Supplementary Notes

Summary of previously published reports using aTFs to activate enhancers or other distantly located sequences

aTF type	Number of gRNAs per target locus	Name of Enhancer/distal sequence	Distance from TSS to Enhancer/distal sequence	Target gene name	Cell type	native expression of target genes	Mean fold-activation by targeting enhancers (mRNA)	Mean fold-activation by targeting promoters (mRNA)	Chromatin status (Target enhancer)	Chromatin status (Target gene promoter)	Reference (paper)
dCas9-p300	4	MYOD1 CE enhancer	20Kb	MYOD1	HEK293T	Lowly expressed	~4	~50	Closed, no H3K27Ac	Open, no H3K27Ac	PMID: 25849900 (Hilton et al.)
	4	MYOD1 DRR enhancer	5Kb	MYOD1	HEK293T	Lowly expressed	~18		Open, no H3K27Ac	Open, no H3K27Ac	
	6	Oct 4 DE enhancer	2.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	~8	~30	Closed, no H3K27Ac	Closed, no H3K27Ac	
	6	Oct 4 PE enhancer	1.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	~30		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	HS2	5Kb, 30Kb, 46Kb, 50Kb	HBE, HBG, HBD, HBB	HEK293T	Lowly expressed	15.5, 269, 7.5, 1		Open, no H3K27Ac	Closed, no H3K27Ac	
	1	MYOD1 CE enhancer	20Kb	MYOD1	HEK293T	Lowly expressed	4 individual gRNAs showed less than 5-fold activation		Closed, no H3K27Ac	Open, no H3K27Ac	
	1	MYOD1 DRR enhancer	5Kb	MYOD1	HEK293T	Lowly expressed	3 individual gRNAs showed less than 5-fold activation and one gRNA showed ~15-fold activation		Open, no H3K27Ac	Open, no H3K27Ac	
	1	Oct 4 DE enhancer	2.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	All 6 individual gRNAs showed less than 3-fold activation		Closed, no H3K27Ac	Closed, no H3K27Ac	
	1	Oct 4 PE enhancer	1.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	5 gRNAs showed less than 10-fold activation and one gRNA showed ~20 fold		Closed, no H3K27Ac	Closed, no H3K27Ac	
	1	HS2	10Kb, 30Kb	HBE, HBG	HEK293T	Lowly expressed	All 4 individual gRNAs showed less than 5-fold activation for HBE.		Open, no H3K27Ac	Closed, no H3K27Ac	
dCas9-VP64	4	MYOD1 CE enhancer	20Kb	MYOD1	HEK293T	Lowly expressed	None	~25	Closed, no H3K27Ac	Open, no H3K27Ac	
	4	MYOD1 DRR enhancer	5Kb	MYOD1	HEK293T	Lowly expressed	None		Open, no H3K27Ac	Open, no H3K27Ac	
	6	Oct 4 DE enhancer	2.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	None	~18	Closed, no H3K27Ac	Closed, no H3K27Ac	
	6	Oct 4 PE enhancer	1.25Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	None		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	HS2	5Kb, 30Kb, 46Kb, 50Kb	HBE, HBG, HBD, HBB	HEK293T	Lowly expressed	None		Open, no H3K27Ac	Closed, no H3K27Ac	
dCas9-VP64	1	Oct4 enhancer	2.4Kb	POU5F1(Oct4)	Mouse embryonic fibroblasts		~3				PMID: 25223790 (Gao et al.)
	1	Nanog enhancer	5Kb	Nanog	Mouse embryonic fibroblasts		~2.5				
dCas9-VP160	1	Oct4 enhancer	2.4Kb	POU5F1(Oct4)	Mouse embryonic fibroblasts		~3				
	1	Nanog enhancer	5Kb	Nanog	Mouse embryonic fibroblasts		~2.5				
TALE-VP64	1	Oct4 enhancer	2.4Kb	POU5F1(Oct4)	Mouse embryonic fibroblasts		~5				
	1	Nanog enhancer	5Kb	Nanog	Mouse embryonic fibroblasts		~4				
dCas9-p300	4	iE1	4Kb	IL1RN	HEK293T	Lowly expressed	~80	>10,000	Closed, no H3K27Ac	Closed, no H3K27Ac	PMID: 30098338 (Kuscu et al.)
	4	iE2	8Kb	IL1RN	HEK293T	Lowly expressed	~30		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE3	24Kb	IL1RN	HEK293T	Lowly expressed	~3		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE1	4Kb	IL1RN	K562	Not expressed	~11	~30	Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE2	8Kb	IL1RN	K562	Not expressed	~45		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE3	24Kb	IL1RN	K562	Not expressed	~22		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE1	4Kb	MyoD1	HEK293T	Lowly expressed	~8	~40	Closed, no H3K27Ac	Open, no H3K27Ac	
	4	iE2	8Kb	MyoD1	HEK293T	Lowly expressed	~6		Closed, no H3K27Ac	Open, no H3K27Ac	
	4	iE3	24Kb	MyoD1	HEK293T	Lowly expressed	~4		Closed, no H3K27Ac	Open, no H3K27Ac	
	4	iE1	4Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	~5	~100	Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE2	8Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	~3		Closed, no H3K27Ac	Closed, no H3K27Ac	
	4	iE3	24Kb	POU5F1(Oct4)	HEK293T	Lowly expressed	~3		Closed, no H3K27Ac	Closed, no H3K27Ac	
dCas9-VP64 + MCP-p300 (enCRISPRa-VP) or dCas9-p300 + MCP-VP64 (enCRISPRa-PV)	the sgRNA with two MS2 hairpins	MYOD1 DRR enhancer	5Kb	MYOD1	HEK293T	Lowly expressed	~30		Open,. No H3K27Ac	Open, no H3K27Ac	PMID: 31980609 (Li et al.)
		HS2	5Kb	HBE	HEK293T	Lowly expressed	23.6		Open, no H3K27Ac	Closed, no H3K27Ac	
		HS2	30Kb	HBB	HEK293T	Lowly expressed	40.6		Open, no H3K27Ac	Closed, no H3K27Ac	
		HS2	50Kb	HBG	HEK293T	Lowly expressed	13		Open, no H3K27Ac	Closed, no H3K27Ac	
dCas9-p300	gRNA library targeting the 4 Mb region around HER2		various	HER2	HEK293T	Expressed	less than ~3	~7	Open	Open, H3K27Ac positive	PMID: 28369033 (Klann et al.)
dCas9-VP64	1	regions identified from H3K27Ac HICHIP		CD69	Jurkat	Lowly expressed	~15		Open, H3K27Ac positive	Open, H3K27Ac positive	PMID: 28945252 (Mumbach et al.)
dCas9-VP64	gRNA libraries targeting 135Kb and 178 Kb at the IL2RA and CD69 loci, respectively		various	IL2RA or CD69	Jurkat	not expressed and lowly expressed, respectively	*N/A		Open, H3K27Ac positive	Open, H3K27Ac positive	PMID: 28854172 (Simeonov et al.)

*protein expression measured by flow cytometry	
	Source
HEK293T RNA	GSM3443142
Jurkat RNA expression	GSM923424
K562 RNA expression	GSM758577
HEK293T Dnase-seq	GSM1008573
K562 Dnase-seq	GSM816655
HEK293T H3K27Ac	GSM1249889
K562 H3K27Ac	SM733656

Impact of heterotopic enhancer activation on promoters of *IL2RA*, *CD69, and MYOD1*

Previously published work has shown that targeting of more than one aTF to a promoter can yield synergistic increases in human gene transcription(*1*). Therefore, we compared the magnitude of synergistic activation resulting from concurrent enhancer-promoter aTF targeting with that observed by targeting two aTFs to only the promoter. As expected, we found that co-expression of various pairs of promoter-targeted gRNAs with the bi-partite p65 aTF led to synergistic increases in gene transcription at the *IL2RA*, *CD69, and MYOD1* promoters (**Supplementary Figs. 1a -1b**). For all three genes, the ranges of fold-activation values observed with aTFs targeted using two promoter gRNAs were more effective in at least half the cases we tested than those obtained using two gRNAs concurrently targeted to enhancer and promoter sites (**Supplementary Fig. 1b**). However, addition of a third enhancer-targeted gRNA to the two promoter-targeted gRNAs led to even greater increases in gene transcription, with mean activation values reaching as high as 1176-fold, 429-fold, and 894-fold for the *IL2RA*, *CD69, and MYOD1* genes, respectively (**Supplementary Fig. 1b**). The impact of adding an enhancer-bound aTF was strongest for the *IL2RA* and *MYOD1* genes but still measurable and significant for the *CD69* gene (**Supplementary Fig. 1b**). Interestingly, for the *IL2RA* and *CD69* genes, the magnitude of the effect of adding an enhancer-bound aTF on gene activation was inversely correlated with the magnitude of the fold-activation induced by the promoter-bound aTF(s) (**Supplementary Fig. 1c**).

