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Highly specific chimeric DNA-RNA-guided genome editing with enhanced CRISPR-Cas12a system

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The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas12a system is composed of a Cas12a effector that acts as a DNA-cleaving endonuclease and a crispr RNA (crRNA) that guides the effector to the target DNA. It is considered a key molecule for inducing target-specific gene editing in various living systems. Here, we improved the efficiency and specificity of the CRISPR-Cas12a system through protein and crRNA engineering. In particular, to optimize the CRISPR-Cas12a system at the molecular level, we used a chimeric DNA-RNA guide chemically similar to crRNA to maximize target sequence specificity. Compared with the wild-type (wt)-Cas12a system, when using enhanced Cas12a system (en-Cas12a), the efficiency and target specificity improved on average by 2.58 and 2.77 times, respectively. In our study, when the chimeric DNA-RNA-guided en-Cas12a effector was used, the gene-editing efficiency and accuracy were simultaneously increased. These findings could contribute to highly accurate genome editing, such as human gene therapy, in the near future.

INTRODUCTION

The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system, which is known to be a bacterial defense system, is composed of Cas endonuclease and guide RNA; it is known to operate in various living organisms.[1–3] Recently, it has been used as a key tool for *in vivo* therapeutics because it can be reprogrammed specifically for a target gene. Thus, it is easy to use the CRISPR-Cas system to access genetic diseases.[4,5] The field of gene therapy is growing into a large market in which these advanced genome-editing tools are frequently employed; thus, it is important to determine whether the CRISPR-Cas system can accurately induce mutations into a target.[6,7] The target sequence specificity of CRISPR occurs because of molecular-level interactions resulting from its intrinsic properties.[8–10] CRISPR-Cas endonuclease recognizes target DNA based on the complementary nucleotide sequence contained in the guide RNA. CRISPR-Cas recognizes the protospacer adjacent motif (PAM) sequence in the target gene through the PAM interaction domain, melts the DNA double helix, and propagates the hybridization of guide RNA and target DNA to form a stable R loop that induces target DNA cleavage.[11–15] It has been reported that the hybridization between the guide RNA and the target DNA, which is formed to aid the CRISPR-Cas system in stably binding to the target DNA, is approximately 20–24 bp; it can have various mismatch tolerances depending on the target sequence.[16–20] Accordingly, the possibility of inducing cleavage to off-targets similar to the target sequence has been reported, and efforts have been made to reduce such errors.[9,21–23]

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Among CRISPR-Cas endonucleases, the CRISPR-Cas12a system, which belongs to class II and type V, has excellent target specificity.[13,24–27] Therefore, it has attracted much attention as an accurate genome-editing tool for use as a therapeutic agent for humans in the future. Unfortunately, the CRISPR-Cas12a system has also been reported to have a tolerance for mismatches in the intermediate region (8–9 bp) or in the PAM distal region inside the protospacer, which is necessary for target recognition.[18] This off-target cleavage effect appears to be more serious for engineered CRISPR-Cas12a, which has enhanced target recognition and improved gene-editing efficiency.[28,29] When considering gene therapy for human systems in the future, efficiency and safety will likely be important issues; they must be addressed simultaneously to improve the CRISPR-Cas12a system.

In this study, we devised a technology that dramatically lowers the induction of off-target mutations while efficiently inducing on-target mutations by effectively recognizing various target nucleotide sequences in human-derived cell lines. When using this enhanced Cas12a system (en-Cas12a) with strong target recognition, the average efficiency of inducing mutations in the target sequence increased (1.7- to 16.9-fold) when guided by a chimeric DNA-RNA guide in comparison with the wild-type Cas12a (wt-Cas12a) system. In addition, the average (0.5%–10.6%) of the mutation induction efficiency of off-target nucleotide sequences was reduced (0.1%–3.6%) by using a chimeric DNA-RNA guide, which increased the target specificity 2.8-fold on average. Using the chimeric DNA-RNA guide-based en-Cas12a system developed in this study, it is possible to induce target-specific, high-efficiency gene editing. Therefore, our proof-of-concept study could contribute in the near future to the fundamental treatment of various incurable human diseases resulting from genetic mutations.

RESULTS

Comparison of target DNA cleavage activity of chimeric DNA-RNA-guided en-AsCas12a and wt-AsCas12a

The CRISPR-Cas12a system uses single-stranded crispr RNA (crRNA) to hybridize target DNA with 20 bases, form a stable R loop, and induce target DNA cleavage. When the amino acid residues (Lys548, Ser542, and Glu174) interacting near the PAM (TTTN) sequence were changed to positive residues for the interaction between Cas12a and the target DNA (Figure 1A, left inset), the target-induced indel ratio (%) was improved for various genes.[28] From these results, we speculate that PAM recognition contributes to the kinetics of the entire Cas12a target recognition and DNA cleavage process, and that it can eventually affect stable R-loop formation through the hybridization of DNA and crRNA. The target specificity (on-target editing/off-target editing) of the Cas12a system has previously been optimized by substituting DNA for the 3′ end of the crRNA to change the hybridization energy between crRNA and target DNA (Figure 1A, right inset).[30] Based on this system, here we attempted to maximize the target specificity and genome-editing efficiency using en-AsCas12a (*Acidaminococcus* sp. Cas12a), which has enhanced target recognition. First, to improve target specificity by changing the binding energy of the target DNA-crRNA hybridization region, we gradually substituted the crRNA with DNA; we then confirmed the influence of this substitution on the target DNA cleavage for wt-AsCas12a and en-AsCas12a effectors (Figure 1B). Amplicon cleavage assays were performed on the target nucleotide sequences of both genes (*DNMT1* and *CCR5*-site2) (Figure S1). When DNA was gradually substituted from the 3′ end of the crRNA (recognized by AsCas12a), en-AsCas12a showed improved target recognition compared with wt-AsCas12a; it demonstrated more tolerance to the DNA substitution of crRNA (Figures 1B and S2). As previously reported,[30] when 12 or more DNAs are substituted from the 3′ end of Cas12a, the cleavage activity of the DNA amplicon is reduced, and almost no activity is shown in substitutions over 16 nt DNAs. However, en-AsCas12a showed robust cleavage activity after 12 nt DNA substitutions at the 3′ end of crRNA, but showed a decrease in activity by more than half following 16 nt or more DNA substitutions. This indicates that en-AsCas12a is more tolerant than wt-AsCas12a to DNA substitution at the 3′ end of crRNA, which is advantageous for target DNA cleavage.

Optimization of the genome-editing activity of en-AsCas12a based on a chimeric DNA-RNA guide to a target nucleotide sequence on the intracellular genome

To check whether the engineered en-AsCas12a effector, based on this chimeric DNA-RNA guide, could effectively induce target-specific gene mutations in human cells, we used various chimeric DNA-RNA guides to induce mutations and analyzed the efficiency in comparison with wt-AsCas12a. Comparative analysis of mutation induction efficiencies for the target nucleotide sequences of three genes (*DNMT1*, *IL2A-AS1*, and *CCR5*-site1) revealed that engineered en-AsCas12a outperformed wt-AsCas12a in terms of editing efficiency (Figures 2 and S1). In particular, the induction of gene mutations targeting intracellular loci showed a different trend from that of amplicon cleavage (Figure 1B). Unlike wt-AsCas12a, which exhibited a significantly lower operating efficiency based on chimeric DNA-RNA guides for the target sequence in the genome, engineered en-AsCas12a allowed up to 8 nt DNA substitutions (8DNA) from the 3′ end of the crRNA while maintaining the editing activity (Figures 2, S3, S4, and S8). Surprisingly, engineered en-AsCas12a showed 1.7- to 16.9-fold improvements in mutation induction efficiency compared with wt-AsCas12a when the 3′ end of crRNA was substituted with 8 nt DNA to increase target specificity. This effect was universally confirmed in various genes (*CCR5*-site2, *FANCF*); on average, a 7.3-fold higher recovery was achieved (Figures S3, S4, and S8). Previous studies have reported that when AsCas12a targets the intracellular genome and operates on the basis of a chimeric DNA-RNA guide it is difficult to induce mutations in the target nucleotide sequence, owing to many restrictions on the topology of the intracellular genome.[30] Accordingly, in this study we attempted to change the genome topology near the target sequence by using nickase. We compared the resulting changes in the genome-editing efficiency of the target sequence

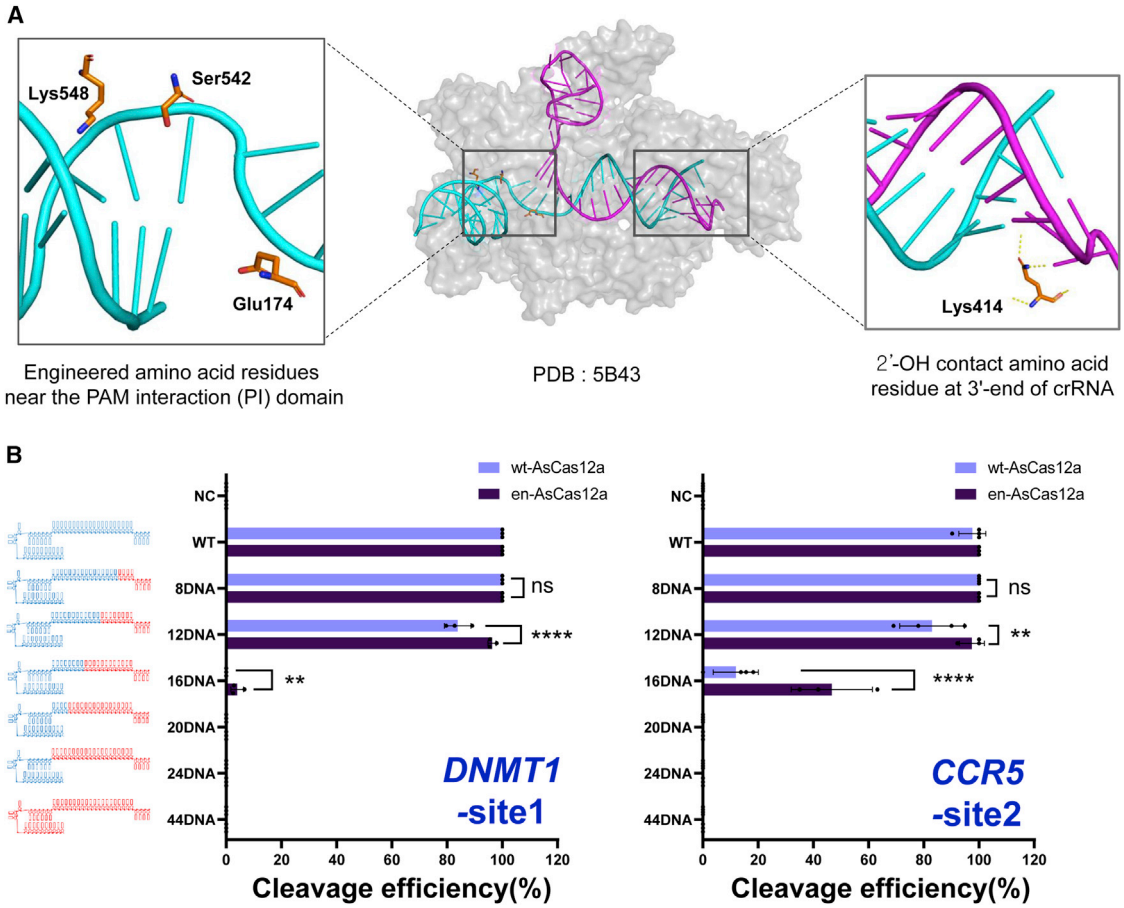

Figure 1. Comparison of target DNA cleavage activity of chimeric DNA-RNA guided en-Cas12a and wt-Cas12a

(A) Structure of the target-strand DNA-crRNA-AsCas12a complex (PDB: 5B43). Left inset: amino acids in AsCas12a interacting with around the PAM sequence in the target DNA. Right inset: amino acid (Lys414) interacting with the target-strand DNA-crRNA duplex in AsCas12a (hydrogen bonding with the 2′-OH group on the crRNA 3′ end region). (B) Comparison of cleavage efficiency of DNA amplicons using chimeric DNA-RNA guided wt-AsCas12a (light purple) and en-AsCas12a (dark purple). Each chimeric DNA-RNA includes target nucleotide sequences (*DNMT1*, *CCR5*-site2) with gradual DNA substitution from the 3′ end of crRNA. NC, negative control; WT, wild-type crRNA was treated with wt- or en-AsCas12a. The RNA region of the (cr)RNA is shown in blue, and the substituted DNA region is shown in red (8DNA to 44DNA indicates number of substituted DNA nucleotides in (cr)RNA). The x axis indicates the efficiency of the target gene (*DNMT1*-site1, *CCR5*-site2) cleavage by wt- and en-AsCas12a using various chimeric DNA-RNA guides (gradual DNA substitution from the 3′ end of the (cr)RNA). All cleavage efficiencies were calculated from band intensity, which was separated on 2% agarose gel (cleaved fragment intensity [%]/total fragment intensity [%]). All calculated values were normalized to wild-type (cr)RNA (Figure S2). The histograms represent means ± SEM from three independent experimental values. p values were calculated using two-way ANOVA with Sidak's multiple comparisons test (ns, not significant; *p < 0.0332, **p < 0.0021, ***p < 0.0002, ****p < 0.0001).

for wt-AsCas12a and en-AsCas12a (Figures S1, S3, S4, and S8). In the case of wt-AsCas12a, the mutation induction efficiency, which was reduced by the use of an 8 nt DNA-substituted chimeric DNA-RNA guide (8DNA), was completely recovered by co-treatment with nickase. In the case of en-AsCas12a, the mutation induction efficiency was maintained at a level similar to that of wt-crRNA using chimeric DNA-RNA (8DNA); it was not significantly affected by nickase (Figures 2, S3, S4, and S8). Therefore, these data indicate that using the en-AsCas12a effector, based on the chimeric DNA-RNA guide (8DNA), enables more effective genome editing than wt-AsCas12a when inducing mutations on the target DNA, without the help of nickase.

Improving target specificity for inducing genetic mutations in the intracellular genome using chimeric DNA-RNA-guided engineered en-AsCas12a

Next, we compared the chimeric DNA-RNA guide-based engineered en-AsCas12a and wt-AsCas12a effectors regarding their target specificities under optimized conditions (3′ end 8DNA substitution of crRNA) that effectively induced mutations in the target sequences (Figures 3, S1, S5, S6, and S9). We performed mutation induction experiments with the Cas12a system for various genes in advance and then selected gene sites (*CCR5*, *AAVS1*, *DNMT1*) with a high off-target indel ratio. For these gene sites, mutations were induced using wt-AsCas12a and en-AsCas12a effectors, after

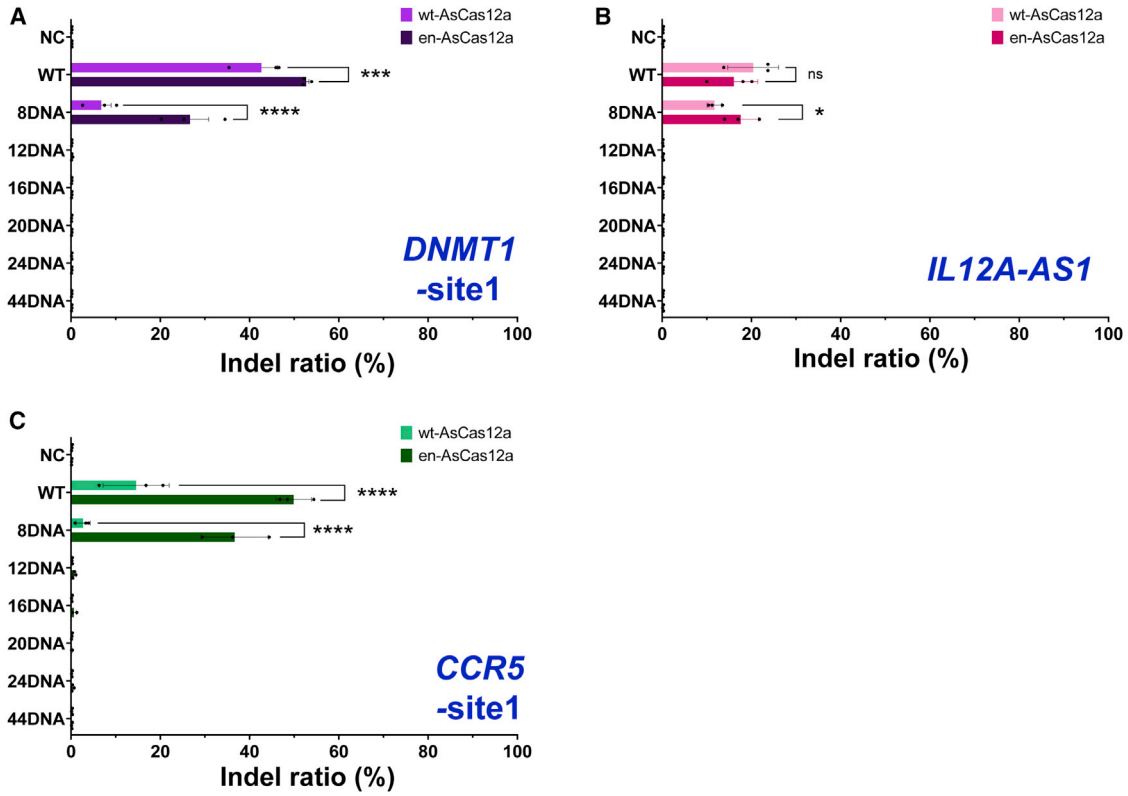

Figure 2. Comparison and optimization of genome-editing efficiency of en-Cas12a and wt-Cas12a based on chimeric DNA-RNA guide targeting endogenous locus in cell lines (HEK293FT)

Comparison of genome-editing efficiency (%) of wt-AsCas12a (light color) and en-AsCas12a (dark color) using a chimeric DNA-RNA guide for human-derived cell line (HEK293FT). Comparison of the indel induction efficiency (%) in intracellular genomic target sequences *DNMT1*-site1 (A), *IL2A-AS1* (B), and *CCR5*-site1 (C) by gradual DNA substitution of the 3′ end of crRNA. All the indel ratios (%) were calculated from targeted amplicon sequencing (indel ratio [%] = mutant DNA read number/total DNA read number). Data are shown as means ± SEM from three independent experiments. p values were calculated using two-way ANOVA with Sidak's multiple comparisons test (ns: not significant; *$p < 0.0332$, **$p < 0.0021$, ***$p < 0.0002$, ****$p < 0.0001$). NC, negative control; WT, wild-type crRNA was treated with wt- or en-AsCas12a, 8DNA to 44DNA. Chimeric crRNA (sequential 8DNA to 44DNA substitution at 3′ end of crRNA) was treated with wt- or en-AsCas12a.

