Supplementary Information

Programmable mammalian translational modulators by CRISPR-associated proteins

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*Corresponding author
**Lead contact

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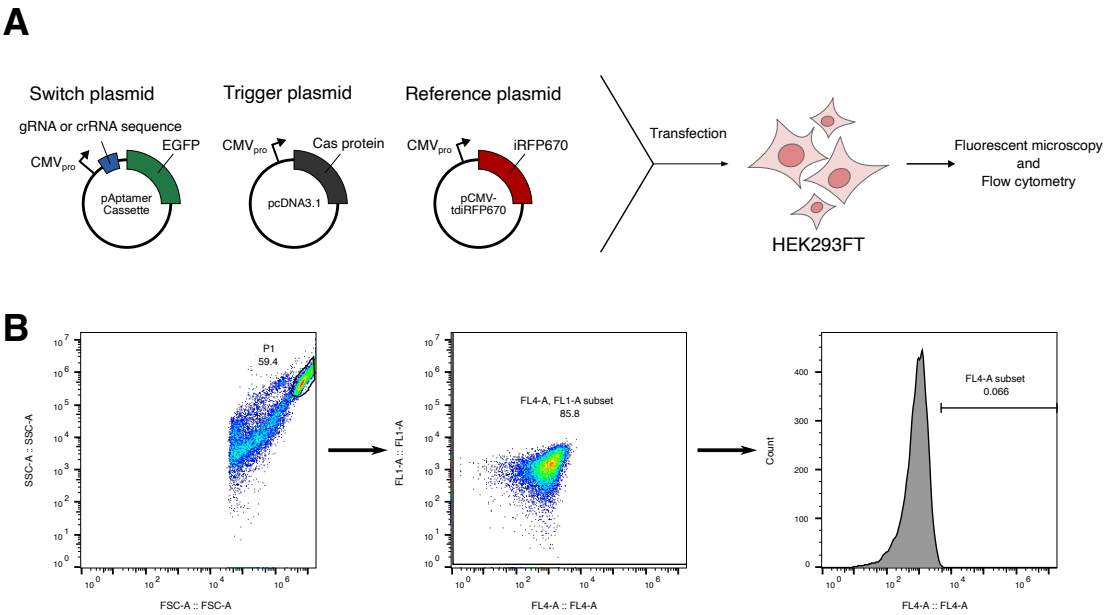
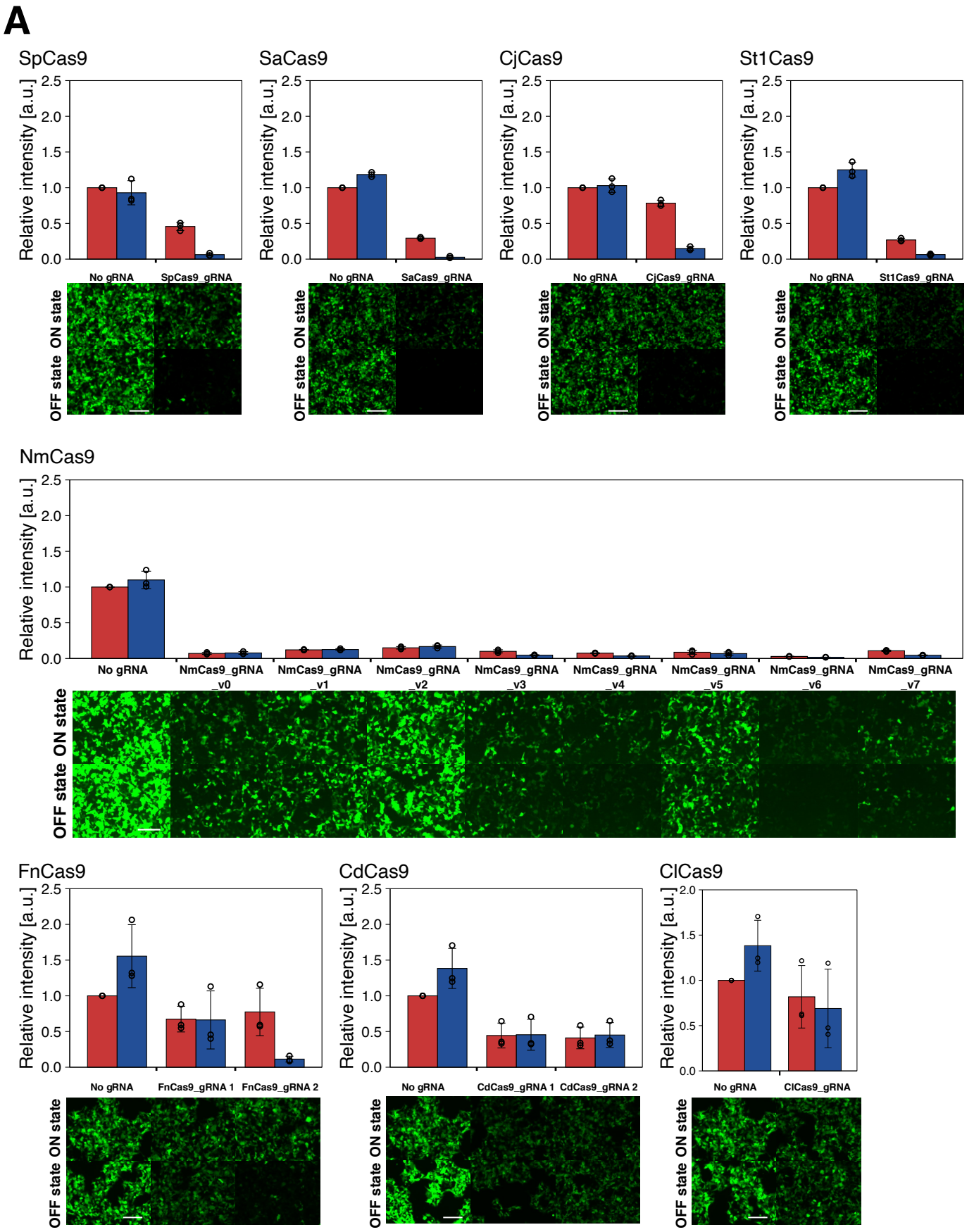


Supplementary Figure 1|

Schematic diagrams of experimental procedures in plasmid transfection.

(A) Schematic diagrams of standard procedure. Three plasmids (switch plasmid, trigger plasmid, and reference plasmid) were transfected into HEK293FT cells. The cells were analyzed by fluorescent microscopy and flow cytometer. (B) Gating procedures of flow cytometry. Flow cytometry datasets were analyzed using FlowJo version10.5.3 (see also Methods section). Live cells were gated in the forward scatter (FSC) versus side scatter (SSC) plot to eliminate debris. The remaining P1-positive events were plotted in the FL1-A (EGFP expression, Y-axis) versus FL4-A (iRFP670 expression, X-axis) and events on each axis line were ruled out by the gate (FL4-A, FL1-A subset). Then, the FL4-A subset gate was generated based on untransfected samples and thereby transfection-positive (FL4-positive) populations were defined.


A
SpCas9
SaCas9
CjCas9
St1Cas9
Relative intensity [a.u.]
2.5
2.0
1.5
1.0
0.5
0.0
No gRNA
SpCas9_gRNA
SaCas9_gRNA
CjCas9_gRNA
St1Cas9_gRNA
OFF state ON state
NmCas9
No gRNA
NmCas9_gRNA _v0
NmCas9_gRNA _v1
NmCas9_gRNA _v2
NmCas9_gRNA _v3
NmCas9_gRNA _v4
NmCas9_gRNA _v5
NmCas9_gRNA _v6
NmCas9_gRNA _v7
FnCas9
CdCas9
ClCas9
No gRNA
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FnCas9_gRNA 2
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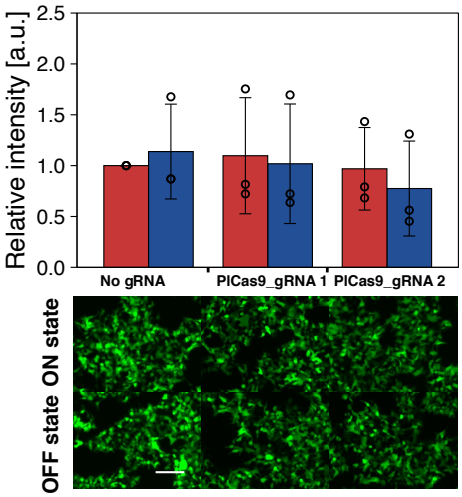
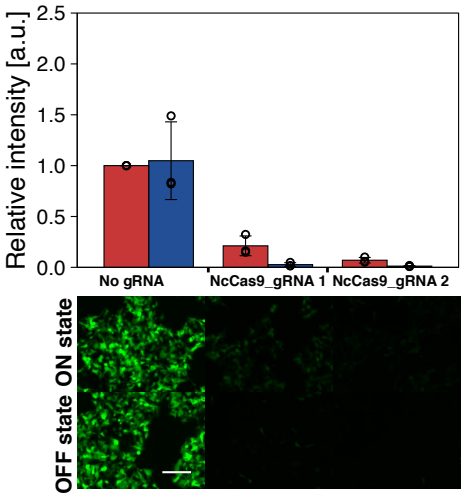
PlCas9



NcCas9



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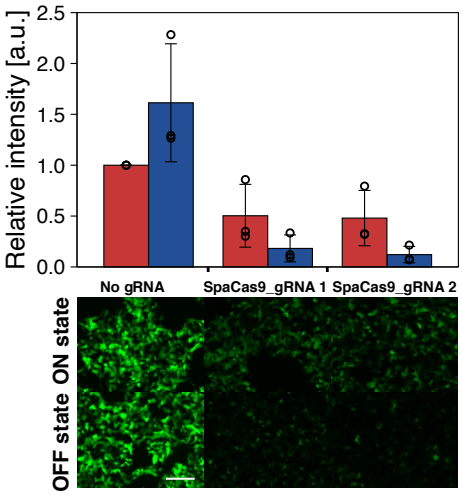

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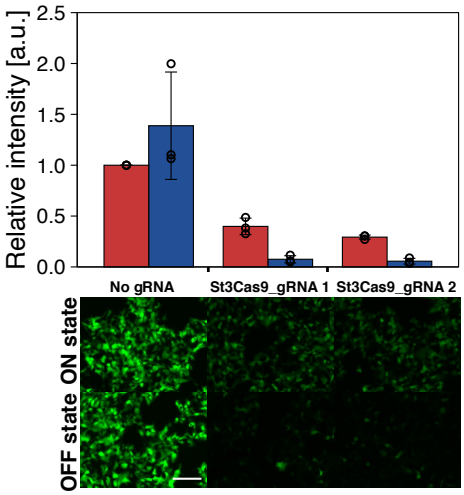

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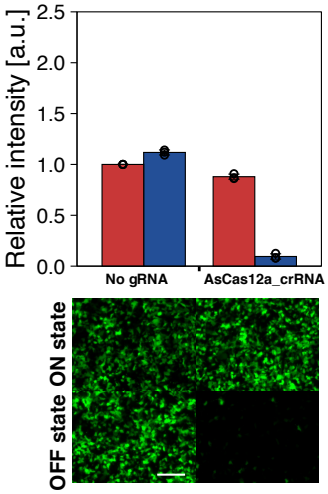

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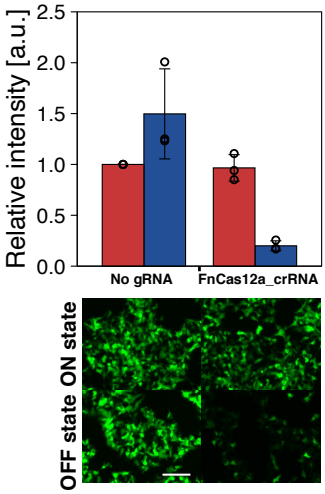

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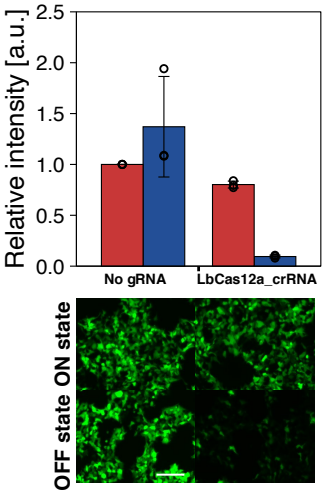