Transcriptome-wide RNA-seq specificity analyses with activation of promoter, enhancer, or both by bi-partite p65 aTF at *CD69*, I*L2RA*, and *MYOD1* loci in HEK293 cells

To evaluate the specificity of transcriptional activation with the simultaneous enhancer-promoter targeting strategy, we performed transcriptome-wide analysis using RNA-seq. For these experiments, we chose the best performing combination of enhancer/promoter gRNAs for the *IL2RA*, *CD69* and *MYOD1* genes in HEK293 cells (**Figs. 1c - 1e**) and performed RNA-seq in HEK293 cells in which we

expressed the bi-partite p65 aTF with the promoter gRNA only, the enhancer gRNA only, and both gRNAs. For all three target genes, we did not observe greater than two-fold activation of any neighboring expressed genes (arbitrarily defined as all those within +/-200 kb of the target gene TSS with an FPKM value >1) with the promoter- or enhancer-targeted gRNAs each alone or together (**Supplementary Table 5**). At a transcriptome-wide level, we did not observe significant activation of any off-target gene with any of the gRNAs targeted to activate the *CD69* gene but did observe significant repression of nine genes (presumably due to either an indirect downstream effect of activating *CD69* or a direct repressive effect of the aTF) (**Supplementary Fig. 2a, Supplementary Table 6**). For the *IL2RA* gene, we observed significant off-target activation of 12 genes and repression of 7 genes with the combined promoter- and enhancer-targeted gRNAs (**Supplementary Fig. 2b**). Six of the 12 upregulated genes were also significantly activated with the promoter-targeted gRNA alone as off-targets, making it difficult to distinguish whether these changes were due to a direct aTF off-target effect or an indirect downstream consequence of activating the *IL2RA* gene; two of the 12 genes were significantly activated with the enhancer-targeted gRNA alone, consistent with an off-target activation effect due to this gRNA (**Supplementary Fig. 2b, Supplementary Table 6**). For some of these off-target genes, we were able to identify potential binding sites with perfectly matched sequences within the 7 bp of seed region (positions 1 to 7) of the gRNA spacer, previously shown to be sufficient for binding by catalytically inactive SpCas9(*2*) (**Supplementary Fig. 2b, Supplementary Table 7**). For the *MYOD1* gene, we observed a very large number of off-target genes (126) transcriptome-wide that were significantly activated and 15 genes that were significantly repressed with individual gRNAs or the combined promoter- and enhancer-targeted gRNAs (**Supplementary Fig. 2c, Supplementary Table 6**). Although some of these off-target gene expression increases may be due to off-target binding of the aTF in conjunction with either the promoter- or enhancer-targeted gRNA (**Supplementary Table 7**), the higher number of genes altered in this case might also be due to indirect effects of increased expression of *MYOD1*, which encodes a transcription factor that can upregulate (or downregulate) muscle-specific gene expression(*3*). Consistent with this, Gene Ontology (**GO**) analysis (http://http://pantherdb.org) revealed that many of the off-target genes we identified are significantly enriched in muscle contraction processes or are previously known targets

of MYOD1-mediated regulation (**Supplementary Fig. 2c**; **Supplementary Table 8**). Taken together, we conclude that the transcriptome-wide specificity of activation with concurrent enhancer-promoter aTF targeting is dependent on the design of the gRNAs and the functional effects of the target genes themselves.

Using orthogonal dCas9- and dCas12a-based fusions to test enhancer-promoter targeting with direct fusion VP64 aTFs and aTFs with heterologous activation domains or no activation domain

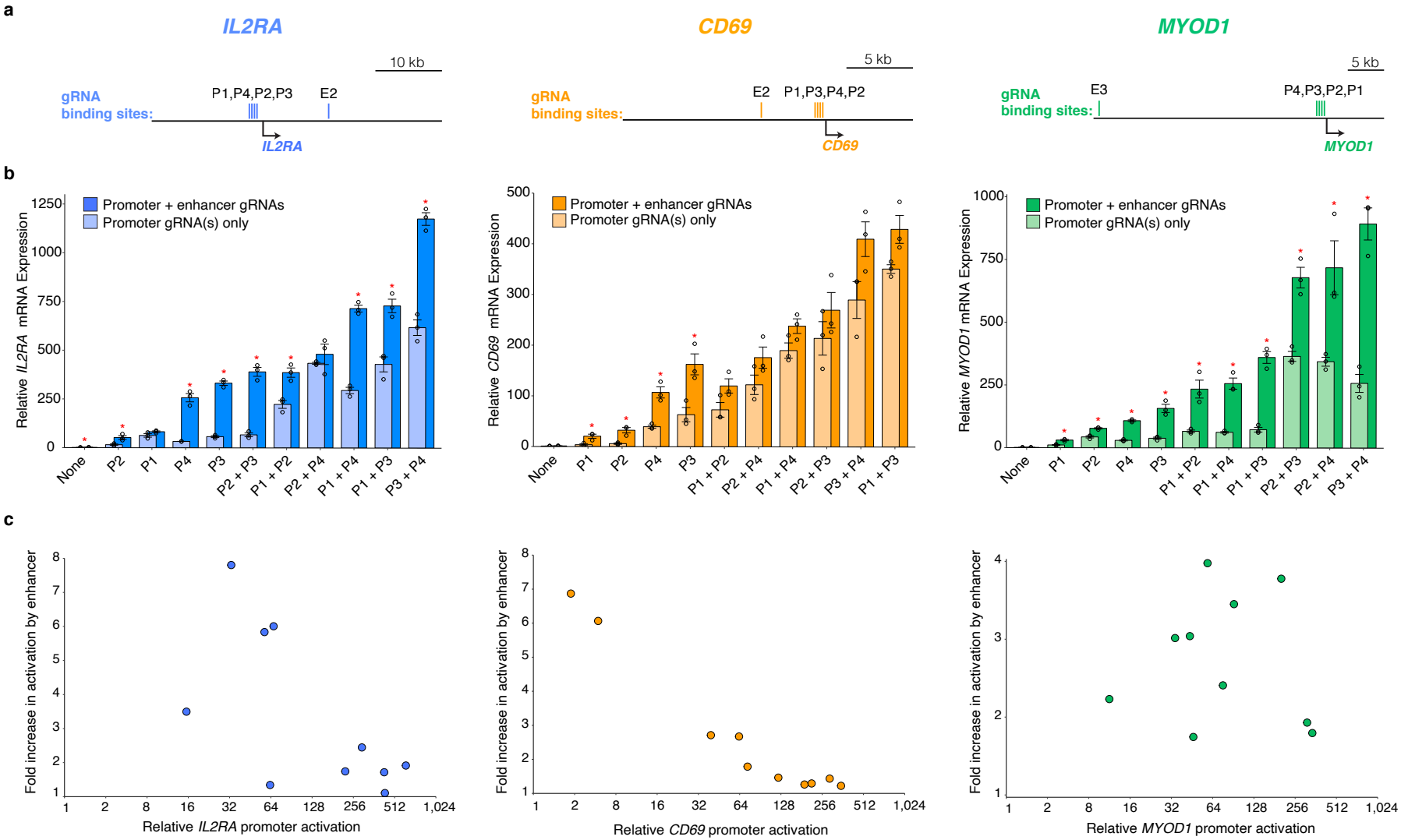
Because the direct VP64 fusion aTF did not work in HEK293 cells, we also tested whether pairing it with an aTF harboring a different activation domain might enable it to activate the *HBB* gene from the LCR. To do this, we constructed a series of aTFs based on catalytically inactive Cas12a nuclease from *Lachnospiraceae bacterium* (**dCas12a**)(*4*) (instead of dCas9) that harbor various activation domains. We targeted these dCas12a-based aTFs to the LCR HS2 enhancer sequence and a dCas9-VP64 aTF to the *HBB* promoter and *vice versa* (**Supplementary Fig. 3**). However, neither configuration resulted in significantly increased activation of the *HBB* gene relative to aTF targeted to only the promoter in HEK293 cells (**Supplementary Fig. 3**). We also used this dCas9/dCas12a system to demonstrate that promoter binding of dCas9 or dCas12a only (i.e., lacking an activation domain) is insufficient to result in robust enhancer activation by a direct fusion VPR aTF in HEK293 cells (**Supplementary Fig. 4**).

