

Table S1. Insulated neighborhoods for Each Gene. Related to Figures 1 and S1.

Table S2. 4C Primers. Related to STAR Methods.

Table S3. Previously Published Datasets Used in this Study. Related to STAR Methods.

Figure S1. Meta-insulated-neighborhood analysis for inactive genes. Related to Figure 1.

Heatmaps showing Smc1, CTCF, TAF2 (TFIID), H3K27ac, Pol II, Med26 (Mediator), H3K27me3 binding and distribution of promoter capture Hi-C interactions (Capture Hi-C) in insulated-neighborhoods of 5,478 inactive genes (Nascent RNA-seq RPKM <0.5). For ChIP-seq, values of $-\log_{10}P$ at significantly enriched windows were used. For Capture Hi-C, values indicating the interaction frequencies (\log_2 ratio of observed over expected number of contacts) were used; these values were obtained directly from the original study cited in the text. These genes are classified as Poised genes and Silent genes based on H3K27me3 binding at their promoters.

Figure S2. Esrrb KD by siRNA and its effects on gene expression and Mediator binding. Related to Figure 3.

(A) Esrrb immunoblot of negative control (siCtrl) versus siRNA knockdown (siEsrrb) normalized via β -Actin levels. The numbers indicate the intensity of signal in siEsrrb normalized to 1 for siCtrl; representative blots are shown.

(B) Browser tracks showing mRNA levels of *Esrrb* and *Actb* (β -Actin) in siCtrl versus siEsrrb. Y axes are normalized read counts.

(C) Relative *Esrrb* and *Actb* mRNA levels in siEsrrb normalized to 1 for siCtrl. Results of two biological replicates are shown.

(D) Venn diagrams showing the proportional overlap of genes down-regulated (Cutoff, 3 fold for the upper, 2 fold for the lower) upon Esrrb KD and Klf4 KD.

(E) Pie-charts showing percentages (numbers in the charts) of enhancers overlapping Esrrb-Med1 co-binding loci of differentially down-regulated genes. FC, fold change (siCtrl/siEsrrb) as in Figure 3F.

(F) Boxplot comparing Esrrb KD effects on decreased Med1 (Mediator) binding in super-enhancers (SEs) and typical enhancers (TEs). Y axis is \log_2 ratio of normalized Med1 ChIP-seq counts in Esrrb KD versus negative control. No significant difference between SE and TE (p -value = 0.15, Wilcoxon Rank-sum test).

(G) Boxplot comparing Esrrb KD effects on downregulation of SE-related and TE-related genes. Y axis is \log_2 ratio of Nascent RNA-seq RPKM (Esrrb KD versus

negative control). No significant difference between SE-related and TE-related genes (p-value = 0.74, Wilcoxon Rank-sum test).

Figure S3. Different types of insulated neighborhoods constrain gene regulation. Related to Figure 4.

(A) Schematic of the major 3 types of insulated neighborhoods observed in our study. Type i, single gene down-regulated by *Esrrb* KD in an insulated neighborhood; Type ii, an insulated neighborhood bearing multiple affected genes; Type iii, an insulated neighborhood bearing active genes where one or more are affected but one or more are not.

(B) Browser plots and bar graph showing a Type i insulated neighborhood. Legends as in Figure 4B.

(C) Browser plots and bar graph showing a Type iii insulated neighborhood.

(D) Browser plots and bar graph showing another Type iii insulated neighborhood.

Figure S4. Depletion of Mediator causes loss of enhancer-promoter interactions. Related to Figure 5.

(A-C) View of genomic regions around *Cdyl2*, *Klf4* and *Tbx3* insulated neighborhoods. *Smc1* ChIA-PET data and binding of Rad21, CTCF, Med1 and H3K27ac are shown. DNaseI hypersensitivity is also shown in (C). Grey bar and VP/Pro, promoter; green bar and Enh, enhancer.

(D) Relative mRNA levels of *Cdyl2*, *Klf4*, *Tbx3* and *Sik1* in si*Esrrb* normalized to 1 for siCtrl. Results of two biological replicates are shown.

(E) Broader view of *Sik1* 4C data comparing control and *Esrrb* KD samples. Gray bar and VP/Pro, promoter; green bar and Enh, enhancer; red bar and Bdr, boundary. P values (Student's t test) for boundaries and enhancer are shown.