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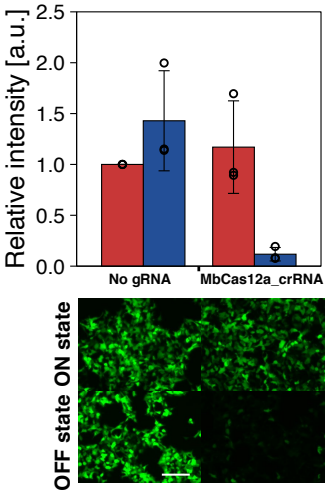
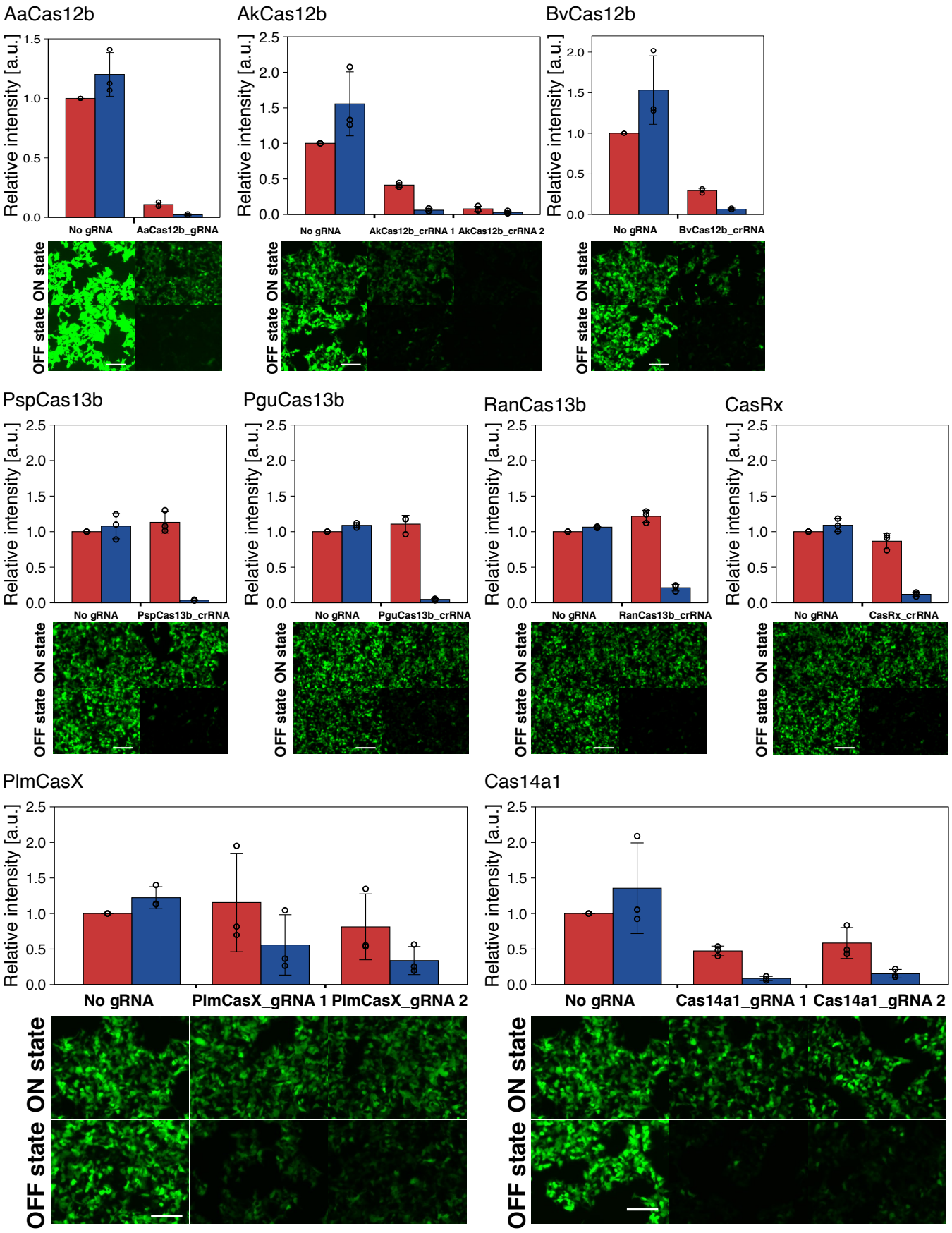
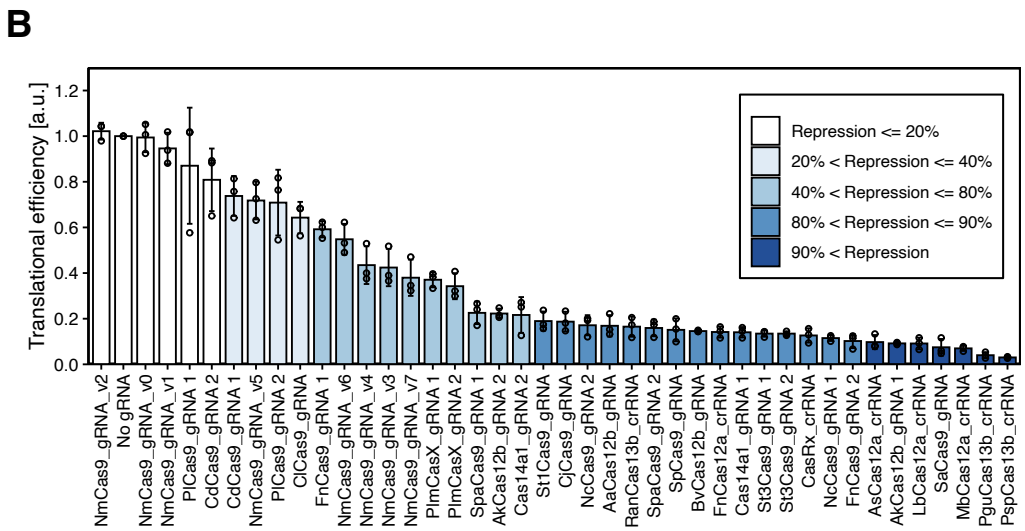


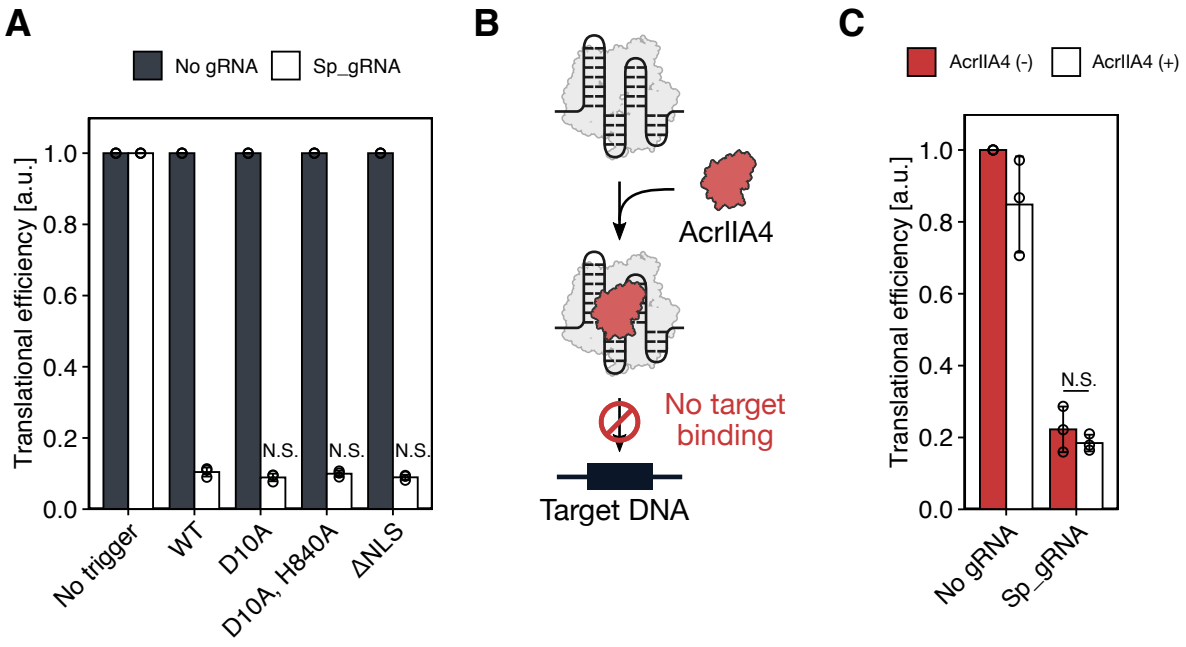

AaCas12b
AkCas12b
BvCas12b
PspCas13b
PguCas13b
RanCas13b
CasRx
PlmCasX
Cas14a1
Relative intensity [a.u.]
No gRNA
AaCas12b_gRNA
AkCas12b_crRNA 1
AkCas12b_crRNA 2
BvCas12b_crRNA
PspCas13b_crRNA
PguCas13b_crRNA
RanCas13b_crRNA
CasRx_crRNA
PlmCasX_gRNA 1
PlmCasX_gRNA 2
Cas14a1_gRNA 1
Cas14a1_gRNA 2
OFF state
ON state



Supplementary Figure 2|

Original validation data of each Cas-responsive switch related to Figure 1C.

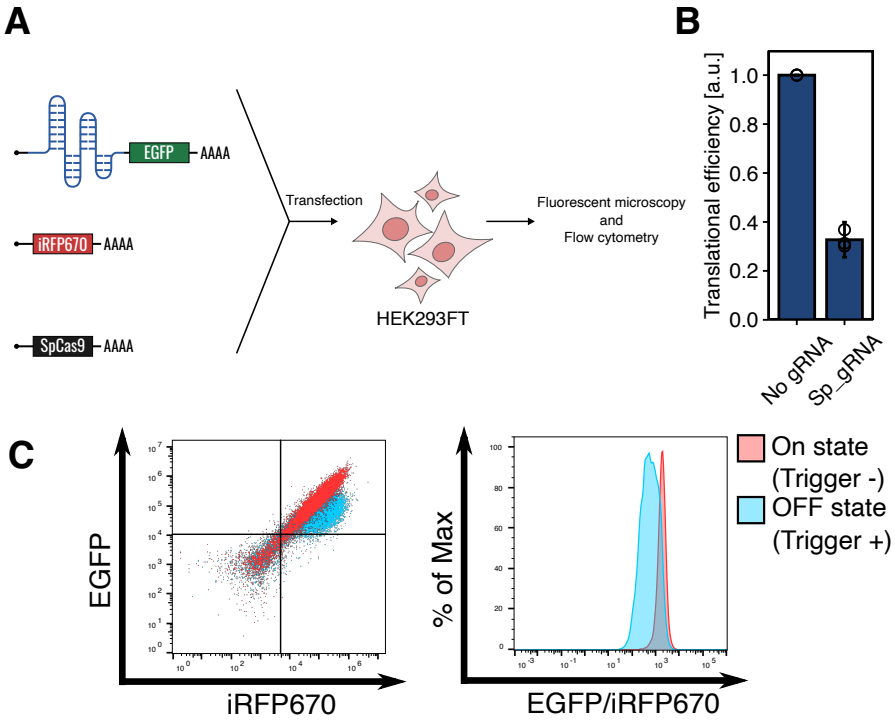
(A) Validated trigger Cas proteins are stated at the top. The bar charts show the calculated reporter expression levels. Error bars represent standard deviations. ON (without trigger) and OFF (with trigger) states are colored as red and blue, respectively. Scale bars in cell images represent 200 μm. (B) Translational efficiencies of the reporter expression levels in all tested Cas-responsive mRNA OFF switches. Data are represented as the mean ± SD from three independent experiments.



Supplementary Figure 3|

SpCas9-responsive reporter reduction is independent of transcriptional regulations.

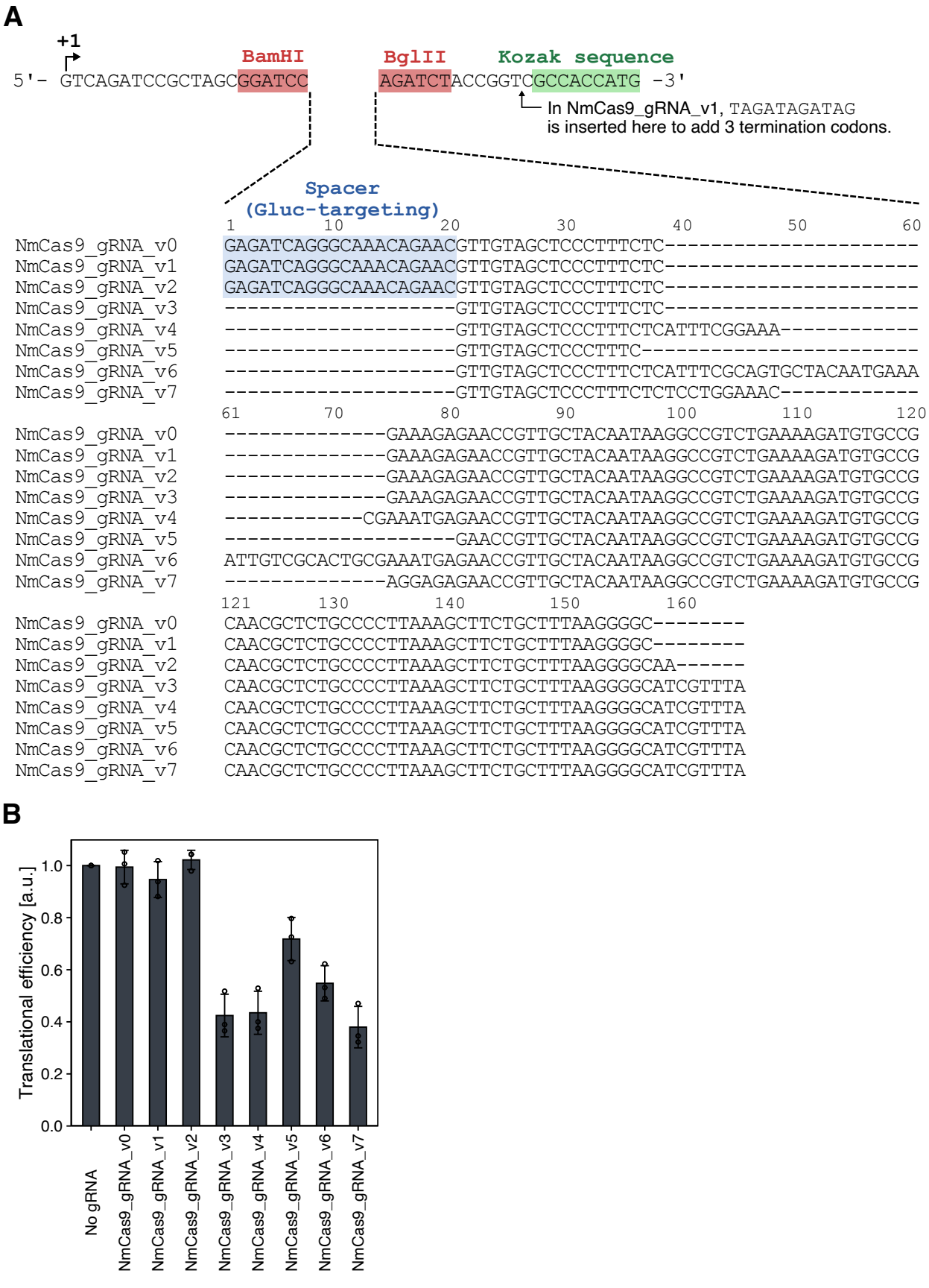
(A) The translational efficiencies in SpCas9 mutants transfection. Each SpCas9 mutant did not affect the translational efficiency of Sp_gRNA compared with wild type (WT). (B) Schematic diagram of AcrIIA4 mediated binding inhibition between DNA and SpCas9 RNP complex. (C) The translational efficiencies in co-transfection of AcrIIA4. Error bars represent standard deviations. Statistical analyses were carried out with unpaired two-tailed Student's *t*-test. N.S., not significant ($p \geq 0.05$).



Supplementary Figure 4|

The performance of SpCas9-responsive switch in RNA transfection.

