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Supplemental Information

Synthetic Oligonucleotides Inhibit CRISPR-Cpf1-Mediated Genome Editing

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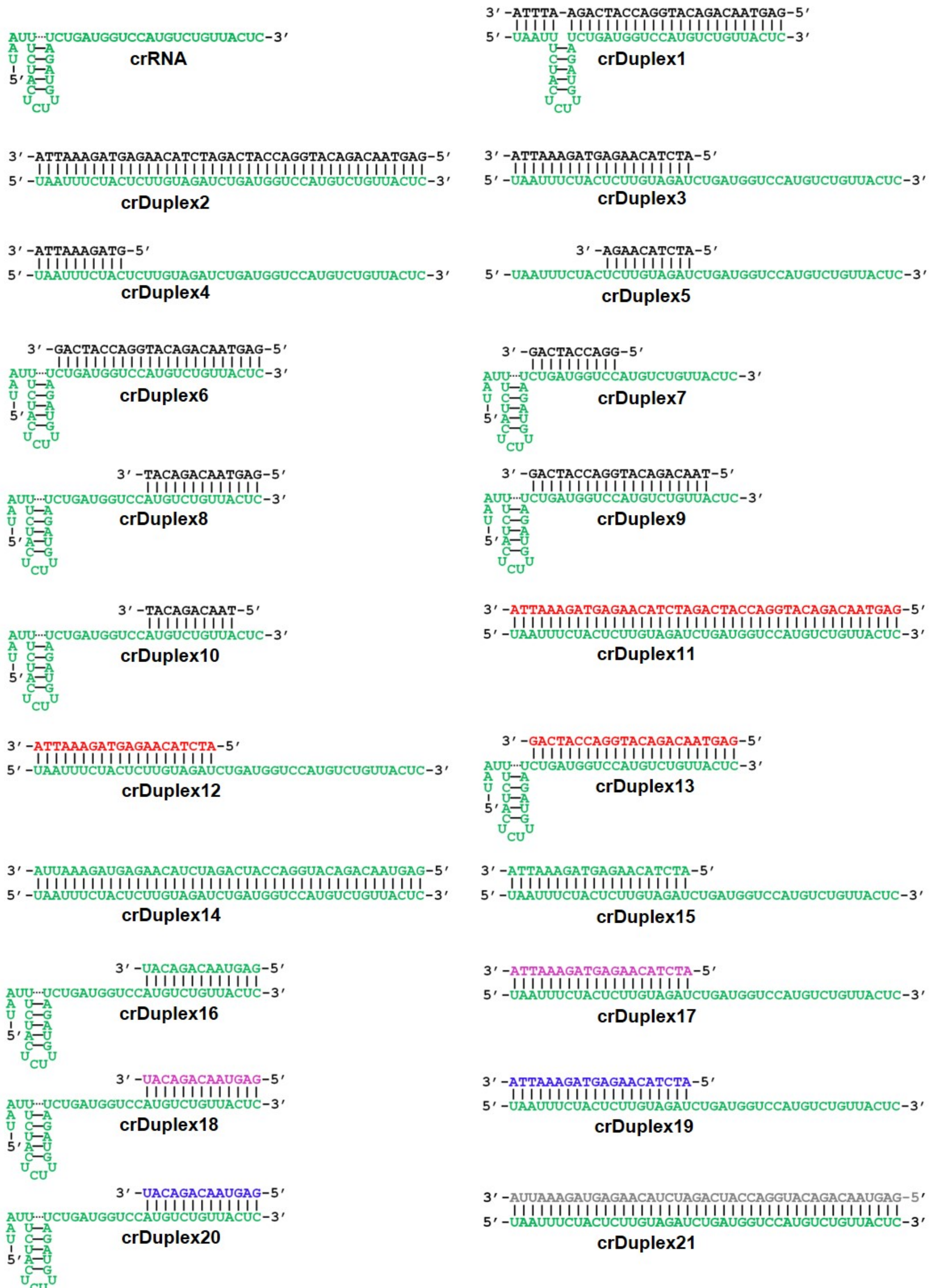

Figure S1. Structures of wild-type crRNA and crRNA duplexes. Related to Figure 1.