Testing activation when concurrently targeting aTFs to a target gene promoter and distal regions within or outside the same TAD

We tested a series of gRNAs for their abilities to activate either the *MYOD1* or *HBB* promoter in K562 cells, with these gRNAs targeting various distally located sites positioned in the same TAD as the target gene or in neighboring TADs (**Supplementary Figs. 6a and 6c**) (using TAD boundaries defined by previously published studies(*5*)). For these experiments, we co-expressed each of these different

gRNAs together with a promoter-targeted gRNA and the bi-partite p65 aTF. Surprisingly, we obtained different results for the two genes: for *MYOD1*, we were able to induce robust activation only when targeting the known CE enhancer site within the same TAD (**Supplementary Fig. 6b**); by contrast, for *HBB*, we observed robust activation from sites both within the same TAD as well as in neighboring TADs (**Supplementary Fig. 6d)**. Interestingly, while our analysis of previously published Hi-C data from K562 cells(*5*) revealed similar normalized 3D interaction frequencies around the *HBB* and *MYOD1* loci (**Supplementary Fig. 6e-6f**), we noted that the two loci appear to be located in spatially distinct nuclear subcompartments. An examination of the Spatial Position Inference of the Nuclear genome (**SPIN**) states of the two loci(*6*) found that the *HBB* TSS is located within a ~600 Kb interior active compartment of the nucleus, while *MYOD1* TSS exists within ~700 Kb interior repressed compartment (**Supplementary Figs. 6a and 6c**), suggesting that perhaps the *HBB* locus might be predisposed to have higher transcriptional activity than the *MYOD1* locus.

SUPPLEMENTARY FIGURES

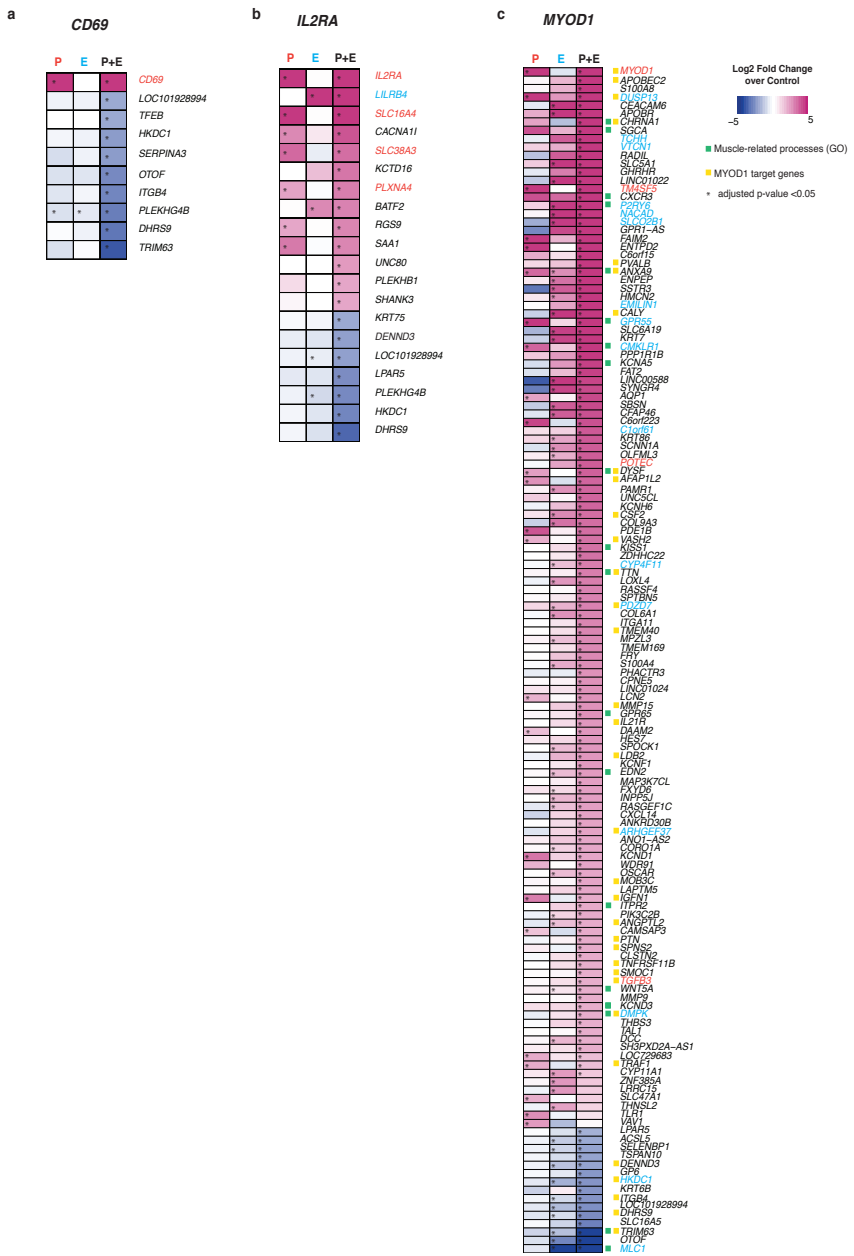


Supplementary Figure 1. Impact of heterotopic enhancer activation on promoters of *IL2RA*, *CD69, and MYOD1*.

a, Schematics illustrating genomic locations of promoter-targeting gRNAs for *IL2RA*, *CD69*, and *MYOD1* and the optimal enhancer-targeting gRNA for each gene in HEK293 cells (from **Fig. 1c-e**).

b, mRNA expression levels of the endogenous *IL2RA*, *CD69* and *MYOD1* genes in HEK293 cells determined by RT-qPCR in the presence of the bi-partite p65 aTF and various combinations of the promoter- and enhancer-targeting gRNAs shown in **a**. A non-targeting gRNA was used for the control samples (labelled as None). Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m. * indicates significantly different from their matched sample lacking the enhancer-targeting gRNA ($p<0.05$) (Student's t-test, two-tailed test assuming equal variance). The exact p-values are in Supplementary Data.

c, Increase in gene expression by heterotopic enhancer activation of the activated promoters of *IL2RA*, *CD69* and *MYOD1* genes. X-axis: the levels of promoter activation (fold-change in gene expression compared to the negative control with non-targeting gRNA) of target genes by bi-partite p65 aTF and gRNAs that target promoters only. Y-axis: the effect of heterotopic enhancer activation by bi-partite p65 aTF (fold-difference in gene expression between promoter with enhancer activation and promoter activation alone).