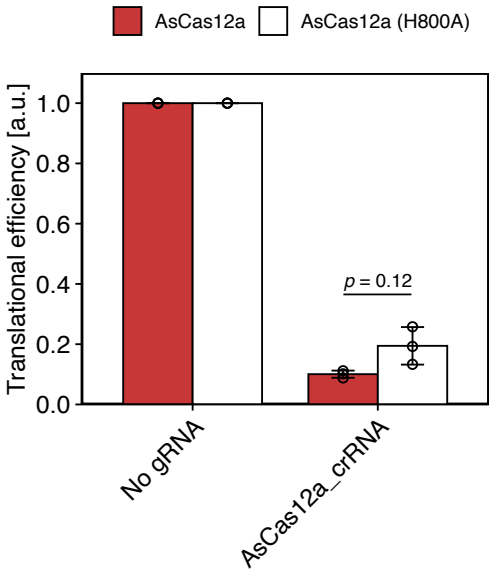
(A) Schematic diagrams of experimental procedures in RNA transfection. Three mRNAs (switch mRNA, trigger mRNA (SpCas9 mRNA), and reference mRNA (iRFP670 mRNA)) were transfected into HEK293FT cells. The cells were analyzed by fluorescent microscopy and flow cytometer. (B) The translational efficiencies in RNA transfection of SpCas9-responsive switches. Error bars represent standard deviations. (C) Representative dot plots and the histograms of expression level from switch mRNA with (blue) and without (red) SpCas9 mRNA.



Supplementary Figure 5|

The performances of NmCas9-responsive switches.

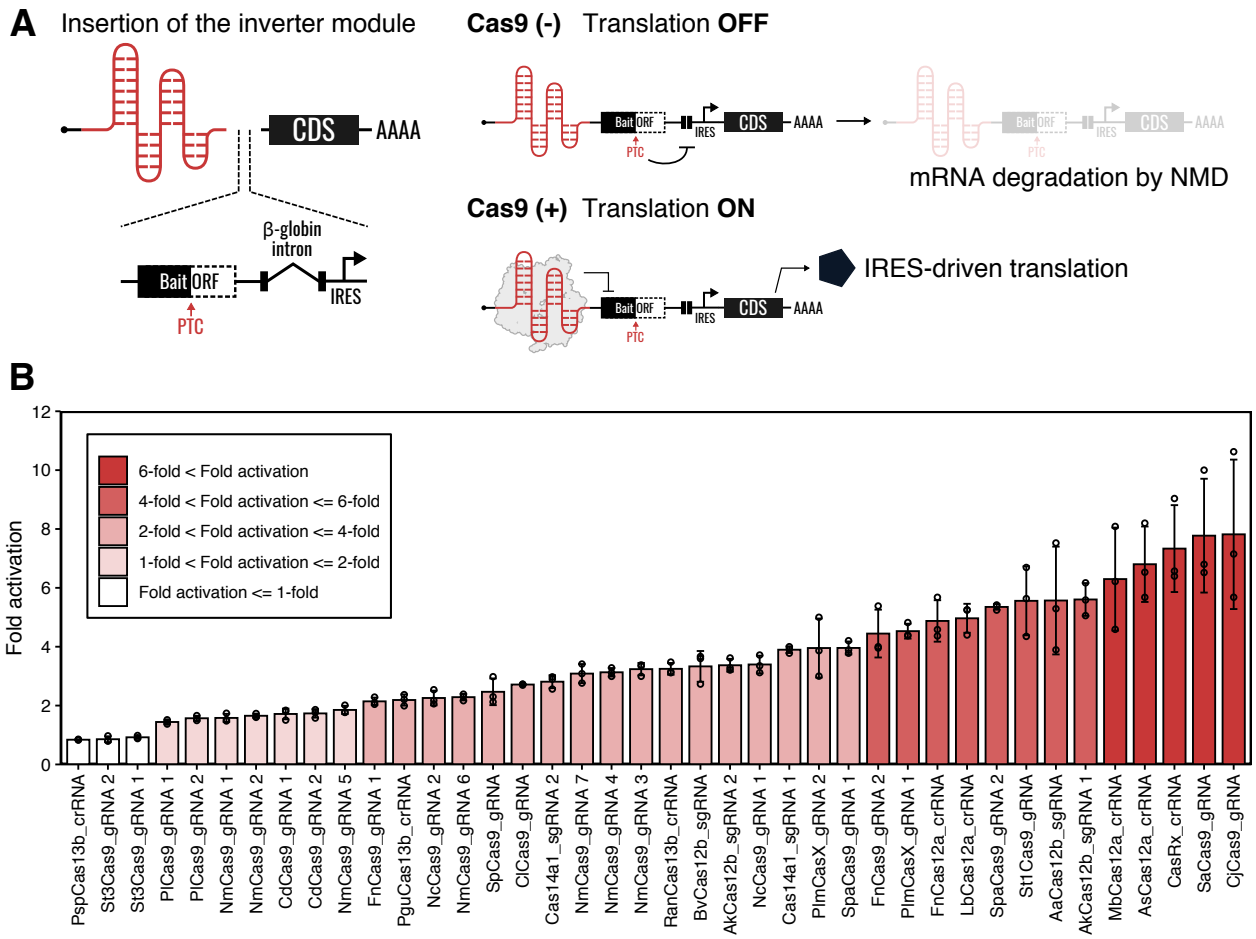
(A) Sequence variations of NmCas9-responsive switches. The surrounding sequence of the inserted gRNA sequence is also shown. (B) The translational efficiencies of NmCas9-responsive switches. The switch plasmid with NmCas9_gRNA_v7 sequence showed the best performance and was used for the following OFF switch experiments. Error bars represent standard deviations.



Supplementary Figure 6|

Effect of RNase ability in AsCas12a-responsive switch

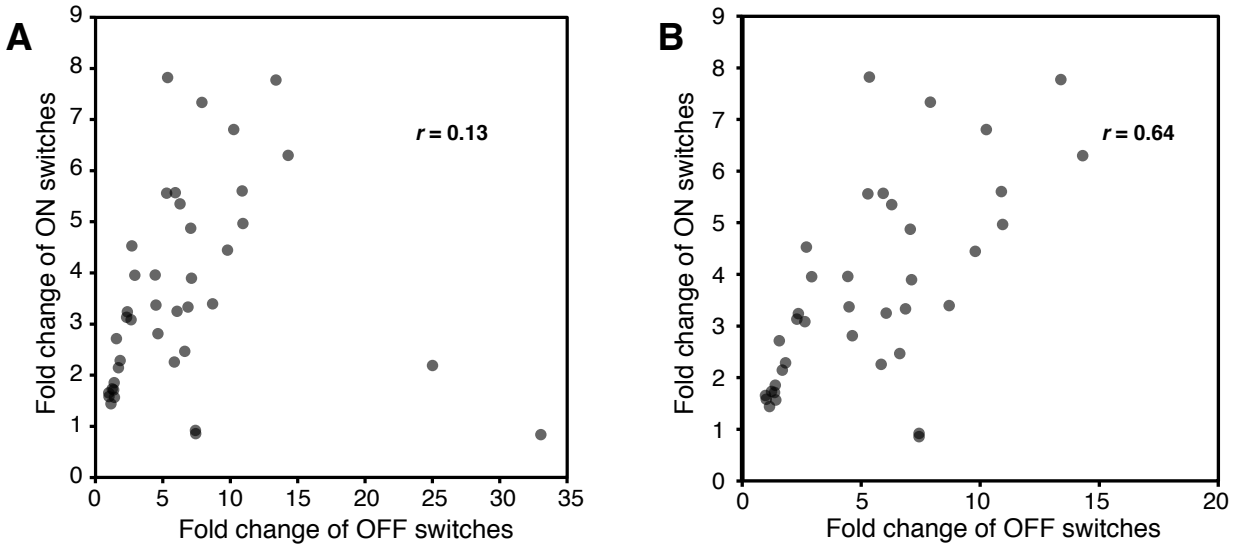
Comparison of translational repression efficiency between wild type AsCas12a and its mutant (H800A). AsCas12a (H800A) lacks RNase activity but maintains other enzyme activity. Values were normalized by the value in the condition when WT and No gRNA reporter were co-transfected. Data are represented as the mean ± SD from three independent experiments. Error bars represent standard deviations. Statistical analyses were carried out with unpaired two-tailed Student's *t*-test.



Supplementary Figure 7|

Cas proteins serve as the triggers of RNA-inverters.

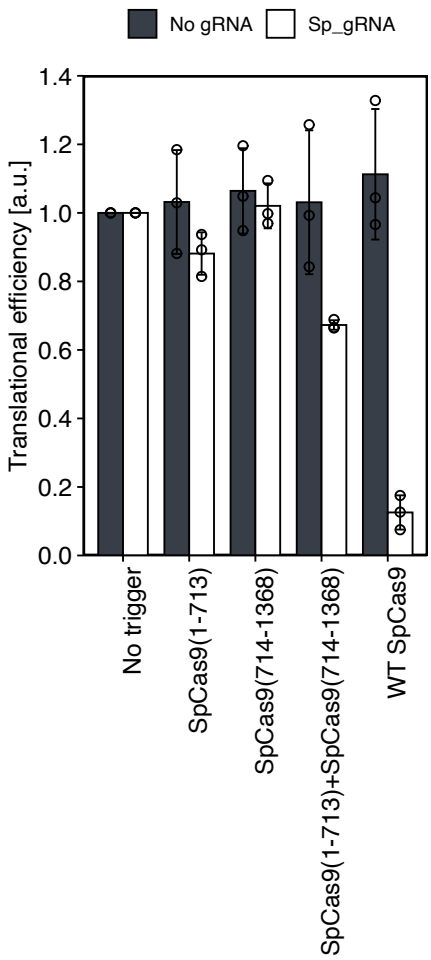
(A) Design of Cas-responsive mRNA ON switches with RNA-inverter. Inverter module was inserted between the protein binding motifs for specific Cas proteins and CDS. The Cas protein binds to the sgRNA or crRNA and protects mRNA switches from the non-sense mediated mRNA decay (NMD). CDS: coding sequence. (B) Fold activations of the reporter expression levels in all tested Cas-responsive mRNA ON switches. Data are represented as the mean ± SD from three independent experiments.



Supplementary Figure 8|

Correlation of the performance in Cas-responsive OFF switches and ON switches

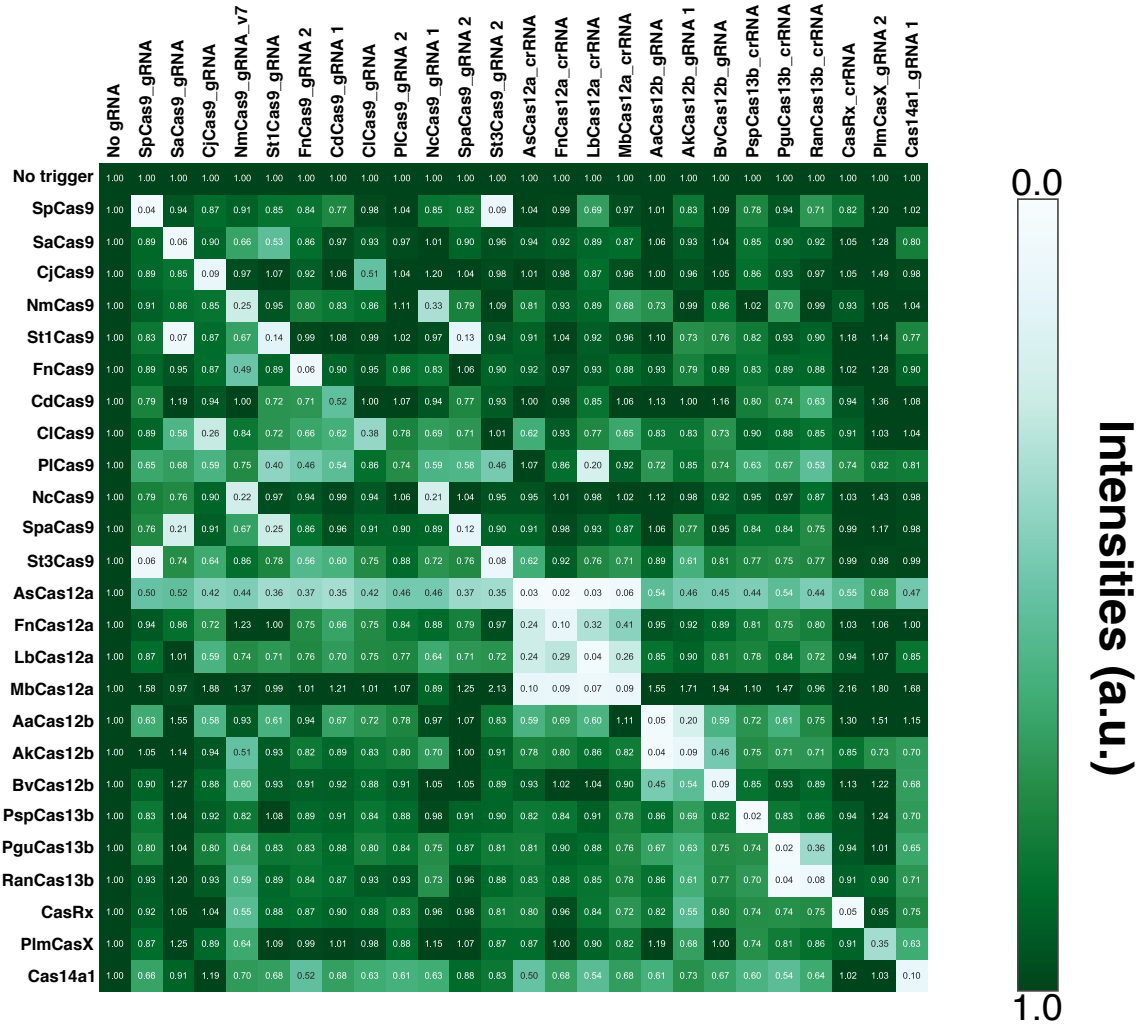
(A) Comparison of all Cas-responsive OFF and ON switches shown in Supplementary Figure 2B and Supplementary Figure 7B. Fold Change of OFF switch was calculated as inverse number of translational efficiencies. (B) Comparison of Cas-responsive OFF and ON switches without PspCas13b- and PguCas13b-responsive ones. Pearson correlation coefficients (*r*) are shown in the charts.



Supplementary Figure 9|

The translational repression with auto-assemble split-Cas9.

