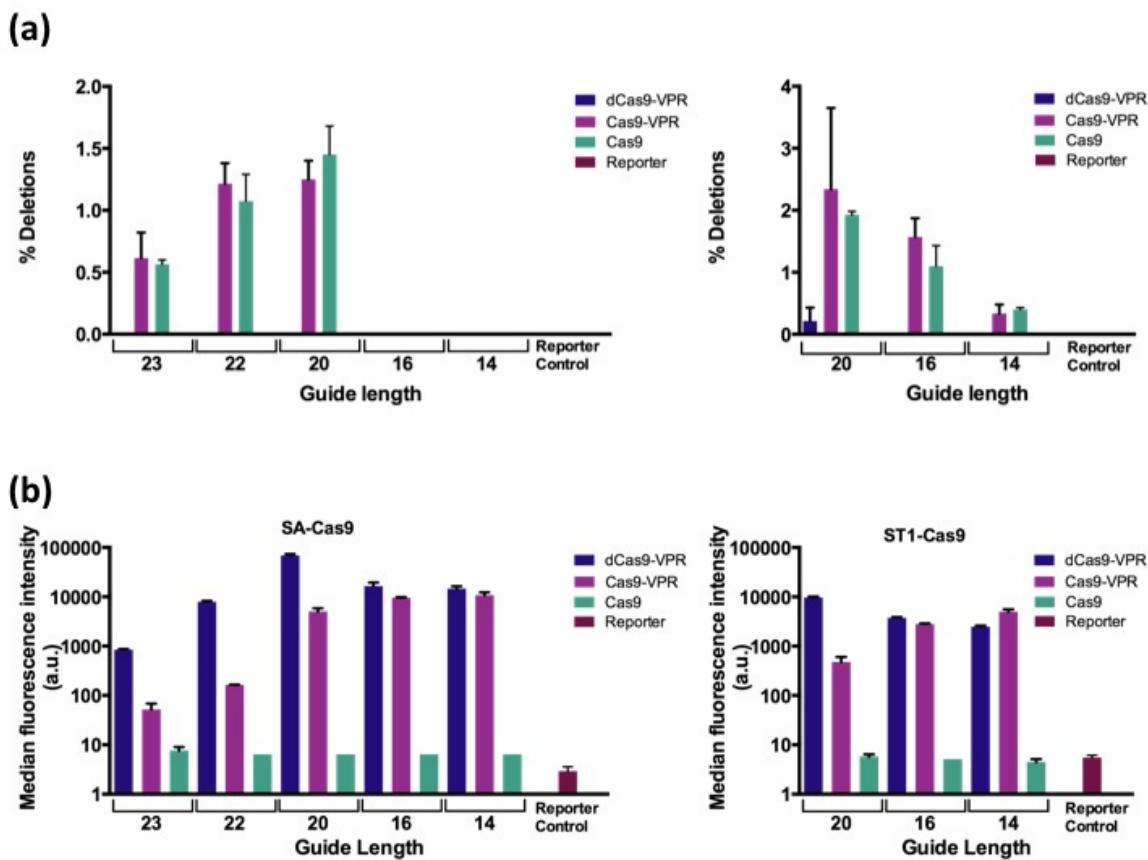


Supplementary Figure 1

**Activation and cutting of a transcriptional reporter using gRNAs with progressively shorter 5' end lengths.**

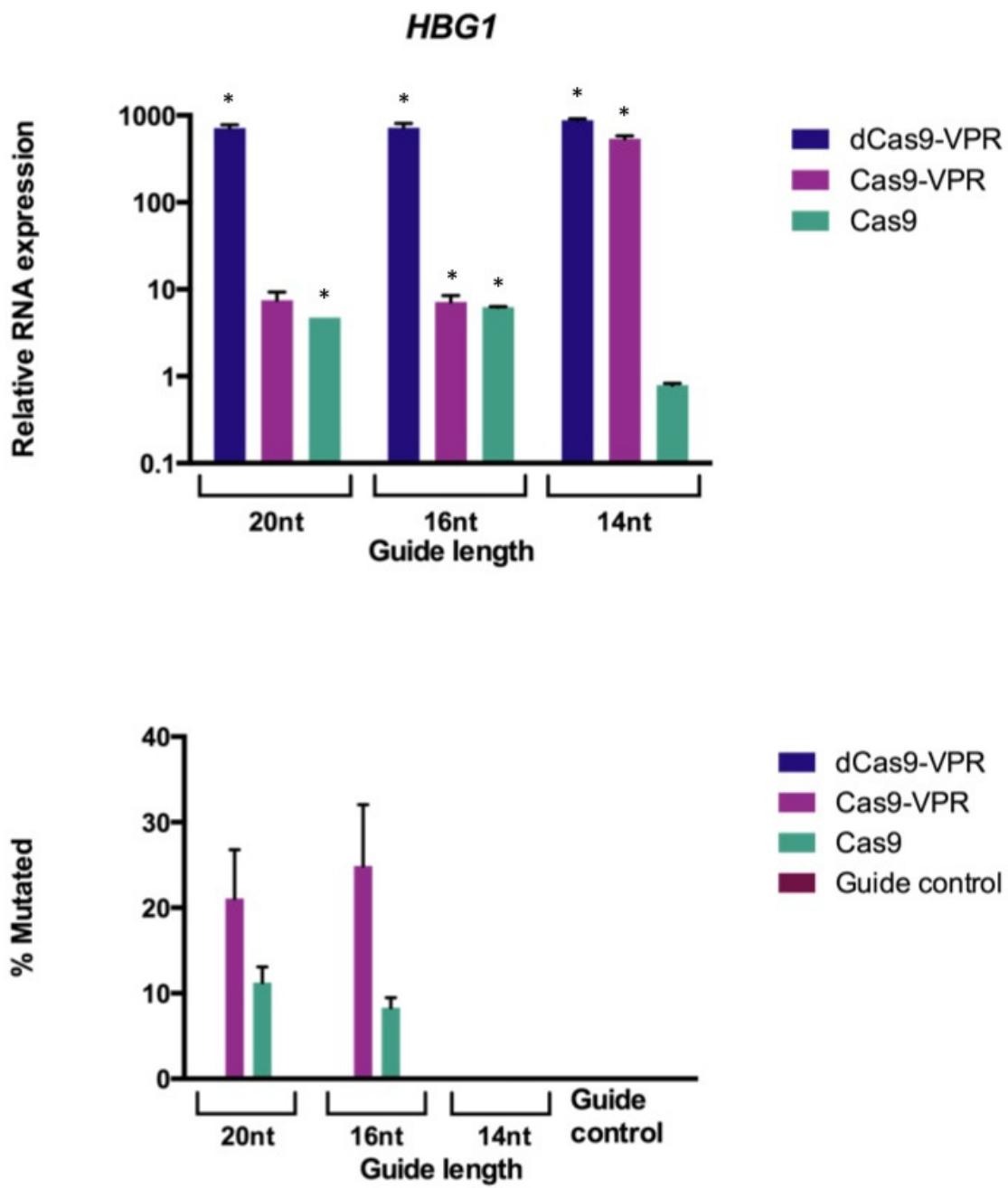
(a) Deletion analysis of truncated gRNAs on a synthetic transcriptional reporter. Samples were transfected with the indicated Cas9 construct and gRNA. Data indicate the mean and s.e.m. ( $n = 2$  independent transfections). (b) Quantification of activation for truncated gRNAs via a fluorescent transcriptional reporter. Data indicate the mean and s.e.m. ( $n = 2$  independent transfections).



Supplementary Figure 2

Activation and cutting of a transcriptional reporter using orthogonal Cas9 proteins.

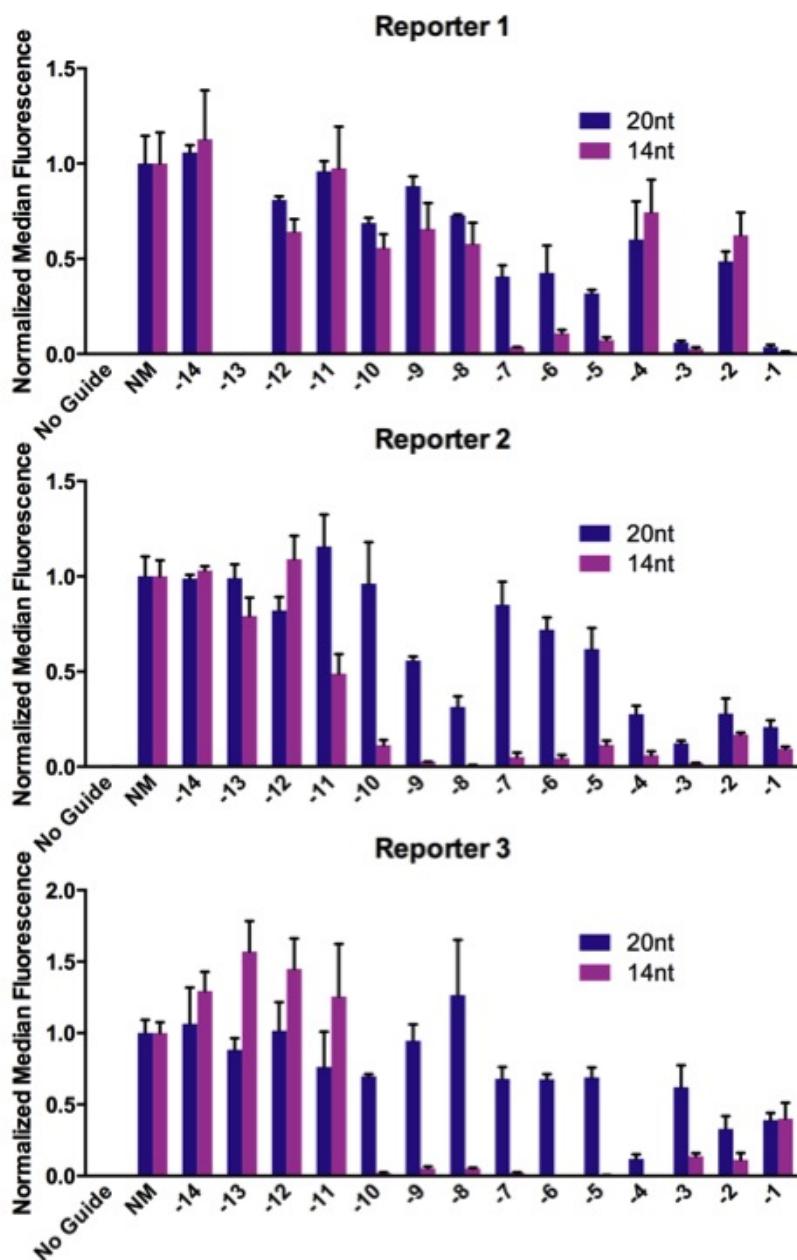
**(a)** Deletion analysis of truncated gRNAs on a synthetic transcriptional reporter using SA and ST1 Cas9. Samples were transfected with the indicated Cas9 construct and gRNA. Data indicate the mean and s.e.m. ( $n = 2$  independent transfections). **(b)** Quantification of activation for truncated gRNAs via a fluorescent transcriptional reporter using SA and ST1 Cas9. Data indicate the mean and s.e.m. ( $n = 2$  independent transfections).



