

A, Seven clusters were extracted from 11,437 read sequences after five rounds of selection with the N17 library. Clusters 1 to 5 share a common sequence motif (#1), whereas clusters 6 and 7 shared motif #2. **B**, Inhibitory activities of representative aptamers of seven clusters were evaluated for Cas9/crGFP1-mediated plasmid cleavage in an in vitro cleavage assay. A streptavidin aptamer (SA-Ap) was used as a negative control. **C**, Fifty-five aptamer candidates belonging to motif 1 were screened at 100 nM by performing the in vitro cleavage assay. S21, S36, and S40 (shown in red) thoroughly inhibited Cas9-mediated plasmid cleavage. SA-Aps and sgRNAs for the different target were used as negative controls. **D**, Putative secondary structures of three aptamers (S21, S36, and S40) are shown on the right. The loop and neck regions are represented in orange and purple, respectively. The stem region corresponding to a fixed primer-binding sequence is represented in blue.

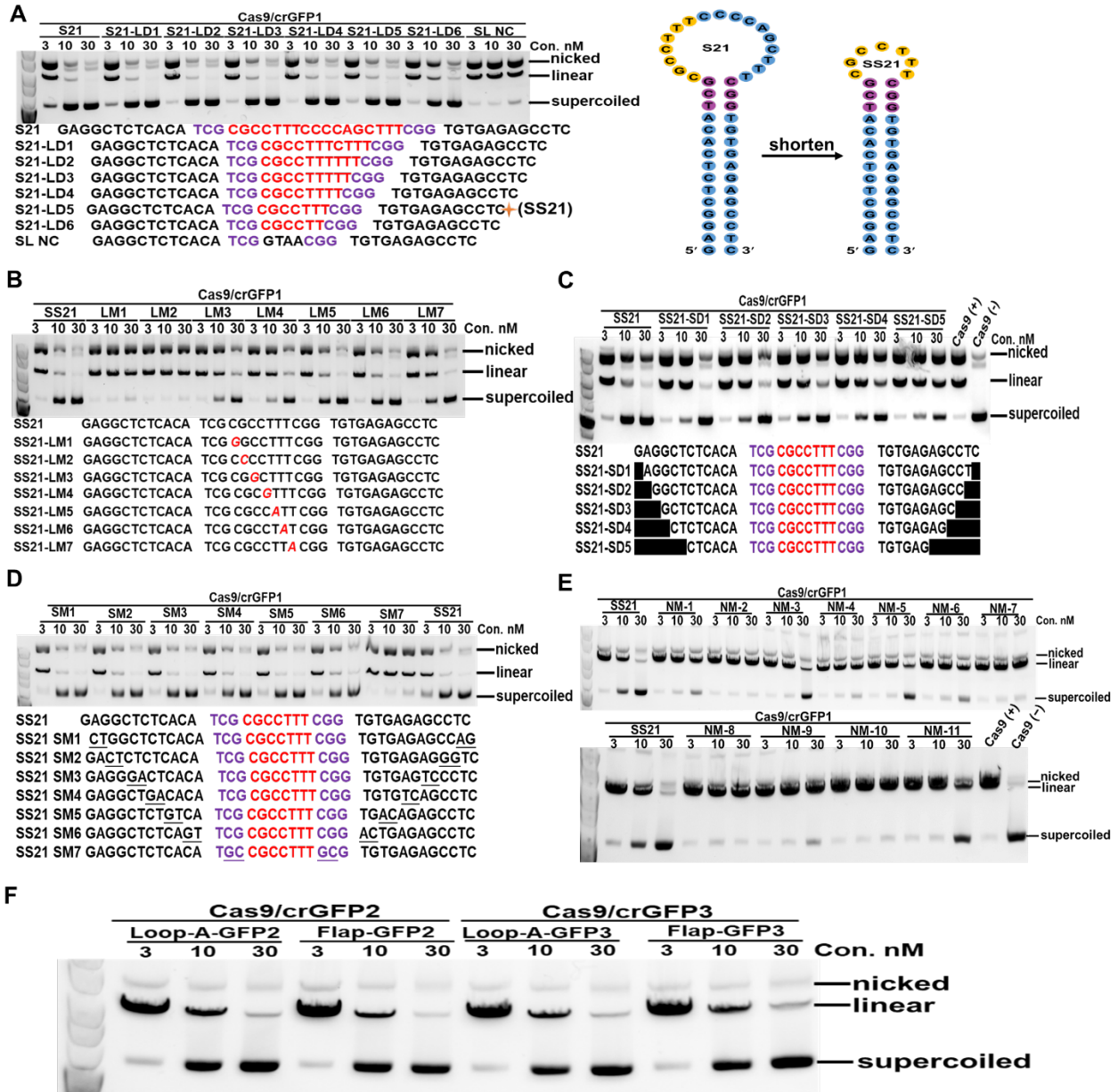


Figure S2. Related to Figure 1 and Figure 2. Deletion and mutation analysis of the S21 and SS21 inhibitory aptamer.

A, Deletion mutants of the S21 aptamer in the loop region were evaluated for inhibitory effects on Cas9/crGFP1-directed plasmid cleavage at the indicated concentrations. The inhibitory activity was not attenuated until the loop region was shortened to seven nucleotides (S21-LD5). The minimal inhibitory aptamer, S21-LD5 (renamed as SS21) was used for further analysis. The

putative secondary structure of SS21 is depicted on the right, inclusive of the loop region (yellow), neck region (purple), and stem region (blue). **B**, Evaluation of mutants of SS21 in the loop region. The mutated nucleotides are shown in red. The corresponding histogram is shown in Figure. 1B. **C**, Deletion mutants of the SS21 aptamer in the stem region were evaluated for inhibitory effects on Cas9/crGFP1-directed plasmid cleavage at the indicated concentrations. **D**, Analysis of the inhibitory activities of SS21 mutant aptamers in the stem and neck regions for Cas9/crGFP1. Only base pairs changed in the neck region caused a serious decrease in inhibitory efficiency. **E**, Analysis of inhibitory activities of mutant aptamers in the neck region. Eleven mutants were generated to determine key nucleotides required for inhibitory ability. The graph of this data is presented in Figure. 1C. **F**, Both the designed Loop-A and flap-type aptamers showed high inhibitory activity for Cas9/crGFP2 and Cas9/crGFP3.