which the on-/off-target activity was directly compared by performing sequencing-based analysis. In the case of wt-Cas12a-based targeting of the *CCR5*-site1, the target mutation induction efficiency was greatly reduced by the use of chimeric DNA-RNA (Figure 3A). Unlike wt-AsCas12a, in which mutation induction efficiency was recovered in a nickase-dependent manner, for en-AsCas12a the target mutation efficiency was maintained in a nickase-independent manner by using chimeric DNA-RNA, in which the 3′ end was substituted with 8 nt DNA (Figure 3B). The nickase dependency was lowered by 11.25 times (Figure 3C) and target specificity was increased by 2.79 times (Figure 3D) using en-AsCas12a. In addition, when the chromosome topology near the target sequence was changed using nickase, the target specificity using chimeric DNA-RNA was further increased by 3.45-fold compared with that of wt-AsCas12a (Figure 3D). We further compared the target specificities of the engineered en-AsCas12a and wt-AsCas12a effectors in the target sequences of two other genes (*AAVS1*-site1 and *DNMT1*-site2) (Figures S5 and S6). When a chimeric DNA-RNA guide was used for *AAVS1*-site1, neither en-AsCas12a nor wt-AsCas12a effectors showed nickase dependence, and gene mutations were induced with similar efficiencies to conditions using wt-crRNA (Figure S5). However, when using the en-AsCas12a effector to target the *AAVS1*-site1 sequence, the indel ratio (%) was significantly higher (3.9-fold) than that of wt-Cas12a. However, there were also more unintentional mutations in the off-target sequence (off-target1) (Figures S5A and S5B). We confirmed that the number of mutations induced in the off-target1 sequence was dramatically reduced by the use of chimeric DNA-RNA, in which the 3′ end was substituted with 8 nt DNA. Therefore, the overall target specificity was increased 3.5-fold compared with that of wt-AsCas12a when chimeric DNA-RNA (8DNA)-guided en-AsCas12a was used (Figure S5D). The *DNMT1*-site2 showed the same trend as the *AAVS1*-site1 locus targeted by the Cas12a system (Figure S6). In the case of the en-AsCas12a effector based on the chimeric DNA-RNA guide with 8 nt DNA substitution at the 3′ end, the indel ratio (%) was significantly increased (1.9-fold) compared with that of wt-AsCas12a. Furthermore, the off-target nucleotide sequence (off-target1, 2)-induced mutations were dramatically

CCR5 -site1

	Chromosome	Position	Mismatch No.	sequence
On-target (On)	Chr3	46372880	0	TTTA TGCACAGGGTGGAACAAGAT
Off-target1 (OT1)	Chr2	96187409	3	TTTC TGCACAGGGTGAAAAAAGAA
Off-target2 (OT2)	Chr6	113590178	3	TTTA TGCACAGGGAGAAAAAAGAT

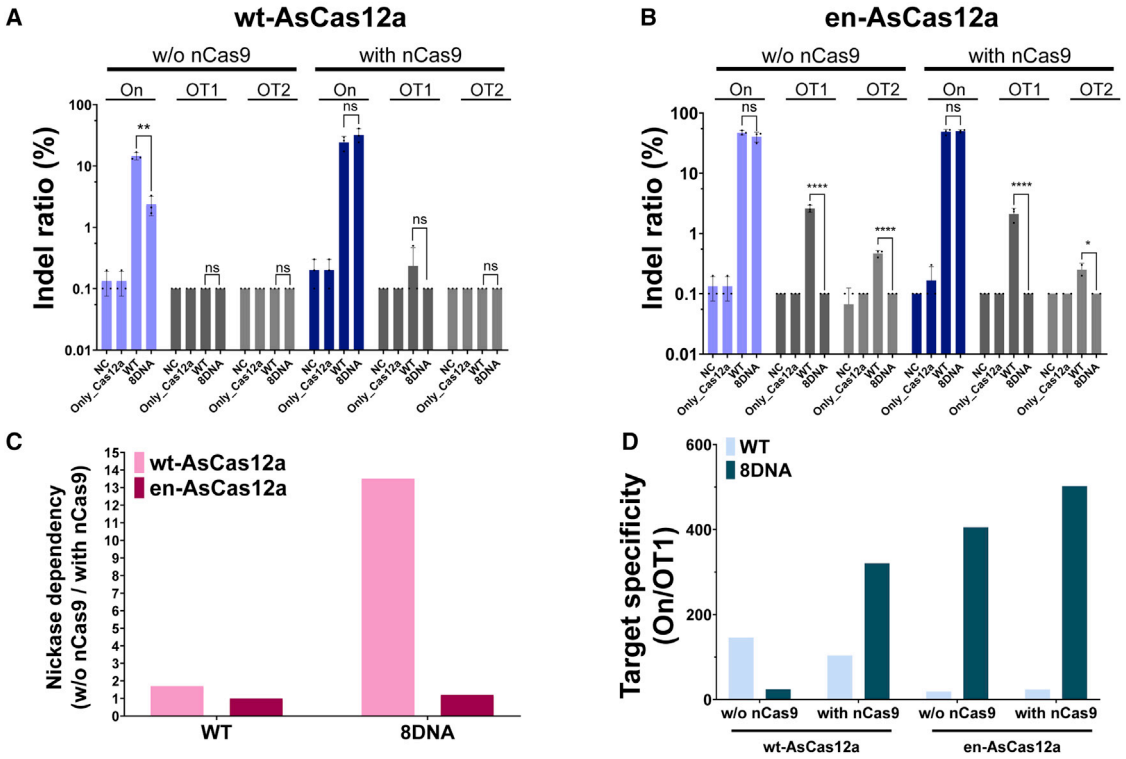

Figure 3. Comparison of genome-editing specificity of en-AsCas12a and wt-AsCas12a based on chimeric DNA-RNA guide targeting endogenous locus in cell lines (HEK293FT)

Upper table shows the on-target nucleotide sequence (On) for target gene (*CCR5*-site1) and the corresponding off-target nucleotide sequence (OT1, OT2) predicted from *in silico* analysis (Bae et al. [31]). Underlining indicates the PAM (TTTN) nucleotide sequence, and the nucleotides mismatched with the target sequence in the off-target are indicated in red. (A and B) Indel ratio (%) of the wt-AsCas12a-based (A) or en-AsCas12a-based (B) editing on the endogenous target sequences (on-/off-target sites for *CCR5*-site1) using wt-crRNA (WT) and 3′ end 8 nt DNA-substituted crRNA (8DNA). NC, negative control; Only_Cas12a, only protein treated; nCas9, nickase Cas9 (D10A). (C and D) Nickase dependency (C) and target specificity (D) were calculated from next-generation sequencing results. Nickase dependency = (without [w/o] nCas9 editing [%]/with [w/] nCas9 editing [%]); Target specificity = (on-target editing [%]/off-target editing [%]). Histograms represent means ± SEM from three independent experimental values. p values were calculated using two-way ANOVA with Sidak's multiple comparisons test (ns, not significant; *p < 0.0332, **p < 0.0021, ***p < 0.0002, ****p < 0.0001).

reduced (Figures S6A and S6B). As a result, the target specificity was increased 2-fold, regardless of the use of nickase (Figures S6C and S6D). We summarized the data to see at a glance the effect of inducing mutations targeting various genes using wt- or en-AsCas12a. These results show that when mutations are induced in target DNA using chimeric-crRNA-based en-AsCas12a, the off-targeting effect can be reduced while maintaining target mutagenesis activity for various genes in different cell lines (Figures 4, S7, and S10). In conclusion, by inducing mutations in multiple genes using chimeric DNA-RNA-guided en-AsCas12a, the targeted indel ratio (%) was improved 2.6-fold on average without the help of nickase. The off-target mutation induction efficiency was also reduced. Eventually, the target specificity was improved 2.8-fold compared with that of wt-AsCas12a.

A model for improving target specificity and genome-editing efficiency of en-Cas12a, based on chimeric DNA-RNA guides

Combining all of the above findings, the results of the working mechanism of en-Cas12a, compared with the existing wt-Cas12a, are presented in Figure 5, based on the chimeric DNA-RNA guide. When wt-Cas12a was used to cleave the target sequence in the genome of the cell, there was tolerance for mismatches between the protospacer (20 bp) middle part and the PAM (TTTN) distal region, so there was a possibility that Cas12a could recognize and cleave off-target sequences. When wt-Cas12a, based on the chimeric DNA-RNA guide, was used (in which the 3′ end was substituted with 8 DNAs), the off-target effect could be reduced by destabilizing the binding of Cas12a to DNA due to crRNA-target strand DNA distortion.[32] For the off-target seqeunce, DNA cleavage property is more severely hampered

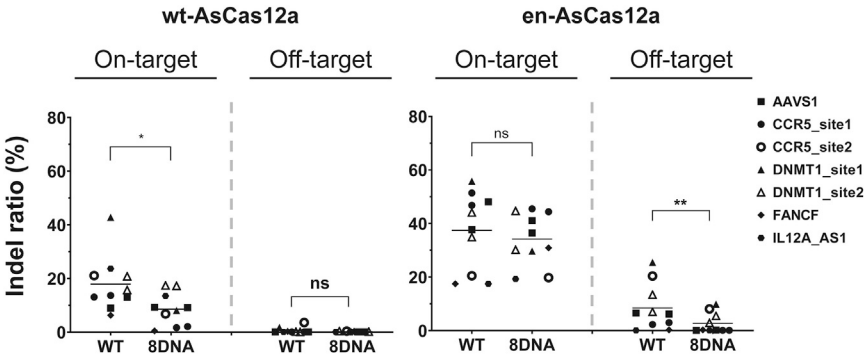

Figure 4. Comparison of the indel ratio (%) of on-target and off-target sites for various genes (*AAVS1*, *CCR5*, *DNMT1*, *FANCF*, *IL12A_AS1*) when wt-AsCas12a- or en-AsCas12a-based editing was attempted in cell lines (HEK293FT)

After inducing genetic mutations based on wt- or en-AsCas12a, the results of targeted amplicon sequencing for various targets (*AAVS1*, *CCR5*, *DNMT1*, *FANCF*, *IL12A_AS1*) and predicted corresponding off-target sites were analyzed and plotted. WT, wild-type crRNA was treated with wt- or en-AsCas12a; 8DNA, chimeric crRNA (sequential 8 nt DNA substitution at 3′ end of crRNA) was treated with wt- or en-AsCas12a. Each horizontal bar indicates the mean value of indel ratio (%). p values were calculated using two-tailed Student's t test (ns, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

by mismatch incorporation in the protospacer region than perfectly matched on-target sequence. However, when chimeric DNA-RNA-based cleavage was performed on the genome in the cell, the mutation-inducing effect on the target nucleotide sequence was largely decreased. In this case, the target specificity was increased only by changing the topology of the genome with the simultaneous use of nickase (Figures 3A–3C). For the en-Cas12a, in which target sequence recognition is reinforced by engineering the interacting amino acids of the PAM sequence recognition part, its efficiency in inducing target sequence mutations for various genes was increased compared with that of Cas12a, but its unintended off-target effects also increased greatly. Using the chimeric DNA-RNA-based en-Cas12a effector to target the intracellular genome can dramatically reduce the effects of off-target mutations. In general, en-Cas12a shows a largely enhanced DNA-targeting property under a chimeric DNA-RNA (3′ end 8 nt DNA substitution) guided condition than wt-Cas12a. Without the help of nickase, it is thus possible to increase the target sequence editing efficiency and dramatically lower the off-target sequence editing efficiency. Regarding the efficient induction of gene mutations with respect to the use of chimeric guides, accurate and high-efficiency gene targeting is possible when using chimeric DNA-RNA-based en-Cas12a.

DISCUSSION

The CRISPR-Cas12a effector is attracting attention as a potential future target-specific genome-editing tool as it is known to be capable of inducing mutations in a target sequence on a desired gene; it also has the highest target specificity among previously known CRISPR systems. However, the Cas12a system has been reported to have lower activity than Cas9 in general, and there remains room to improve the properties of the endonuclease itself for applications in various *in vivo* conditions. Efforts have been made to more effectively recognize the target DNA sequence and induce cleavage by engineering the Cas12a system.[28,29] These studies have shown overall improved activity compared with wt-Cas12a in various genes, through enhanced binding, by changing amino acids around the domain, within Cas12a, and by recognizing the PAM sequence in the target DNA. However, most CRISPR endonucleases induce double-stranded cleavage by forming an R loop through the complementary binding of target-strand DNA and crRNA. In general, as the tolerance of mismatch recognition increases due to enhanced binding affinity, the chance of off-target binding also increases, and we believe that the effects of off-target binding would be maximized when using engineered Cas12a systems. In the future, if a genome-editing technology is directly applied to the human body, the target specificity of the CRISPR system can be a very important issue. For the gene-editing system to work efficiently in the human body, safety issues must also be considered in parallel with editing efficiency. Therefore, a method for increasing target specificity is also required, in parallel with methods to enhance activity.

Previous studies have improved target specificity by applying crRNA engineering to the Cas12a system; target-specific gene mutations have been effectively induced by optimizing the length of DNA substitution. Mismatch tolerance has been reduced by changing the complementary binding energy of target-strand DNA and crRNA through sequential 8 nt DNA substitutions at the 3′ end of crRNA. This principle confirms that the induction of off-target mutations can be reduced while maintaining the efficiency of inducing target mutations. Based on this, here we used chimeric DNA-RNA crRNA to engineer en-AsCas12a, which displayed maximized target sequence recognition and improved mutation induction efficiency in various target nucleotide sequences in comparison with wt-AsCas12a. Surprisingly, the induction of off-target mutations was dramatically decreased, and eventually the target specificity was largely improved. Interestingly, the observed discrepancy between amplicon cleavage (Figure 1B) and genome editing inside the cell (Figure 2) showed that the cleavage activity of Cas12a endonuclease is greatly influenced by DNA topology. Chimeric DNA-RNA crRNA-based en-AsCas12a and wt-AsCas12a displayed differing sensitivities to the intracellular genome, so en-AsCas12a showed higher gene mutation induction efficiency than wt-AsCas12a. These results suggest that en-AsCas12a can, to some extent, overcome structures that are unfavorable to target DNA cleavage due to unstable R-loop formation (due to the DNA substitution of crRNA in the genomic sequence). This is achievable by enhancing PAM recognition through protein engineering. In general,

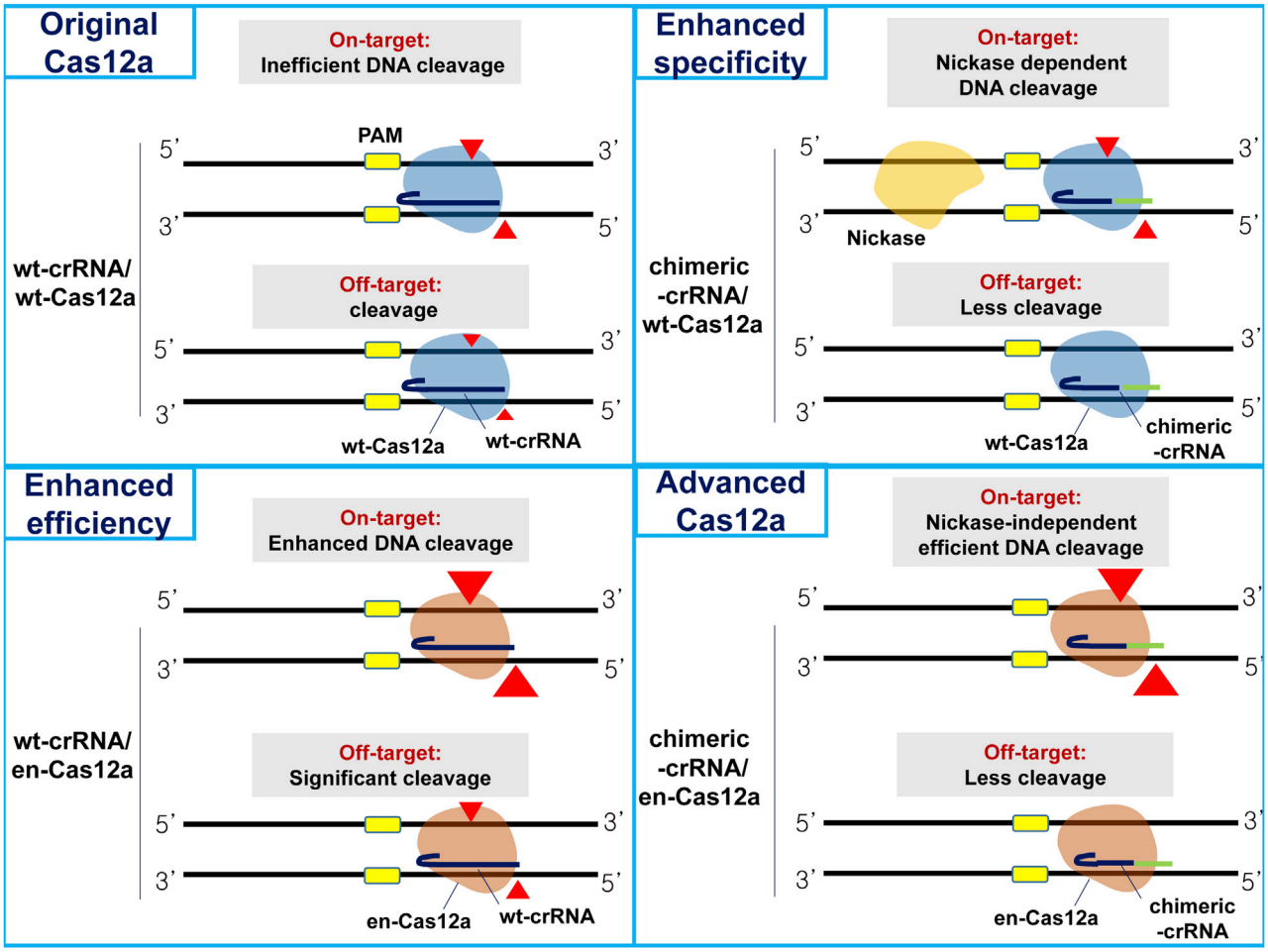

Figure 5. A model for enhancing target specificity and editing efficiency of en-Cas12a based on chimeric DNA-RNA-guided engineering