The green letter denotes the unmodified RNA nucleotide. The black letter denotes unmodified DNA nucleotide. The red letter denotes PS-linkage modified DNA. The violet letter denotes 2'-fluoro modified RNA. The blue letter denotes 2'-*O*-methyl modified RNA. The gray letter denotes PS-linkage modified RNA. Vertical lines represent complementary binding between paired bases.

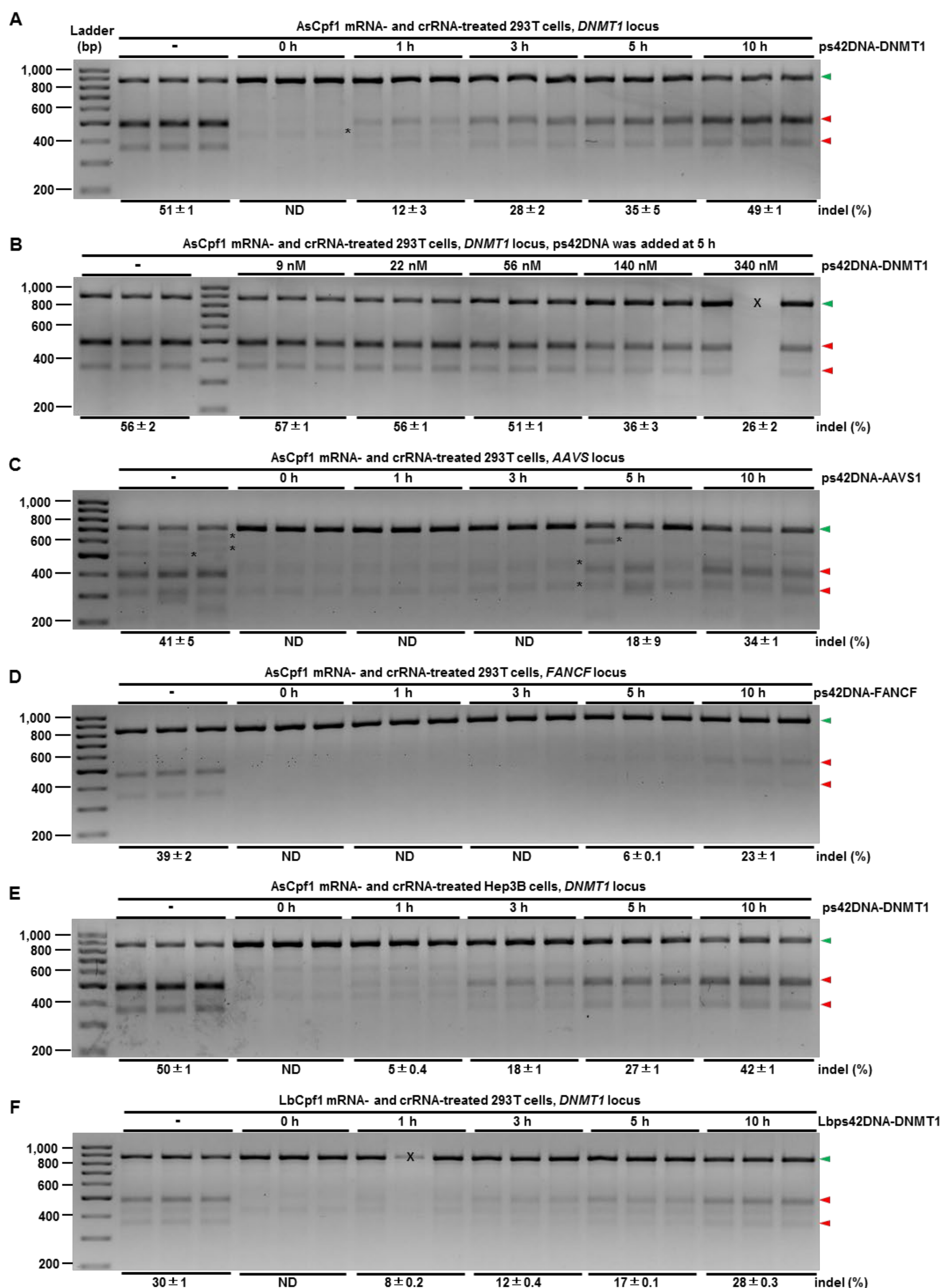

Figure S2. Inhibition effects of phosphorothioated DNA oligonucleotides on Cpf1-mediated genome editing in mammalian cells. Related to Figure 2.

(A) Time-dependent inhibitory effects of ps42DNA-DNMT1 on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. ps42DNA-DNMT1 was added at various time points after treatment with AsCpf1 mRNA and

crRNA targeting *DNMT1* locus. Related to Figure 2B.
(B) Dose-dependent inhibitory effects of ps42DNA on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. ps42DNA-DNMT1 was added at 5 h after treatment with CRISPR-Cpf1 mRNA and crRNA targeting *DNMT1* locus. Related to Figure 2C.
(C) Time-dependent inhibitory effects of ps42DNA-AAVS1 on AsCpf1-mediated genome editing at the *AAVS1* locus in 293T cells. ps42DNA-AAVS1 was added at various time points after treatment with AsCpf1 mRNA and crRNA targeting *AAVS1* locus. Related to Figure 2D.
