**Supplementary Information**

# Engineered CRISPR-OsCas12f1 and RhCas12f1 with robust activities and expanded target range for genome editing


Xiangfeng Kong[1,2,5], Hainan Zhang[1,2,5], Guoling Li[2,5], Zikang Wang[1,2,5], Xuqiang Kong[1,2,5], Lecong Wang[1,2], Mingxing Xue[1,2], Weihong Zhang[1,2], Yao Wang[1,2], Jiajia Lin[4], Jingxing Zhou[1,2], Xiaowen Shen[1,2], Yinghui Wei[1,2], Na Zhong[1,2], Weiya Bai[2], Yuan Yuan[2], Linyu Shi[2], Yingsi Zhou[1,2,#], Hui Yang[1, 2, 3,#]

[1]HUIEDIT Therapeutics Co., Ltd., Shanghai 200031, China.
[2]HUIDAGENE Therapeutics Co., Ltd., Shanghai 200131, China.
[3]Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai 200031, China.
[4]Department of Neurology, First Affiliated Hospital, Fujian Medical University, Fuzhou, China.
[5]These authors contributed equally to this work.
[#]Correspondence to: yingsizhou@huidagene.com (Y.Z.), huiyang@huidagene.com (H.Y.)

This PDF includes:

**Supplementary Figure 1 to 15**



**Supplementary Table 1 to 3**

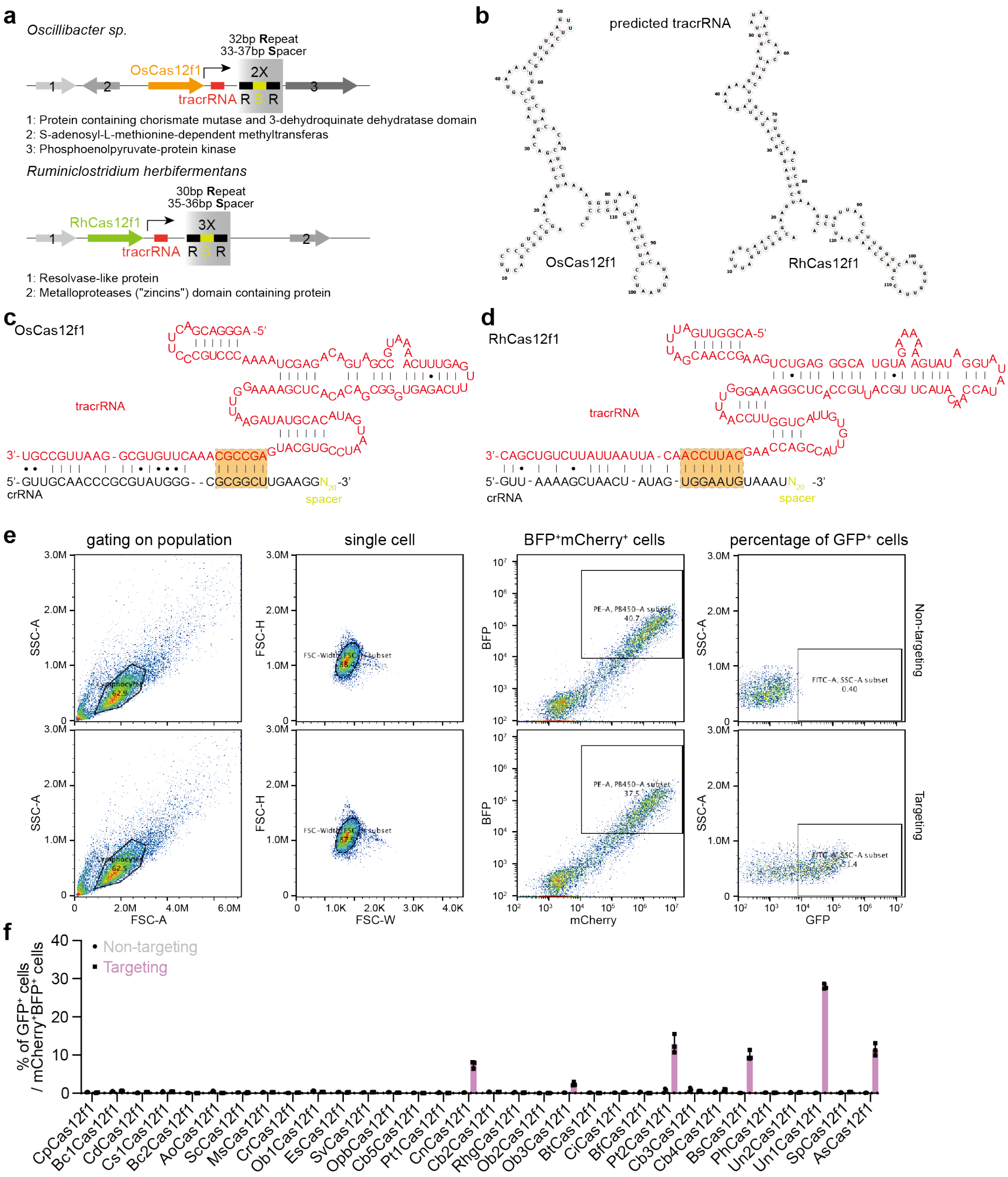


**Supplementary Note 1**

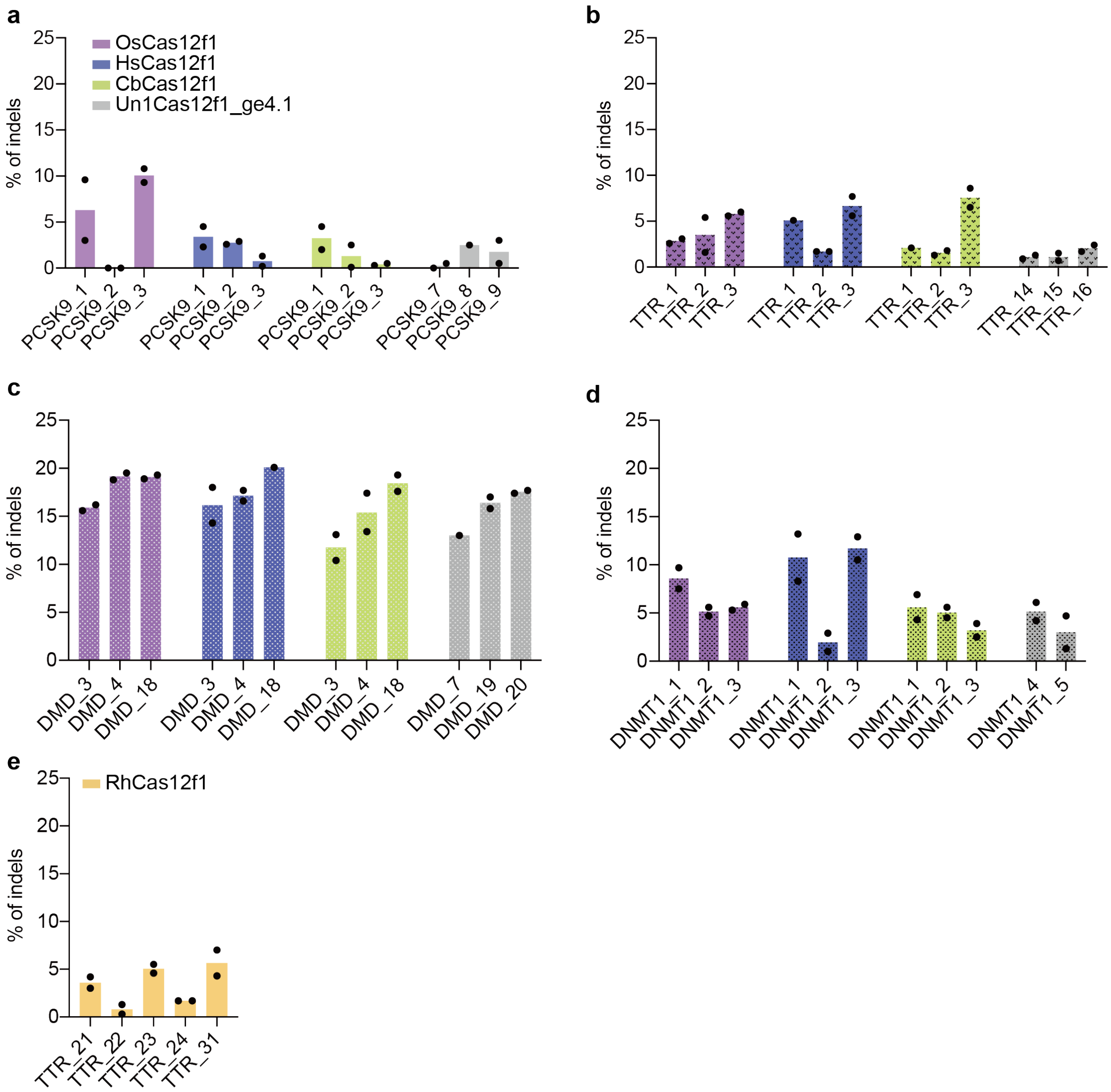


**Supplementary References**

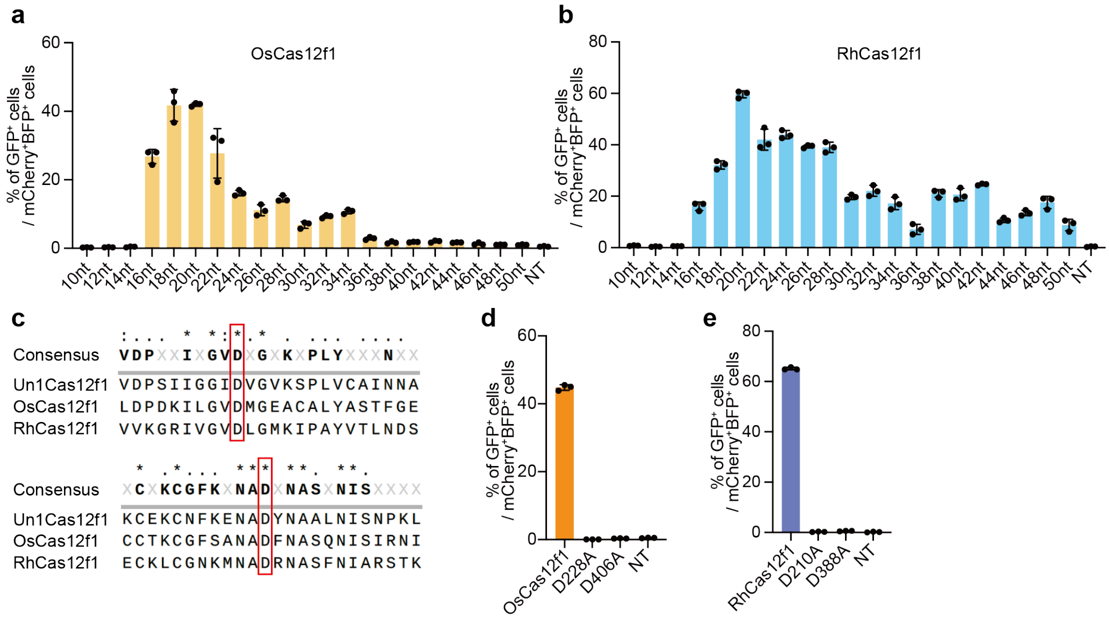
## Supplementary Figures



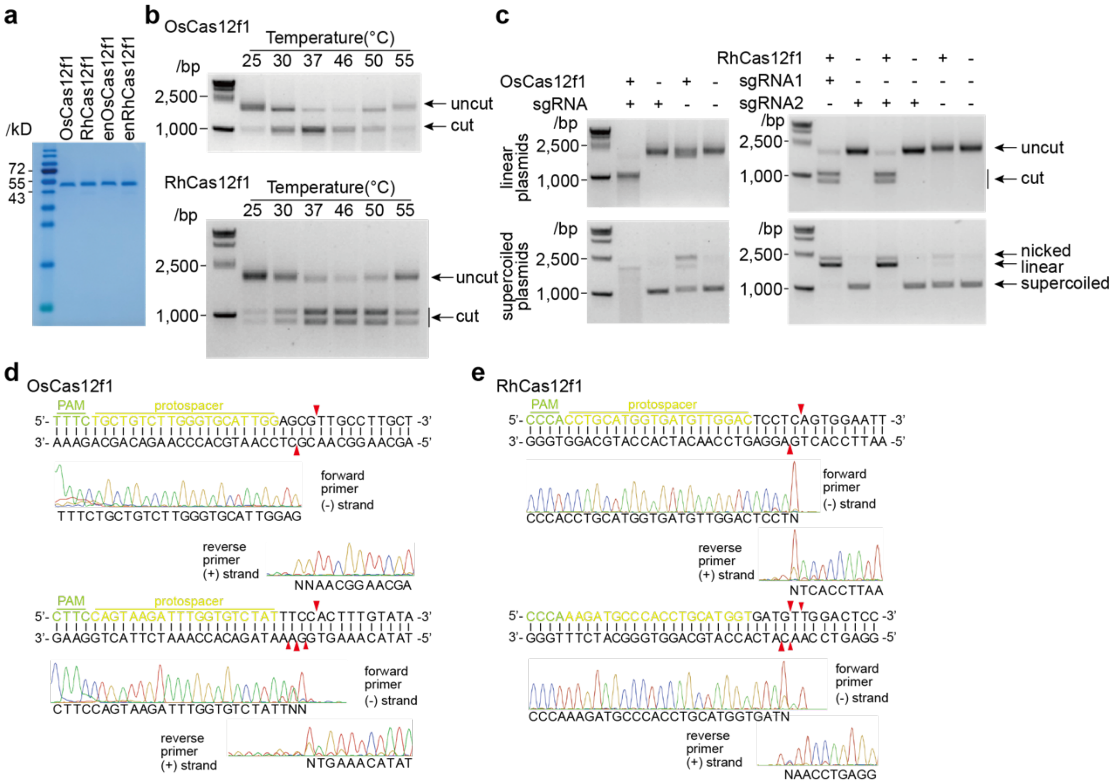

**Supplementary Figure 1. Strategy for flow cytometry gating and Cas12f1 candidates prediction. a**, Scheme representing native CRISPR-Cas loci encoding OsCas12f1 and RhCas12f1. **b**, Predicted tracrRNA structure by RNAfold. *In silico* prediction of base paring between tracrRNA and crRNA of OsCas12f1 (**c**) and RhCas12f1 (**d**). **e**, Gating strategy used for evaluating the EGFP activation efficiency. Gate set on the non-targeting control was used to analyze the EGFP activation efficiency of targeting group. **f**, Screen for functional Cas12f1 in HEK293T cells. Target sequence: CCATTACAGTAGGAGCATAC. Values and error bars represent mean and s.d. (n=3). Source data are provided as a Source Data file.



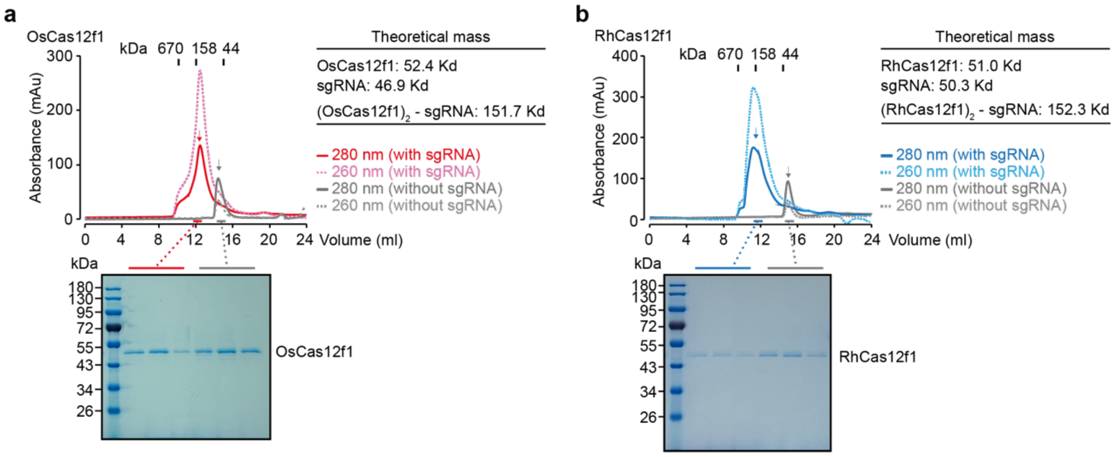