(F) Boxplot showing DNase I hypersensitivity at active and inactive promoters. Raw counts were used.

Figure S5. Depletion of Mediator causes loss of PIC assembly. Related to Figure 6.

(A) Immunoblots of Mock and Mediator depleted nuclear extracts showing specific depletion of Mediator subunits.

(B) Bar graphs of mRNA levels of Group A and Group B genes in siCtrl and si*Esrrb*-treated cells from Figure 6C.

Table S1. Insulated neighborhoods for Each Gene. Related to Figures 1 and S1. (See attached Excel file)

Table S2. 4C Primers. Related to STAR Methods.

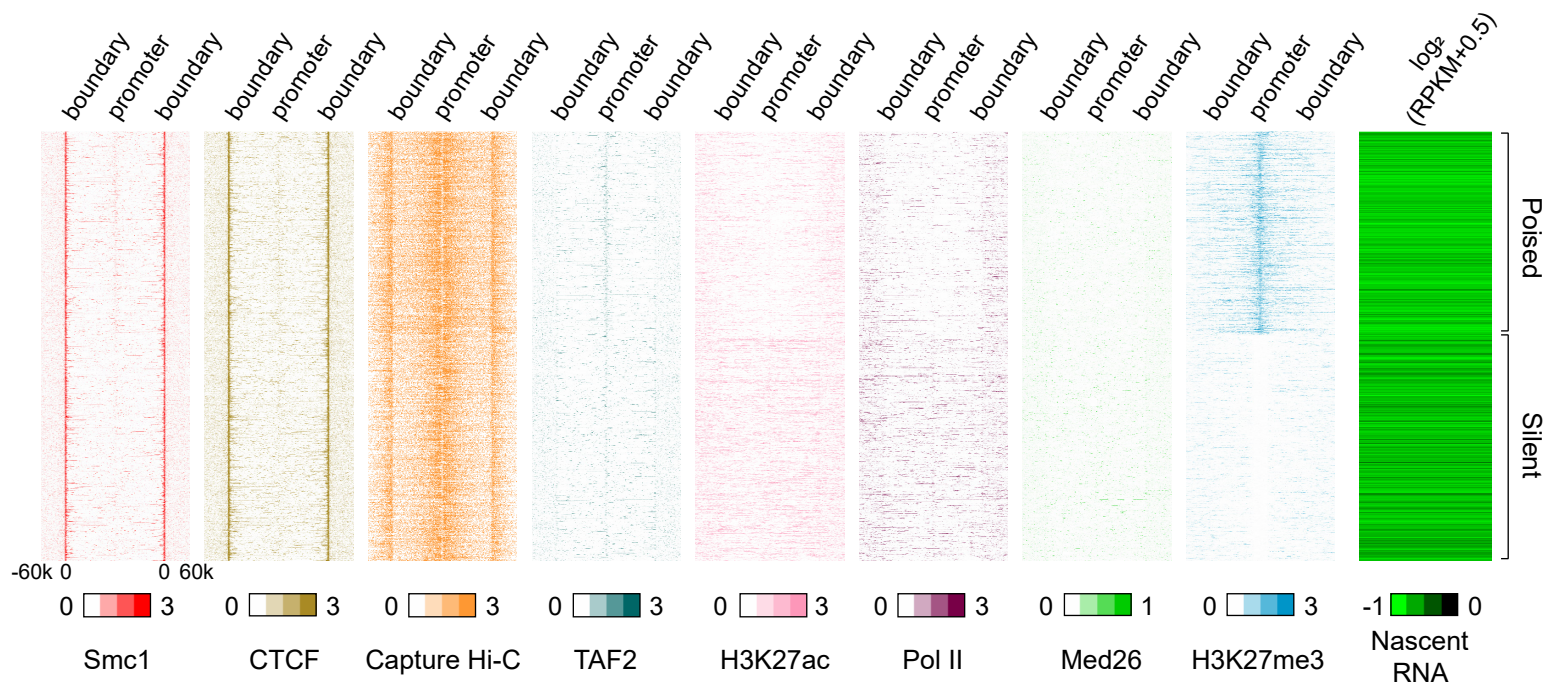
View point	1 st RE*	2 nd RE**	Reading primer	Non-reading primer
Mitf_promoter	DpnII	NlaIII	5'- AATGATACGGCGACCACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT <u>NN</u> ACCGGACAGGGACTGT GATC-3'***	5'- CAAGCAGAAGACGGCATA CGAGACTGACTGTTCTCC TCCCAGG-3'
Sik1_promoter	DpnII	NlaIII	5'- AATGATACGGCGACCACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT <u>NN</u> CACTGAGGGAACCTGG GATC-3'	5'- CAAGCAGAAGACGGCATA CGATCGGCTGCTAGCTCT GCTC-3'
Cdyl2_promoter	DpnII	NlaIII	5'- AATGATACGGCGACCACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT <u>NN</u> GTGGGTTGGGTGGGT GATC-3'	5'- CAAGCAGAAGACGGCATA CGACTTCTCCGTACCTCG TAAAGGTC-3'
Klf4_promoter	DpnII	NlaIII	5'- AATGATACGGCGACCACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT <u>NN</u> TTGAATCAAAGAAGA AGGATC-3'	5'- CAAGCAGAAGACGGCATA CGAGCCGCCAGGTGAGA ATG-3'
Tbx3_promoter	NlaIII	CviQI	5'- AATGATACGGCGACCACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT <u>NN</u> TGGCTGGGCGGTTCCA TG-3'	5'- CAAGCAGAAGACGGCATA CGAGCAGGTTCAAACCTAG CAGCTATTGG-3'