(D) Time-dependent inhibitory effects of ps42DNA-FANCF on AsCpf1-mediated genome editing at the *FANCF* locus in 293T cells. ps42DNA-FANCF was added at various time points after treatment with AsCpf1 mRNA and crRNA targeting *FANCF* locus. Related to Figure 2E.
(E) Time-dependent inhibitory effects of ps42DNA on AsCpf1-mediated genome editing at the *DNMT1* locus in Hep3B cells. ps42DNA-DNMT1 was added at various time points after treatment with AsCpf1 mRNA and crRNA targeting *DNMT1* locus. Related to Figure 2F.
(F) Time-dependent inhibitory effects of Lbps42DNA-DNMT1 on LbCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. Lbps42DNA-DNMT1 was added at various time points after treatment with LbCpf1 mRNA and LbCpf1 crRNA targeting *DNMT1* locus. Related to Figure 2G.
The control group (-) was treated only with AsCpf1 mRNA and crRNA. The concentration of inhibitors in (B) and (D)–(G) is 140 nM (2.5-fold molar excess relative to crRNA). Relative genome-editing efficiency in (B)–(G) was determined using the T7E1 cleavage assay from three biological replicates 48 hr post-treatment. The lane marked with "×" is excluded for calculation of genome editing efficiency. The time points above gels denote the time to add inhibitors, and the asterisks denote the non-specific bands. The green and red arrowheads denote the intact DNA substrate and cleaved DNA fragments, respectively.

