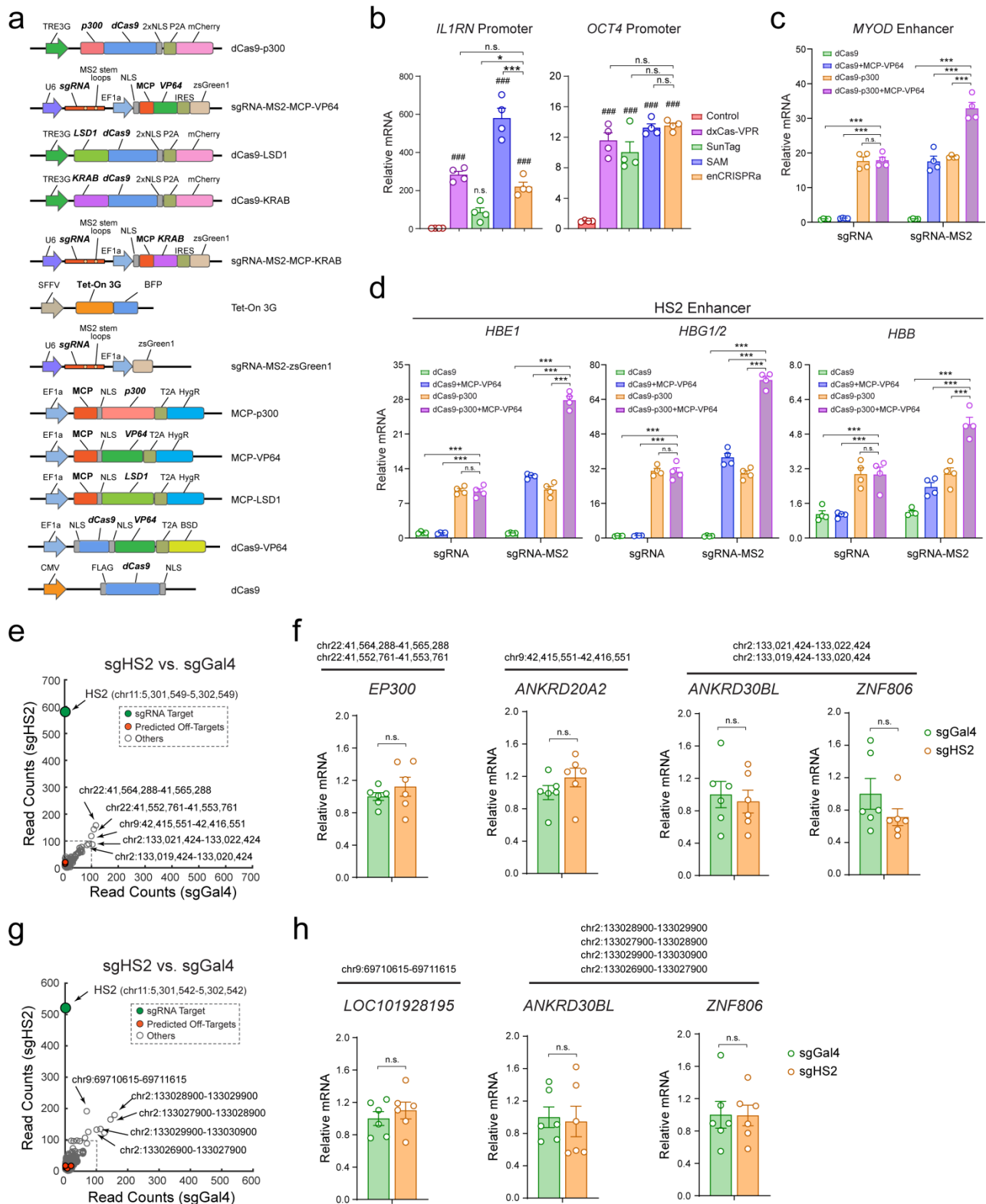


Supplementary Information

**Interrogation of Enhancer Function by Enhancer-Targeting
CRISPR Epigenetic Editing**

Li and Liu et al.



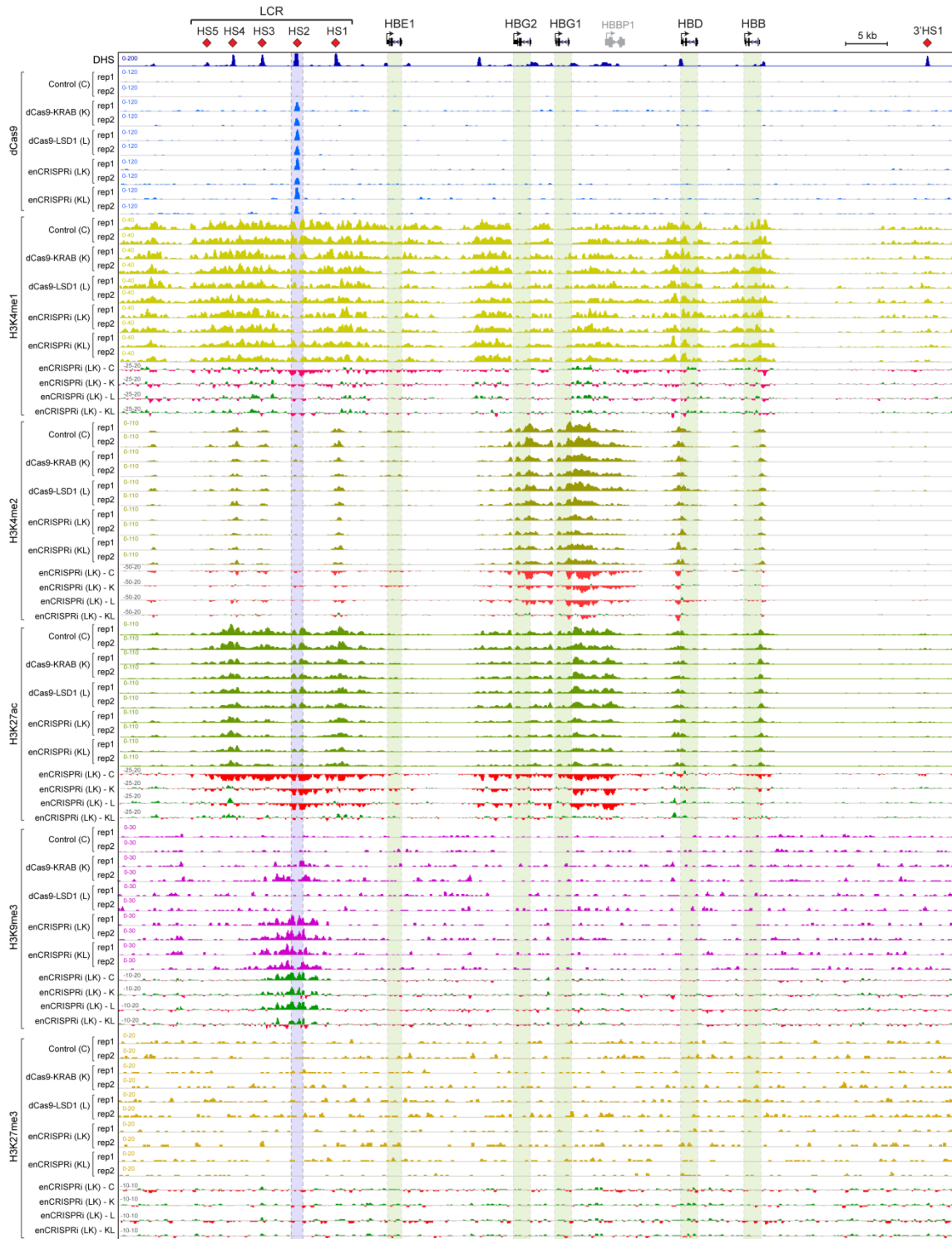
Supplementary Figure 1. Evaluation of enCRISPRa in Target Gene Activation

(a) Schematic of the constructs used in this study.

(b) Expression of *IL1RN* and *OCT4* upon dxCas9-VPR, SunTag, SAM or enCRISPRa-mediated promoter activation in HEK293T cells. mRNA expression relative to non-transduced cells is shown as mean \pm SEM ($N = 4$ experiments). The differences between control and dCas9 activators were analyzed by a one-way ANOVA. * $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$.

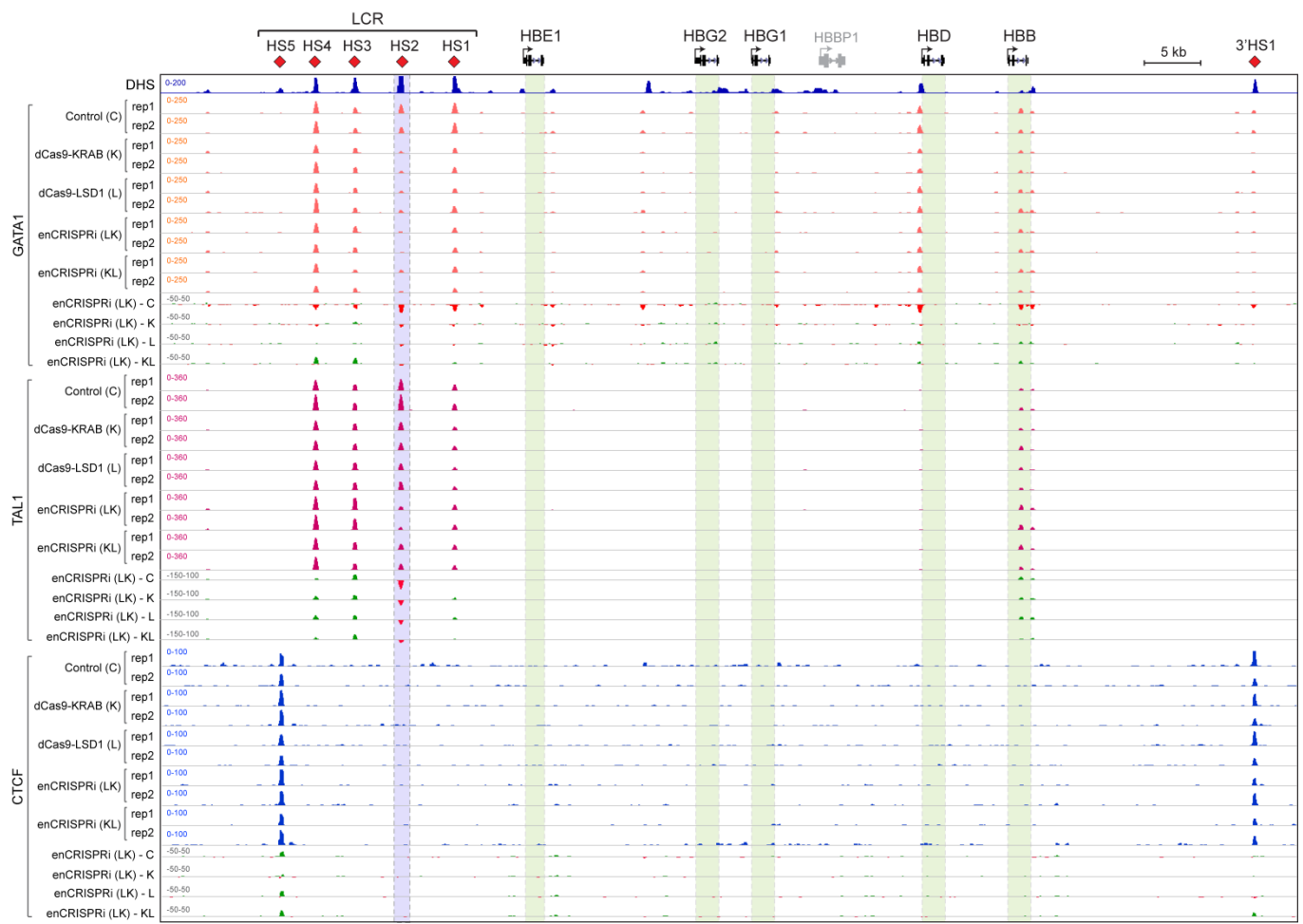
0.001. The difference between enCRISPRa and other dCas9 activators were analyzed by a one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant.

