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Supplementary Table 1. Oligonucleotides used in this study.

Name	Assay	Sequence (5' to 3')
oGS1 (dsODN Fwd)	GUIDE-seq	/5Phos/GTTTAATTGAGTTGTCATATGTTAATAACGGT*A*T
oGS2 (dsODN Rev)		/5Phos/ATACCGTTATTAACATATGACAACTCAATTAA*A*C
T_6-RT primer	Quantify sgRNA expression	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTTTTT
sgRNA Fwd		GGCCTTTTTACGGTTCCTGGCCTT
sgRNA Rev		TGGAGTTCAGACGTGTGCTCTTCC
HBB Fwd	CRISPRa qPCR	GCACGTGGATCCTGAGAACT
HBB Rev		ATTGGACAGCAAGAAAGCGAG
HBG Fwd		GCTGAGTGAACTGCACTGTGA
HBG Rev		GAATTCTTTGCCGAAATGGA
IL1RN Fwd		GGAATCCATGGAGGGAAGAT
IL1RN Rev		TGTTCTCGCTCAGGTCAGTG
GAPDH Fwd	qPCR	GTCTCCTCTGACTTCAACAGCG
GAPDH Rev		ACCACCCTGTTGCTGTAGCCAA
DNA-Fwd	Cryo-EM	AAAACAGGTTTTTGCTCTCAAGACCCACAATCCAGGCCGGAA
DNA-Rev		TTCCGGCCTGGATTGTGGGTCTTGAGAGCAAAAACCTGTTTT
Full-length sgRNA	Cryo-EM and gene editing in cells	GGGAUUCGUCGGUUCAGCGACGAUAAGCCGAGAAGUGCCAAUAAAACUGUUAAGUGGUUUGGUAACGCUCGGUAAGGUAGCCAAAAGGCUGAAACUCCGUGCACAAAGACCGCACGGACGCUUCACAUAUAGCUCAUAAACAAGGGUUUGCGAGCUAGCUUGUGGAGUGUGAACNNNNNNNNNNNNNNNNNNN
sgRNA-v2	Gene editing in cells	GGGAUUCGUCGGUUCAGCGACGAUAAGCCGAGAAGUGCCAAUAAAACUGUUAAGUGGUUUGGUAACGCUCGGUAAGGUAGCCUUCGGGCAAGACCACUGAACNNNNNNNNNNNNNNNNNNNN

/5Phos/ indicates 5′ phosphorylation. Asterisks indicate phosphorothioate linkages.

Supplementary Table 2. Cas12f target sequences.

Target name	Sequence (5' to 3')	Assay	5' PAM
HBB	GTAGCAATTTGTACTGATGG	CRISPRa	TTTA
HBG	CATTGAGATAGTGTGGGGAA	CRISPRa	TTTG
IL1RN	GTTTCTGCTAGCCTGAGTCA	CRISPRa	TTTG
APOB	CTGTCGACACCCAGAATCAT	DNA Cleavage	TTTG
CARS	CAACAGCCTCACCAGGAACA	DNA Cleavage	TTTA
CLTA4	CCTGGAGATGCATACTCACA	DNA Cleavage	TTTG
DNMT1	TGTGGCCACAAGGCTCAGTT	DNA Cleavage	TTTG
HEXA	AGTATACGCTTCCACAGAAA	DNA Cleavage	TTTG
INIP	AGAGCAGCGATTGTAAGGAG	DNA Cleavage	TTTA
MRPL39	ATTTCACAGGACTTTGTTAA	DNA Cleavage	TTTA
NOTCH1	GCATCAGCTGGCACTCGTCC	DNA Cleavage	TTTG
PDCD1	CTGTGAGCTCTAGTCCCCAC	DNA Cleavage	TTTG
POLRMT	AGGACTATGTGTGGCCAGTG	DNA Cleavage	TTTA
PRNP	TGGCCACATGGAGTGACCTG	DNA Cleavage	TTTG
TP53-1	AGGCATCACTGCCCCCTGAT	DNA Cleavage	TTTG
TP53-2	ATAAGAGGTCCCAAGACTTA	DNA Cleavage	CTTG
TP53-3	CTTACCTCGCTTAGTGCTCC	DNA Cleavage	CTTG
TP53-4	CCTCTTTCCTAGCACTGCCC	DNA Cleavage	CTTG
VEGFA-1	CTCTCAAGACCCACAATCCA	DNA Cleavage	TTTG
VEGFA-2	AAGAAGGGATGTGGTGCATT	DNA Cleavage	ATTG

Supplementary Table 3. Cas12a target sequences.