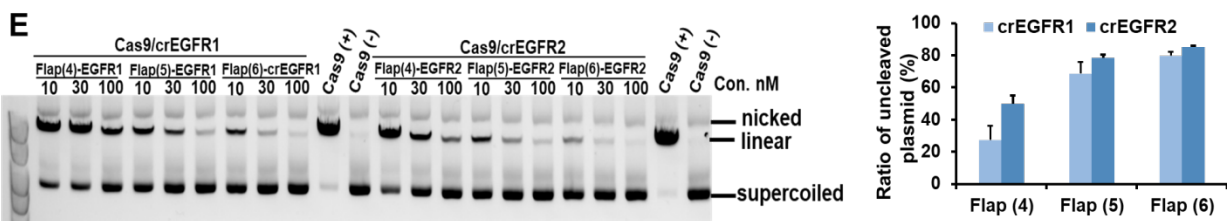
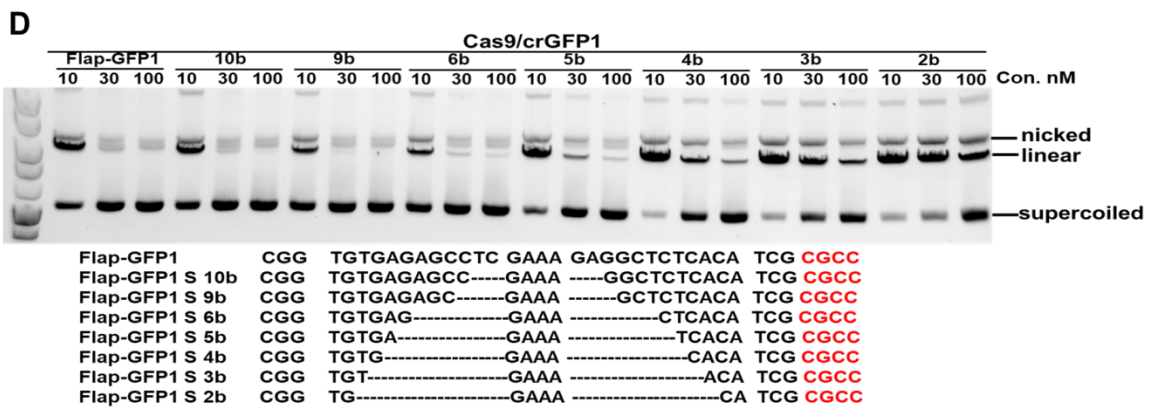
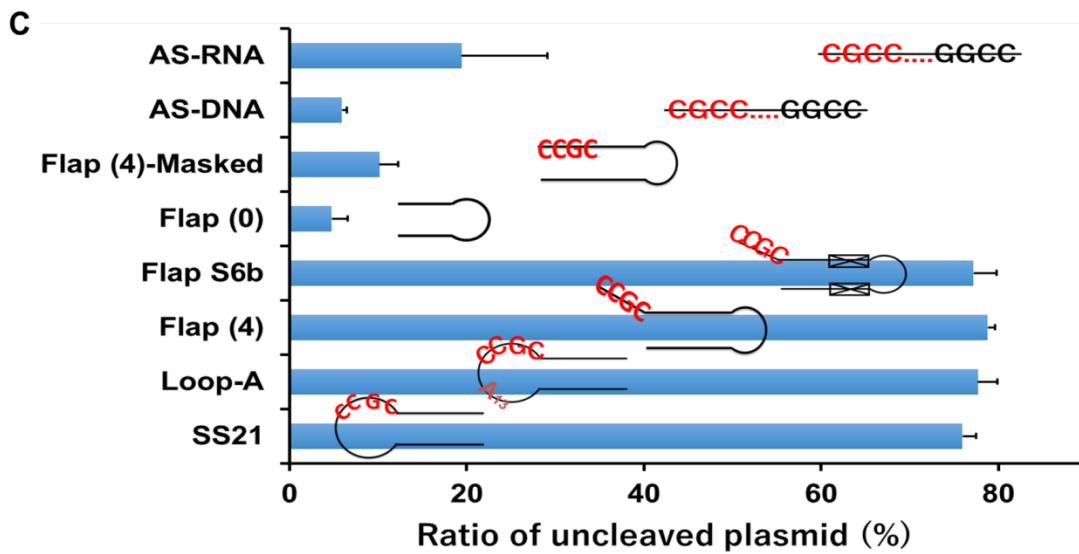
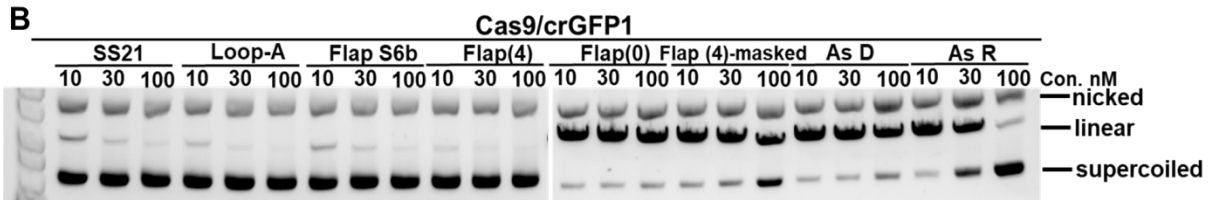
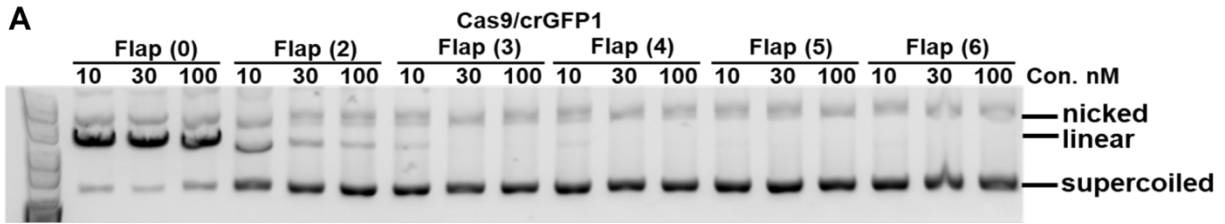


Figure S3. Related to Figure 3. Comparison between flap length and various kinds of inhibitors, evaluated by performing in vitro cleavage assays for Cas9/crGFP1.