a
CD69
P
E
P+E
CD69
LOC101928994
TFEB
HKDC1
SERPINA3
OTOF
ITGB4
PLEKHG4B
DHRS9
TRIM63
b
IL2RA
P
E
P+E
IL2RA
LILRB4
SLC16A4
CACNA1I
SLC38A3
KCTD16
PLXNA4
BATF2
RGS9
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APOBR
CHRNA1
SGCA
TCF4
LYZN1
RADIL
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SLC22R1
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FAIM2
ENTPD2
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PVALB
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EMILIN1
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FAT2
LINC00588
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GPR68
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DAAM2
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SPOCK1
LDB2
KCNF1
EDN2
MAP3K7CL
RYDR
INPP5J
RASGEF1C
CXCL14
ANKRD30B
ARHGEF37
ANO1-AS2
CORO1A
KCND1
WDR91
OSCAR
MOB3C
LAPTM5
IGFN1
ITPR2
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ANGPTL2
CAMSAP3
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SELENBP1
TSPAN10
DENND3
GP6
HKDC1
KRT6B
ITGB4
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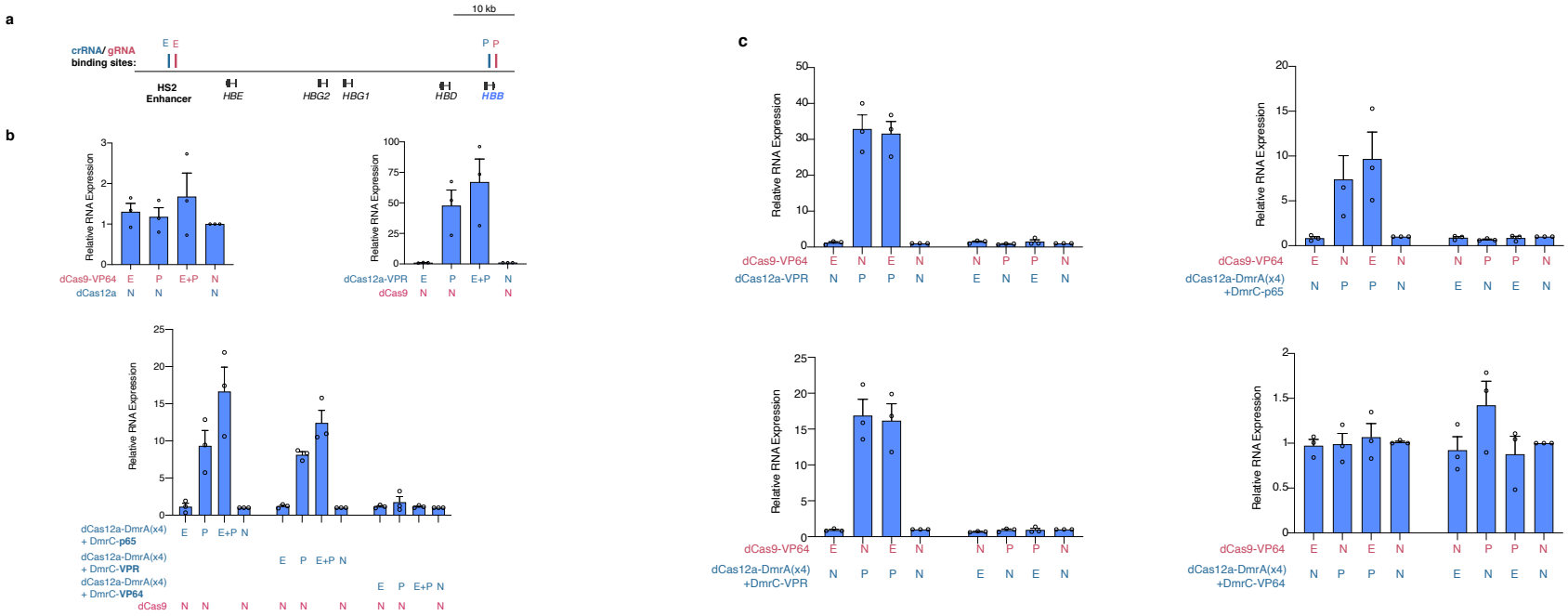
DHRS9
SLC16A5
TRIM63
OTOF
MLC1
Log2 Fold Change over Control
−5
5
Muscle-related processes (GO)
MYOD1 target genes
adjusted p-value <0.05

Supplementary Figure 2. Transcriptomic analyses after activation of promoter, enhancer, or both by bi-partite p65 aTF at *CD69*, I*L2RA*, and *MYOD1* loci in HEK293 cells.

a-c, Heat maps of the genes that were differentially expressed relative to control (log2 fold change) using the bi-partite p65 aTF (P: promoter gRNA transfected; E: enhancer gRNA transfected; E + P: enhancer and promoter gRNA co-transfected), ranked by fold change of E + P over control. All the genes that were significantly up- or down-regulated (adjusted p-value < 0.05, p-values (attained by the Wald test) are corrected for multiple testing using the Benjamini and Hochberg method) by 4-fold or more are listed. Red-colored and blue-colored genes have perfectly matched sequences within the 7 bp of seed region (positions 1 to 7) of the promoter-targeted gRNA spacer and enhancer-targeted gRNAs spacer, respectively.

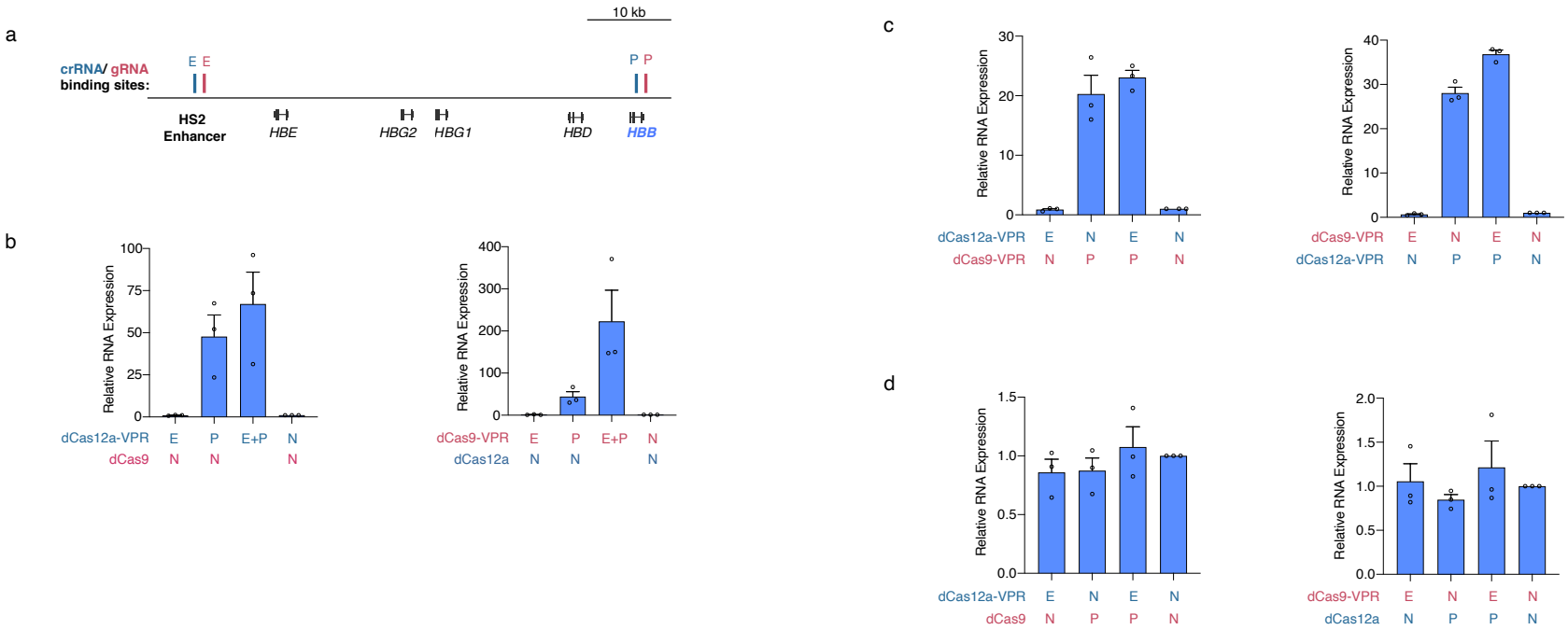


Supplementary Figure 3. Pairing dCas9-VP64 with dCas12a-based aTFs for concurrent targeting of LCR and *HBB* promoter in HEK293 cells.

a, Genomic locations of gRNAs or crRNAs targeting the LCR HS2 region (E) and the promoter regions of *HBB* (P).

b, mRNA expression levels of the endogenous *HBB* genes in HEK293 cells in the presence of 1) dCas9-VP64 and dCas12a (serves as a loading control) with Cas9 gRNAs targeting *HBB* promoter, LCR HS2 enhancer, both or none (top left panel) dCas12a-based aTFs and dCas9 (serves as a loading control) with Cas12a crRNAs targeting *HBB* promoter, LCR HS2 enhancer, both or none (top right and bottom panel). N indicates non-targeting gRNAs or crRNA. Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

c, mRNA expression levels of the endogenous *HBB* genes when dCas9-VP64 is targeted to HS2 LCR enhancer and dCas12a-based aTF is targeted to *HBB* promoter or vice versa. N indicates non-targeting gRNAs or crRNA. Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.