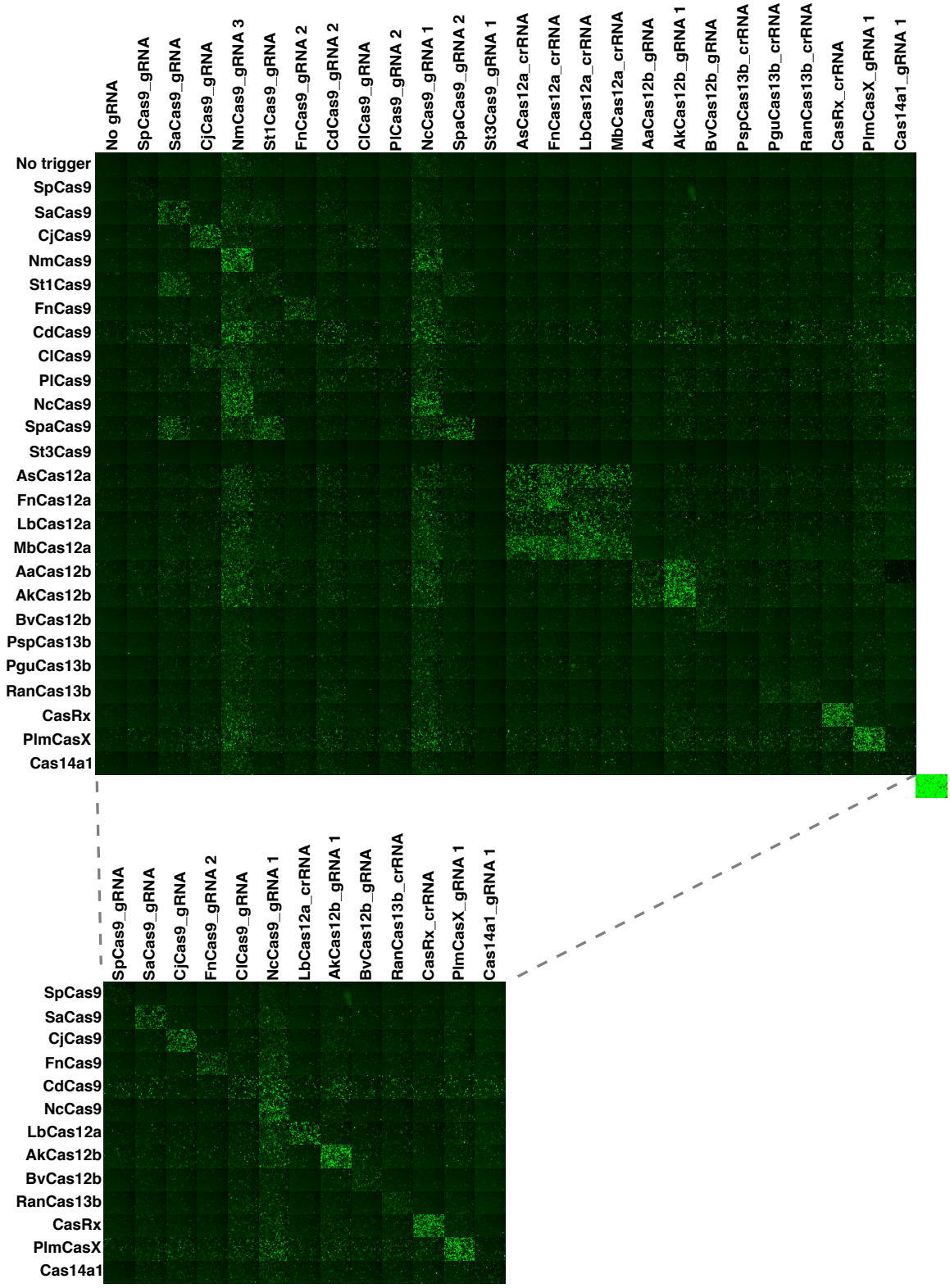
The translational repression is conducted only when both protein fragments exist. The reporter assay resulted in 33% repression when both of the input proteins exist. Data are represented as the mean ± SD from three independent experiments.



Supplementary Figure 10|

Orthogonality heat-map among representative Cas-responsive OFF switches related to Figure 3A.

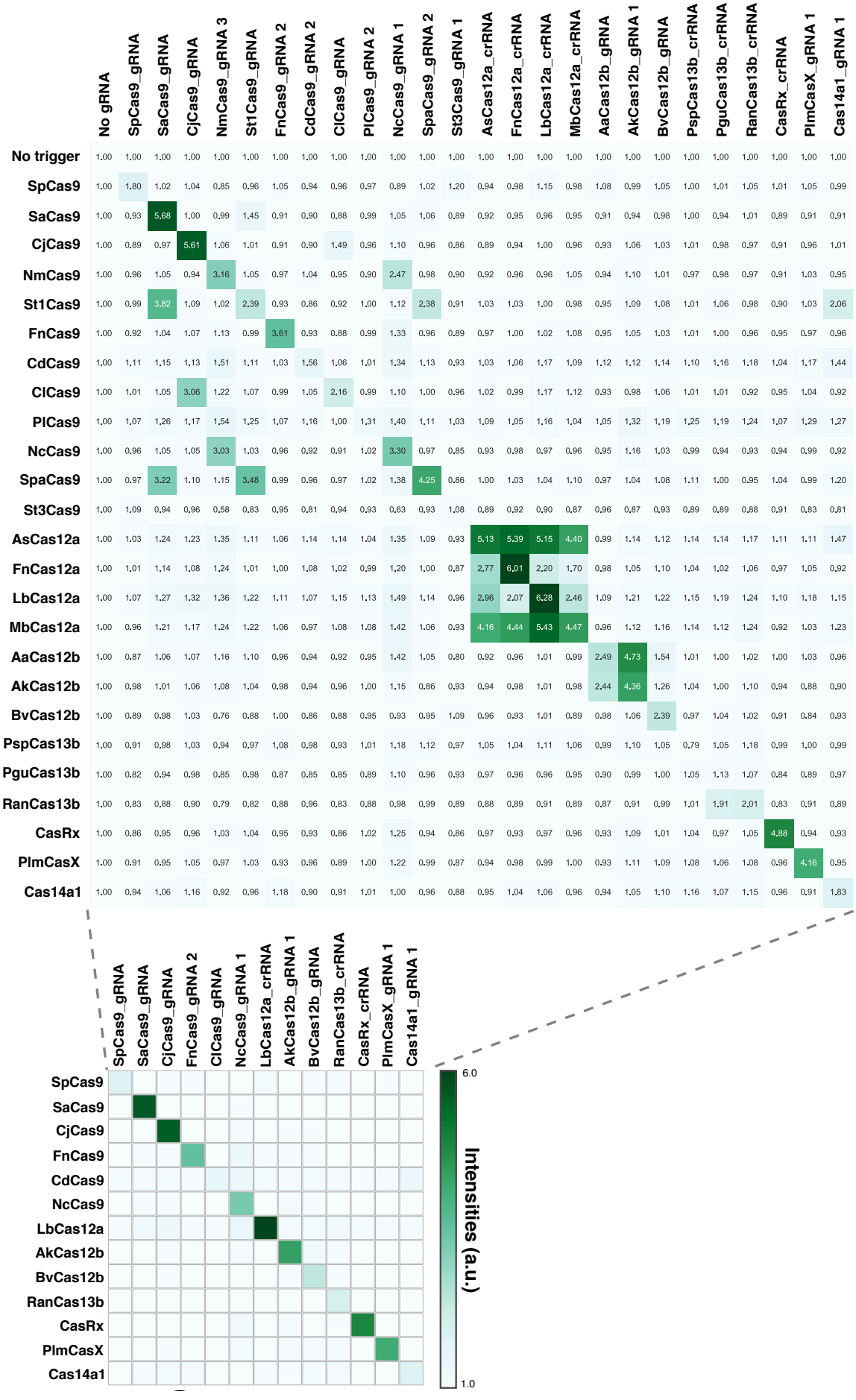
Heat-map of 25 × 25 orthogonality matrix of representative Cas proteins and Cas-responsive switches with the indicated mean values from Imaging analysis.



Supplementary Figure 11|

Fluorescent cell images of 25 × 25 orthogonality matrix of representative Cas proteins and Cas-responsive ON switches.

A fluorescent image of cells transfected with a plasmid constitutively expressing EGFP is shown at the bottom right of the upper panel. Pruned images in the bottom indicate mutually orthogonal sets.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SpCas9_gRNA
SaCas9_gRNA
CjCas9_gRNA
NmCas9_gRNA 3
St1Cas9_gRNA
FnCas9_gRNA 2
CdCas9_gRNA 2
ClCas9_gRNA
PlCas9_gRNA 2
NcCas9_gRNA 1
SpaCas9_gRNA 2
St3Cas9_gRNA 1
AsCas12a_crRNA
FnCas12a_crRNA
LbCas12a_crRNA
MbCas12a_crRNA
AaCas12b_gRNA
AkCas12b_gRNA 1
BvCas12b_gRNA
PspCas13b_crRNA
PguCas13b_crRNA
RanCas13b_crRNA
CasRx_crRNA
PlmCasX_gRNA 1
Cas14a1_gRNA 1
No trigger 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
SpCas9 1.00 1.80 1.02 1.04 0.85 0.96 1.05 0.94 0.96 0.97 0.89 1.02 1.20 0.94 0.98 1.15 0.98 1.08 0.99 1.05 1.00 1.01 1.05 1.01 1.05 0.99
SaCas9 1.00 0.93 5.68 1.00 0.99 1.45 0.91 0.90 0.88 0.99 1.05 1.06 0.89 0.92 0.95 0.96 0.95 0.91 0.94 0.98 1.00 0.94 1.01 0.89 0.91 0.91
CjCas9 1.00 0.89 0.97 5.61 1.06 1.01 0.91 0.90 1.49 0.96 1.10 0.96 0.86 0.89 0.94 1.00 0.96 0.93 1.06 1.03 1.01 0.98 0.97 0.91 0.96 1.01
NmCas9 1.00 0.96 1.05 0.94 3.16 1.05 0.97 1.04 0.95 0.90 2.47 0.98 0.90 0.92 0.96 0.96 1.05 0.94 1.10 1.01 0.97 0.98 0.97 0.91 1.03 0.95
St1Cas9 1.00 0.99 3.82 1.09 1.02 2.39 0.93 0.86 0.92 1.00 1.12 2.38 0.91 1.03 1.03 1.00 0.98 0.95 1.09 1.08 1.01 1.06 0.98 0.90 1.03 2.06
FnCas9 1.00 0.92 1.04 1.07 1.13 0.99 3.61 0.93 0.88 0.99 1.33 0.96 0.89 0.97 1.00 1.02 1.08 0.95 1.05 1.03 1.01 1.00 0.96 0.95 0.97 0.96
CdCas9 1.00 1.11 1.15 1.13 1.51 1.11 1.03 1.56 1.06 1.01 1.34 1.13 0.93 1.03 1.06 1.17 1.09 1.12 1.12 1.14 1.10 1.16 1.18 1.04 1.17 1.44
ClCas9 1.00 1.01 1.05 3.06 1.22 1.07 0.99 1.05 2.16 0.99 1.10 1.00 0.96 1.02 0.99 1.17 1.12 0.93 0.98 1.06 1.01 1.01 0.92 0.95 1.04 0.92
PlCas9 1.00 1.07 1.26 1.17 1.54 1.25 1.07 1.16 1.00 1.31 1.40 1.11 1.03 1.09 1.05 1.16 1.04 1.05 1.32 1.19 1.25 1.19 1.24 1.07 1.29 1.27
NcCas9 1.00 0.96 1.05 1.05 3.03 1.03 0.96 0.92 0.91 1.02 3.30 0.97 0.85 0.93 0.98 0.97 0.96 0.95 1.16 1.03 0.99 0.94 0.93 0.94 0.99 0.92
SpaCas9 1.00 0.97 3.22 1.10 1.15 3.48 0.99 0.96 0.97 1.02 1.38 4.25 0.86 1.00 1.03 1.04 1.10 0.97 1.04 1.08 1.11 1.00 0.95 1.04 0.99 1.20
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AsCas12a 1.00 1.03 1.24 1.23 1.35 1.11 1.06 1.14 1.14 1.04 1.35 1.09 0.93 5.13 5.39 5.15 4.40 0.99 1.14 1.12 1.14 1.14 1.17 1.11 1.11 1.47
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AaCas12b 1.00 0.87 1.06 1.07 1.16 1.10 0.96 0.94 0.92 0.95 1.42 1.05 0.80 0.92 0.96 1.01 0.99 2.49 4.73 1.54 1.01 1.00 1.02 1.00 1.03 0.96
AkCas12b 1.00 0.98 1.01 1.06 1.08 1.04 0.98 0.94 0.96 1.00 1.15 0.86 0.93 0.94 0.98 1.01 0.98 2.44 4.36 1.26 1.04 1.00 1.10 0.94 0.88 0.90
BvCas12b 1.00 0.89 0.98 1.03 0.76 0.88 1.00 0.86 0.88 0.95 0.93 0.95 1.09 0.96 0.93 1.01 0.89 0.98 1.06 2.39 0.97 1.04 1.02 0.91 0.84 0.93
PspCas13b 1.00 0.91 0.98 1.03 0.94 0.97 1.08 0.98 0.93 1.01 1.18 1.12 0.97 1.05 1.04 1.11 1.06 0.99 1.10 1.05 0.79 1.05 1.18 0.99 1.00 0.99
PguCas13b 1.00 0.82 0.94 0.98 0.85 0.98 0.87 0.85 0.85 0.89 1.10 0.96 0.93 0.97 0.96 0.96 0.95 0.90 0.99 1.00 1.05 1.13 1.07 0.84 0.89 0.97
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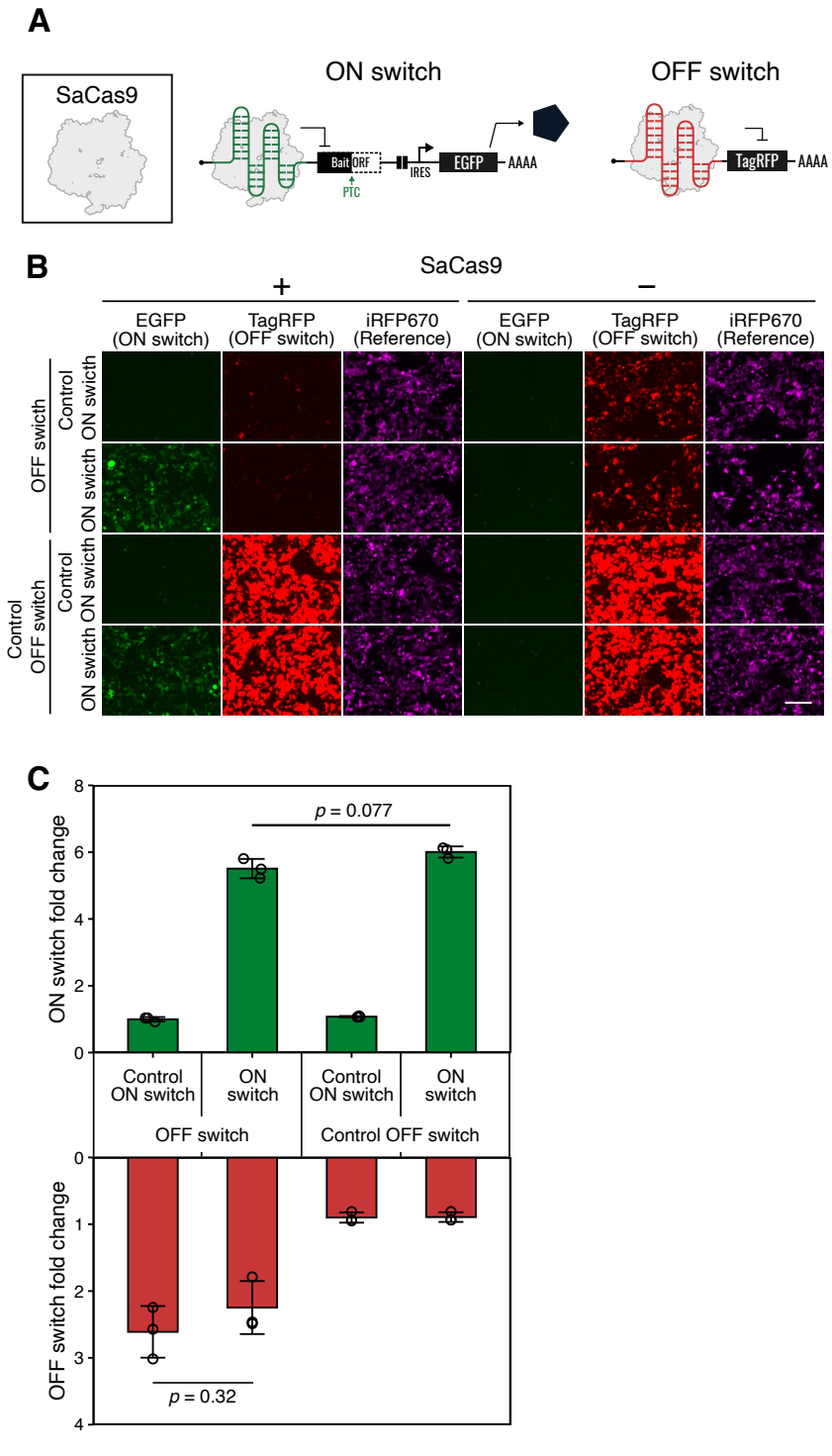
CasRx 1.00 0.86 0.95 0.96 1.03 1.04 0.95 0.93 0.86 1.02 1.25 0.94 0.86 0.97 0.93 0.97 0.96 0.93 1.09 1.01 1.04 0.97 1.05 4.88 0.94 0.93
PlmCasX 1.00 0.91 0.95 1.05 0.97 1.03 0.93 0.96 0.89 1.00 1.22 0.99 0.87 0.94 0.98 0.99 1.00 0.93 1.11 1.09 1.08 1.06 1.08 0.96 4.16 0.95
Cas14a1 1.00 0.94 1.06 1.16 0.92 0.96 1.18 0.90 0.91 1.01 1.00 0.96 0.88 0.95 1.04 1.06 0.96 0.94 1.05 1.10 1.16 1.07 1.15 0.96 0.91 1.83
SpCas9_gRNA
SaCas9_gRNA
CjCas9_gRNA
FnCas9_gRNA 2
ClCas9_gRNA
NcCas9_gRNA 1
LbCas12a_crRNA
AkCas12b_gRNA 1
BvCas12b_gRNA
RanCas13b_crRNA
CasRx_crRNA
PlmCasX_gRNA 1
Cas14a1_gRNA 1
SpCas9
SaCas9
CjCas9
FnCas9
CdCas9
NcCas9
LbCas12a
AkCas12b
BvCas12b
RanCas13b
CasRx
PlmCasX
Cas14a1
6.0
Intensities (a.u.)
1.0