- (c) Expression of *MYOD* upon dCas9 alone, dCas9 + MCP-VP64, dCas9-p300, or dCas9-p300 + MCP-VP64 mediated enhancer activation together with the *MYOD* enhancer-targeting sgRNA with or with MS2 loops in HEK293T cells. Results are mean \pm SEM ($N = 4$ experiments). The differences between different groups were analyzed by a two-way ANOVA. *** $P < 0.001$, n.s. not significant.
- (d) Expression of *HBE1*, *HBG1/2* and *HBB* upon dCas9 alone, dCas9 + MCP-VP64, dCas9-p300, or dCas9-p300 + MCP-VP64 mediated enhancer activation with the HS2 enhancer-targeting sgRNA with or with MS2 in HEK293T cells. Results are mean \pm SEM ($N = 4$ experiments) and analyzed by a two-way ANOVA. *** $P < 0.001$, n.s. not significant.
- (e) Genome-wide analysis of dCas9 binding in HEK293T cells expressing sgHS2 or sgGal4. The same plot as Fig. 1f is shown except that the top 5 potential dCas9 off-targets are labeled.
- (f) Expression of the nearest neighbor genes of the top 5 potential dCas9 off-targets in HEK293T cells. Results are mean \pm SEM ($N = 6$ experiments) and analyzed by a two-sided t -test. n.s. not significant.
- (g) Genome-wide analysis of dCas9 binding in K562 cells expressing sgHS2 or sgGal4. The same plot as Fig. 2d is shown except that the top 5 potential dCas9 off-targets are labeled.
- (h) Expression of the nearest neighbor genes of the top 5 potential dCas9 off-targets in K562 cells. Results are mean \pm SEM ($N = 6$ experiments) and analyzed by a two-sided t -test. Source data are provided as a Source Data file.



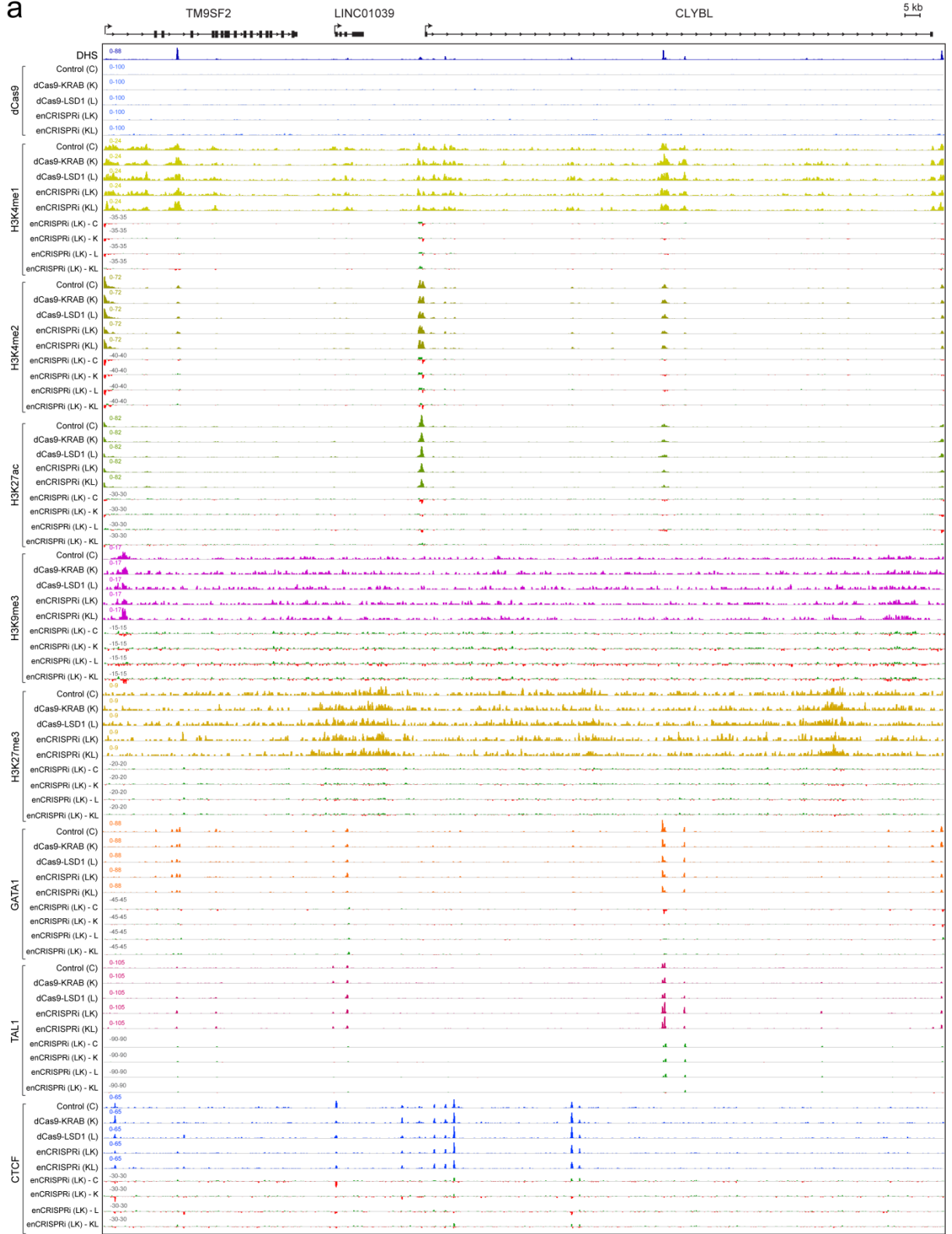
Supplementary Figure 2. enCRISPRi Leads to Locus-Wide Epigenetic Reprogramming

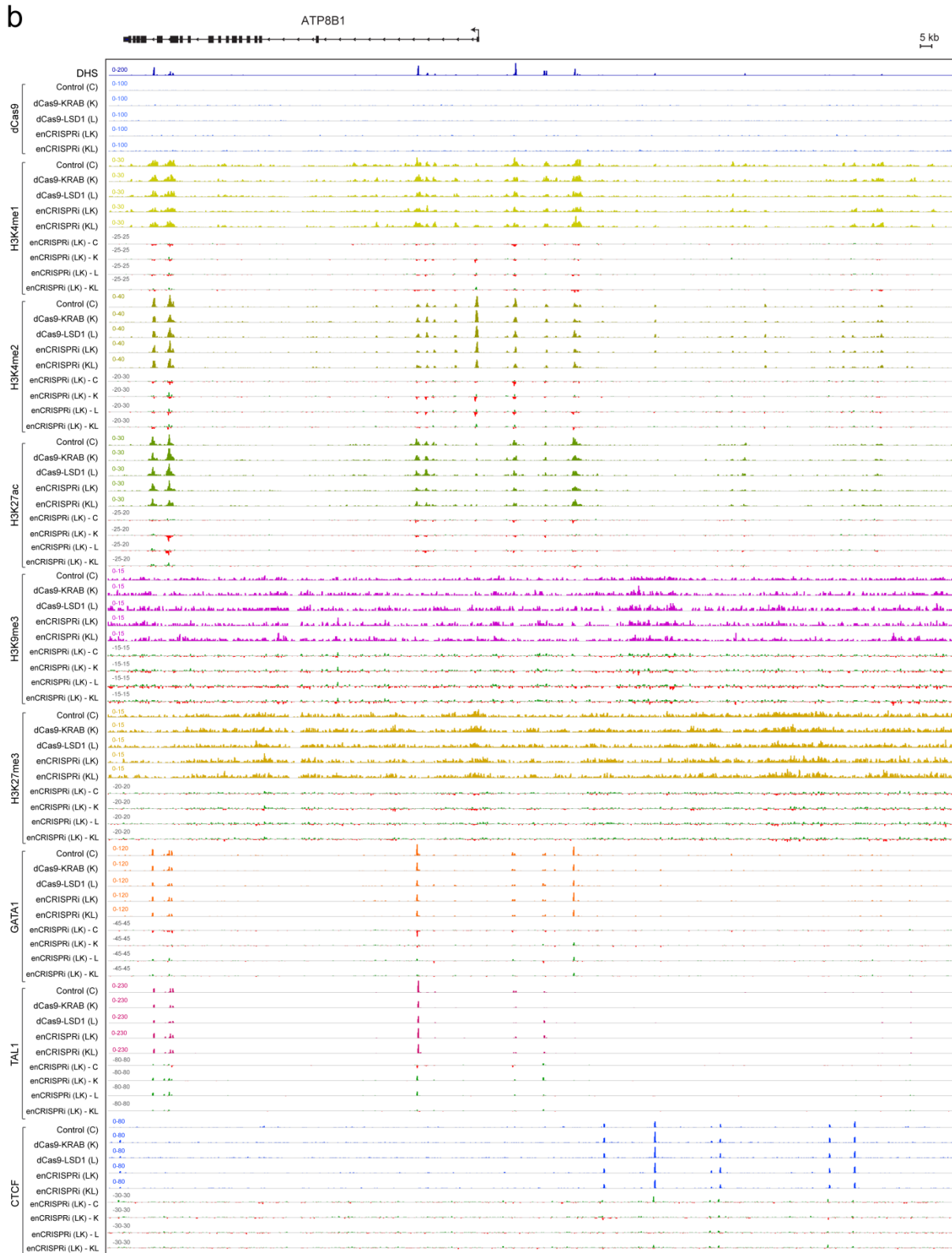
Similar to Fig. 3, density maps are shown for ChIP-seq of dCas9, H3K4me1, H3K4me2, H3K27ac, H3K9me3 and H3K27me3 at the β -globin cluster (chr11: 5,222,500-5,323,700; hg19) in K562 cells co-expressing non-targeting sgGal4 (control or C) or sgHS2 with dCas9-KRAB (K), dCas9-LSD1 (L) or enCRISPRi (LK and KL). Regions showing increased or decreased ChIP-seq signals in enCRISPRi (LK) relative to control, dCas9-KRAB, dCas9-LSD1 or enCRISPRi (KL) (enCRISPRi – C, enCRISPRi – K, enCRISPRi – L, or enCRISPRi – KL) are depicted in *green* and *red*, respectively. Blue bars denote the sgRNA-targeted HS2 enhancer. Green bars denote the β -globin genes. Independent replicates (rep1 and rep2) are shown for all ChIP-seq experiments.



Supplementary Figure 3. Targeting enCRISPRi to the β -Globin Gene Cluster Interferes with TF Binding
 Similar to Fig. 3, density maps are shown for ChIP-seq of GATA1, TAL1, and CTCF at the β -globin cluster (chr11: 5,222,500-5,323,700; hg19) in K562 cells. Independent ChIP-seq replicates (rep1 and rep2) are shown.