Supplementary Figure 4. Lack of heterotopic enhancer activation when promoter is targeted by aTF without activation domains

a, Genomic locations of gRNAs or crRNAs targeting the LCR HS2 region (E) and the promoter regions of *HBB* (P).

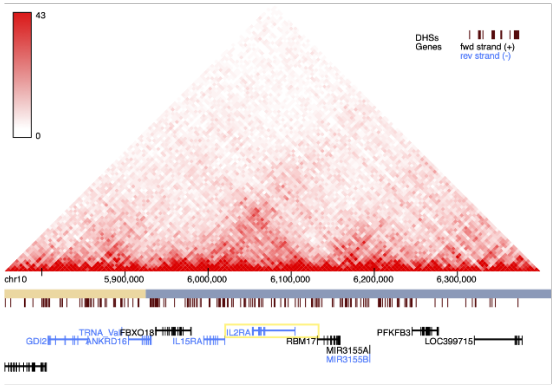
b, mRNA expression levels of the endogenous *HBB* genes in HEK293 cells in the presence of 1) dCas12a-VPR and dCas9 with Cas12a crRNAs targeting *HBB* promoter, LCR HS2 enhancer, both or none (left panel); 2) dCas9-VPR and dCas9 with Cas9 gRNAs targeting *HBB* promoter, LCR HS2 enhancer, both or none (right panel). Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

c, mRNA expression levels of the endogenous *HBB* genes when dCas12a-VPR is targeted to LCR HS2 enhancer and dCas9-VPR is targeted to *HBB* promoter or vice versa. Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

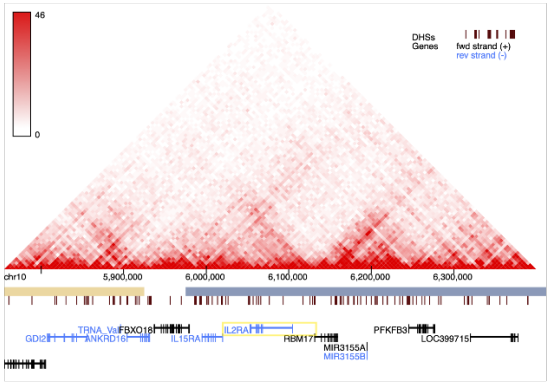
d, mRNA expression levels of the endogenous *HBB* genes 1) when dCas12a-VPR is targeted to enhancer and dCas9 is targeted to *HBB* promoter and 2) when dCas9-VPR is targeted to enhancer and dCas12a is targeted to *HBB* promoter. Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