Target name	Sequence (5' to 3')	Assay	5' PAM
HBB	GTAGCAATTTGTACTGATGGTAT	CRISPRa	TTTA
HBG	CATTGAGATAGTGTGGGGAAGGG	CRISPRa	TTTG
IL1RN	GTTTCTGCTAGCCTGAGTCACCC	CRISPRa	TTTG
APOB	CTGTCGACACCCAGAATCATGGC	DNA Cleavage	TTTG
CARS	CAACAGCCTCACCAGGAACAAGG	DNA Cleavage	TTTA
CLTA4	CCTGGAGATGCATACTCACACAC	DNA Cleavage	TTTG
DNMT1	TGTGGCCACAAGGCTCAGTTCTC	DNA Cleavage	TTTG
HEXA	AGTATACGCTTCCACAGAAAGGA	DNA Cleavage	TTTG
INIP	AGAGCAGCGATTGTAAGGAGAGG	DNA Cleavage	TTTA
MRPL39	ATTTCACAGGACTTTGTTAAAGG	DNA Cleavage	TTTA
NOTCH1	GCATCAGCTGGCACTCGTCCACA	DNA Cleavage	TTTG
PDCD1	CTGTGAGCTCTAGTCCCCACTGT	DNA Cleavage	TTTG
POLRMT	AGGACTATGTGTGGCCAGTGAGG	DNA Cleavage	TTTA
PRNP	TGGCCACATGGAGTGACCTGGGC	DNA Cleavage	TTTG
TP53-1	AGGCATCACTGCCCCCTGATGGC	DNA Cleavage	TTTG
TP53-2	ATAAGAGGTCCCAAGACTTAGTA	DNA Cleavage	CTTG
TP53-3	CTTACCTCGCTTAGTGCTCCCTG	DNA Cleavage	CTTG
TP53-4	CCTCTTTCCTAGCACTGCCCAAC	DNA Cleavage	CTTG
VEGFA-1	CTCTCAAGACCCACAATCCAGGC	DNA Cleavage	TTTG
VEGFA-2	AAGAAGGGATGTGGTGCATTTGG	DNA Cleavage	ATTG

Supplementary Table 4. Cas9 target sequences.

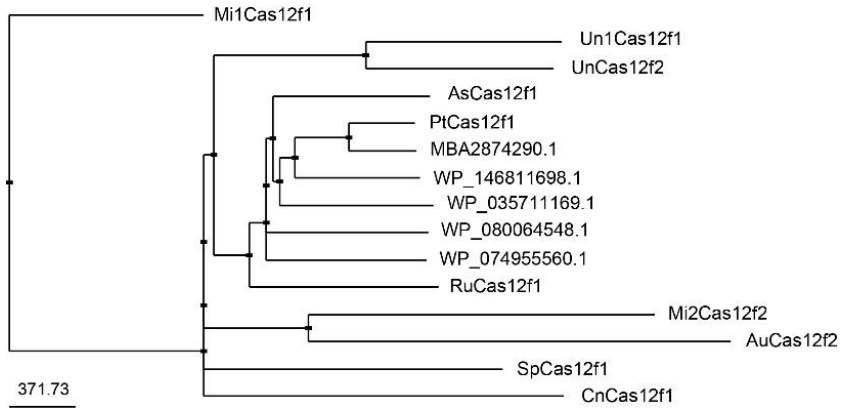
Target name	Sequence (5' to 3')	Assay	3' PAM
HBB	AGCAATTTGTACTGATGGTA	CRISPRa	TGG
HBG	CATTGAGATAGTGTGGGGAA	CRISPRa	GGG
IL1RN	GCTAGCCTGAGTCACCCTCC	CRISPRa	TGG
CARS	CAACAGCCTCACCAGGAACA	DNA Cleavage	AGG
HEXA	GAGTATACGCTTCCACAGAA	DNA Cleavage	AGG
INIP	AGAGCAGCGATTGTAAGGAG	DNA Cleavage	AGG
PDCD1	TGTCTTGCTGGAAAATGTGG	DNA Cleavage	AGG
POLRMT	AGGACTATGTGTGGCCAGTG	DNA Cleavage	AGG
TP53-1	ATGGCAAATGCCCCAATTGC	DNA Cleavage	AGG
VEGFA-1	GACCCAGAGATGCATAAAAC	DNA Cleavage	AGG
VEGFA-2	AAGAAGGGATGTGGTGCATT	DNA Cleavage	TGG

Supplementary Table 5. Cryo-EM data collection, refinement, and validation statistics.