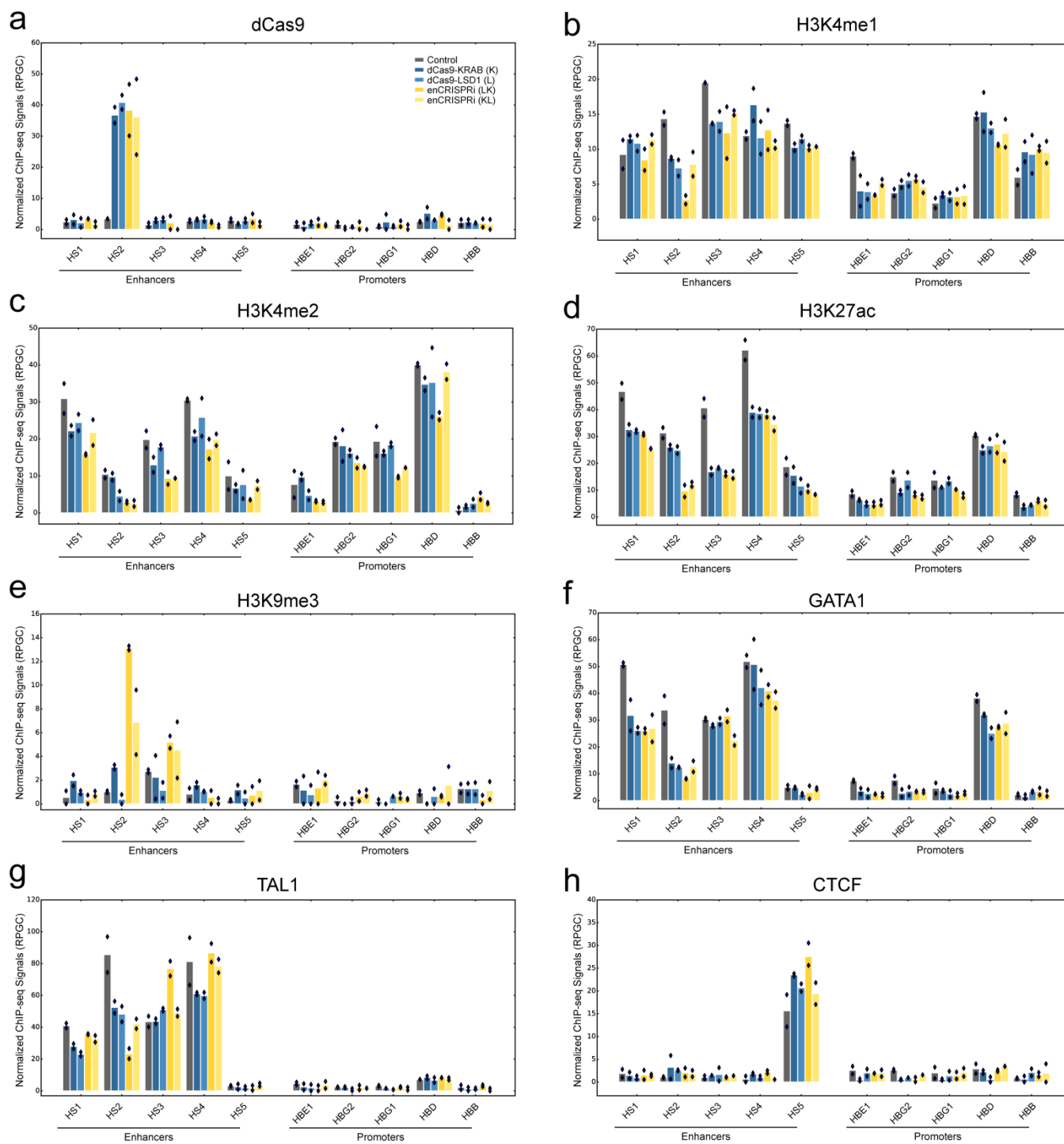
a





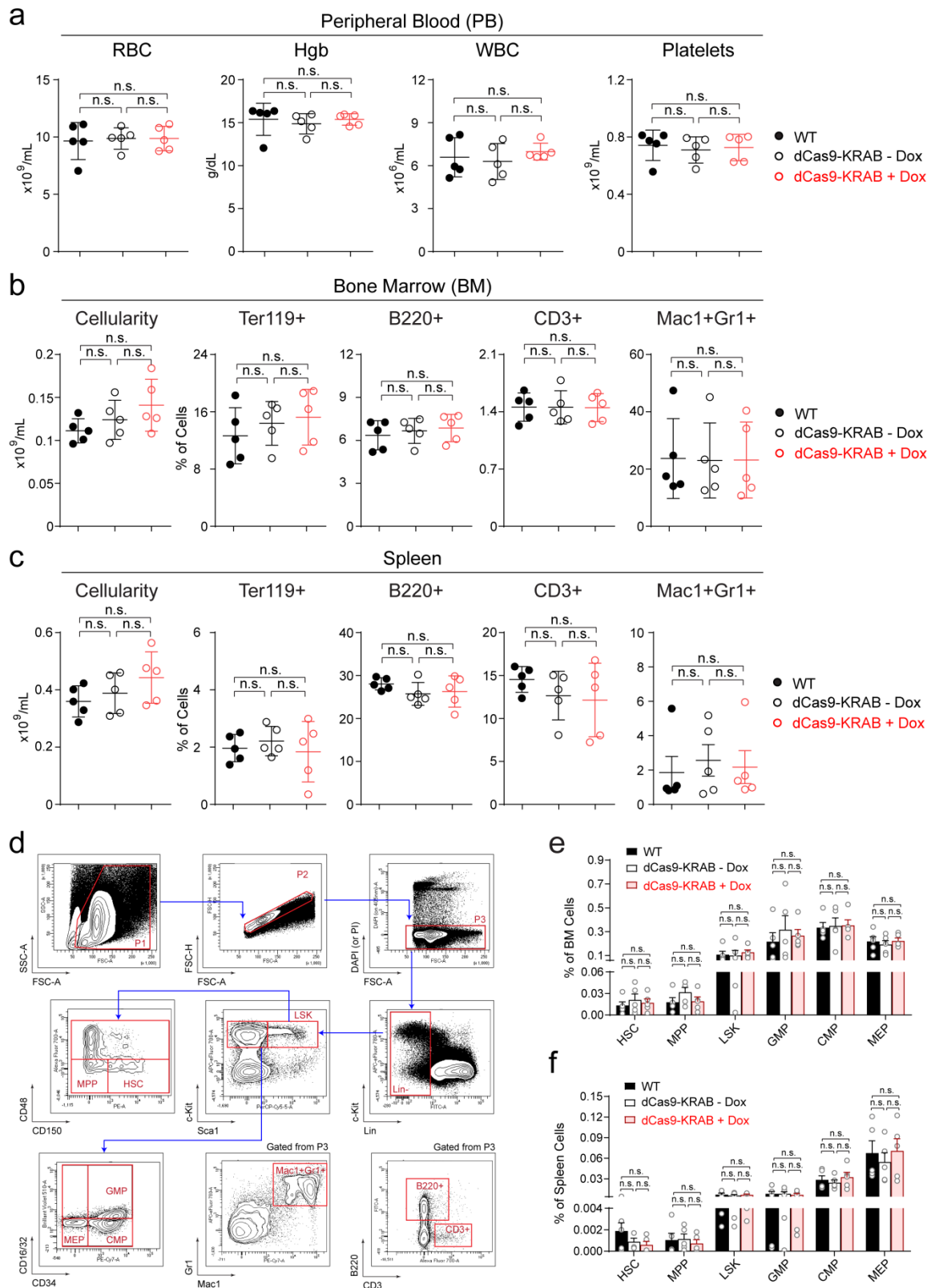
Supplementary Figure 4. Representative Non-Targeted Genomic Loci Upon HS2-Targeted enCRISPRi

- (a) Similar to Fig. 3, density maps are shown for ChIP-seq of dCas9, H3K4me1, H3K4me2, H3K27ac, H3K9me3, H3K27me3, GATA1, TAL1, and CTCF at the *TM9SF2-LINC01039-CLYBL* locus (chr13: 100,154,607-100,430,801; hg19) in K562 cells.
- (b) Similar to Fig. 3, density maps are shown for ChIP-seq of dCas9, H3K4me1, H3K4me2, H3K27ac, H3K9me3, H3K27me3, GATA1, TAL1, and CTCF at the *ATP8B1* locus (chr18: 55,292,001-55,668,879; hg19) in K562 cells.



Supplementary Figure 5. Analysis of ChIP-seq Signals at the β -Globin Enhancers and Promoters

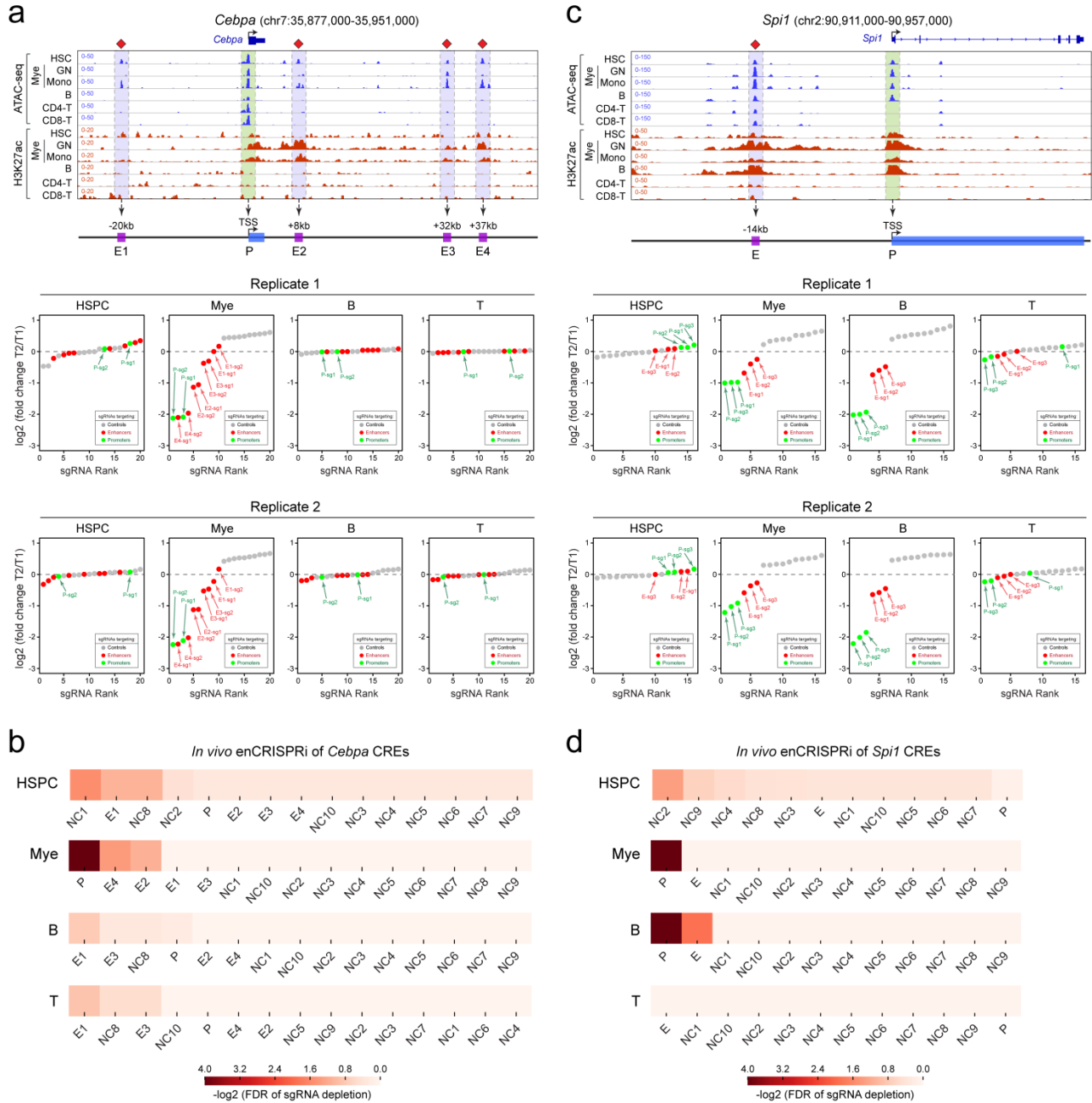
Bar graphs are shown for the normalized ChIP-seq signals as reads per genomic content (PRGC) at the β -globin enhancers (HS1 to HS5) and promoters (*HBE1*, *HBG2*, *HBG1*, *HBD* and *HBB*) for dCas9 (a), H3K4me1 (b), H3K4me2 (c), H3K27ac (d), H3K9me3 (e), GATA1 (f), TAL1 (g), and CTCF (h) in K562 cells co-expressing non-targeting sgGal4 (control) or sgHS2 with dCas9-KRAB (K), dCas9-LSD1 (L) or enCRISPRi (LK and KL). Dots indicate values from the independent ChIP-seq replicates (rep1 and rep2). Source data are provided as a Source Data file.