(Upper left) wt-crRNA guided wt-Cas12a system. In general, the wt-Cas12a effector can induce genetic mutations in target sequences but can also induce mutations in similar off-target sequences. (Bottom left) wt-crRNA-guided en-Cas12a system. Recognition of a target sequence is enhanced by an effector engineered by amino acid substitution, and thus the efficiency of inducing gene mutations is increased compared with that of the wt-Cas12a effector. However, due to the same principle as target sequence recognition, there is a problem in that the mutation induction efficiency of off-target sequences is also increased. (Upper right) Chimeric-crRNA-guided wt-Cas12a system. Effectively reduced off-target mutation induction efficiency when using a chimeric DNA-RNA guide with 8 nt DNA substituted at the 3′ end. However, the efficiency of mutation induction for the target nucleotide sequence in the genome is also reduced, and the efficiency is restored only when there is the action of nickase on the nucleotide sequence near the target. (Bottom right) Chimeric-crRNA-guided en-Cas12a system. This maximizes the target sequence indel ratio (%) and minimizes the off-target indel ratio (%) when using a chimeric DNA-RNA (8DNA)-guided en-Cas12a effector. It can induce more accurate and high-efficiency gene editing than wt-Cas12a on genomic DNA. PAM (TTTN) sequence for Cas12a effector is indicated by the yellow box. DNA cleavage points are indicated by red arrowheads, and the degree of cleavage is indicated by arrowhesd size according to the Cas12a activity. The wt-Cas12a and en-Cas12a effectors are shown in blue and brown, respectively. In the wt-crRNA and chimeric DNA-RNA guides, RNA is indicated in dark blue and nucleotides replaced with DNA in green.

the low operating efficiency induced in the intracellular genome by wt-Cas12a (based on the DNA substitution of crRNA to improve target specificity) could be recovered by changing the genome topology by using nickase around the target sequence. However, in the case of en-Cas12a, it was possible to induce a significant amount of mutation following up to eight DNA substitutions at the 3′ end of the crRNA, without the help of nickase. Therefore, when using chimeric DNA-RNA guide-based en-AsCas12a, it was possible to simultaneously improve the genome-editing efficiency (%) and the target specificity (on-target editing [%]/off-target editing [%]) compared with those of wt-AsCas12a by changing the hybridization energy of the target DNA strand and crRNA. In addition, when analyzing the results in our study, although weak compared with wt-Cas12a, the editing function of en-Cas12a is likely to be slightly increased by the topology change by nickase treatment. Although the engineered Cas12a system shows improved target specificity while maintaining high efficiency, genome editing was only effectively conducted by sequential delivery of an en-Cas12a expression plasmid into human-derived cell lines and a synthesized chimeric DNA-RNA guide rather than delivery with mixture of purified protein and

chimeric DNA-RNA guide. To enable effective gene editing in the future by applying this technology to living tissues, it is thought that the technology such as the mRNA form expression system of the en-Cas12a and the highly efficient lipid carrier must be fused for more effective delivery.

In this study, we developed a technology that maximizes the safety and efficiency of genome editing using chimeric DNA-RNA crRNA-based en-Cas12a. In the future, many improvements are needed in terms of efficiency and safety regarding the application of gene therapy to humans using the CRISPR system or for precise gene editing in *in vivo* systems. Through this technology, it is expected that the safety and efficacy of various CRISPR endonucleases can be optimized in a similar way when applied *in vivo*.

MATERIALS AND METHODS

Preparation of the CRISPR-Cas12a recombinant protein and chimeric guides

wt- and en-AsCas12a recombinant proteins were prepared for the *in vitro* DNA cleavage assay. Codon-optimized AsCas12a (*Acidaminococcus* sp. Cas12a) coding sequence was cloned into a pET28a bacterial expression vector and then transformed into BL21 (DE3) *Escherichia coli* cells. Transformed bacterial colonies were cultured at 37°C until the optical density reached 0.6, after which isopropylthio-β-galactoside (IPTG) inoculation was performed. After 48 h, *E. coli* cells were precipitated at 4°C and 5,000 rpm, following which the culture medium of the upper layer was removed. The precipitated *E. coli* cell pellet was resuspended in lysis buffer (10 mM β-mercaptoethanol, 300 mM NaCl, 20 mM Tris-HCl [pH 8.0], 1 mM PMSF, and 1% Triton X-100). To disturb the bacterial cell membrane, we performed sonication on ice water for 3 min, following which the cell lysate was precipitated for 10 min at 5,000 rpm at 4°C. Next, the nitrilotriacetic acid (Ni-NTA) resin was pre-washed with wash buffer (20 mM Tris-HCl [pH 8.0], 300 mM NaCl), and the precipitated cell lysate was stirred at 4°C for 90 min. Washing was performed with 10× the volume of wash buffer to remove non-specific binding components in the mixed cell lysate solution. For the elution of AsCas12a protein, an elution buffer (20 mM Tris-HCl [pH 8.0], 300 mM NaCl, 200 mM imidazole) was used and finally exchanged against the storage buffer (200 mM NaCl, 50 mM HEPES [pH 7.5], 1 mM dithiothreitol [DTT], 40% glycerol) using a Centricon (Millipore, Amicon Ultra-15), and stored at −80°C. Chimeric DNA-RNA oligonucleotides (Bioneer) were synthesized for each target gene sequence and dissolved in diethyl pyrocarbonate (DEPC) water, then stored at −80°C (Table S1).

Preparation of the guide RNA for Cas12a and nCas9 (D10A)

Guide RNAs for Cas12a and nCas9 (D10A) were generated by *in vitro* transcription (Tables S1 and S2). A DNA template for *in vitro* transcription was constructed using annealing or extension PCR with sense and antisense DNA oligonucleotides (Macrogen) containing the target DNA sequence (Table S3). DNA templates were mixed with T7 RNA polymerase (New England Biolabs, M0251L) and reaction buffer mixture (50 mM $MgCl_2$, 100 mM ribonucleoside triphosphate [Jena Bio, NU-1014], 10× RNA polymerase reaction buffer, 100 mM DTT, RNase inhibitor Murine, DEPC), and incubated at 37°C. After 16 h, to remove the original DNA template, DNase I was added and the mixture was incubated for another 1 h at 37°C. The transcribed RNA was purified using a column (GENECLEAN Turbo Kit; MP Biomedicals). The purified RNA was concentrated through lyophilization (2,000 rpm) at −55°C for 1 h.

In vitro DNA cleavage assay

On-/off-target site PCR amplicons of each gene (*DNMT1*, *CCR5*, *IL2A-AS1*, and *AAVS1*) were obtained from purified human genomic DNA using DNA primers (Table S3). The purified target PCR amplicon was incubated with purified recombinant wt- or en-Cas12a protein and crRNA (RNA guides or chimeric DNA-RNA guides) in 10× buffer (NEBuffer3.1; New England Biolabs) for 1 h. After adding a stop buffer (100 mM EDTA, 1.2% SDS) to stop the reaction, the cleaved fragment was separated using 2% agarose gel electrophoresis. DNA cleavage efficiency (cleaved fragment intensity [%]/total fragment intensity [%]) was measured using ImageJ software (NIH).

Cell culture and transfection

The HEK293FT (ATCC) cell line was cultured in Dulbecco's modified Eagle medium (DMEM; Gibco) with 10% fetal bovine serum (Gibco) at 37°C and in 5% CO_2. To ensure efficient chimeric DNA-RNA guide delivery, we performed sequential transfection with wt- or en-Cas12a expression vectors and crRNAs. For the primary transfection, 10^5 cells were mixed with a plasmid vector (AsCas12a, n-SpCas9 (D10A, H840A)) and 20 μL of electroporation buffer (Lonza, V4XC-2032) and were nucleofected according to the manufacturer's instructions (program: CM-137). The transfected cells were transferred to a 24-well plate with 500 μL of medium and incubated at 37°C in 5% CO_2. Twenty-four hours after primary transfection, for secondary transfection crRNA (200 pmol), single guide RNA (30 pmol), 1 μL of P3000, and 1.5 μL of Lipofectamine 3000 reagent (Thermo Fisher Scientific) were mixed in 50 μL of Opti-MEM (Gibco), incubated for 10 min, and added to DMEM medium. Forty-eight hours after the second transfection, cells were harvested and genomic DNA was extracted using a genomic DNA purification kit (DNeasy Blood & Tissue Kit; Qiagen).

Targeted amplicon sequencing and data analysis

To analyze the indel frequency of the on-/off-target locus of each gene, we performed targeted deep sequencing using PCR amplicons. The Cas-OFFinder (http://www.rgenome.net/cas-offinder/) web tool was used to select potential off-target sites corresponding to each on-target site. For the preparation of PCR amplicons, PCR amplification was performed using DNA primers corresponding to each endogenous locus (Table S3). To add adapter and index sequences to each 5′ and 3′ end, we performed nested PCR using Phusion High-Fidelity DNA Polymerase (Thermo Fisher). After

index tagging, the PCR amplicon mixture was analyzed using a Mini-Seq (Illumina, SY-420-1001) according to the manufacturer's guidelines. Sequencing read fatstq files were analyzed using Cas-Analyzer (http://www.rgenome.net/cas-analyzer/), and the indel ratio (mutant DNA read number/total DNA read number) was calculated from the read number.

DATA AND CODE AVAILABILITY

CRISPR RGEN Tools is an open-source collaborative initiative available in the repository (http://www.rgenome.net/). Targeted deep-sequencing data are available at the NCBI Sequence Read Archive under accession number SRP334002.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2022.03.021.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.K., W.L., and S.H.L.; methodology, H.K., W.L., and S.H.L.; software, H.K., W.L., and S.H.L.; validation and formal analysis, H.K., W.L., C.H.K., Y.O., L.W.G., H.L., W.J.S., and J.K.H.; investigation, H.K., W.L., and S.H.L.; resources, K.-S.L., K.J.J., K.-H.N., Y.-S.W., K.-R.L., Y.L., Y.-H.K., J.-W.H., B.-H.J., and S.H.L.; data curation, H.K., W.L., and S.H.L.; writing –original draft, H.K., W.L., B.-H.J., D.-S.L., and S.H.L.; writing – review and editing, H.K., W.L., B.-H.J., D.-S.L., and S.H.L.; visualization, H.K., W.L., and S.H.L.; supervision, D.-S.L. and S.H.L.; project administration, B.-H.J., D.-S.L., and S.H.L.; funding acquisition, Y.-H.K., Y.L., J.-W.H., and S.H.L.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental information

Highly specific chimeric DNA-RNA-guided genome editing with enhanced CRISPR-Cas12a system

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Supplemental Material

Supplementary Tables

Table S1. Sequence information of the synthesized wt- and chimeric crRNAs for AsCas12a.

Target gene **guide ID**	Target sequence (5′-3′)	crRNA sequence for AsCas12a (5′-3′)
hDNMT1_site1 **WT**	TTTCCTGATGGTCCATGTCTGTTACTCG	**5'UAAUUUCUACUCUUGUAGAUCUGAUGGUC CAUGUCUGUUACUCG** 3'
hDNMT1_site1 **8DNA**		**5'UAAUUUCUACUCUUGUAGAUCUGAUGGUC CAUGUCUGTTACTCG 3'**
hDNMT1_site1 **12DNA**		**5'UAAUUUCUACUCUUGUAGAUCUGAUGGUC CAUGTCTGTTACTCG 3'**
hDNMT1_site1 **16DNA**		**5'UAAUUUCUACUCUUGUAGAUCUGAUGGUC CATGTCTGTTACTCG 3**
hDNMT1_site1 **20DNA**		**5'UAAUUUCUACUCUUGUAGAUCUGATGGTCC ATGTCTGTTACTCG 3**
hDNMT1_site1 **24DNA**		**5'UAAUUUCUACUCUUGUAGAUCTGATGGTCC ATGTCTGTTACTCG 3**
hDNMT1_site1 **44DNA**		**5'TAATTTCTACTCTTGTAGATCTGATGGTCCAT GTCTGTTACTCG 3'**
hDNMT1_site2 **WT**	TTTGGCTCAGCAGGCACCTGCCTCAGCT	**5'UAAUUUCUACUCUUGUAGAUGCUCAGCAG GCACCUGCCUCAGCU** 3'
hDNMT1_site2 **8DNA**		**5'UAAUUUCUACUCUUGUAGAUGCUCAGCAG GCACCUGCCTCAGCT 3'**
hCCR5_site1 **WT**	TTTATGCACAGGGTGGAACAAGATGGAT	**5'UAAUUUCUACUCUUGUAGAUUGCACAGGG UGGAACAAGAUGGAU** 3'
hCCR5_site1 **8DNA**		**5'UAAUUUCUACUCUUGUAGAUUGCACAGGG UGGAACAAGATGGAT** 3'
hCCR5_site1 **12DNA**		**5'UAAUUUCUACUCUUGUAGAUUGCACAGGG UGGAACAAGATGGAT** 3'
hCCR5_site1 **16DNA**		**5'UAAUUUCUACUCUUGUAGAUUGCACAGGG TGGAACAAGATGGAT** 3'
hCCR5_site1 **20DNA**		**5'UAAUUUCUACUCUUGUAGAUUGCACAGGG TGGAACAAGATGGAT** 3'
hCCR5_site1 **24DNA**		**5'UAAUUUCUACUCUUGUAGAUTGCACAGGG TGGAACAAGATGGAT** 3'
hCCR5_site1 **44DNA**		**5'TAATTTCTACTCTTGTAGATTGCACAGGGTG GAACAAGATGGAT 3'**
hCCR5_site2 **WT**	TTTTGTGGGCAACATGCTGGTCATCCTC	**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCUGGUCAUCCUC** 3'
hCCR5_site2 **8DNA**		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCUGGTCATCCTC** 3'
hCCR5_site2 **9DNA**		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCUGGTCATCCTC** 3'
hCCR5_site2 **10DNA**		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCUGGTCATCCTC** 3'
hCCR5_site2 **11DNA**		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCTGGTCATCCTC** 3'
hCCR5_site2 **12DNA**		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC AUGCTGGTCATCCTC** 3'
hCCR5_site2		**5'UAAUUUCUACUCUUGUAGAUGUGGGCAAC**

16DNA		ATGCTGGTCATCCTC 3’
hCCR5_site2 **20DNA**		5’UAAUUUCUACUCUUGUAGAUGUGGGCAAC ATGCTGGTCATCCTC 3’
hCCR5_site2 **24DNA**		5’UAAUUUCUACUCUUGUAGAUGTGGGCAAC ATGCTGGTCATCCTC 3’
hCCR5_site2 **44DNA**		5’TAATTTCTACTCTTGTAGATGTGGGCAACATG CTGGTCATCCTC 3’
hIL12A-AS1 **WT**	TTTAGGATGCCACTAAAAGGGAAAGGGG	5’UAAUUUCUACUCUUGUAGAUGGAUGCCAC UAAAAGGGAAAGGGG 3’
hIL12A-AS1 **8DNA**		5’UAAUUUCUACUCUUGUAGAUGGAUGCCAC UAAAAGGGAAAGGGG 3’
hIL12A-AS1 **12DNA**		5’UAAUUUCUACUCUUGUAGAUGGAUGCCAC UAAAAGGGAAAGGGG 3’
hIL12A-AS1 **16DNA**		5’UAAUUUCUACUCUUGUAGAUGGAUGCCAC TAAAAGGGAAAGGGG 3’
hIL12A-AS1 **20DNA**		5’UAAUUUCUACUCUUGUAGAUGGAUGCCAC TAAAAGGGAAAGGGG 3’
hIL12A-AS1 **24DNA**		5’UAAUUUCUACUCUUGUAGAUGGATGCCACT AAAAGGGAAAGGGG 3’
hIL12A-AS1 **44DNA**		5’TAATTTCTACTCTTGTAGATGGATGCCACTAA AAGGGAAAGGGG 3’
hAAVS1 **WT**	TTTGCTTACGATGGAGCCAGAGAGGATC	5’UAAUUUCUACUCUUGUAGAUCUUACGAUG GAGCCAGAGAGGAUC 3’
hAAVS1 **8DNA**		5’UAAUUUCUACUCUUGUAGAUCUUACGAUG GAGCCAGAGAGGATC 3’
hFANCF **WT**	TTTGGGCGGGGTCCAGTTCCGGGATTAG	5’UAAUUUCUACUCUUGUAGAUGGCGGGGUC CAGUUCCGGGAUUAG 3’
hFANCF **8DNA**		5’UAAUUUCUACUCUUGUAGAUGGCGGGGUC CAGUUCCGGGATTAG 3’

†PAM sequences (TTTN) for AsCpf1 in the target DNA are shown in blue and substituted DNA sequences in (cr)RNA are shown in red, respectively.

Table S2. Sequence information of the sgRNA for nickase SpCas9(D10A) used in this study.

Target gene	CRISPR-Cas9 target sequence (5′-3′)	sgRNA sequence for dead or nickase SpCas9 (5′-3′)
hDNMT1_site1 **sgRNA**	GAGTGCTAAGGGAACGTTCACGG	5’GGAGUGCUAAGGGAACGUUCAGUUUUAGAGCU AGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’
hDNMT1_site2 **sgRNA**	CCAGCAGCCAACCTGACCAAAGG	5’GCCAGCAGCCAACCUGACCAAGUUUUAGAGCUA GAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’
hCCR5_site1 **sgRNA**	TAATAATTGATGTCATAGATTGG	5’GUAAUAAUUGAUGUCAUAGAUGUUUUAGAGCUA GAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’
hCCR5_site2 **sgRNA**	AACACCAGTGAGTAGAGCGGAGG	5’GAACACCAGUGAGUAGAGCGGGUUUUAGAGCU AGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’
AAVS1 **sgRNA**	GCAAGGAGAGAGATGGCTCCAGG	5’GGCAAGGAGAGAGAUGGCUCCGUUUUAGAGCU AGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’
IL12A-AS1 **sgRNA**	TTCTGGGGTCAACATCTTGGTGG	5’GUUCUGGGGUCAACAUCUUGGGUUUUAGAGCUA GAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGAGUCGGUGC 3’

FANCF **sgRNA**	**CCGCTCCAGAGCCGTGCGAA**TGG	**5'GCCGCUCCAGAGCCGUGCGAA**GUUUUAGAGCUA GAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGAGUCGGUGC** 3'

†PAM sequence (NGG) in the target DNA for the SpCas9 nickase (D10A) is shown in blue. Underlined sequence in sgRNA indicates the target sequence.