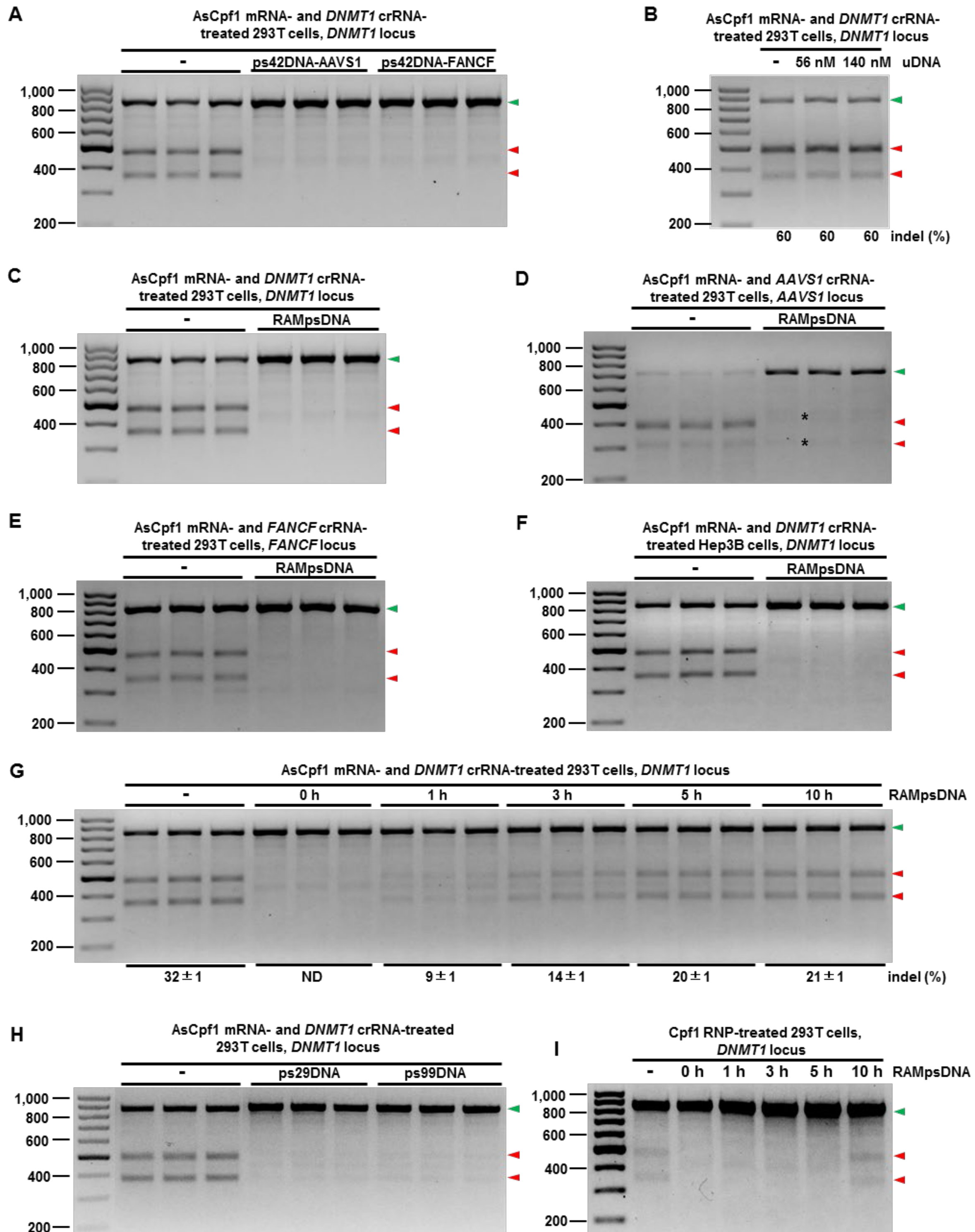

Figure S3. Inhibitory effects of RAMpsDNA against Cpf1 in human cells. Related to Figure 3.

(A) Inhibitory effects of ps42DNA-AAVS1 and ps42DNA-FANCF on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. ps42DNA-AAVS1 or ps42DNA-FANCF was added simultaneously with AsCpf1 mRNA and AsCpf1 crRNA targeting *DNMT1* locus. Related to Figure 3A.

(B) A representative gel image for assessment of the impact of uDNA on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. uDNA was added at 0 h after treatment with CRISPR-Cpf1 mRNA and crRNA targeting the *DNMT1* locus.