Supplementary Figure 6. Inducible dCas9-KRAB Expression Has No Effect on Hematopoietic Differentiation

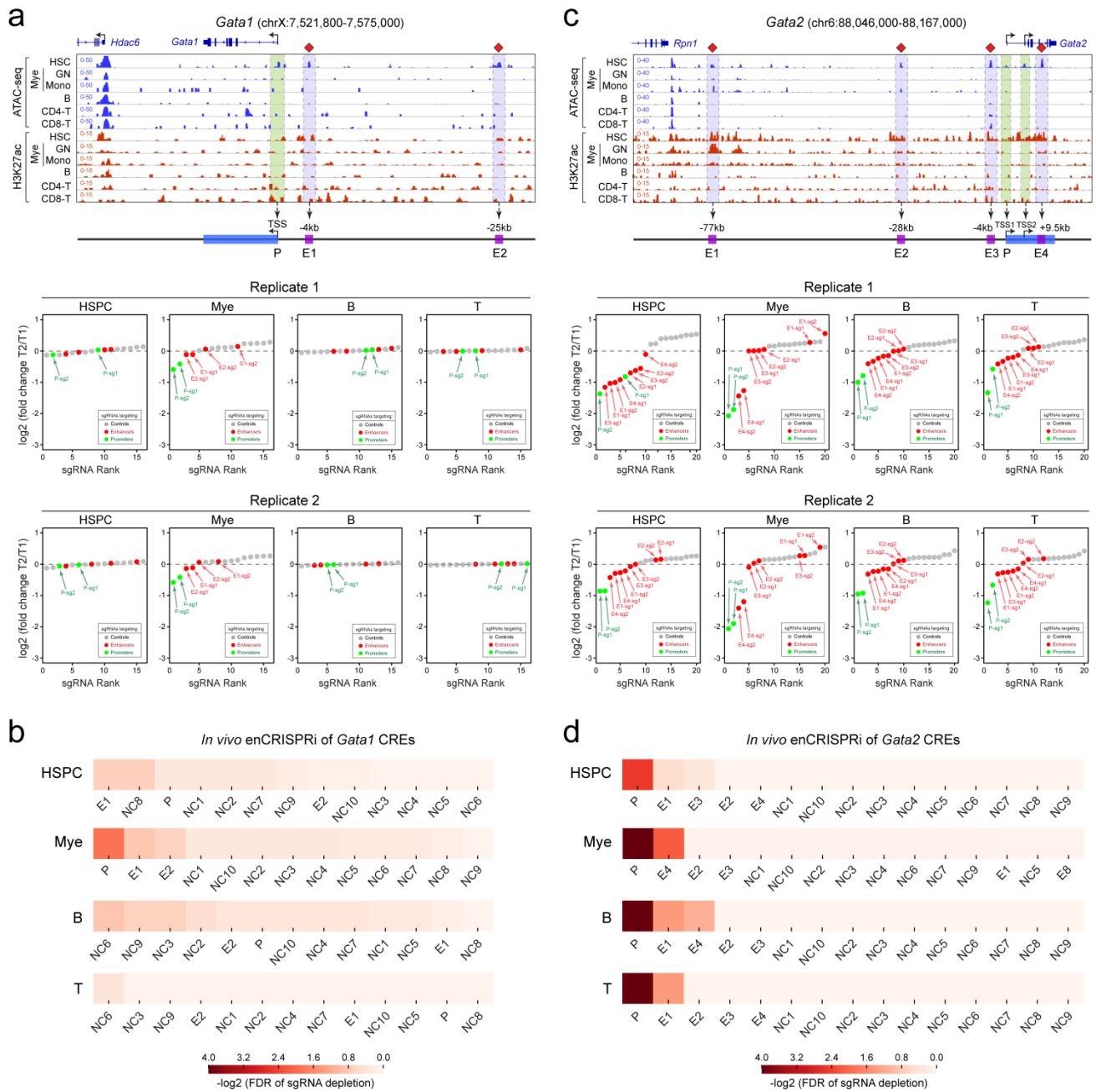
(a) Complete blood count (CBC) of peripheral blood (PB) red blood cells (RBC), hemoglobin (Hgb), white blood cells (WBC) and platelets in mice 8 weeks after Dox treatment. $N = 5$ mice per group. Results are mean \pm SD and analyzed by a repeated-measures one-way ANOVA with multiple comparisons. n.s. not significant.

- (b)** BM cellularity and frequencies of erythroid (Ter119⁺), B-lymphoid (B220⁺), T-lymphoid (CD3⁺) and myeloid (Mac1⁺Gr1⁺) cells in mice 8 weeks after Dox treatment.
- (c)** Spleen cellularity and frequencies of erythroid, B-lymphoid, T-lymphoid and myeloid cells 8 weeks after Dox treatment.
- (d)** Representative flow cytometry gates are shown for the analysis of various hematopoietic stem/progenitor cells (HSPCs) and mature lineages in mouse bone marrow.
- (e)** Frequencies of HSPCs in BM 8 weeks after Dox treatment. *N* = 5 mice per group. Results are mean ± SEM and analyzed by a two-way ANOVA with multiple comparisons. n.s. not significant.
- (f)** Frequencies of HSPCs in spleen 8 weeks after Dox treatment. *N* = 5 mice per group. Source data are provided as a Source Data file.



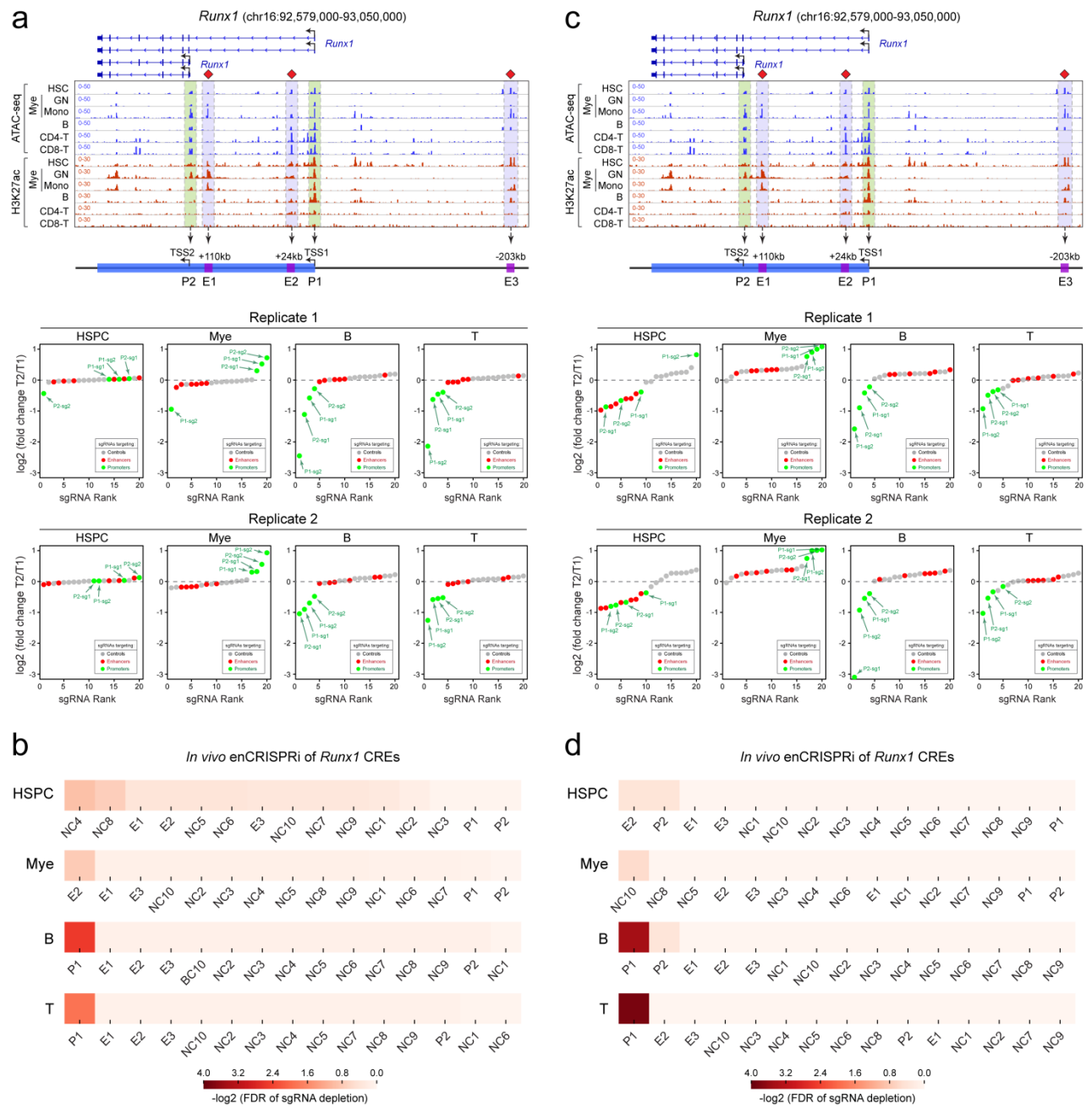
Supplementary Figure 7. Locus-Specific Enhancer Perturbation at *Cebpa* and *Spi1* Loci

- (a) *In vivo* enCRISPRi perturbation of *Cebpa* CREs during hematopoiesis. Similar to Fig. 5e, waterfall plots are shown for target-specific sgRNAs (green and red dots) and non-targeting control sgRNAs (grey dots) by the normalized \log_2 fold changes in HSPCs, myeloid, T or B cells 16-weeks post-BMT (T2) relative to pooled sgRNA-transduced HSPCs (T1) in two independent replicate screens ($N = 3$ recipient mice per screen). The annotated *Cebpa* promoter (P) and enhancers (E1 to E4) are indicated by green and blue shaded lines.
- (b) Ranking of the top depleted *Cebpa* CREs by the $-\log_2$ (FDR of sgRNA depletion). The significance (FDR) of depletion for each targeted enhancer, promoter or non-targeting control (NC) region in each cell type was calculated using the MAGECK test. P, promoter; E, enhancer; NC, non-targeting control.
- (c) *In vivo* enCRISPRi perturbation of *Spi1* CREs during hematopoiesis. Similar to Fig. 5f, waterfall plots are shown for two independent replicate screens ($N = 3$ recipient mice per screen). The annotated *Spi1* promoter (P) and enhancer (E) are indicated by green and blue shaded lines.
- (d) Ranking of the top depleted *Spi1* CREs by the $-\log_2$ (FDR of sgRNA depletion).