Table S3. Sequence information for DNA primers used in this study.

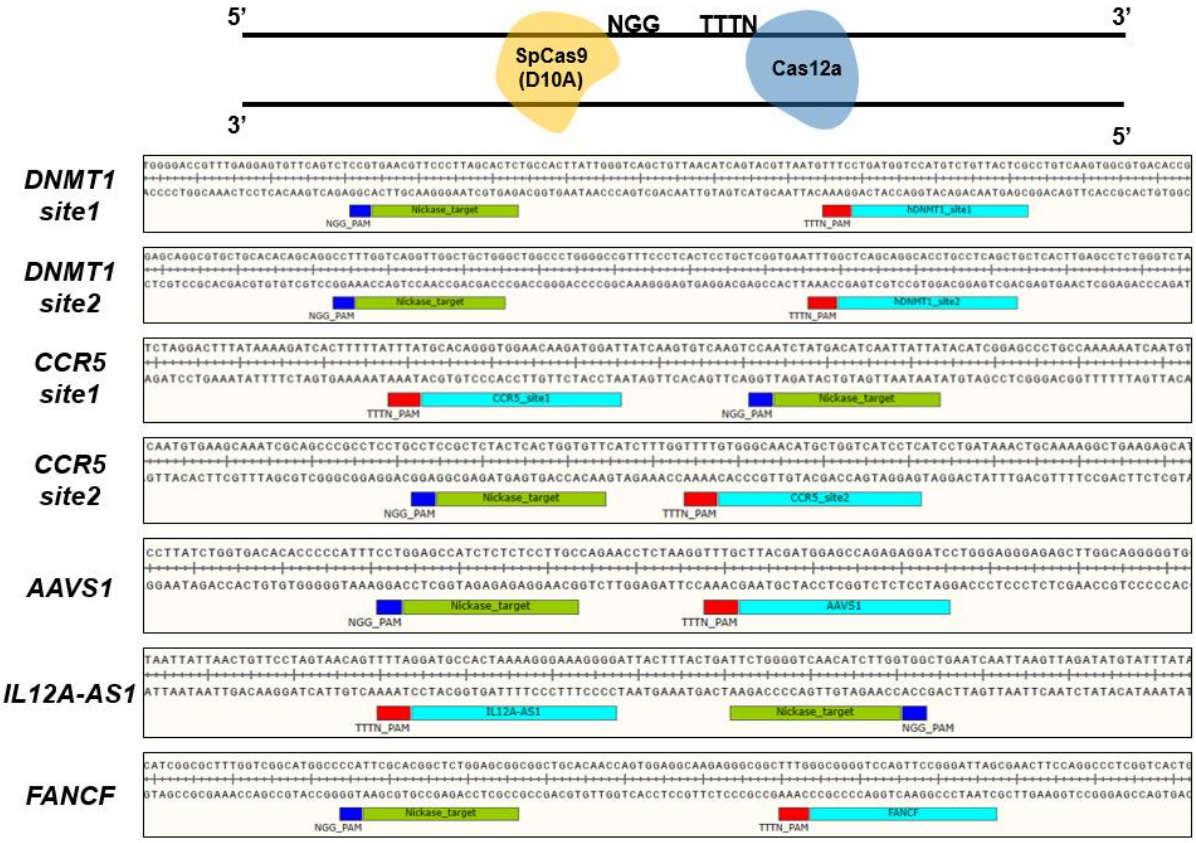
Target gene (primer direction)	DNA sequence (5′ to 3′)
hDNMT1_site1_On_F	GGAGATCAAGCTTTGTATGTTG
hDNMT1_site1_On_R	CCAGAATGCACAAAGTACTGC
hDNMT1_site1_On_F2	CTGTGAGGATTGAGTGAGTTG
hDNMT1_site1_On_R2	CACACATGTGAACGGACAGA
hDNMT1_site1_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAGTG TTCAGTCTCCGTGA
hDNMT1_site1_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTA GCAGCTTCCTCCTC
hDNMT1_site1_OT1_F	CAGGGGTATTTTCCTTCAAGA
hDNMT1_site1_OT1_R	TCAGGAATACCAACATGGAAAA
hDNMT1_site1_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTGTG TCTGCTGGAAGCTC
hDNMT1_site1_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCA GATAGGGTCTGTGCTC
hDNMT1_site2_On_F	ACACAACAGCTTCATGTCAG
hDNMT1_site2_On_R	TTGGCTTGGAGATCAAGCTT
hDNMT1_site2_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAG TGCTAAGGGAACGT
hDNMT1_site2_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTG CTTAGAGCAGGCGTG
hDNMT1_site2_OT1_F	CTGAGCTGGTATCCAAGATGC
hDNMT1_site2_OT1_R	GCATTGTCATTAGAACCACAAATC
hDNMT1_site2_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAA GTGAGTCTTGCTGAG
hDNMT1_site2_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAA TCTGTGCACTCGGAG
hDNMT1_site2_OT2_F	GTTGCAGTGAGCCAAGATCA
hDNMT1_site2_OT2_R	TCTTGGAACCAATCCTCTGC
hDNMT1_site2_OT2_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGCAG TGCTTCTCCATTGAG
hDNMT1_site2_OT2_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTACG CCATGGGTGATAGTG
hDNMT1_site2_OT3_F	GCAACCAGATTTTTCCTCCA
hDNMT1_site2_OT3_R	CCAAGCCGTTACAGATGGTT
hDNMT1_site2_OT3_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCAAT GGACTCTGGGATAG
hDNMT1_site2_OT3_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGG TTGTGAACAGGAAACT
hCCR5_site1_On_F	ACCATGCTTGACCCAGTTTC
hCCR5_site1_On_R	AAACACAGCATGGACGACAG
hCCR5_site1_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAATGTA GACATCTATGTAGGCAA
hCCR5_site1_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCG ATTTGCTTCACATTG
hCCR5_site1_OT1_F	CAAGCAATTCTTGTGCCTCA

hCCR5_site1_OT1_R	TCCAGGCCCTGTATACTTGC
hCCR5_site1_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGGTC AACATTGCAAGGAG
hCCR5_site1_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAA GCCATTCTGGAAAAGA
hCCR5_site1_OT2_F	CATGGTGAAACCCCAACTCT
hCCR5_site1_OT2_R	CCAAATCCCACACTTTGCTT
hCCR5_site1_OT2_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCAA CTGTATTGAGAGGAAGC
hCCR5_site1_OT2_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTG GTGGATAAGAAGGAATTTT
hCCR5_site2_On_F	TGAGATGGTGCTTTCATGAAT
hCCR5_site2_On_R	GAAAATGAGAGCTGCAGGTG
hCCR5_site2_On_F2	AAACTTCATTGCTTGGCCAA
hCCR5_site2_On_R2	GAAGATTCCAGAGAAGAAGCC
hCCR5_site2_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTGCC AAAAAATCAATGTGAAG
hCCR5_site2_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAG GGGACAGTAAGAAGGAA
hCCR5_site2_OT1_F	GAAAATGGCTGTTGGGTAAATC
hCCR5_site2_OT1_R	TAAGGGCCACAGACATAAAC
hCCR5_site2_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCCTG TCATAAATTTGACGTG
hCCR5_site2_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAAA AGCAGATCAGAGATGGC
hAAVS1_On_F	CCTGGTGAACACCTAGGACG
hAAVS1_On_R	CTATGTCCACTTCAGGACAGC
hAAVS1_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTCCCT CCCACCCCCTG
hAAVS1_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCCA TCCTTAGGCCTCCT
hAAVS1_OT1_F	AGCAGGTTGGGTATCCTGTG
hAAVS1_OT1_R	AGGCTGTTTCTGCCTCCATA
hAAVS1_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATCTC CTGGTCTGCACAA
hAAVS1_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAG GGGCTATTCAGATGT
hAAVS1_OT2_F	ATCCAGGGGGTTGGAATATC
hAAVS1_OT2_R	TGCCTGAGAGCAGGTCTTTT
hAAVS1_OT2_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTATC TGTTAATGATAGCCTG
hAAVS1_OT2_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAA GCCCATGAAGACTGG
hIL12A-AS1_On_F	GCTTGCTGTATACACAAGGC
hIL12A-AS1_On_R	CTGATCTTGAGAACAGAAGACC
hIL12A-AS1_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAAAG GTGTTGCTTATTGCCC
hIL12A-AS1_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCT CCTTCCATCTGGGTTTC
hIL12A-AS1_OT1_F	ATTCTGCCTCCTCTCCCACT
hIL12A-AS1_OT1_R	CAGGAGGAGCAAATTCCAGA
hIL12A-AS1_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTCCT AACAGAGATTTACTTTCTC
hIL12A-AS1_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCC TCTCTCCCTTCCTCTC

hIL12A-AS1_OT2_F	GCATCAACAAACTGGCTCATT
hIL12A-AS1_OT2_R	CCTTTGGGATGGTGTCATCT
hIL12A-AS1_OT2_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCACT GCTAATGTTTAAAATTC
hIL12A-AS1_OT2_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTA GGGCAGCATTTTGTAG
hFANCF_On_F	CACGGATAAAGACGCTGGGA
hFANCF_On_R	CACAGGCTGCTGAGAAACCT
hFANCF_On_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACATC CATCGGCGCTTTG
hFANCF_On_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTG GTAACGAGCTGCATCC
hFANCF_OT1_F	TGGGAGGAAACCCTAAAGAG
hFANCF_OT1_R	TGCAGGCCCAAGTATTTTGA
hFANCF_OT1_Adapter_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGCTG ACTCAGCTGAACTG
hFANCF_OT1_Adapter_R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTG GTGTGTTATGCCTGT
IVT_AsCas12a_hDNMT1_site1_sense	CGAGTAACAGACATGGACCATCAGATCTACAAGAGTAGA AATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hDNMT1_site2_sense	AGCTGAGGCAGGTGCCTGCTGAGCATCTACAAGAGTAG AAATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hCCR5_site1_sense	ATCCATCTTGTTCCACCCTGTGCAATCTACAAGAGTAGAA ATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hCCR5_site2_sense	GAGGATGACCAGCATGTTGCCCACATCTACAAGAGTAGA AATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hAAVS1_sense	GATCCTCTCTGGCTCCATCGTAAGATCTACAAGAGTAGAA ATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hIL12A-AS1_sense	CCCCTTTCCCTTTTAGTGGCATCCATCTACAAGAGTAGAA ATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_hFANCF_sense	CTAATCCCGGAACTGGACCCCGCCATCTACAAGAGTAGA AATTACCCTATAGTGAGTCGTATTAATTTC
IVT_AsCas12a_universial_antisense	GAAATTAATACGACTCACTATAGGG
IVT_SpCas9_hDNMT1_site1_sgRNA_sense	GAAATTAATACGACTCACTATAGGAGTGCTAAGGGAACGT TCAGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hDNMT1_site2_sgRNA_sense	GAAATTAATACGACTCACTATAGCCAGCAGCCAACCTGAC CAAGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hCCR5_site1_sgRNA_sense	GAAATTAATACGACTCACTATAGTAATAATTGATGTCATAG ATGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hCCR5_site2_sgRNA_sense	GAAATTAATACGACTCACTATAGAACACCAGTGAGTAGAG CGGGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hAAVS1_sgRNA_sense	GAAATTAATACGACTCACTATAGGCAAGGAGAGAGATGG CTCCGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hIL12A-AS1_sgRNA_sense	GAAATTAATACGACTCACTATAGTTCTGGGGTCAACATCT TGGGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_hFANCF_sgRNA_sense	GAAATTAATACGACTCACTATAGCCGCTCCAGAGCCGTG CGAAGTTTTAGAGCTAGAAATAGCAAG
IVT_SpCas9_universial_antisense	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATA ACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAA AC

† Sequence information of the forward and reverse adapter primers used in targeted amplicon sequencing is shown in green and blue, respectively.

Supplementary Figures



[Figure S1] Schematics of the target sequence for each gene locus which is targeted by Cas12a or SpCas9 nickase in this study. Within each locus (*DNMT1* site1-2, *CCR5* site1-2, *AAVS1, IL12A-AS1, FANCF*), the targeted sequences either alone or simultaneously by the AsCas12a and SpCas9 (D10A) nickases are displayed in different colors. The protospacer and PAM (TTTN) sequence of the Cas12a is highlighted in cyan and red, respectively. The protospacer and PAM (NGG) sequence of the SpCas9 (D10A) nickase is highlighted in green and blue, respectively.

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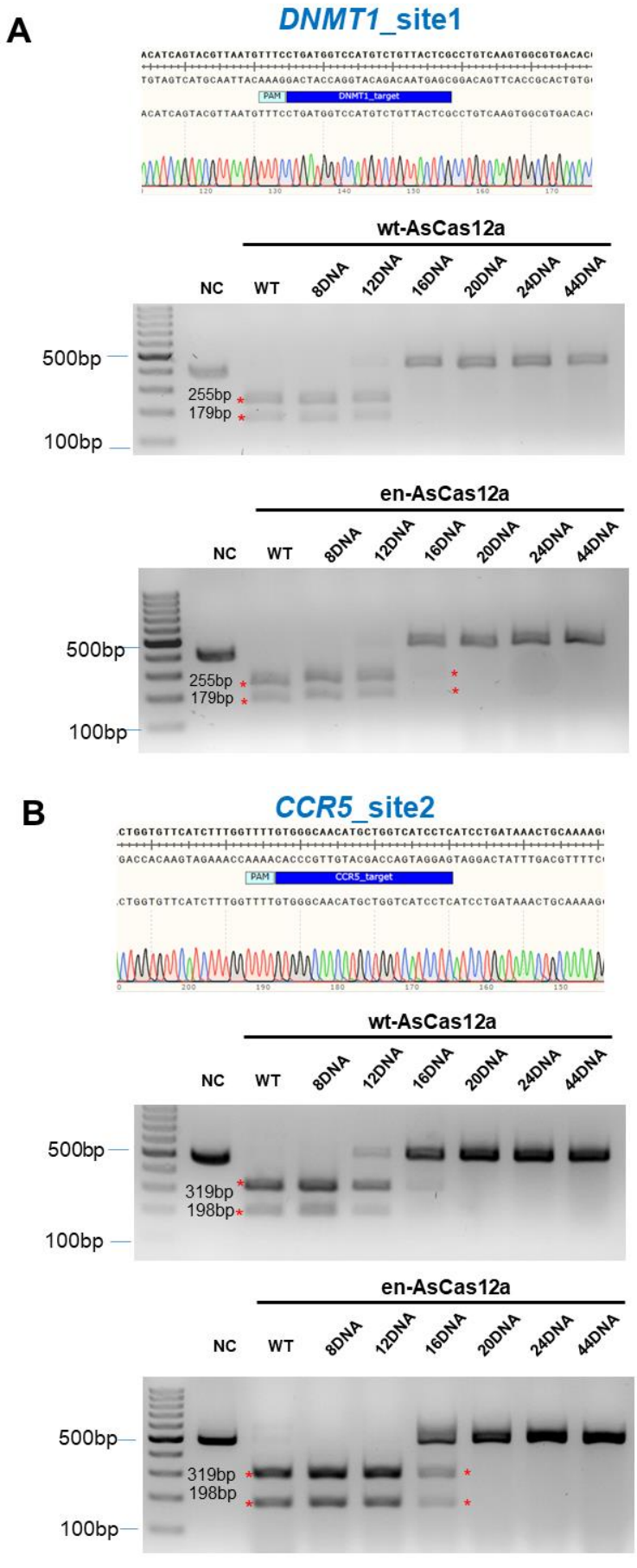
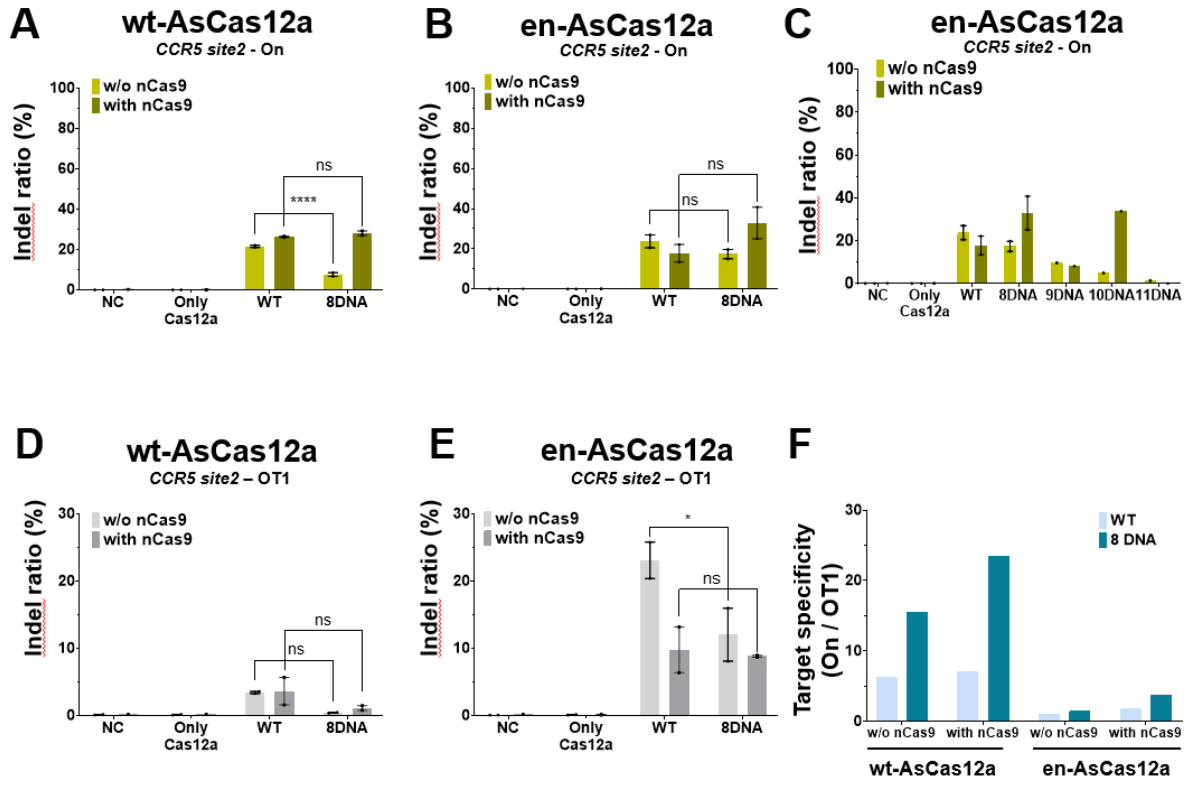
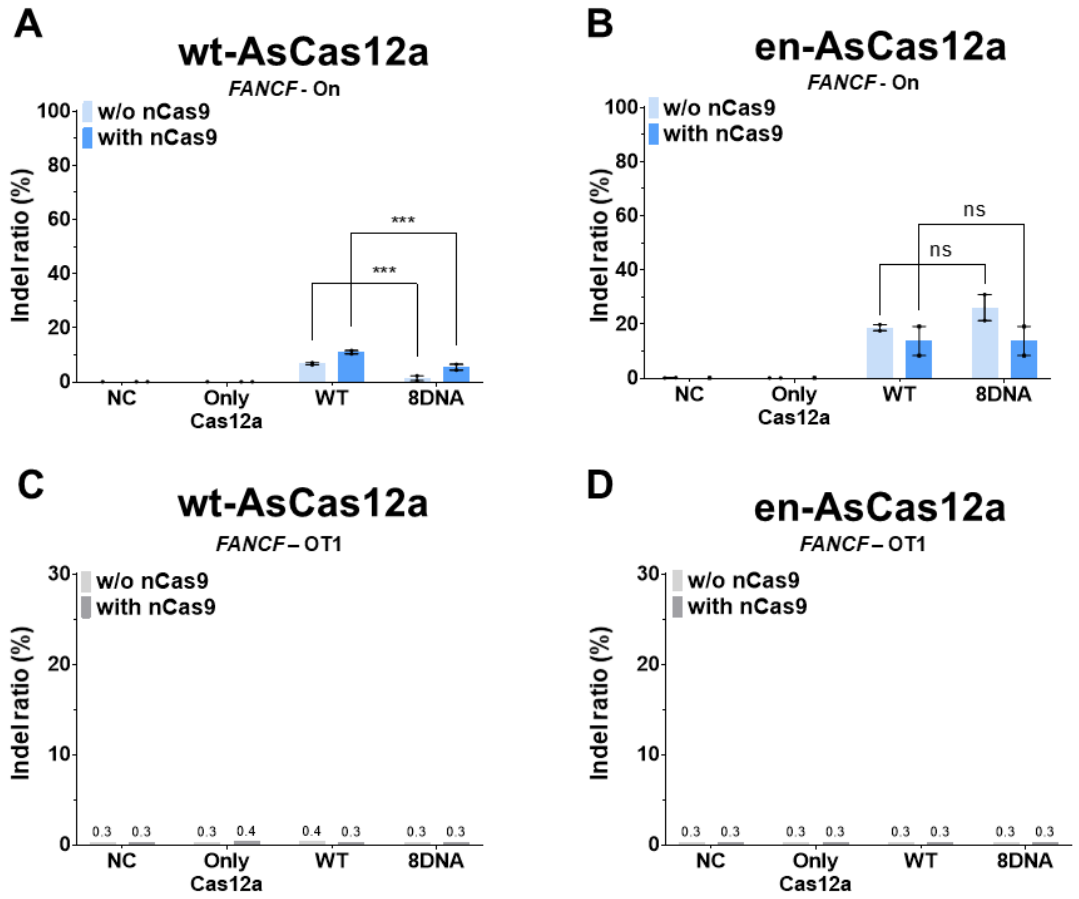