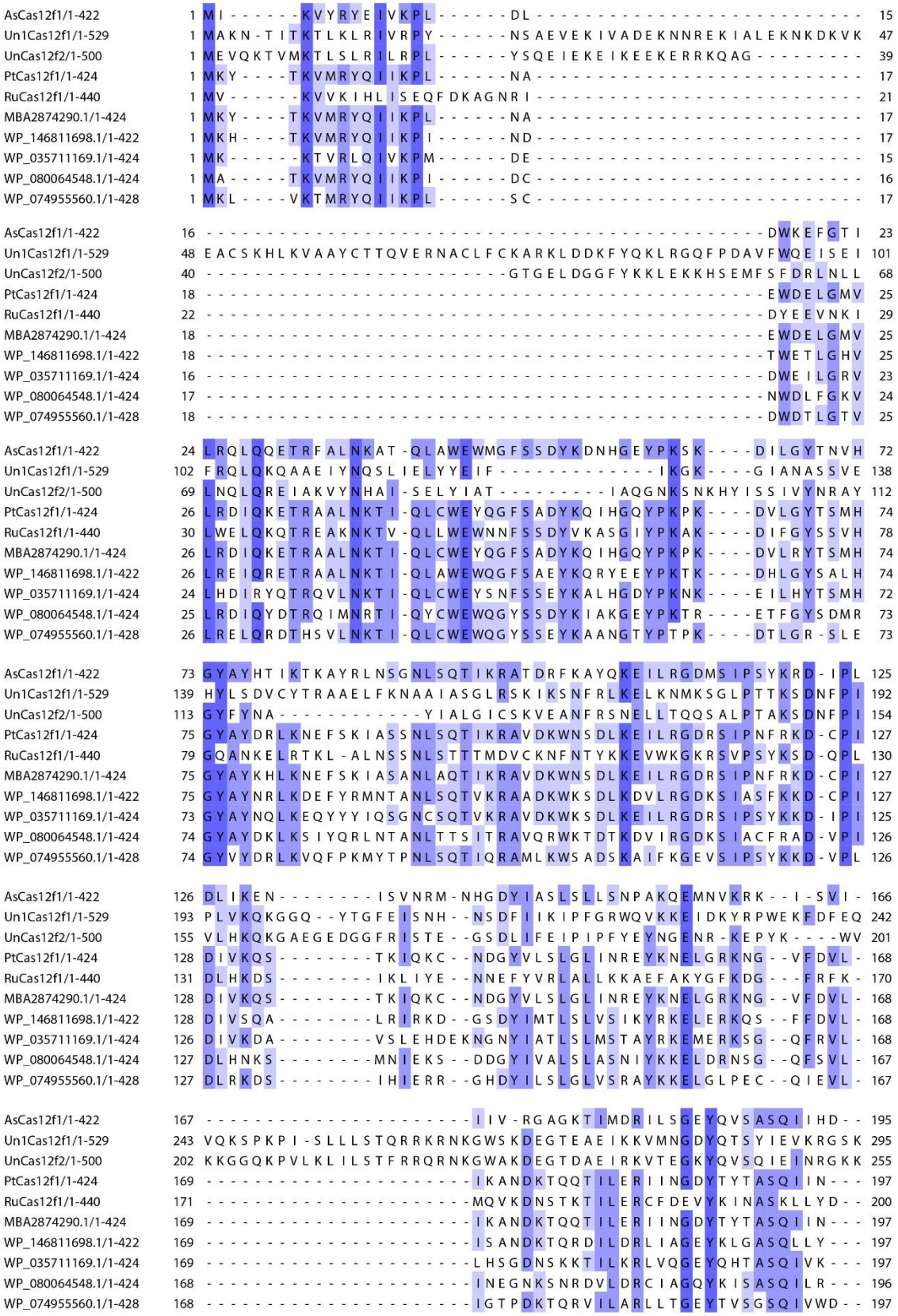
	AsCas12f-gRNA-DNA complex (EMDB-27801) (PDB 8DZJ)
Data collection and processing	
Magnification	81,000
Voltage (kV)	300
Electron exposure (e–/Å^2)	50
Defocus range (μm)	-1.0 to -2.5
Pixel size (Å)	0.5323
Symmetry imposed	C1
Initial particle images (no.)	5,285,777
Final particle images (no.)	490,190
Map resolution (Å)	2.9
FSC threshold	0.143
Map resolution range (Å)	2.7 to 7.1
Refinement	
Model resolution (Å)	3.1
FSC threshold	0.5
Map sharpening *B* factor (Å^2)	-78.8
Model composition	
Non-hydrogen atoms	9030
Protein residues	613
Nucleotide	190
Ligands	Zn: 1
B factors (Å^2)	
Protein	19.0
Nucleotide	46.3
Ligand	57.1
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles ( )	0.603
Validation	
MolProbity score	1.61
Clashscore	12.59
Poor rotamers (%)	0.38
Ramachandran plot	
Favored (%)	98.41
Allowed (%)	1.49
Disallowed (%)	0.00

Supplementary Table 6. Numbers of potential off-target sites predicted by Cas-OFFinder for all AsCas12f targets.

Rank	Target site	Number of potential off-target sites
1	*MRPL39*	1538095
2	*TP53-2*	1113028
3	*APOB*	898574
4	*HEXA*	771008
5	*PDCD1*	551945
6	*INIP*	537243
7	*NOTCH1*	505045
8	*PRNP*	503098
9	*CLTA4*	479644
10	*CARS*	461355
11	*POLRMT*	444226
12	*VEGFA-2*	404473
13	*VEGFA-1*	373137
14	*TP53-1*	348930
15	*DNMT1*	334536
16	*TP53-3*	325648
17	*TP53-4*	240052



