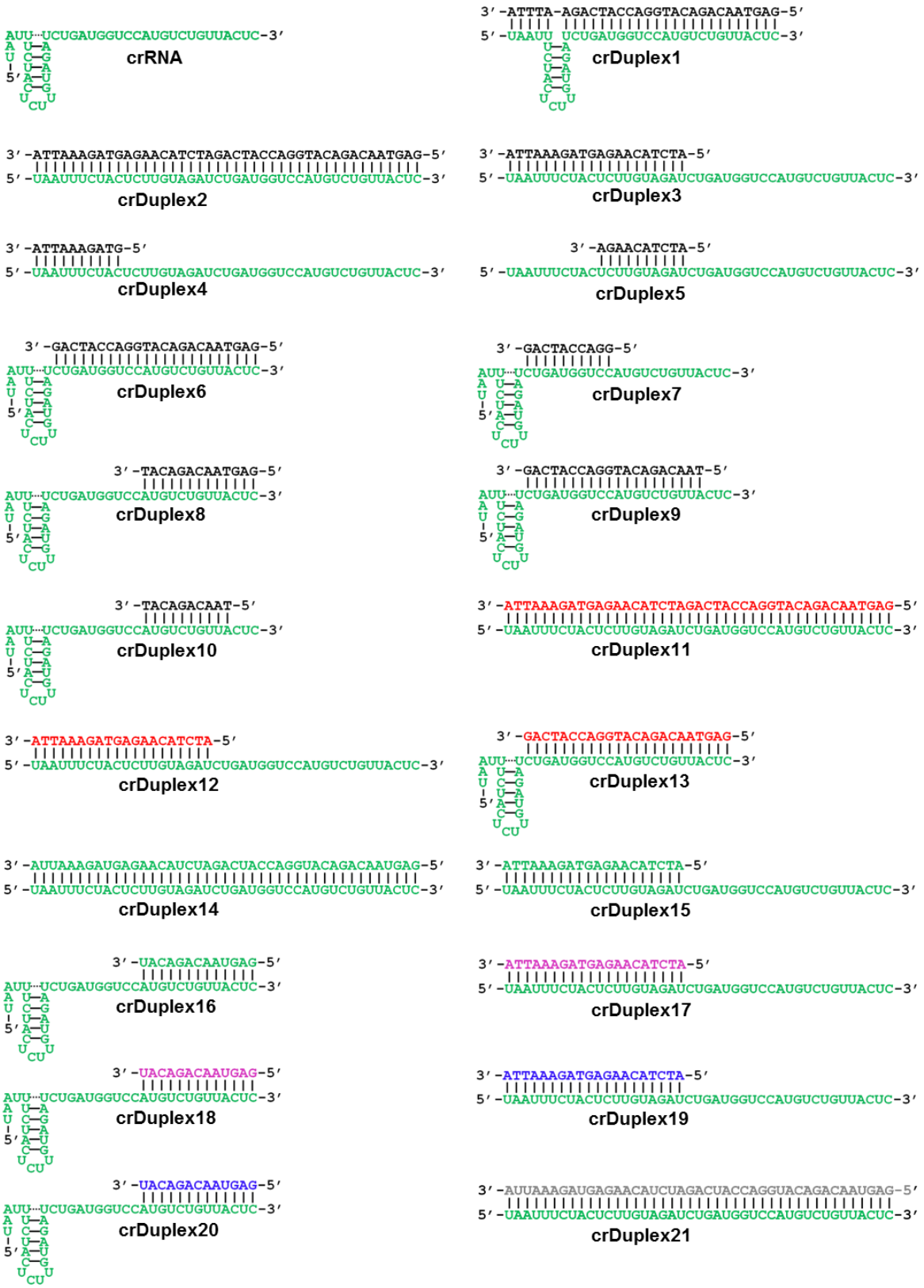
Supplementary Information

Phosphorothioate-modified DNA oligonucleotides inactivate CRISPR-Cpf1 mediated genome editing