A
DNMT1_site1
PAM
DNMT1_target
wt-AsCas12a
NC
WT
8DNA
12DNA
16DNA
20DNA
24DNA
44DNA
500bp
100bp
255bp
179bp
en-AsCas12a
B
CCR5_site2
CCR5_target
319bp
198bp

[Figure S2] Results of the *in-vitro* DNA amplicon cleavage assay to compare the activity of wt- and en-AsCas12a based on a chimeric DNA-RNA guide. (A, B) Results of the Sanger sequencing (upper) and target DNA amplicon cleavage assay (bottom) of *DNMT1*-site1 (A) and *CCR5*-site2 (B) sequences. DNA amplicons for each gene were obtained by PCR using the corresponding DNA primers (Table S3). (NC: negative control, WT: wild-type crRNA, 8-44DNA: chimeric crRNA which has sequential 8 to 44 nt DNA substitution from 3'-end of crRNA). The cleaved amplicons were separated on 2% agarose gel. The protospacer and PAM (TTTN) sequences in a sequencing data are indicated by dark blue and light blue, respectively. Red asterisks on the gel picture indicates a cleaved DNA fragments.



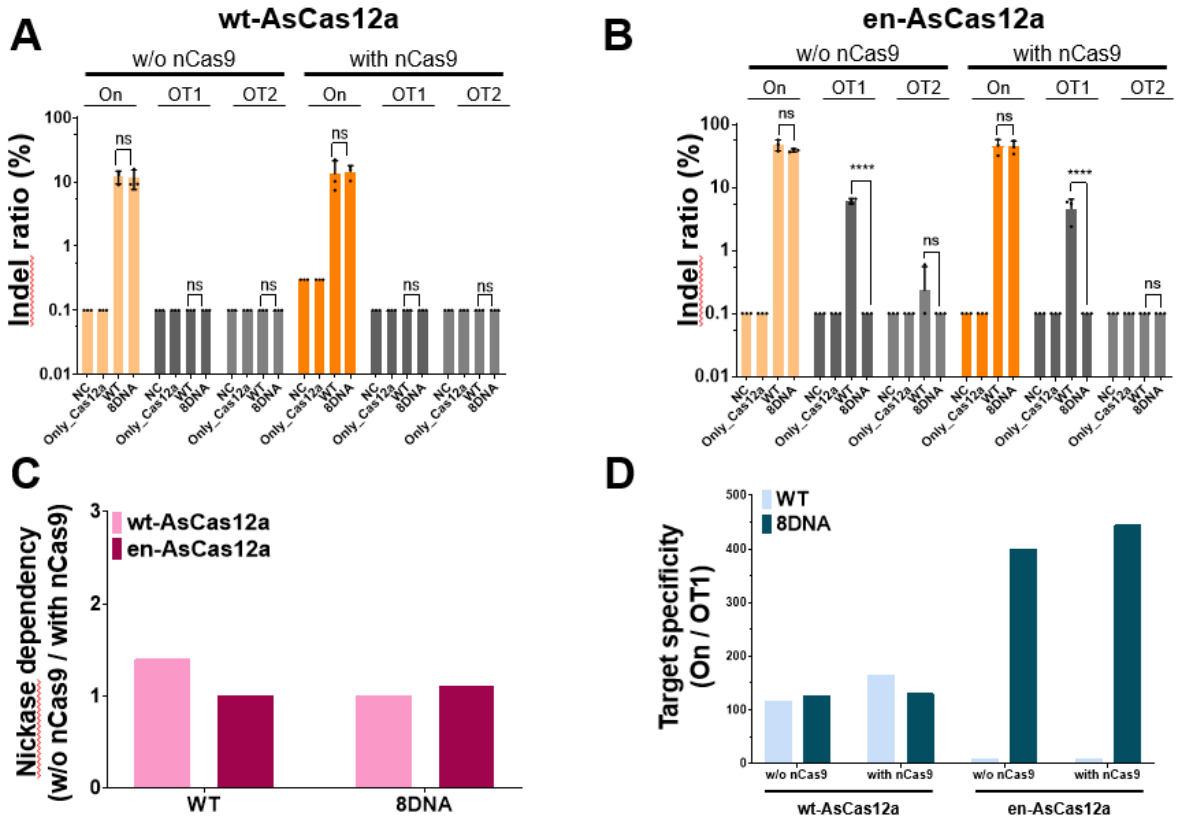
[Figure S3] Comparison of chimeric DNA-RNA guide-based genome editing efficiency and specificity between wt-Cas12a and en-Cas12a for *CCR5* target seqence. (A, B) Comparison of indel ratio (%) for the target nucleotide sequence (On, *CCR5*-site2) using wt-Cas12a (A) and en-Cas12a (B) based on a wt- and chimeric guide, respectively. Indel ratio (%) according to the presence (dark green) / absence (pale green) of simultaneous treatment of nCas9 was also compared. **(C)** Analysis of the indel ratio (%) of en-Cas12a according to the increase in the number of DNA substitutions at the 3'-end of the chimeric guide. **(D, E)** Comparison of indel ratio (%) for off-target sequence (OT1) using wt- and chimeric guide-based wt-Cas12a (D) and en-Cas12a (E). **(F)** Comparison of target specificity between wt- and chimeric guide (8DNA) based on wt-Cas12a and en-Cas12a. Histograms are shown as means ± s.e.m. from two independent experiments. P-values are calculated using a two-way anova with sidak's multiple comparisons test (ns: not significant, P*: <0.0332, P**: <0.0021, P***: <0.0002, P****: <0.0001). NC: negative control, only Cas12a: only protein treated, WT: wild-type crRNA was treated with wt- or en-AsCas12a, 8DNA: chimeric crRNA (sequential 8DNA substitution at 3'-end of crRNA) was treated with wt- or en-AsCas12a. nCas9: nickase Cas9 (D10A)).



[Figure S4] Comparison of chimeric DNA-RNA guide-based genome editing efficiency and specificity between wt-Cas12a and en-Cas12a for *FANCF* target sequence. (A, B) Comparison of indel ratio (%) for the target nucleotide sequence (On, *FANCF*) using wt-Cas12a (A) and en-Cas12a (B) based on a wt- and chimeric guide, respectively. Indel ratio (%) according to the presence (blue) / absence (light blue) of simultaneous treatment of nCas9 was also compared. **(C, D)** Comparison of indel ratio (%) for the off-target sequence (OT1) using wt- and chimeric guide-based wt-Cas12a (C) and en-Cas12a (D). Histograms are shown as means ± s.e.m. from two independent experiments. P-values are calculated using a two-way anova with sidak's multiple comparisons test (ns: not significant, P*: <0.0332, P**: <0.0021, P***: <0.0002, P****: <0.0001). NC: negative control, only Cas12a: only protein treated, WT: wild-type crRNA was treated with wt- or en-AsCas12a, 8DNA: chimeric crRNA (sequential 8DNA substitution at 3'-end of crRNA) was treated with wt- or en-AsCas12a. nCas9: nickase Cas9 (D10A)).

AAVS1

	Chromosome	Position	Bulge	Mismatch No.	Sequence
On-target (On)	Chr19	55115578	0	0	TTTG CTTACGATGGAGCCAGAGAG
Off-target1 (OT1)	Chr4	95395972	0	3	TTTC CTTATGATGAAGCCAGAGAA
Off-target1 (OT2)	Chr1	182157128	RNA - 1	2	TTTA CTTA-GATGAAGCCACAGAG



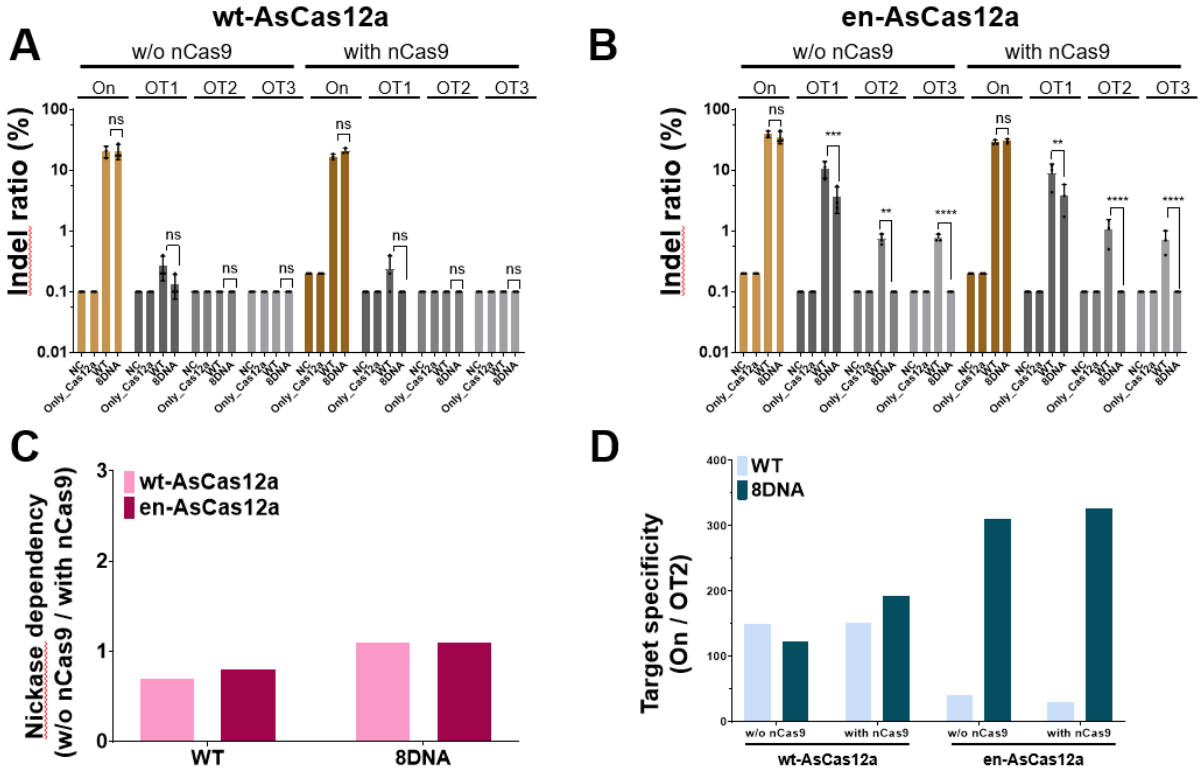
[Figure S5] Comparison of genome editing target specificity (on-target editing (%) / off-target editing (%)) of wt-AsCas12a and en-AsCas12a on endogenous target (*AAVS1*) in human cell line (HEK293FT) using an wt- (WT) or optimized chimeric DNA-RNA guide (8 DNA). Upper table shows the on-target nucleotide sequence (On) for target gene (*AAVS1*) and the corresponding off-target nucleotide sequence (OT1-2) predicted from *in-silico* analysis.[1] The underline indicates the PAM (TTTN) nucleotide sequence, and the nucleotides mismatched with the target sequence in the off-target is indicated in red. **(A, B)** Indel ratio (%) of the wt-AsCas12a (A) or en-AsCas12a (B) based editing on the endogenous target sequences (on-/off-target sites for *AAVS1*) using wt-crRNA (WT) and 3'-end 8-nt DNA substituted crRNA (8 DNA). NC: negative control, only Cas12a: only protein treated, nCas9: nickase Cas9 (D10A). **(C, D)** Nickase dependency (C) and target specificity (D) were calculated from NGS results, respectively. Nickase dependency = (without (w/o) nCas9 editing (%) / with (w/) nCas9 editing (%)), Target specificity = (on-target editing (%) / off-target editing (%)). Each histogram is shown as means ± s.e.m. from three independent experimental values. *P*-values are calculated using a two-way ANOVA with sidak's multiple comparisons test (ns: not

significant, P*: <0.0332, P**: <0.0021,

P***: <0.0002, P****: <0.0001).