A, The inhibitory efficiencies of the aptamers depended on the length of the flap sequence. When increasing the length of the flap sequence, the inhibitory activities increased and plateaued as the length of Flap region reaches four nucleotides. **B**, Three dosages of each inhibitor were assessed for Cas9/crGFP1 by performing in vitro cleavage assays. **C**, The quantification of **B** was represented for each inhibitor (30 nM). The data shown are presented as means \pm SD from three independent experiments. SS21: minimal stem-loop aptamer (S21). Loop-A: sequences in the loop region were changed to 13 “As”, except for four nucleotides “CGCC.” Flap (4): flap-type inhibitor with a 4-nucleotide flap sequence. Flap S6b: the length of the stem region of Flap (4) was shortened to six base pairs. Flap (0): the flap sequence was deleted as a negative control. Flap (4)-masked: double-stranded oligonucleotide. Four nucleotides were extended to make a flap sequence duplex. AS-DNA: antisense DNA for the crRNA guide sequence. AS-RNA: antisense RNA for the crRNA guide sequence. **D**, Effect of the length of the stem region of Flap-type inhibitory aptamer on Cas9/crRNA/tracrRNA-directed plasmid cleavage. The inhibitory activities Flap-type inhibitory aptamer with various lengths of stem region were examined for Cas9/crGFP1 in the in vitro cleavage assay. No significant difference was found when the stem region was reduced from 12 to 6 base pairs. Once five base pairs were left, the inhibitory activity began to decline, especially at 10 nM. **E**, Length-dependent activities of aptamer were assessed for other target sites in *EGFR*. A significant difference was observed between Flap (4) and Flap (6). Two Flap (6) aptamers for *EGFR1* and *EGFR2* sites performed best.

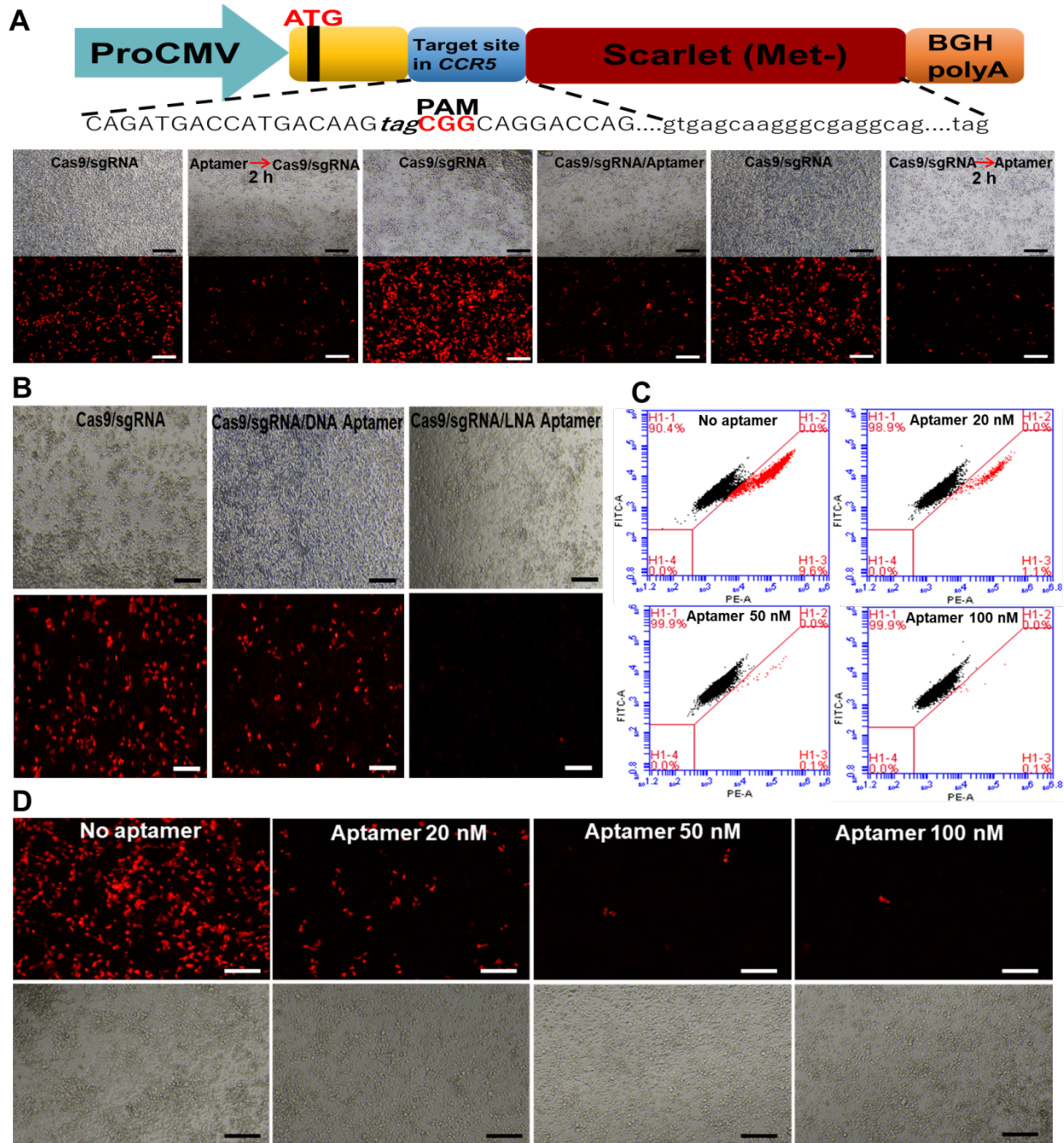


Figure S4. Related to Figure 4. Flap-type inhibitory aptamer blocked genome editing in 293-stop-mScarlet cells.