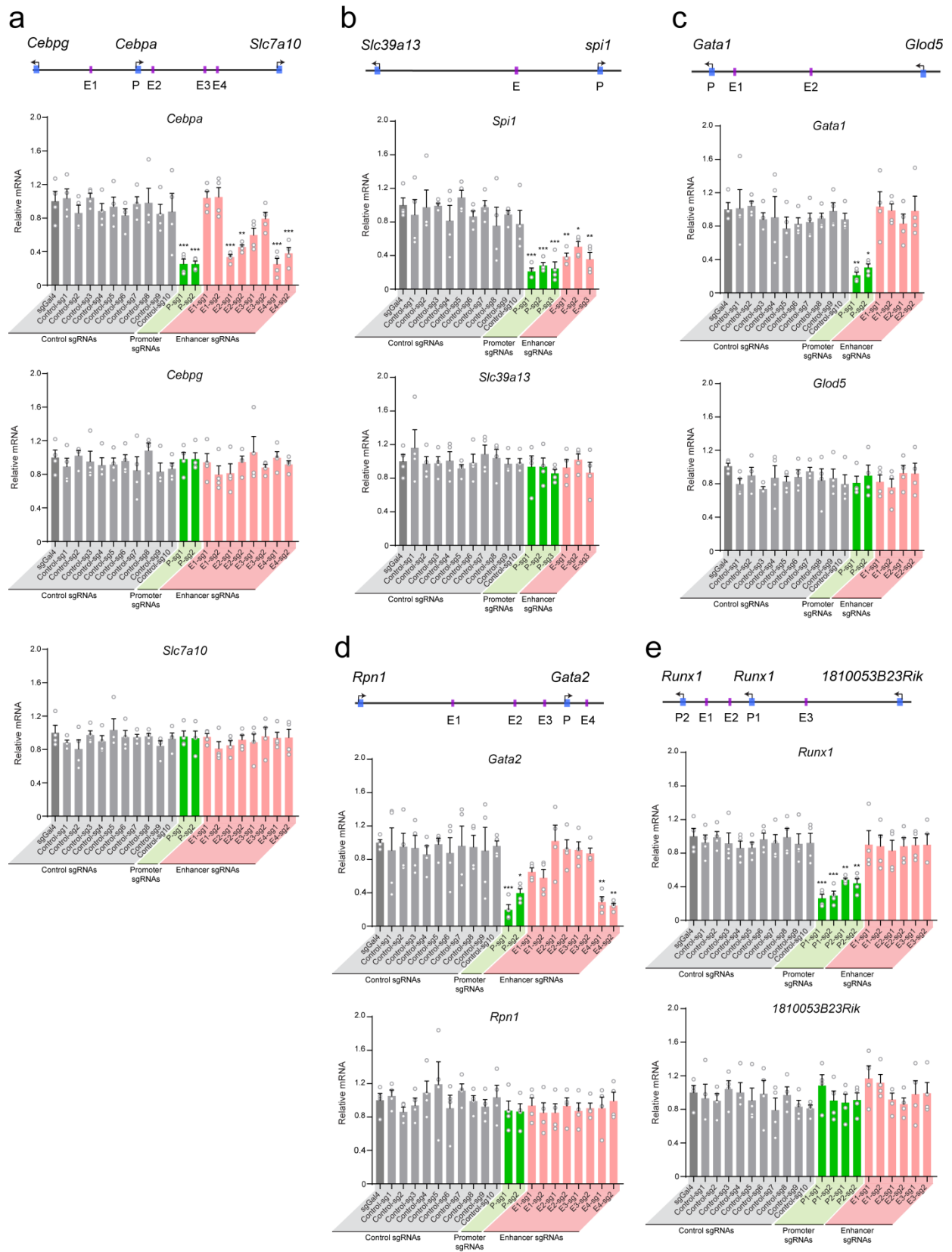
Supplementary Figure 8. Locus-Specific Enhancer Perturbation at *Gata1* and *Gata2* Loci

- (a) *In vivo* enCRISPRi perturbation of *Gata1* CREs during hematopoiesis. Density maps are shown for ATAC-seq and H3K27ac ChIP-seq at the *Gata1* locus (chrX:7,521,800-7,575,000; mm9) in bone marrow HSC, granulocytes (GN), monocytes (Mono), B, CD4⁺ and CD8⁺ T cells, respectively. Waterfall plots are shown for target-specific sgRNAs (green and red dots) and non-targeting control sgRNAs (grey dots) by the normalized log₂ fold changes in HSPCs, myeloid, T or B cells 16-weeks post-BMT (T2) relative to pooled sgRNA-transduced HSPCs (T1) in two independent replicate screens (*N* = 3 recipient mice per screen). The annotated *Gata1* promoter (P) and enhancers (E1 and E2) are indicated by green and blue shaded lines.
- (b) Ranking of the top depleted *Gata1* CREs by the $-\log_2$ (FDR of sgRNA depletion). The significance (FDR) of depletion for each targeted enhancer, promoter or non-targeting control (NC) region in each cell type was calculated using the MAGECK test. P, promoter; E, enhancer; NC, non-targeting control.
- (c) *In vivo* enCRISPRi perturbation of *Gata2* CREs during hematopoiesis. Density maps are shown for ATAC-seq and H3K27ac ChIP-seq at the *Gata2* locus (chr6:88,046,000-88,167,000; mm9) in bone marrow HSC, GN, Mono, B, CD4⁺ and CD8⁺ T cells, respectively. Waterfall plots are shown for two independent replicate screens (*N* = 3 recipient mice per screen). The annotated *Gata2* promoter (P) and enhancers (E1 to E4) are indicated by green and blue shaded lines.
- (d) Ranking of the top depleted *Gata2* CREs by the $-\log_2$ (FDR of sgRNA depletion).



Supplementary Figure 9. Locus-Specific and Multi-Loci Perturbations of *Runx1* CREs

- (a) *In vivo* enCRISPRi perturbation of *Runx1* CREs by locus-specific enCRISPRi screen during hematopoiesis. Similar to Fig. 6c, waterfall plots are shown for two independent replicate screens ($N = 3$ recipient mice per screen). The annotated *Runx1* promoters (P1 and P2) and enhancers (E1 to E3) are indicated by green and blue shaded lines.
- (b) Ranking of the top depleted *Runx1* CREs by the $-\log_2$ (FDR of sgRNA depletion) by locus-specific enCRISPRi screen. The significance (FDR) of depletion for each targeted enhancer, promoter or non-targeting control (NC) region in each cell type was calculated using the MAGECK test. P, promoter; E, enhancer; NC, non-targeting control.
- (c) *In vivo* enCRISPRi perturbation of *Runx1* CREs by multi-loci enCRISPRi screen during hematopoiesis. Similar to Fig. 6d, waterfall plots are shown for two independent replicate screens ($N = 3$ recipient mice per experiment).
- (d) Ranking of the top depleted *Runx1* CREs by the $-\log_2$ (FDR of sgRNA depletion) in the multi-loci enCRISPRi screen.

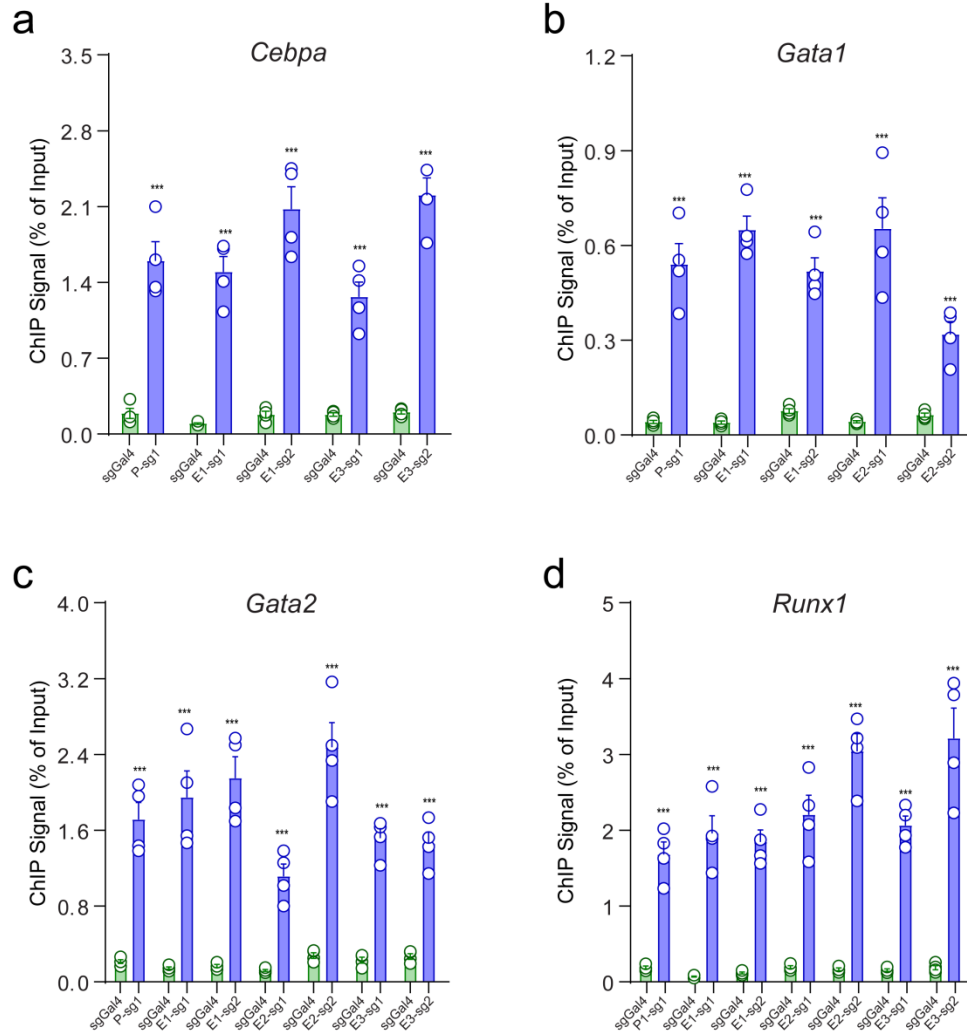


Supplementary Figure 10. In Vivo enCRISPRi of Promoters and Enhancers Impairs Target Gene Expression

(a) enCRISPRi-mediated perturbation of *Cebpa* CREs impaired the mRNA expression of *Cebpa* but not the other nearby genes in BM HSPCs. Independent sgRNAs (sg1 and sg2) for each targeted enhancer or promoter are shown. Cells

transduced with non-targeting sgGal4 were analyzed as the control. Results are mean \pm SEM and analyzed by a one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant.