**Supplementary Figure 2. Efficiency validation of genome editing by Cas12f1 in human cells. a~e**, Indel efficiencies at endogenous genes in HEK293T cells, determined by TIDER. All values represent means of results from n=2 biologically independent experiments. Source data are provided as a Source Data file.



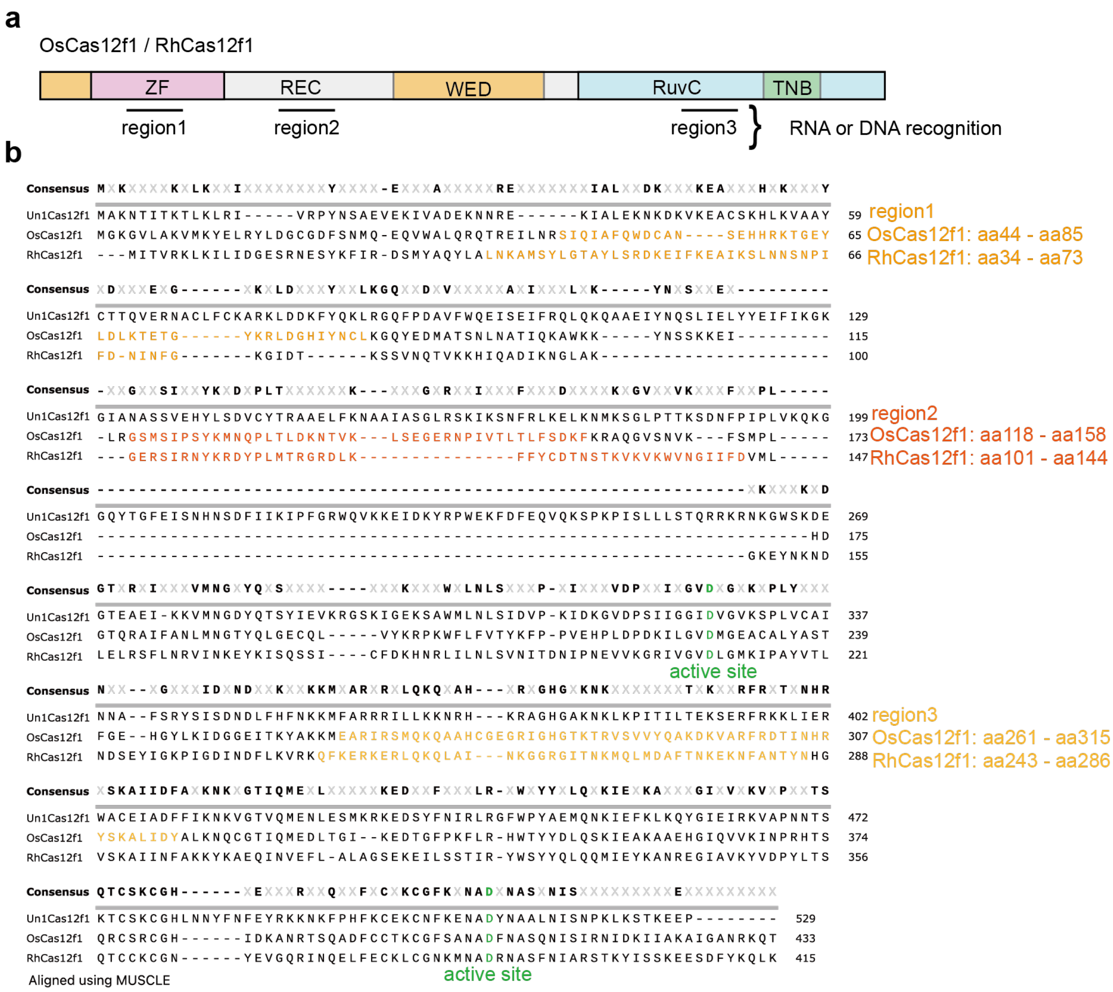

**Supplementary Figure 3. Optimal parameter sets of OsCas12f1 and RhCas12f1.** Optimal spacer length for OsCas12f1 (**a**) and RhCas12f1 (**b**). Values and error bars represent mean and s.d. (n=3). **c**, Alignment of OsCas12f1 and RhCas12f1 with Un1Cas12f1 to identify the conserved residues of RuvC active site, which is marked by red box. Validation of the enzymatic activity sites of OsCas12f1 (**d**) and RhCas12f1 (**e**). TTTC-CCATTACAGTAGGAGCATAC and CCCA-CCATTACAGTAGGAGCATAC target sequence used for assessing the GFP activation efficiencies of OsCas12f1 and RhCas12f1, respectively. Values and error bars represent mean and s.d. (n=3). Source data are provided as a Source Data file.



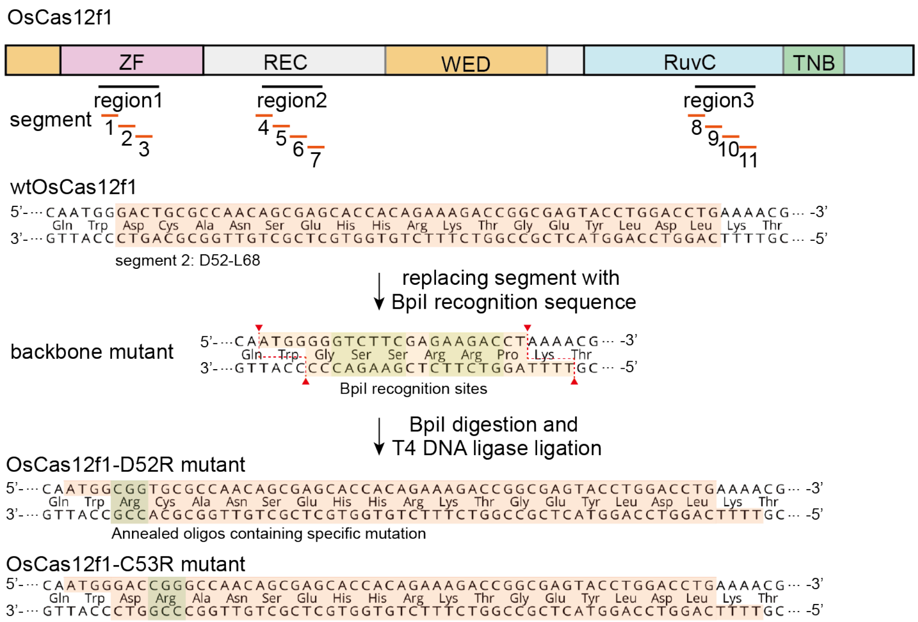

**Supplementary Figure 4. Characterization of OsCas12f1- and RhCas12f1-mediated cleavage. a**, SDS-PAGE analysis of purified OsCas12f1, RhCas12f1, enOsCas12f1 and enRhCas12f1 proteins. **b**, Linear plasmids cleavage at different temperature by OsCas12f1 and RhCas12f1. **c**, OsCas12f1 and RhCas12f1 cut both supercoiled and linear plasmids *in vitro*. Run-off sequencing of OsCas12f1- (**d**) and RhCas12f1- (**e**) cleaved products. Red triangle indicate the cleavage sites. These experiments were repeated at lease two times showing similar results.