**Supplementary Figure 3**

**Activation and cutting of endogenous *HBG1* gene.**

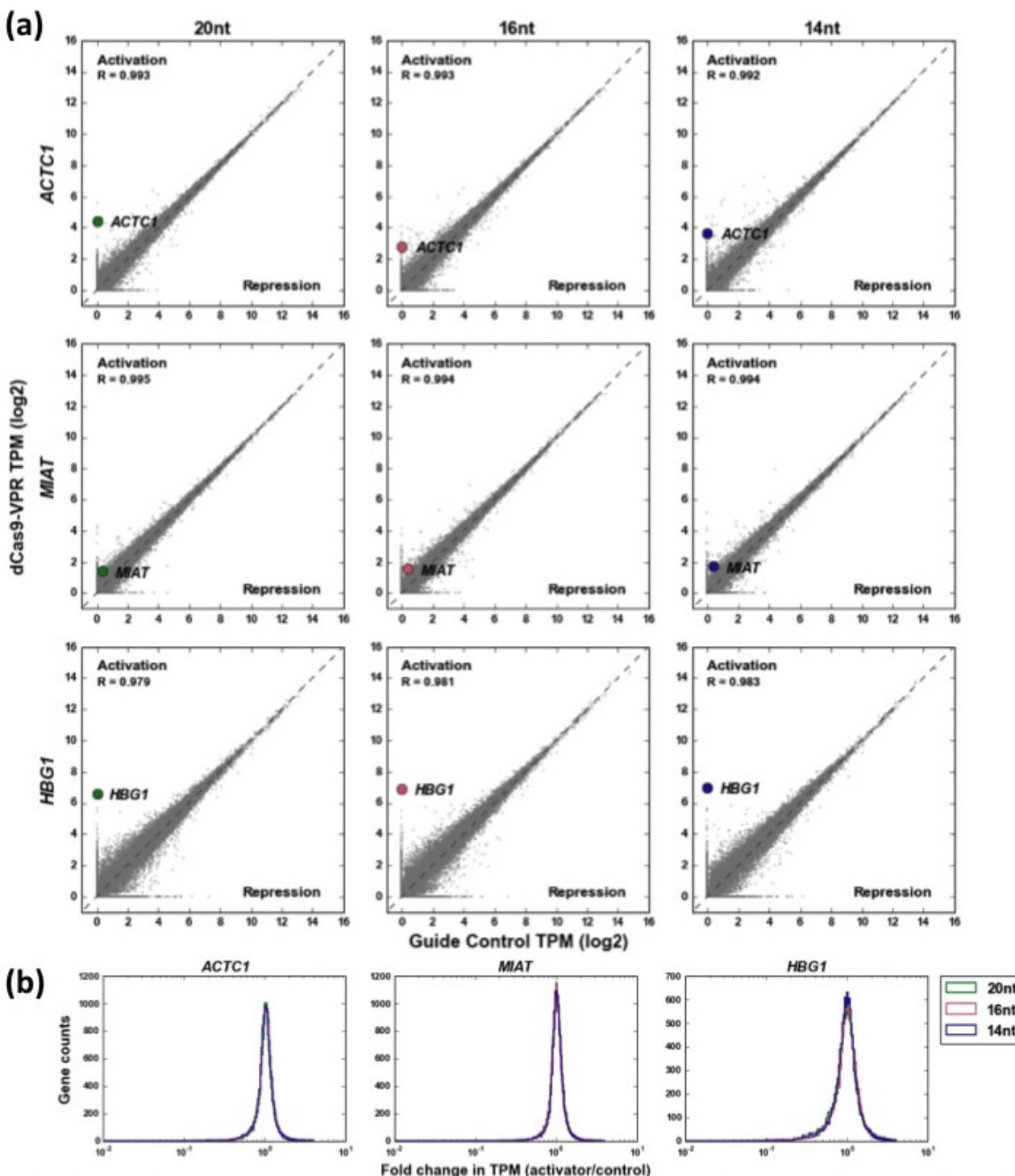
RNA expression and mutagenesis analysis of the gene *HBG1*. Each sample was transfected with the indicated Cas9 construct and gRNA. Data indicate the mean and s.e.m ( $n = 2$  independent transfections). \* $P < 0.05$  when compared to the guide control for activation experiments.



Supplementary Figure 4

Mismatch comparison between 20-nt and 14-nt sgRNA activation using Cas9-VPR.

Comparison of activation of Cas9-VPR targeted to three tdTomato reporters using 20-nt or 14-nt gRNA with single mismatches at each position. Single-mismatch mutations are Watson-Crick transversions. The -1 position is adjacent to the PAM sequence. tdTomato reporter activation by Cas9-VPR was measured using flow cytometry, and values shown represent the ratio of the activation signal observed from each gRNA to fully matched gRNA. In other words, the 20-nt mismatched guides were normalized to the fully matched 20-nt gRNA and the 14-nt mismatched guides were normalized to the fully matched 14-nt gRNA. NM, no mismatches, or the fully matched gRNA samples.  $n = 2$  independent biological replicates. Data represent the normalized median with error bars representing s.e.m.

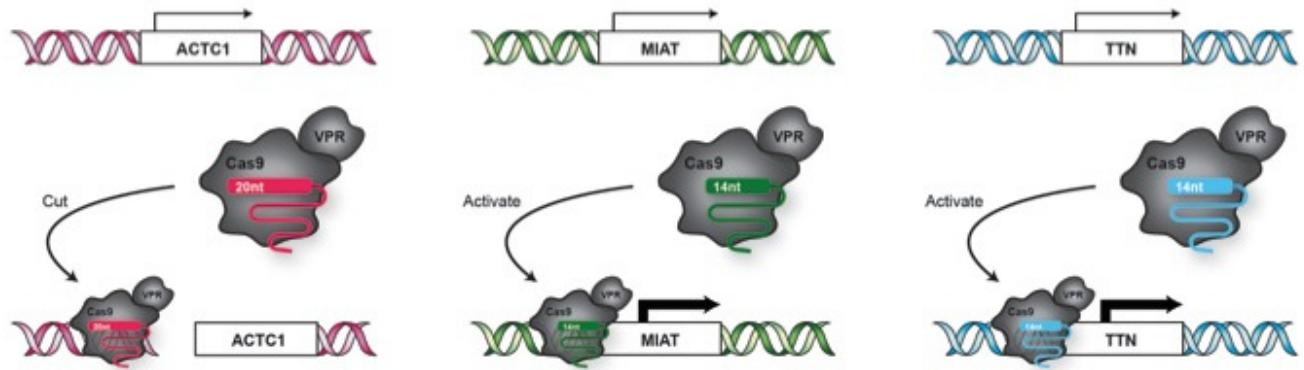


**Supplementary Figure 5**

**Off-target expression analysis.**

**(a)** Gene expression levels ( $\log_2$  TPM (transcripts per million)) in cells transfected with dCas9-VPR targeting the indicated genes with gRNAs of indicated lengths (y-axis) versus expression in cells transfected with gRNA only (x-axis). R indicates Pearson's correlation coefficient calculated for log-transformed values on all genes except the target. A pseudocount of 1 TPM was added to each gene

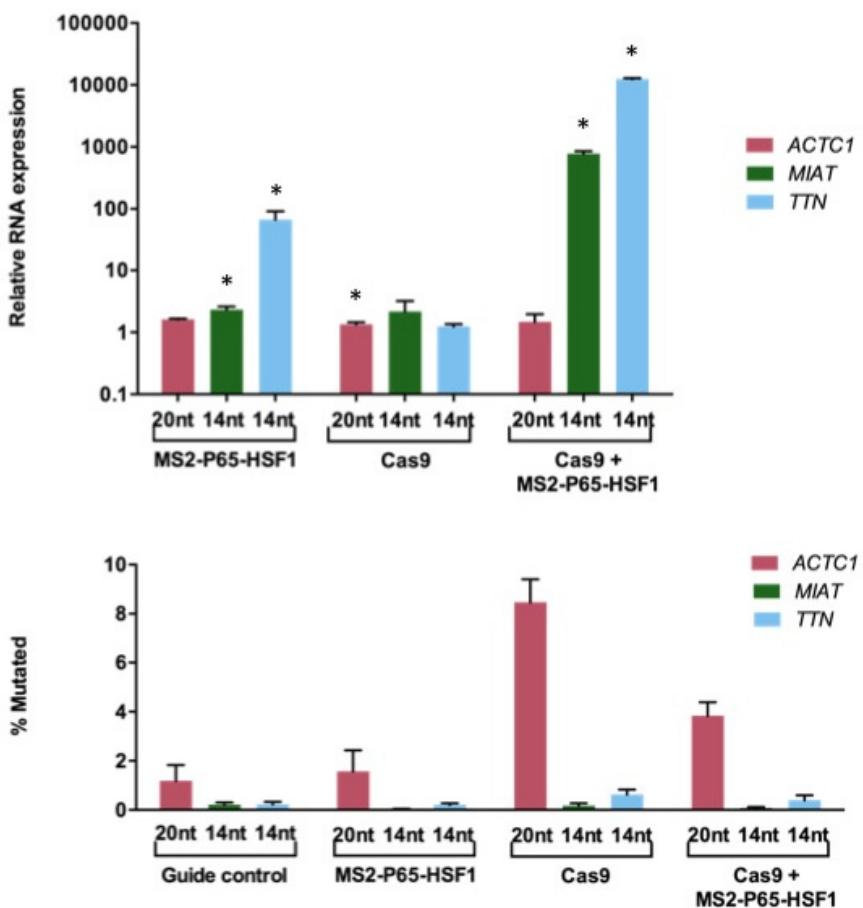
before log transformation. Average of two biological replicates shown. A one-way within-sample ANOVA was performed and demonstrated no significant difference in expression of nontargeted transcripts for truncated guides versus the full-length guide ( $P = 0.316$ ). Correlation between samples and controls was also high ( $R \geq 0.979$ ). **(b)** Histograms showing the distribution of fold changes in gene expression (activator/guide control). Genes were filtered to include only those with TPM > 1. Average of two biological replicates shown.



**Supplementary Figure 6**

**Pictorial representation of Figure 1d.**

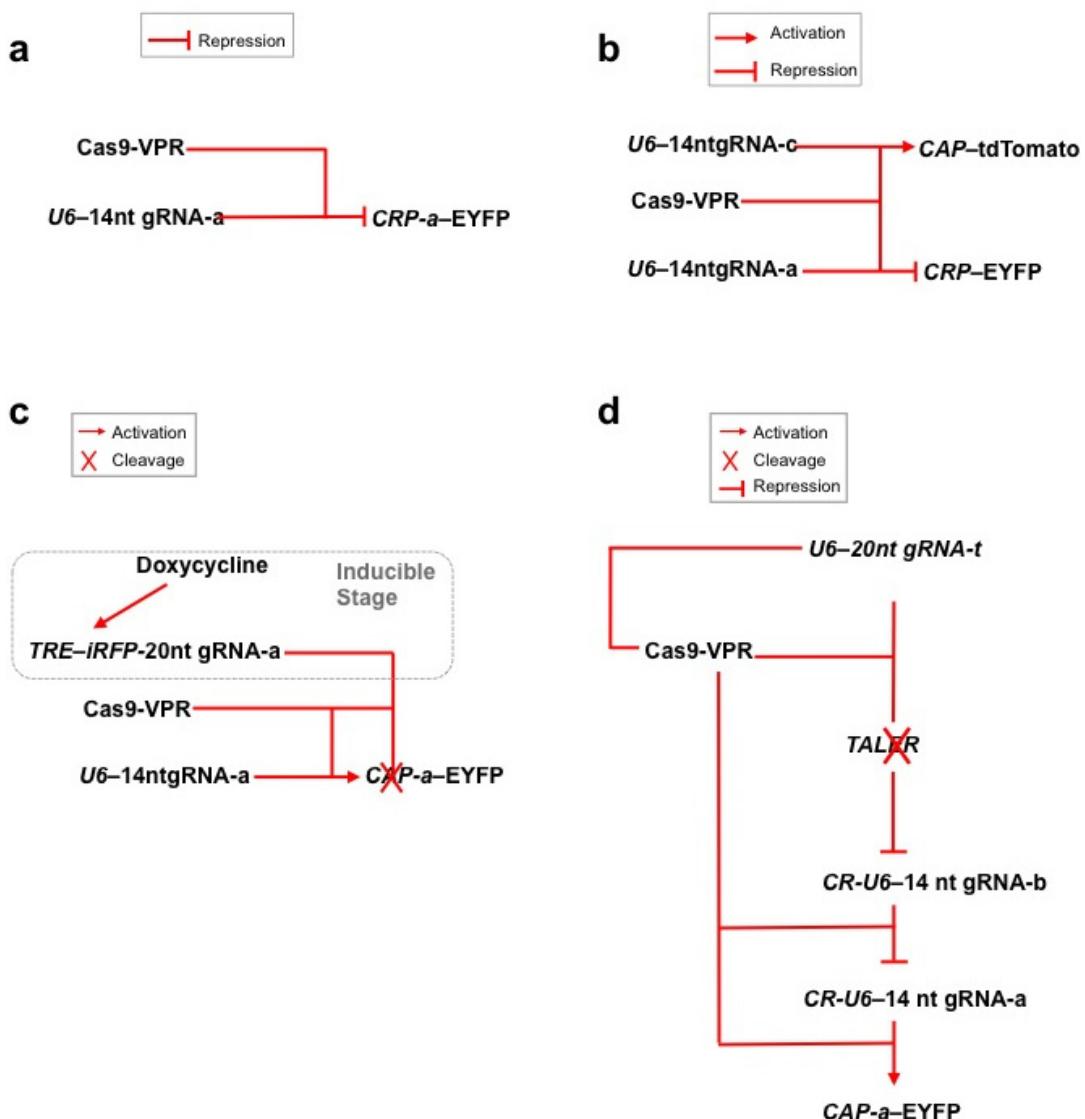
Cells were transfected with a 20-nt guide directed toward *ACTC1*, 14-nt guides directed toward *MIAT* and *TTN* and either Cas9-VPR or Cas9. This picture represents the expected behavior of Cas9-VPR. The *ACTC1* locus should be cut, while transcription occurs for the genes *MIAT* and *ACTC1*.