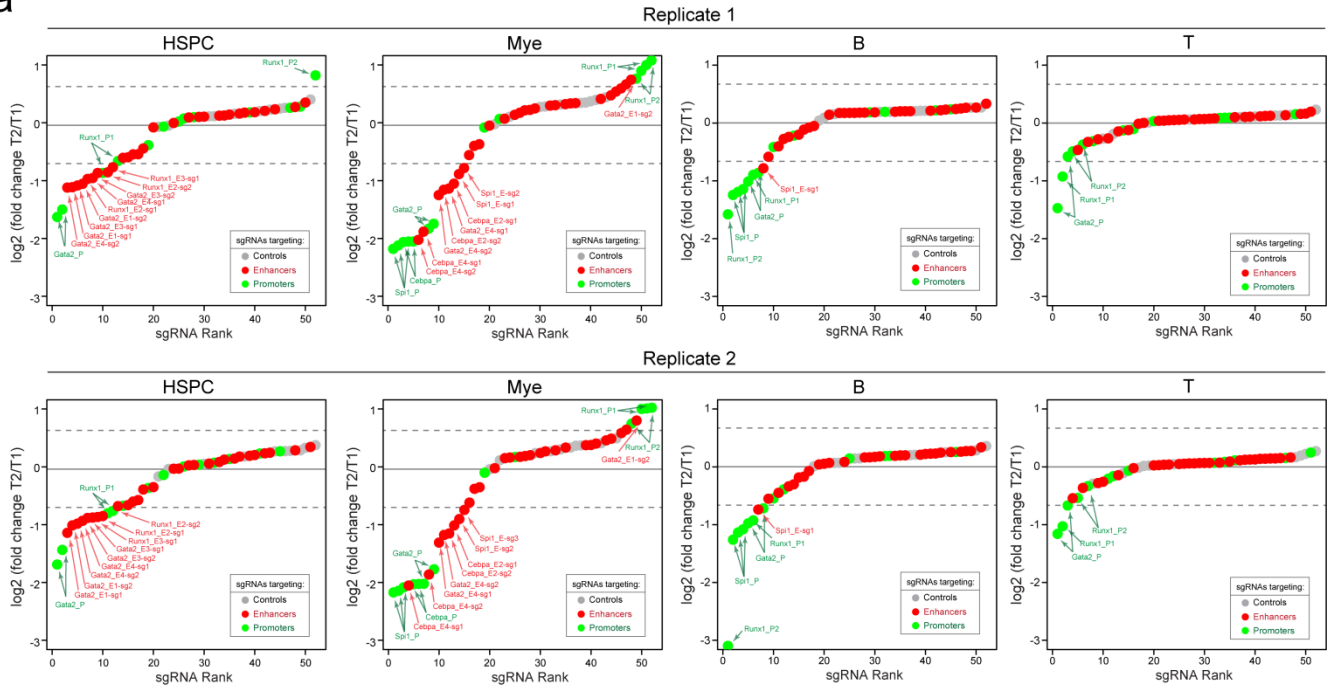
- (b) Expression of *Spi1* and the nearby gene upon enCRISPRi-mediated perturbation of CREs in HSPCs.
- (c) Expression of *Gata1* and the nearby gene upon enCRISPRi-mediated perturbation of CREs in HSPCs.
- (d) Expression of *Gata2* and the nearby gene upon enCRISPRi-mediated perturbation of CREs in HSPCs.
- (e) Expression of *Runx1* and the nearby gene upon enCRISPRi-mediated perturbation of CREs in HSPCs. Source data are provided as a Source Data file.



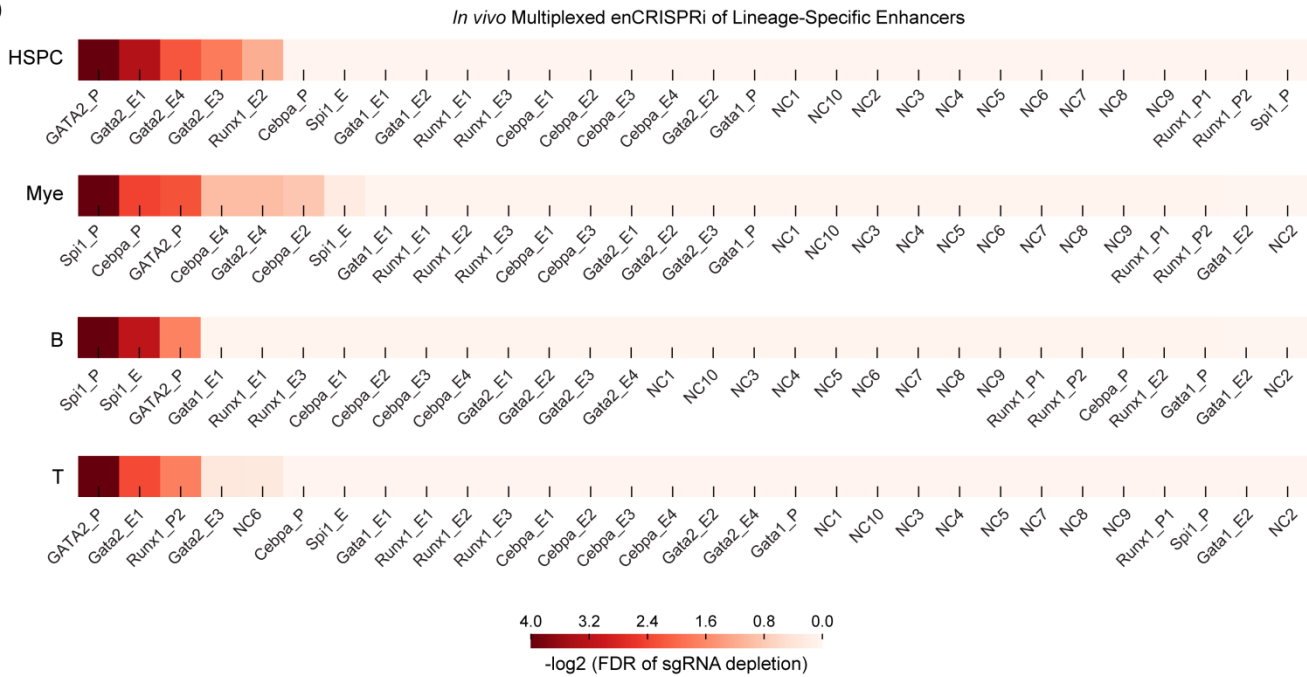
Supplementary Figure 11. Chromatin Occupancy of dCas9 in enCRISPRi-Expressing HSPCs

- (a) ChIP-qPCR analysis of dCas9 occupancy in the presence of non-targeting sgRNA (sgGal4) or sgRNAs targeting *Cebpa* promoter or enhancers in BM HSPCs. Independent sgRNAs (sg1 and sg2) for each targeted enhancer or promoter are shown. Results are mean \pm SEM and analyzed by a two-sided *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
- (b) ChIP-qPCR analysis of dCas9 occupancy for sgRNAs targeting *Gata1* promoter or enhancers in HSPCs.
- (c) ChIP-qPCR analysis of dCas9 occupancy for sgRNAs targeting *Gata2* promoter or enhancers in HSPCs.
- (d) ChIP-qPCR analysis of dCas9 occupancy for sgRNAs targeting *Runx1* promoter or enhancers in HSPCs. Source data are provided as a Source Data file.

a



b



Supplementary Figure 12. Pooled sgRNA-Mediated *In Vivo* enCRISPRi Perturbations of Promoters and Enhancers during Hematopoiesis

- (a) *In vivo* perturbation of annotated CREs for five key hematopoietic TFs. Similar to Fig. 6b, waterfall plots are shown for two independent replicate screens ($N = 15$ recipient mice per screen).
- (b) Ranking of the top depleted CREs by the $-\log_2(\text{FDR of sgRNA depletion})$ by multiplexed enCRISPRi screens. The significance (FDR) of depletion for each targeted enhancer, promoter or non-targeting control (NC) region in each cell type was calculated using the MAGECK test. P, promoter; E, enhancer; NC, non-targeting control.

SUPPLEMENTARY TABLES

Supplementary Table 1. List of Genomic Datasets Used in This Study

The name, data type, cell type, GEO accession number and citation for each dataset are shown.

Supplementary Table 2. Sequences of Primers and sgRNAs

The name and sequence of each primer or sgRNA are shown.

Supplementary Table 1. List of Genomic Datasets Used in This Study

Datasets	Data Type	Cell Type	GEO ID	Citation
ChIP-seq_Jurkat-H3K27ac	ChIP-seq	Jurkat	GSM3854054	This study
ChIP-seq_293T_enCRISPRa_sgGal4_HA_rep1	ChIP-seq	293T	GSM3854055	This study
ChIP-seq_293T_enCRISPRa_sgGal4_HA_rep2	ChIP-seq	293T	GSM3854056	This study
ChIP-seq_293T_enCRISPRa_sgHS2_HA_rep1	ChIP-seq	293T	GSM3854057	This study
ChIP-seq_293T_enCRISPRa_sgHS2_HA_rep2	ChIP-seq	293T	GSM3854058	This study
ChIP-seq_K562_enCRISPRi-LK_sgGal4_HA_rep1	ChIP-seq	K562	GSM3854059	This study
ChIP-seq_K562_enCRISPRi-LK_sgGal4_HA_rep2	ChIP-seq	K562	GSM3854060	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_HA_rep1	ChIP-seq	K562	GSM3854061	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_HA_rep2	ChIP-seq	K562	GSM3854062	This study
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ChIP-seq_K562_dCas9-KRAB_sgHS2_cas9_rep2	ChIP-seq	K562	GSM3854064	This study
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ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K4me2_rep2	ChIP-seq	K562	GSM3854120	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K4me_rep1	ChIP-seq	K562	GSM3854121	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K4me_rep2	ChIP-seq	K562	GSM3854122	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K9me3_rep1	ChIP-seq	K562	GSM3854123	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K9me3_rep2	ChIP-seq	K562	GSM3854124	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_TAL1_rep1	ChIP-seq	K562	GSM3854125	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_TAL1_rep2	ChIP-seq	K562	GSM3854126	This study
ChIP-seq_K562_sgGal4_Cas9_rep1	ChIP-seq	K562	GSM3854127	This study
ChIP-seq_K562_sgGal4_Cas9_rep2	ChIP-seq	K562	GSM3854128	This study
ChIP-seq_K562_sgGal4_CTCF_rep1	ChIP-seq	K562	GSM3854129	This study
ChIP-seq_K562_sgGal4_CTCF_rep2	ChIP-seq	K562	GSM3854130	This study
ChIP-seq_K562_sgGal4_GATA1_rep1	ChIP-seq	K562	GSM3854131	This study
ChIP-seq_K562_sgGal4_GATA1_rep2	ChIP-seq	K562	GSM3854132	This study
ChIP-seq_K562_sgGal4_H3K27ac_rep1	ChIP-seq	K562	GSM3854133	This study
ChIP-seq_K562_sgGal4_H3K27ac_rep2	ChIP-seq	K562	GSM3854134	This study
ChIP-seq_K562_sgGal4_H3K4me2_rep1	ChIP-seq	K562	GSM3854135	This study
ChIP-seq_K562_sgGal4_H3K4me2_rep2	ChIP-seq	K562	GSM3854136	This study
ChIP-seq_K562_sgGal4_H3K4me_rep1	ChIP-seq	K562	GSM3854137	This study
ChIP-seq_K562_sgGal4_H3K4me_rep2	ChIP-seq	K562	GSM3854138	This study
ChIP-seq_K562_sgGal4_H3K9me3_rep1	ChIP-seq	K562	GSM3854139	This study
ChIP-seq_K562_sgGal4_H3K9me3_rep2	ChIP-seq	K562	GSM3854140	This study
ChIP-seq_K562_sgGal4_TAL1_rep1	ChIP-seq	K562	GSM3854141	This study
ChIP-seq_K562_sgGal4_TAL1_rep2	ChIP-seq	K562	GSM3854142	This study
ChIP-seq_K562_dCas9-KRAB_sgHS2_H3K27me3_rep1	ChIP-seq	K562	GSM4190316	This study
ChIP-seq_K562_dCas9-KRAB_sgHS2_H3K27me3_rep2	ChIP-seq	K562	GSM4190317	This study
ChIP-seq_K562_dCas9-LSD1_sgHS2_H3K27me3_rep1	ChIP-seq	K562	GSM4190318	This study
ChIP-seq_K562_dCas9-LSD1_sgHS2_H3K27me3_rep2	ChIP-seq	K562	GSM4190319	This study
ChIP-seq_K562_enCRISPRi-KL_sgHS2_H3K27me3_rep1	ChIP-seq	K562	GSM4190320	This study
ChIP-seq_K562_enCRISPRi-KL_sgHS2_H3K27me3_rep2	ChIP-seq	K562	GSM4190321	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K27me3_rep1	ChIP-seq	K562	GSM4190322	This study
ChIP-seq_K562_enCRISPRi-LK_sgHS2_H3K27me3_rep2	ChIP-seq	K562	GSM4190323	This study
ChIP-seq_K562_sgGal4_H3K27me3_rep1	ChIP-seq	K562	GSM4190324	This study
ChIP-seq_K562_sgGal4_H3K27me3_rep2	ChIP-seq	K562	GSM4190325	This study
RNA-seq_K562_dCas9-KRAB_sgHS2_rep1	RNA-seq	K562	GSM3854143	This study
RNA-seq_K562_dCas9-KRAB_sgHS2_rep2	RNA-seq	K562	GSM3854144	This study
RNA-seq_K562_dCas9-LSD1_sgHS2_rep1	RNA-seq	K562	GSM3854145	This study
RNA-seq_K562_dCas9-LSD1_sgHS2_rep2	RNA-seq	K562	GSM3854146	This study
RNA-seq_K562_enCRISPRi-KL_sgHS2_rep1	RNA-seq	K562	GSM3854147	This study
RNA-seq_K562_enCRISPRi-KL_sgHS2_rep2	RNA-seq	K562	GSM3854148	This study
RNA-seq_K562_enCRISPRi-LK_sgHS2_rep1	RNA-seq	K562	GSM3854149	This study
RNA-seq_K562_enCRISPRi-LK_sgHS2_rep2	RNA-seq	K562	GSM3854150	This study
RNA-seq_K562_sgGal4_rep1	RNA-seq	K562	GSM3854151	This study
RNA-seq_K562_sgGal4_rep2	RNA-seq	K562	GSM3854152	This study
ATAC-seq_Jurkat	ATAC-seq	Jurkat	GSM3854041	This study
ATAC_seq_B	ATAC-seq	B cell	GSM1463172	Lara-Astiaso et al, Science 2014
ATAC_seq_Monocytes	ATAC-seq	Monocytes	GSM1463174	Lara-Astiaso et al, Science 2014
ATAC_seq_CD4	ATAC-seq	T cell	GSM1463175	Lara-Astiaso et al, Science 2014
ATAC_seq_Granulocytes	ATAC-seq	Granulocytes	GSM1463176	Lara-Astiaso et al, Science 2014
ATAC_seq_CD8	ATAC-seq	T cell	GSM1463178	Lara-Astiaso et al, Science 2014
ATAC_seq_LSK	ATAC-seq	LSK cell	GSM1463179	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_LT_HSC	ChIP-seq	LT-HSC	GSM1441269	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_GN	ChIP-seq	Granulocytes	GSM1441277	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_Mono	ChIP-seq	Monocytes	GSM1441278	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_B	ChIP-seq	B cell	GSM1441280	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_CD4	ChIP-seq	T cell	GSM1441281	Lara-Astiaso et al, Science 2014
ChIP-seq_H3K27Ac_CD8	ChIP-seq	T cell	GSM1441282	Lara-Astiaso et al, Science 2014