IL2RA

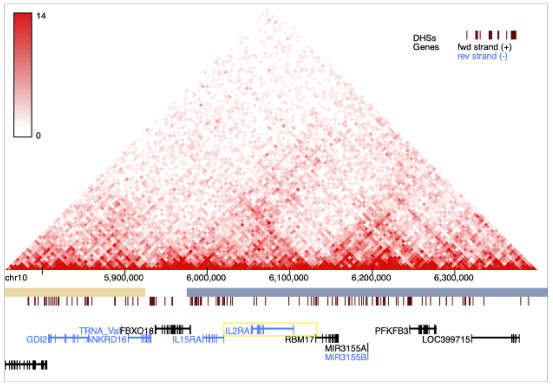
K562



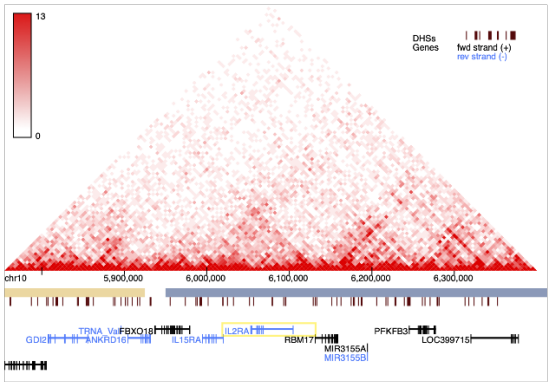
IMR90



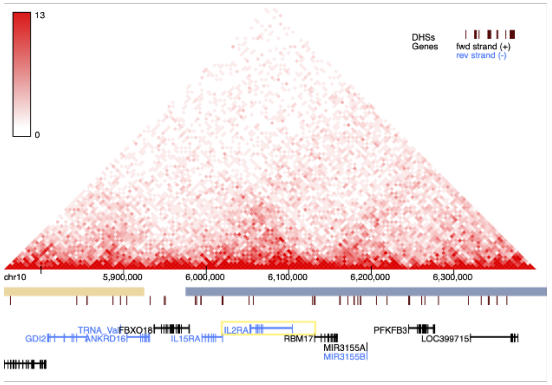
HUVEC



HMEC

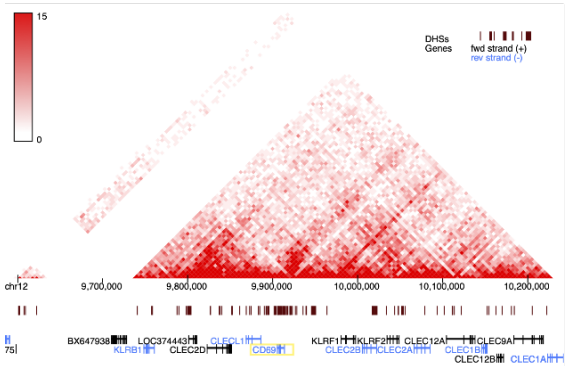


NHEK

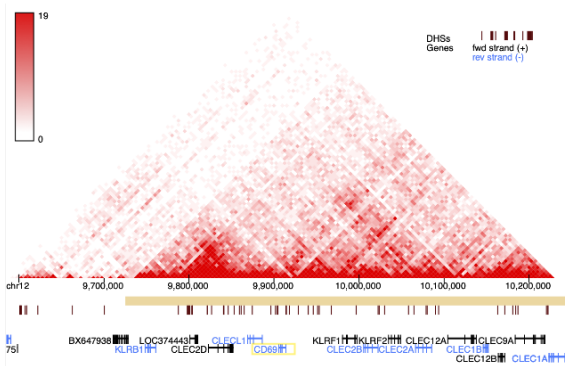


CD69

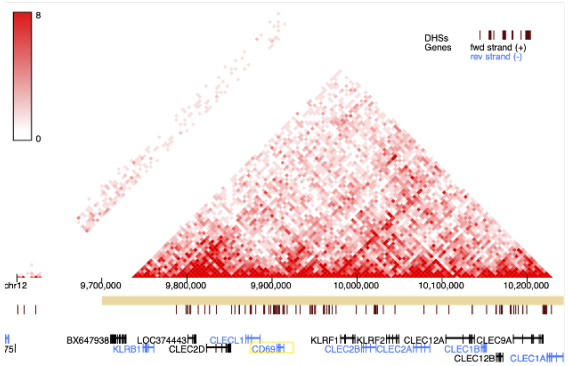
K562



IMR90



HUVEC



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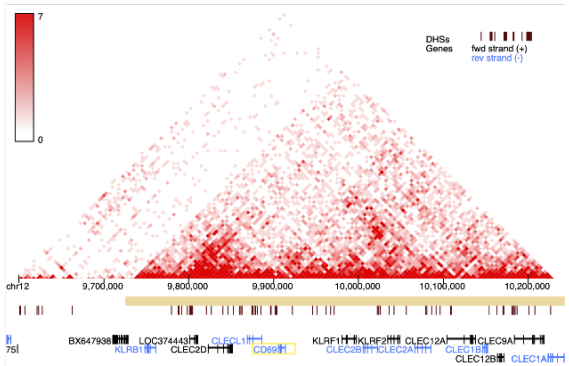
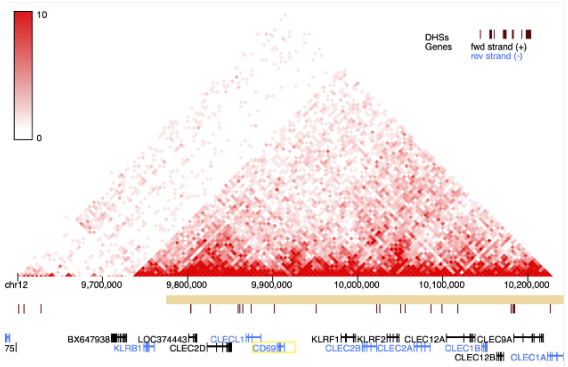
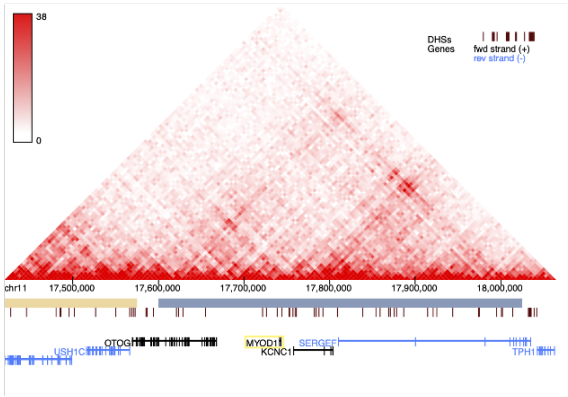


NHEK



MYOD1

K562



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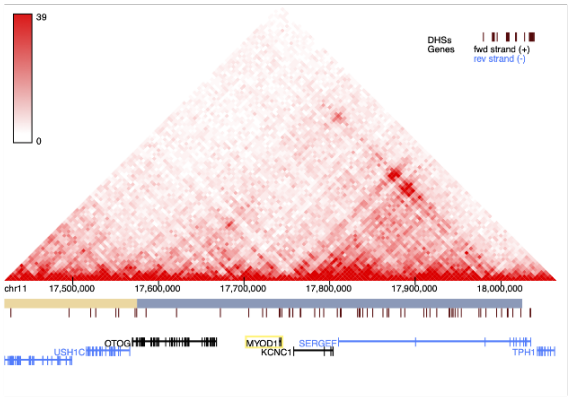

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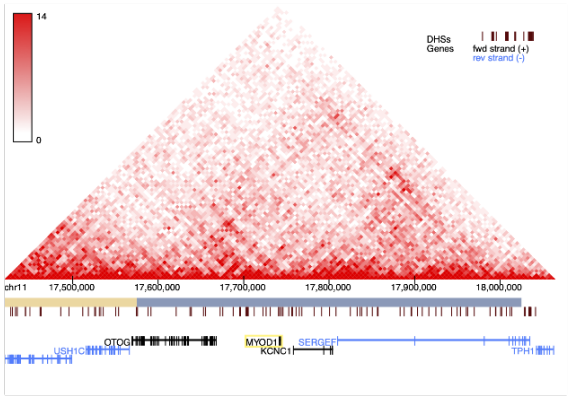

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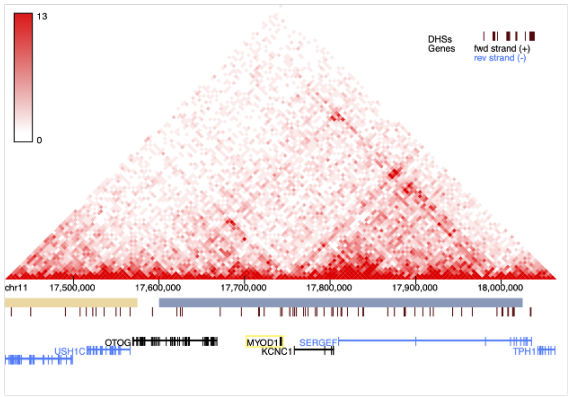

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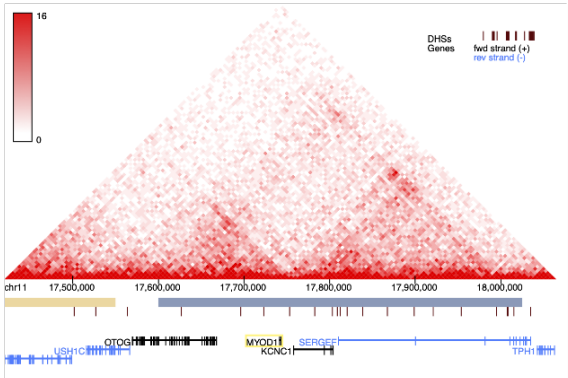
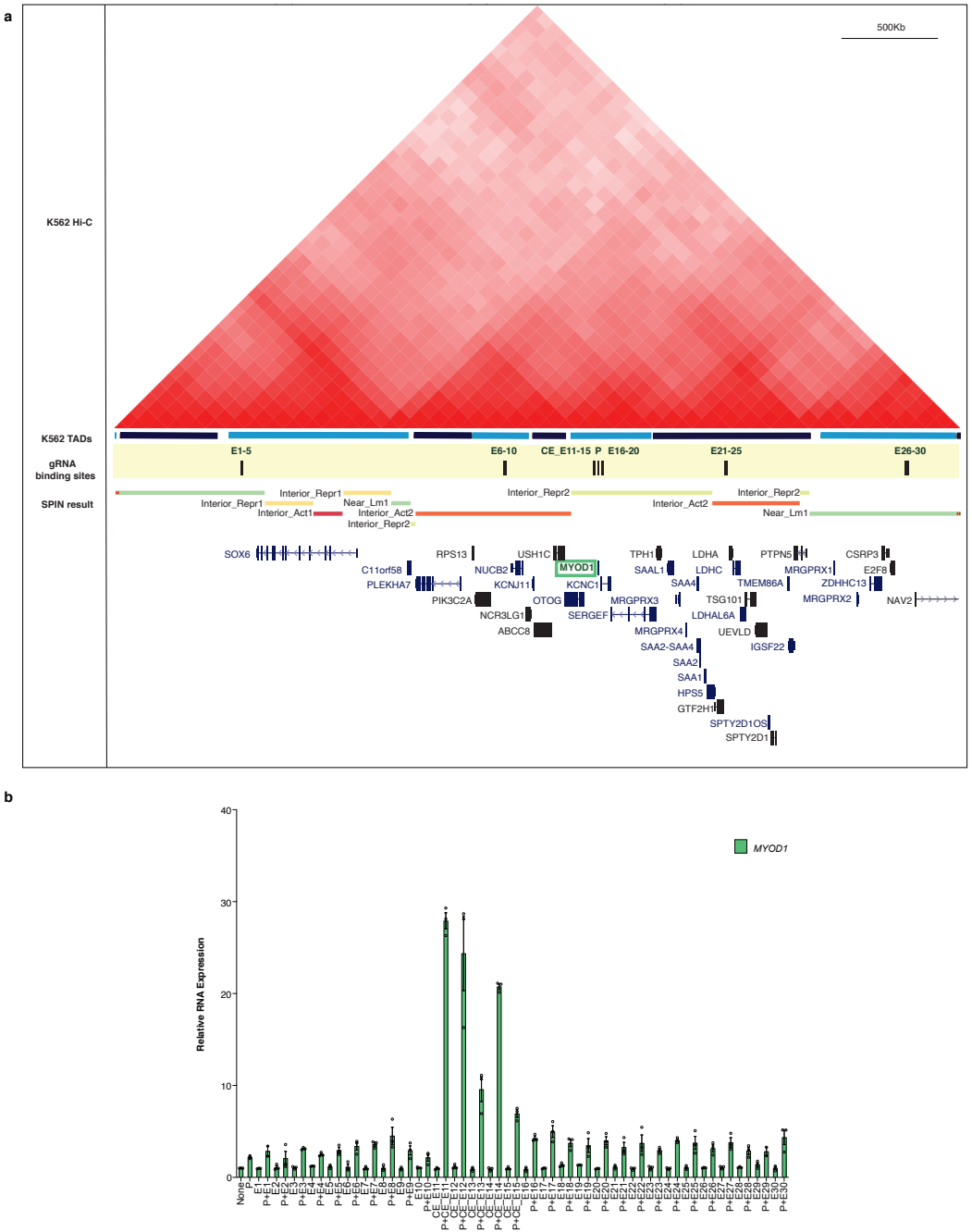