**Supplementary Figure 7**

**gRNA-mediated recruitment of an activator using Cas9.**

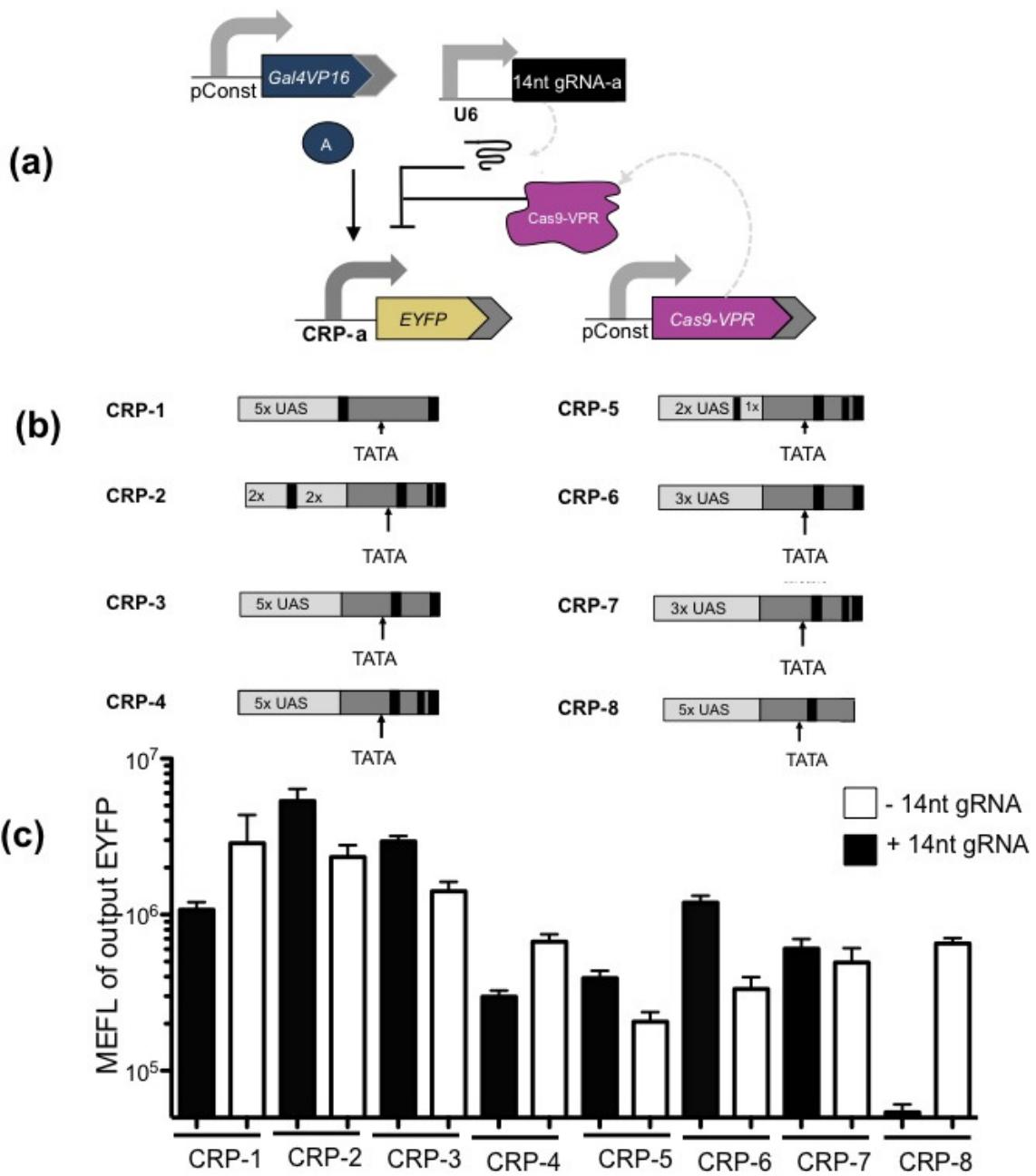
The indicated constructs were transfected with a 20-nt *ACTC1* gRNA and 14-nt *MIAT* and *TTN* gRNAs simultaneously with the indicated Cas9 and/or AD. Activation in this experiment occurred not through direct fusion to Cas9, but through an aptamer-based system in which the effector was recruited to the gRNA. Data indicate the mean and s.e.m. ( $n = 2$  independent transfections). \* $P < 0.05$  when compared to the guide control.



**Supplementary Figure 8**

**Simplified schematics of circuits in Figure 2.**

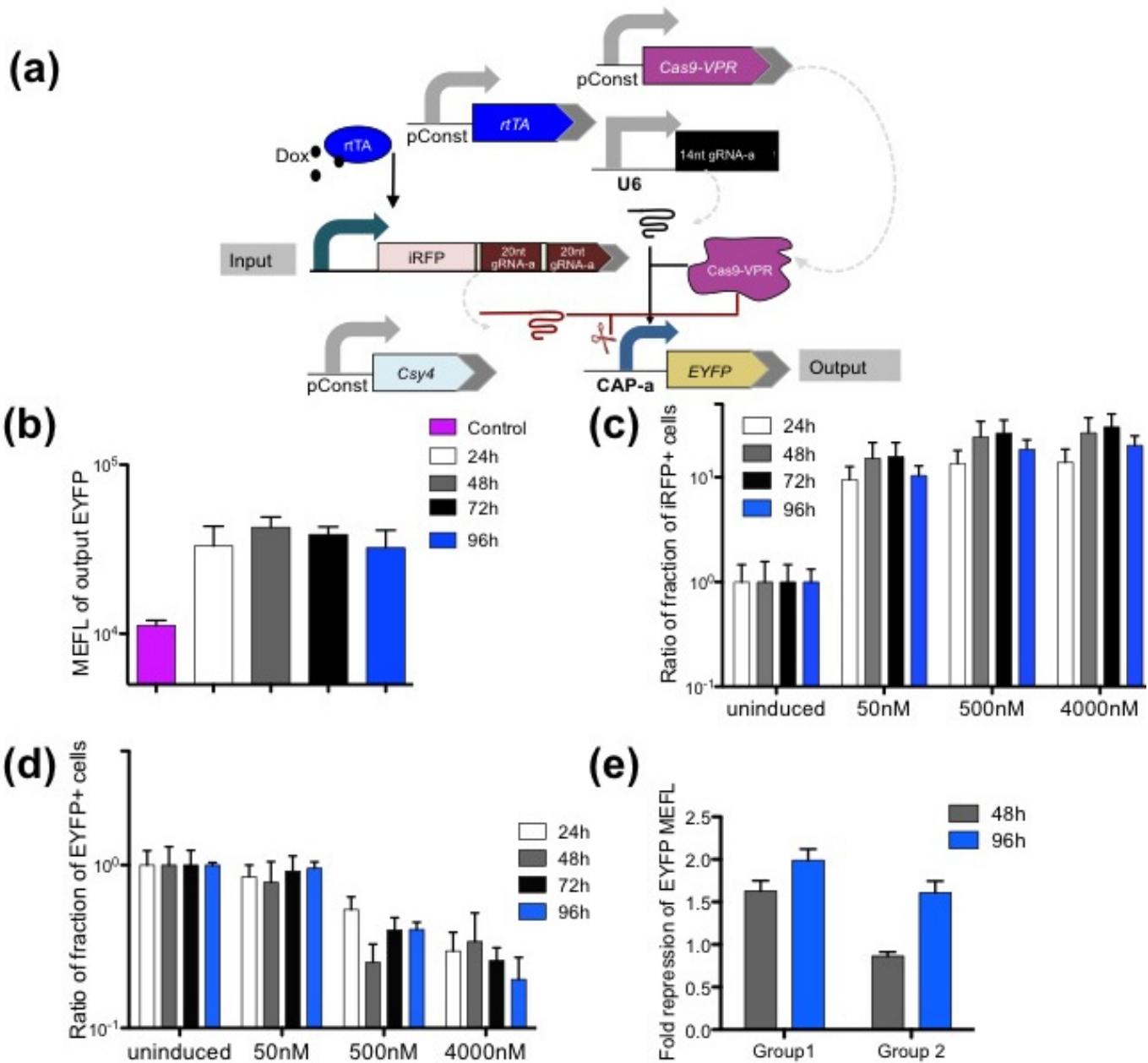
(a) Schematic of a Cas9-VPR and 14-nt gRNA repression device. (b) Schematic of parallel Cas9-VPR/14-nt gRNA-based transcriptional repression and activation devices in a single cell. A 14-nt gRNA-c drives Cas9-VPR to a CRISPR-activatable promoter (CAP) and mediates the activation of tdTomato while another 14-nt gRNA targets Cas9-VPR to a CRISPR repressible promoter (CRP) to repress EYFP expression. (c) Schematics of a genetic kill switch designed to incorporate Cas9-VPR DNA cleavage and transcriptional activation functions. A 14-nt gRNA directs Cas9-VPR to a CAP to activate output EYFP expression. Addition of doxycycline generates a 20-nt gRNA that directs Cas9-VPR to the same region in the promoter but cuts within the promoter, thereby decreasing EYFP output. (d) Genetic kill circuit that incorporates all three functions of Cas9-VPR: DNA cleavage, transcriptional activation and repression. Input gRNA that cuts within TALER coding sequences decreases available gRNA-a and reduces output expression.



**Supplementary Figure 9**

**Building different promoter architectures to analyze Cas9-VPR-mediated transcriptional repression.**

**(a)** Schematics of Cas9-VPR/14-nt gRNA-based transcriptional repression control unit. **(b)** Architecture of different CRISPR repressible promoters (CRPs). We developed a library of CRPs containing various numbers of gRNA target sites at different locations relative to the transcriptional start site in minimal CMV promoter or various numbers of upstream activation sites (UAS), in order to identify promoter architectures that allowed us to achieve efficient Cas9-VPR-mediated transcriptional repression. **(c)** Geometric mean and s.d. of means of EYFP for cells expressing  $>10^7$  MEFL of transfection marker EBFP ( $n = 3$  technical replicates). The highest repression was achieved with CRP-8. Some of the promoters designed for repression purposes unexpectedly led to activation, and further analysis is required to understand the effect of spacing between Cas9-VPR target sites at the promoters or location of targeting (downstream of the promoter) on this observation. Note CRP-a in the schematics of (a) is CRP-8.