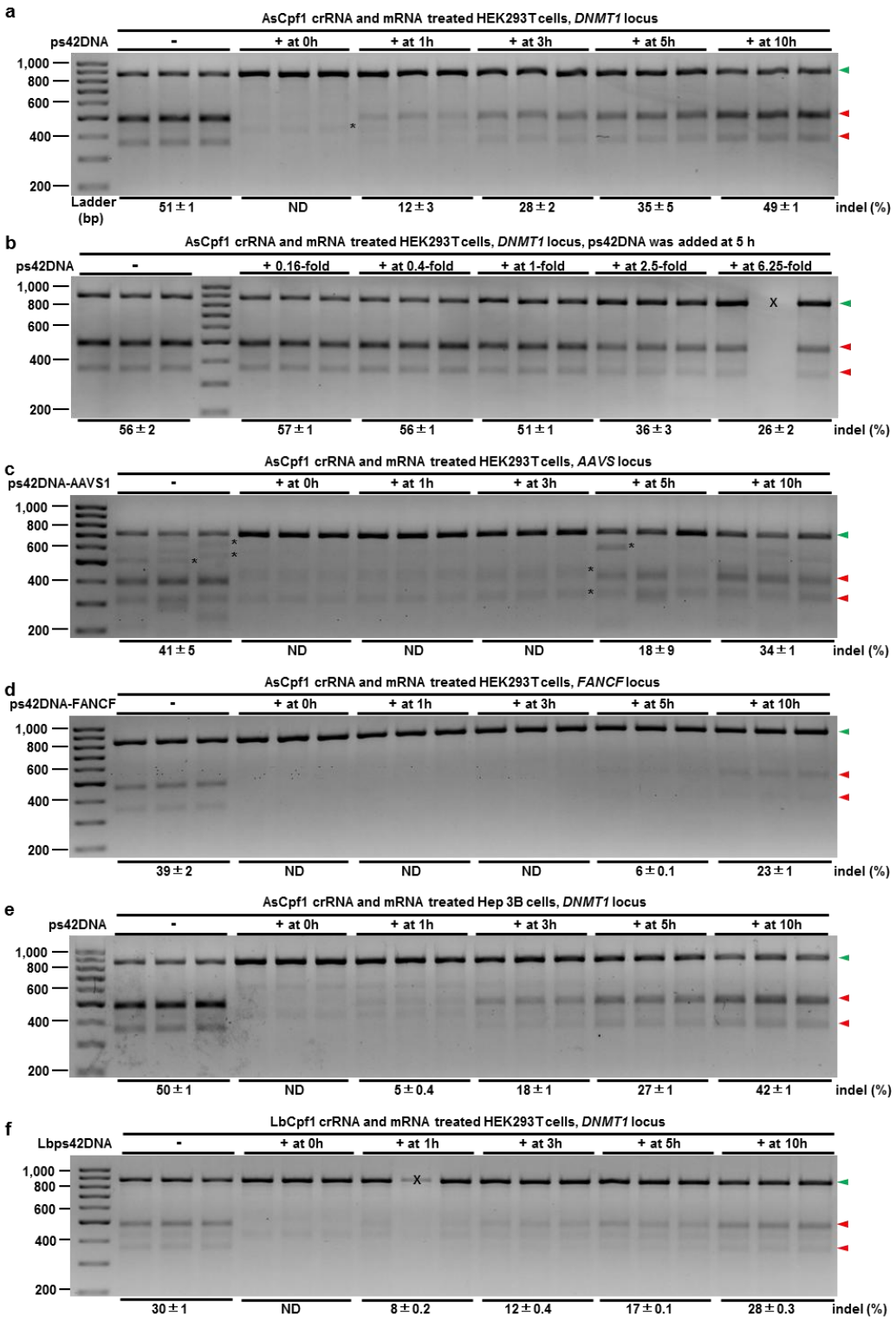
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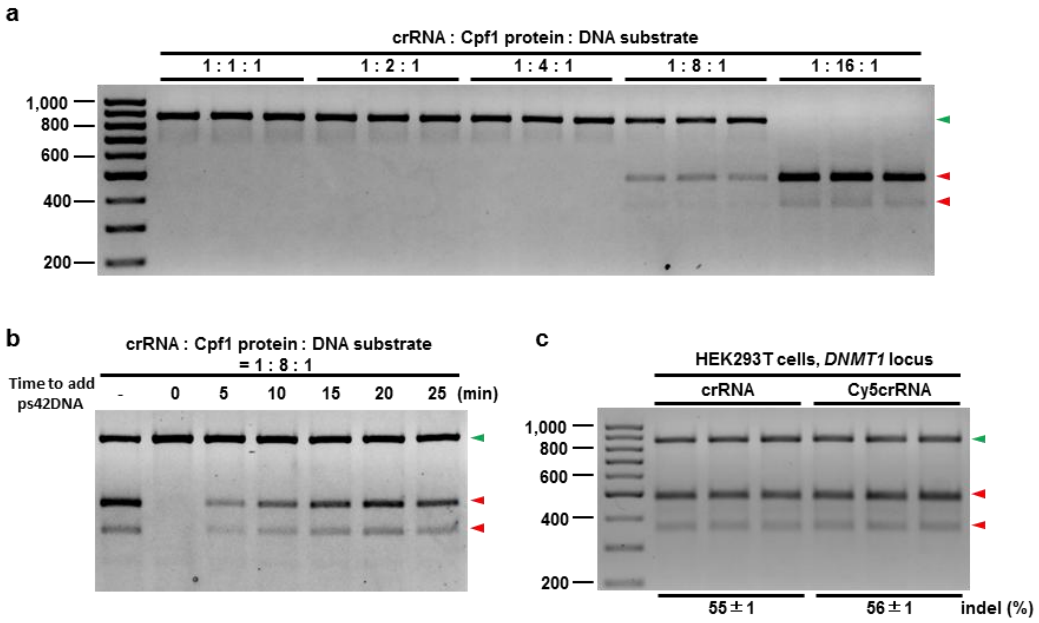
Supplementary Fig. 1 Structures of wild-type crRNA and crRNA duplexes. The green letter

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.