(C) Inhibitory effects of RAMpsDNA on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. RAMpsDNA was added simultaneously with CRISPR-Cpf1 mRNA and crRNA targeting the *DNMT1* locus. Related to Figure 3B.
(D) Inhibitory effects of RAMpsDNA on AsCpf1-mediated genome editing at the *AAVS1* locus in 293T cells. RAMpsDNA was added simultaneously with CRISPR-Cpf1 mRNA and crRNA targeting the *AAVS1* locus. Related to Figure 3B.
(E) Inhibitory effects of RAMpsDNA on AsCpf1-mediated genome editing at the *FANCF* locus in 293T cells. RAMpsDNA was added simultaneously with CRISPR-Cpf1 mRNA and crRNA targeting the *FANCF* locus. Related to Figure 3B.
(F) Inhibitory activity of RAMpsDNA on AsCpf1-mediated genome editing at the *DNMT1* locus in Hep3B cells. RAMpsDNA was added simultaneously with CRISPR-Cpf1 mRNA and crRNA targeting *DNMT1* locus. Related to Figure 3C.
(G) Time-dependent inhibitory effects of RAMpsDNA on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. The time points above gels denote the time to add RAMpsDNA. Genome editing efficiency was determined by the T7E1 cleavage assay three biological replicates. Related to Figure 3D.
(H) The effects of RAMpsDNA length on on AsCpf1-mediated genome editing at the *DNMT1* locus in 293T cells. Phosphorothioated DNA oligonucleotides with different length was added simultaneously with CRISPR-Cpf1 mRNA and crRNA targeting *DNMT1* locus.
(I) Inhibitory activity of RAMpsDNA on Cpf1-RNP mediated genome editing at the *DNMT1* locus in 293T cells. RAMpsDNA was added at various time points after treatment with AsCpf1 mRNA and crRNA targeting *DNMT1* locus.
The control group (-) was treated only with AsCpf1 mRNA and crRNA or Cpf1 RNP. The concentration of inhibitors in (A) and (C)-(I) is 140 nM (2.5-fold molar excess relative to crRNA). T7E1 cleavage assays were conducted 48 hr post-treatment. The asterisks denote the non-specific bands. The green and red arrowheads denote the intact DNA substrate and cleaved DNA fragments, respectively.