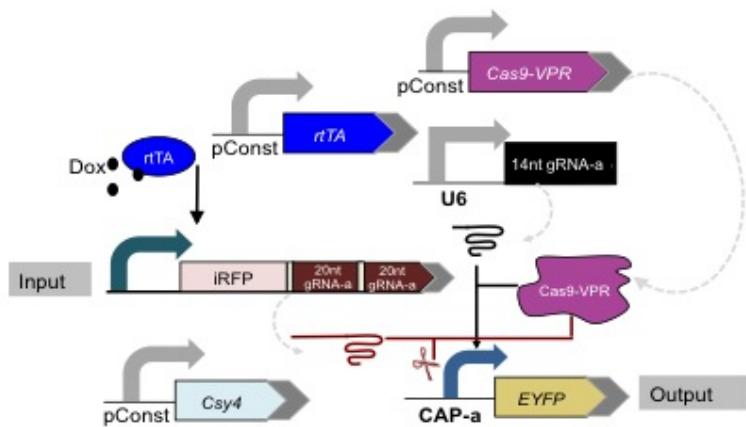
Supplementary Figure 10

**Analysis of dynamics of a genetic kill-switch circuit.**

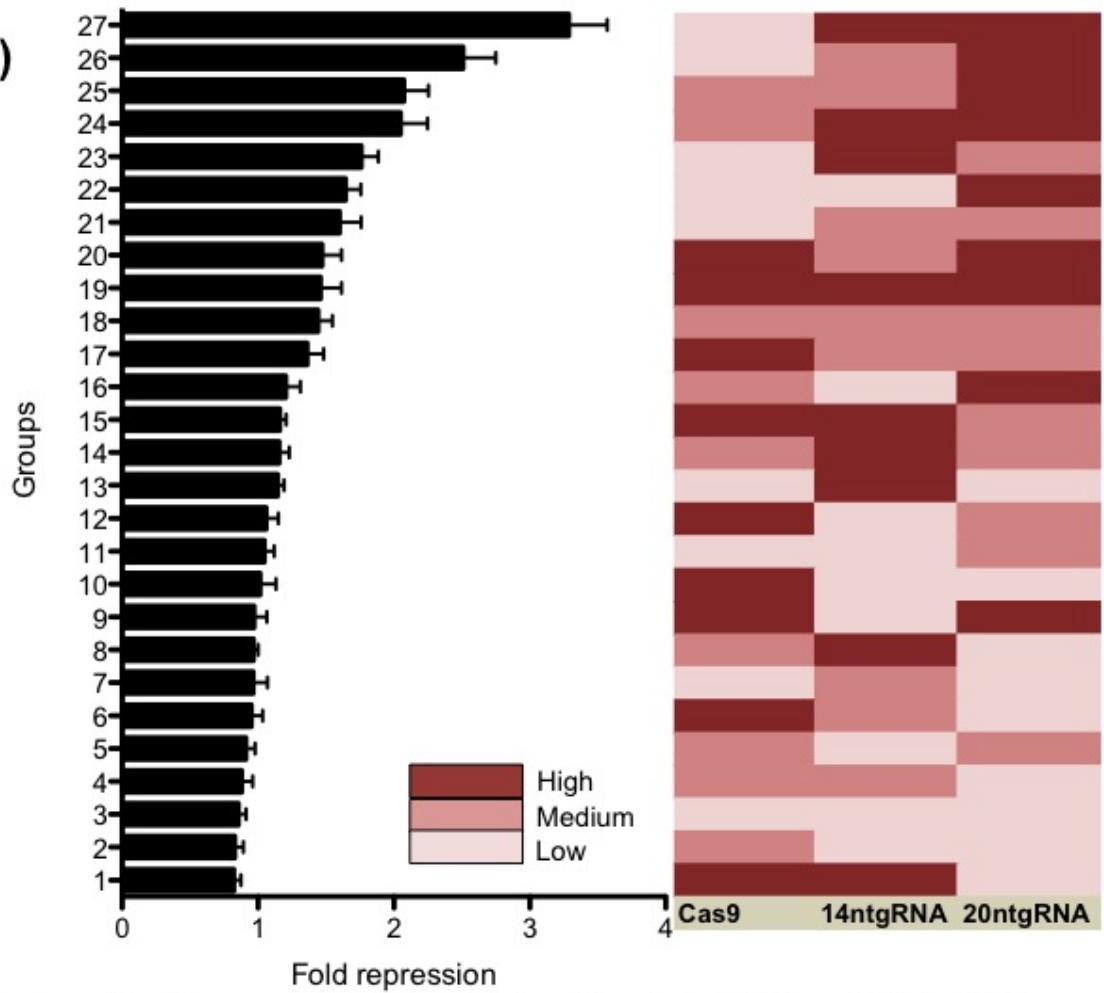
(a) A schematic of a genetic kill switch designed such that 20-nt and 14-nt gRNAs compete for same target site within a CAP (CRISPR-activatable promoter). Upon induction of 20-nt gRNA and infrared fluorescent protein (iRFP) with doxycycline, a reduction in EYFP expression is expected as a result of cleavage mediated by Cas9-VPR and 20-nt gRNA within the CAP. (b) 14-nt gRNA and Cas9-VPR-mediated activation of EYFP is detectable around 24 h after transfection and continues through 96 h. The control group received only the transfection marker EBFP and was measured 48 h after transfection. Data are the geometric mean and s.d. of means of EYFP for cells expressing  $>2 \times 10^7$  MEFL of transfection marker EBFP. (c) After the addition of doxycycline, cells positive for iRFP and 20-nt gRNA expression were detectable around 24 h after transfection and remained high in iRFP expression until 96 h. Shown are the percentages of cells expressing EBFP $>10^7$  MEFL and iRFP $>10^{6.5}$  relative to the uninduced population. (d) Fraction of cells that had EYFP above autofluorescence relative to the uninduced population in different treatment conditions and over time. Shown are the percentages of cells expressing EBFP $>10^7$  MEFL and EYFP $>10^{5.5}$  relative to the uninduced population. (e) Bars show the geometric

mean ratio and s.d. of the mean ratio of uninduced versus fully induced samples, for cells expressing  $>10^7$  MEFs of transfection marker EBFP. Group 1 includes cells that received doxycycline (4,000 nM) at the time of transfection, and group 2 includes cells that received doxycycline 24 h after the transfection. We observed a slower dynamic in group 2, possibly because of the initial accumulation of EYFP protein. For all figures,  $n = 3$  independent technical replicates combined from three experiments.

(a)



(b)

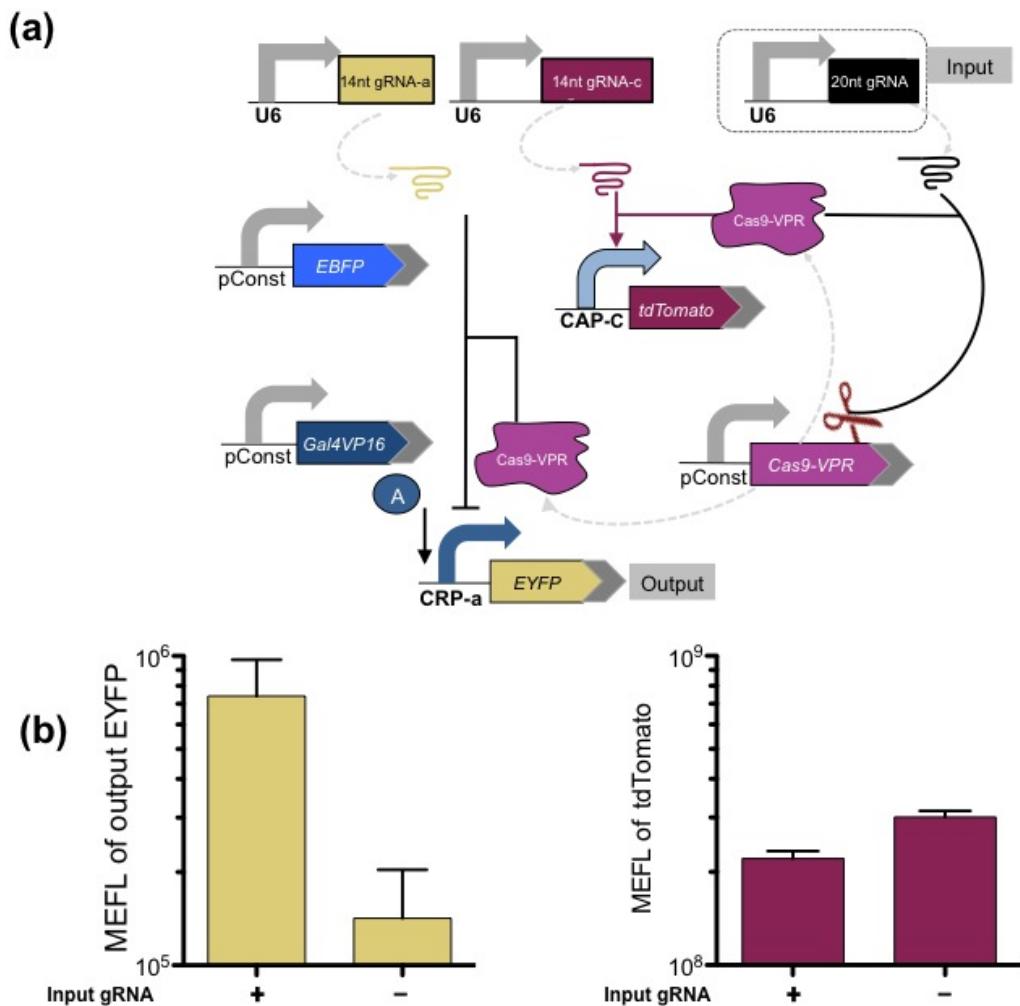


Supplementary Figure 11

**Understanding the design rules based on the concentrations of Cas9-VPR, 14-nt gRNA and 20-nt gRNA.**

(a) A schematic of a genetic kill switch designed such that 20-nt and 14-nt gRNAs compete for same target site within a CAP. (b) Varying the dosages of transfected plasmids encoding Cas9-VPR, 14-nt gRNA, and 20-nt gRNA between low (5 ng), medium (25 ng for

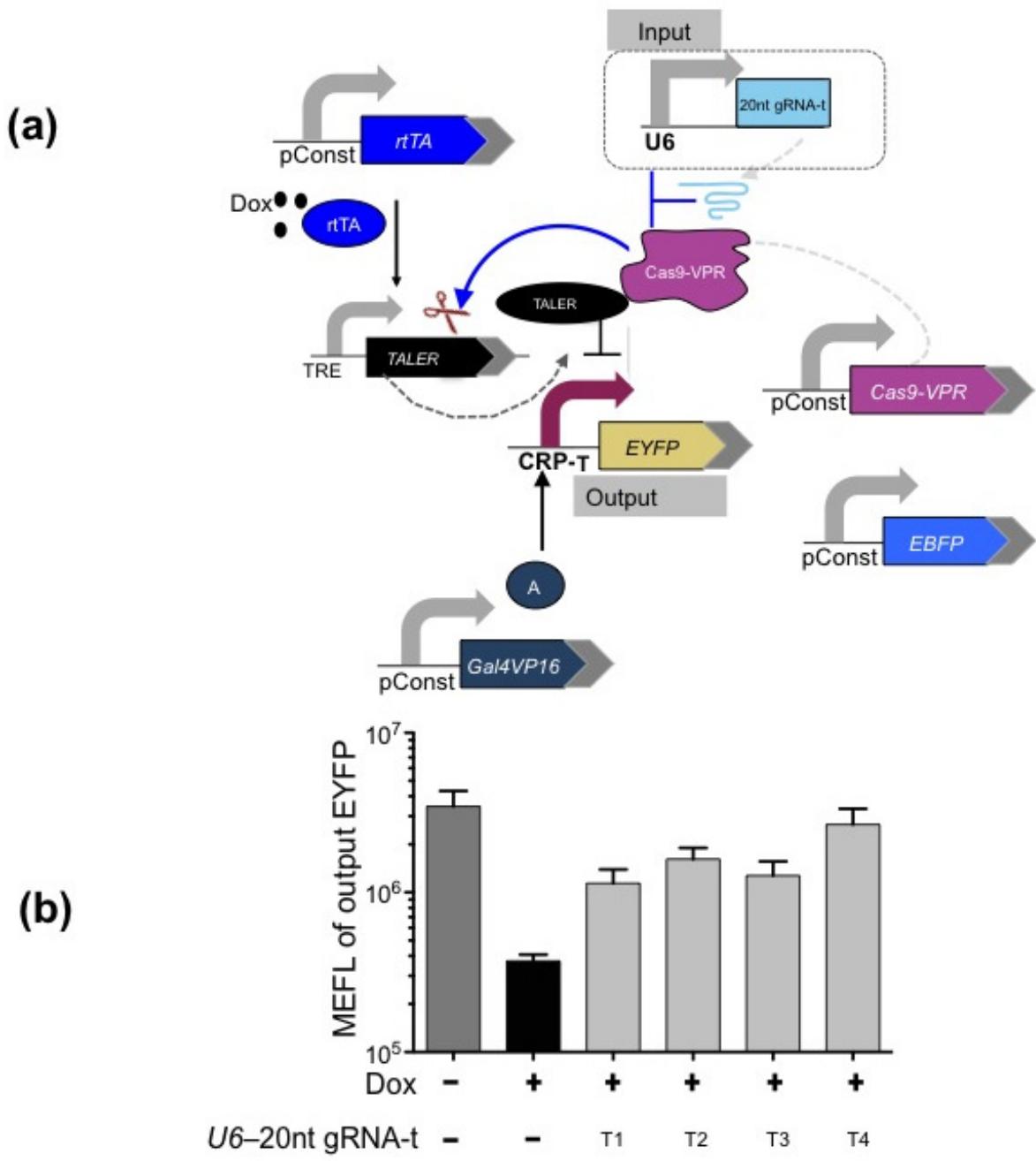
14-nt gRNA and 50 nt for 20-nt gRNA) and high (250 ng) helped us unravel some design rules. Each line represents a single condition of transfection with corresponding Cas9, 14-nt gRNA and 20-nt gRNA plasmid levels in front of the fold change observed upon the addition of doxycycline. Bar graphs show the fold change of the geometric mean and s.d. of means of EYFP over uninduced cells for cells expressing  $>3 \times 10^7$  MEFL transfection marker EBFP.  $n = 3$  independent technical replicates combined from three experiments. Cas9 in the footnote of the colored map refers to the concentration of Cas9-VPR complex.