Supplementary Fig. 1 | Phylogenetic tree of Cas12f family proteins. Hypothetical proteins are denoted by Genbank protein accession numbers.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Un1Cas12f1/1-529 1 MAKN-TITKTLKLRIVRPY------NSAEVEKIVADEKNNREKIALEKNKDKVK 47
UnCas12f2/1-500 1 MEVQKTVMKTLSLRILRPL------YSQEIEKEIKEEKERRKQAG--------- 39
PtCas12f1/1-424 1 MKY----TKVMRYQIIKPL------NA---------- 17
RuCas12f1/1-440 1 MV------KVVKIHLISEQFDKAGNRI---------- 21
MBA2874290.1/1-424 1 MKY----TKVMRYQIIKPL------NA---------- 17
WP_146811698.1/1-422 1 MKH----TKVMRYQIIKPI------ND---------- 17
WP_035711169.1/1-424 1 MK------KTVRLQIVKPM------DE---------- 15
WP_080064548.1/1-424 1 MA-----TKVMRYQIIKPI------DC---------- 16
WP_074955560.1/1-428 1 MKL----VKTMRYQIIKPL------SC---------- 17
AsCas12f1/1-422 16 ----------DWKEFGTI 23
Un1Cas12f1/1-529 48 EACSKHLKVAAYCTTQVERNACLFCKARKLDDKFYQKLRGQFPDAVFWQEISEI 101
UnCas12f2/1-500 40 ----------GTGELDGGFYKKLEKKHSEMFSFDRLNLL 68
PtCas12f1/1-424 18 ----------EWDELGMV 25
RuCas12f1/1-440 22 ----------DYEEVNKI 29
MBA2874290.1/1-424 18 ----------EWDELGMV 25
WP_146811698.1/1-422 18 ----------TWETLGHV 25
WP_035711169.1/1-424 16 ----------DWEILGRV 23
WP_080064548.1/1-424 17 ----------NWDLFGKV 24
WP_074955560.1/1-428 18 ----------DWDTLGTV 25
AsCas12f1/1-422 24 LRQLQQETRFALNKAT-QLAWEWMGFSSDYKDNHGEYPKSK----DILGYTNVH 72
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UnCas12f2/1-500 69 LNQLQREIAKVYNHAI-SELYIAT---------IAQGNKSNKHYISSIVYNRAY 112
PtCas12f1/1-424 26 LRDIQKETRAALNKTI-QLCWEYQGFSADYKQIHGQYPKPK----DVLGYTSMH 74
RuCas12f1/1-440 30 LWELQKQTREAKNKTV-QLLWEWNNFSSDYVKASGIYPKAK----DIFGYSSVH 78
MBA2874290.1/1-424 26 LRDIQKETRAALNKTI-QLCWEYQGFSADYKQIHGQYPKPK----DVLRYTSMH 74
WP_146811698.1/1-422 26 LREIQRETRAALNKTI-QLAWEWQGFSAEYKQRYEEYPKTK----DHLGYSALH 74
WP_035711169.1/1-424 24 LHDIRYQTRQVLNKTI-QLCWEYSNFSSEYKALHGDYPKNK----EILHYTSMH 72
WP_080064548.1/1-424 25 LRDIQYDTRQIMNRTI-QYCWEWQGYSSDYKIAKGEYPKTR----ETFGYSDMR 73
WP_074955560.1/1-428 26 LRELQRDTHSVLNKTI-QLCWEWQGYSSEYKAANGTYPTPK----DTLGR-SLE 73
AsCas12f1/1-422 73 GYAYHTIKTKAYRLNSGNLSQTIKRATDRFKAYQKEILRGDMSIPSYKRD-IPL 125
Un1Cas12f1/1-529 139 HYLSDVCYTRAAELFKNAAIASGLRSKIKSNFRLKELKNMKSGLPTTKSDNFPI 192
UnCas12f2/1-500 113 GYFYNA----------YIALGICSKVEANFRSNELLTQQSALPTAKSDNFPI 154
PtCas12f1/1-424 75 GYAYDRLKNEFSKIASSNLSQTIKRAVDKWNSDLKEILRGDRSIPNFRKD-CPI 127
RuCas12f1/1-440 79 GQANKELRTKL-ALNSSNLSTTTMDVCKNFNTYKKEVWKGKRSVPSYKSD-QPL 130
MBA2874290.1/1-424 75 GYAYKHLKNEFSKIASANLAQTIKRAVDKWNSDLKEILRGDRSIPNFRKD-CPI 127
WP_146811698.1/1-422 75 GYAYNRLKDEFYRMNTANLSQTVKRAADKWKSDLKDVLRGDKSIASFKKD-CPI 127
WP_035711169.1/1-424 73 GYAYNQLKEQYYYIQSGNCSQTVKRAVDKWKSDLKEILRGDRSIPSYKKD-IPI 125
WP_080064548.1/1-424 74 GYAYDKLKSIYQRLNTANLTTSITRAVQRWKTDTKDVIRGDKSIACFRAD-VPI 126
WP_074955560.1/1-428 74 GYVYDRLKVQFPKMYTPNLSQTIQRAMLKWSADSKAIFKGEVSIPSYKKD-VPL 126
AsCas12f1/1-422 126 DLIKEN--------ISVNRM-NHGDYIASLSLLSNPAKQEMNVKRK--I-SVI- 166
Un1Cas12f1/1-529 193 PLVKQKGGQ--YTGFEISNH--NSDFIIKIPFGRWQVKKEIDKYRPWEKFDFEQ 242
UnCas12f2/1-500 155 VLHKQKGAEGEDGGFRISTE--GSDLIFEIPIPFYEYNGENR-KEPYK----WV 201
PtCas12f1/1-424 128 DIVKQS--------TKIQKC--NDGYVLSLGLINREYKNELGRKNG--VFDVL- 168
RuCas12f1/1-440 131 DLHKDS--------IKLIYE--NNEFYVRLALLKKAEFAKYGFKDG---FRFK- 170
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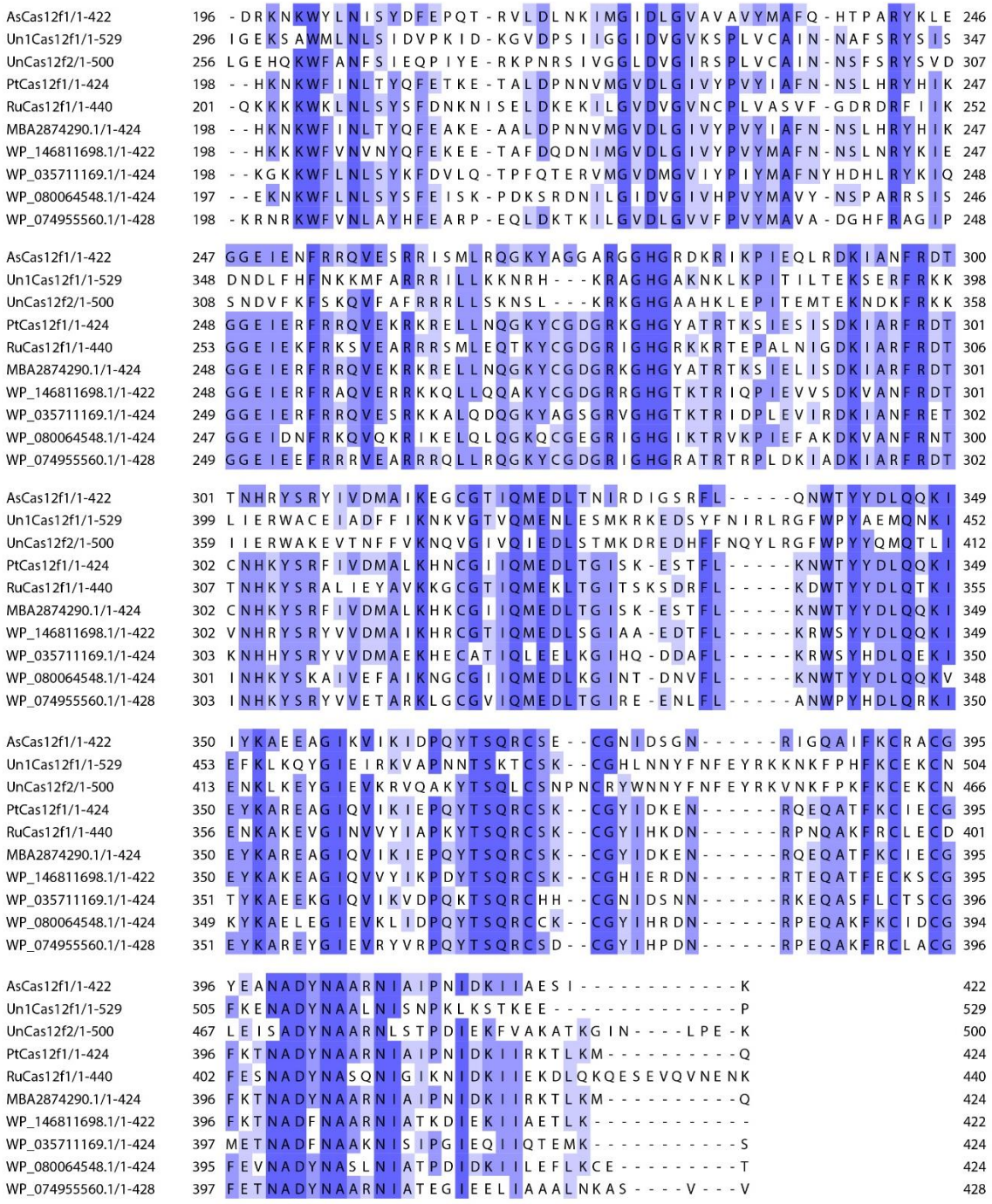
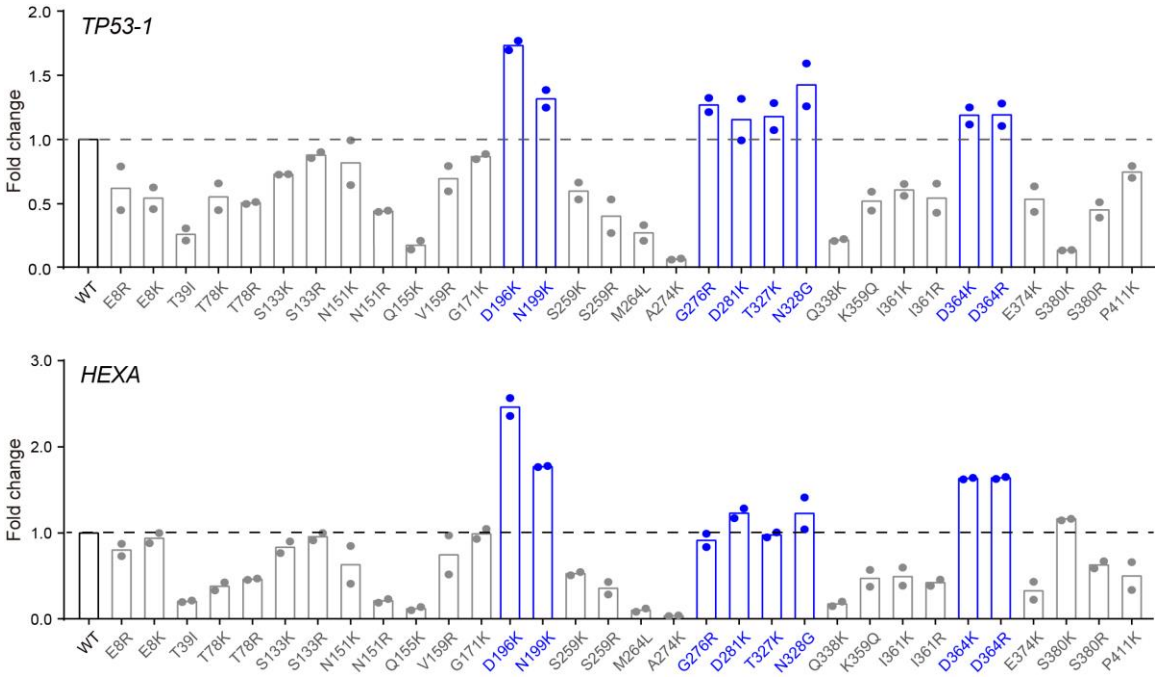
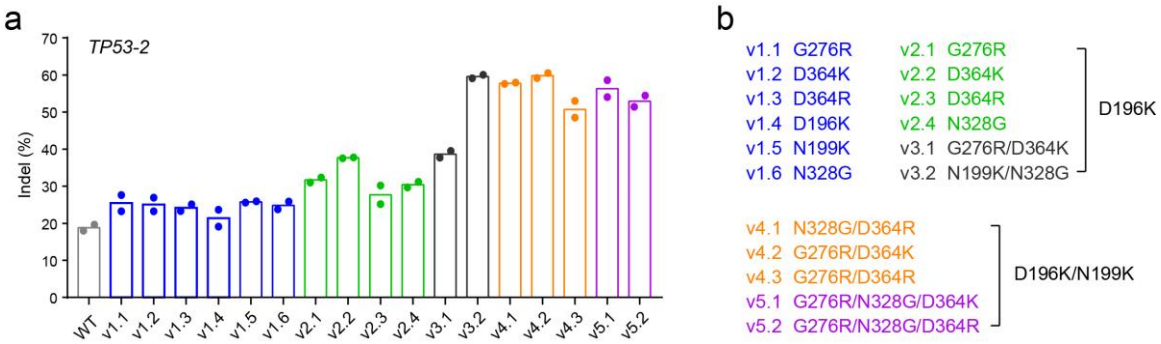
WP_035711169.1/1-424 126 DIVKDA--------VSLEHDEKNGNYIATLSLMSTAYRKEMERKSG--QFRVL- 168
WP_080064548.1/1-424 127 DLHNKS--------MNIEKS--DDGYIVALSLASNIYKKELDRNSG--QFSVL- 167
WP_074955560.1/1-428 127 DLRKDS--------IHIERR--GHDYILSLGLVSRAYKKELGLPEC--QIEVL- 167
AsCas12f1/1-422 167 ----------IIV-RGAGKTIMDRILSGEYQVSASQIIHD-- 195
Un1Cas12f1/1-529 243 VQKSPKPI-SLLLSTQRRKRNKGWSKDEGTEAEIKKVMNGDYQTSYIEVKRGSK 295
UnCas12f2/1-500 202 KKGGQKPVLKLILSTFRRQRNKGWAKDEGTDAEIRKVTEGKYQVSQIEINRGKK 255
PtCas12f1/1-424 169 ----------IKANDKTQQTILERIINGDYTYTASQIIN--- 197
RuCas12f1/1-440 171 ----------MQVKDNSTKTILERCFDEVYKINASKLLYD-- 200
MBA2874290.1/1-424 169 ----------IKANDKTQQTILERIINGDYTYTASQIIN--- 197
WP_146811698.1/1-422 169 ----------ISANDKTQRDILDRLIAGEYKLGASQLLY--- 197
WP_035711169.1/1-424 169 ----------LHSGDNSKKTILKRLVQGEYQHTASQIVK--- 197
WP_080064548.1/1-424 168 ----------INEGNKSNRDVLDRCIAGQYKISASQILR--- 196
WP_074955560.1/1-428 168 ----------IGTPDKTQRVILARLLTGEYTVSGSQIVWD-- 197