*1st RE, primary restriction enzyme; **2nd RE, secondary restriction enzyme; ***NN, barcode.

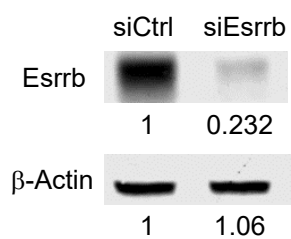
Table S3. Previously Published Datasets Used in this Study. Related to STAR Methods.

Dataset	Data Access	Source
Promoter Capture Hi-C	E-MTAB-2414	Schoenfelder et al., 2015
Smc1 ChIA-PET	GSM1397342, GSM1480237	Downen et al., 2014
Smc3 ChIP-seq	GSM560344, GSM560343	Kagey et al., 2010
ChIP-seq input of Kagey et al., 2010	GSM560357	Kagey et al., 2010
Rad21 ChIP-seq	GSM2418859	Hansen et al., 2016
CTCF ChIP-seq	GSM2418860	Hansen et al., 2016
ChIP-seq input of Hansen et al., 2010	GSM2418857	Hansen et al., 2016
H3K27ac ChIP-seq	GSM2417096	Chronis et al., 2017
ChIP-seq input of Chronis et al., 2010	GSM2417127	Chronis et al., 2017
DNase I HS	GSM1014154	ENCODE Project

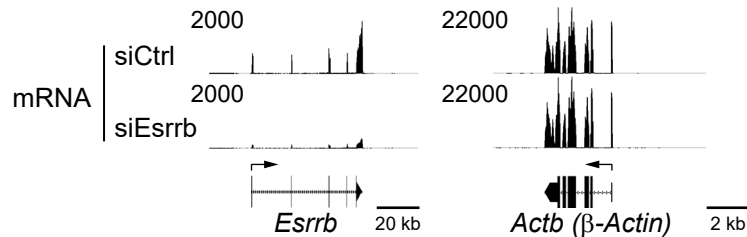
Fig S1 (related to Fig 1)