**Supplementary Figure 12**

**Design and analysis of a genetic kill switch that functions based on DNA cleavage in the Cas9-VPR coding sequence.**

(a) A schematic of a genetic kill switch designed such that the presence of 20-nt gRNAs leads to Cas9-VPR-mediated cleavage within its own coding sequence and thereby reverses the output EYFP and tdTomato levels. Cas9-VPR targets a CAP by means of 14-nt gRNA-c, leading to activation of tdTomato expression. In parallel, Cas9-VPR and 14-nt gRNA-a also target a CRP, where Cas9-VPR binding provides a steric barrier to EYFP transcription. In the presence of a pair of full-length 20-nt gRNAs targeting the middle of the Cas9-VPR coding sequence, the guides direct Cas9-VPR to cut and disable itself and, by doing so, decrease the available pool of Cas9-VPR in the cell, ultimately causing a reduction of reporter inhibition or activation. Comparing cells that either received two pairs of 20-nt gRNAs that cuts with Cas9-VPR coding region or did not, we observed about fivefold de-repression of EYFP and about a 1.4-fold decrease in tdTomato expression. Shown are the geometric mean and s.d. of means of EYFP and tdTomato for cells expressing  $>10^7$  MEFL transfection marker EBFP.  $n = 4$  independent technical replicates combined from three experiments.

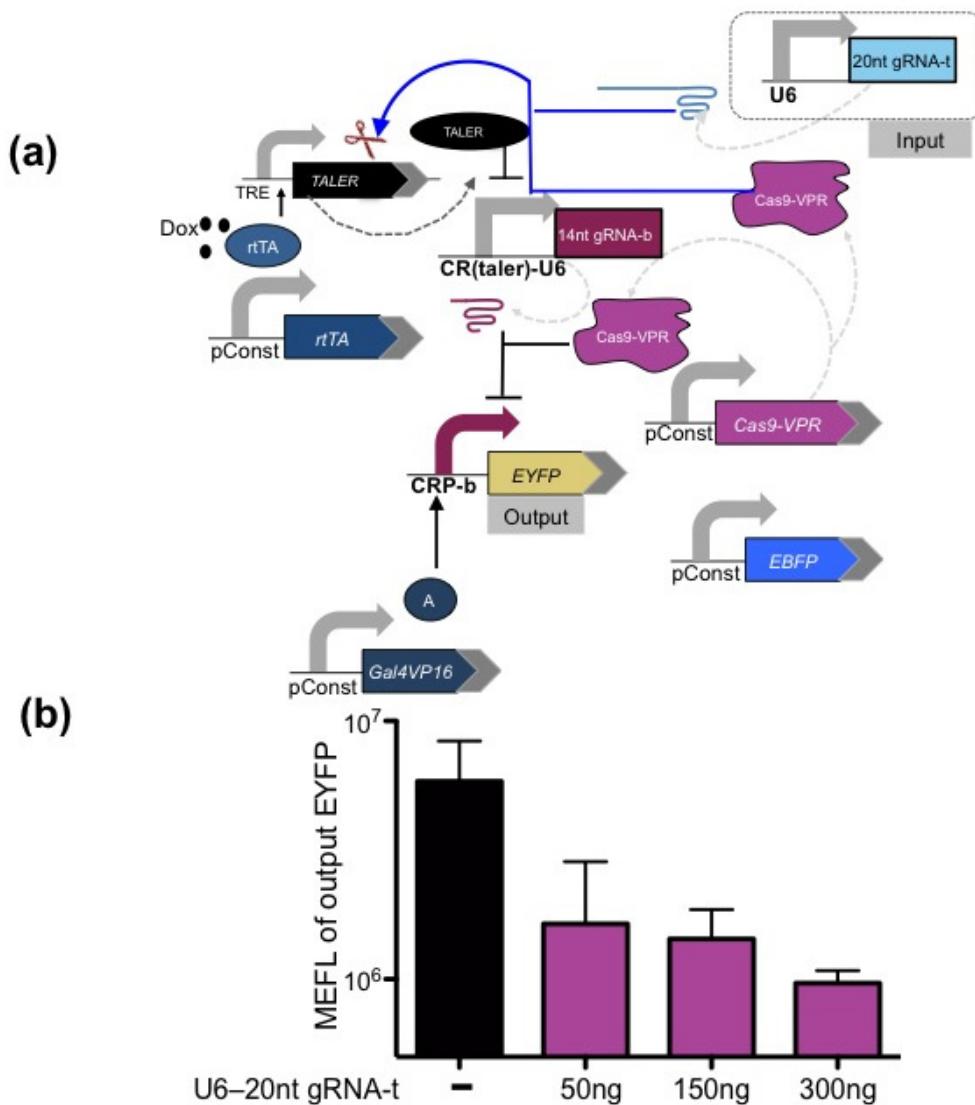


Supplementary Figure 13

**Design and analysis of a genetic kill switch that operates based on DNA cleavage in the TALER coding sequence and reversal of a transcriptional repression device.**

**(a)** Schematics of the kill switch involving a TALER-based transcriptional repression device and Cas9-VPR-mediated DNA mutation within the TALER DNA sequence. We tested whether Cas9-VPR can cleave within the TALER coding sequence and, by doing so, decrease available TALER, thus removing its repression of EYFP. Analysis of this switch using 20-nt gRNA against different regions

within the TALER sequence allowed us to achieve a moderately functional kill switch (**b**). Shown are the geometric mean and s.d. of means of EYFP for cells expressing  $>10^7$  MEFL of transfection marker EBFP ( $n = 3$  technical replicates). T1–4 refer to 20-nt gRNAs that cut at different regions in the TALER coding sequence.



Supplementary Figure 14

**Design and analysis of a genetic kill switch that operates based on DNA cleavage in the TALER coding sequence and reversal of a transcriptional repression device.**

(a) Schematics of the layered kill switch. We generated a modified U6 promoter, regulated by TALER, enabling us to connect the genetic kill switch in **Supplementary Figure 13** with a Cas9-VPR 14-nt gRNA repression device. Transfection of this circuit in HEK293FT cells exhibited repression of output EYFP upon addition of a pair of input 20-nt gRNAs that cut within the TALER coding sequence. (b) Shown are the geometric mean and s.d. of means of EYFP for cells expressing  $>10^7$  MEFL of transfection marker EBFP. Output is compared between cells with or without gRNA encoding plasmids that cut within TALER coding sequences ( $n = 3$  technical replicates).

### **Supplementary note 1:**

Due to the interesting features in the circuit of figure 2c, such as competition of the 20ntgRNA and 14ntgRNA for the same target site in the promoter of EYFP and inducibility of 20ntgRNA with doxycycline, we performed further characterization experiments with this circuit. In the absence of 20nt gRNA, 14nt gRNA-a/Cas9-VPR mediated activation of EYFP is detectable around 24 h after transfection and remains high until 96h after transfection (**Supplementary Fig. 10b**). When 20nt gRNA is induced to outcompete 14nt by addition of doxycycline at the time of transfection (**Supplementary Fig. 10c**), we observed an increase in infrared fluorescent protein (iFRP) positive cells relative to the uninduced population 24h after transfection through 96h. Simultaneously, in groups that received higher doxycycline concentrations (500- 4000nM), the fraction of EYFP expressing cells reduces 24h after transfection relative to uninduced population and this reduction continues towards 96h after transfection (Supplementary Fig. 10d). Increasing dosage of doxycycline increases iRFP signal, which is indirectly correlated with higher level of 20nt gRNA expression and subsequently better repression of EYFP signal (**Supplementary Fig. 10 c,d**). Addition of doxycycline 24h after transfection resulted in a slower dynamic of repression as compared to induction at the time of transfection. In the former case, reduction in EYFP was detectable towards 96h after transfection (**Supplementary Fig. 10e**) possibly due to the effect of initial EYFP protein accumulation. To further characterize circuit behavior, we varied the concentration of transfected plasmids encoding Cas9-VPR, 20nt gRNA or 14nt gRNA and studied the fold repression of the output upon addition of doxycycline (**Supplementary Fig. 11**). Our data indicate that increased 20nt gRNA concentration is associated with higher repression and the highest fold repression is achieved in the presence of high 20nt gRNA and low Cas9-VPR encoding plasmids. Future analysis of this and other multifunctional circuits depicted in this study should quantify finer grained input/output behaviors of the circuits, determine if and when the shared resources of the Cas9-VPR protein complex in this context limits our ability to develop complex circuitry, investigate the circuits in genomically integrated contexts to develop additional optimization needed for improved behavior in above settings.