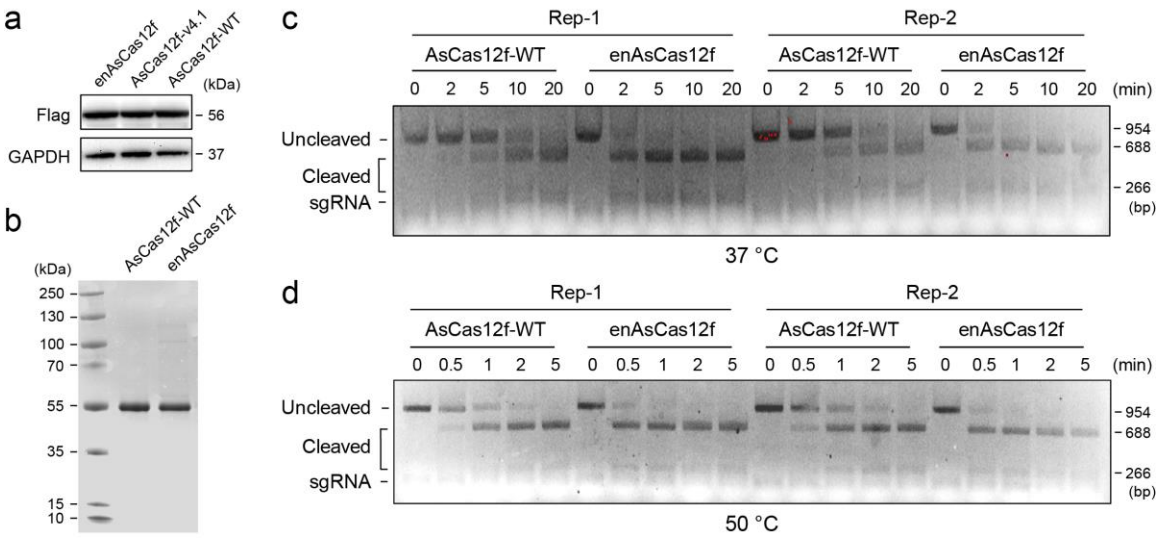
Supplementary Fig. 2 | Full sequence alignment of AsCas12f and other Cas12f family proteins. The alignment was performed using the T-Coffee multiple sequence alignment program.