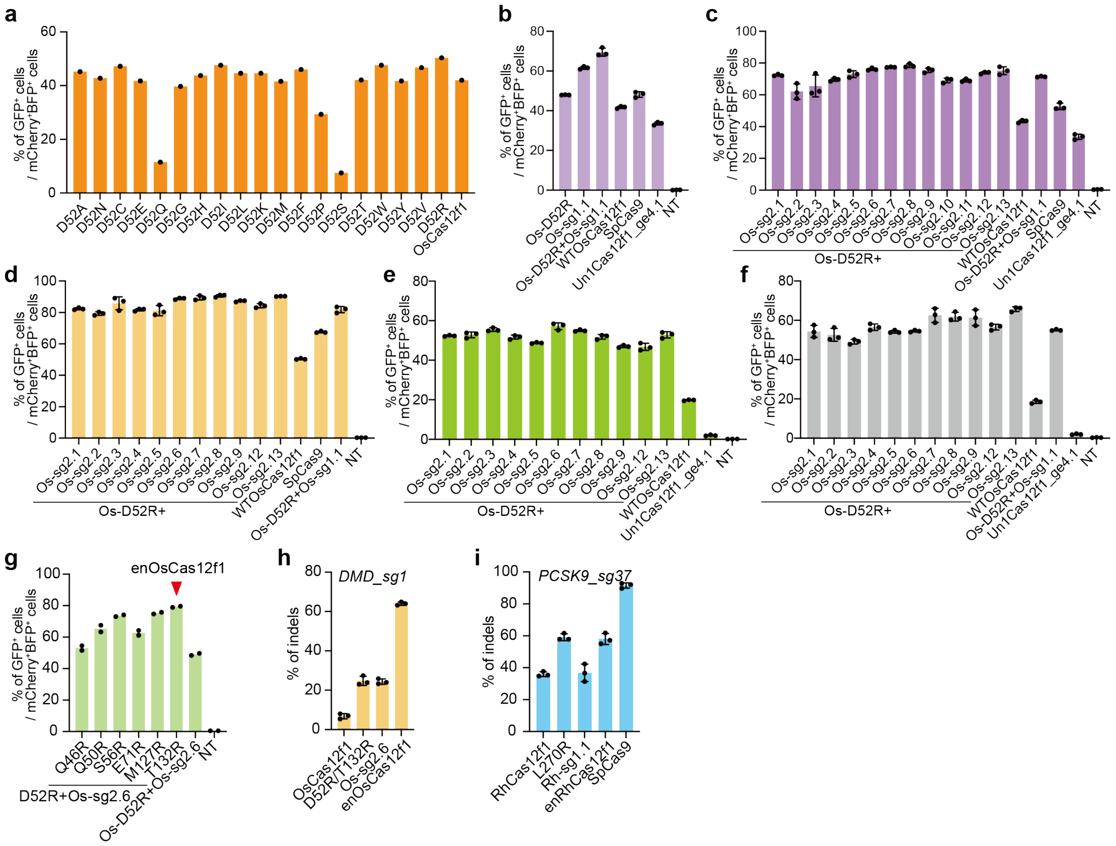

**Supplementary Figure 5. OsCas12f1-sgRNA and RhCas12f1-sgRNA complex formation.** Size-exclusion chromatography profiles of the OsCas12f1 (**a**) and RhCas12f1 (**b**) with or without its sgRNA. UV absorbance at 280 nm and 260 nm were shown in solid and dashed lines, respectively. The molecular weights of standard marker proteins are indicated. Both OsCas12f1 and RhCas12f1 could form dimer with its sgRNA, which was respectively indicated by pink and blue arrow, at least in the test condition. The peak fractions were analyzed by SDS-PAGE shown blow. These experiments were repeated three times showing similar results.



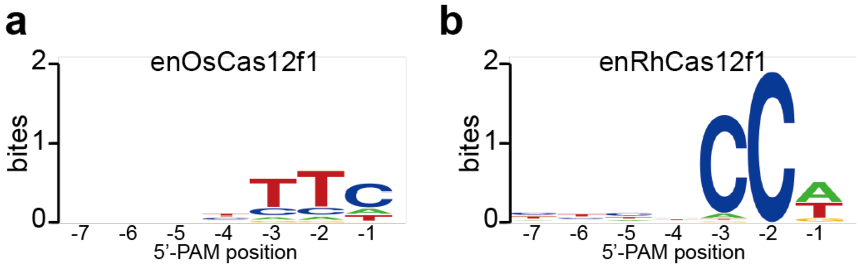

**Supplementary Figure 6. Protein alignment of OsCas12f1 and RhCas12f1 with Un1Cas12f1. a**, Predicted domain architecture of OsCas12f1 and RhCas12f1 by alignment with Un1Cas12f1. ZF, zinc finger domain; REC, recognition domain; WED, wedge domain; RuvC, RuvC nuclease domain; TNB, target nucleic acid-binding domain. The maximum-likelihood region of OsCas12f1 and RhCas12f1 for RNA and/or DNA recognition (region1~3) are indicated. **b**, protein alignment of OsCas12f1, RhCas12f1 and Un1Cas12f1.



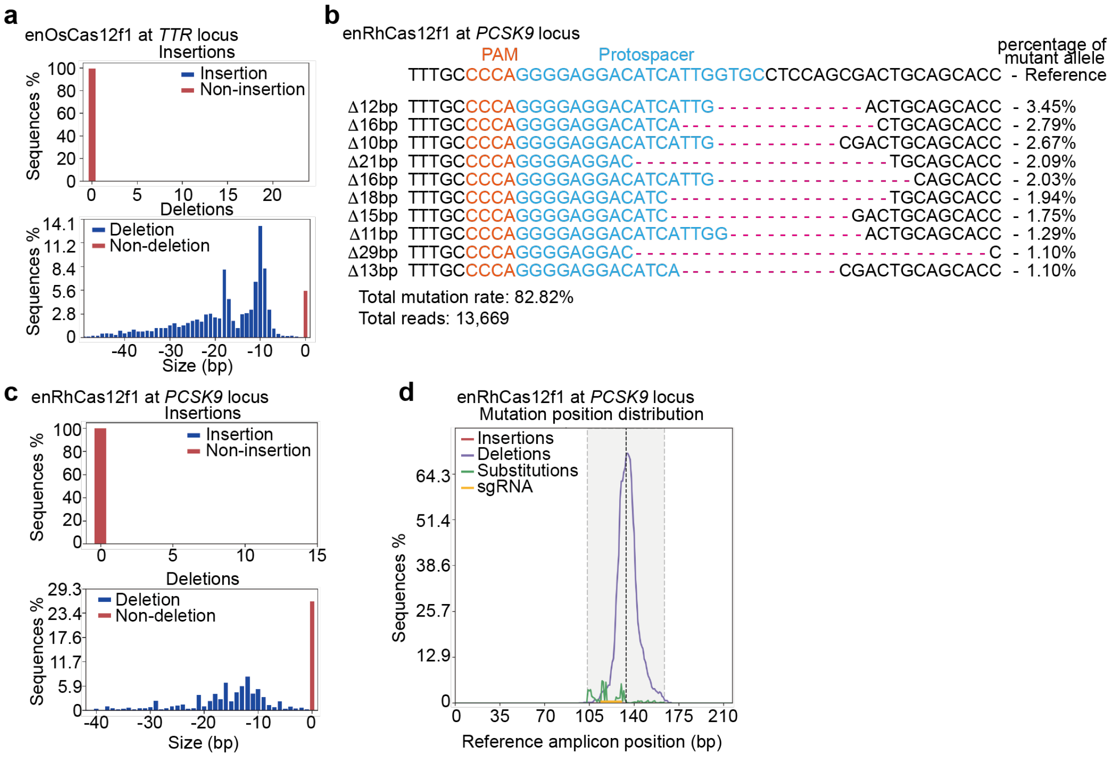

**Supplementary Figure 7. Mutagenesis strategy for screening of enOsCas12f1 and enRhCas12f1.** enOsCas12f1 was shown as example. Region1~3 of OsCas12f1 were divided into 11 segments containing 17 amino acid residues in length. Eleven backbone mutants for OsCas12f1 were generated by replacing the above mentioned segments with BpiI recognition sequence by PCR and Gibson assembly method using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs). The specific mutation is then introduced by incorporation of annealed oligos containing mutation by BpiI digestion and T4 DNA ligase ligation.



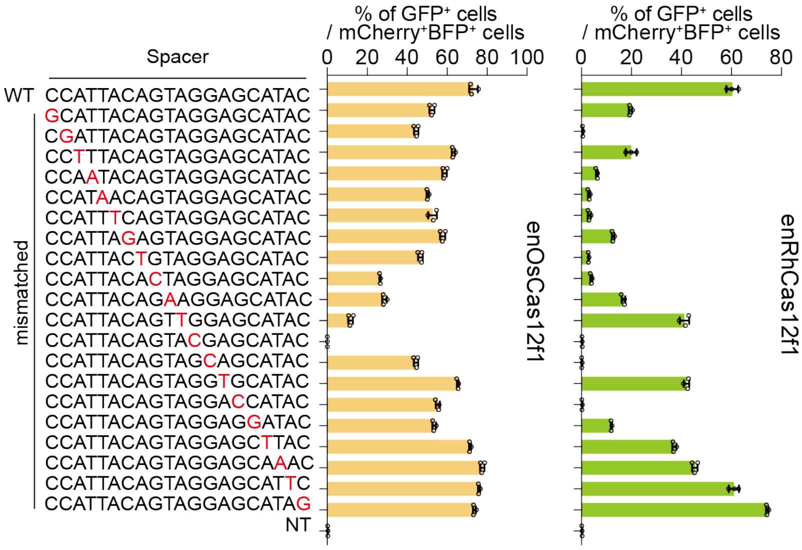
**Supplementary Figure 8. Engineering and optimization for enCas12f1. a**, Saturation mutagenesis analysis at D52 site of OsCas12f1. Target: TTTC–CCATTACAGTAGGAGCATAC. Data shown here represent values of results from n=1 experiment. **b**, Evaluating the efficiency of OsCas12f1-D52R+Os-sg1.1 combination variant. Target: TTTC–CCATTACAGTAGGAGCATAC. Values and error bars represent mean and s.d. (n=3). **c**, Increased EGFP activation efficiency by Os-sg2.6. Target: TTTC–CCATTACAGTAGGAGCATAC. Values and error bars represent mean and s.d. (n=3). **d~f**, Validation of OsCas12f1-D52R+Os-sg2.6 by reporter containing different endogenous protospacer sequences. Target-d: CTTC-ttgtgctggacggtgacgta; target-e: TTTC-attggctttgatttccctag; target-f: TTTC-cctagggtccagcttcaaat. Values and error bars represent mean and s.d. (n=3). **g**, Increased EGFP activation efficiency of enOsCas12f variant by combining Cas12f1 protein mutant and sgRNA variant. The best combination is represented as enOsCas12f1. Reporter containing *DMD_2* protospacer sequence was used. Values represent means of results from n=2 biologically independent experiments. **h**, Efficiency of enOsCas12f1 at endogenous *DMD* locus in HEK293T cells. Values and error bars represent mean and s.d. (n=3). **i**, Efficiency enRhCas12f1 at endogenous *PCSK9* locus in HEK293T cells. Values and error bars represent mean and s.d. (n=3). Source data are provided as a Source Data file.