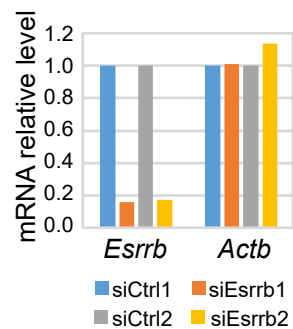
A



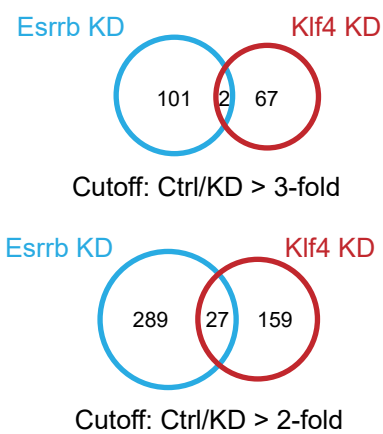
B



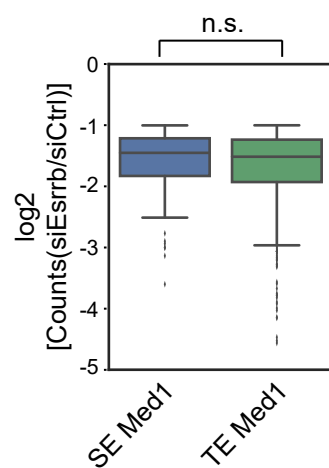
C