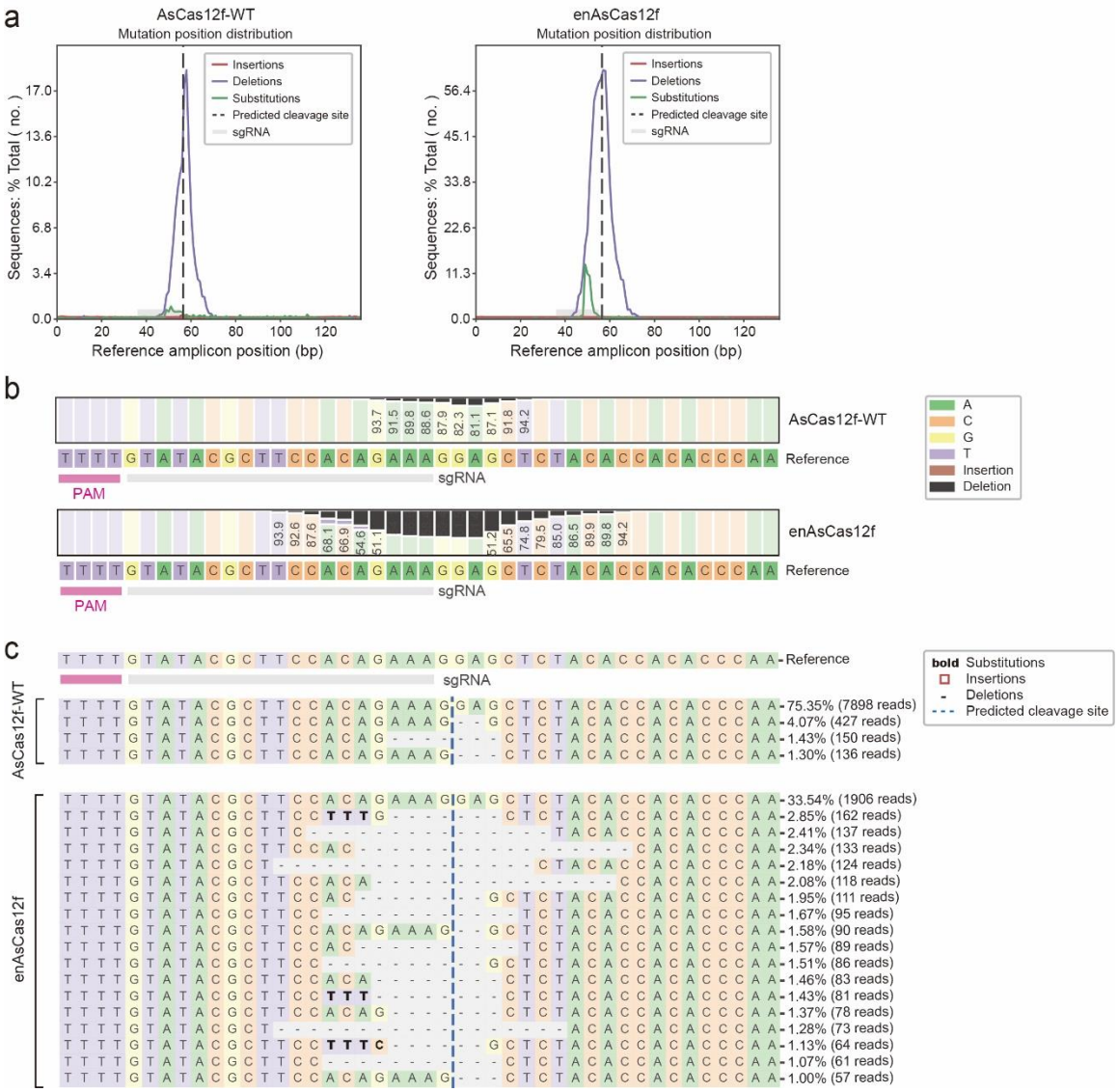
Supplementary Fig. 3 | Gene-editing performance of AsCas12f variants that bear single-point mutations at *TP53-1* (top) and *HEXA* (bottom) loci in HEK293T cells. Fold changes are calculated relative to the indel levels mediated by the wild-type protein.