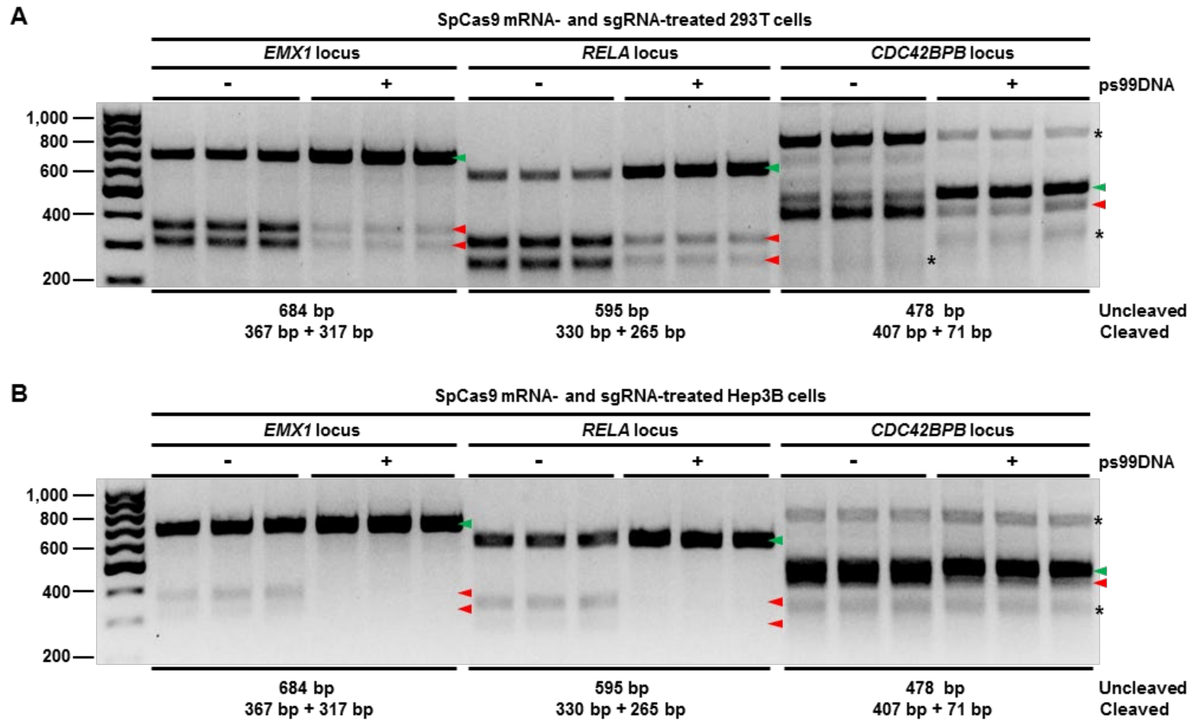

Figure S4. Inhibitory effects of phosphorothioated DNA oligonucleotides on Cas9-mediated genome editing in mammalian cells. Related to STAR Methods.

(A) Inhibitory effects of ps99DNA on SpCas9-mediated genome editing at the *EMX1*, *RELA*, and *CDC42BPB* loci in 293T cells. ps99DNA was added simultaneously with CRISPR-Cas9 mRNA and sgRNA targeting the *EMX1*, *RELA*, or *CDC42BPB* loci.

(B) Inhibitory effects of ps99DNA on SpCas9-mediated genome editing at the *EMX1*, *RELA*, and *CDC42BPB* loci in Hep3B cells. ps99DNA was added simultaneously with CRISPR-Cas9 mRNA and sgRNA targeting the *EMX1*, *RELA*, or *CDC42BPB* loci.

The control group (-) was treated only with SpCas9 mRNA and sgRNA. T7E1 cleavage assays were conducted 48 hr post-treatment. Asterisks denote the non-specific bands. The green and red arrowheads denote the intact DNA substrate and cleaved DNA fragments, respectively.