A, Schematic diagram of the mScarlet expression cassette. The 293 cells with a stably integrated DNA fragment of *CCR5* including stop codon upstream of the *mScarlet* reporter gene. The PAM

region was followed by a *TAG* stop codon, leading to no expression of the fluorescent protein. Transfection of Cas9/crRNA/tracrRNA complexes targeting *CCR5* site resulted in recovering mScarlet fluorescence by destroying the stop codon, as measured by flow cytometry. Scale bar = 200 μm . **B**, Inhibitory effect of an LNA-modified aptamer. Compared with DNA inhibitory aptamer, a great improvement was shown when replacing the flap sequence with an LNA-modified sequence. Scale bar = 200 μm . **C**, LNA-modified Flap-type inhibitory aptamer has higher inhibitory activities in 293-stop-mScarlet cells. Cells expressing mScarlet were detected by flow cytometry three days post-transfection. The minimal aptamer concentration required to prevent genomic editing in the cells was about 50 nM. **D**, Inhibitory activity was monitored by fluorescence microscopy. Fluorescence images of 293-stop-mScarlet cells were taken on the third day after co-transfection with Cas9/crRNA/tracrRNA and the aptamer. Scale bar = 200 μm .

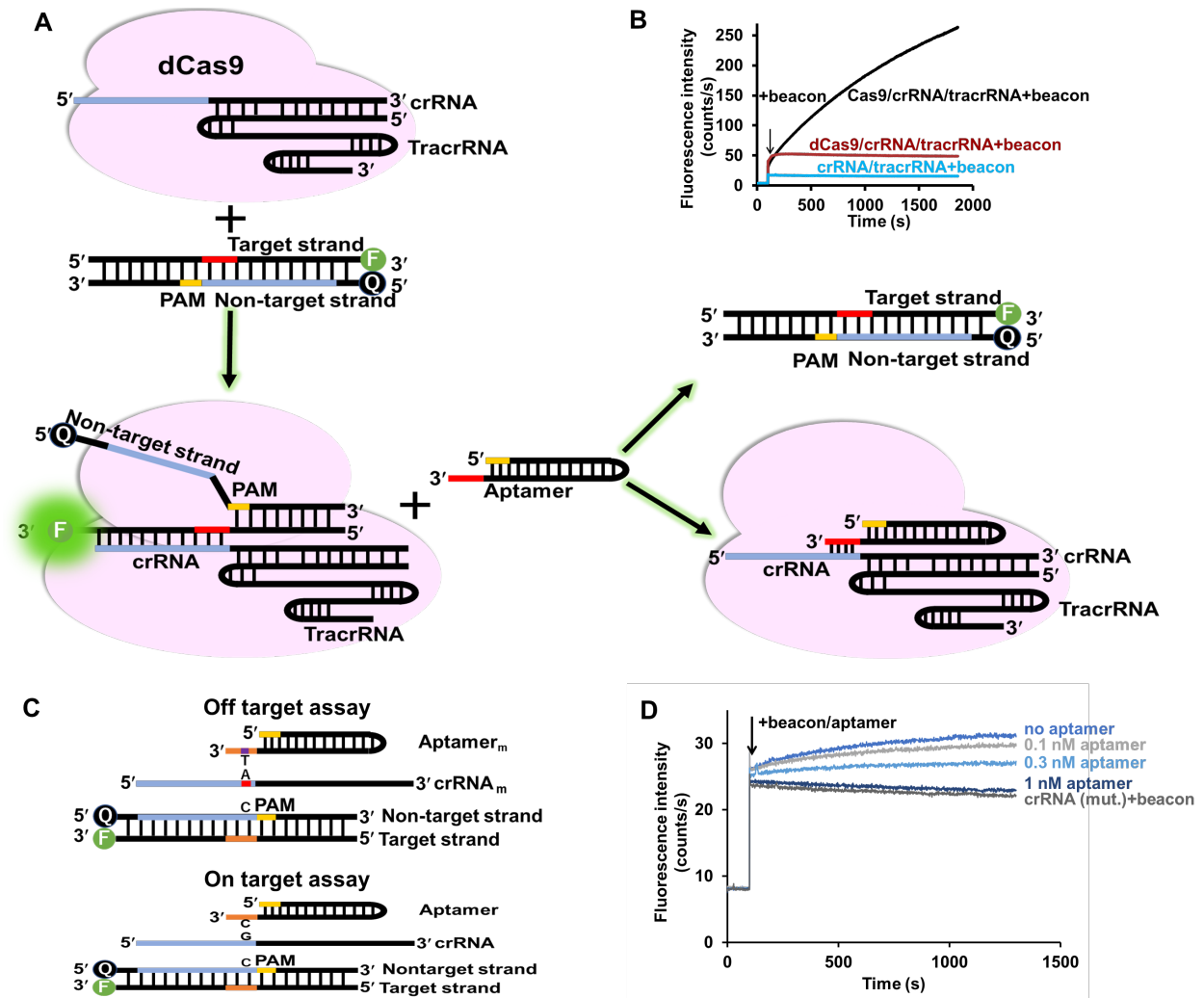


Figure S5. Related to Figure 5. Kinetic analysis of the interaction between the inhibitory aptamer and the Cas9/crRNA/tracrRNA complex by molecular beacon assay.

A, Schematic representation of a fluorometric molecular beacon assay. The beacon consists of three double-stranded DNA regions, the protospacer region (corresponding to the target sequence of Cas9/crGFP1), PAM region and surrounding sustained double-stranded sequences. Fluorescent label (F) and quencher (Q) are added at the PAM-distal ends of the beacon target and non-target strands, respectively. The fluorescence label is quenched by the nearby quencher via FRET mechanism. The 5'-NGG-3' PAM trinucleotide in the non-target strand is highlighted in yellow. The complementary sequence of crRNA in flap region is colored red, protospacer sequences in

crRNA in light blue. **B**, Measurement for the binding between Cas9 (dCas9)/crRNA/tracrRNA complex and the beacon. Time dependence of the fluorescence intensities after the addition of 1 nM beacon to Cas9/crRNA/tracrRNA. For dCas9, after a rapid increase of fluorescence, the kinetic curve reached a plateau. The fluorescence intensity was obtained from the addition of beacon to crRNA/tracrRNA without Cas9 as background. **C**, Schematic representation of beacon assay for on-target and off-target sequences. The circles labeled F and Q indicate the fluorophore and quencher, respectively. The 5'-NGG-3' PAM trinucleotide in the non-target strand is highlighted in yellow. The complementary sequence of crRNA in Flap region is coloured orange, protospacer sequences in crRNA in light blue. Mutations in crRNA_m and aptamer_m are highlighted in red and purple. "G" to "A" and "C" to "T" mutations were annotated in crRNA_m and aptamer_m strands separately. **D**, Effect of the aptamers with mutation (off-target) on the kinetics of beacon binding to dCas9/crRNA/tracrRNA.