## Supplementary Note 2: PCR primers and gRNA sequences

Reporter Guides

gRNA	Sequence
Reporter 1 20nt	GTCCCCCTCCACCCCCCACAGTG
Reporter 1 18nt	GCCCCCTCCACCCCCCACAGTG
Reporter 1 16nt	GCCTCCACCCCCCACAGTG
Reporter 1 14nt	GTCCACCCCCCACAGTG
Reporter 1 12nt	GCACCCCCCACAGTG
Reporter 1 10nt	GCCCCCACAGTG
Reporter 1 8nt	GCCACAGTG
Reporter 1 20nt -1	GTCCCC TCCACCCCCCACAGTc
Reporter 1 20nt -2	GTCCCC TCCACCCCCCACAGaG
Reporter 1 20nt -3	GTCCCC TCCACCCCCCACAcTG
Reporter 1 20nt -4	GTCCCC TCCACCCCCCACtGTG
Reporter 1 20nt -5	GTCCCC TCCACCCCCAgAGTG
Reporter 1 20nt -6	GTCCCC TCCACCCCCtCAGTG
Reporter 1 20nt -7	GTCCCC TCCACCCCCgACAGTG
Reporter 1 20nt -8	GTCCCC TCCACCCgCACAGTG
Reporter 1 20nt -9	GTCCCC TCCACGgCACAGTG
Reporter 1 20nt -10	GTCCCC TCCAggCCCCCACAGTG
Reporter 1 20nt -11	GTCCCC TCCTCCCCCACAGTG
Reporter 1 20nt -12	GTCCCC TCgACCCCCCACAGTG
Reporter 1 20nt -13	GTCCCC TgCACCCCCCACAGTG
Reporter 1 20nt -14	GTCCCC aCACCCCCCACAGTG
Reporter 1 14nt -1	GTCCACCCCCCACAGTc
Reporter 1 14nt -2	GTCCACCCCCCACAGaG
Reporter 1 14nt -3	GTCCACCCCCCACAcTG
Reporter 1 14nt -4	GTCCACCCCCCACtGTG
Reporter 1 14nt -5	GTCCACCCCCAgAGTG
Reporter 1 14nt -6	GTCCACCCCCtCAGTG
Reporter 1 14nt -7	GTCCACCCgACAGTG
Reporter 1 14nt -8	GTCCACCCgCACAGTG
Reporter 1 14nt -9	GTCCACCGgCACAGTG
Reporter 1 14nt -10	GTCCAgCCCACAGTG
Reporter 1 14nt -11	GTCCtCCCCCACAGTG
Reporter 1 14nt -12	GTcgACCCCCCACAGTG
Reporter 1 14nt -13	GTgCACCCCCCACAGTG
Reporter 1 14nt -14	GaCACCCCCCACAGTG
Reporter 2 20nt	GGGGCC ACTAGGGACAGGAT
Reporter 2 20nt -1	GGGGCC ACTAGGGACAGGAa
Reporter 2 20nt -2	GGGGCC ACTAGGGACAGGTt
Reporter 2 20nt -3	GGGGCC ACTAGGGACAGCaT
Reporter 2 20nt -4	GGGGCC ACTAGGGACAcGAT
Reporter 2 20nt -5	GGGGCC ACTAGGGACtGGAT
Reporter 2 20nt -6	GGGGCC ACTAGGGAgAGGAT
Reporter 2 20nt -7	GGGGCC ACTAGGGtCAGGAT
Reporter 2 20nt -8	GGGGCC ACTAGGgCACAGGAT
Reporter 2 20nt -9	GGGGCC ACTAGGgGACACAGGAT
Reporter 2 20nt -10	GGGGCC ACTAcGGACACAGGAT
Reporter 2 20nt -11	GGGGCC ACTtGGGACACAGGAT
Reporter 2 20nt -12	GGGGCC ACaAGGGACAGGAT
Reporter 2 20nt -13	GGGGCC AgTAGGGACACAGGAT
Reporter 2 20nt -14	GGGGCC tCTAGGGACACAGGAT
Reporter 2 14nt	ACTAGGGACAGGAT
Reporter 2 14nt -1	ACTAGGGACAGGAa
Reporter 2 14nt -2	ACTAGGGACAGGtT
Reporter 2 14nt -3	ACTAGGGACACGcAT
Reporter 2 14nt -4	ACTAGGGACAcGAT
Reporter 2 14nt -5	ACTAGGGACtGGAT
Reporter 2 14nt -6	ACTAGGGAgAGGAT
Reporter 2 14nt -7	ACTAGGGtCAGGAT
Reporter 2 14nt -8	ACTAGGgACAGGAT
Reporter 2 14nt -9	ACTAGGcGACAGGAT
Reporter 2 14nt -10	ACTAcGGACAGGAT

Reporter 2 14nt -11	ACTtGGGACAGGAT
Reporter 2 14nt -12	ACaAGGGACAGGAT
Reporter 2 14nt -13	AgTAGGGACAGGAT
Reporter 2 14nt -14	tCTAGGGACAGGAT
Reporter 3 20nt	GAAGAGAGACAGTACATGCC
Reporter 3 20nt -1	G AAGAGA GACAGTACATGCCg
Reporter 3 20nt -2	G AAGAGA GACAGTACATGCgC
Reporter 3 20nt -3	G AAGAGA GACAGTACATGgCC
Reporter 3 20nt -4	G AAGAGA GACAGTACATcCCC
Reporter 3 20nt -5	G AAGAGA GACAGTACAaGCC
Reporter 3 20nt -6	G AAGAGA GACAGTAcTGCC
Reporter 3 20nt -7	G AAGAGA GACAGTAgATGCC
Reporter 3 20nt -8	G AAGAGA GACAGTtCATGCC
Reporter 3 20nt -9	G AAGAGA GACAGaACATGCC
Reporter 3 20nt -10	G AAGAGA GACAcTACATGCC
Reporter 3 20nt -11	G AAGAGA GAActGTACATGCC
Reporter 3 20nt -12	G AAGAGA GAgAGTACATGCC
Reporter 3 20nt -13	G AAGAGA GtCAGTACATGCC
Reporter 3 20nt -14	G AAGAGA cACAGTACATGCC
Reporter 3 14nt	GGACAGTACATGCC
Reporter 3 14nt -1	G GACAGTACATGCCg
Reporter 3 14nt -2	G GACAGTACATGCgC
Reporter 3 14nt -3	G GACAGTACATGgCC
Reporter 3 14nt -4	G GACAGTACATcCCC
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Reporter 3 14nt -8	G GACAGTtCATGCC
Reporter 3 14nt -9	G GACAGaACATGCC
Reporter 3 14nt -10	G GACAcTACATGCC
Reporter 3 14nt -11	G GACtGTACATGCC
Reporter 3 14nt -12	G GAgAGTACATGCC
Reporter 3 14nt -13	G GtCAGTACATGCC
Reporter 3 14nt -14	G cACAGTACATGCC

#### Reporter PCR Primers

TGAAGCGCATGAACTCTTT  
GTTGAAAACGACGGCCAGT

#### qPCR Primers

Target	Forward Primer	Reverse Primer
Beta-actin	CATGTACGTTGCTATCCAGCCTCCTTAATGTCACGCACGAT	
MIAT	TGGCTGGGGTTGAACCTT	AGGAAGCTGTTCCAGACTGC
ACTC1	ATGTGTGACGACGAGGAG/	CACGATGGACGGGAAGAC
TTN	TGTTGCCACTGGTCTAAAC	ACAGCAGTCTCTCCGCTTC
HBG	AGATGCCACAAAGCACCTG	CTGCAGTCACCATTCTGC

#### Endogenous Indel PCR

Primers for PCR 1:

CTACACGACGCTTCCGATCTTAAGGCGAactcgatgtgcgtgcgg ACTC1.N701.Adapter.F  
 CTACACGACGCTTCCGATCTCGTACTAGactcgatgtgcgtgcgg ACTC1.N702.Adapter.F  
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 CTACACGACGCTTCCGATCT TAGGCATG actcgatgtgcgtgcgg ACTC1.N706.Adapter.F  
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 CTACACGACGCTTCCGATCTCGTACTAGGGTTCAAGGCTTCTGCG MIAT.N702.Adapter.F  
 CTACACGACGCTTCCGATCTAGGCAGAAGGTTCAAGGCTTCTGCG MIAT.N703.Adapter.F

CTACACGACGCTTCCGATCTCCTGAGCGGTTCAAGGCTCTGCG ( MIAT.N704.Adapter.F  
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 CTACACGACGCTTCCGATCTTAGGCATGGGTTCAAGGCTCTGCG ( MIAT.N706.Adapter.F  
 GCTGAACCGCTTCCGATCTTAGATCGCCTCCTAACCGCGCCCCAC MIAT.N501.Adapter.R  
 GCTGAACCGCTTCCGATCTCTCTATCTCTAACCGCGCCCCAG MIAT.N502.Adapter.R  
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 GCTGAACCGCTTCCGATCT TATCTCT CGTTCAGAACGAGT ( HBG1.N503.R  
 GCTGAACCGCTTCCGATCT AGAGTAGA CGTTCAGAACGAGT ( HBG1.N504.R  
 GCTGAACCGCTTCCGATCT GTAAGGAG CGTTCAGAACGAGT ( HBG1.N505.R  
 GCTGAACCGCTTCCGATCT ACTGCATA CGTTCAGAACGAGT ( HBG1.N506.R

Primers for PCR 2:

NGS_F	AATGATAACGGCGACCACCGAGATCTACACTTTCCCTACAGACGCTTCCGATCT
NGS_R	CAAGCAGAACGGCATACGAGATCGGTCTGGCATTCTGCTGAACCGCTTCCGATCT
Amplicon primers	
ACTC1 223 bp	
ACTC1.F	actcagatgtgctgctgcgg
ACTC1.R	CCCTTCTGGGTGTTGGTG
MIAT 242 bp	
MIAT.F	GGTCAGGCTTCTGCGCCC
MIAT.R	CTCCTAACCGCGCCCCAGA
TTN 244 bp	
TTN.F	GCCCACAAGTGTATCTACTGTC
TTN.R	GCCCTGGGTGATTGGCTGTC
HBG	
HBG1.F	tagcagtatcccttgggg
HBG1.R	CGTTCAGAACGAGTGTgt

#### Endogenous gRNA

ACTC1 20nt -249	TGGCGCCCTGCCCTCTGCTG
ACTC1 16nt -249	GCCCTGCCCTCTGCTG
ACTC1 14nt -249	CCTGCCCTCTGCTG
MIAT 20nt -220	ATGCAGGAGGCTGAGCGCAC
MIAT 16nt -220	GGGAGGCTGAGCGCAC
MIAT 14nt -220	GAGGCTGAGCGCAC
TTN 20nt -172	CCTTGGTGAAGTCTCCTTG
TTN 16nt -172	GGTGAAGTCTCCTTG
TTN 14nt -172	TGAAGTCTCCTTG
HBG 20nt -100	CTTGACCAATAGCCTTGACA
HBG 16nt -100	ACCAATAGCCTTGACA
HBG 14nt -100	CAATAGCCTTGACA

**Kill switch gRNA**

5' Cas9 cut in ORF5

5' Cas9 cut in ORF6

GTACTGATAAGGCTGACTTG

CTAGGCTGTCAAATCCGG

**Supplementary Note 3: Plasmid dosages and combinations used in synthetic circuits experiments**

**Plasmids Fig 2a**

1	2	3	4	5
50	200	100	50	70

ng

pEmPTY up to 500 ng or as needed between groups

pConst_EBFP	1
U6_14n gRNA-a	2
pConst_Gal4VP16	3
CRP8 (a)_EYFP	4
pConst_Cas9VPR	5
pConst_dCas9	6

**Plasmids Fig 2b**

1	2	3	4	5	6	7
50	100	50	100	150	100	50

ng

pEmPTY up to 600 ng or as needed between groups

pConst_Gal4VP16	1
U6_14nt gRNA-a	2
CRP8(or CRP-a)_EYFP	3
U6_14nt gRNA-c	4
CAP(c)_tdTomato	5
pConst_Cas9VPR	6
pConst_EBFP	7

**Plasmids Fig 2c (similar with Supp Fig 10, 11)**

1	2	3	4	5	6	7
100	50	50	25	25	250	200

ng

pEmPTY up to 700 ng or as needed between groups

pConst_Csy4	1
TRE_irFP-20nt gRNA-a	2
pConst_EBFP	3
pConst_Gal4vp16-2A-rtta	4
U6_14ntgRNA-a	5
CAP-8(a)_EYFP	6
pConst_Cas9VPR	7
pConst_dCas9VPR	8

Plasmids	<i>Fig 2d</i>	1	2	3	4	5	6
ng		50	50	50	100	100	150
ng		7	8				
<b>pEmPTY up to 650 ng or as needed between groups</b>							
pConst_rtTA3			1				
pConst_mKate			2				
TRE_TALER14			3				
CR(taler)U6_14nt gRNA-b			4				
CR(b)U6_14nt gRNA-a			5				
CAPa_EYFP			6				
pConst_Cas9VPR			7				
U6_20nt gRNA T1			8				

Plasmids	<i>Supp Fig 9</i>	1	2	3	4	5
ng		50	200	100	50	70
<b>pEmPTY up to 500 ng or as needed between groups</b>						
pConst_EBFP		1				
U6_14n gRNA-a		2				
pConst_Gal4VP16		3				
CRP(1 to 8)_EYFP		4				
pConst_Cas9VPR		5				

Plasmids	<i>Supp Fig 12</i>	1	2	3	4	5	6
ng		50	100	50	100	150	100
ng		7	8	9			
pConst_Gal4VP16		1					
U6_14nt gRNA-a		2	<b>pEmPTY up to 800 ng or as needed between groups</b>				
CRPa_EYFP		3					
U6_14nt gRNA-c		4					
CAP(c)_tdTomato		5					
pConst_Cas9VPR		6					
pConst_EBFP		7					
U6_20nt gRNA( Cas9-5)		8					

U6\_20nt gRNA (Cas9-6) 9

Plasmids Supp Fig 13

	<b>1</b> 100 ng	<b>2</b> 50	<b>3</b> 100	<b>4</b> 50	<b>5</b> 50	<b>6</b> 200
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**pEmPTY up to 550 ng or as needed between groups**

pConst_Gal4VP16-2A-rtTA3	1
pConst_EBFP	2
TRE_TALER	3
CRP-T_EYFP	4
pConst_Cas9VPR	5
U6_20nt gRNA T (gRNAs that cleave within TALER14 Sequences)	6

Plasmids Supp Fig 14

	<b>1</b> 50	<b>2</b> 50	<b>3</b> 100	<b>4</b> 50	<b>5</b> 50	<b>6</b> 200
<b>ng</b>	<b>7</b>	<b>8</b>				
<b>ng</b>	25	25				
	50	50				
	75	75				

**pEmPTY as needed between groups**

pConst_Gal4VP16-2A-rtTA3	1
pConst_EBFP	2
TRE_TALER14	3
CR(taler)U6_14nt gRNA-b	4
CRP-b_EYFP	5
pConst_Cas9VPR	6
U6_20nt gRNA T1	7
U6_20nt gRNA T4	8

#### **Supplementary Note 4 :**

Sequences of DNA constructed and used in for synthetic circuits in this study.