Supplementary Table 2. Sequences of Primers and sgRNAs

Name	Forward	Reverse	Application	
HS2-1-sgRNA	CACCGAATATGTCACATTCTGTCTC	AAACGAGACAGAATGTGACATATTC	sgRNA for enhancer or promoter perturbation	
HS2-2-sgRNA	CACCGGAGCTATGGGAGGTCACTAA	AAACTAGTGACCTCCCATAGTCCC		
HS2-3-sgRNA	CACCGGAAAGGTATACAGAACCAAGA	AAACTCTGGTCTGTGTACCTCTCC		
HS2-4-sgRNA	CACCGGCGCTGTAAAGCATCCTGTG	AAACGAGCAGATGCTACAGGGCC		
IL1RN-sgRNA1	CACCGTGTACTCTCTGAGGTGCTC	AAACGACACCTCAGAGAGTACAC		
IL1RN-sgRNA2	CACCGCATCAAGTCAGCCATCAGC	AAACGCTGATGCTGACTTGTATG		
OCT4-sgRNA1	CACCGACTCCACTGCACCTCCAGTCT	AAACAGACTGGAGTGCAGTGGAGTC		
OCT4-sgRNA2	CACCGACACCATTTGCCACCACCAT	AAACATGGTGGTGGCAATGGTGTCT		
MYOD-sgRNA1	CACCGGGCCACATTTCCCTTCCAG	AAACCTGGAAAGGAATGGGGCCCC		
MYOD-sgRNA2	CACCGGGGCTGGATTTGGGTTCDBAG	AAACCTGGAAACCCATTCACCTGCC		
Gata4-sgRNA	CACCGGAGCTGTAGTATAGCCGTGT	AAACTAGCAGGTAAGTACTAGTGTCT		
Chf5-sgRNA	CACCGTAGCGGTAAAGATGTAGAC	AAAGTCTACATCTTACCOCCTAC		
TAD1-sgRNA	CACCGTTGAAGTGTCCGATAGGA	AAACTCTATCGGGACTTTGTAAC		
CTCF1-sgRNA	CACCGACCAAGAAACACATCCAGAT	AAACATCTGGGATGTGTTCTGTGTC		
CTCF2-sgRNA	CACCGTTAGACAGCAATGTTATCC	AAACGGATAAACATTTGCTCTAAC		
TAD2-sgRNA	CACCGCAGCGAGACTCCGCTGGCGT	AAACACGCCGACGGAGTCTCGCTGG		
HS1-sgRNA	CACCGCAATAGTATATGAGGAGAGC	AAACGCTCTCTCATATACCTATGTC		
HS2-sgRNA	CACCGGAGCTGTAAAGCATCCTGTG	AAACTAGCAGGTAAGTACTAGTGTCT		
HS3-sgRNA	CACCGTTTGGAGGAGGAGTCTCTTA	AAACTAGAGACTCTGTCCCAAAC		
HS4-sgRNA	CACCGCCCACTCAGCAGCTATGAGA	AAACTCTATAGCTGTGAGTGGGCG		
HS5-sgRNA	CACCGTCCCCACCTTACAGGGAC	AAACCTCCCTGAAGGTGGGGGAC		
HS2+2.5k-sgRNA	CACCGCAGCTCTCTACTCAGAAT	AAACATCTGAGGTAGAGACTGTGC		
HS2+0.5k-sgRNA	CACCGGGCCACCTTGGACAGCTAGG	AAACCTAGCTGCCGGGGTGGCC		
HS2-0k-sgRNA	CACCGGAAAGTATACAGAACCAAGA	AAACTCTGGTCTGTGTAACTTCC		
HS2-2.5k-sgRNA	CACCGGAGCTGTAAAGCATCCTGTG	AAACCCAGAGTAAAGCTAGTGTCT		
HS2+0.5k-sgRNA	CACCGAATAGAGGATTTATGGGAT	AAACTCCGATAAATACCTCTATTC		
HBG-sgRNA	CACCGGCTAAACTCCACCCATGGGT	AAACCCATCGGTGGGATTTAGCC		
TAL1-Mut-sgRNA1	CACCGAGGTCACAGAAAGCGGTT	AAACACCCCTTCTTCTGTGAGCCCTC		
TAL1-Mut-sgRNA2	CACCGACAGAAAGCGGTTAGGAAA	AAACTTCTTAAACCGCTTCTGTCTC		
TAL1-WT-sgRNA1	CACCGGAATGGGGTGGGGCAACCAC	AAACGCTGGTTCGCCCAACCCATCTC		
TAL1-WT-sgRNA2	CACCGAAAGACTATCCACTCTCTCC	AAACCGAAAGTAGGGTTACCTTTTC		
Sp1-P-sg1	ccacgcCAAGTCTCTGATTTTATCGA	aaacCGATGTCAAGAGAGTGTCTC		
Sp1-P-sg2	ccacgcCCCTCTCTTGGATGATGTC	aaacCGATGTCAAGAGAGTGTCTC		
Sp1-P-sg3	ccacgcAGGCTGTGGGTGACAGCGCA	aaacTGCGTCTGACCCAGACCGCTC		
Sp1-Enh-sg1	ccacgcAGGAAGCGCCAGTCCACCAC	aaacTGGTGTACTGGCGCTCTCTC		
Sp1-Enh-sg2	ccacgcTGCCACGCCCTGGAGCGGCC	aaacGGGCGGCTCCAGCTGGGCAc		
Sp1-Enh-sg3	ccacgcAGTGCCTCTTTCCACAT	aaacGTGGAAACGGGGGAGCTC		
Cebpa-P-sg1	ccacgcGTGCTAGTGAGAGAGATGCG	aaacCGATCTCTCCACTAGCAc		
Cebpa-P-sg2	ccacgcGCTTCCAAAGCGGTGGAGTGT	aaacCACTCAGCTGTGGAAGATG		
Cebpa-Enh1-sg1	ccacgcTCTCCTCTCTTTAATAGAGC	aaacCGATTTAAAGAGAGTGTCTC		
Cebpa-Enh1-sg2	ccacgcGTGCTGCTTTCTACAC	aaacTGGTGAGAAAGCAGCAGCAc		
Cebpa-Enh2-sg1	ccacgcGTAATGCCCGGCAAGAACT	aaacAGTCTGCCGGGCAACTCac		
Cebpa-Enh2-sg2	ccacgcTCTGACGGCTCTAACCTGA	aaacTCAGGTTAGGACCCGTCAc		
Cebpa-Enh3-sg1	ccacgcCTTCTATCAGGATCAAAA	aaacTTTGATCCGTGTGAGAAc		
Cebpa-Enh3-sg2	ccacgcTGTCTTGTGTTGGGGCCAC	aaacTGCGCCCAAAACAGAAc		
Cebpa-Enh4-sg1	ccacgcAAACGGAGTACTGAGCTA	aaacTAGCTCAGTACTCCCTGTC		
Cebpa-Enh4-sg2	ccacgcCGGAGTGTGACATATATCC	aaacGAGATATTGAGAGTGTCTC		
Gata2-P-sg1	ccacgcGTCTCGGCTCCCGCCGCC	aaacGGGCGGCCGAGGCGGAGCAc		
Gata2-P-sg2	ccacgcCTGCACAGCTGGAAGCGG	aaacCGCTTCACTGTGTGAGCAGG		
Gata2-Enh1-sg1	ccacgcAGGTGGCCACGGGGTGGCG	aaacCGCCACCCCTGGGCGACCTC		
Gata2-Enh1-sg2	ccacgcCCTGCAGCAGGGGTTCTGC	aaacCGAGAACCCCTGCTCGAGGc		
Gata2-Enh2-sg1	ccacgcAACTTACGGAACCAACTTGT	aaacCAAGGTGTTTCCGTAAGTc		
Gata2-Enh2-sg2	ccacgcAGGTGGATGGCCGGCCACT	aaacAGTGGCCCGGCTCCAGCTC		
Gata2-Enh3-sg1	ccacgcTGGCGACAGAGATAGAGC	aaacCGATCTGATGCTCCAGTGTc		
Gata2-Enh3-sg2	ccacgcCCCGAGCTTATTATACAG	aaacSGCTATAATAAGACTCCGGc		
Gata2-Enh4-sg1	ccacgcACTCCGGGAGAGATCCGAA	aaacTCGATCTCTGCGGAGTc		
Gata2-Enh4-sg2	ccacgcGCTCTGAAAACCTTGCCTGTC	aaacGACCGCAAGTTTTCAGAGC		
Gata1-P-sg1	ccacgcCGGAGGACTAGAGCTTAA	aaacTTTAGGCTAGTCCCTCCGc		
Gata1-P-sg2	ccacgcAAGGGATCCCAACAAGCTGC	aaacCGAGGTTTGGATCCCTCTc		
Gata1-Enh1-sg1	ccacgcGTAGATTAAGGGAATGAGCGG	aaacCGGCTGATCCCTTATGATc		
Gata1-Enh1-sg2	ccacgcCACAGAGAGAGCGCTTCAAC	aaacCGTTCAGGCTCTCTCTCTc		
Gata1-Enh2-sg1	ccacgcACTATTGTGGGAAAGTCTC	aaacGAGACTTCCGCAAAATGTC		
Gata1-Enh2-sg2	ccacgcTAAAGTGACAGTAAAGCGA	aaacTGCTTTACTGTCACTTTACc		
Runx1-P1-sg1	ccacgcGGAATGGAATCTCCGGGTC	aaacGACCCGAGGAATCCACTCCc		
Runx1-P1-sg2	ccacgcGTAAGGACAGTCTCATTTCC	aaacGAAATGAACTGCTTTTACc		
Runx1-P2-sg1	ccacgcGAACCAAGTTGGGTAGCC	aaacGGCTACCCAACTTGTGGTCTc		
Runx1-P2-sg2	ccacgcGGTGTGTGTAAGAACTTCTT	aaacAAGAAATTTTACACAACTCCc		
Runx1-Enh1-sg1	ccacgcGCATTTCTGCTCTCCACAG	aaacCTGTGAGGAGCAATATGTC		
Runx1-Enh1-sg2	ccacgcATCCCGCCCGCAGCAGACT	aaacCACTCTGCTGGCGGGGATc		
Runx1-Enh2-sg1	ccacgcTCAAGGAGCGGAAACCCCG	aaacCGGTTTCTGCTCTTGCAC		
Runx1-Enh2-sg2	ccacgcATGCTGCAGGCTCAGATGG	aaacCACTGAGGCTGTCAAGATc		
Runx1-Enh3-sg1	ccacgcTGTGCGCAGTTCGCCACGC	aaacGCTGGGCAATGCTCGACAC		
Runx1-Enh3-sg2	ccacgcGATCTGAGTTTGTACTCTT	aaacAAAGTGAATAAAGTCAAGTc		
NC-sg1	ccacgcGAGTGTGCAAGGACCCGGT	aaacACCGGCTCCCTTGAACTCTc		
NC-sg2	ccacgcGCATTTGAGCAGACTGAGAG	aaacCTGTGAGGAGCAATATGTC		
NC-sg3	ccacgcCCAGTTCACAGATGATGAC	aaacGTGATCTGTGCTGAGTCTc		
