAGCTACAGATATGCTGGCTGAG
E12 ChIP	GGAACTAGAGGTAGTCCTGGCTACTTGGG	CACCTCACCCTATCTTGAAGGCAGGCCACACT
E17 ChIP	GAATGGCAATTGCGGCAACCATG	GTATCGTCTCCTTCCTCCACAATCC
E21 ChIP	TGGGTCCTATATAAACCTTCTTC	CACAGACACTTAGGCACACACAT

Oligos used for Surveyor Assay:

Oligo Name	Forward Oligo (5' – 3')	Reverse Oligo (5' – 3')
E8	TACTGTTGGAAGGATCCGTTAGC	CCCAAAACCATGTAGAGAGCATC
E12	GCTATTAGGATGGCCAATGATC	CTAGCGCAATACCACAGTGAACAT
E17	TGACACTGCCAATCAGTTGTAGG	AGGGGACTAAAGAAGACTCCACA
E21	CTGCCACGATATTTAGCAATC	GCTAGATGTTGCTGTGATGCT

Oligos used for 4C:

CDKN2A Viewpoint	TGGGAGGAGCTAGGGCAAGCTT
E12 Viewpoint	AAAGAGGTGAACTAAGCTT

Table S2 (Related to Figure 3): List of gRNAs used

gRNAs used for CRISPRi:

gRNA Name	gRNA Sequence
E8 gRNA 1	AGTGTTGCCCTGCTAAGATC
E8 gRNA 2	CACATATCCCAACTATGACT
E12 gRNA 1	CGTGGAGTCTAGCCATGTCA
E12 gRNA 2	GTGAGGTGTTTTATGACCAC
E17 gRNA 1	TGGAACTTATTCTAGGGCGT
E17 gRNA 2	GCCCTCACTGCTACAACTGC
E21 gRNA 1	ATACATCAACAGAAAGAAGA
E21 gRNA 2	GGACCTCAACTCACACATGC

gRNAs used for enhancer KOs:

gRNA Name	gRNA Sequence
E8 KO gRNA 1	AACTGATCGTTTCAAAGCCG
E8 KO gRNA 2	ATGGCATTGCCATATCGTGG
E12 KO gRNA 1	CGTAAACAATGACAACGGAA
E12 KO gRNA 2	ATCTTGCTTACCTCTGCGAG
E17 KO gRNA 1	GATGTGGGTTAGCGTTTCAG
E17 KO gRNA 2	TAGTAACAAGGCATCTCATG
E21 KO gRNA 1	CCCAAATCCAAGAGTAGAGC
E21 KO gRNA 2	TGTTACAGCCTCCCACTGAT

Table S3 (Related to Figure 2 and Figure 4): 4C reads and experiments

Sample Name	Total Reads	Aligned Reads	Alignment Percentage
E9_vp_E8_ED_rep1	8894272	6159675	69.25440328
E9_vp_E8_ED_rep2	5321831	4228668	79.458893
E9_vp_E8_WT_rep1	7534258	5463650	72.5174264
E9_vp_E8_WT_rep2	7395234	4645476	62.81716035
Promoter_HeLa_rep1	6796753	6176852	90.87945376
Promoter_HeLa_rep2	4587697	4156286	90.59634932