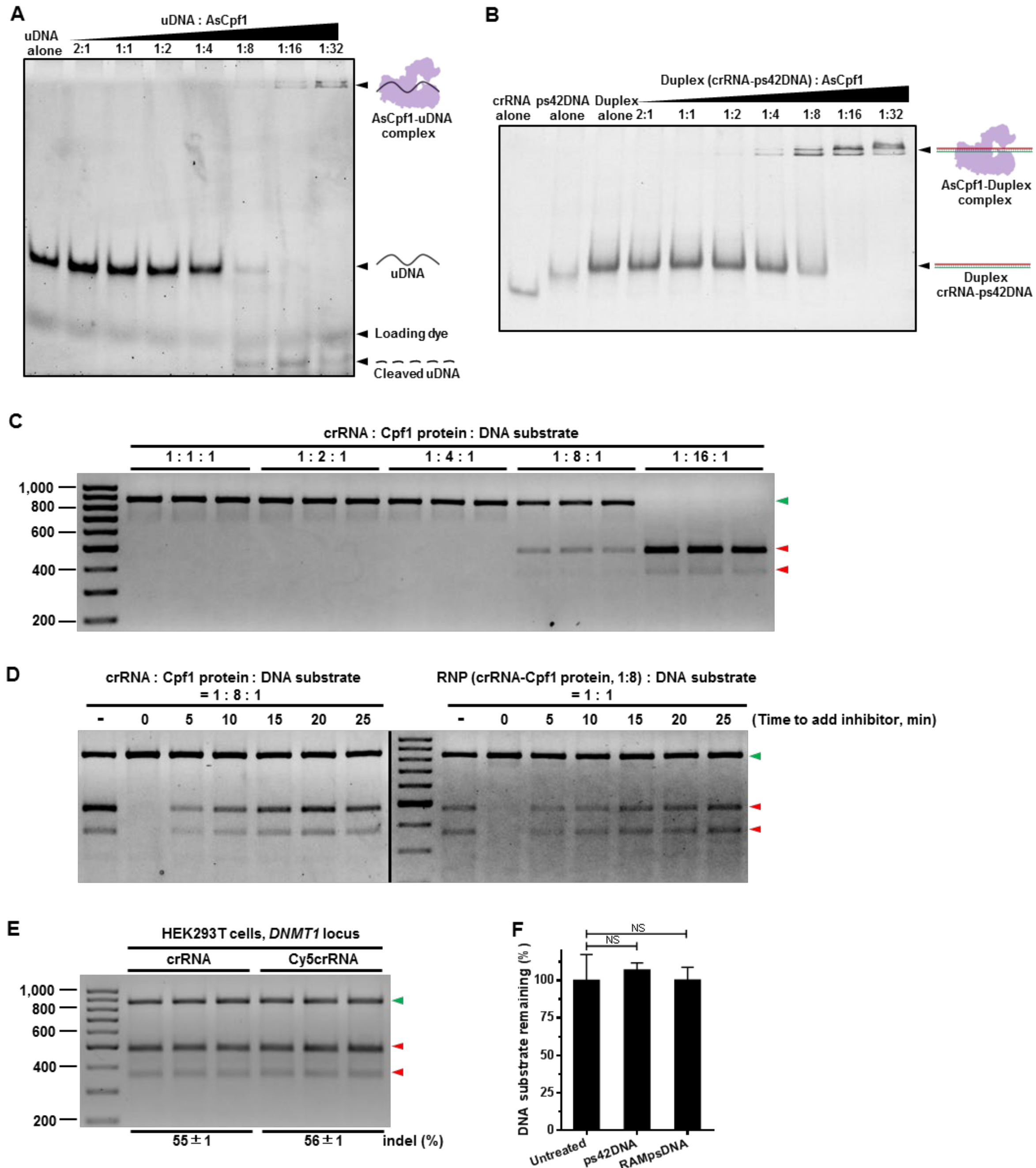

Figure S5. Cpf1-mediated *in vitro* cleavage assay. Related to Figure 4 and Figure 5.

(A) AsCpf1 protein-induced unmodified DNA (uDNA) cleavage in the absence of crRNA.

(B) Interactions between AsCpf1 protein and duplex crRNA-ps42DNA.

(C) AsCpf1-mediated DNA cleavage in vitro in the presence of wild-type crRNA at the indicated molar ratio of crRNA : AsCpf1 protein: DNA substrate. The reaction mixtures were treated with proteinase K to release the short cleaved fragment complexed with AsCpf1 protein.

(D) The time course of the inhibitory effect of ps42DNA. Left, three components including crRNA, AsCpf1 protein, and the target DNA substrate were incubated at 37°C for 30 min. Right, crRNA and AsCpf1 protein were pre-assembled into RNP at 37°C for 30 min, and then incubated with the target DNA substrate at 37°C for 30 min. In both panels. The inhibitor ps42DNA was added to the reaction at the indicated time points. The vertical line

indicates the border between two separate gels.

(E) Comparison of genome editing efficiency of crRNA and Cy5crRNA at the *DNMT1* locus in the presence of AsCpf1 plasmid in 293T cells. Genome editing efficiency (%) was determined by the T7E1 cleavage assay 48 h post-transfection.

(F) Quantification of DNA substrate remaining (%) in the presence of AsCpf1 protein, crRNA and ps42DNA (or RAMpsDNA). The uncleaved DNA substrate was quantified by densitometric analysis and normalized to the DNA substrate alone group. Data are expressed as the mean ± SD from three biological replicates (NS, not significant; two-tailed t-test).

Gels in (A) and (B) were resolved on 15% non-denaturing TBE polyacrylamide gels and stained by SYBR Gold. Gels in (C)-(E) were separated on 2% agarose gels and stained by EZ-Vision In-Gel staining. The green and red arrowheads denote the intact DNA substrate and cleaved DNA fragments, respectively.

Table S1. crRNAs and oligonucleotides used in this study. Related to Figures 1-5.