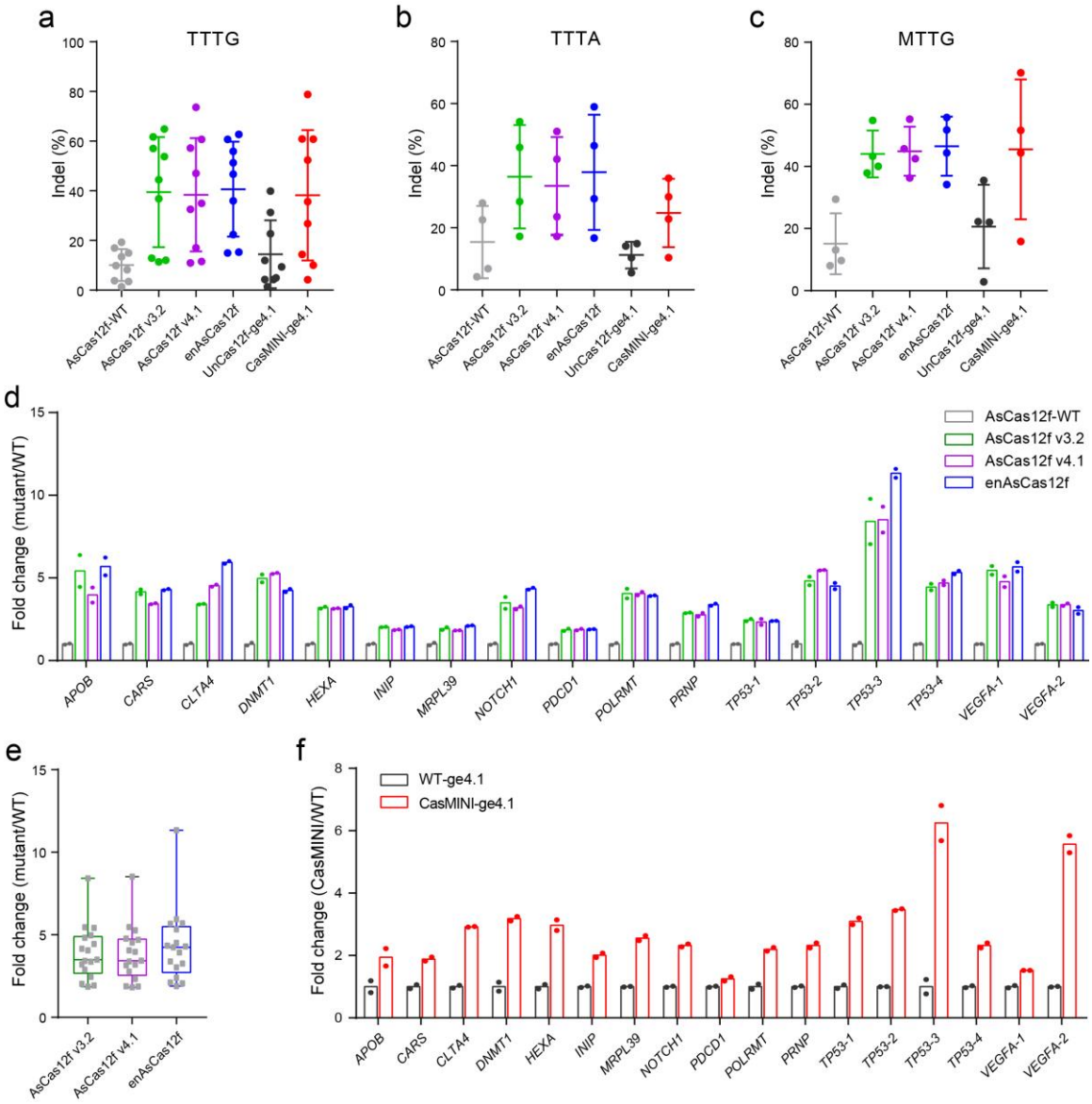
Supplementary Fig. 4 | AsCas12f variants with increased gene-editing efficiency. a, Indel level at the *TP53-2* locus generated by AsCas12 variants that bear one, two, three, four, or five single-point mutations. **b**, List of mutations included in each AsCas12f variant in (**a**) and Fig. 1 (**d**)-(**e**).