DNMT1 -site2

	Chromosome	Position	Mismatch No.	Sequence
On-target (On)	Chr19	10133920	0	TTTG GCTCAGCAGGCACCTGCCTC
Off-target1 (OT1)	Chr21	25149419	2	CTTA GCTCAGCAGGCACCTGCCCA
Off-target2 (OT2)	Chr12	57638266	4	TTTA GCTCAGCTGACACCTGCCCA
Off-target3 (OT3)	Chr6	107959600	2	TCCA GCTCAGCAGACACCAGCCTC



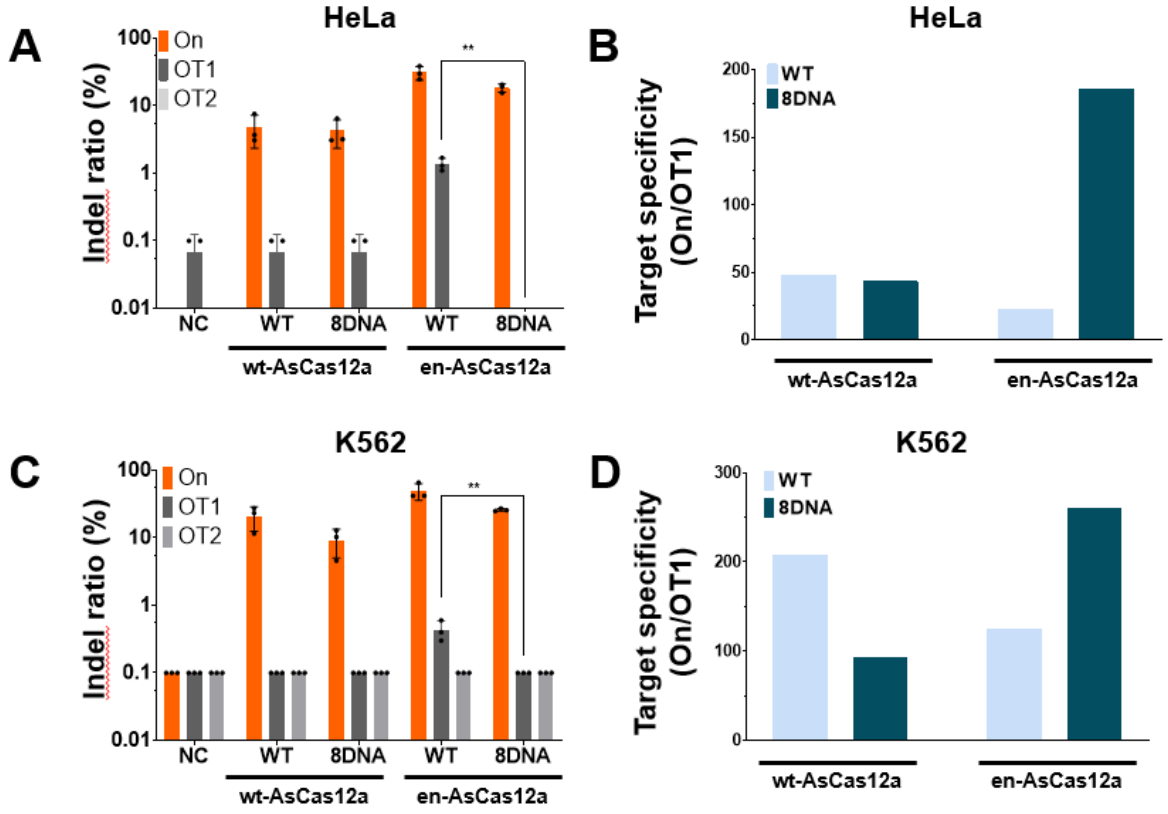
[Figure S6] Comparison of genome editing target specificity (on-target editing (%) / off-target editing (%)) of wt-AsCas12a and en-AsCas12a on endogenous target (*DNMT1*-site2) in human cell line (HEK293FT) using an wt- (WT) or optimized chimeric DNA-RNA guide (8 DNA). Upper table shows the on-target nucleotide sequence (On) for target gene (*DNMT1*-site2) and the corresponding off-target nucleotide sequence (OT1-2) predicted from *in-silico* analysis.[1] The underline indicates the PAM (TTTN) nucleotide sequence, and the nucleotides mismatched with the target sequence in the off-target is indicated in red. **(A, B)** Indel ratio (%) of the wt-AsCas12a (A) or en-AsCas12a (B) based editing on the endogenous target sequences (on-/off-target sites for *DNMT1*-site2) using wt-crRNA (WT) and 3'-end 8-nt DNA substituted crRNA (8 DNA). NC: negative control, only Cas12a: only protein treated, nCas9: nickase Cas9 (D10A). **(C, D)** Nickase dependency (C) and target specificity (D) were calculated from NGS results, respectively. Nickase dependency = (without (w/o) nCas9 editing (%) / with (w/) nCas9 editing (%)), Target specificity = (on-target editing (%) / off-target

editing (%)). Each histogram is shown as means ± s.e.m. from three independent experimental values. *P*-values are calculated using a two-way ANOVA with sidak's multiple comparisons test (ns: not significant, P*: <0.0332,

P**: <0.0021, P***: <0.0002, P****: <0.0001).

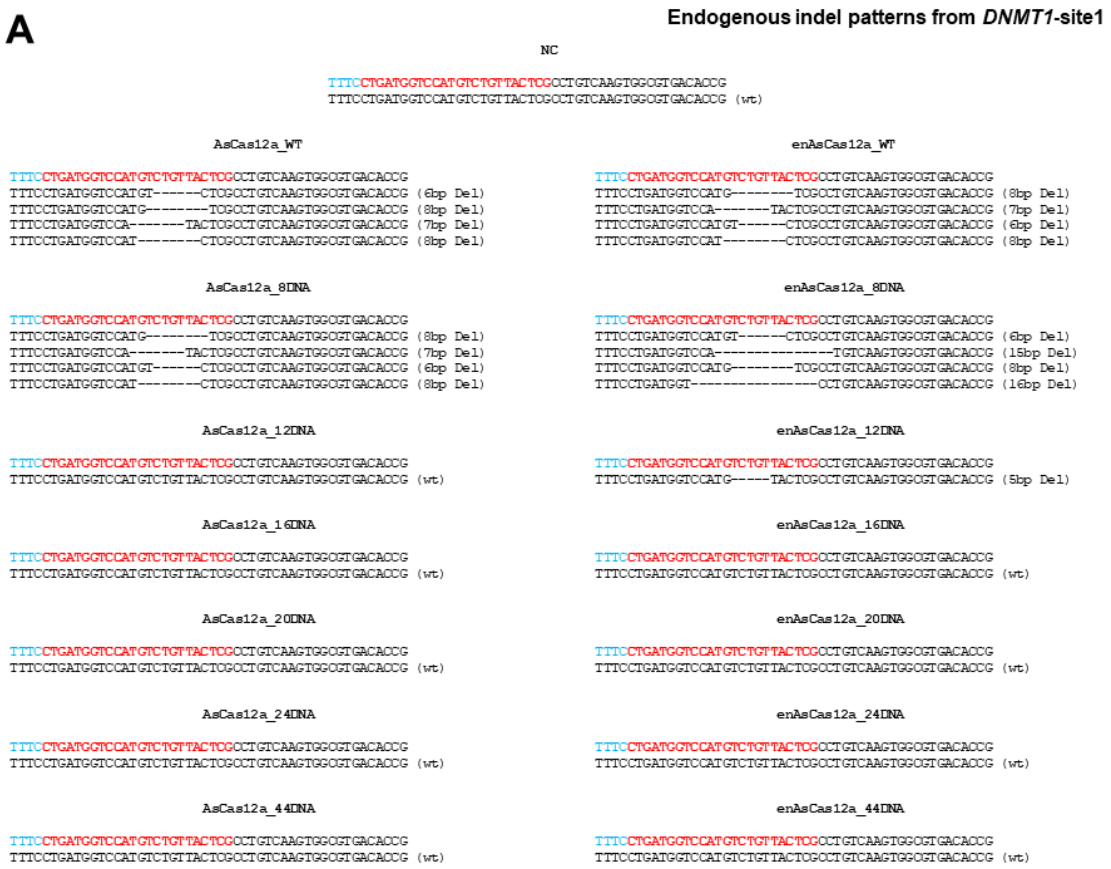
AAVS1

	Chromosome	Position	Bulge	Mismatch No.	Sequence
On-target (On)	Chr19	55115578	0	0	TTTG CTTACGATGGAGCCAGAGAG
Off-target1 (OT1)	Chr4	95395972	0	3	TTTC CTTATGATGAAGCCAGAGAA
Off-target1 (OT2)	Chr1	182157128	RNA - 1	2	TTTA CTTA-GATGAAGCCACAGAG



[Figure S7] Comparison of genome editing target specificity (on-target editing (%) / off-target editing (%)) of wt-AsCas12a and en-AsCas12a on endogenous target (*AAVS1*) in human cell lines (HeLa, K562) using an wt- (WT) or optimized chimeric DNA-RNA guide (8 DNA). Upper table shows the on-target nucleotide sequence (On) for target gene (*AAVS1*) and the corresponding off-target nucleotide sequence (OT1-2) predicted from *in-silico* analysis.[1] The underline indicates the PAM (TTTN) nucleotide sequence, and the nucleotides mismatched with the target sequence in the off-target is indicated in red. **(A, B)** Indel ratio (A) and target specificity (B) of the wt- or en-AsCas12a based editing on target sequences (on-/off-target sites for *AAVS1*) in HeLa cell using wt-crRNA (WT) and 3'-end 8-nt DNA substituted crRNA (8 DNA). **(C, D)** Indel ratio (C) and target specificity (D) of the wt- or en-AsCas12a

based editing on target sequences (on-/off-target sites for *AAVS1*) in K562 cell using wt-crRNA (WT) and 3'-end 8-nt DNA substituted crRNA (8 DNA). NC: negative control. Target specificity = (on-target editing (%) / off-target editing (%)). Each histogram is shown as means ± s.e.m. from three independent experimental values. *P*-values are calculated using a two-way ANOVA with sidak's multiple comparisons test (ns: not significant, P*: <0.0332, P**: <0.0021, P***: <0.0002, P****: <0.0001).



B

Endogenous indel patterns from *CCR5*-site2

NC

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

AsCas12a_WT

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATG---TATCAAGTGTCAAGTCCAATCT (3bp Del)

enAsCas12a_WT

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAAC-------ATTATCAAGTGTCAAGTCCAATCT (7bp Del)
TTTATGCACAGG----------GTGTCAAGTCCAATCT (22bp Del)
TTTATGCACAGGGTGGAACA------ATTATCAAGTGTCAAGTCCAATCT (6bp Del)
TTTATGCACAGGGTGGAACATG----------GTGTCAAGTCCAATCT (12bp Del)

AsCas12a_8DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTG--------TTTTTTATCAAGTGTCAAGTCCAATCT (8bp Del)

enAsCas12a_8DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACA----GGATTATCAAGTGTCAAGTCCAATCT (4bp Del)
TTTATGCACAGGGTGGAA----------CAAGTGTCAAGTCCAATCT (13bp Del)
TTTATGCACAGGGTGGAAC-------ATTATCAAGTGTCAAGTCCAATCT (7bp Del)
TTTATGCACAGGGTGGAACAAG--------TCAAGTGTCAAGTCCAATCT (8bp Del)

AsCas12a_12DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

enAsCas12a_12DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

AsCas12a_16DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

enAsCas12a_16DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

AsCas12a_20DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

enAsCas12a_20DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

AsCas12a_24DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

enAsCas12a_24DNA

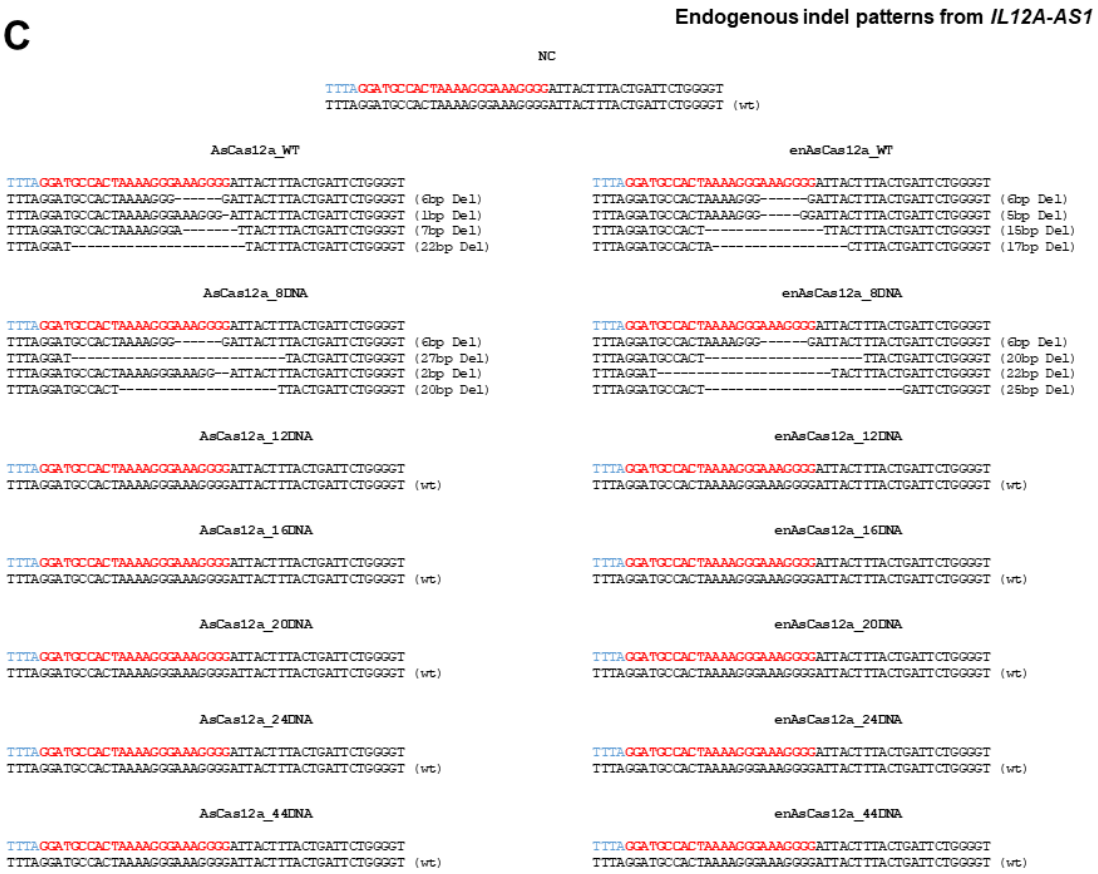
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

AsCas12a_44DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)

enAsCas12a_44DNA

TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT
TTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCT (wt)



[Figure S8] Representative indel pattern from NGS analysis of endogenous genomic locus edited by wt-Cas12a or en-Cas12a using various chimeric guides. (A-C) List of indel patterns induced by wt-Cas12a, en-Cas12a using various chimeric guides on *DNMT1*-site1 (A), *CCR5*-site2 (B), and *IL12A-AS1* (C) locus in HEK293FT cell line. PAM sequence (TTTN) for AsCas12a is shown in blue and protospacer is shown in red, respectively. The dashed line indicates deleted sequence relative to the wild-type reference sequence. NC: negative control, WT: wild-type crRNA was treated with wt- or en-AsCas12a, 8-44DNA: chimeric crRNA (sequential 8-44DNA substitution at 3'-end of crRNA) was treated with wt- or en-AsCas12a.

A

AsCas12a - Endogenous indel patterns from *CCR5*-site1 on-target

```
AsCas12a_w/o_nCas9_NC

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

AsCas12a_w/o_nCas9_only_Cas12a

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

AsCas12a_w/o_nCas9_WT

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGA----------TATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (11bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACA------ATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (6bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAA----GATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (4bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGG----------GTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (22bp Del)

AsCas12a_w/o_nCas9_8DNA

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAAC-------ATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (7bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAA----------GTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (15bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGG----------GTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (22bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATG----------GTCCAATCTATGACATCAATTATTATACATCGGA (16bp Del)


AsCas12a_with_nCas9_NC

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

AsCas12a_with_nCas9_only_Cas12a

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

AsCas12a_with_nCas9_WT

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGG----------ATTATTATACATCGGA (43bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGA----------TCAAGTCCAATCTATGACATCAATTATTATACATCGGA (20bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------CAAGTCCAATCTATGACATCAATTATTATACATCGGA (20bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGA----------TATGACATCAATTATTATACATCGGA (26bp Del)

AsCas12a_with_nCas9_8DNA

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------CAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (13bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------TATTATACATCGGA (43bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGG----------TATTATACATCGGA (35bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACA----------TATTATACATCGGA (41bp Del)
```

enAsCas12a - Endogenous indel patterns on *CCR5 site1 on*

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enAsCas12a_w/o_nCas9_NC

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

enAsCas12a_w/o_nCas9_WT

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGG----------GTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (22bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGG----------AGTCCAATCTATGACATCAATTATTATACATCGGA (24bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGG----------AGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (17bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGG----------TGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (10bp Del)

enAsCas12a_w/o_nCas9_8DNA

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGG----------GTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (22bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------CAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (13bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGA----------CAAGTCCAATCTATGACATCAATTATTATACATCGGA (20bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGG----------AAGTCCAATCTATGACATCAATTATTATACATCGGA (23bp Del)


enAsCas12a_with_nCas9_NC

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

enAsCas12a_with_nCas9_only_Cas12a

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (wt)

enAsCas12a_with_nCas9_WT

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCAC----------AGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (39bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGG----------ATCAATTATTATACATCGGA (39bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAAC----------TATTATACATCGGA (42bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGA----------GTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (19bp Del)

enAsCas12a_with_nCas9_8DNA

GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------CAATTATTATACATCGGA (39bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------CAAGTCCAATCTATGACATCAATTATTATACATCGGA (20bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGG----------TGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGA (10bp Del)
GGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAA----------TATTATACATCGGA (43bp Del)
```

AsCas12a - Endogenous indel patterns on *CCR5 site1 OT1*

AsCas12a_w/o_nCas9_NC

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_w/o_nCas9_only_Cas12a

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_w/o_nCas9_WT

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_w/o_nCas9_8DNA

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_with_nCas9_NC

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_with_nCas9_only_Cas12a

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_with_nCas9_WT

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a_with_nCas9_8DNA

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a - Endogenous indel patterns on *CCR5 site1 OT1*

enAsCas12a_w/o_nCas9_NC

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a_w/o_nCas9_WT

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAA-----GACACTCCCTGCTCCAGAATCAGATCATAGTTAT (5bp Del)
TGTTAACTCTTTTCTGCACAGGGTG----AAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (4bp Del)
TGTTAACTCTTTTCTGCACAGGGTGAA-----AAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (5bp Del)

enAsCas12a_w/o_nCas9_8DNA

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a_with_nCas9_NC

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a_with_nCas9_only_Cas12a

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

enAsCas12a_with_nCas9_WT

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAA--------ACACTCCCTGCTCCAGAATCAGATCATAGTTAT (8bp Del)
TGTTAACTCTTTTCTGCACAGGGTGAAAAA-------ACACTCCCTGCTCCAGAATCAGATCATAGTTAT (7bp Del)
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAG----GACACTCCCTGCTCCAGAATCAGATCATAGTTAT (4bp Del)
TGTTAACTCTTTTCTGCACAGGGTGAAAAAA-----GACACTCCCTGCTCCAGAATCAGATCATAGTTAT (5bp Del)

enAsCas12a_with_nCas9_8DNA

TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT
TGTTAACTCTTTTCTGCACAGGGTGAAAAAAGAAATGACACTCCCTGCTCCAGAATCAGATCATAGTTAT (wt)

AsCas12a - Endogenous indel patterns on *CCR5 site1 OT2*

AsCas12a_w/o_nCas9_NC

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_w/o_nCas9_only_Cas12a

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_w/o_nCas9_WT

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_w/o_nCas9_8DNA

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_with_nCas9_NC

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_with_nCas9_only_Cas12a

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_with_nCas9_WT

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

AsCas12a_with_nCas9_8DNA

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a - Endogenous indel patterns on *CCR5 site1 OT2*

enAsCas12a_w/o_nCas9_NC

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_w/o_nCas9_WT

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGAT--GAGGCCCACTGGCTATCAGATGATAGAGGTAACA (2bp Del)

enAsCas12a_w/o_nCas9_8DNA

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_with_nCas9_NC

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_with_nCas9_only_Cas12a

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_with_nCas9_WT

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

enAsCas12a_with_nCas9_8DNA

CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA
CACAGTGTGTTTTATGCACAGGGAGAAAAAAGATGAGAGGCCCACTGGCTATCAGATGATAGAGGTAACA (wt)

B AsCas12a - Endogenous indel patterns from *AAVS1* on-target

AsCas12a_w/o_nCas9_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_w/o_nCas9_only_Cas12a

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_w/o_nCas9_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGAT---GGGAGGGAGAGCTTGGCAGG (3bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
```