Supplementary Fig. 2 Inhibition effects of phosphorothioated DNA oligonucleotides on Cpf1-mediated genome editing in mammalian cells. **a** Time-dependent inhibitory effects of ps42DNA on AsCpf1-mediated *DNMT1* genome editing in human 293T cells. ps42DNA was added at various time points after treatment with CRISPR-Cpf1 components. **b**

Dose-dependent inhibitory effects of ps42DNA on AsCpf1-mediated *DNMT1* genome editing in human 293T cells. ps42DNA was added at 5 h time point after treatment with CRISPR-Cpf1 components. **c** Time-dependent inhibitory effects of ps42DNA-AAVS1 on AsCpf1-mediated genome editing at the *AAVS1* locus in human 293T cells. **d** Time-dependent inhibitory effects of ps42DNA-FANCF on AsCpf1-mediated genome editing at the *FANCF* locus in human 293T cells. **e** Time-dependent inhibitory effects of ps42DNA on AsCpf1-mediated genome editing at the *DNMT1* locus in human Hep3B cells. **f** Time-dependent inhibitory effects of Lbps42DNA on LbCpf1-mediated genome editing at the *DNMT1* locus in human 293T cells. Relative genome editing efficiency (indel) was determined by the T7E1 cleavage assay and normalized to that of the treatment with wild-type crRNA and AsCpf1 mRNA. The lane marked with "×" is excluded for calculation of genome editing efficiency. The asterisk denotes the non-specific bands. The green and red arrowhead denote the intact DNA substrate and cleaved DNA fragments, respectively.



Supplementary Fig. 3 Mechanistic studies for phosphorothioated DNA oligonucleotides on Cpf1-mediated genome editing. **a** AsCpf1-mediated DNA cleavage *in vitro* in the presence of wild-type crRNA at the indicated molar ratio of crRNA : AsCpf1 : DNA substrate. The reaction mixtures were treated with proteinase K to release the short cleaved fragment complexed with Cpf1. **b** Inhibitory effects of ps42DNA oligonucleotides on Cpf1-mediated *in vitro* cleavage reaction. **c** Genome editing efficiency of Cy5crRNA at the *DNMT1* locus in HEK293T cells. All gels were resolved on 2% agarose gels, and visualized by EZ-Vision In-Gel staining on ChemiDoc MP imaging system. The green and red arrowhead denote the intact DNA substrate and cleaved DNA fragments, respectively.

Supplementary Table 1 crRNAs and oligonucleotides used in this study.

Name of oligonucleotides	Length (nt)	Sequence (5' to 3')
crRNA targeting human *DNMT1*	43	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC
Oligo 1	29	GAGTAACAGACATGGACCATCAGAAATTA
Oligo 2 (uDNA)	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGAAATTA
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)	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGAAATTA
Oligo 12	20	ATCTACAAGAGTAGAAATTA
Oligo 13	23	GAGTAACAGACATGGACCATCAG
Oligo 14	43	GAGUAACAGACAUGGACCAUCAGAUCUACAAGAGUAGAAAUUA
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 (ps42RNA)	13	GAGUAACAGACAUGGACCAUCAGAUCUACAAGAGUAGAAAUUA
crRNA targeting human *AAVS*	43	UAAUUUCUACUCUUGUAGAUCUUACGAUGGAGCCAGAGAGGAU
ps42DNA-AAVS1	43	ATCCTCTCTGGCTCCATCGTAAGATCTACAAGAGTAGAAATTA
crRNA targeting human *FANCF*	43	UAAUUUCUACUCUUGUAGAUGUCGGCAUGGCCCCAUUCGCACG
ps42DNA-FANCF	43	CGTGCGAATGGGGCCATGCCGACATCTACAAGAGTAGAAATTA
LbcrRNA targeting human *DNMT1*	43	AAUUUCUACUAAGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC
Lbps42DNA	43	GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGAAATTA

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.

Supplementary Table 2 A list of primers used for T7E1 assay and in vitro cleavage reaction.

Locus	Forward primer	Reverse primer
Human DNMT1	CTGGGACTCAGGCGGGTCAC	CCTCAGCCAGAAGTCCCGTGC
Human AAVS1	GGGCTGGCTACTGGCCTTAT	ATGGCATCTTCCAGGGGTCC
Human FANCF	AGCTCCGCCTGGGTCTTCAT	GCGGAGACGTTCATGACTGG