Supplementary Figure 12|

Heat-map of 25 × 25 orthogonality matrix of representative Cas proteins and Cas-responsive ON switches.

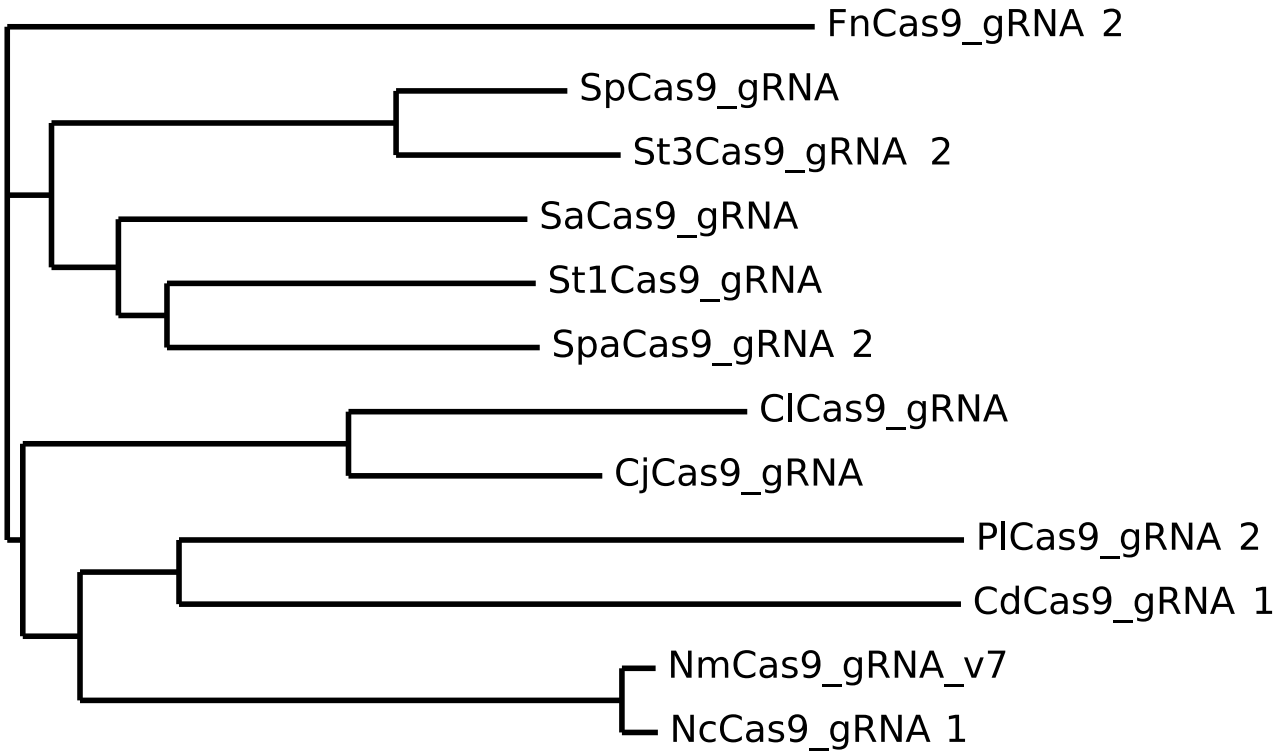
Values indicated the mean values in imaging analysis from three independent experiments performed on different days. Pruned images in the bottom indicate mutually orthogonal sets.


A
SaCas9
ON switch
Bait ORF
PTC
IRES
EGFP
AAAA
OFF switch
TagRFP
AAAA
B
SaCas9
+
−
EGFP (ON switch)
TagRFP (OFF switch)
iRFP670 (Reference)
EGFP (ON switch)
TagRFP (OFF switch)
iRFP670 (Reference)
OFF swicth
Control ON swicth
ON swicth ON swicth
Control OFF switch
Control ON swicth
ON swicth ON swicth
C
ON switch fold change
p = 0.077
Control ON switch
ON switch
Control ON switch
ON switch
OFF switch
Control OFF switch
OFF switch fold change
p = 0.32

Supplementary Figure 13|

Simultaneous regulation of translational activation and repression with SaCas9.

(A) The schematic diagram of the simultaneous translational regulation system. SaCas9 was used as the trigger protein. Translational activation and repression were monitored with EGFP and TagRFP, respectively. (B) Fluorescent microscopic images in each condition. Translational activation and repression were observed simultaneously when SaCas9 was transfected. Scale bar, 200 μm. (C) Quantitative data of reporter expression levels. The data were obtained by imaging analysis. iRFP670 was co-transfected as a reference and used to define a transfection-positive area. OFF switch: a plasmid for expressing TagRFP whose translation is repressed by SaCas9. ON switch: a plasmid for expressing EGFP whose translation is activated by SaCas9. Control OFF and ON switch: plasmids without the gRNA sequence for SaCas9. Error bars represent mean ± SD from three independent experiments. Statistical analyses were carried out with unpaired two-tailed Student's t-test.



Supplementary Figure 14|

Phylogenetic tree of Cas9 gRNA used in OFF switch orthogonality.

The sequences were aligned with the MUSCLE web tool. The pairs that showed crosstalk were grouped in closer clusters.

Supplementary Table 1

Information of Cas proteins used in this study.

	Name	Origin	RNA-guided nuclease activity in mammalian cells	Repression efficiency (%)	Size (bp)*1	gRNA length (nt)*2
1	SpCas9	*Streptococcus pyogenes*	+	85	4140	97
2	SaCas9	*Staphylococcus aureus*	+	93	3345	97
3	CjCas9	*Campylobacter jejuni*	+	81	3012	93
4	NmCas9	*Neisseria meningitidis*	+	v0: 1, v1: 5, v2: 0, v3: 58, v4: 57, v5: 28, v6: 45, v7: 62	3333	121
5	St1Cas9	*Streptococcus thermophilus*	+	81	3402	147
6	FnCas9	*Francisella novicida*	+ (with proxy-CRISPR)	1: 41, 2: 90	4950	1: 75, 2: 111
7	CdCas9	*Corynebacterium diphtheriae*	+	1:26, 2:19	3318	1: 98, 2: 97
8	ClCas9	*Campylobacter lari* CF89-12	-	36	3075	113
9	PlCas9	*Parvibaculum lavamentivorans*	-	1: 13, 2: 29	3177	1: 122, 2: 124
10	NcCas9	*Neisseria cinerea*	+ (with proxy-CRISPR)	1: 88, 2: 83	3312	1: 108, 2: 162
11	SpaCas9	*Streptococcus pasteurianus*	+	1: 77, 2: 84	3456	1: 83, 2: 82
12	St3Cas9	*Streptococcus thermophilus*	+	1: 87, 2: 87	4260	1: 80, 2: 94

	Name	Origin	RNA-guided nuclease activity in mammalian cells	Repression efficiency (%)	Size (bp)*1	gRNA length (nt)*2
1	AsCpf1 (AsCas12a)	*Acidaminococcus sp.* BV3L6	+	90	4062	43
2	FnCpf1 (FnCas12a)	*Francisella novicida* U112	+	86	4038	20
3	LbCpf1 (LbCas12a)	*Lachnospiraceae bacterium* ND2006	+	91	3822	21
4	MbCpf1 (MbCas12a)	*Moraxella bovoculi* 237	+	93	4257	21
5	AaCas12b	*Alicyclobacillus acidiphilus*	+	83	3447	137
6	AkCas12b	*Alicyclobacillus kakegawensis*	+	1: 91, 2: 78	3597	1: 117, 2: 137
7	BvCas12b	*Bacillus sp.* NSP2.1	+	85	3522	98

	Name	Origin	RNA-guided nuclease activity in mammalian cells	Repression efficiency (%)	Size (bp)*1	gRNA length (nt)*2
1	PspCas13b	*Prevotella sp.*	+	97	3429	66
2	PguCas13b	*Porphyromonas gulae*	+	96	3657	66
3	RanCas13b	*Riemerella anatipestifer*	+	83	3417	66
4	CasRx (RfxCas13d)	*Ruminococcus flavefaciens XPD3002*	+	87	3003	58

	Name	Origin	RNA-guided nuclease activity in mammalian cells	Repression efficiency (%)	Size (bp)*1	gRNA length (nt)*2
1	PlmCasX	*Planctomycetes*	+	1: 63, 2: 66	2937	1: 122, 2: 102
2	Cas14a1	Uncultured archaeron	+	1: 86, 2: 78	1590	1: 163, 2: 204

*1 The length of the ORF used in this study (including stop codon)

*2 The length of the gRNA/crRNA used in this study

Supplementary Table 2

Profile of the 60 AND gates in Figure 5.

AND #	Input A	Input B	Cas protein C
1	PguCas13b	MbCas12a	PspCas13b
2	PguCas13b	SaCas9	PspCas13b
3	PguCas13b	AkCas12b	PspCas13b
4	PguCas13b	NcCas9	PspCas13b
5	MbCas12a	SaCas9	PspCas13b
6	MbCas12a	AkCas12b	PspCas13b
7	MbCas12a	NcCas9	PspCas13b
8	SaCas9	AkCas12b	PspCas13b
9	SaCas9	NcCas9	PspCas13b
10	AkCas12b	NcCas9	PguCas13b
11	PspCas13b	MbCas12a	PguCas13b
12	PspCas13b	SaCas9	PguCas13b
13	PspCas13b	AkCas12b	PguCas13b
14	PspCas13b	NcCas9	PguCas13b
15	MbCas12a	SaCas9	PguCas13b
16	MbCas12a	AkCas12b	PguCas13b
17	MbCas12a	NcCas9	PguCas13b
18	SaCas9	AkCas12b	PguCas13b
19	SaCas9	NcCas9	PguCas13b
20	AkCas12b	NcCas9	PguCas13b
21	PspCas13b	PguCas13b	MbCas12a
22	PspCas13b	SaCas9	MbCas12a
23	PspCas13b	AkCas12b	MbCas12a
24	PspCas13b	NcCas9	MbCas12a
25	PguCas13b	SaCas9	MbCas12a
26	PguCas13b	AkCas12b	MbCas12a
27	PguCas13b	NcCas9	MbCas12a
28	SaCas9	AkCas12b	MbCas12a
29	SaCas9	NcCas9	MbCas12a
30	AkCas12b	NcCas9	MbCas12a

AND #	Input A	Input B	Cas protein C
31	PspCas13b	PguCas13b	SaCas9
32	PspCas13b	MbCas12a	SaCas9
33	PspCas13b	AkCas12b	SaCas9
34	PspCas13b	NcCas9	SaCas9
35	PguCas13b	MbCas12a	SaCas9
36	PguCas13b	AkCas12b	SaCas9
37	PguCas13b	NcCas9	SaCas9
38	MbCas12a	AkCas12b	SaCas9
39	MbCas12a	NcCas9	SaCas9
40	AkCas12b	NcCas9	SaCas9
41	PspCas13b	PguCas13b	AkCas12b
42	PspCas13b	MbCas12a	AkCas12b
43	PspCas13b	SaCas9	AkCas12b
44	PspCas13b	NcCas9	AkCas12b
45	PguCas13b	MbCas12a	AkCas12b
46	PguCas13b	SaCas9	AkCas12b
47	PguCas13b	NcCas9	AkCas12b
48	MbCas12a	SaCas9	AkCas12b
49	MbCas12a	NcCas9	AkCas12b
50	SaCas9	NcCas9	AkCas12b
51	PspCas13b	PguCas13b	NcCas9
52	PspCas13b	MbCas12a	NcCas9
53	PspCas13b	SaCas9	NcCas9
54	PspCas13b	AkCas12b	NcCas9
55	PguCas13b	MbCas12a	NcCas9
56	PguCas13b	SaCas9	NcCas9
57	PguCas13b	AkCas12b	NcCas9
58	MbCas12a	SaCas9	NcCas9
59	MbCas12a	AkCas12b	NcCas9
60	SaCas9	AkCas12b	NcCas9

Supplementary Table 3

Key plasmids used in this study.