AsCas12a_w/o_nCas9_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAG--TCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGG-------------------GAGGGAGAGCTTGGCAGG (18bp Del)
```

AsCas12a_with_nCas9_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_with_nCas9_only_Cas12a

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_with_nCas9_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG----GATCCTGGGAGGGAGAGCTTGGCAGG (4bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG---------------GGAGGGAGAGCTTGGCAGG (15bp Del)
```

AsCas12a_with_nCas9_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
CTCCT----------------------------------------------GGGAGGGAGAGCTTGGCAGG (47bp Del)
CTCCTTGCC------------------------------------------TGGGAGGGAGAGCTTGGCAGG (42bp Del)
```

enAsCas12a - Endogenous indel patterns on *AAVS1 on*

enAsCas12a_w/o_nCas9_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

enAsCas12a_w/o_nCas9_only_Cas12a

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

enAsCas12a_w/o_nCas9_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG----------------------AGCTTGGCAGG (23bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (26bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
```

enAsCas12a_w/o_nCas9_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGGAGAGCTTGGCAGG (18bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG----------------GAGGGAGAGCTTGGCAGG (16bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGG-------------------GAGGGAGAGCTTGGCAGG (18bp Del)
```

enAsCas12a_with_nCas9_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

enAsCas12a_with_nCas9_only_Cas12a

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

enAsCas12a_with_nCas9_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG-------------------GAGCTTGGCAGG (18bp Del)
CTC-------------------------------------------TCCTGGGAGGGAGAGCTTGGCAGG (45bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------GAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCT-------------------------------------GGGAGGGAGAGCTTGGCAGG (36bp Del)
```

enAsCas12a_with_nCas9_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG---------------GGAGGGAGAGCTTGGCAGG (15bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG----GATCCTGGGAGGGAGAGCTTGGCAGG (4bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
```

AsCas12a - Endogenous indel patterns on *AAVS1 OT1*

AsCas12a_w/o_nCas9_NC

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_w/o_nCas9_only_Cas12a

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_w/o_nCas9_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_w/o_nCas9_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_with_nCas9_NC

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_with_nCas9_only_Cas12a

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_with_nCas9_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_with_nCas9_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a - Endogenous indel patterns on *AAVS1 OT1*

enAsCas12a_w/o_nCas9_NC

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a_w/o_nCas9_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCC-----AGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (5bp Del)
AACATAGAAGTTTCCTTATGATGAAGCC--AGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (2bp Del)
AACATAGAAGTTTCCTTATGATGAAGCCA--------GTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (8bp Del)
AACATAGAAGTTTCCTTATGAT---------GAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (9bp Del)

enAsCas12a_w/o_nCas9_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a_with_nCas9_NC

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a_with_nCas9_only_Cas12a

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

enAsCas12a_with_nCas9_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAG---AGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (3bp Del)
AACATAGAAGTTTCCTTATGATGAAGCCAGAGA----------CCCCATCTGTGTACTC (21bp Del)
AACATAGAAGTTTCCTTATGATGAAGCC-----AGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (5bp Del)
AACATAGAAGTTTCCTTATGATGAAGCCAG------TGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (6bp Del)

enAsCas12a_with_nCas9_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

C

AsCas12a - Endogenous indel patterns from *DNMT1*-site2 on-target

AsCas12a_w/o_nCas9_NC

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

AsCas12a_w/o_nCas9_only_Cas12a

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

AsCas12a_w/o_nCas9_WT

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC---------TCACTTGAGCCTCTGGGTCTAGAAC (9bp Del)
```

AsCas12a_w/o_nCas9_8DNA

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGAC--AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (2bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
```

AsCas12a_with_nCas9_NC

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

AsCas12a_with_nCas9_only_Cas12a

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

AsCas12a_with_nCas9_WT

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCAC-------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (7bp Del)
```

AsCas12a_with_nCas9_8DNA

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGAC--AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (2bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
```

enAsCas12a - Endogenous indel patterns on *DNMT1 site2 on*

enAsCas12a_w/o_nCas9_NC

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

enAsCas12a_w/o_nCas9_only_Cas12a

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

enAsCas12a_w/o_nCas9_WT

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCAC-------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (7bp Del)
```

enAsCas12a_w/o_nCas9_8DNA

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGAC--AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (2bp Del)
```

enAsCas12a_with_nCas9_NC

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

enAsCas12a_with_nCas9_only_Cas12a

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (wt)
```

enAsCas12a_with_nCas9_WT

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCAC-------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (7bp Del)
```

enAsCas12a_with_nCas9_8DNA

```
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGCCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACC------AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGC------TGCTCACTTGAGCCTCTGGGTCTAGAAC (6bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCT----CAGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (4bp Del)
TGGGCTGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGCACCTGAC--AGCTGCTCACTTGAGCCTCTGGGTCTAGAAC (2bp Del)
```

AsCas12a - Endogenous indel patterns on *DNMT1 site2 OT1*

AsCas12a_w/o_nCas9_NC

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_w/o_nCas9_only_Cas12a

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_w/o_nCas9_WT

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_w/o_nCas9_8DNA

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_with_nCas9_NC

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_with_nCas9_only_Cas12a

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_with_nCas9_WT

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

AsCas12a_with_nCas9_8DNA

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

enAsCas12a - Endogenous indel patterns on *DNMT1 site2 OT1*

enAsCas12a_w/o_nCas9_NC

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

enAsCas12a_w/o_nCas9_WT

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC-----ATGGAGAAAACACTTGGGCTGGCCCTCC (5bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCAC------ATGGAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTG------GAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC----CATGGAGAAAACACTTGGGCTGGCCCTCC (4bp Del)

enAsCas12a_w/o_nCas9_8DNA

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC-----ATGGAGAAAACACTTGGGCTGGCCCTCC (5bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTG------GAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCAC------ATGGAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAG----------AAAACACTTGGGCTGGCCCTCC (24bp Del)

enAsCas12a_with_nCas9_NC

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

enAsCas12a_with_nCas9_only_Cas12a

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC (wt)

enAsCas12a_with_nCas9_WT

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC-----ATGGAGAAAACACTTGGGCTGGCCCTCC (5bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTG------GAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCAC------ATGGAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC----CATGGAGAAAACACTTGGGCTGGCCCTCC (4bp Del)

enAsCas12a_with_nCas9_8DNA

ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTGCCCATGGAGAAAACACTTGGGCTGGCCCTCC
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACC-----ATGGAGAAAACACTTGGGCTGGCCCTCC (5bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCAC------ATGGAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAGGCACCTG------GAGAAAACACTTGGGCTGGCCCTCC (6bp Del)
ACAGTCAAGAGCAAAGTTGTGCTTAGCTCAGCAG----------AAAACACTTGGGCTGGCCCTCC (16bp Del)

AsCas12a - Endogenous indel patterns on *DNMT1 site2 OT2*

AsCas12a_w/o_nCas9_NC

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_w/o_nCas9_only_Cas12a

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_w/o_nCas9_WT

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_w/o_nCas9_8DNA

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_with_nCas9_NC

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_with_nCas9_only_Cas12a

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_with_nCas9_WT

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a_with_nCas9_8DNA

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a - Endogenous indel patterns on *DNMT1 site2 OT2*

enAsCas12a_w/o_nCas9_NC

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a_w/o_nCas9_WT

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACA----CCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (4bp Del)
TCATAGGACATTTAGCTCAGCTGACACCTGC---CCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (3bp Del)
TCATAGGACATTTAGCTCAGCTGACA-----CCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (5bp Del)
TCATAGGACATTTAGCTCA----------GCTGGCAGCTGGCCCAGCTATTATGACAAC (21bp Del)

enAsCas12a_w/o_nCas9_8DNA

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a_with_nCas9_NC

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a_with_nCas9_only_Cas12a

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

enAsCas12a_with_nCas9_WT

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACA----CCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (4bp Del)
TCATAGGACATTTAGCTCAGCTGACACCTGC---CCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (3bp Del)
TCATAGGACATTTAGCTCAGCTGACA-----CCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (5bp Del)
TCATAGGACATTTA----------------------------GGCAGCTGGCCCAGCTATTATGACAAC (29bp Del)

enAsCas12a_with_nCas9_8DNA

TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC
TCATAGGACATTTAGCTCAGCTGACACCTGCCCACCAATTGCTGGCAGCTGGCCCAGCTATTATGACAAC (wt)

AsCas12a - Endogenous indel patterns on *DNMT1 site2 OT3*

AsCas12a_w/o_nCas9_NC

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_w/o_nCas9_only_Cas12a

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_w/o_nCas9_WT

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_w/o_nCas9_8DNA

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_with_nCas9_NC

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_with_nCas9_only_Cas12a

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_with_nCas9_WT

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

AsCas12a_with_nCas9_8DNA

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a - Endogenous indel patterns on *DNMT1 site2 OT3*

enAsCas12a_w/o_nCas9_NC

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a_w/o_nCas9_only_Cas12a

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a_w/o_nCas9_WT

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACA-------CTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (7bp Del)
AAGTCCAGTTTCCAGCTCAGCAGA----------CACAGCCAGGGGAAGGAGAGGCTCCCGA (18bp Del)
AAGTCCAGTTTCCAGCTCAGCAGACAC------CTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (6bp Del)
AAGTCCAGTTTCCAGCTCAGCAGACA------TCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (6bp Del)

enAsCas12a_w/o_nCas9_8DNA

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a_with_nCas9_NC

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a_with_nCas9_only_Cas12a

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

enAsCas12a_with_nCas9_WT

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACA-------CTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (7bp Del)
AAGTCCAGTTTCCAGCTCAGCAGACA-----CTC---GGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (8bp Del)
AAGTCCAGTTTCCAGCTCAGCAGACACCAGC--CTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (2bp Del)
AAGTCCAGTTTCCAGCTCAGCAGACACTA----------ACCCACAGCCAGGGGAAGGAGAGGCTCCCGA (10bp Del)

enAsCas12a_with_nCas9_8DNA

AAGTCCAGTTTCCAGCTCAGCGGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA
AAGTCCAGTTTCCAGCTCAGCAGACACCAGCCTCTAGGGTCCCACAGCCAGGGGAAGGAGAGGCTCCCGA (wt)

[Figure S9] Representative indel patterns from NGS analysis of each on-/off-target sites on genomic DNA edited by single or co-transfection of chimeric crRNA guided Cas12a and SpCas9 nickase (D10A). (A-C) List of indel patterns induced by the combination of Cas12a and SpCas9 nickase (D10A) at each on-/off-target sites of *CCR5*-site1 (A), *AAVS1* (B), and *DNMT1*-site2 (C) locus in HEK293FT cell line. PAM sequences (NGG, TTTN) for SpCas9 and Cas12a effector are shown in orange and blue, and each protospacer region is shown in purple and red color, respectively. The dashed line indicates deleted sequence relative to the wild-type reference sequence. NC: negative control, only Cas12a: only protein treated, WT: wild-type crRNA was treated with wt- or en-AsCas12a, 8DNA: chimeric crRNA (sequential 8DNA substitution at 3'-end of crRNA) was treated with wt- or en-AsCas12a. nCas9: nickase Cas9 (D10A)), w/o: without.

A **Endogenous indel patterns from *AAVS1* on-target (HeLa)**

HeLa_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTA-------------------------------------------GCTTGGCAGG (43bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATG--------------------AGGGAGAGCTTGGCAGG (20bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGG-------------------GAGGGAGAGCTTGGCAGG (18bp Del)
```

AsCas12a_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA-----TCCTGGGAGGGAGAGCTTGGCAGG (5bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTT-----------------------------------GGCAGG (37bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATG-------------------------AGCTTGGCAGG (26bp Del)
```

en-AsCas12a_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG-----------GGAGGGAGAGCTTGGCAGG (11bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGAT--------------TCCTGGGAGGGAGAGCTTGGCAGG (14bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-----------------------AGCTTGGCAGG (23bp Del)
```

en-AsCas12a_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-----------------------AGCTTGGCAGG (23bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGA------------------------------CAGG (31bp Del)
```

Endogenous indel patterns from *AAVS1* OT1 (HeLa)

HeLa_NC

```
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)
```

AsCas12a_WT

```
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)
```

AsCas12a_8DNA

```
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)
```

en-AsCas12a_WT

```
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGAT----------GAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (9bp Del)
AACATAGAAGTTTCCTTATGATGAAGCC--AGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (2bp Del)
AACATAGAAGTTTCCTTATGAT-----------AGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (11bp Del)
AACATAGAAGTTTCCTTATGA----------------------------------GCCCATCTGTGTACTC (33bp Del)
```

en-AsCas12a_8DNA

```
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)
```

B

Endogenous indel patterns from *AAVS1* on-target (K562)

K562_NC

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG (wt)
```

AsCas12a_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG----GATCCTGGGAGGGAGAGCTTGGCAGG (4bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGA--GGATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAG--AGGATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
```

AsCas12a_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG----------------------AGCTTGGCAGG (23bp Del)
```

en-AsCas12a_WT

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG----------------------AGCTTGGCAGG (23bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGG------------------GAGGGAGAGCTTGGCAGG (18bp Del)
```

en-AsCas12a_8DNA