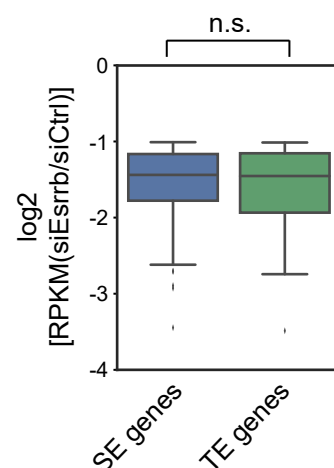
D



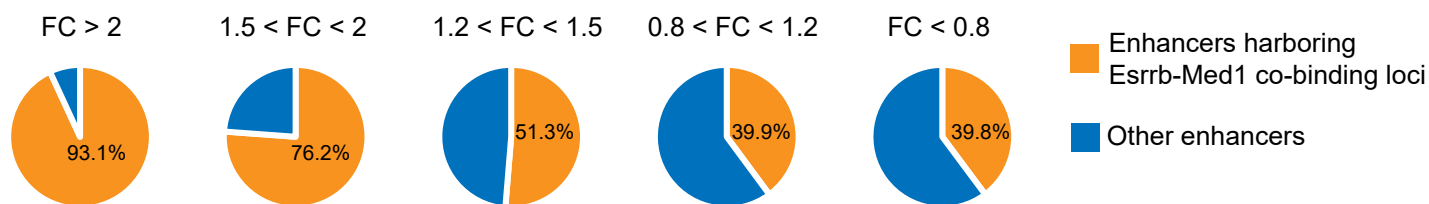
F



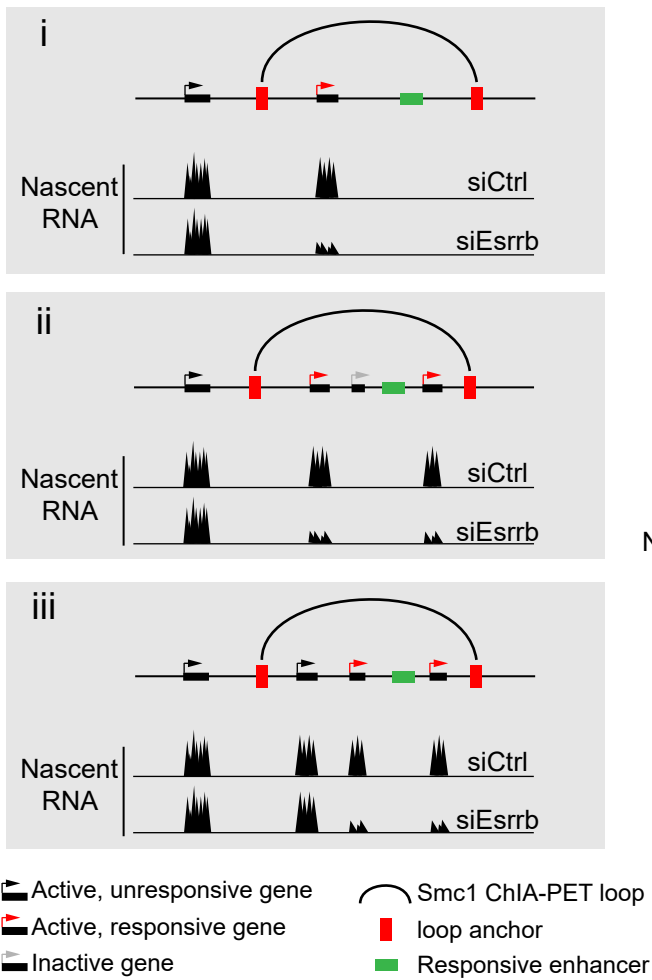
G