Description	Original name	Benchling link	Short name	Figure
OFF switch	pGluc-Sp_gRNA-EGFP	https://benchling.com/s/seq-YB5Dr4uWTfanMOKvIUsY	Sp_gRNA or SpCas9_gRNA	Fig1, 2, 3, FigS2, S3, S9, S10
OFF switch	pGluc-Sa_gRNA-EGFP	https://benchling.com/s/seq-M1VrldwdMbyqmlAdm2TA	Sa_gRNA or SaCas9_gRNA	Fig1, 2, 3, 5, FigS2, S10
OFF switch	pGluc-Cj_gRNA-EGFP	https://benchling.com/s/seq-i683ARLbCYEjQdq5Cnh0	CjCas9_gRNA	Fig1, 3, FigS2, S10
OFF switch	pGluc-St1_gRNA-EGFP	https://benchling.com/s/seq-TskVXFHofI9E2pxSQWkM	St1Cas9_gRNA	Fig1, 3, FigS2, S10
OFF switch	pFn_gRNA_v1-EGFP	https://benchling.com/s/seq-ovf2dJM05eLri2RCfAHh	FnCas9_gRNA 1	Fig1, FigS2
OFF switch	pFn_gRNA_v2-EGFP	https://benchling.com/s/seq-m3xNCSEq42TLjiLL293S	FnCas9_gRNA 2	Fig1, 3, FigS2, S10
OFF switch	pCd_gRNA_v1-EGFP	https://benchling.com/s/seq-K6uhc21sAPxxOEK6PZuw	CdCas9_gRNA 1	Fig1, 3, FigS2, S10
OFF switch	pCd_gRNA_v2-EGFP	https://benchling.com/s/seq-KZVPW44Dk4V6u4zafopV	CdCas9_gRNA 2	Fig1, FigS2
OFF switch	pCl_gRNA-EGFP	https://benchling.com/s/seq-H4UxWLOhgNvA0YR4Szip	ClCas9_gRNA	Fig1, 3, FigS2, S10
OFF switch	pPl_gRNA_v1-EGFP	https://benchling.com/s/seq-VfDteXn2JGE7dYPfy9D3	PlCas9_gRNA 1	Fig1, FigS2
OFF switch	pPl_gRNA_v2-EGFP	https://benchling.com/s/seq-wilfLbHEW4cECDThdcwt	PlCas9_gRNA 2	Fig1, 3, FigS2, S10
OFF switch	pNc_gRNA_v1-EGFP	https://benchling.com/s/seq-9GsfCiNp0dlL6B6UFmpP	NcCas9_gRNA 1	Fig1, 3, 5, FigS2
OFF switch	pNc_gRNA_v2-EGFP	https://benchling.com/s/seq-oF6McTKt84TdwsJ5Wt1H	NcCas9_gRNA 2	Fig1, FigS2
OFF switch	pSpa_gRNA_v1-EGFP	https://benchling.com/s/seq-PRk8eHaoV3JHCtXrX3BE	SpaCas9_gRNA 1	Fig1, FigS2
OFF switch	pSpa_gRNA_v2-EGFP	https://benchling.com/s/seq-Oh0lQ13b1xRVbmpRBqYH	SpaCas9_gRNA 2	Fig1, 3, FigS2, S10
OFF switch	pGluc-St3_gRNA_v1-EGFP	https://benchling.com/s/seq-N17o8zbHO751wm0LPAsa	St3Cas9_gRNA 1	Fig1, FigS2
OFF switch	pGluc-St3_gRNA_v2-EGFP	https://benchling.com/s/seq-BwCIYQ2YCuw2O3GdfvUV	St3Cas9_gRNA 2	Fig1, 3, FigS2, S10
OFF switch	pGluc-AsCas12a_crRNA-EGFP	https://benchling.com/s/seq-glNfU5jZtiKihoYUIYbg	AsCas12a_crRNA	Fig1, 3, FigS2, S6, S10
OFF switch	pFnCas12a_crRNA-EGFP	https://benchling.com/s/seq-V8aaKQn6IXZBvwOrjF4e	FnCas12a_crRNA	Fig1, 3, FigS2, S10
OFF switch	pLbCas12a_crRNA-EGFP	https://benchling.com/s/seq-AjWByTr164WVX6iEujJH	LbCas12a_crRNA	Fig1, 3, FigS2, S10
OFF switch	pMbCas12a_crRNA-EGFP	https://benchling.com/s/seq-hbYhHvSP2IG6F4WpK4IN	MbCas12a_crRNA	Fig1, 3, 5, FigS2, S10
OFF switch	pAaCas12b_sgRNA-EGFP	https://benchling.com/s/seq-0ah7ki0EgntdfZahBDPU	AaCas12a_crRNA	Fig1, 3, FigS2, S10
OFF switch	pAkCas12b_sgRNA_v1-EGFP	https://benchling.com/s/seq-7ZiHjayLdyOwjiBwW9qe	AkCas12b_gRNA 1	Fig1, 3, 5, FigS2, S10
OFF switch	pAkCas12b_sgRNA_v2-EGFP	https://benchling.com/s/seq-WhxCmn2hJG2pYh5T4JaK	AkCas12b_gRNA 2	Fig1, FigS2
OFF switch	pBvCas12b_sgRNA-EGFP	https://benchling.com/s/seq-ERy3edQQ4oRn27cBoE1F	BvCas12b_gRNA	Fig1, 3, FigS2, S10
OFF switch	pGluc-Psp_crRNA-EGFP	https://benchling.com/s/seq-WJlEdGEGMXhOOPGzuGHZ	PspCas13b_crRNA	Fig1, 3, 5, FigS2, S10
OFF switch	pGluc-Pgu_crRNA-EGFP	https://benchling.com/s/seq-q7r7cKeYiylzuEg4k7bP	PguCas13b_crRNA	Fig1, 3, 5, FigS2, S10
OFF switch	pGluc-Ran_crRNA-EGFP	https://benchling.com/s/seq-dR5ZevWPdwURmwnkXMsA	RanCas13b_crRNA	Fig1, 3, FigS2, S10
OFF switch	pGluc-CasRX_crRNA-EGFP	https://benchling.com/s/seq-E6RZl3LyVrpYHNZuhz06	CasRx_crRNA	Fig1, 3, FigS2, S10

OFF switch	pPlmCasX_gRNA-EGFP	https://benchling.com/s/seq-CN4z7nmpzAYbyQPomM4H	PlmCasX_gRNA 1	Fig1, FigS2
OFF switch	pPlmCasX_gRNA(dSpacer)-EGFP	https://benchling.com/s/seq-XSQeSp220dxaYoc5BkL9	PlmCasX_gRNA 2	Fig1, 3, FigS2, S10
OFF switch	pCas14a1_sgRNA1-EGFP	https://benchling.com/s/seq-nL3klfKUsOsPT2W8iuz7	Cas14a1_gRNA 1	Fig1, 3, FigS2, S10
OFF switch	pCas14a1_sgRNA2-EGFP	https://benchling.com/s/seq-oIcaD1LAx1CS6wGriPSc	Cas14a1_gRNA 2	Fig1, FigS2
OFF switch	pGluc-Nm_gRNA-EGFP	https://benchling.com/s/seq-9ShtAQING48KU8WRPZl0	NmCas9_gRNA_v0	Fig1, FigS2, S5
OFF switch	pGluc-Nm_gRNA-STOP-EGFP	https://benchling.com/s/seq-l3yoBcMcdBdqRQRUwmXg	NmCas9_gRNA_v1	Fig1, FigS2, S5
OFF switch	pGluc-Nm_gRNA_v2-EGFP	https://benchling.com/s/seq-4QVuutAH6V2urfJllMRy	NmCas9_gRNA_v2	Fig1, FigS2, S5
OFF switch	pGluc-Nm_gRNA_v3-EGFP	https://benchling.com/s/seq-wbDRU1SvlenI79ZS1H2F	NmCas9_gRNA_v3	Fig1, FigS2, S5
OFF switch	pNm_gRNA_v4-EGFP	https://benchling.com/s/seq-b1iBbNY6afS8lW4wlZMq	NmCas9_gRNA_v4	Fig1, FigS2, S5
OFF switch	pNm_gRNA_v5-EGFP	https://benchling.com/s/seq-mPrZo0zK3tP8fOo0fXtN	NmCas9_gRNA_v5	Fig1, FigS2, S5
OFF switch	pNm_gRNA_v6-EGFP	https://benchling.com/s/seq-tHODiWmkQAncUaHAP2Et	NmCas9_gRNA_v6	Fig1, FigS2, S5
OFF switch	pNm_gRNA_v7-EGFP	https://benchling.com/s/seq-CXq80mUzT3gHGyDvJXiX	NmCas9_gRNA_v7	Fig1, 3, FigS2, S5, S10
Trigger	pcDNA3.1-SpCas9	https://benchling.com/s/seq-EmEEkWHXs3Jf4rcdpaTg	SpCas9/WT	Fig1, 2, 3, Fig S2, S3, S7, S9, S10
Trigger	pcDNA3.1+-SaCas9	https://benchling.com/s/seq-Sj4R81pj6RXIZ6Jly9p4	SaCas9	Fig1, 2, 3, 5, Fig S2, S7, S10, S13
Trigger	pcDNA3.1+-CjCas9	https://benchling.com/s/seq-6eXuV9JcFQeQbmYwfJX8	CjCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-NmCas9	https://benchling.com/s/seq-Wfg75vjX32YQQJkiyuYE	NmCas9	Fig1, 3, Fig S2, S5, S7, S10
Trigger	pcDNA3.1+-st1Cas9	https://benchling.com/s/seq-VRWF7oQowjQbAvG8mYce	St1Cas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-FnCas9	https://benchling.com/s/seq-T1yYZahdAytxkLbQf5Jw	FnCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-CdCas9	https://benchling.com/s/seq-kC3slahy2rNyCbP39Uls	CdCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-ClCas9	https://benchling.com/s/seq-QMBrgPUM4wQ6nJFQp2Jv	ClCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-PlCas9	https://benchling.com/s/seq-ibuHG9biYiK2zC93XPUq	PlCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-NcCas9	https://benchling.com/s/seq-ifoU1HSjWp7QaJ2rnz4O	NcCas9	Fig1, 3, 5, Fig S2, S7, S10
Trigger	pcDNA3.1+-SpaCas9	https://benchling.com/s/seq-hmk13W570AKm7GQ0roes	SpaCas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-St3Cas9	https://benchling.com/s/seq-2q6Vcj3AwAHrO3ZXoP3Y	St3Cas9	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-AsCpf1	https://benchling.com/s/seq-7cpBK5lcPWKG0wk88fqZ	AsCas12a	Fig1, 3, Fig S2, S6, S7, S10
Trigger	pcDNA3.1-hMbCpf1	https://benchling.com/s/seq-Ap2WTz89rTXvcdSrQkgT	MbCas12a	Fig1, 3, 5, Fig S2, S7, S10
Trigger	pcDNA3.1+-AaCas12b	https://benchling.com/s/seq-Cg0Aurq89nwhVyoOf7cz	AaCas12b	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1-hFnCpf1	https://benchling.com/s/seq-NQNXT0q4aU2jFTtNf3Oq	FnCas12a	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1-hLbCpf1	https://benchling.com/s/seq-LRXskpS65geRNfMcOMZT	LbCas12a	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-AkCas12b	https://benchling.com/s/seq-9CJWprWiG5TviPN6Zh4R	AkCas12b	Fig1, 3, 5, Fig S2, S7, S10