Library of CRPs:

UAS

gRNA-a target site

minimal CMV promoter

CRP1

CTCCGAATTCTCGACAGATCTCATGTGATTACGCCAAGCTACGGGCGGAG  
TAATGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGA  
GCGGAGTACTGTCCTCCGAGCGGAGTTCTGTCCTCCGAGCGGAGACTCTA  
GATACCTCATCAGGAACATGTTGGATTCTAGGCCTGTACGGTGGGAGGCC  
TATATAAGCAGAGCTCGTTAGTGAACCCTCAGATCGCTCGAGTACCTCAT  
CAGGAACATGTTGGATCCAATTCA

CRP2

GGCTCCGAATTCACCTGCTGACAGGTGCTCCGAATTCTCGACAGATCTCAT  
GTGATTACGCCAAGCTACGGGCGGAGTACTGTCCTCCGAGCGGAGTACTG  
TCCTCCGATACCTCATCAGGAACATGTTGGCGGAGTACTGTCCTCCGAGC  
GGAGTACTGTCCTCCGAGAGCGGAGACTCTAGAGAATTCTAGGCCTGTACG  
GTGGGAGGCCTATAAATACCTCATCAGGAACATGTTGGTCGTTAGTGAAC  
CGTCAGATCGCTACCTCATCAGGAACATGTTGGGATCCAATTCTACCTCAT  
CAGGAACATGTTGGGACCGCTTCAGTGCAGGTGAGCTT

CRP3

GCTGACAGGTGCTCCGAATTCTCGACAGATCTCATGTGATTACGCCAAGCT  
ACGGGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTA  
CTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTTCTGTCCTCCGAGC  
GGAGACTCTAGAGAATTCTAGGCCTGTACGGTGGGAGGCCTATAAATAC  
TCATCAGGAACATGTTGGTCGTTAGTGAACCCTCAGATCGCTACCTCATC  
AGGAACATGTTGGGATCCAATTCAACCCTCAGTGCAGGTGAGCTTCAA  
GTTTGTACAAAAAGCAG

CRP-4

TCCGAATTCTCGACAGATCTCATGTGATTACGCCAAGCTACGGGCGGAGT  
ACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAG  
CGGAGTACTGTCCTCCGAGCGGAGTTCTGTCCTCCGAGCGGAGACTCTAGA  
GAATTCTAGGCCTGTACGGTGGGAGGCCTATAAATACCTCATCAGGAACA  
TGTTGGTCGTTAGTGAACCCTCAGATCGCTACCTCATCAGGAACATGTTG

**G**GATCCAATTCTACCTCATCAGGAACATGTTGGGACCGCTTCAGTGCAGGT  
GAGCT

CRP-5

TCTCATGTGATTACGCCAAGCTACGGGCGGAGTACTGTCCTCCAGCAGGAG  
TAATGTCCTCCGAGTACCTCATCAGGAACATGTTGGAGCAGGAGTTCTGTCC  
TCCGAGCGGAGACTCTAGAGAATTCTAGGCGTGTACGGTGGGAGGCCTATA  
**TAATACCTCATCAGGAACATGTTGGCTTAGTGAACCGTCAGATGCC**TA  
CCTCATCAGGAACATGTTGGGATCCAATTCTACCTCATCAGGAACATGTTGG  
GACC

CRP-6

TCCGAATTCTCGACAGATCTCATGTGATTACGCCAAGCTACGGGCGGAGT  
ACTGTCCTCCGAGCGGAGTACTGTCCTCCAGAGCGGAGTTCTGTCCCTCCG  
AGCGGAGACTCTAGAGAATTCTAGGCGTGTACGGTGGGAGGCCTATAAT  
ACCTCATCAGGAACATGTTGGTCGTTAGTGAACCGTCAGATGCC**TACCTC**  
ATCAGGAACATGTTGGGATCCAATTGACCGCTTCAGTGCAGGTGA

CRP-7

GAATTCTCGACAGATCTCATGTGATTACGCCAAGCTACGGGCGGAGTACT  
GTCCTCCGAGCGGAGTACTGTCCTCCGAGAGCGGAGTTCTGTCCCTCCGAG  
CGGAGACTCTAGAGAATTCTAGGCGTGTACGGTGGGAGGCCTATAATAC  
**CTCATCAGGAACATGTTGGCTTAGTGAACCGTCAGATGCC****TACCTCAT**  
CAGGAACATGTTGGGATCCAATTCTACCTCATCAGGAACATGTTGGGACCG  
CTTCAGTGCAGGTGAGCTA

CRP-8

AGATCTCATGTGATTACGCCAAGCTACGGGCGGAGTACTGTCCTCCGAGCG  
GAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTC  
CGAGCGGAGTTCTGTCCCTCCGAGCGGAGACTCTAGAGAATTCTAGGCGTGT  
**ACGGTGGGAGGCCTATAA****TACCTCATCAGGAACATGTTGG**TCGTTAGT  
GAACCGTCAGATGCCCTCGAGGGATCCAATT

**U6-14ntgRNA-a**

AAGGTCGGGCAGGAAGAGGGCCTATTCCATGATTCTCATATTGCATA  
TACGATACAAGGCTGTTAGAGAGATAATTAGAATTAAATTGACTGTAAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAACCT  
GAAAGTATTCGATTCTTGCTTATATATCTTGTGGAAAGGACGAAACAC  
CG**CATCAGGAACATGT**GTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCT  
AGTCGTTATCAACTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

**CRP-8(or CRP-a)\_EYFP**

GATATCAACTTGTATAGAAAAGTTGGCTCGAATTCTCGACAGATCTCAT  
GTGATTACGCCAAGCTACGGG**CGGAGTACTGTCCTCCGAGCGGAGTACTG**  
**TCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGG**  
**AGTTCTGTCCTCCGAGCGGAGACTCTAGAGAATTCTAGGCCTGTACGGTGG**  
**GAGGCCTATAATACCTCATCAGGAACATGTTGGTCGTTAGTGAACCGTC**  
**AGATCGCCTCGAGATCCAATTGACCCAAGTTGTACAAAAAAAGCAGGCTG**  
AATCCACCGGTGCGCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGG  
GGTGGTGCCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGT  
TCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC  
CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCTGGCCCACCC  
CGTGACCAACCTCGCTACGGCCTGCAGTGCTGCCCGTACCCCGACC  
ACATGAAGCAGCACGACTTCAAGTCCGCCATGCCGAAGGCTACGTCC  
AGGAGCGCACCATCTTCAAGGACGACGGCAACTACAAGACCCCGGCC  
GAGGTGAAGTCGAGGGCGACACCCTGGTAACCGCATCGAGCTGAAGGG  
CATCGACTTCAAGGAGGACGGCAACATCCTGGGGACAAGCTGGAGTACA  
ACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCA  
TCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG  
CTCGCCGACCACTACCAGCAGAACACCCCCATCGCGACGGCCCCGTGCT  
GCTGCCGACAACCAACTACCTGAGCTACCAAGCTGAGCAAAGACCC  
CAACGAGAACCGCGATCACATGGCCTGCTGGAGTTCGTGACCGCCGCCG  
GGATCACTCTGGCATGGACGAGCTGTACAAGTAATACCCAGCTTCTTGT  
CAAAGTGGTACCGTGAATTCACTCCTCAGGTGCAGGCTGCCTATCAGAAG  
GTGGTGGCTGGTGGCCAATGCCCTGGCTACAAATACCAACTGAGATCTT  
TTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCTTGAGCATCTGA  
CTTCTGGCTAATAAAAGGAAATTATTTTCAATTGCAATAGTGTGTTGGAATT  
TTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTAAAACATC  
AGAATGAGTATTGGTTAGAGTTGGCAACATATGCCCATATGCTGGCTGC  
CATGAACAAAGGTTGGCTATAAAGAGGTATCAGTATATGAAACAGCCCC  
GCTGTCCATTCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTT  
TATATTTGTTTGTGTTATTTTTCTTAACATCCCTAAATTTCTTACAT  
GTTTACTAGCCAGATTTTCCCTCTCCTGACTACTCCAGTCAGTGT  
CCCTCTCTTATGGAGATCCCTGACCTGCAGCCAGCTGGCTAAT  
CATGGTCATAGCTGTTCTGTGAAATTGTTATCCGCTACAATTCCA

Sequences of 14ntgRNA-c and CAP-c are provided in Supplementary Note 1 (Reporter 1-14).

#### TRE\_irFP-csy4-20nt gRNA-a-csy4-PolyA

GCTCCGAATTCTCGAGTTACTCCCTATCAGTGATAGAGAACGTATGTCGA  
GTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTACTCCCTATCAGT  
GATAGAGAACGTATGTCGAGTTACTCCCTATCAGTGATAGAGAACGTATGT  
CGAGTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTATCCCTATC  
AGTGATAGAGAACGTATGTCGAGTTACTCCCTATCAGTGATAGAGAACGTA  
TGTGAGGTAGGCCTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTA  
GTGAACCGTCAGATCGCCTGGAGATTGAGCTCGGTACCCGGGATCCTC  
TAGTCAGCTGACGCGTGCTAGTGGCGCGCCGAATCGACCCAAGTTGTAC

AAAAAAAGCAGGCTGAGGATCCCCGCCACCATGGCTGAAGGATCCGTCGCC  
AGGCAGCCTGACCTCTTGACCTGCGACGATGAGCCGATCCATATCCCCGGT  
GCCATCCAACCGCATGGACTGCTGCTGCCCTGCCGCCGACATGACGAT  
CGTTGCCGGCAGCGACAACCTTCCGAACTCACCGGACTGGCGATCGGCG  
CCCTGATCGGCCGCTCTGCCGCCGATGTCTCGACTCGGAGACGCACAAAC  
CGTCTGACGATGCCCTGGCCGAGCCCGGGCCGTCGGAGCACCGA  
TCACTGTGGCTTCACGATGCGAAAGGACGCAGGCTCATCGGCTCCTGGC  
ATCGCCATGATCAGCTCATCTTCCTCGAGCTCGAGCCTCCCCAGCGGGACG  
TCGCCGAGCCGCAGGCAGTCTTCCGCCGACCAACAGCGCCATCCGCCGC  
CTGCAGGCCCGAAACCTTGGAAAGCGCCTGCGCCGCCGGCGCAAG  
AGGTGCGGAAGATTACCGGCTCGATCGGGTGTGATGATCTATCGCTCGCCT  
CCGACTTCAGCGGCGAAGTGTGATCGCAGAGGATCGGTGCGCCGAGGTGAG  
TCAAAACTAGGCCTGCACTATCCTGCCTCAACC GTGCCGCCAGGCCCGT  
CGGCTCTATACCATAACCCGGTACGGATCATTCCGATATCAATTATCGGC  
CGGTGCCGGTCACCCAGACCTCAATCCGGTCACCGGGGCCGATTGAT  
CTTAGCTTCGCCATCCTGCGCAGCGTCTGCCCGTCCATCTGGAATTATG  
CGCAACATAGGCATGCACGGCACGATGTCGATCTCGATTTGCGCGGCCGA  
GCGACTGTGGGGATTGATCGTTGCCATACCGAACGCCGTACTACGTCGA  
TCTCGATGGCCGCCAACGCTGCGAGCTAGTCGCCAGGTTCTGGCCTGGC  
AGATCGGCGTGATGGAAGAGTGAGTTTAGAGCTAGAAATAGCAAGTTAAA  
ATAAGGCTAGTCGTTATCAACTTGGAAAAAGTGGCACCGAGTCGGTGCTTTT  
TTTCGTTCACTGCCGTAGGCAGCTAACGAAACAGGTACCTCATCAGGAACA  
TGTGTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCGTTATCAA  
CTTGAAAAAGTGGCACCGAGTCGGTCTTTTCTGTTCACTGCCGTATAGG  
CAGCTAAGAAТААССАГСТТСТГТААААГТGGTACGCGTGAATTCACT  
СТСАГГТGCAGGСТGCСТАСАГАААГГТGGTGGCTGGTGTGGCCAATGCC  
СТGGСТСААААААССААСТGAGАТСТТТСССТСТGССААААААТATGGGG  
АААТCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAААААААГГАААААА  
TTTCATTGCAATAGTGTGTTGGATTTTGTGTCTCACTCGGAAGGACAT  
ATGGGAGGGCAAATCATTAAAACATCAGAATGAGTATTGGTTAGAGTT  
GGCAACATATGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAG  
AGGTCACTAGTATATGAAACAGCCCCCTGCTGTCCATTCTTATTCCATAGA  
AAAGCCTTGACTTGAGGTTAGATTTTATATTTGTTGTGTTATTTTT  
CTTAACATCCCTAAAATTTCTTACATGTTTACTAGCCAGATTTCTCC  
TCTCCTGACTACTCCAGTCAGCTGTCCTCTTCTTATGGAGATCCCT  
CGACCTGCAGCC