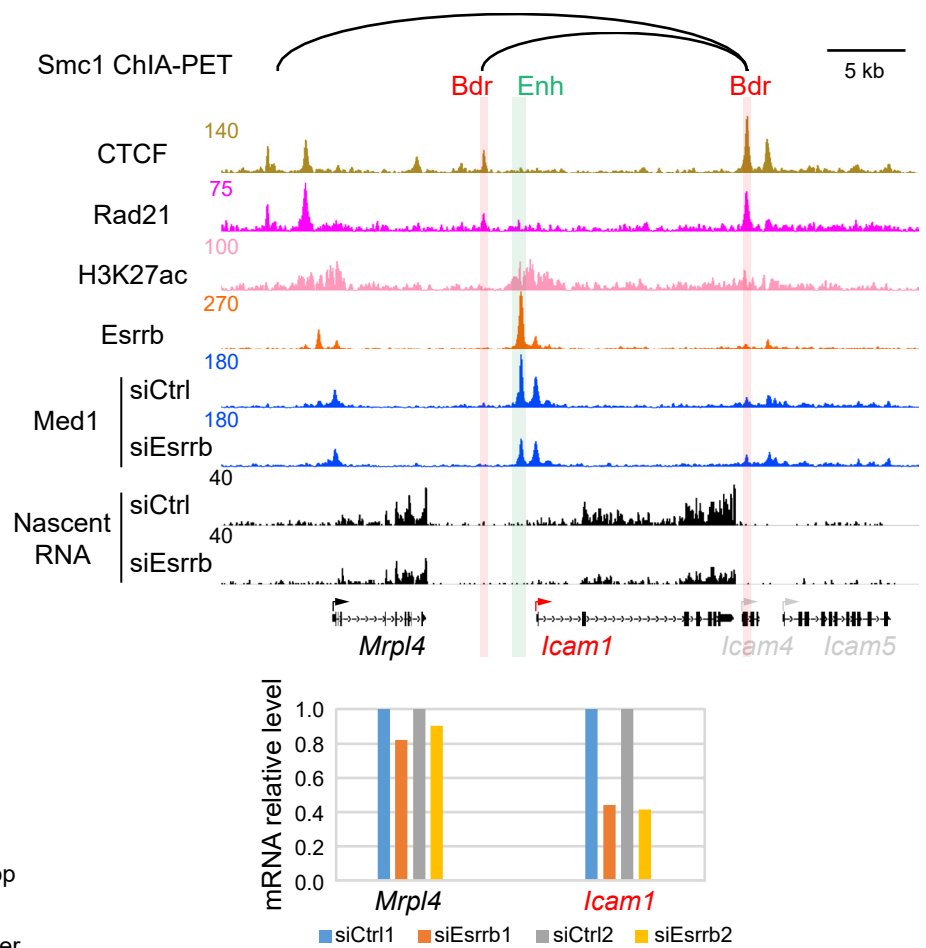
E



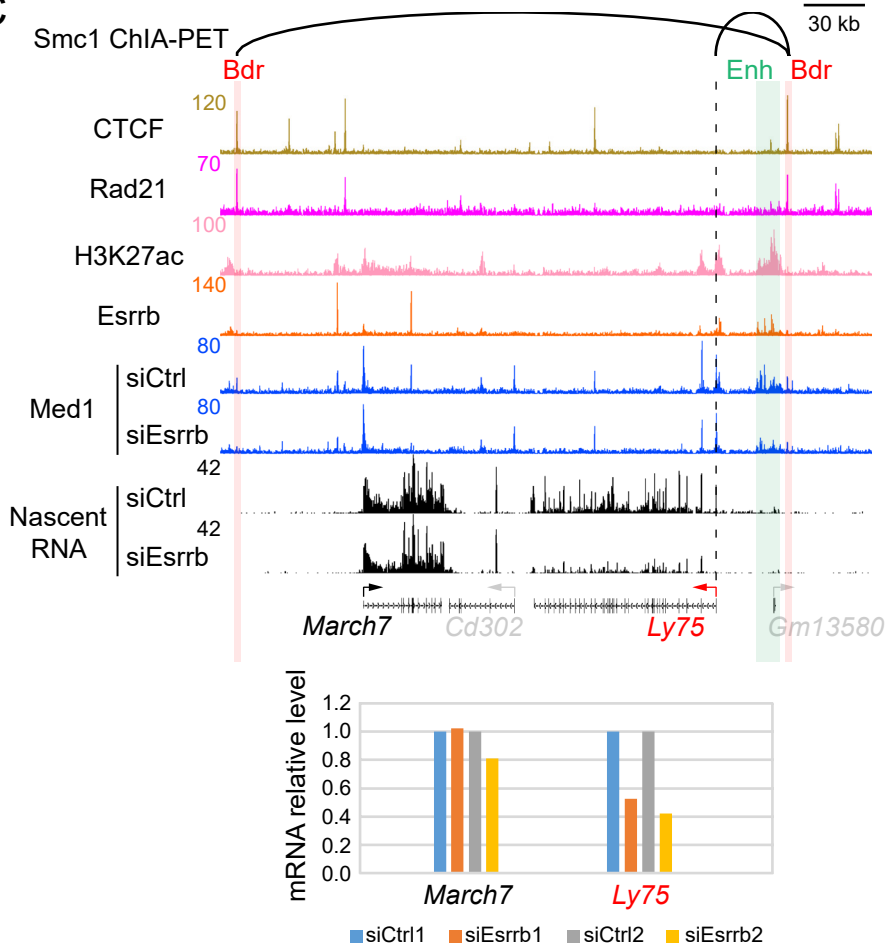
A



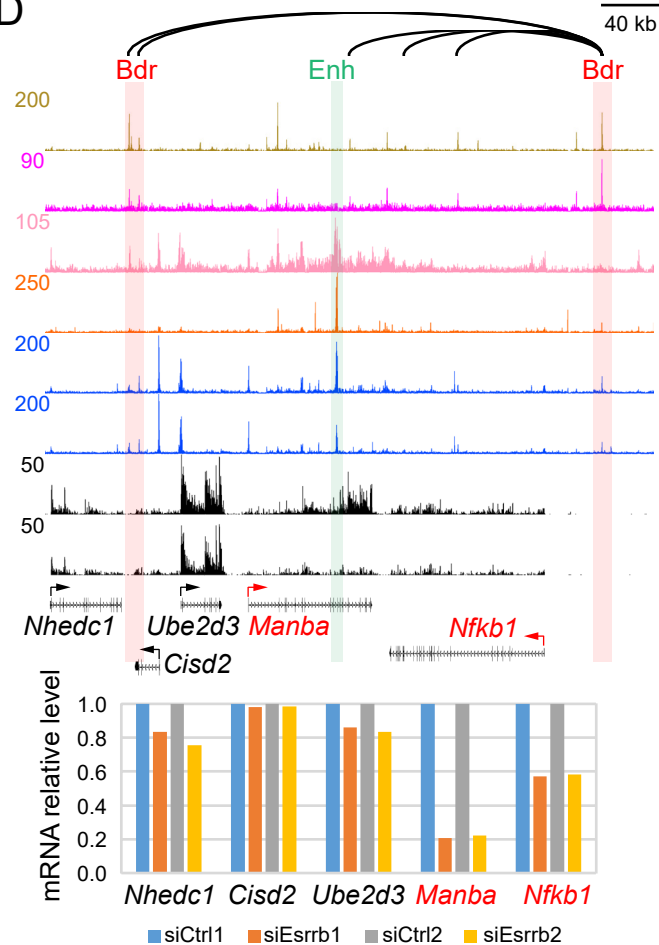
B



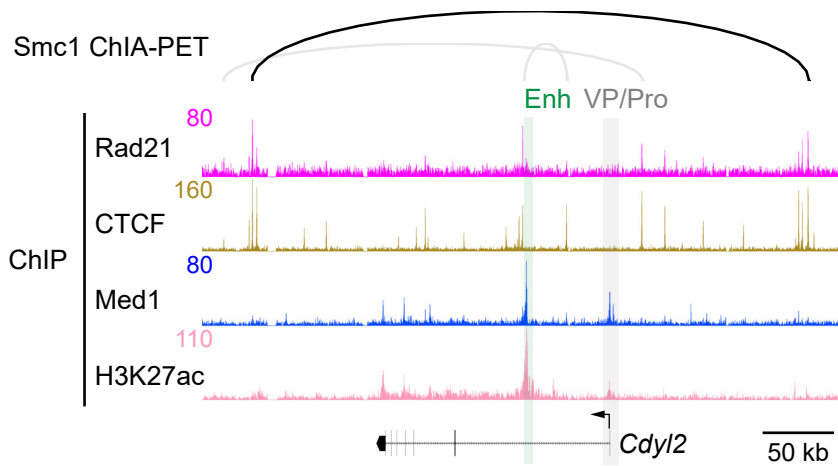