NC-sg4	ccacgcGGTGTGCTGATGTGTCAGA	aaacCTGACACTCAGCAGCACCCc		
NC-sg5	ccacgcGCTATGCTACGAGACTATAG	aaacCTATAGTCCGTAGCATAGGC		
NC-sg6	ccacgcGTGGCTGTGACAGCGCAG	aaacCTGCGCTTACCAGGCCACCc		
NC-sg7	ccacgcGGGGCTGACCCAGATAGCA	aaacTGCTATCGTGGTCCGGCCCCc		
NC-sg8	ccacgcGAGAGGTTTAAAGAGCGCG	aaacCGCTGCTTGAACCTCTCTc		
NC-sg9	ccacgcCGCGAAGGCGAGATTAAAC	aaacCTTTAATCTGCTTCTGCTC		
NC-sg10	ccacgcCCCAACCAACAAAGAGCGG	aaacCGCTCTTTGCTTGGCTGc		
IL1RN-RT	GGATCCATGGAGGGAAGAT	TGTTCTCGCTCAGTCTAGT	RT-qPCR primers	
MYOD-RT	TCCTCTTTACCGTCTCAC	AACACCCGACTGCTGTATCC		
OCT4-RT	CGAAAGGAAAGCGAACCAGTATCGAGAAC	CGTTGTGCATGTCTGCTTGTATCCG		
HBE1-RT	GCAAGAAAGTGTGCTGACTTCC	ACCATCAGTACCAGGAG		
HBG-RT	TGGATGATCTCAAGGGCAC	TCAGTGGTATCTGGAGGACA		
HBβ-RT	CTGAGGAGAAGTCTGGCGTTA	AGCATCAGAGAGTGGACAGAT		
GAPDH-RT	AGCTCAAGATCTGCAGATGATCC	GATAGATGCTGGATGACACT		
TAL1-RT	GTCCAGCTGATCTCCGCCCC	CCGCGTCCCAAGAACCCCG		
Sp1-RT	TACCAAGTCCAAATGCATGACTAC	GAGTATCGAGAGCTGCATCTGTT		
Runx1-RT	CCAGCAAGCTGAGGAGCGGGC	TGACGGTGACCAAGAGT		
Gata1-RT	CAGAACCCGCCCTCTCATCC	TAGTGCATTGGGTGCTCTG		
Gata2-RT	GCAGAAAGCAAGGCTGCG	CAGTTGACCACTCCCGG		
Cebpa-RT	CGCGGAAAGCAACAACATC	GTCACTGGTCAACTCCAGAC		
EP300-RT	AGCCAAAGGCTCTAACTC	TCACACCATGTTAGTCCG		
ANKRD20A2-RT	CAATATAAGAAAGATGTGAAGC	TCCATCAGCACTTTCATCTTC		
ANKRD30B-RT	TGCTGTCTGTGGATTAATCTC	TTACGCCGTGACGGTGTCTTTC		
ZNF806-RT	GATAAACCCTGACCAAGACGTTG	TCCTTTGTTTCAAATCTCCAG		
LOC101928195-RT	AAGATAAGGACCATTTGCCAGG	AGGTAAAGTAAAGTCCCTGGCAC		
Cebpg-RT	AGCGGCTTACAGCAGGTTT	GGCGGATTTGCTCACTATCC		
Slc7a10-RT	GGGTTTGCCCTCTTCGATC	GACATAGGCGTATGTCGCC		
Slc39a19-RT	AGTGGCTATCTCAACTGCTT	CCCGATCTTTTGTCTGAGCA		
Glo1b-RT	CCCTTCCCGTGTCTTACTG	CCCGAGATCTTGAATAAACA		
Rpn1-RT	GCTCCACATCAGAGCCAG	CAGTTTCCACAGCACCGAGA		
1810053B23Rik-RT	CTTCACTCTGGGATGACCTTCTCTC	CCTTATGTTTGGAGGTTGGCAT		
Cebpa-P-ChIP	TTGCGCCACGATCTCTCTCCAC	TTTATAGAGGTCGGGATCGCC		
Cebpa-Enh1-ChIP	CCAAGCTGTCTTCC TGCTGCTTC	GTATAGAGAGGAAACAGATGACC		
Cebpa-Enh3-ChIP	CCGTTGAGAAAGAGGAGTCTG	GATAGCATCGGATTTGGTGTAG		
Gata2-P-ChIP	CGGGCTCTCGTGGTCTCTACTC	CCACAGATTCCTCCACACTAG		
Gata2-Enh1-ChIP	TGTTCTCAGATGCTGCAGTCC	GATAGATGTTTGGTCAAGT		
Gata2-Enh2-ChIP	CGCGACTACCACTTATGCTTCC	TCCGGCATGGCAGTTGSTGAG		
Gata2-Enh3-ChIP	CGCCAGGTTGTGTGACTTTGG	ATGGTCCCATGGGAGCGAGCA		
Gata1-P-ChIP	CCCTTGTCTTGGCTCTGTGGAG	GCATACAGTCCAAACCCACATAG		
Gata1-Enh1-ChIP	AAGGGCTCAGTAAAGAAATGAAGG	CTGATTCCTTATCTATGCTTCT		
Gata1-Enh2-ChIP	ACTGGAGTTAAGGATGGTGGGAGC	CCTAAGTGTGGGCAACCTC		
Runx1-P1-ChIP	AAAGCCTTGGTGTGATTCAGTGT	CAA TGCGCTGGGCTGGAAG		
Runx1-Enh1-ChIP	TTCTCAGAGAGTGTCTGATC	AGACCCAGCAACCTCTGGAG		
Runx1-Enh2-ChIP	CGATGAGGAGTGTGTGGAGG	GATGGAGCATCCTGTTTCTC		
Runx1-Enh3-ChIP	AGAGCAGAAAGAACTCAGAAGCGAG	AAATGCTGCTGCTGAAGATC		
rTA-A	AAAGTGCCTCTGATTTGTTAT			Mouse genotyping primers
rTA-B	GCGAAGAGTTTGTCTCAACC			
rTA-C	GGAGCGGGAGAAATGGATATG			
dCas9-KRAB-A	GCACAGATTCGGCAGATC			
dCas9-KRAB-B	CCCTCACTGTGTGACCAAGG			
dCas9-KRAB-C	GCAGAAAGCCCGCTCTGG			
TAL1-WT-ChIP	GAAGCAGAGTAAAGAGATGATAAG	TC TGCCAGGAAGTGGTTTACCTG		
TAL1-Mut-ChIP	GAAGCAGAGTAAAGAGATGATAAG	AGTAGGTTTACGTTTCTCTAAC		
gDNA-sgRNA-NGS-LCreadoutPRC1-F	AATGGACTATCATATGCTTACCCTAAGTAAAGTATTTCC	AAAAAAAGcagcagctgggtgctactggccctGGCAGACA		

sgRNApool_NGS_F1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCTAAGTAGAGctttggaaggacgaacacccg	
sgRNApool_NGS_F2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#ACACGATCctttggaaggacgaacacccg	
sgRNApool_NGS_F3	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#atCGCGCGTctttggaaggacgaacacccg	
sgRNApool_NGS_F4	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCTcgaatCATGATCctttggaaggacgaacacccg	
sgRNApool_NGS_F5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCTcgaatCGTTACCctttggaaggacgaacacccg	
sgRNApool_NGS_F6	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#acgaatTCCTTGGTctttggaaggacgaacacccg	
sgRNApool_NGS_F7	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#atcgatAACGCATTctttggaaggacgaacacccg	
sgRNApool_NGS_F8	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCTacgaatcgaatACAGGATCctttggaaggacgaacacccg	
sgRNApool_NGS_F9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCTacgaatcgaatAGGTAAGGctttggaaggacgaacacccg	
sgRNApool_NGS_F10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#AACAAATGGctttggaaggacgaacacccg	
sgRNApool_NGS_F11	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#ACTGTATCctttggaaggacgaacacccg	
sgRNApool_NGS_F12	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTCCGATCT#atAGGTCCACTctttggaaggacgaacacccg	
PMLS_POOL_NGS_R1	CAAGCAGAAAGCGGCATACGAGATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCTcgaatTCGGTGCCACTTGGCCCTGCAGACA	
PMLS_POOL_NGS_R2	CAAGCAGAAAGCGGCATACGAGATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCT#atTCGGTGCCACTTGGCCCTGCAGACA	
PMLS_POOL_NGS_R3	CAAGCAGAAAGCGGCATACGAGATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCT#atTCGGTGCCACTTGGCCCTGCAGACA	
PMLS_POOL_NGS_R4	CAAGCAGAAAGCGGCATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCTcgaatTCGGTGCCACTTGGCCCTGCAGACA	
PMLS_POOL_NGS_R5	CAAGCAGAAAGCGGCATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCTcgaatTCGGTGCCACTTGGCCCTGCAGACA	
PMLS_POOL_NGS_R6	CAAGCAGAAAGCGGCATACGAGATGCTGACTGGAGTTTCAGACGTGTGCTCTTCCGATCTcgaatTCGGTGCCACTTGGCCCTGCAGACA	

sgRNA pooled screen primers with barcodes and staggers