Supplementary Figure 5. TADs centered on target loci from different cell types.

The individual TADs where *IL2RA, CD69, MYOD1, and APOC3* loci are located, are maintained across various cell types for which these data are available, including the K562 cell line used in this study. The triangle heat maps for TADs were obtained from 3D genome browser.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K562 Hi-C
500Kb
K562 TADs
gRNA binding sites
SPIN result
E1-5
E6-10
CE_E11-15
P
E16-20
E21-25
E26-30
Interior_Repr1
Interior_Repr2
Interior_Act1
Interior_Act2
Near_Lm1
SOX6
RPS13
USH1C
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LDHA
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CSRP3
C11orf58
NUCB2
MYOD1
SAAL1
LDHC
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PLEKHA7
KCNJ11
KCNC1
SAA4
TMEM86A
ZDHHC13
PIK3C2A
OTOG
MRGPRX3
TSG101
MRGPRX2
NAV2
NCR3LG1
SERGEF
LDHAL6A
ABCC8
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UEVLD
SAA2-SAA4
IGSF22
SAA2
SAA1
HPS5
GTF2H1
SPTY2D1OS
SPTY2D1
b
MYOD1
Relative RNA Expression
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30
20
10
0
None
P
E1
P+E1
E2
P+E2
E3
P+E3
E4
P+E4
E5
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E6
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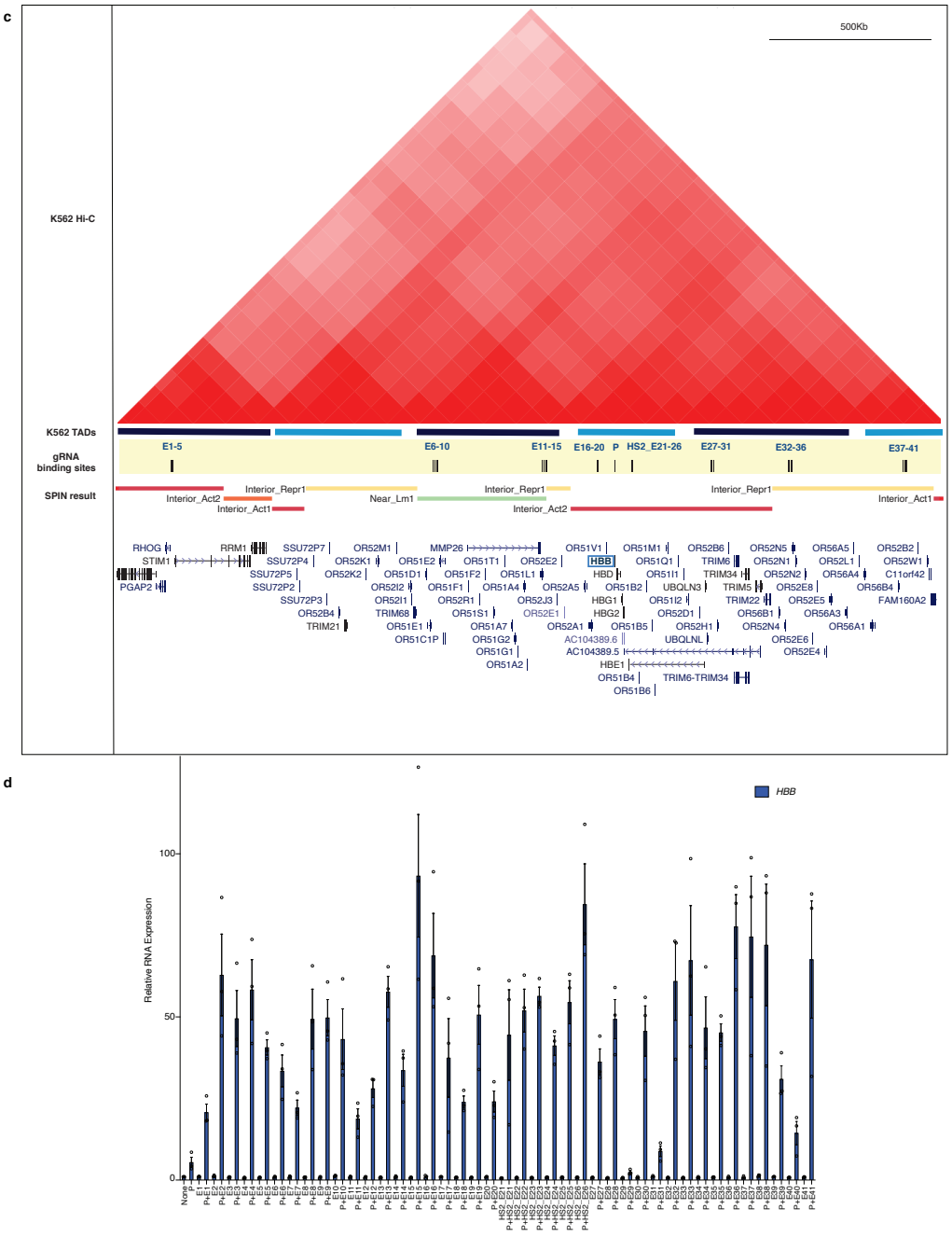
c
K562 Hi-C
500Kb
K562 TADs
gRNA binding sites
E1-5
E6-10
E11-15
E16-20
P
HS2_E21-26
E27-31
E32-36
E37-41
SPIN result
Interior_Act2
Interior_Repr1
Interior_Act1
Near_Lm1
Interior_Repr1
Interior_Act2
Interior_Repr1
Interior_Act1
RHOG
STIM1
PGAP2
RRM1
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SSU72P4
SSU72P5
SSU72P2
SSU72P3
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TRIM21
OR52M1
OR52K1
OR52K2
OR51D1
OR52I2
OR52I1
TRIM68
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OR51F2
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OR52R1
OR51S1
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OR51G2
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OR51A2
OR51V1
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OR51L1
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AC104389.5
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HBG1
HBG2
OR51B5
TRIM6-TRIM34
HBB
HBD
OR51B2
OR51Q1
OR51I2
OR51I1
OR52D1
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OR52N2
TRIM22
TRIM5
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OR52N4
OR52E6
OR52E4
OR56A5
OR52L1
OR56A4
OR52E5
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OR56A3
OR56A1
OR52B2
OR52W1
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FAM160A2
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d
HBB
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50
0
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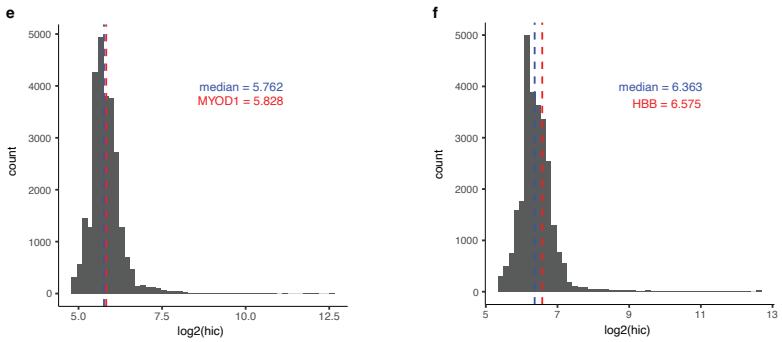