Trigger	pcDNA3.1+-BvCas12b	https://benchling.com/s/seq-lwRpPTKuL6fB3m0XNzK6	BvCas12b	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1-PspCas13b(WT)-NES-myc-His6	https://benchling.com/s/seq-X0bNQN8K5SsJBN6F3OOX	PspCas13b	Fig1, 3, 5, Fig S2, S7, S10
Trigger	pcDNA3.1+-PguCas13b-NES	https://benchling.com/s/seq-BPSiSwCOpWBOxS0GGgcD	PguCas13b	Fig1, 3, 5, Fig S2, S7, S10
Trigger	pcDNA3.1+-RanCas13b-NES	https://benchling.com/s/seq-j8EjvpKgsQntC2aumCLE	RanCas13b	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-CasRx	https://benchling.com/s/seq-9sXxgMXbXXXfw9qoAtLo	CasRx	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-PlmCasX	https://benchling.com/s/seq-iwnSWt8Fk44t9U9QBnqa	PlmCasX	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1+-Cas14a1	https://benchling.com/s/seq-omwxft6FEDKBWlKxejS4	Cas14a1	Fig1, 3, Fig S2, S7, S10
Trigger	pcDNA3.1-SpCas9(D10A)	https://benchling.com/s/seq-rglwwC4Z2fEgTNhxNCmX	D10A	Fig S3
Trigger	pcDNA3.1-SpCas9(D10A_H840)	https://benchling.com/s/seq-oTpkJKDiTUBXWxa45FDX	D10A, H840A	Fig S3
Trigger	pcDNA3.1-SpCas9(dNLS)	https://benchling.com/s/seq-xIMuZsE3gLNDmcQDstVq	ΔNLS	Fig S3
Acr	pcDNA3.1+-AcrIIA4	https://benchling.com/s/seq-gGe8r2DZoDQvxqDhar3j	AcrIIA4	Fig S3
Trigger	pcDNA3.1+-AsCpf1(H800A)	https://benchling.com/s/seq-9U2OSQeiLx0biaTx8INe	AsCas12a(H800A)	Fig S6
ON switch	pNMD-ON-Gluc-Sp_gRNA-EGFP	https://benchling.com/s/seq-rBiY9xY8IxCQlmCDzb2v	SpCas9_gRNA	Fig 1, Fig S7, S11, S12, S13
ON switch	pNMD-ON-Gluc-Sa_gRNA	https://benchling.com/s/seq-Rkh4phoiHfg2TfGdl4LP	SaCas9_gRNA	Fig 1, Fig S7, S11, S12, S13
ON switch	pNMD-ON-Gluc-Cj_gRNA	https://benchling.com/s/seq-ytclQFC5hKdeTzpgVAgg	CjCas9_gRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc-St1_gRNA	https://benchling.com/s/seq-edpM2PVwBHJPhFaMwMGg	St1Cas9_gRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc_Nm_gRNA_v1	https://benchling.com/s/seq-j8yTztzbcE5l6BnnaYcV	NmCas9_gRNA 1	Fig S7
ON switch	pNMD-ON-Gluc_Nm_gRNA_v2	https://benchling.com/s/seq-4JkdlEgacfZVliRF9dFW	NmCas9_gRNA 2	Fig S7
ON switch	pNMD-ON-Gluc_Nm_gRNA_v3	https://benchling.com/s/seq-AYCFLt1uYmg1tnfU7TLk	NmCas9_gRNA 3	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc_Nm_gRNA_v4	https://benchling.com/s/seq-lg2jkJ3G8H1fd4MMQ4CO	NmCas9_gRNA 4	Fig S7
ON switch	pNMD-ON-Gluc_Nm_gRNA_v5	https://benchling.com/s/seq-fXdYnMeOXbnz6Whw4Jed	NmCas9_gRNA 5	Fig S7
ON switch	pNMD-ON-Gluc_Nm_gRNA_v6	https://benchling.com/s/seq-VX81PClbzWVwRpJdbQNJ	NmCas9_gRNA 6	Fig S7
ON switch	pNMD-ON-Gluc_Nm_gRNA_v7	https://benchling.com/s/seq-gzSPC4lFm9ll9ShoyHlr	NmCas9_gRNA 7	Fig S7
ON switch	pNMD-ON-Fn_gRNA_v1	https://benchling.com/s/seq-UpTK1Av0CTE5Qzgf5OSR	FnCas9_gRNA 1	Fig S7
ON switch	pNMD-ON-Fn_gRNA_v2	https://benchling.com/s/seq-wnexJ24ICiNDjWyIaPjl	FnCas9_gRNA 2	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Cd_gRNA_v1	https://benchling.com/s/seq-aZ03Yf3zGnKi9y1qrP35	CdCas9_gRNA 1	Fig S7
ON switch	pNMD-ON-Cd_gRNA_v2	https://benchling.com/s/seq-Mssi3187k0LiwSpbSY1g	CdCas9_gRNA 2	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Cl_gRNA	https://benchling.com/s/seq-gkRuH0CD6I8VFkLfGTuN	ClCas9_gRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Pl_gRNA_v1	https://benchling.com/s/seq-neQ5XOaKZayZxaEnBpBJ	PlCas9_gRNA 1	Fig S7

ON switch	pNMD-ON-Pl_gRNA_v2	https://benchling.com/s/seq-uo12MN8p9ELYe4KBwKPy	PlCas9_gRNA 2	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Nc_gRNA_v1	https://benchling.com/s/seq-57djv04vQeZYOqLdpZB9	NcCas9_gRNA 1	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Nc_gRNA_v2	https://benchling.com/s/seq-5Kd4BkOffc6EGynJb7Xf	NcCas9_gRNA 2	Fig S7
ON switch	pNMD-ON-Spa_gRNA_v1	https://benchling.com/s/seq-fnoQ3X1Qs6sCtgmAhZQ6	SpaCas9_gRNA 1	Fig S7
ON switch	pNMD-ON-Spa_gRNA_v2	https://benchling.com/s/seq-OmmTbhPsOewdpW8J7vJh	SpaCas9_gRNA 2	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-St3_gRNA_v1	https://benchling.com/s/seq-vvJqP4bzkFr0jzUDum9m	St3Cas9_gRNA 1	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-St3_gRNA_v2	https://benchling.com/s/seq-0hRIB3bEUrvRH6pStcVP	St3Cas9_gRNA 2	Fig S7
ON switch	pNMD-ON-Gluc-AsCas12a_crRNA	https://benchling.com/s/seq-uMWXFaSnWI4Tj569dVR3	AsCas12a_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-FnCas12a_crRNA	https://benchling.com/s/seq-zCLlzSzy9FGZVE8LqSGw	FnCas12a_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-LbCas12a_crRNA	https://benchling.com/s/seq-097cK15zRAFJnNdSzJrU	LbCas12a_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-MbCas12a_crRNA	https://benchling.com/s/seq-2MgLIE9wFI3d5ljtMnbC	MbCas12a_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-AaCas12b_sgRNA	https://benchling.com/s/seq-omJehIz6xnx7yEdD9GMw	AaCas12a_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-AkCas12b_sgRNA_v1	https://benchling.com/s/seq-eNnHdLLBxiWLCq3zFjEl	AkCas12b_gRNA 1	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-AkCas12b_sgRNA_v2	https://benchling.com/s/seq-9AOlST8qZ7TUMkhdqs9K	AkCas12b_gRNA 2	Fig S7
ON switch	pNMD-ON-BvCas12b_sgRNA	https://benchling.com/s/seq-y5azslDwpaymVWAunhno	BvCas12b_gRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc-Psp_crRNA-EGFP	https://benchling.com/s/seq-6A2RLRkhHstAACjndh3T	PspCas13b_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc-Pgu_crRNA	https://benchling.com/s/seq-A8zYUC0tnz18biHZwwih	PguCas13b_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc_Ran_crRNA	https://benchling.com/s/seq-INRGbAowneL4z9J9NlpK	RanCas13b_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Gluc-CasRx_crRNA	https://benchling.com/s/seq-bHrydKbzUXtMT2df0vvF	CasRx_crRNA	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-PlmCasX_gRNA	https://benchling.com/s/seq-1ASZJgt3yMozw41vGMC4	PlmCasX_gRNA 1	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-PlmCasX_gRNA(dSpacer)	https://benchling.com/s/seq-2vhdu4FdsBUepnpg8sbH	PlmCasX_gRNA 2	Fig S7
ON switch	pNMD-ON-Cas14a1_sgRNA_v1	https://benchling.com/s/seq-X7M8REytCChUfrThKnd0	Cas14a1_gRNA 1	Fig 1, Fig S7, S11, S12
ON switch	pNMD-ON-Cas14a1_sgRNA_v2	https://benchling.com/s/seq-gcwfVBOq93RuFqMvzOZm	Cas14a1_gRNA 2	Fig S7
Trigger	pcDNA3.1-SpCas9(1-713)	https://benchling.com/s/seq-WhnSX12uuWktk1Ptnl6N	SpCas9(1-713)	Fig S9
Trigger	pcDNA3.1-SpCas9(714-1368)	https://benchling.com/s/seq-EgutSzE4sOjkDFpvAga6	SpCas9(714-1368)	Fig S9
Trigger	pcDNA3.1-SpCas9(1-713)-N_intein	https://benchling.com/s/seq-LznvtjWCo3gRuQTQLrFV	N-Cas9	Fig 2
Trigger	pcDNA3.1-C_intein-SpCas9(714-1368)	https://benchling.com/s/seq-IQZPP14tzFCrLOAcMehv	C-Cas9	Fig 2
Trigger	pcDNA3.1-SpCas9(1-713)-(GGGGS)3-DmrA	https://benchling.com/s/seq-dv1jMt9Ay11JeCJLUQVH		Fig 2
Trigger	pcDNA3.1-DmrC-(GGGGS)3-SpCas9(714-1368)	https://benchling.com/s/seq-Fm4hweFErSPt3TEBtD2O		Fig 2
Acr	pcDNA3.1+-AcrIIC2_Nm	https://benchling.com/s/seq-q7VMJRLxbI80KzAUR0GI	AcrIIC2	Fig 2

OFF switch	pGluc-Sa_gRNA-tagRFP	https://benchling.com/s/seq-CdGyAKcK4BLcaxnwoJqB		Fig S13
TX-TL	pGluc-Sp_gRNA-tagRFP	https://benchling.com/s/seq-lDi3fB9tlGStqMMVnkq5	pSp_gRNA-RFP or Sp_gRNA-RFP	Fig 4
TX-TL	pTRE-Tight-hmAG1	https://benchling.com/s/seq-2cqCEKIUhTzatrtUhv0P	pTRE-hmAG1	Fig 4
TX-TL	SP-dCas9-VPR	https://benchling.com/s/seq-ZElwRvTTCW7jt0rU9sK6	dSpCas9-VPR	Fig 4, 6
TX-TL	pHL-gRNA[TRE new]-iRFP-RIH	https://benchling.com/s/seq-q7JGwy2sJVyYMaulUoN3	gRNA	Fig 4
60 AND	pAkCas12b_sgRNA_v1-hMbCpf1	https://benchling.com/s/seq-nCZ2RO20R6Aumlh4YjgK		Fig5
60 AND	pAkCas12b_sgRNA_v1-NcCas9	https://benchling.com/s/seq-1a7DesFlHNILsHpSu4w9		Fig5
60 AND	pAkCas12b_sgRNA_v1-PguCas13b-NES	https://benchling.com/s/seq-B67ojjSjELQKJys2zkGN		Fig5
60 AND	pAkCas12b_sgRNA_v1-PspCas13b-NES	https://benchling.com/s/seq-qz4qlieBfHwUzOWjC5e0		Fig5
60 AND	pAkCas12b_sgRNA_v1-SaCas9	https://benchling.com/s/seq-bzPeWMvq81ltvXucSkKQ		Fig5
60 AND	pGluc-Pgu_crRNA-AkCas12b	https://benchling.com/s/seq-9jNJRU5L8trZNL9Ea1Fk		Fig5
60 AND	pGluc-Pgu_crRNA-hMbCpf1	https://benchling.com/s/seq-K4EDhN81LGr1ZKzR7Zv4		Fig5
60 AND	pGluc-Pgu_crRNA-NcCas9	https://benchling.com/s/seq-aLCsxbyWI32xJbcDvUvE		Fig5
60 AND	pGluc-Pgu_crRNA-PspCas13b	https://benchling.com/s/seq-SjvgeWN3ybsNDiRJL3bI		Fig5
60 AND	pGluc-Pgu_crRNA-SaCas9	https://benchling.com/s/seq-xwARaxvsdDQ0dPAR5yLI		Fig5
60 AND	pGluc-Psp_crRNA-AkCas12b	https://benchling.com/s/seq-6uVhkXlqnt3fl0fTPK9b		Fig5
60 AND	pGluc-Psp_crRNA-hMbCpf1	https://benchling.com/s/seq-exsrOWx6k6to67kEyp9I		Fig5
60 AND	pGluc-Psp_crRNA-NcCas9	https://benchling.com/s/seq-RfV6xht046zxli5hD6kJ		Fig5
60 AND	pGluc-Psp_crRNA-PguCas13b-NES	https://benchling.com/s/seq-ErjJA7ofbgNchGonVhRw		Fig5
60 AND	pGluc-Psp_crRNA-SaCas9	https://benchling.com/s/seq-vMQG9zSxZXBfRrJbHaUu		Fig5
60 AND	pGluc-Sa_gRNA-AkCas12b	https://benchling.com/s/seq-gsFRzqnDG9GMjHBUxcXK		Fig5
60 AND	pGluc-Sa_gRNA-MbCas12a	https://benchling.com/s/seq-pcMlOudTctmDddOgJqYx		Fig5
60 AND	pGluc-Sa_gRNA-NcCas9	https://benchling.com/s/seq-PKfTqfWkEK0qDtiR6NaT		Fig5
60 AND	pGluc-Sa_gRNA-PguCas13b-NES	https://benchling.com/s/seq-1nJKeWZjZPKnwLvLAXw7		Fig5
60 AND	pGluc-Sa_gRNA-PspCas13b-NES	https://benchling.com/s/seq-vZdLDuRUj7lEkoSsrUus		Fig5
60 AND	pMbCas12a_crRNA-AkCas12b	https://benchling.com/s/seq-ij4dn2iIGnGvaZlxhdfJ		Fig5
60 AND	pMbCas12a_crRNA-NcCas9	https://benchling.com/s/seq-blf5FyEY59ZpzSoStpet		Fig5
60 AND	pMbCas12a_crRNA-PguCas13b-NES	https://benchling.com/s/seq-3xdNZDrleFaTH8dagqKG		Fig5
60 AND	pMbCas12a_crRNA-PspCas13b-NES	https://benchling.com/s/seq-qHKPavIjaz79DIq4Mk5f		Fig5

60 AND	pMbCas12a_crRNA-SaCas9	https://benchling.com/s/seq-n012CwEHH9uvfaQ0gvj5		Fig5
60 AND	pNc_gRNA_v1-AkCas12b	https://benchling.com/s/seq-uM9EVPlutz8XRqTR19bt		Fig5
60 AND	pNc_gRNA_v1-MbCpf1	https://benchling.com/s/seq-PY2fTqoxv4KsMFs6I2GF		Fig5
60 AND	pNc_gRNA_v1-PguCas13b-NES	https://benchling.com/s/seq-HdyvKPFRGxGzxA18WgRv		Fig5
60 AND	pNc_gRNA_v1-PspCas13b-NES	https://benchling.com/s/seq-QHZAHBtI1644ZiJJs246		Fig5
60 AND	pNc_gRNA_v1-SaCas9	https://benchling.com/s/seq-uO9Q7cbMhicDBiKJg0l8		Fig5
Half-subtractor	pSa_lgRNA_a-CMVmin-Gluc_Sp_gRNA-tagBFP-Triplex-HHR-Sa_gRNA[TRE]-HDVR	https://benchling.com/s/seq-uf65bSQ0kDL47n4kCUnW		Fig6
Half-subtractor	pSp_lgRNA_a-CMVmin-Gluc_Sa_gRNA-tagBFP	https://benchling.com/s/seq-alXjZwBUovuZrMfD2Xm3		Fig6
Half-subtractor	pSp_lgRNA_ax2-CMVmin-Gluc_Sa_gRNA-tagBFP	https://benchling.com/s/seq-KpJNkbiOwhNvphA7kTqG		Fig6
Half-subtractor	pTRE-Tight-Gluc_Sp_gRNA-hmAG1	https://benchling.com/s/seq-RtHU0UdngTZHkwAhHqPP		Fig6
Half-subtractor	pcDNA3.1+-dSaCas9-VPR	https://benchling.com/s/seq-VOVsrV43YHMX6DoRTtEC		Fig6
Half-subtractor	pHL-Sa_lgRNA_a-iRFP-RIH	https://benchling.com/s/seq-Ay5dnk1zINPfqphQkY9X		Fig6
Half-subtractor	pHL-Sp_lgRNA_a-iRFP-RIH	https://benchling.com/s/seq-BNfF7C046rnblBDNHpsC		Fig6
	pcDNA3.1+-myc-HisA	https://benchling.com/s/seq-wZkotHbe8PB30KDVPJqa	Control/ No trigger	

Supplementary Table 4

Primers, template oligo DNA for generating synthetic mRNAs.