CR(b)U6\_14nt gRNA-a

AAGGTGGGCAGGAAGAGGGCCTATTCCATGATTCTTCATATTGCATA  
TACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTGACTGTAAACACA  
AAGATATTAGTACAAACACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAACCT  
GAAATATAGAACCGATCCTCCCATTGGTATATATTATAGAACCGATCCTCCC  
ATTGGCTTGTGGAAAGGACGAAACACCGCATCAGGAACATGTGTTAAGAG

CTATGCTGGAAACAGCAGAAATAGCAAGTTAAATAAGGCTAGTCGTTATC  
AACTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

CR(taler)U6\_14nt gRNA-b

AAGGTGGGCAGGAAGAGGGCCTATTCCCATGATTCCCTCATATTGCATA  
TACGATACAAGGCTGTTAGAGAGATAATTAGAATTAAATTGACTGTAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTAAAATGGACTATCATGCTTACCGTAACCT  
GAAATACCCCTCTCCGCTTATATATTACCCCTCTCCGCTTCTCTTGTGGA  
AAGGACGAAACACCGACCGATCCTCCATGTTAAGAGCTATGCTGGAAAC  
AGCAGAAATAGCAAGTTAAATAAGGCTAGTCGTTATCAACTTGAAAAAGT  
GGCACCGAGTCGGTGCTTTTT

TRE\_TALER14\_PolyA

CTCCGAATTGCCCTCAGGTCCGAGGTCTAGACGAGTTACTCCCTATCA  
GTGATAGAGAACGATGTCGAGTTACTCCCTATCAGTGATAGAGAACGTATG  
TCGAGTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTACTCCCTA  
TCAGTGATAGAGAACGTATGTCGAGTTATCCCTATCAGTGATAGAGAACGT  
ATGTCGAGTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGGTAGGCG  
TGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTAGTGAACCGTCAGAT  
CGCAAAGGGCGAATTGACCCAAGTTGTACAAAAAAAGCAGGCTGATTACC  
GGAGAATTCCAATTGACCATGACCGGTTGTCGACCATTGTCGCCGCGCAGG  
CCAAGTCCTGCCCGCGAGCTCTGCCCGGACCCCAACCGGATAGGGTTCA  
GCCGACTGCAGATCGTGGGTGTCGCGCTGCTGGCAGCCCTGATGGGATG  
GCTTGCCCGCTCGCGGACGGTGCCCAGCAGCTCAGCGATCTGCTCCGTCC  
GCGCCCTCACCTGCGTTCTGGCGGGCAGCTCAGCGATCTGCTCCGTCC  
GTTCGATCCGTCGCTTCTTGATACATCGCTTGTGATTCGATGCCCTGCCGTC  
GGCACGCCGCATACAGCGGCTGCCAGCAGAGTGGATGAGGCCAATC  
GGCTCTGCGTGCAGCCGATGACCCGCCACCCACCGTGCCTGCTGTCA  
CTGCCCGCGGCCGCCGCGGCCAAGCCGGCCCCGCGACGGCGTGC  
GCAACCCCTCCGACGCTTCGCCGCCGCGCAGGTGGATCTACGCACGCTCG  
GCTACAGTCAGCAGCAGCAAGAGAAGATCAAACCGAAGGTGCGTTGACA  
GTGGCGCAGCACCACGAGGCACTGGTGGCCATGGTTACACACCGCGCA  
CATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCA  
CGTATCAGCACATAATCACGGCGTTGCCAGAGGCACACACGAAGACATCG  
TTGGCGTCGGCAAACAGTGGTCCGGCGACCGCCCTGGAGGCCCTGCTC  
ACGGATGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACACAGGCCA  
ACTTGTGAAGATTGCAAAACGTGGCGCGTGACCGCAATGGAGGCAGTGC  
ATGCATCGCGCAATGCACTGACGGGTGCCCTGAACCTGACCCGGAC  
CAAGTGGTGGCTATGCCAGCAACATTGGCGGCAAGCAAGCGCTCGAAC  
GGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCCGG  
ACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAA  
ACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCC  
GGACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCAAGCAAGCGCTCG  
AAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACT

CCGGACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCAAGCAAGCGCT  
CGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGACCATGGCCTGA  
CTCCGGACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCAAGCAAGCG  
CTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGACCATGGCCT  
GACCCGGACCAAGTGGTGGCTATGCCAGCAACGGTGGCGGCAAGCAAG  
CGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGACCATGGC  
CTGACTCCGGACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCAAGCA  
AGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGACCATG  
GCCTGACCCCGGACCAAGTGGTGGCTATGCCAGCAACGGTGGCGGCAAG  
CAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGACCA  
TGGCCTGACTCCGGACCAAGTGGTGGCTATGCCAGCCACGATGGCGGCA  
AGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGGAC  
CATGGCCTGACTCCGGACCAAGTGGTGGCTATGCCAGCCACGATGGCGG  
CAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCAGG  
ACCATGGCCTGACCCCGGACCAAGTGGTGGCTATGCCAGCAACAATGGC  
GGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGCCA  
GGACCATGGCCTGACTCCGGACCAAGTGGTGGCTATGCCAGCCACGATG  
GCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTGTC  
CAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATGCCAGCAACGG  
TGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGTG  
GCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATGCCAGCAAC  
GGTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGT  
GTGCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTATGCCAGGCC  
ACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGT  
CTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATGCCAG  
CAACGGTGGCGGCAAGCAAGCGCTCGAAAGCATTGTGGCCCAGCTGAGCC  
GGCCTGATCCGGCGTTGGCCGCGTTGACCAACGACCACCTCGTCGCCCTG  
GCCTGCCCTGGCGGACGTCTGCCATGGATGCAGTGAAAAGGGATTGCC  
GCACGCGCCGGATTGATCAGAAGAGTCATGCCGTATTGGCGAACGCA  
CGTCCCCTCGCGTTGCCGACTACCGCAAGTGGTCGCGTGTGGAGTTT  
TCCAGTGCCACTCCCACCCAGCGTACGCATTGATGAGGCCATGACGCAGT  
TCGGGATGAGCAGGAACGGGTTGGTACAGCTCTTCGCAAGAGTGGCGTC  
ACCGAACTCGAAGCCCGCGGTGGAACGCTCCCCCAGCCTCGCAGCGTTG  
GGACCGTATCCTCCAGGCATCAGGGATGAAAAGGGCAAACCGTCCCCTA  
CTTCAGCTAAACACCGGATCAGCGTCTTGCATGCATTGCCGATTGCG  
TGGAGCGTGACCTTGATGCCCGCCAGCCAATGCACGAGGGAGATCAGACG  
CGGGCAAGCAGCCGTAACCGTCCGATCGGATCGTGTGTGTACCGGCC  
CTCCGCACAGCAGGCTGTGAGGTGCGCGTCCCGAACAGCGCGATGCGC  
TGCATTGCCCTCAGCTGGAGGGAAAACGCCCGCGTACCGGATCTGG  
GGCGGCCTCCGGATCCCAGCCCCAAGAAGAAGAGAAAGGTGGAGGCCA  
GCGGTGGCGGCTCAAAGCTTGGTGGCGGCTCAACTAGTTAAGGGCCCGGC  
GCGCCTAAGGTACCCCGGGTAAC TGAAAATCCACCGGATCTAGATAACTG  
ATCTACCCAGCTTCTTGATCAAAGTGGTACCGCGTGAATTCACTCCTCAGGT  
GCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTC  
ACAAATACCACTGAGATCTTTCCCTCTGCCAAAAATTATGGGACATCAT  
GAAGCCCCTTGAGCATCTGACTCTGGCTAATAAAGGAATTATTTATTGATTG

CAATAGTGTGTTGGAATTGGTGTCTCTCACTCGGAAGGACATATGGGAG  
GGCAAATCATTAAAACATCAGAATGAGTATTGGTTAGAGTTGGCAACAT  
ATGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTCATC  
AGTATATGAAACAGCCCCCTGCTGTCCATTCCATTAGAAAAGCCTT  
GACTTGAGGTTAGATTTTATATTTGTTGTATTGTTCTTAACA  
TCCCTAAAATTCCTACATGTTACTAGCCAGATTTCTCCTCCTG  
ACTACTCCCAGTCATAGCTGCCCTCTTATGGAGATC

**U6\_20nt gRNA-T1**

AAGGTCGGGCAGGAAGAGGGCCTATTCCATGATTCCATATTGCATA  
TACGATACAAGGCTTAGAGAGAGATAATTAGAATTAAATTGACTGTAAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAACCT  
GAAAGTATTCGATTCTGGCTTATATATCTTGTGGAAAGGACGAAACAC  
CGCTTGGTCCGGAGTCAGGCCAGTTAGAGCTAGAAATAGCAAGTTAAA  
TAAGGCTAGTCGTTATCAACTGAAAAAGTGGCACCGAGTCGGTGCTTTT  
TT

**U6\_20nt gRNA-T2**

AAGGTCGGGCAGGAAGAGGGCCTATTCCATGATTCCATATTGCATA  
TACGATACAAGGCTTAGAGAGAGATAATTAGAATTAAATTGACTGTAAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAACCT  
GAAAGTATTCGATTCTGGCTTATATATCTTGTGGAAAGGACGAAACAC  
CGGAGGCCTTGCTACGGATGCGTTAGAGCTAGAAATAGCAAGTTAAA  
TAAGGCTAGTCGTTATCAACTGAAAAAGTGGCACCGAGTCGGTGCTTTT  
TT

**U6\_20nt gRNA-T3**

AAGGTCGGGCAGGAAGAGGGCCTATTCCATGATTCCATATTGCATA  
TACGATACAAGGCTTAGAGAGAGATAATTAGAATTAAATTGACTGTAAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAACCT  
GAAAGTATTCGATTCTGGCTTATATATCTTGTGGAAAGGACGAAACAC  
CGGTGGCTATGCCAGCAACATGTTTAGAGCTAGAAATAGCAAGTTAAA  
AAGGCTAGTCGTTATCAACTGAAAAAGTGGCACCGAGTCGGTGCTTTT  
T

**U6\_20nt gRNA-T4**

AAGGTCGGGCAGGAAGAGGGCCTATTCCCATGATTCCCTCATATTCATA  
TACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTGACTGTAAACACA  
AAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTCTGGTAGTT  
TGCAGTTTAAAATTATGTTTAAAATGGACTATCATATGCTTACCGTAACCTT  
GAAAGTATTCGATTTCTGGCTTATATATCTTGTGGAAAGGACGAAACAC  
**CGCGTGGGGTGTCTGCCCTCGCTTTAGAGCTAGAAATAGCAAGTTAAA**  
**TAAGGCTAGTCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT**  
TT