```
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGG
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG-------------------GGAGAGCTTGGCAGG (19bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC-------------------------TTGGCAGG (25bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAG--GATCCTGGGAGGGAGAGCTTGGCAGG (2bp Del)
CTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAG----------------------AGCTTGGCAGG (23bp Del)
```

Endogenous indel patterns from *AAVS1* OT1 (K562)

K562_NC

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

AsCas12a_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

en-AsCas12a_WT

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAA----------GCTGCTCTGAAAGCTGCCCATCTGTGTACTC (14bp Del)
AACATAGAAGTTTCCTTATGATGAA----------GCTCTGAAAGCTGCCCATCTGTGTACTC (17bp Del)
AACATAGAAGTTTCCTTATGATGAAGCC--AGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (2bp Del)
AACATAGAAGTTTCCTTATGA----------TGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (17bp Del)

en-AsCas12a_8DNA

AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC
AACATAGAAGTTTCCTTATGATGAAGCCAGAGAAGCTGTGCTGCTCTGAAAGCTGCCCATCTGTGTACTC (wt)

[Figure S10] Representative indel pattern from NGS analysis of endogenous genomic locus in various cell lines edited by wt-Cas12a or en-Cas12a using chimeric DNA-RNA guide. (A, B) List of indel patterns induced by wt-AsCas12a and en-AsCas12a using wt- or 8 DNA chimeric guide on *AAVS1* locus in HeLa (A) or K562 (B) cell lines. PAM sequence (TTTN) for AsCas12a is shown in blue and protospacer is shown in red, respectively. The dashed line indicates deleted sequence relative to the wild-type reference sequence. NC: negative control, WT: wild-type crRNA was treated with wt- or en-AsCas12a, 8 DNA: chimeric crRNA (sequential 8 DNA substitution at 3'-end of crRNA) was treated with wt- or en-AsCas12a.

Information of the nucleotide sequence for AsCas12a used in this study

1. CMV-wt-AsCas12a

ATGACACAGTTCGAGGGCTTTACCAACCTGTATCAGGTGAGCAAGACACTGCGGTTTGAGCTGAT
CCCACAGGGCAAGACCCTGAAGCACATCCAGGAGCAGGGCTTCATCGAGGAGGACAAGGCCCG
CAATGATCACTACAAGGAGCTGAAGCCCATCATCGATCGGATCTACAAGACCTATGCCGACCAGT
GCCTGCAGCTGGTGCAGCTGGATTGGGAGAACCTGAGCGCCGCCATCGACTCCTATAGAAAGGA
GAAAACCGAGGAGACAAGGAACGCCCTGATCGAGGAGCAGGCCACATATCGCAATGCCATCCAC
GACTACTTCATCGGCCGGACAGACAACCTGACCGATGCCATCAATAAGAGACACGCCGAGATCTA
CAAGGGCCTGTTCAAGGCCGAGCTGTTTAATGGCAAGGTGCTGAAGCAGCTGGGCACCGTGAC

CACAACCGAGCACGAGAACGCCCTGCTGCGGAGCTTCGACAAGTTTACAACCTACTTCTCCGGC
TTTTATGAGAACAGGAAGAACGTGTTCAGCGCCGAGGATATCAGCACAGCCATCCCACACCGCAT
CGTGCAGGACAACTTCCCCAAGTTTAAGGAGAATTGTCACATCTTCACACGCCTGATCACCGCCG
TGCCCAGCCTGCGGGAGCACTTTGAGAACGTGAAGAAGGCCATCGGCATCTTCGTGAGCACCTC
CATCGAGGAGGTGTTTTCCTTCCCTTTTTATAACCAGCTGCTGACACAGACCCAGATCGACCTGTA
TAACCAGCTGCTGGGAGGAATCTCTCGGGAGGCAGGCACCGAGAAGATCAAGGGCCTGAACGA
GGTGCTGAATCTGGCCATCCAGAAGAATGATGAGACAGCCCACATCATCGCCTCCCTGCCACACA
GATTCATCCCCCTGTTTAAGCAGATCCTGTCCGATAGGAACACCCTGTCTTTCATCCTGGAGGAGT
TTAAGAGCGACGAGGAAGTGATCCAGTCCTTCTGCAAGTACAAGACACTGCTGAGAAACGAGAA
CGTGCTGGAGACAGCCGAGGCCCTGTTTAACGAGCTGAACAGCATCGACCTGACACACATCTTC
ATCAGCCACAAGAAGCTGGAGACAATCAGCAGCGCCCTGTGCGACCACTGGGATACACTGAGGA
ATGCCCTGTATGAGCGGAGAATCTCCGAGCTGACAGGCAAGATCACCAAGTCTGCCAAGGAGAA
GGTGCAGCGCAGCCTGAAGCACGAGGATATCAACCTGCAGGAGATCATCTCTGCCGCAGGCAAG
GAGCTGAGCGAGGCCTTCAAGCAGAAAACCAGCGAGATCCTGTCCCACGCACACGCCGCCCTG
GATCAGCCACTGCCTACAACCCTGAAGAAGCAGGAGGAGAAGGAGATCCTGAAGTCTCAGCTGG
ACAGCCTGCTGGGCCTGTACCACCTGCTGGACTGGTTTGCCGTGGATGAGTCCAACGAGGTGGA
CCCCGAGTTCTCTGCCCGGCTGACCGGCATCAAGCTGGAGATGGAGCCTTCTCTGAGCTTCTAC
AACAAGGCCAGAAATTATGCCACCAAGAAGCCCTACTCCGTGGAGAAGTTCAAGCTGAACTTTCA
GATGCCTACACTGGCCTCTGGCTGGGACGTGAATAAGGAGAAGAACAATGGCGCCATCCTGTTT
GTGAAGAACGGCCTGTACTATCTGGGCATCATGCCAAAGCAGAAGGGCAGGTATAAGGCCCTGA
GCTTCGAGCCCACAGAGAAAACCAGCGAGGGCTTTGATAAGATGTACTATGACTACTTCCCTGAT
GCCGCCAAGATGATCCCAAAGTGCAGCACCCAGCTGAAGGCCGTGACAGCCCACTTTCAGACCC
ACACAACCCCCATCCTGCTGTCCAACAATTTCATCGAGCCTCTGGAGATCACAAAGGAGATCTAC
GACCTGAACAATCCTGAGAAGGAGCCAAAGAAGTTTCAGACAGCCTACGCCAAGAAAACCGGCG
ACCAGAAGGGCTACAGAGAGGCCCTGTGCAAGTGGATCGACTTCACAAGGGATTTTCTGTCCAA
GTATACCAAGACAACCTCTATCGATCTGTCTAGCCTGCGGCCATCCTCTCAGTATAAGGACCTGGG
CGAGTACTATGCCGAGCTGAATCCCCTGCTGTACCACATCAGCTTCCAGAGAATCGCCGAGAAGG
AGATCATGGATGCCGTGGAGACAGGCAAGCTGTACCTGTTCCAGATCTATAACAAGGACTTTGCC
AAGGGCCACCACGGCAAGCCTAATCTGCACACACTGTATTGGACCGGCCTGTTTTCTCCAGAGAA
CCTGGCCAAGACAAGCATCAAGCTGAATGGCCAGGCCGAGCTGTTCTACCGCCCTAAGTCCAGG
ATGAAGAGGATGGCACACCGGCTGGGAGAGAAGATGCTGAACAAGAAGCTGAAGGATCAGAAAA
CCCCAATCCCCGACACCCTGTACCAGGAGCTGTACGACTATGTGAATCACAGACTGTCCCACGAC
CTGTCTGATGAGGCCAGGGCCCTGCTGCCCAACGTGATCACCAAGGAGGTGTCTCACGAGATCA
TCAAGGATAGGCGCTTTACCAGCGACAAGTTCTTTTTCCACGTGCCTATCACACTGAACTATCAGG
CCGCCAATTCCCCATCTAAGTTCAACCAGAGGGTGAATGCCTACCTGAAGGAGCACCCCGAGAC
ACCTATCATCGGCATCGATCGGGGCGAGAGAAACCTGATCTATATCACAGTGATCGACTCCACCG
GCAAGATCCTGGAGCAGCGGAGCCTGAACACCATCCAGCAGTTTGATTACCAGAAGAAGCTGGA
CAACAGGGAGAAGGAGAGGGTGGCAGCAAGGCAGGCCTGGTCTGTGGTGGGCACAATCAAGG
ATCTGAAGCAGGGCTATCTGAGCCAGGTCATCCACGAGATCGTGGACCTGATGATCCACTACCAG
GCCGTGGTGGTGCTGGAGAACCTGAATTTCGGCTTTAAGAGCAAGAGGACCGGCATCGCCGAGA
AGGCCGTGTACCAGCAGTTCGAGAAGATGCTGATCGATAAGCTGAATTGCCTGGTGCTGAAGGA
CTATCCAGCAGAGAAAGTGGGAGGCGTGCTGAACCCATACCAGCTGACAGACCAGTTCACCTCC
TTTGCCAAGATGGGCACCCAGTCTGGCTTCCTGTTTTACGTGCCTGCCCCATATACATCTAAGATC
GATCCCCTGACCGGCTTCGTGGACCCCTTCGTGTGGAAAACCATCAAGAATCACGAGAGCCGCA
AGCACTTCCTGGAGGGCTTCGACTTTCTGCACTACGACGTGAAAACCGGCGACTTCATCCTGCA
CTTTAAGATGAACAGAAATCTGTCCTTCCAGAGGGGCCTGCCCGGCTTTATGCCTGCATGGGATAT
CGTGTTCGAGAAGAACGAGACACAGTTTGACGCCAAGGGCACCCCTTTCATCGCCGGCAAGAGA
ATCGTGCCAGTGATCGAGAATCACAGATTCACCGGCAGATACCGGGACCTGTATCCTGCCAACGA
GCTGATCGCCCTGCTGGAGGAGAAGGGCATCGTGTTCAGGGATGGCTCCAACATCCTGCCAAAG
CTGCTGGAGAATGACGATTCTCACGCCATCGACACCATGGTGGCCCTGATCCGCAGCGTGCTGC
AGATGCGGAACTCCAATGCCGCCACAGGCGAGGACTATATCAACAGCCCCGTGCGCGATCTGAA
TGGCGTGTGCTTCGACTCCCGGTTTCAGAACCCAGAGTGGCCCATGGACGCCGATGCCAATGGC
GCCTACCACATCGCCCTGAAGGGCCAGCTGCTGCTGAATCACCTGAAGGAGAGCAAGGATCTGA
AGCTGCAGAACGGCATCTCCAATCAGGACTGGCTGGCCTACATCCAGGAGCTGCGCAACAAAAG
GCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGGGATCCTACCCATACGATGTT

CCAGATTACGCTTATCCCTACGACGTGCCTGATTATGCATACCCATATGATGTCCCCGACTATGCC

Cyon: wt-AsCas12a (WT), Yellow: nucleoplasmin NLS, Green: linker, Gray: HA-tag

2. pET28-wt-AsCas12a

CATCATCATCATCATCATGTGTACCCCTACGACGTGCCCGACTACGCCGAATTGCCTCCAAAAAAG
AAGAGAAAGGTAATGACACAGTTTGAAGGCTTCACCAATCTCTACCAGGTCAGCAAGACGCTACG
TTTTGAGCTTATCCCGCAGGGAAAAACCCTGAAACACATTCAGGAACAGGGGTTCATAGAGGAAG
ATAAGGCGCGTAACGACCATTATAAAGAACTGAAGCCTATAATCGACCGTATTTATAAAACGTACGC
GGATCAGTGCCTGCAGCTGGTTCAGCTGGATTGGGAGAATCTGTCCGCGGCTATTGATAGCTATC
GCAAAGAGAAGACCGAGGAAACCCGTAACGCACTGATTGAAGAGCAGGCGACCTATCGGAATGC
GATCCATGATTACTTCATCGGCCGCACCGACAACCTGACCGATGCAATTAACAAACGTCACGCAG
AGATTTACAAAGGTCTGTTTAAAGCAGAGTTATTCAATGGCAAGGTTCTGAAACAGCTGGGTACGG
TCACCACCACCGAACACGAAAACGCACTGCTGAGGAGCTTTGATAAATTTACCACATATTTCAGCG
GTTTCTATGAAAATCGTAAGAATGTATTTAGCGCCGAAGATATTTCCACCGCAATTCCTCATCGTATT
GTGCAGGATAATTTTCCGAAGTTTAAAGAAAATTGTCATATTTTTACCCGTCTGATCACCGCGGTAC
CGAGCCTGCGAGAGCATTTTGAAAACGTTAAGAAAGCCATTGGAATTTTTGTCAGTACCAGCATTG
AAGAAGTGTTTTCGTTCCCGTTCTATAACCAACTGCTGACCCAGACCCAGATTGATCTGTACAATC
AGCTGCTGGGGGGCATAAGCCGCGAGGCAGGTACCGAAAAGATAAAGGGACTCAATGAGGTGCT
GAATCTGGCAATTCAGAAGAATGATGAgACGGCTCATATCATTGCTAGCCTGCCGCATCGTTTCATT
CCCCTGTTTAAGCAAATCCTGAGCGATCGCAATACACTGAGCTTTATCCTCGAAGAGTTTAAATCG
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GACCGCTGAAGCACTGTTTAATGAACTGAACTCGATCGACCTCACCCATATTTTTATATCCCACAAA
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ATTCAAACAGAAAACCAGCGAAATCCTGAGCCATGCCCATGCTGCACTGGATCAGCCGCTGCCG
ACCACCCTGAAAAAACAGGAGGAAAAGGAGATTCTGAAAAGCCAACTGGACAGCCTGCTGGGCC
TGTATCACCTGCTGGACTGGTTTGCAGTCGATGAGAGCAACGAGGTTGATCCTGAGTTCTCCGCT
CGTCTGACCGGAATCAAGCTGGAGATGGAACCGAGTCTGTCGTTTTACAATAAAGCGCGTAATTA
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GCGGATGGGACGTTAACAAAGAAAAAAACAATGGGGCAATTCTGTTTGTGAAAAATGGCCTCTATT
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ACCTCAGAGGGTTTCGACAAGATGTACTACGATTATTTCCCGGATGCGGCAAAAATGATACCCAAA
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GTGCAAATGGATTGACTTTACCCGTGACTTTCTGTCAAAATATACCAAAACGACGAGCATTGATCT
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TGCTCTACCATATTTCCTTCCAACGAATCGCTGAAAAAGAAATAATGGACGCCGTTGAAACCGGCA
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GGACAGGCAGAACTGTTTTACCGCCCCAAAAGCCGTATGAAAAGGATGGCACACCGCCTGGGCG
AAAAAATGCTGAATAAGAAACTCAAAGATCAGAAAACGCCGATACCGGATACCCTTTATCAGGAGC
TGTATGATTATGTTAACCACCGGCTGAGCCATGACCTGAGCGACGAAGCGCGTGCACTGCTGCC
GAACGTGATTACCAAGGAAGTCTCGCATGAAATTATTAAAGATCGGCGCTTCACCAGTGATAAATTT
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AGCAGTTTGACTACCAGAAAAAGCTGGACAACAGAGAAAAGGAACGTGTAGCCGCCCGGCAGGC
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TGTCGATCTAATGATTCACTATCAAGCAGTAGTGGTACTGGAAAATCTGAATTTCGGTTTCAAAAGC
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ATGCCGGCGTGGGACATTGTTTTTGAAAAGAATGAGACACAGTTTGATGCCAAAGGTACCCCCTT
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TGTACCCGGCAAATGAATTGATAGCGCTGCTGGAGGAGAAAGGTATTGTCTTTCGGGATGGATCA
AACATCCTGCCGAAGCTGCTGGAGAACGATGACAGCCACGCAATAGACACCATGGTAGCGCTGA
TCCGAAGCGTGCTGCAGATGCGTAACAGTAATGCGGCTACGGGGGAAGACTACATTAATAGCCCG
GTCCGTGATCTGAACGGCGTTTGTTTCGATAGCAGATTTCAAAATCCGGAGTGGCCGATGGATGC
CGATGCCAATGGAGCTTACCATATCGCTCTCAAAGGTCAGCTCCTACTGAACCATTTGAAAGAATC
AAAAGATCTGAAACTGCAGAACGGCATCTCGAATCAGGACTGGCTGGCCTACATTCAAGAACTGA
GAAACGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATG
ACAAG

Green: 6His-tag, Magenta : HA-tag, Yellow: SV40 NLS, Cyon: enAsCas12a (WT), Gray: 3X FLAG

Information of the amino acid sequence for AsCas12a used in this study

3. CMV-wt-AsCas12a

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQ
LDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNG
KVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTR
LITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLN
LAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFN
ELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAA
GKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEF
SARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYY
LGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFI
EPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQ
YKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPE
NLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSD
EARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGE
RNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVD
LMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFT
SFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFK
MNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLE
EKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQN
PEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNKRPAATKKAGQAKKK
KGSYPYDVPDYAYPYDVPDYAYPYDVPDYA

4. pET28-wt-AsCas12a

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RNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIG
RTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFS
AEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQT
QIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSD
EEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELT
GKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKS
QLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQM
PTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKM
IPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCK

WIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIY
NKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKD
QKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAAN
SPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAA
RQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKL
NCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNH
ESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKR
IVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQD
WLAYIQELRNDYKDHDGDYKDHDIDYKDDDDK

Information of the nucleotide sequence for en-AsCas12a used in this study

5. CMV-en-AsCas12a

ATGACACAGTTCGAGGGCTTTACCAACCTGTATCAGGTGAGCAAGACACTGCGGTTTGAGCTGAT
CCCACAGGGCAAGACCCTGAAGCACATCCAGGAGCAGGGCTTCATCGAGGAGGACAAGGCCCG
CAATGATCACTACAAGGAGCTGAAGCCCATCATCGATCGGATCTACAAGACCTATGCCGACCAGT
GCCTGCAGCTGGTGCAGCTGGATTGGGAGAACCTGAGCGCCGCCATCGACTCCTATAGAAAGGA
GAAAACCGAGGAGACAAGGAACGCCCTGATCGAGGAGCAGGCCACATATCGCAATGCCATCCAC
GACTACTTCATCGGCCGGACAGACAACCTGACCGATGCCATCAATAAGAGACACGCCGAGATCTA
CAAGGGCCTGTTCAAGGCCGAGCTGTTTAATGGCAAGGTGCTGAAGCAGCTGGGCACCGTGAC
CACAACCGAGCACGAGAACGCCCTGCTGCGGAGCTTCGACAAGTTTACAACCTACTTCTCCGGC
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CGTGCAGGACAACTTCCCCAAGTTTAAGGAGAATTGTCACATCTTCACACGCCTGATCACCGCCG
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GGTGCTGAATCTGGCCATCCAGAAGAATGATGAGACAGCCCACATCATCGCCTCCCTGCCACACA
GATTCATCCCCCTGTTTAAGCAGATCCTGTCCGATAGGAACACCCTGTCTTTCATCCTGGAGGAGT
TTAAGAGCGACGAGGAAGTGATCCAGTCCTTCTGCAAGTACAAGACACTGCTGAGAAACGAGAA
CGTGCTGGAGACAGCCGAGGCCCTGTTTAACGAGCTGAACAGCATCGACCTGACACACATCTTC
ATCAGCCACAAGAAGCTGGAGACAATCAGCAGCGCCCTGTGCGACCACTGGGATACACTGAGGA
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GGTGCAGCGCAGCCTGAAGCACGAGGATATCAACCTGCAGGAGATCATCTCTGCCGCAGGCAAG
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GCTTCGAGCCCACAGAGAAAACCAGCGAGGGCTTTGATAAGATGTACTATGACTACTTCCCTGAT
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TCAAGGATAGGCGCTTTACCAGCGACAAGTTCTTTTTCCACGTGCCTATCACACTGAACTATCAGG
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CAACAGGGAGAAGGAGAGGGTGGCAGCAAGGCAGGCCTGGTCTGTGGTGGGCACAATCAAGG
ATCTGAAGCAGGGCTATCTGAGCCAGGTCATCCACGAGATCGTGGACCTGATGATCCACTACCAG
GCCGTGGTGGTGCTGGAGAACCTGAATTTCGGCTTTAAGAGCAAGAGGACCGGCATCGCCGAGA
AGGCCGTGTACCAGCAGTTCGAGAAGATGCTGATCGATAAGCTGAATTGCCTGGTGCTGAAGGA
CTATCCAGCAGAGAAAGTGGGAGGCGTGCTGAACCCATACCAGCTGACAGACCAGTTCACCTCC
TTTGCCAAGATGGGCACCCAGTCTGGCTTCCTGTTTTACGTGCCTGCCCCATATACATCTAAGATC
GATCCCCTGACCGGCTTCGTGGACCCCTTCGTGTGGAAAACCATCAAGAATCACGAGAGCCGCA

AGCACTTCCTGGAGGGCTTCGACTTTCTGCACTACGACGTGAAAACCGGCGACTTCATCCTGCA
CTTTAAGATGAACAGAAATCTGTCCTTCCAGAGGGGCCTGCCCGGCTTTATGCCTGCATGGGATAT
CGTGTTCGAGAAGAACGAGACACAGTTTGACGCCAAGGGCACCCCTTTCATCGCCGGCAAGAGA
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Cyon: en-AsCas12a (WT), Yellow: nucleoplasmin NLS, Green: linker, Gray: HA-tag

6. pET28-en-AsCas12a

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CTGTATGATTATGTTAACCACCGGCTGAGCCATGACCTGAGCGACGAAGCGCGTGCACTGCTGCC
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AACATCCTGCCGAAGCTGCTGGAGAACGATGACAGCCACGCAATAGACACCATGGTAGCGCTGA
TCCGAAGCGTGCTGCAGATGCGTAACAGTAATGCGGCTACGGGGGAAGACTACATTAATAGCCCG
GTCCGTGATCTGAACGGCGTTTGTTTCGATAGCAGATTTCAAAATCCGGAGTGGCCGATGGATGC
CGATGCCAATGGAGCTTACCATATCGCTCTCAAAGGTCAGCTCCTACTGAACCATTTGAAAGAATC
AAAAGATCTGAAACTGCAGAACGGCATCTCGAATCAGGACTGGCTGGCCTACATTCAAGAACTGA
GAAACGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATG
ACAAG

Green: 6His-tag, Magenta : HA-tag, Yellow: SV40 NLS, Cyon: en-AsCas12a (WT), Gray: 3X FLAG

Information of the amino acid sequence for en-AsCas12a used in this study

7. CMV-en-AsCas12a

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQ
LDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNG
KVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTR
LITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLN
LAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFN
ELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAA
GKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEF
SARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLARGWDVNREKNNGAILFVKNGLYY
LGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFI
EPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQ
YKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPE
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EARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGE
RNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVD
LMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFT
SFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFK
MNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLE
EKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQN
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8. pET28-en-AsCas12a

HHHHHHVYPYDVPDYAELPPKKKRKVMTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKA
RNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIG
RTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFS
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RQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKL
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ESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKR
IVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQD
WLAYIQELRNDYKDHDGDYKDHDIDYKDDDDK

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