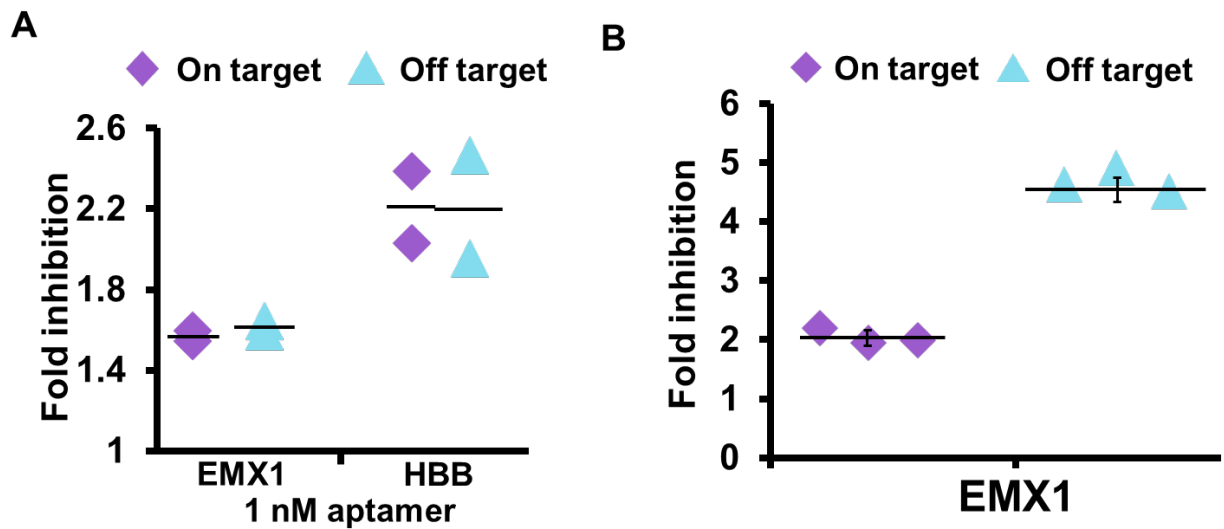


Figure S6. Related to Figure 6. The inhibitory aptamer inhibits both on- and off-target genomic editing in 293FT cells.

A, Fold inhibitions of on- and off-target were at a similar level by co-transfection. Data are presented with two biological replicates. **B**, Aptamer differentially reduced on- and off-target editing. 293FT cells were transfected with plasmids encoding Cas9 and sgRNA targeting *EMX1* locus. After 12 hours and 24 hours, 100 nM aptamers were delivered to cells. Data are presented as means \pm SD from three biological replicates. A significant difference between on-target and off-target was determined by student's t test, p-value=0.004.

Supplementary Table 1. Aptamers in seven clusters

cluster 1	cluster 4
TTGGGGCGCGCTTACGG	TCGGGCTCCTTTAACGG
TCGGGGCGTGCTTACGG	TCGGGCTCCTTTATCGG
TCGGGGCGTGCTGACGG	TCGGCCTCCTTTATCGG
TTGGGGCGTGCTTACGG	
TTGGGGCGTGCTGACGG	cluster 5
	TCACCCACCTTCAATGG
cluster 2	TCACCCCTCCTTCAATGG
TTGGGGCGCGCTTACGG	TCACCCACCTTCTATGG
TCGGGGCGTGCTTACGG	
TCGGGGCGTGCTGACGG	cluster 6
TTGGGGCGTGCTTACGG	CTTTGCATTGCGGACCT
TTGGGGCGTGCTGACGG	CTTTGCCTTGC GGACCT
	CTTTGACTTGC GGACCT
cluster 3	
TCGCCCTCCCTTGACGG	cluster 7
TTGCCCTCCCTTGACGG	CTTTGCTGGGGCGGACT
TCGCCCTCTCTTGACGG	CTTTGCTTGGGCGGACT
	CTTTGCGTGGGCGGACT

Only randomized regions were shown.