Supplementary Figure 6. Comparison of heterotopic gene activation when targeting aTFs to known enhancers or other regions inside and outside of the TAD of the target gene.

a, A Hi-C interaction map of chromosome 11 in K562 cells at 100 Kb resolution from Nucleome genome browser (https://vis.nucleome.org/entry/), shows the TAD containing *MYOD1* locus (*MYOD1* TAD) and adjacent TADs along a ~3.7 Mb region. P (bold) indicates the gRNA designed to target the *MYOD1* promoter, E1-E10 and E21-E30 indicate gRNAs targeting regions outside the *MYOD1* TAD, CE_E11 -E15 indicates gRNAs targeting known *MYOD1* CE enhancer region, and E16-E20 indicates gRNAs targeting an unknown region equidistant from the TSS as the known enhancer within the *MYOD1* TAD. All gRNAs are targeted to non-coding regions with inactive chromatin state in K562.

b, *MYOD1* expression in K562 cells resulting from the bi-partite p65 aTF targeting the promoter (P), outside the *MYOD1* TAD region (E1-E10, E21-E30) and within the *MYOD1* TAD region harboring the known enhancer (CE_E11 -E15) and the equidistant region as the known enhancer from *MYOD1* TSS (E16-E20). Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

c, A Hi-C interaction map of chromosome 11 in K562 cells at 100 Kb resolution from Nucleome genome browser (https://vis.nucleome.org/entry/), shows the TAD containing *HBB* locus (*HBB* TAD) and adjacent TADs along a ~2.6 Mb region. P (bold) indicates the gRNA designed to target the *HBB* promoter, E1-E15 and E27-E41 indicate gRNAs targeting regions outside the *HBB* TAD, HS2_E21 –E26 indicates gRNAs targeting the known *HBB* LCR HS2 enhancer region, and E16-E20 indicates gRNAs targeting an unknown region equidistant from the TSS as the known enhancer within the *HBB* TAD. All gRNAs are targeted to non-coding regions with inactive chromatin state in K562.

d, *HBB* expression in K562 cells resulting from the bi-partite p65 aTF targeting the promoter (P), outside the *HBB* TAD region (E1-E15, E27-E41) and within the *HBB* TAD region harboring the known enhancer (HS2_E21 –E26) and the equidistant region as the known enhancer from *HBB* TSS (E16-E20). Open circles indicate biological replicates (n=3), bars the mean of replicates and error bars the s.e.m.

e-f, Histograms of average $\log_2$(Hi-C) contacts within genome-wide sliding windows of size 4.35 Mb and 2.575 Mb, chosen to match the sizes of the *MYOD1* and *HBB* loci, respectively. Dashed blue line corresponds to the median value for that distribution, and dashed red line corresponds to the values for *MYOD1* and *HBB*.

Information about plasmids used in this study

	Name	Addgene #	Description
1	BPK1179	TBD	pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xFLAG-DmrA-DmrA-DmrA-DmrA
2	BPK617	TBD	pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xFLAG-VP64
3	BPK1160	TBD	pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xFLAG-p65
4	JEH127	TBD	pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xHA-VPR(VP64-p65-RTA)
5	BPK880	TBD	BPK880: pCAG-DmrC-NLS-3xFLAG-VP64
6	BPK1169	104564	BPK1169: pCAG-DmrC-NLS-3xFLAG-p65
7	MMW948	104565	MMW948: pCAG-DmrC-NLS-3xFLAG-VPR(VP64-p65-RTA)
8	BPK1520	65777	BPK1520 (pU6-BsmBICassette-S.pyogenes.sgRNA)
9	JG1211	104567	pCAG-human dLbCpf1(D832A)-NLS-3xHA-VPR
10	YET1000	104571	pCAG-human dLbCpf1(D832A)-NLS-3xHA-DmrA(X4)
11	BPK3082	78742	pU6-LbCpf1-crRNA-BsmBI cassette

BPK1179: pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xFLAG-DmrA-DmrA-DmrA-DmrA

NNNN NLS

NNNN dSpCas9(D10A, H840A)

NNNN 3x FLAG Tag

NNNN DmrA

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NNNN NLS

NNNN dSpCas9(D10A, H840A)

NNNN 3x FLAG Tag

NNNN VP64

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BPK1160: pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xFLAG-p65

NNNN NLS

NNNN dSpCas9(D10A, H840A)

NNNN 3x FLAG Tag

NNNN p65

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JEH127: pCAG-NLS-dSpCas9(D10A,H840A)-NLS-3xHA-VPR(VP64-p65-RTA)

NNNN NLS

NNNN dSpCas9(D10A, H840A)

NNNN 3x HA Tag

NNNN VPR

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BPK880: pCAG-DmrC-NLS-3xFLAG-VP64

NNNN NLS

NNNN DmrC

NNNN 3x FLAG Tag

NNNN VP64

ATGGGATCCAGA**ATCCTCTGGCATGAGATGTGGCATGAAGGCCTGGAAGAGGCATCTCGTTTGTACTTTGGGGAAAGGAACGTGAAAGGCATG**
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CGATATGTTATAA

BPK1169: pCAG-DmrC-NLS-3xFLAG-p65

NNNN NLS

NNNN DmrC

NNNN 3x FLAG Tag

NNNN p65

ATGGGATCCAGA**ATCCTCTGGCATGAGATGTGGCATGAAGGCCTGGAAGAGGCATCTCGTTTGTACTTTGGGGAAAGGAACGTGAAAGGCATG**
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MMW948: pCAG-DmrC-NLS-3xFLAG-VPR(VP64-p65-RTA)

NNNN NLS

NNNN DmrC

NNNN 3x FLAG Tag

NNNN VPR

ATGGGATCCAGA**ATCCTCTGGCATGAGATGTGGCATGAAGGCCTGGAAGAGGCATCTCGTTTGTACTTTGGGGAAAGGAACGTGAAAGGCATG**
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BPK1520 (pU6-BsmBICassette-S.pyogenes.sgRNA-U6 Terminator)

NNNN U6 Promoter

NNNN BsmBI Restriction Sites

NNNN *S. pyogenes* sgRNA

NNNN U6 Terminator

TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCA
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JG1211: CAG-human dLbCpf1(D832A)-NLS-3xHA-VPR

Human codon optimized dLbCpf1: **bold**, NLS: italic, 3xHA: lower case, VPR: lower case and **bold**

ATG**AGCAAGCTGGAGAAGTTTACAAACTGCTACTCCCTGTCTAAGACCCTGAGGTTCAAGGCCATCCCTGTGGGCAAGACCCAGGAGAACATCG**
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YET1000: CAG-human dLbCpf1(D832A)-NLS-3xHA-DmrA(X4)

Human codon optimized dLbCpf1: **bold**, NLS: *italic*, 3xHA: lower case, DmrA: lowercase and **bold**

ATG**AGCAAGCTGGAGAAGTTTACAAACTGCTACTCCCTGTCTAAGACCCTGAGGTTCAAGGCCATCCCTGTGGGCAAGACCCAGGAGAACATCG**
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BPK3082: U6-Lb-crRNA-BsmBIcassette

U6 promoter: **bold**, Lb crRNA: *italic*, BsmBI sites: lower case, U6 terminator: *italic* and **bold**

TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTT
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GCTTTATATATCTTGTGGAAAGGACGAAACACC*G**AATTTCTACTAAGTGTAGAT*GgagacgATTAATGcgtctcC***TTTTTTT***

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