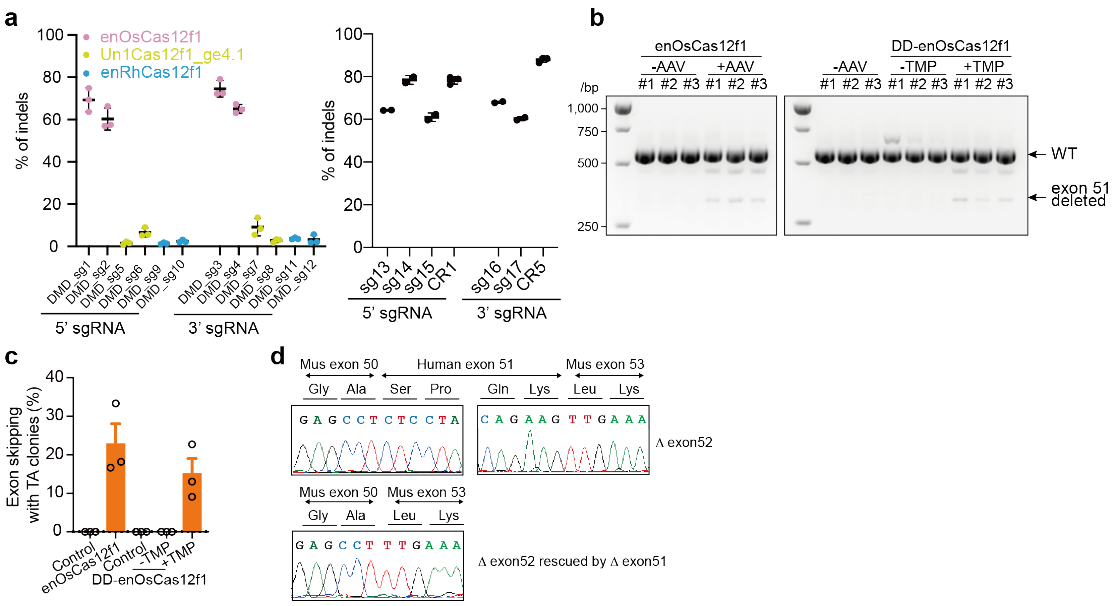

**Supplementary Figure 9. In vitro PAM preferences of enOsCas12f1 and enRhCas12f1.** WebLogos of the in vitro PAM sequences for enOsCas12f1 (**a**) and enRhCas12f1 (**b**).



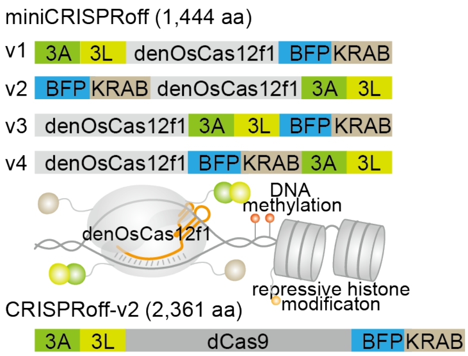

**Supplementary Figure 10. enCas12f1-mediated gene disruption in human cells. a,** Size and position distribution of indels induced by enOsCas12f1. **b,** The top 10 mutant alleles by enRhCas12f1 mediated disruption at PCSK9 locus. **c-d,** Size and position distribution of indels induced by enRhCas12f1.



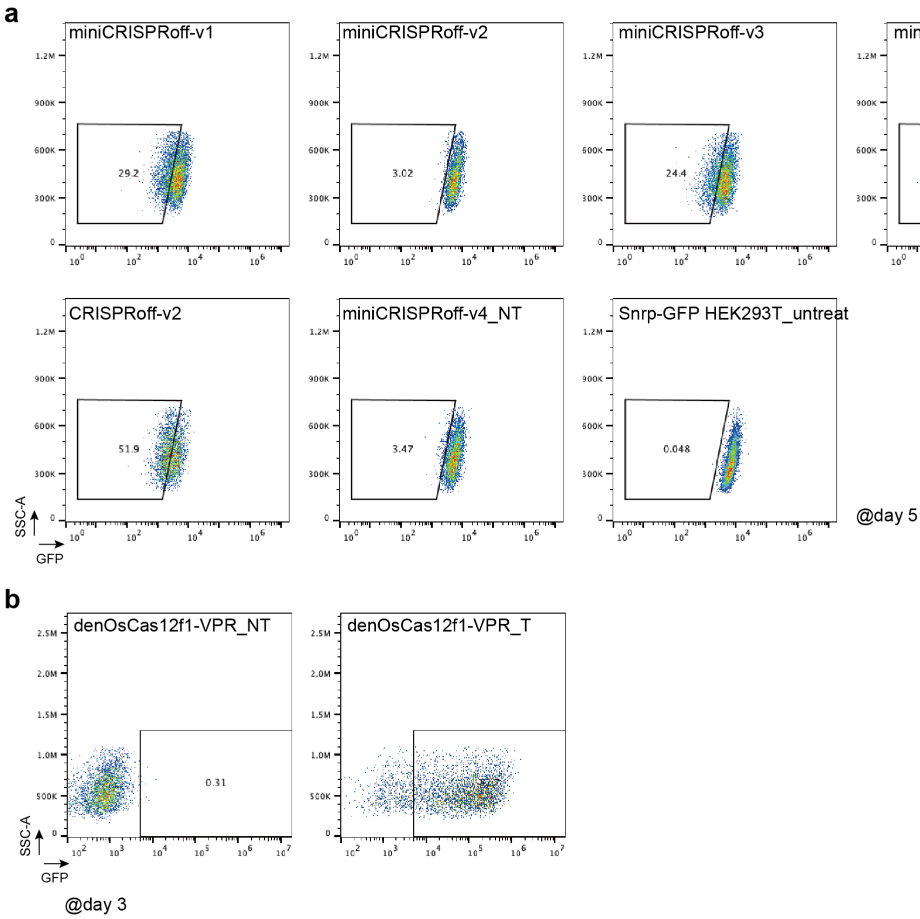

**Supplementary Figure 11. Mismatch tolerance of enOsCas12f1 and enRhCas12f1.** Impact of 1bp mismatched sgRNA on the GFP activation efficiencies of enOsCas12f1 and enRhCas12f1. The 5'-TTTC PAM- and 5'-CCCA PAM- target sequence was used for OsCas12f1 and RhCas12f1, respectively. Values and error bars represent mean and s.d. (n=3). Source data are provided as a Source Data file.



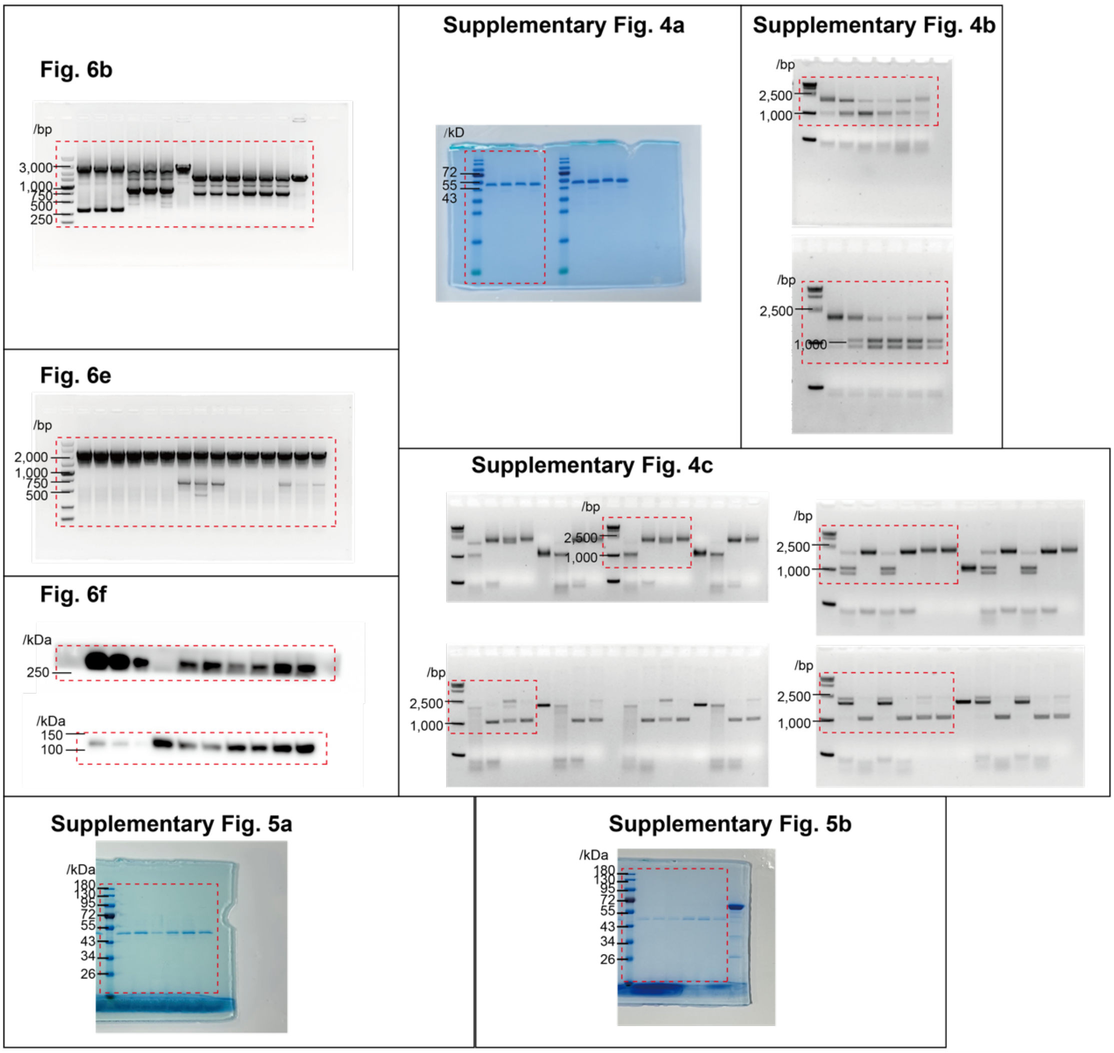

**Supplementary Figure 12. Deletion of DMD exon 51 by DD-enOsCas12f1. a,** Indel frequencies induced by enOsCas12f1 and SpCas9 at the 5' and 3' region flanking DMD exon 51 in HEK293T cells. Target sites for SpCas9 from Ousterout *et al*[1]. Values and error bars represent mean and s.d. (n>=2). **b**, RT-PCR across DMD exon 51 sowed a smaller band with exon 51 deletion in treated muscle. **c**, Percentage of exon 51 deletion calculated by TA cloning of RT-PCR product. Values and error bars represent mean and s.d. (n=3). **d**, Representative chromatogram of the expected deletion PCR product. Source data are provided as a Source Data file.




**Supplementary Figure 13. Cloning strategy for enOsCas12f1-mediated epigenome editing (miniCRISPRoff).** Scheme of denOsCas12f1 fusing with epigenetic editors (miniCRISPRoff) for gene silencing. CRISPRoff-v2 design from Nuñez *et al*[2].




**Supplementary Figure 14. Gating strategy used for assessing the efficiency of miniCRISPRoff and denOsCas12f1-VPR. a,** GFP repression efficiency of miniCRISPRoffs and CRISPRoff-v2 at 5 days post transfection in Snrp-GFP HEK293T cells. **b**, GFP activation efficiency induced by denOsCas12f1-VPR at 3 days post transfection in TRE3G-GFP HEK293T cells.