C



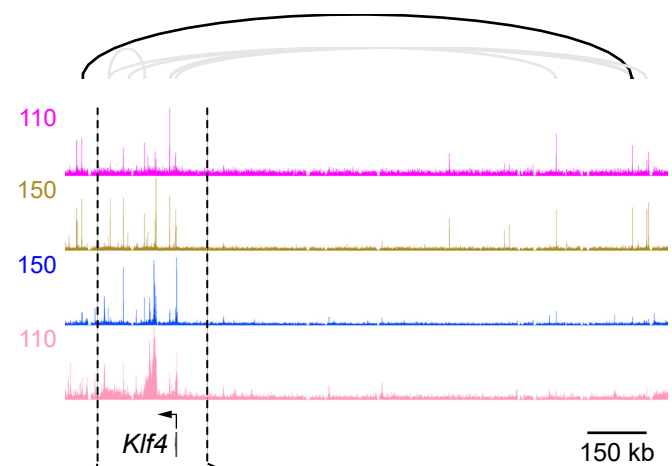
D



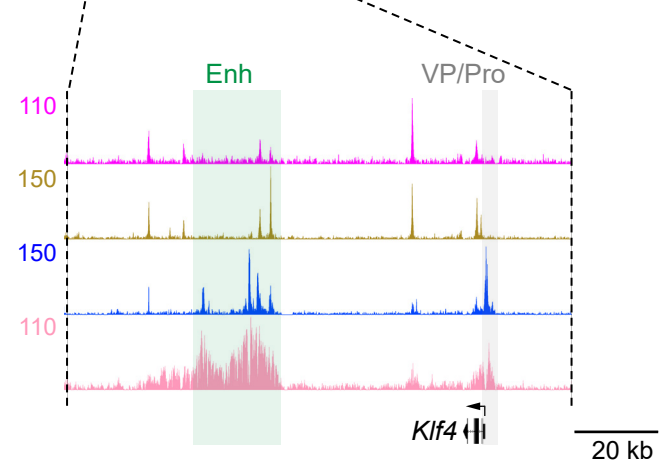
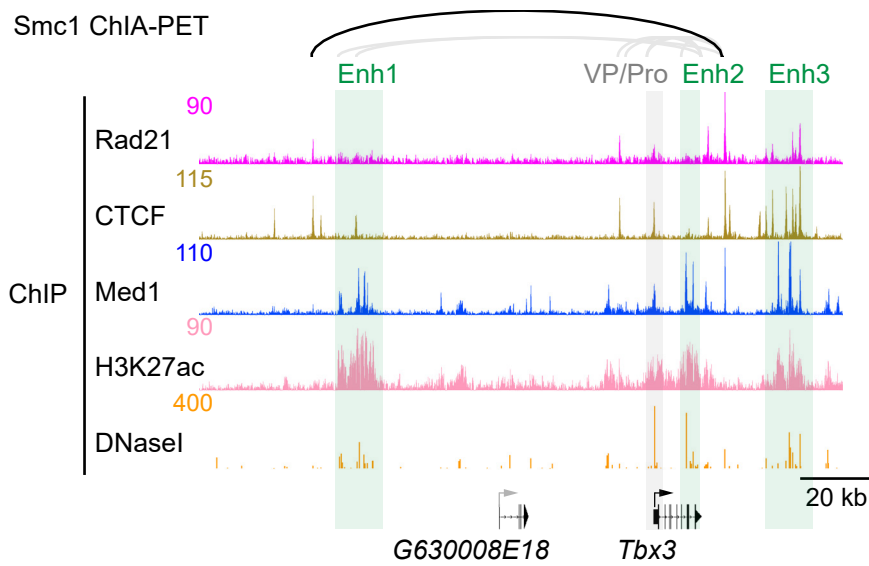
A