PCR primers

No.	Primer/Oligo DNA name	Sequence (5' → 3')
1	TAP_T7_G3C fwd primer	CAGTGAATTGTAATACGACTCACTATAGGGC
2	IVT_5prime_UTR primer	CAGTGAATTGTAATACGACTCACTATAGGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATA AGACACCGGTCGCCACCATG
3	Rev5UTR primer	CATGGTGGCGACCGGTGTCTTATATTTCTTCTTACTC
4	IVT_3prime_UTR primer	TCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTT GGTCTTTGAATAAAGCCTGAGTAGG
5	Fwd3UTR primer	TCTAGACCTTCTGCGGGGC
6	Rev3UTR2T20	TTTTTTTTTTTTTTTTTTTTCCTACTCAGGCTTTATTCAAAGACCAAG
7	SphcCas9 ORF fwd primer	CACCGGTCGCCACCATGGATAAGAAATACAGCATTGGAC
8	SphcCas9 ORF rev primer	GCCCCGCAGAAGGTCTAGACTATCACACCTTCCTCTTCTTCTTGG
9	T7-5UTR for cassette1	TAATACGACTCACTATAggTCAGATCCGCTAGCGGATCC
10	EGFP ORF_Rv	GCCCCGCAGAAGGTCTAGACTACTTGTACAGCTCGTCCATGCCGAGAG
11	EGFP mRNA1 ORF_Fw	CACCGGTCGCCACCATGGTGAGCAAGGGCGAG
12	EGFP mRNA1 ORF_Rv	CCTACTCAGGCTTTATTCATGTGAAATTTGTGATGCTATTGCTTTATTTGTAAC
13	YF771_T7_5UTR_fwd	ATTGTAATACGACTCACTATAGGGCGAATTAAGAGAGAAAAGAAGAGTAAG
14	3UTR120A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTACTCAGGCTTTATTCA

Key PCR products

No.	Name	Type	Templates/Plasmids	Forward primer	Reverse primer
1	5'-UTR	UTR	IVT_5prime_UTR primer	TAP_T7_G3C fwd primer	Rev5UTR primer
2	3'-UTR	UTR	IVT_3prime_UTR primer	Fwd3UTR primer	Rev3UTR2T20
3	Cas9 mRNA ORF	ORF	pHL-EF1a-SphcCas9-iC-A	SphcCas9 ORF fwd primer	SphcCas9 ORF rev primer
5	Gluc-Sp_gRNA-EGFP ORF	5'UTR-ORF	pGluc-Sp_gRNA-EGFP	T7-5UTR for cassette1	EGFP ORF_Rv
7	EGFP mRNA ORF	5'UTR-ORF	pAptamerCassette-EGFP	EGFP mRNA1 ORF_Fw	EGFP mRNA1 ORF_Rv
4	Cas9 mRNA Template	IVT template	Cas9 mRNA ORF, 5'-UTR, 3'-UTR	TAP_T7_G3C fwd primer	3UTR120A
6	Gluc-Sp_gRNA-EGFP Template	IVT template	Gluc-Sp_gRNA-EGFP ORF, 3'-UTR	T7-5UTR for cassette1	3UTR120A
8	EGFP mRNA Template	IVT template	EGFP mRNA ORF, 5'-UTR	TAP_T7_G3C fwd primer	3UTR120A
9	iRFP670 mRNA Template	IVT template	pUC19-iRFP670woT7f	YF771_T7_5UTR_fwd	3UTR120A

Supplementary Table 5

Transfection tables of all experiments performed in this study.

Figure 1, Figure S2, S3A, S5B, S6, S7 (24-well plate)

Switch plasmid	100 ng
Trigger plasmid	400 ng
Reference plasmid	100 ng
Opti-MEM	up to 100 μL
Lipofectamine 2000	2 μL

Figure 2B (24-well plate)

	[N-Cas9, C-Cas9] [-, -]	[N-Cas9, C-Cas9] [+, -]	[N-Cas9, C-Cas9] [-, +]	[N-Cas9, C-Cas9] [+, +]	WT
pGluc-Sp_gRNA-EGFP	100 ng	100 ng	100 ng	100 ng	100 ng
pcDNA3.1-myc-His6	800 ng	400 ng	400 ng		400 ng
pcDNA3.1-SpCas9					400 ng
pcDNA3.1-SpCas9(1-713)-N_intein		400 ng		400 ng	
pcDNA3.1-C_intein-SpCas9(714-1368)			400 ng	400 ng	
pCMV-tdiRFP670	100 ng	100 ng	100 ng	100 ng	100 ng

Figure 2D (24-well plate)

	A/C heterodimerizer (-)					
	WT		Split		No trigger	
	No_gRNA	Sp_gRNA	No_gRNA	Sp_gRNA	No_gRNA	Sp_gRNA
pAptamerCassette-EGFP	100 ng		100 ng		100 ng	
pGluc-Sp_gRNA-EGFP		100 ng		100 ng		100 ng
pcDNA3.1-SpCas9(1-713)-(GGGGS)3-DmrA			400 ng	400 ng		
pcDNA3.1-DmrC-(GGGGS)3-SpCas9(714-1368)			400 ng	400 ng		
pcDNA3.1-myc-His6	400 ng	400 ng			800 ng	800 ng
pcDNA3.1-SpCas9	400 ng	400 ng				
	A/C heterodimerizer (+)					
	WT		Split		No trigger	
	No_gRNA	Sp_gRNA	No_gRNA	Sp_gRNA	No_gRNA	Sp_gRNA
pAptamerCassette-EGFP	100 ng		100 ng		100 ng	
pGluc-Sp_gRNA-EGFP		100 ng		100 ng		100 ng
pcDNA3.1-SpCas9(1-713)-(GGGGS)3-DmrA			400 ng	400 ng		
pcDNA3.1-DmrC-(GGGGS)3-SpCas9(714-1368)			400 ng	400 ng		
pcDNA3.1-myc-His6	400 ng	400 ng			800 ng	800 ng
pcDNA3.1-SpCas9	400 ng	400 ng				

Figure 2F (24-well plate)

	0 ng	200 ng	400 ng	1000 ng	2000 ng
pGluc-Sa_gRNA-EGFP or pAptamerCassette-EGFP	100 ng	100 ng	100 ng	100 ng	100 ng
pcDNA3.1-SaCas9	200 ng	200 ng	200 ng	200 ng	200 ng
pcDNA3.1-myc-His6	2000 ng	1000 ng	400 ng	200 ng	0 ng
pcDNA3.1+-AcrIIC2_Nm	0 ng	200 ng	400 ng	1000 ng	2000 ng
pCMV-tdiRFP670	100 ng	100 ng	100 ng	100 ng	100 ng

Figure 3, S10-S12 (384-well plate)

Switch plasmid	31.25 ng
Trigger plasmid	125 ng
Reference plasmid	31.25 ng
Opti-MEM	up to 10 μL
Lipofectamine 2000	0.4 μL

Figure 4 (24-well plate)

notation	plasmid name	
dSpCas9-VPR	Sp_dCas9-VPR or pcDNA3.1+-myc-HisA	400 [ng]
gRNA	pHL-gRNA[TRE new]-iRFP-RIH or pHL-Sp_lgRNA_Nluc-iRFP-RIH	100 [ng]
pTRE-hmAG1	pTRE-Tight-hmAG1	100 [ng]
pSp_gRNA-RFP	pGluc-Sp_gRNA-tagRFP or pcDNA3.1+-myc-HisA	100 [ng]
reference	pAptamerCassette-tagBFP	100 [ng]

Figure 5 (96-well plate)

Trigger plasmid 1	200 ng
Trigger plasmid 2	200 ng
Mediator plasmid 1	50 ng
Mediator plasmid 2	50 ng
Reporter plasmid	12.5 ng
Reference plasmid	25 ng
Opti-MEM	up to 20 μL
Lipofectamine 2000	0.5 μL

Figure 6 (24-well plate)	[0, 0]	[0, 1]	[1, 0]	[1, 1]
pTRE-Gluc_Sa_gRNA-hmA1	400 ng			
pHL-Sp_lgRNA_a-iRFP-RIH	100 ng			
pHL-Sa_lgRNA_a-iRFP-RIH	100 ng			
pcDNA3.1-myc-HisA	800 ng	400 ng	400 ng	0 ng
pcDNA3.1-dSpCas9-VPR	0 ng	400 ng	0 ng	400 ng
pcDNA3.1-dSaCas9-VPR	0 ng	0 ng	400 ng	400 ng
pSa_lgRNA_a-CMVmin-Gluc_Sp_gRNA-tagBFP	100 ng			
pSp_lgRNA_ax2-CMVmin-Gluc_Sa_gRNA-tagBFP-Tri-HHR-Sp_gRNA[TRE]-HDVR	400 ng			
Opti-MEM	Up to 100 μL			
Lipofectamine 2000	2 μL			

Figure S3C (24-well plate)

Switch/control plasmid	100 ng
Trigger plasmid	100 ng
Acr (AcrllA4) plasmid	100 ng
Reference plasmid	100 ng
Opti-MEM	up to 20 μL
Lipofectamine 2000	0.5 μL

Figure S4 (24-well plate)

Switch/control mRNA	100 ng
Trigger mRNA	100 ng
Reference mRNA	100 ng
Opti-MEM	up to 50 μL
Lipofectamine MessengerMax	1 μL

Figure S9 (24-well plate)

	[N-Cas9, C-Cas9] [-, -]	[N-Cas9, C-Cas9] [+, -]	[N-Cas9, C-Cas9] [-, +]	[N-Cas9, C-Cas9] [+, +]	WT
pGluc-Sp_gRNA-EGFP	100 ng	100 ng	100 ng	100 ng	100 ng
pcDNA3.1-myc-His6	800 ng	400 ng	400 ng		400 ng
pcDNA3.1-SpCas9					400 ng
pcDNA3.1-SpCas9(1-713)		400 ng		400 ng	
pcDNA3.1-SpCas9(714-1368)			400 ng	400 ng	
pCMV-tdiRFP670	100 ng	100 ng	100 ng	100 ng	100 ng

Figure S13 (24-well plate)

notation	plasmid name	
OFF switch	pGluc-Sa_gRNA-tagRFP or pAptamerCassette-tagRFP2	100 ng
ON switch	pNMD-ON-Gluc-Sa_gRNA or pNMD-ON-Gluc-Sa_gRNA	100 ng
trigger	pcDNA3.1＋-SaCas9 or pcDNA3.1＋-myc-HisA	400 ng
reference	pCMV-tdiRFP	100 ng

Supplementary Sequences

RNA sequences used in this study.

1. The 5’ terminus of mRNA is capped with ARCA.
2. The protein coding regions are shown as bold letters.
3. The start sites and stop codons are underlined.

Cas9 mRNA

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC**AUG**
GAUAAGAAAUAC
AGCAUUGGACUGGACAUUGGGACAAACUCCGUGGGAUGGGCCGUGAUUACAGACGAAUACAAAGUGCCUU
CAAAGAAGUUCAAGGUGCUGGGCAACACCGAUAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUG
UUCGACUCCGGCGAGACAGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGUAUACCCGCCGAA
AGAAUAGGAUCUGCUACCUGCAGGAGAUUUUCAGCAACGAAAUGGCCAAGGUGGACGAUAGUUUCUUUCAC
CGCCUGGAGGAAUCAUUCCUGGUCGAGGAAGAUAAGAAACACGAGCGGCAUCCCAUCUUUGGCAACAUUG
UGGACGAGGUCGCUUAUCACGAAAAGUACCCUACCAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACA
GAUAAAGCAGACCUGCGCCUGAUCUAUCUGGCCCUGGCUCACAUGAUUAAGUUCCGGGGCCAUUUUCUGAU
CGAGGGGGAUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAAUC
AGCUGUUUGAGGAAAACCCCAUUAAUGCAUCUGGCGUGGACGCAAAAGCCAUCCUGAGUGCCAGACUGUCU
AAGAGUCGGAGACUGGAGAACCUGAUCGCUCAGCUGCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAAUC
UGAUUGCACUGUCACUGGGACUGACUCCCAACUUCAAGAGCAAUUUUGAUCUGGCCGAGGACGCUAAACUG
CAGCUGUCCAAGGACACCUAUGACGAUGACCUGGAUAACCUGCUGGCUCAGAUCGGGGAUCAGUACGCAG
ACCUGUUCCUGGCCGCUAAGAAUCUGUCUGACGCCAUCCUGCUGAGUGAUAUUCUGCGCGUGAACACCGAG
AUUACAAAAGCCCCCCUGUCAGCUAGCAUGAUCAAGAGAUAUGACGAGCACCAUCAGGAUCUGACCCUGCU
GAAGGCUCUGGUGAGGCAGCAGCUGCCUGAGAAGUACAAGGAAAUCUUCUUUGAUCAGUCUAAGAACGGA
UACGCCGGCUAUAUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUAUCAAACCCAUUCUGGAGA
AGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUCGGGAAGACCUGCUGAGGAAGCAGCGCACUUU
UGAUAACGGAAGCAUCCCUCACCAGAUUCAUCUGGGAGAGCUGCACGCAAUCCUGAGGCGCCAGGAAGAC
UUCUACCCAUUUCUGAAGGAUAACAGGGAGAAGAUCGAAAAAAUUCUGACAUUCCGCAUCCCCUACUAUGU
GGGCCCUCUGGCAAGAGGCAACAGCCGGUUUGCCUGGAUGACUCGCAAAUCUGAGGAAACAAUCACUCCC
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AAAAAAAAAAAAAAAAAAAAAAAAAA

Gluc-Sp_gRNA-EGFP
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EGFP mRNA
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AAAAAAAAAAAAAAAAAAAA

iRFP670 mRNA
GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC**<u>AUG</u>GCGCGUAAGGUC**
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