**Supplementary Figure 15. Uncropped images.** The red rectangles indicate the cropping location.

**Supplementary Table 1. Enhanced OsCas12f1 and RhCas12f1 screen data**

| enOsCas12f1 viriants | | |
|---|---|---|
| **Mutant (amino acids position)** | **% of GFP+ cell** | **mutant / WT** |
| Os-aa44 | 1.13 | 0.03 |
| Os-aa45 | 18.6 | 0.48 |
| Os-aa46 | 44.2 | 1.14 |
| Os-aa47 | 35.1 | 0.91 |
| Os-aa48 | 34.7 | 0.90 |
| Os-aa49 | 47 | 1.21 |
| Os-aa50 | 44.2 | 1.14 |
| Os-aa51 | 32.1 | 0.83 |
| Os-aa52 | 52.8 | 1.36 |
| Os-aa53 | 51.7 | 1.34 |
| Os-aa54 | 45.5 | 1.18 |
| Os-aa55 | 37.1 | 0.96 |
| Os-aa56 | 50.2 | 1.30 |
| Os-aa57 | 39.8 | 1.03 |
| Os-aa62 | 41.6 | 1.07 |
| Os-aa63 | 44.3 | 1.14 |
| Os-aa64 | 37.5 | 0.97 |
| Os-aa65 | 37.7 | 0.97 |
| Os-aa66 | 42.8 | 1.11 |
| Os-aa67 | 36.7 | 0.95 |
| Os-aa68 | 34.6 | 0.89 |
| Os-aa70 | 42.2 | 1.09 |
| Os-aa71 | 44.7 | 1.16 |
| Os-aa72 | 40.6 | 1.05 |
| Os-aa73 | 38.3 | 0.99 |
| Os-aa74 | 11 | 0.28 |
| Os-aa77 | 24.7 | 0.64 |
| Os-aa78 | 6.18 | 0.16 |
| Os-aa79 | 1.4 | 0.04 |
| Os-aa81 | 0.82 | 0.02 |
| Os-aa82 | 2.97 | 0.08 |
| Os-aa83 | 34.9 | 0.90 |
| Os-aa84 | 36.4 | 0.94 |
| Os-aa85 | 7.67 | 0.20 |
| Os-aa118 | 31.9 | 0.82 |
| Os-aa119 | 46.3 | 1.20 |
| Os-aa120 | 45.4 | 1.17 |
| Os-aa121 | 21.6 | 0.56 |
| Os-aa122 | 9.8 | 0.25 |
| Os-aa123 | 9.08 | 0.23 |
| Os-aa124 | 34.7 | 0.90 |
| Os-aa125 | 2.12 | 0.05 |
| Os-aa127 | 49.7 | 1.28 |
| Os-aa128 | 33.3 | 0.86 |
| Os-aa129 | 16.6 | 0.43 |
| Os-aa130 | 19.4 | 0.50 |
| Os-aa131 | 1.47 | 0.04 |
| Os-aa132 | 48.4 | 1.25 |
| Os-aa133 | 1.26 | 0.03 |
| Os-aa134 | 14.8 | 0.38 |
| Os-aa136 | 40.3 | 1.04 |
| Os-aa137 | 28.9 | 0.75 |
| Os-aa138 | 2.69 | 0.07 |
| Os-aa140 | 35.5 | 0.92 |
| Os-aa141 | 46.6 | 1.20 |
| Os-aa142 | 38.5 | 0.99 |

| | | |
|---|---|---|
| Os-aa143 | 37.4 | 0.97 |
| Os-aa144 | 44.2 | 1.14 |
| Os-aa146 | 41.3 | 1.07 |
| Os-aa147 | 41.4 | 1.07 |
| Os-aa148 | 42.1 | 1.09 |
| Os-aa149 | 0.95 | 0.02 |
| Os-aa150 | 41.3 | 1.07 |
| Os-aa151 | 2.48 | 0.06 |
| Os-aa152 | 27.1 | 0.70 |
| Os-aa153 | 4.88 | 0.13 |
| Os-aa154 | 26.7 | 0.69 |
| Os-aa155 | 28.3 | 0.73 |
| Os-aa156 | 25.6 | 0.66 |
| Os-aa158 | 37.3 | 0.96 |
| Os-aa261 | 17.7 | 0.46 |
| Os-aa262 | 36.5 | 0.94 |
| Os-aa264 | 44.2 | 1.14 |
| Os-aa266 | 33.3 | 0.86 |
| Os-aa267 | 30.5 | 0.79 |
| Os-aa268 | 2.55 | 0.07 |
| Os-aa270 | 4.27 | 0.11 |
| Os-aa271 | 3.74 | 0.10 |
| Os-aa272 | 38.6 | 1.00 |
| Os-aa274 | 0.66 | 0.02 |
| Os-aa275 | 4.16 | 0.11 |
| Os-aa276 | 37.4 | 0.97 |
| Os-aa277 | 0.75 | 0.02 |
| Os-aa279 | 1.52 | 0.04 |
| Os-aa280 | 20 | 0.52 |
| Os-aa282 | 1.13 | 0.03 |
| Os-aa283 | 30.8 | 0.80 |
| Os-aa285 | 30.7 | 0.79 |
| Os-aa287 | 0.32 | 0.01 |
| Os-aa288 | 33.5 | 0.87 |
| Os-aa289 | 30.9 | 0.80 |
| Os-aa290 | 18.1 | 0.47 |
| Os-aa291 | 1.02 | 0.03 |
| Os-aa292 | 40.6 | 1.05 |
| Os-aa293 | 39.9 | 1.03 |
| Os-aa295 | 24.8 | 0.64 |
| Os-aa297 | 28.4 | 0.73 |
| Os-aa298 | 33.3 | 0.86 |
| Os-aa300 | 30.7 | 0.79 |
| Os-aa302 | 19.5 | 0.50 |
| Os-aa303 | 28.4 | 0.73 |
| Os-aa304 | 34.6 | 0.89 |
| Os-aa305 | 4.9 | 0.13 |
| Os-aa308 | 3.65 | 0.09 |
| Os-aa309 | 26 | 0.67 |
| Os-aa311 | 42.5 | 1.10 |
| Os-aa312 | 1.79 | 0.05 |
| Os-aa313 | 40.9 | 1.06 |
| Os-aa314 | 43 | 1.11 |
| Os-aa315 | 41.9 | 1.08 |
| SpCas9 | 53.9 | |
| Un1Cas12f1_ge4.1 | 36.5 | |
| WTOsCas12f1 | 38.7 | |
| WTOsCas12f1_NT | 0.55 | |

| **enRhCas12f1 viriants** | | |
|---|---|---|
| **Mutant (amino acids position)** | **% of GFP+ cell** | **mutant / WT** |

| | | |
|---|---|---|
| Rh-aa34 | 0.15 | 0.00 |
| Rh-aa35 | 0.12 | 0.00 |
| Rh-aa37 | 0.28 | 0.00 |
| Rh-aa38 | 0.083 | 0.00 |
| Rh-aa39 | 0.042 | 0.00 |
| Rh-aa40 | 66.2 | 1.08 |
| Rh-aa41 | 0.044 | 0.00 |
| Rh-aa42 | 0.047 | 0.00 |
| Rh-aa43 | 20.7 | 0.34 |
| Rh-aa44 | 70.5 | 1.15 |
| Rh-aa45 | 58.2 | 0.95 |
| Rh-aa46 | 0.02 | 0.00 |
| Rh-aa47 | 71.8 | 1.17 |
| Rh-aa49 | 65.3 | 1.06 |
| Rh-aa51 | 64.3 | 1.05 |
| Rh-aa52 | 71.3 | 1.16 |
| Rh-aa53 | 54.2 | 0.88 |
| Rh-aa55 | 67.1 | 1.09 |
| Rh-aa56 | 73 | 1.19 |
| Rh-aa59 | 66.3 | 1.08 |
| Rh-aa60 | 4.22 | 0.07 |
| Rh-aa61 | 62.2 | 1.01 |
| Rh-aa62 | 0.023 | 0.00 |
| Rh-aa63 | 69.6 | 1.13 |
| Rh-aa64 | 60.2 | 0.98 |
| Rh-aa65 | 72.4 | 1.18 |
| Rh-aa66 | 29.5 | 0.48 |
| Rh-aa67 | 0.091 | 0.00 |
| Rh-aa68 | 69.3 | 1.13 |
| Rh-aa69 | 0.12 | 0.00 |
| Rh-aa70 | 22.4 | 0.36 |
| Rh-aa71 | 69 | 1.12 |
| Rh-aa72 | 0.35 | 0.01 |
| Rh-aa73 | 11.6 | 0.19 |
| Rh-aa101 | 0.78 | 0.01 |
| Rh-aa102 | 55.9 | 0.91 |
| Rh-aa104 | 29.2 | 0.47 |
| Rh-aa105 | 0.024 | 0.00 |
| Rh-aa107 | 0.16 | 0.00 |
| Rh-aa108 | 0.055 | 0.00 |
| Rh-aa111 | 69.5 | 1.13 |
| Rh-aa112 | 62.6 | 1.02 |
| Rh-aa113 | 28.5 | 0.46 |
| Rh-aa114 | 0.15 | 0.00 |
| Rh-aa115 | 27.4 | 0.45 |
| Rh-aa116 | 0.025 | 0.00 |
| Rh-aa118 | 49 | 0.80 |
| Rh-aa120 | 25 | 0.41 |
| Rh-aa121 | 0.046 | 0.00 |
| Rh-aa123 | 0.1 | 0.00 |
| Rh-aa124 | 69.3 | 1.13 |
| Rh-aa125 | 73.3 | 1.19 |
| Rh-aa126 | 69.5 | 1.13 |
| Rh-aa127 | 67 | 1.09 |
| Rh-aa128 | 66.9 | 1.09 |
| Rh-aa129 | 72.2 | 1.17 |
| Rh-aa130 | 76.1 | 1.24 |
| Rh-aa131 | 77.2 | 1.26 |
| Rh-aa133 | 27.2 | 0.44 |
| Rh-aa135 | 0 | 0.00 |
| Rh-aa137 | 0.087 | 0.00 |

| | | |
|---|---|---|
| Rh-aa138 | 55.6 | 0.90 |
| Rh-aa139 | 72.1 | 1.17 |
| Rh-aa140 | 69.6 | 1.13 |
| Rh-aa141 | 66 | 1.07 |
| Rh-aa142 | 51.8 | 0.84 |
| Rh-aa143 | 0 | 0.00 |
| Rh-aa144 | 56 | 0.91 |
| Rh-aa243 | 3.84 | 0.06 |
| Rh-aa244 | 6 | 0.10 |
| Rh-aa246 | 57.2 | 0.93 |
| Rh-aa249 | 70.1 | 1.14 |
| Rh-aa251 | 49.8 | 0.81 |
| Rh-aa252 | 41.1 | 0.67 |
| Rh-aa254 | 75.3 | 1.22 |
| Rh-aa255 | 49.3 | 0.80 |
| Rh-aa256 | 69.1 | 1.12 |
| Rh-aa257 | 67.6 | 1.10 |
| Rh-aa258 | 45.1 | 0.73 |
| Rh-aa260 | 39.7 | 0.65 |
| Rh-aa261 | 45.2 | 0.73 |
| Rh-aa263 | 34 | 0.55 |
| Rh-aa264 | 75 | 1.22 |
| Rh-aa265 | 70.8 | 1.15 |
| Rh-aa266 | 70.2 | 1.14 |
| Rh-aa268 | 27.4 | 0.45 |
| Rh-aa269 | 66.1 | 1.07 |
| Rh-aa270 | 73.8 | 1.20 |
| Rh-aa271 | 51.3 | 0.83 |
| Rh-aa272 | 71.7 | 1.17 |
| Rh-aa273 | 75.3 | 1.22 |
| Rh-aa274 | 13.8 | 0.22 |
| Rh-aa275 | 50.8 | 0.83 |
| Rh-aa276 | 63.2 | 1.03 |
| Rh-aa278 | 0.21 | 0.00 |
| Rh-aa280 | 72.3 | 1.18 |
| Rh-aa281 | 9.5 | 0.15 |
| Rh-aa282 | 58.9 | 0.96 |
| Rh-aa283 | 79.7 | 1.30 |
| Rh-aa284 | 42.4 | 0.69 |
| Rh-aa285 | 25.8 | 0.42 |
| Rh-aa286 | 10.5 | 0.17 |
| SpCas9 | 49.7 | |
| Un1Cas12f1_ge4.1 | 36.1 | |
| WTRhCas12f1 | 61.5 | |
| WTRhCas12f1_NT | 0.62 | |