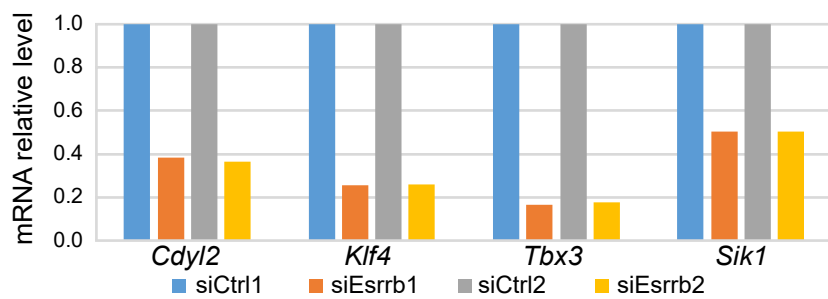
B



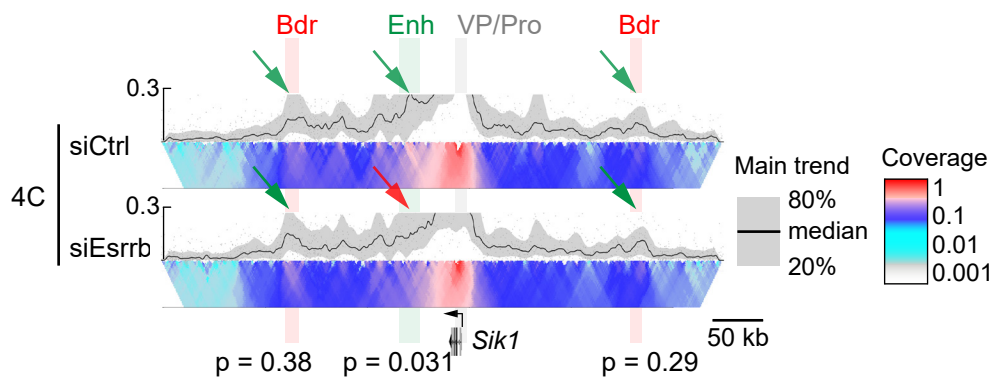
C



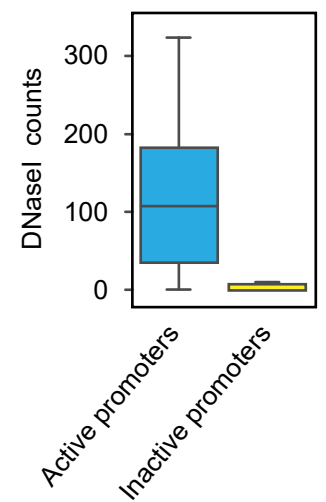
D



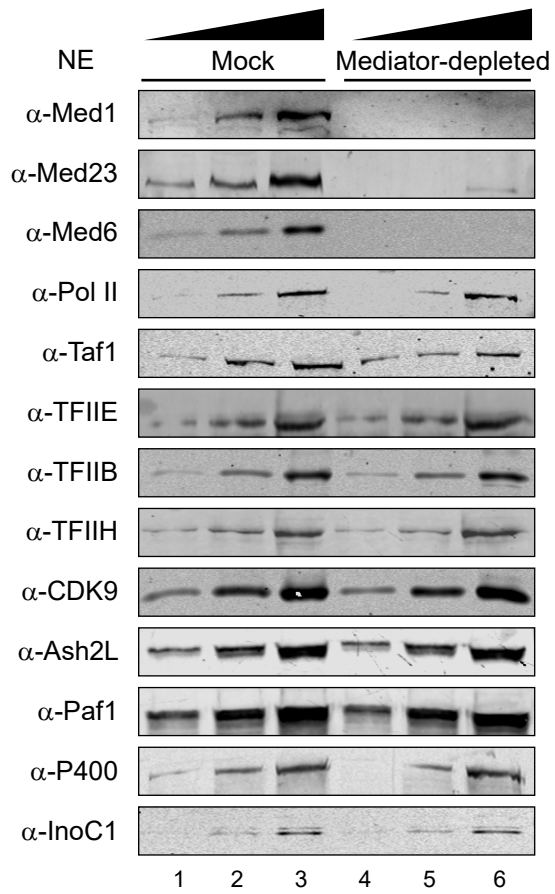
E



F



A



B