Supplementary Table 2. Candidate aptamers for the in vitro cleavage assay

Name	Sequence	Name	Sequence
S1	TCGCCCTCCCTTGACGG	S28	CACTCCTTCATACTCCCTCGGCC
S2	TCACCCACCTTCAATGG	S29	ACTATGCGCTGGCACCTCTTGTC
S3	CTTTGCCTTGCGGACCT	S30	ACGCTCCCTCCCAAGTATTATGG
S4	TCATTAGGCGTAATTGG	S31	TCTTCGGCTCCCTCCTCTCAGAC
S5	TCTATCGGCTTTACAGG	S32	TCGTTCTTTGGTGCGGTGAATGG
S6	TAAAAGGGGCAGGGTGG	S33	TCGGGGGCGCTCTTTAATATTGG
S7	TTGGTCCCCTTTATCGG	S34	TAAGTGTGATCGAGCCCTCCTGG
S8	TTGGGGTGTACTTACGG	S35	TTTACTCTCGCCATCGATCACGG
S9	TTAGGCGGCACCTCTAG	S36	ACGCGCCTCCCGTCCGAATTCGG
S10	TTGGGGTGTACTTACGG	S37	CTGTCGCGCCTCTCCGGATATGG
S11	TTCATAACCTTGAT	S38	TGCGCAGTCCCCTCACGTTACCT
S12	TGTCCTAACCTCTCCGG	S39	ACCACGTTCCCGGCATGTCATTA
S13	ACTCAGCCCTCCCAGGG	S40	TCGCGGTAGTCCCTTTTTTCGG
S14	CTATCGGACGCGGTACT	S41	CGTTCGCTGTTTCGTGGTAATA
S15	GGAATCCAAGCTCGGCCTCCCGG	S42	TTGTGCGATCCCTTTATACGG
S16	CGGTTACGGTCACCCAAGCGCAT	S43	TATGCCAGCTTTCATCACGG
S17	TCCACCCTCCGCGATGACATGG	S44	ACTCCGCGCCGACCCATTA
S18	TCACTGATCACAGCTCTTTTTGG	S45	CGCCGGATTCCCCTGTATT
S19	TCGCAAAAAGGGTCAGAATTCGG	S46	TCTGGGGGCGGTCATTAAGG
S20	TCGCCCCATTCCCTGTTGCTCGG	S47	TCGGTCCCCCTTTAAACGG
S21	TCGCGCCTTTCCCCAGCTTTCGG	S48	TCGGCCTCTCCTTGTTTGG
S22	TCGATGCCTCCTTTACTATACGG	S49	TCGCCCTCTCGGCACTCGG
S23	TCGTTCCACCCTTTCTGTTTCGG	S50	TCGCACAGGTTTAGTACGG
S24	TCGTGGTCAGGTTGATTTGCTGG	S51	TCAGGCTCCTCCTTATTGG
S25	TCGGTGTGGCAGGTTTATTACGG	S52	TCTGTTGCCTCTCCGGAAC
S26	TCGGATACACACCAACTGCTTGG	S53	ATCATACTCCCCGCTTTGG
S27	TCGGACGACCTAAGGCAAAACGG	S54	GGGGTTCCGTAGGGAGTGG
		S55	TTGGCACATGGCGTTACGG

The constant 5' (i.e., 5'-GAG GCT CTC ACA-3') and 3' (i.e., 5'-TGT GAG AGC CTC-3') sequences were trimmed and only randomized region was shown.

Supplementary Table 3. Sequences of oligonucleotides used in this study

Library used for SELEX experiments	
N17	GUGGAGAGGUTCTUACANNNNNNNNNNNNNNNNNNNTGTGAGAGCCTCTCCGC
N19	GUGGAGAGGUTCTUACANNNNNNNNNNNNNNNNNNNTGTGAGAGCCTCTCCGC
N21	GUGGAGAGGUTCTUACANNNNNNNNNNNNNNNNNNNTGTGAGAGCCTCTCCGC
N23	GUGGAGAGGUTCTUACANNNNNNNNNNNNNNNNNNNTGTGAGAGCCTCTCCGC
Primers used for amplification	
Library-F	CCGATCTAACCAAGTGGAGAGGTTCTTACA
Library-R	TGCGTAGAGCGATTGGCGGAGAGGCTCTCACA
EMX1-F	GCCATCCCCTTCTGTGAATGTTAGAC
EMX1-R	CGGAATCTACCACCCAGGCTCT
HPRT-F	ACATCAGCAGCTGTTCTG
HPRT-R	GGCTGAAAGGAGAGAACT
sgCCR5 target-F	GGAGACCCAAGCTGGCTAGCGTTT A
sgCCR5 target-R	TTGCTCACCTCCTCGCCCTTGCTCACCGT
mScarlet-F	CGAGGAGGTGAGCAAGGGCGAGGCAGTG
mScarlet-R	CAGCGGGTTTAAACGGGCCGAATTCTACTTGTACAGCTC
EMX1-on-seq-F	GTGGAGAGGTTCTTACA TTCCAGAACCGGAGGACAAAG
EMX1-on-seq-R	CGGAGAGGCTCTCACA AGGTGACATCGATGTCCTCCC
EMX1-off-seq-F	GTGGAGAGGTTCTTACA ACTATCACCTATTTTTTCTGA
EMX1-off-seq-R	CGGAGAGGCTCTCACA ATCTTGGGGTTACAGAAAGAA
HBB-on-seq-F	GTGGAGAGGTTCTTACA TAACCTTGATACCAACCTGCC
HBB-on-seq-R	CGGAGAGGCTCTCACA CAACTGTGTTCACTAGCAACC
HBB-off-seq-F	GTGGAGAGGTTCTTACA AAAGGGGAAGATCCCAGAGAA
HBB-off-seq-R	CGGAGAGGCTCTCACA GTATGTCCAACCTCCAAATTG
BssHII-U6F	CCTTTCGTCGGCGCG AATCAAGGTCGGGCAGGAAGAG
U6R	GGTGTTCGTCCTTTCCACAAG
TRE-RNA2.0F-U6r	AAAGGACGAAACACCATACGTTCTCTATCACTGATGTTTAAGAGCTAG
RNA2.0rev-BssHII	GGCGGCCGCGCGCGAAAAAGCACCGACTCGGTGCCACTTGGCCCTGCAGACATGGGTGATCCTCA TGTTGGCCAAGTTGATAACGGACTAGCCTTATTTAACTTGCTAGGCCCTGCAGACATGGGTGATCCTC

ATGTTGGCCTAGCTCTTAAAC

sgEMX1-F

GTGGAAAGGACGAAACACCGAGTCCGAGCAGAAGAAGAAGTTTAAGAGCTAGAAATAGCA

sgEMX1-R

TGCTATTTCTAGCTCTTAAACTTCTTCTTCTGCTCGGACTCGGTGTTTCGTCTTTCCAC

sgHBB-F

GTGGAAAGGACGAAACACCGCTTGCCCCACAGGGCAGTAAGTTTAAGAGCTAGAAATAGCA

sgHBB-R

TGCTATTTCTAGCTCTTAAACTTACTGCCCTGTGGGGCAAGCGGTGTTTCGTCTTTCCAC

Aptamers used in this study

C1 GAGGCTCTCACA TTGGGGCGTGCTGACGG TGTGAGAGCCTC

C2 GAGGCTCTCACA TTGCACTCCTTCATCGG TGTGAGAGCCTC

C3 GAGGCTCTCACA TCGCCCTCCCTTGACGG TGTGAGAGCCTC

C4 GAGGCTCTCACA TCGGGCTCCTTTAACGG TGTGAGAGCCTC

C5 GAGGCTCTCACA TCACCCACCTTCAATGG TGTGAGAGCCTC

C6 GAGGCTCTCACA CTTTGCTGGGGCGGACT TGTGAGAGCCTC

C7 GAGGCTCTCACA CTTTGCCTTGCGGACCT TGTGAGAGCCTC

S21 GAGGCTCTCACA TCG CGCCTTTCCCCAGCTTTCGG TGTGAGAGCCTC

S21-LD1 GAGGCTCTCACA TCG CGCCTTTCTTTCGG TGTGAGAGCCTC

S21-LD2 GAGGCTCTCACA TCG CGCCTTTTTTCGG TGTGAGAGCCTC

S21-LD3 GAGGCTCTCACA TCG CGCCTTTTTTCGG TGTGAGAGCCTC

S21-LD4 GAGGCTCTCACA TCG CGCCTTTTTTCGG TGTGAGAGCCTC

S21-LD5 GAGGCTCTCACA TCG CGCCTTTCGG TGTGAGAGCCTC (SS21)