| Supplementary Table 2. Sequence of target loci for indel frequency. | | | | |
|---|---|---|---|---|
| Genomic loci | sgRNA | PAM | protospacer sequences | Figure |
| PAM preference analysis at endogenous loci for enOsCas12f1 & Un1Cas12f1_ge4.1 | | ATTA | TAGGCATGAGCCGCTGCACC | Fig. 3c and 3e |
| | | ATTA | TGCGGATCAAACCTCACCAA | Fig. 3c and 3e |
| | | ATTT | CACATCTGAGCTGGCTTTCC | Fig. 3c and 3e |
| | | ATTT | TAAGGGAGAAAATAGGTCCC | Fig. 3c and 3e |
| | | ATTT | GTTGTGCTGTAGGAAGCTCA | Fig. 3c and 3e |
| | | ATTC | CTCCTCAGTTGTGAGCCCAT | Fig. 3c and 3e |
| | | ATTC | ACAGCCAACGACTCCGGCCC | Fig. 3c and 3e |
| | | ATTC | tacatcttcacccaccaggg | Fig. 3c and 3e |
| | | ATTG | TGTGGACAGCATGTATATGT | Fig. 3c and 3e |
| | | ATTG | CAGCAGCCCCCGCATCGCAT | Fig. 3c and 3e |
| | | TTTA | CCCTGGCTACCTCCCCTACC | Fig. 3c and 3e |
| | | TTTA | GAGGGAGACACAAGTTGATA | Fig. 3c and 3e |
| | | TTTT | CTGTGTCAGTTTGTGCCACC | Fig. 3c and 3e |
| | | TTTT | AAGGGAGAAAATAGGTCCCC | Fig. 3c and 3e |
| | | TTTT | CGTCCAACTTCTGGGCTGTT | Fig. 3c and 3e |
| | | TTTC | CTCTGCCCCAGGCTGCAGCT | Fig. 3c and 3e |
| | | TTTC | TGCCTCCAGACACACTGCTA | Fig. 3c and 3e |
| | | TTTC | GTCCAACTTCTGGGCTGTTC | Fig. 3c and 3e |
| | | TTTG | TACTTTGTCCTCCGGTTCTG | Fig. 3c and 3e |
| | | TTTG | ACTTTAGTGACTAGCCGCCA | Fig. 3c and 3e |
| | | TTTG | GGTTCTCTCTATAGCCATTG | Fig. 3c and 3e |
| | | CTTA | CTGATCTGGACAAAAGCAAA | Fig. 3c and 3e |
| | | CTTA | CTGGAAGGCACTTGGCATCT | Fig. 3c and 3e |
| | | CTTA | CCTTGGCATGGTGGAGGTAG | Fig. 3c and 3e |
| | | CTTT | CCTCTGCCCCAGGCTGCAGC | Fig. 3c and 3e |
| | | CTTT | CTGAACACATGCACGGCCAC | Fig. 3c and 3e |
| | | CTTT | CTGCTGTCTTGGGTGCATTG | Fig. 3c and 3e |
| | | CTTC | CAGTAAGATTTGGTGTCTAT | Fig. 3c and 3e |
| | | CTTC | TCTCATAGGTGGTATTCACA | Fig. 3c and 3e |
| | | CTTC | atggtcctaggtggcttcac | Fig. 3c and 3e |
| | | CTTG | TGGGTGCCAAGGTCCTCCAC | Fig. 3c and 3e |
| | | CTTG | GATTCACCGGTGCCCTGGGT | Fig. 3c and 3e |
| | | CTTG | GGTGCATTGGAGCCTTGCCT | Fig. 3c and 3e |
| | | GTTA | AATAGATCAGAGAGGCCAGG | Fig. 3c and 3e |
| | | GTTA | GTGACCCAGCCAGCCATACC | Fig. 3c and 3e |
| | | GTTT | GTGCCACCACCATACCGCCA | Fig. 3c and 3e |
| | | GTTT | GATCCGCATAATCTGGAAAG | Fig. 3c and 3e |
| | | GTTC | TAGATGCTGTCCGAGGCAGT | Fig. 3c and 3e |
| | | GTTC | AGAAAGGCTGCTGATGACAC | Fig. 3c and 3e |
| | | GTTC | cggaactgcatgctcaccac | Fig. 3c and 3e |
| | | GTTG | GGCTGACCTCGTGGCCTCAG | Fig. 3c and 3e |
| | | GTTG | CCAAAGAACCCTCCCACAGG | Fig. 3c and 3e |
| | | GTTG | TGCTGTAGGAAGCTCATCTC | Fig. 3c and 3e |
| PAM preference analysis at endogenous loci for enRhCas12f1 | | ACCA | ATGATGTCCTCCCCTGGGGC | Fig. 3d and 3f |
| | | ACCA | AATCTTACTGGAAGGCACTT | Fig. 3d and 3f |
| | | ACCA | CGGCTCCTCCGAAGCGAGAA | Fig. 3d and 3f |
| | | ACCT | CTTTGCCCCAGGGGAGGACA | Fig. 3d and 3f |
| | | ACCT | CTGCATGCTCATGGAATGGG | Fig. 3d and 3f |
| | | ACCT | TGGCATGGTGGAGGTAGAGC | Fig. 3d and 3f |
| | | ACCC | TGGGGACTTTGGGGACCAAC | Fig. 3d and 3f |
| | | ACCC | TCGAAGGTCTGTATACTCAC | Fig. 3d and 3f |
| | | ACCC | TGGTGGACATCTTCCAGGAG | Fig. 3d and 3f |
| | | ACCG | GCTGGTCTTGGGCATTGGTG | Fig. 3d and 3f |
| | | ACCG | GTGAATCCAAGTGTCCTCTG | Fig. 3d and 3f |
| | | ACCG | CTTACCTTGGCATGGTGGAG | Fig. 3d and 3f |
| | | TCCA | GACTTTCACACCTTATAGGA | Fig. 3d and 3f |
| | | TCCA | CTTTGTATATCCCTTCTACA | Fig. 3d and 3f |
| | | TCCA | GCAAGGCAGAGGAGGAGCAG | Fig. 3d and 3f |
| | | TCCT | CCCCTGGGGCAAAGAGGTCC | Fig. 3d and 3f |
| | | TCCT | CCTCAGTTGTGAGCCCATGC | Fig. 3d and 3f |
| | | TCCT | CCGAAGCGAGAACAGCCCAG | Fig. 3d and 3f |
| | | TCCC | CTGGGGCAAAGAGGTCCACA | Fig. 3d and 3f |
| | | TCCC | TTCTACAAATTCCTCCTCAG | Fig. 3d and 3f |
| | | TCCC | GGCCCGAGCTAGCACTTCTC | Fig. 3d and 3f |
| | | TCCG | TGGAGGTTGCCTGGCACCTA | Fig. 3d and 3f |
| | | TCCG | AGGCAGTCCTGCCATCAATG | Fig. 3d and 3f |
| | | TCCG | AAGCGAGAACAGCCCAGAAG | Fig. 3d and 3f |
| | | CCCA | GGGGAGGACATCATTGGTGC | Fig. 3d and 3f |
| | | CCCA | CCTGCATGGTGATGTTGGAC | Fig. 3d and 3f |
| | | CCCA | AAGATGCCCACCTGCATGGT | Fig. 3d and 3f |
| | | CCCT | GGGGACTTTGGGGACCAACT | Fig. 3d and 3f |

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| | | CCCT | TCTACAAATTCCTCCTCAGT | Fig. 3d and 3f |
| | | CCCT | GGTGGACATCTTCCAGGAGT | Fig. 3d and 3f |
| | | CCCC | TGGGGCAAAGAGGTCCACAC | Fig. 3d and 3f |
| | | CCCC | GCATCGCATCAGGGGCACAC | Fig. 3d and 3f |
| | | CCCG | CTGGTCCTCAGGGAACCAGG | Fig. 3d and 3f |
| | | CCCG | TTTGCCCCTCACTTGGTAGA | Fig. 3d and 3f |
| | | CCCG | CATCGCATCAGGGGCACACA | Fig. 3d and 3f |
| | | GCCA | CCAGGTTGGGGGTCAGTACC | Fig. 3d and 3f |
| | | GCCA | AGTGCCTTCCAGTAAGATTT | Fig. 3d and 3f |
| | | GCCA | TCCAATCGAGACCCTGGTGG | Fig. 3d and 3f |
| | | GCCT | CAACTCGGCCAGGGTGAGCT | Fig. 3d and 3f |
| | | GCCT | TCCAGTAAGATTTGGTGTCT | Fig. 3d and 3f |
| | | GCCC | CAGGCTGCAGCTCCCACTGG | Fig. 3d and 3f |
| | | GCCC | ATGCAGCTCTCCAGACTCAC | Fig. 3d and 3f |
| | | GCCC | TCCTCCTTCTGCCATGGGTG | Fig. 3d and 3f |
| | | GCCG | CCTGTGCTGAGGCCACGAGG | Fig. 3d and 3f |
| | | GCCG | TGCATGTGTTCAGAAAGGCT | Fig. 3d and 3f |
| PCSK9 | PCSK9_sg1 | TTTC | CCGGTGGTCACTCTGTATGC | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg2 | TTTC | CGTCTTTGACTCTAAGGCCC | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg3 | TTTC | CTCTGCCCCAGGCTGCAGCT | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg4 | TTTC | CAGGTCATCACAGTTGGGGC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg5 | TTTC | TCCAGGAGTGGGAAGCGGCG | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg6 | TTTC | CTCGGGCTCTGGCAGGTGAC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg7 | TTTG | ACTCTAAGGCCCAAGGGGGC | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg8 | TTTG | GGGGTGAGGGTGTCTACGCC | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg9 | TTTG | CATTCCAGACCTGGGGCATG | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | PCSK9_sg10 | TTTA | TTCGGAAAAGCCAGCTGGTC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg11 | TTTG | CCCAGAGCATCCCGTGGAAC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg12 | TTTG | TTCCTCCCAGGCCTGGAGTT | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg13 | TTTG | GGGACCAACTTTGGCCGCTG | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg14 | TTTG | GCCGCTGTGTGGACCTCTTT | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg15 | TTTG | CCCCAGGGGAGGACATCATT | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg16 | TTTG | TGTCACAGAGTGGGACATCA | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg17 | TTTG | GCAGAGAAGTGGATCAGTCT | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg18 | TTTG | CAGGTTGGCAGCTGTTTTGC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg19 | TTTG | CAGGACTGTATGGTCAGCAC | Fig. 4a, 4b, 4c and 4d |
| | PCSK9_sg20 | CCCA | TCCCTACACCCGCACCTTGG | Fig. 4a and 4b |
| | PCSK9_sg21 | CCCA | CCTCTCGCAGTCAGAGCGCA | Fig. 4a and 4b |
| | PCSK9_sg22 | CCCA | GGCTGCCCGCCGGGGATACC | Fig. 4a and 4b |
| | PCSK9_sg23 | CCCA | AAAAGGGTGGCTCACCAGCT | Fig. 4a and 4b |
| | PCSK9_sg24 | CCCA | TGTCGACTACATCGAGGAGG | Fig. 4a and 4b |
| | PCSK9_sg25 | CCCA | GAGCATCCCGTGGAACCTGG | Fig. 4a and 4b |
| | PCSK9_sg26 | CCCA | CAAATGTCGCCTTGGAAAGA | Fig. 4a and 4b |
| | PCSK9_sg27 | CCCA | TCAGACGGCCGTGCTTACCT | Fig. 4a and 4b |
| | PCSK9_sg28 | CCCA | CCTGGCAGGGGTGGTCAGCG | Fig. 4a and 4b |
| | PCSK9_sg29 | CCCA | GGCCTGGAGTTTATTCGGAA | Fig. 4a and 4b |
| | PCSK9_sg30 | CCCA | CCCGCCAGGGGCAGCAGCAC | Fig. 4a and 4b |
| | PCSK9_sg31 | CCCA | GCCCTCGCCAGGCGCTGGCA | Fig. 4a and 4b |
| | PCSK9_sg32 | CCCA | GCACCTACCTCGGGAGCTGA | Fig. 4a and 4b |
| | PCSK9_sg33 | CCCA | CCTCCTCACCTTTCCAGGTC | Fig. 4a and 4b |
| | PCSK9_sg34 | CCCA | AGACCAGCCGGTGACCCTGG | Fig. 4a and 4b |
| | PCSK9_sg35 | CCCA | GGGTCACCGGCTGGTCTTGG | Fig. 4a and 4b |
| | PCSK9_sg36 | CCCA | AAGTCCCCAGGGTCACCGGC | Fig. 4a and 4b |
| | PCSK9_sg37 | CCCA | GGGGAGGACATCATTGGTGC | Fig. 4a and 4b |
| | PCSK9_sg38 | CCCA | CTCTGTGACACAAAGCAGGT | Fig. 4a and 4b |
| | PCSK9_sg39 | CCCA | ACCTGGTGGCCGCCCTGCCC | Fig. 4a and 4b |
| | PCSK9_sg40 | CCCA | TGGGTGCTGGGGGGCAGGGC | Fig. 4a and 4b |
| | PCSK9_sg41 | CCCA | CCCTGCCATCCTGCTTACCT | Fig. 4a and 4b |
| | PCSK9_sg42 | CCCA | GGCCCTTTTTGCAGGTTGGC | Fig. 4a and 4b |
| | PCSK9_sg43 | CCCA | GATGAGGAGCTGCTGAGCTG | Fig. 4a and 4b |
| | PCSK9_sg44 | CCCA | CTCCTGGAGAAACTGGAGCA | Fig. 4a and 4b |
| | PCSK9_sg45 | CCCA | TTTCCGTCTTTGACTCTAAG | Fig. 4a and 4b |
| | PCSK9_sg46 | CCCA | AGGGGGCAAGCTGGTCTGCC | Fig. 4a and 4b |
| | PCSK9_sg47 | CCCA | CAACGCTTTTGGGGGTGAGG | Fig. 4a and 4b |
| | PCSK9_sg48 | CCCA | AAAGCGTTGTGGGCCCGGCA | Fig. 4a and 4b |
| | PCSK9_sg49 | CCCA | GGCCAACTGCAGCGTCCACA | Fig. 4a and 4b |
| | PCSK9_sg50 | CCCA | TGCTGGCCTCAGCTGGTGGA | Fig. 4a and 4b |
| | PCSK9_sg51 | CCCA | GCCTCCTACCTGTGAGGACG | Fig. 4a and 4b |
| | PCSK9_sg52 | CCCA | GGGCAAGCCCAGCCTCCTAC | Fig. 4a and 4b |
| | PCSK9_sg53 | CCCA | GGCTGCAGCTCCCACTGGGA | Fig. 4a and 4b |
| | PCSK9_sg54 | CCCA | CTGGGAGGTGGAGGACCTTG | Fig. 4a and 4b |
| | PCSK9_sg55 | CCCA | CAAGCCGCCTGTGCTGAGGC | Fig. 4a and 4b |
| | PCSK9_sg56 | CCCA | CGCACTGGTTGGGCTGACCT | Fig. 4a and 4b |