Oligonucleotides	Length (nt)	Sequence (5' to 3')
AsCpf1 crRNA targeting *DNMT1*	43	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUG UUACUC
Oligo 1	29	GAGTAACAGACATGGACCATCAGAAATTA
Oligo 2 (uDNA)	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGA AATTA
Oligo 3	20	ATCTACAAGAGTAGAAATTA
Oligo 4	10	GTAGAAATTA
Oligo 5	10	ATCTACAAGA
Oligo 6	23	GAGTAACAGACATGGACCATCAG
Oligo 7	10	GGACCATCAG
Oligo 8	13	GAGTAACAGACAT
Oligo 9	20	TAACAGACATGGACCATCAG
Oligo 10	10	TAACAGACAT
Oligo 11 (ps42DNA-DNMT1)	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGA AATTA
Oligo 12	20	ATCTACAAGAGTAGAAATTA
Oligo 13	23	GAGTAACAGACATGGACCATCAG
Oligo 14	43	GAGUAACAGACAUGGACCAUCAGAUCUACAAGAGUAG AAAUUA
Oligo 15	20	AUCUACAAGAGUAGAAAUUA
Oligo 16	13	GAGUAACAGACAU
Oligo 17	20	AUCUACAAGAGUAGAAAUUA
Oligo 18	13	GAGUAACAGACAU
Oligo 19	20	AUCUACAAGAGUAGAAAUUA
Oligo 20	13	GAGUAACAGACAU
Oligo 21	13	GAGUAACAGACAUGGACCAUCAGAUCUACAAGAGUAG AAAUUA
Randomized ps42DNA (RAMpsDNA)	43	AGACGTACAGTAAGACAATAAGACCGAACTTAGCATAT GGAAT
Randomized ps29DNA	30	AGACGTACAGTAAGACAATAAGACCGAACT
AsCpf1 crRNA targeting *AAVS*	43	UAAUUUCUACUCUUGUAGAUCUUACGAUGGAGCCAGA GAGGAU
ps42DNA-AAVS1	43	ATCCTCTCTGGCTCCATCGTAAGATCTACAAGAGTAGAA ATTA
AsCpf1 crRNA targeting *FANCF*	43	UAAUUUCUACUCUUGUAGAUGUCGGCAUGGCCCCAUU CGCACG
ps42DNA-FANCF	43	CGTGCGAATGGGGCCATGCCGACATCTACAAGAGTAGA AATTA
LbCpf1 crRNA targeting *DNMT1*	43	AAUUUCUACUAAGUGUAGAUCUGAUGGUCCAUGUCUG UUACUC
Lbps42DNA-DNMT1	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGA

		AATTA
SpCas9 sgRNA targeting *EMX1*	100	GAGUCCGAGCAGAAGAAGAA + scaffold
ps99DNA	100	Reversely complement of scaffold + TTCTTCTTCTGCTCGGACTC
SpCas9 sgRNA targeting *RELA*	100	GAUCUCCACAUAGGGGCCAG + scaffold
SpCas9 sgRNA targeting *CDC42BPB*	100	GAGCCGCACCUUGGCCGACA + scaffold

Unmodified RNA nucleotides are shown in green. Unmodified DNA nucleotides are shown in black. PS-linkage modified DNA nucleotides are shown in red. 2'-fluoro modified RNA nucleotides are shown in violet. 2'-*O*-methyl modified RNA nucleotides are shown in blue. PS-linkage modified RNA nucleotides are shown in gray.

Table S2. A list of primers used for T7E1 assay and *in vitro* cleavage reaction. Related to Figures 1-3, and 5.

Locus	**Forward primer**	**Reverse primer**
DNMT1	CTGGGACTCAGGCGGGTCAC	CCTCAGCCAGAAGTCCCGTGC
AAVS1	GGGCTGGCTACTGGCCTTAT	ATGGCATCTTCCAGGGGTCC
FANCF	AGCTCCGCCTGGGTCTTCAT	GCGGAGACGTTCATGACTGG
EMX1	AAAACCACCCTTCTCTCTGGC	GGAGATTGGAGACACGGAGAG
RELA	TTCTAGGGAGCAGGTCCTGACT	TCCTTTCCTACAAGCTCGTGGG
CDC42BPB	GCGCCCTGACGGACTGGCCGA	GGAGGGCAAGGAGGGATGAAAA