S21-LD6 GAGGCTCTCACA TCG CGCCTTCGG TGTGAGAGCCTC

SL NC GAGGCTCTCACA TCG GTAACGG TGTGAGAGCCTC

SS21-LM1 GAGGCTCTCACA TCG **G**GCCTTT CGG TGTGAGAGCCTC

SS21-LM2 GAGGCTCTCACA TCG **CC**CCTTT CGG TGTGAGAGCCTC

SS21-LM3 GAGGCTCTCACA TCG CG**G**CCTTT CGG TGTGAGAGCCTC

SS21-LM4 GAGGCTCTCACA TCG CGC**G**TTT CGG TGTGAGAGCCTC

SS21-LM5 GAGGCTCTCACA TCG CGCC**A**TT CGG TGTGAGAGCCTC

SS21-LM6 GAGGCTCTCACA TCG CGCCT**A**T CGG TGTGAGAGCCTC

SS21-LM7 GAGGCTCTCACA TCG CGCCT**A** CGG TGTGAGAGCCTC

SS21-SD1	AGGCTCTCACA	TCG CGCCTTT CGG	TGTGAGAGCCT
SS21-SD2	GGCTCTCACA	TCG CGCCTTT CGG	TGTGAGAGCC
SS21-SD3	GCTCTCACA	TCG CGCCTTT CGG	TGTGAGAGC
SS21-SD4	CTCTCACA	TCG CGCCTTT CGG	TGTGAGAG
SS21-SD5	CTCACA	TCG CGCCTTT CGG	TGTGAG
SS21 SM1	<u>CTGGCTCTCACA</u>	TCG CGCCTTT CGG	<u>TGTGAGAGCCAG</u>
SS21 SM2	<u>GACTCTCTCACA</u>	TCG CGCCTTT CGG	<u>TGTGAGAGGGTC</u>
SS21 SM3	<u>GAGG<u>G</u>ACTCACA</u>	TCG CGCCTTT CGG	<u>TGTGAG<u>T</u>CCCTC</u>
SS21 SM4	<u>GAGGCT<u>G</u>ACACA</u>	TCG CGCCTTT CGG	<u>TGTG<u>T</u>CAGCCTC</u>
SS21 SM5	<u>GAGGCTCT<u>G</u>TCA</u>	TCG CGCCTTT CGG	<u>TG<u>A</u>CAGAGCCTC</u>
SS21 SM6	<u>GAGGCTCTCAG<u>T</u></u>	TCG CGCCTTT CGG	<u><u>A</u>CTGAGAGCCTC</u>
SS21 SM7	<u>GAGGCTCTCACA</u>	<u>TGC</u> CGCCTTT <u>GCG</u>	<u>TGTGAGAGCCTC</u>
SS21 NM1	GAGGCTCTCACA	<u>A</u> CG CGCCTTT CGG	TGTGAGAGCCTC
SS21 NM2	GAGGCTCTCACA	<u>G</u> CG CGCCTTT <u>CG</u> <u>T</u>	TGTGAGAGCCTC
SS21 NM3	GAGGCTCTCACA	<u>C</u> CG CGCCTTT CGG	TGTGAGAGCCTC
SS21 NM4	GAGGCTCTCACA	TCG CGCCTTT <u>TGG</u>	TGTGAGAGCCTC
SS21 NM5	GAGGCTCTCACA	<u>TC</u> <u>A</u> CGCCTTT <u>TGG</u>	TGTGAGAGCCTC
SS21 NM6	GAGGCTCTCACA	<u>TC</u> <u>C</u> CGCCTTT <u>G</u> GG	TGTGAGAGCCTC
SS21 NM7	GAGGCTCTCACA	<u>TC</u> <u>A</u> CGCCTTT CGG	TGTGAGAGCCTC
SS21 NM8	GAGGCTCTCACA	<u>TGG</u> CGCCTTT <u>CCG</u>	TGTGAGAGCCTC
SS21 NM9	GAGGCTCTCACA	<u>TTG</u> CGCCTTT CGG	TGTGAGAGCCTC
SS21 NM10	GAGGCTCTCACA	<u>TAG</u> CGCCTTT CGG	TGTGAGAGCCTC
SS21 NM11	GAGGCTCTCACA	<u>TC</u> <u>CA</u> CGCCTTT <u>TGG</u>	TGTGAGAGCCTC
Flap (4)-crGFP1m	CGG	TGTGAGAGCCTC	GAAA GAGGCTCTCACA TCG CTCC
Flap-crGFP1 S 10b	CGG	TGTGAGAGCC	----GAAA ----GGCTCTCACA TCG CGCC
Flap-crGFP1 S 9b	CGG	TGTGAGAGC	-----GAAA -----GCTCTCACA TCG CGCC
Flap-crGFP1 S 6b	CGG	TGTGAG	-----GAAA -----CTCACA TCG CGCC
Flap-crGFP1 S 5b	CGG	TGTGA	-----GAAA -----TCACA TCG CGCC
Flap-crGFP1 S 4b	CGG	TGTG	-----GAAA -----CACACA TCG CGCC
Flap-crGFP1 S 3b	CGG	TGT	-----GAAA -----ACA TCG CGCC