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| | PCSK9_sg57 | CCCA | GGTCTGGAATGCAAAGTCAA | Fig. 4a and 4b |
| | PCSK9_sg58 | CCCA | GGACGTGGGAGGTCCCAGGG | Fig. 4a and 4b |
| TTR | TTR_sg1 | TTTC | TGAACACATGCACGGCCACA | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | TTR_sg2 | TTTC | GCTCCAGATTTCTAATACCA | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | TTR_sg3 | TTTC | TGCCTCCAGACACACTGCTA | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | TTR_sg4 | TTTC | ACACCTTATAGGAAAACCAG | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg5 | CTTC | TCATCGTCTGCTCCTCCTCT | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg6 | GTTC | TAGATGCTGTCCGAGGCAGT | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg7 | GTTC | AGAAAGGCTGCTGATGACAC | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg8 | GTTC | TTTGGCAACTTACCCAGAGG | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg9 | ATTC | CTCCTCAGTTGTGAGCCCAT | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg10 | CTTC | TACAAATTCCTCCTCAGTTG | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg11 | CTTC | CAGTAAGATTTGGTGTCTAT | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg12 | CTTC | TCTCATAGGTGGTATTCACA | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg13 | ATTC | ACAGCCAACGACTCCGGCCC | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg14 | TTTG | TGGTATTAGAAATCTGGAGC | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | TTR_sg15 | TTTG | TTAACTTCTCACGTGTCTTC | Fig. 4a, 4b and Supplementary Fig. 2b |
| | TTR_sg16 | TTTG | ACCATCAGAGGACACTTGGA | Fig. 4a, 4b, 4c, 4d and Supplementary Fig. 2a |
| | TTR_sg17 | TTTG | GCAACTTACCCAGAGGCAAA | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg18 | TTTG | TAGAAGGGATATACAAAGTG | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg19 | TTTG | TATATCCCTTCTACAAATTC | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg20 | TTTG | GTGTCTATTTCCACTTTGTA | Fig. 4a, 4b, 4c and 4d |
| | TTR_sg21 | TCCA | GACTTTCACACCTTATAGGA | Fig. 4a, 4b and Supplementary Fig. 2e |
| | TTR_sg22 | TCCA | GACTCACTGGTTTTCCTATA | Fig. 4a, 4b and Supplementary Fig. 2e |
| | TTR_sg23 | TCCA | CTTTGTATATCCCTTCTACA | Fig. 4a, 4b and Supplementary Fig. 2e |
| | TTR_sg24 | TCCA | GTAAGATTTGGTGTCTATTT | Fig. 4a, 4b and Supplementary Fig. 2e |
| | TTR_sg25 | TCCA | GCAAGGCAGAGGAGGAGCAG | Fig. 4a and 4b |
| | TTR_sg26 | TCCA | AGTGTCCTCTGATGGTCAAA | Fig. 4a and 4b |
| | TTR_sg27 | CCCA | GGGCACCGGTGAATCCAAGT | Fig. 4a and 4b |
| | TTR_sg28 | CCCA | GGTGTCATCAGCAGCCTTTC | Fig. 4a and 4b |
| | TTR_sg29 | CCCA | GAGGCAAATGGCTCCCAGGT | Fig. 4a and 4b |
| | TTR_sg30 | CCCA | TGCAGCTCTCCAGACTCACT | Fig. 4a and 4b |
| | TTR_sg31 | TCCA | TGAGCATGCAGAGGTGAGTA | Supplementary Fig. 2e |
| VEGFA | VEGFA_sg2 | TTTC | GTCCAACTTCTGGGCTGTTC | Fig. 4a and 4b |
| | VEGFA_sg3 | TTTC | GGAGGCCCGACCGGGGCCGG | Fig. 4a and 4b |
| | VEGFA_sg4 | TTTC | TGCTGTCTTGGGTGCATTGG | Fig. 4a and 4b |
| | VEGFA_sg5 | TTTC | TGTCCTCAGTGGTCCCAGGC | Fig. 4a and 4b |
| | VEGFA_sg6 | TTTC | CAGATTATGCGGATCAAACC | Fig. 4a and 4b |
| | VEGFA_sg7 | TTTC | CAGAAAATCAGTTCGAGGAA | Fig. 4a and 4b |
| | VEGFA_sg8 | TTTC | CCTTTCCTCGAACTGATTTT | Fig. 4a and 4b |
| | VEGFA_sg9 | TTTC | GTTTTTGCCCCTTTCCCTTT | Fig. 4a and 4b |
| | VEGFA_sg10 | TTTC | TTGCGCTTTCGTTTTTGCCC | Fig. 4a and 4b |
| | VEGFA_sg11 | TTTC | CTTTTGCCTTTTTGCAGTCC | Fig. 4a and 4b |
| | VEGFA_sg12 | TTTC | TCCGCTCTGAGCAAGGCCCA | Fig. 4a and 4b |
| | VEGFA_sg13 | TTTG | TTGTGCTGTAGGAAGCTCAT | Fig. 4a and 4b |
| | VEGFA_sg14 | TTTG | CCCCTTTCCCTTTCCTCGAA | Fig. 4a and 4b |
| | VEGFA_sg15 | TTTG | CCTTTTTGCAGTCCCTGTGG | Fig. 4a and 4b |
| | VEGFA_sg16 | TTTG | CAGTCCCTGTGGGCCTTGCT | Fig. 4a and 4b |
| | VEGFA_sg17 | TTTG | TTTGTACAAGATCCGCAGAC | Fig. 4a and 4b |
| | VEGFA_sg18 | TTTG | TACAAGATCCGCAGACGTGT | Fig. 4a and 4b |
| | VEGFA_sg19 | TTTG | CAGGAACATTTACACGTCTG | Fig. 4a and 4b |
| | VEGFA_sg20 | CCCA | GCCCCAGCTACCACCTCCTC | Fig. 4a and 4b |
| | VEGFA_sg21 | CCCA | GCTACCACCTCCTCCCCGGC | Fig. 4a and 4b |
| | VEGFA_sg22 | CCCA | GAAGTTGGACGAAAAGTTTC | Fig. 4a and 4b |
| | VEGFA_sg23 | CCCA | GGCCCTGGCCCGGGCCTCGG | Fig. 4a and 4b |
| | VEGFA_sg24 | CCCA | CAGCCCGAGCCGGAGAGGGA | Fig. 4a and 4b |
| | VEGFA_sg25 | CCCA | AGACAGCAGAAAGTTCATGG | Fig. 4a and 4b |
| | VEGFA_sg26 | CCCA | GGCTGCACCCATGGCAGAAG | Fig. 4a and 4b |
| | VEGFA_sg27 | CCCA | TGGCAGAAGGAGGAGGGCAG | Fig. 4a and 4b |
| | VEGFA_sg28 | CCCA | CTGAGGAGTCCAACATCACC | Fig. 4a and 4b |
| | VEGFA_sg29 | CCCA | CCTGCATGGTGATGTTGGAC | Fig. 4a and 4b |
| | VEGFA_sg30 | CCCA | AAGATGCCCACCTGCATGGT | Fig. 4a and 4b |
| | VEGFA_sg31 | CCCA | CTTCCCAAAGATGCCCACCT | Fig. 4a and 4b |
| P2RX5-TAX1BP3 on-target | | TTTA | CACATAGGCCATTCAGAAAC | Fig. 5b |
| NLRC4 on-target | | TTTA | GAGGGAGACACAAGTTGATA | Fig. 5b |
| intergene on-target | | TTTA | AGAACACATACCCCTGGGCC | Fig. 5b |
| CLIC4 on-target | | TTTA | CCCTGGCTACCTCCCCTACC | Fig. 5b |
| P2RX5-TAX1BP3 OT1 | | TTTG | CATATGGGTCATTCAGAAAC | Fig. 5c |
| P2RX5-TAX1BP3 OT2 | | TTTA | CACATAGGCCTTTGAGAAAG | Fig. 5c |
| P2RX5-TAX1BP3 OT3 | | TTTC | CACACAGGCCTTTCAGCAAC | Fig. 5c |
| P2RX5-TAX1BP3 OT4 | | TTTA | CACAGATGCCATTCAGATAC | Fig. 5c |
| P2RX5-TAX1BP3 OT5 | | TTTA | CACATAGGTCATTCACAAAT | Fig. 5c |
| P2RX5-TAX1BP3 OT6 | | TTTG | CAGATAGTCCTTTCAGAAAC | Fig. 5c |

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| P2RX5-TAX1BP3 OT7 | | TTTA | CACATAGGCTATTCAAAATC | Fig. 5c |
| P2RX5-TAX1BP3 OT8 | | TTTA | CCCAAAAGCCATTCAGAAAC | Fig. 5c |
| P2RX5-TAX1BP3 OT9 | | TTTC | CACATGGCCCATACAGAAAC | Fig. 5c |
| P2RX5-TAX1BP3 OT10 | | TTTA | CTCAAAGGCCATTCAGGAAC | Fig. 5c |
| NLRC4 OT1 | | TTTG | GAGGGAGACACGGGTTGGTA | Fig. 5c |
| NLRC4 OT2 | | TTTA | GAGGGAGACAGATGTGGATA | Fig. 5c |
| NLRC4 OT3 | | TTTG | GAGGGAGAGACAGGTTGGTA | Fig. 5c |
| NLRC4 OT4 | | TTTG | GAGAGAGACAGAAGTTGAAA | Fig. 5c |
| NLRC4 OT5 | | TTTC | CAGAGAGACACAAGATGATA | Fig. 5c |
| intergene OT1 | | TTTC | AGAACAGATAGCCCTGGACC | Fig. 5c |
| intergene OT2 | | TTTA | AGAACACAGACGCCTGGGTC | Fig. 5c |
| intergene OT3 | | TTTA | AGAACACCTACCCATGGGAC | Fig. 5c |
| intergene OT4 | | TTTA | AGAACACATACTCCGGTGCC | Fig. 5c |
| CLIC4 OT1 | | TTTG | CCCTGGCTCCCTCACCCACC | Fig. 5c |
| CLIC4 OT2 | | TTTA | CCCTGGCAATCTCCTCTACC | Fig. 5c |
| CLIC4 OT3 | | TTTC | CCCTTTCTTCCTCCCCTACC | Fig. 5c |
| CLIC4 OT4 | | TTTG | CCCTGGCTACGCCCTCTACC | Fig. 5c |
| CLIC4 OT5 | | TTTA | CCCTGACGGCCTCCCCTACC | Fig. 5c |
| CLIC4 OT6 | | TTTC | CCTAGGCTGCCTCCCCTACC | Fig. 5c |
| CLIC4 OT7 | | TTTA | CCCTGTCTACCTCACCTCCC | Fig. 5c |
| Target 36 for PEM-seq | | TTTA | AGAACACATACCCCTGGGCC | Fig. 5d |
| PCSK9 for PEM-seq | | CCCA | GGGGAGGACATCATTGGTGC | Fig. 5d |
| miniCRISPRoff at Snrp | | CTTC | TTGTGCAGTGCCAGGTGAAA | Fig. 6j |
| CRISPRoff at Snrp | | 3'-TGG | CTCCTCAGAACCAAGCGTC | Fig. 6j |
| DNMT1 | DNMT1_sg1 | TTTC | CCTCACTCCTGCTCGGTGAA | Supplementary Fig. 2d |
| | DNMT1_sg2 | TTTC | TCAAGGGGCTGCTGTGAGGA | Supplementary Fig. 2d |
| | DNMT1_sg3 | TTTC | CCTTCAGCTAAAATAAAGGA | Supplementary Fig. 2d |
| | DNMT1_sg4 | TTTG | GCTCAGCAGGCACCTGCCTC | Supplementary Fig. 2d |
| | DNMT1_sg5 | TTTG | GTCAGGTTGGCTGCTGGGCT | Supplementary Fig. 2d |
| DMD | DMD_sg1 | TTTC | attggctttgatttccctag | Supplementary Fig. 11a |
| | DMD_sg2 | TTTC | cctagggtccagcttcaaat | Supplementary Fig. 11a |
| | DMD_sg3 | TTTC | ccaccagttcttaggcaact | Supplementary Fig. 11a |
| | DMD_sg4 | TTTC | tctctcagcaaacacattac | Supplementary Fig. 11a |
| | DMD_sg5 | TTTG | atttccctagggtccagctt | Supplementary Fig. 11a |
| | DMD_sg6 | TTTG | aagctggaccctagggaaat | Supplementary Fig. 11a |
| | DMD_sg7 | TTTG | ctgagagagaaacagttgcc | Supplementary Fig. 11a |
| | DMD_sg8 | TTTA | ctctcctagaccatttccca | Supplementary Fig. 11a |
| | DMD_sg9 | GCCA | atgaaacgttcttgtcttag | Supplementary Fig. 11a |
| | DMD_sg10 | CCCA | gtataaaatacagagctaag | Supplementary Fig. 11a |
| | DMD_sg11 | CCCA | ccagttcttaggcaactgtt | Supplementary Fig. 11a |
| | DMD_sg12 | TCCA | ccaatcactttactctccta | Supplementary Fig. 11a |
| | DMD_sg13 | GTTC | CTAGGGCAGAGAACAGGATT | Supplementary Fig. 11a |
| | DMD_sg14 | TTTC | TGGCATTGTCATACGTGTAT | Supplementary Fig. 11a |
| | DMD_sg15 | CTTC | AATCAATATAGGGCCACACA | Supplementary Fig. 11a |
| | DMD_sg16 | CTTC | TGTATTCAAGCTCAAGGCCT | Supplementary Fig. 11a |
| | DMD_sg17 | GTTC | TGCTACTTACTGGGAATTTG | Supplementary Fig. 11a |
| | DMD_sg18 | TTTC | caccaatcactttactctcc | Supplementary Fig. 2c |
| | DMD_sg19 | TTTG | gactacgcagcccaacatat | Supplementary Fig. 2c |
| | DMD_sg20 | TTTG | attctgcaatatgttgggct | Supplementary Fig. 2c |

**Supplementary Table 3. Sequences of sgRNA for IVT and Primers & Oligos for bisulfite sequencing and PEM-seq**

| **sgRNA for IVT** | **sequences** |
|---|---|
| OsCas12f1 sg1 | AGGGACGACTTCCCGTCCCAAAATCGAGACAGTAGCCGTAAAACTTTGAGTTTCAGAGTGGGCGACACACTCGAAAAGGTTAAGATATGCACATAGTAATCCGTGCATGAGCCGCGAAAGCGGCTTGAAGGTGCTGTCTTGGGTGCATTGG |
| OsCas12f1 sg2 | AGGGACGACTTCCCGTCCCAAAATCGAGACAGTAGCCGTAAAACTTTGAGTTTCAGAGTGGGCGACACACTCGAAAAGGTTAAGATATGCACATAGTAATCCGTGCATGAGCCGCGAAAGCGGCTTGAAGGCAGTAAGATTTGGTGTCTAT |
| enOsCas12f1 sg1 | AGGGCCGACTTCCCGGCCCAAAATCGAGACAGTAGCCGTAAAACGTTGAGTTTCAGCGTGGGCGACACACTCGAAAAGGTTAAGATATGCACATAGTAATCCGTGCATGAGCCGCGAAAGCGGCTTGAAGGTGCTGTCTTGGGTGCATTGG |
| enOsCas12f1 sg2 | AGGGCCGACTTCCCGGCCCAAAATCGAGACAGTAGCCGTAAAACGTTGAGTTTCAGCGTGGGCGACACACTCGAAAAGGTTAAGATATGCACATAGTAATCCGTGCATGAGCCGCGAAAGCGGCTTGAAGGCAGTAAGATTTGGTGTCTAT |
| RhCas12f1 sg1 | ACGGTTGATTTAGCAACCGAAGTCTGAGGGCATGTAGAAAAAAGTATAGGTATATACCAACATACTTGCATTGCCACTCGGAAAGGGTTAACCTTGGTCATTGTGTTACCGACCAAGCATTCCAGAAATGGAATGTAAATCCTGCATGGTGATGTTGGAC |
| RhCas12f1 sg2 | ACGGTTGATTTAGCAACCGAAGTCTGAGGGCATGTAGAAAAAAGTATAGGTATATACCAACATACTTGCATTGCCACTCGGAAAGGGTTAACCTTGGTCATTGTGTTACCGACCAAGCATTCCAGAAATGGAATGTAAATAAGATGCCCACCTGCATGGT |
| enRhCas12f1 sg1 | ACGGCTGATTTAGCAGCCGAAGTCTGAGGGCATGTAGAAAAAAGTATAGGTATATACCAACATACTTGCATTGCCACTCGGAAAGGGTTAACCTTGGTCATTGTGTTACCGACCAAGCATTCCAGAAATGGAATGTAAATCCTGCATGGTGATGTTGGAC |
| enRhCas12f1 sg2 | ACGGCTGATTTAGCAGCCGAAGTCTGAGGGCATGTAGAAAAAAGTATAGGTATATACCAACATACTTGCATTGCCACTCGGAAAGGGTTAACCTTGGTCATTGTGTTACCGACCAAGCATTCCAGAAATGGAATGTAAATAAGATGCCCACCTGCATGGT |

| **Bis-seq primers** | **sequences** |
|---|---|
| Bis-F1 | TTAGAGGGATAGAGATTTTTGTATTG |
| Bis-R1 | ACTCACCATACTAACAAAATCCACA |
| Bis-F2 | TAGAGGGATAGAGATTTTTGTATTG |
| Bis-R2 | CAAAATCCACAAACCCAACTAAC |

**Primers and oligos for PEM-seq**

| | |
|---|---|
| Bridge adaptor-up | /5phos/CCACGCGTGCTCTACANNNNTNNNANNNTNNNNAGATCGGAAGAGCACACGTCTGAACTCCAGT-NH2(C7) |
| Bridge adaptor-down | TGTAGAGCACGCGTGGNNNNNN-NH2(C7) |
| P5-I5 | AATGATACGGCGACCACCGAGATCTACACACACTCTTTCCCTACACGACGC |
| P7-tag | CAAGCAGAAGACGGCATACGAGAT |
| Biotin-target36 | Biotin/gcaacagggctctgagttataataacttaga |
| I5-target36-enOsCas12f1 | ACTCTTTCCCTACACGACGCTCTTCCGATCTAGCGggcctgtgagaagtcctttа |
| I5-target36-Un1Cas12f1_ge4.1 | ACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTggcctgtgagaagtcctttа |

| | |
|---|---|
| I5-target36-LbCas12a | ACTCTTTCCCTACACGACGCTCTTCCGATCTACCAggcctgtgagaagtcctttа |
| I5-target36-SpCas9 | ACTCTTTCCCTACACGACGCTCTTCCGATCTCGTAggcctgtgagaagtcctttа |
| I7-target36-enOsCas12f1 | CAGAAGACGGCATACGAGATCATTGCGTGACTGGAGTTCAGACGTGTGC |
| I7-target36-Un1Cas12f1_ge4.1 | CAGAAGACGGCATACGAGATAGGACAGTGACTGGAGTTCAGACGTGTGC |
| I7-target36-LbCas12a | CAGAAGACGGCATACGAGATGGATCTGTGACTGGAGTTCAGACGTGTGC |
| I7-target36-SpCas9 | CAGAAGACGGCATACGAGATGCAATCGTGACTGGAGTTCAGACGTGTGC |
| Biotin-PCSK9-sg37 | Biotin/CTCCTCACCTTTCCAGGTCATCACAG |
| I5-PCSK9-sg37_SpCas9 | ACTCTTTCCCTACACGACGCTCTTCCGATCTAGCGCAATGCCCAAGACCAGCCGGTGAC |
| I5-PCSK9-sg37_enRhCas12f1 | ACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTCAATGCCCAAGACCAGCCGGTGAC |
| I7-PCSK9-sg37_SpCas9 | CAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGC |
| I7-PCSK9-sg37_RhCas | CAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC |

**Supplementary Note 1.** Sequences of OsCas12f1, RhCas12f1 and OsCas12f1-derived gene editing tools.

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**Supplementary References**


1. Ousterout, D.G. et al. Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. *Nat Commun* **6**, 6244 (2015).
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