Flap-crGFP1 S 2b	CGG	TG-----GAAA -----CA	TCG CGCC
Flap (0)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG
Flap (2)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CG
Flap (3)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CGC
Flap (4)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CGCC
Flap (5)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CGCCG
Flap (6)-crGFP1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CGCCGA
Loop NC for EMSA		GAGGCTCTCACAGTAATGTGAGAGCCT	
Loop NC for Biacore	Bio-	GAGGCTCTCACAGTAATGTGAGAGCCT	
S21 for Biacore	Bio-	GAGGCTCTCACA	TCG CGCCTTTCCCCAGCTTTCGG TGTGAGAGCCTC
SA-Ap		GGTCCTCACACCGCTGTGTGCCGCAACACTGTGAGGACC	
SL-crGFP2	GAGGCTCTCACA	TCG CGGCTTT CGG	TGTGAGAGCCTC
SL-crGFP3	GAGGCTCTCACA	TCG CATCTTT CGG	TGTGAGAGCCTC
Loop A crGFP1	GAGGCTCTCACA	TCG CGCAAAAAAAAAAAAAA CGG	TGTGAGAGCCTC
Loop A crGFP2	GAGGCTCTCACA	TCG CGGCAAAAAAAAAAAAAA CGG	TGTGAGAGCCTC
Loop A crGFP3	GAGGCTCTCACA	TCG CATCAAAAAAAAAAAAAA CGG	TGTGAGAGCCTC
Flap crGFP2	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CGGC
Flap (4) crGFP3	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CATC
Flap (4)-crEGFR1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG ATGT
Flap (5)-crEGFR1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG ATGTG
Flap (6)-crEGFR1	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG ATGTGC
Flap (4)-crEGFR2	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CATT
Flap (5)-crEGFR2	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CATTG
Flap (6v)-crEGFR2	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG CATTGC
Flap (4)-crEPCAM	CGG	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG GTGT
Flap-crCCR5	C^G^G	TGTGAGAGCCTC GAAAGAGGCTCTCACA	TCG CTAC^T^T
Flap-crCCR5-L	C^G^G	GTGTGAGAGCCTC GAAAGAGGCTCTCACA	TCG mC(L)T(L)A(L)mC(L)^T(L)^T(L)
Flap-crEMX1	C^G^G	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG TTCT^T^C
Flap-crEMX-L6	C^G^G	TGTGAGAGCCTC GAAA GAGGCTCTCACA	TCG

	T(L)T(L)mC(L)T(L)^T(L)^mC(L)
Flap-crHPRT	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG TGTT^T^A
Flap-HBB-L6	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG T(L)T(L)A(L)5(L)^T(L)^G(L)
Flap-crHPRT-L6	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG T(L)G(L)T(L)T(L)^T(L)^A(L)
Flap-crGFP-L4	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG mC(L)G(L)mC(L)mC(L)
Flap-crGFP-L6	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG mC(L)G(L)mC(L)mC(L) ^G(L) ^A(L)
Flap-crTRE-L6	C^G^G TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG G(L)G(L)T(L)G(L)*T(L)*T(L)
DS (37 bp)-S	GAGGCTCTCACA CCG CGCCGAGGTGAAGTTCGAGGCC
DS (37 bp)-AS	GGCCTCGAACTTCACCTCGGCG CGGTGTGAGAGCCTC
Flap (4)-masked	GGCG CGG TGTGAGAGCCTC GAAA GAGGCTCTCACA TCG CGCC
As RNA	cgccgaggugaaguucgaggcc
As DNA	CGCCGAGGTGAAGTTCGAGGCC
cpfl-rr1	GACG CAAA GTGAGAGCCTC GAAA GAGGCTCTCAC TTTG
cpfl-rr2	GACG CGAA GTGAGAGCCTC GAAA GAGGCTCTCAC TTTG
cpfl-rr3	GACG CAGA GTGAGAGCCTC GAAA GAGGCTCTCAC TTTG
cpfl-rr4	GACG CAAG GTGAGAGCCTC GAAA GAGGCTCTCAC TTTG
Cpfl-F-EGFR1	GCAC CAA AGTGAGAGCCTC GAAA GAGGCTCTCACT TTG
Cpfl-F-EGFR2	GCCG CAA AGTGAGAGCCTC GAAA GAGGCTCTCACT TTG
Cpfl-F-EGFR3	GCCA CAA AGTGAGAGCCTC GAAA GAGGCTCTCACT TTG
Molecular Beacon	
EGFmb-F	Q-T CGC CCTCGAACTTCACCTCGGCG CGG GTCTTGTAGTTG
EGFmb-R	CAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCG A-FAM
crRNA and tracrRNA	
crGFP1	ggccucgaacuaccucggcg guuuuagagcuaugcuguuuug
crGFP2	caactacaagacccgccc guuuuagagcuaugcuguuuug
crGFP3	cgatgcccttcagctcgatg guuuuagagcuaugcuguuuug

crGFP4	catgccgagagtgatcccgg guuuuagagcuauugcuguuuug
crGFP1m	ggccucgaacuaccucggag guuuuagagcuauugcuguuuug
crEGFR1	aucauaauuccucugcacat guuuuagagcuauugcuguuuug
crEGFR2	ccacuguguugagggaug guuuuagagcuauugcuguuuug
crEPCAM	gugcaccaacugaaguacac guuuuagagcuauugcuguuuug
crCCR5	cagaugaccaugacaaguag guuuuagagcuauugcuguuuug
crEMX1	gaguccgagcagaagaaga guuuuagagcuauugcuguuuug
crHPRT	gcuuuuctcagtccuaaaca guuuuagagcuauugcuguuuug
crHBB:	ctgccccacaggcagtaa guuuuagagcuauugcuguuuug
Tracr RNA	aaacagcauagcaaguuaaaauaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu
Cpf1 crGFP	uaauuucuacucuuguagau cgucgccguccagcucgacc
Cpf1 crEGFR1	uaauuucuacucuuguagau gugccaccugcgugaagaag
Cpf1 crEGFR2	uaauuucuacucuuguagau cggcagaccaggcagucgcu
Cpf1 crEGFR3	uaauuucuacucuuguagau uggcaguucuccucuccugc

N(L) = LNA, ^ = phosphorothioated, N = DNA, n = RNA, *N* = mutated nucleotide