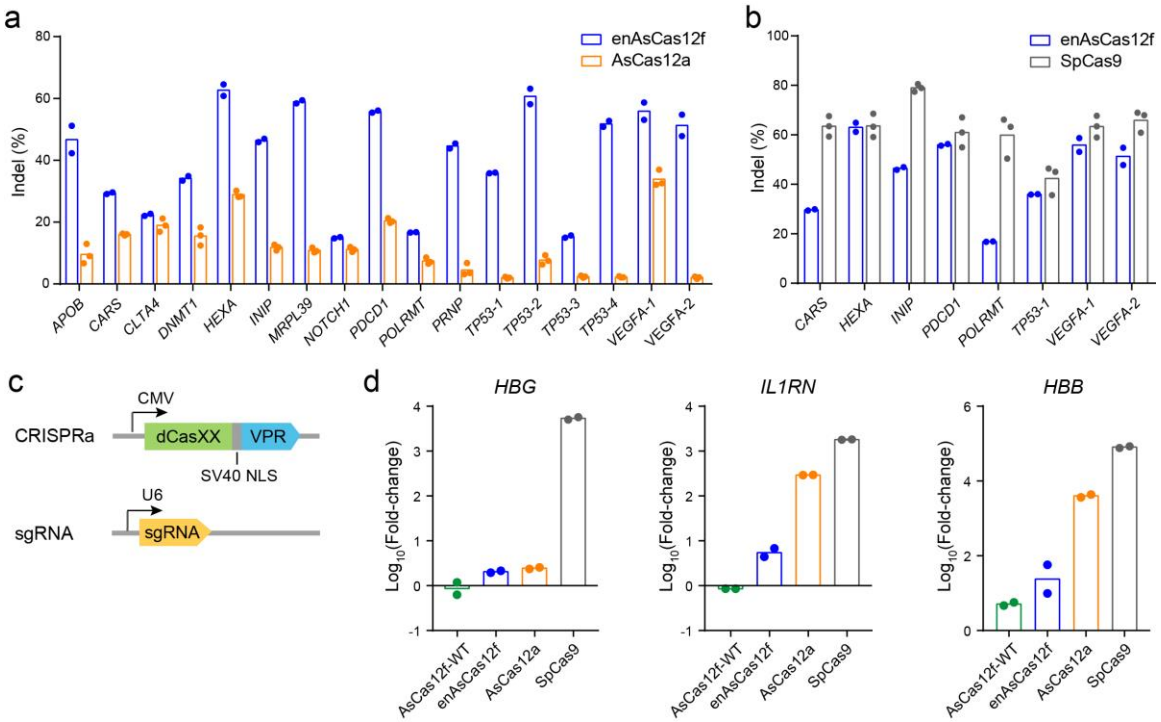
Supplementary Fig. 5 | *In vitro* DNA cleavage by wild-type AsCas12f and enAsCas12f. **a**, Western blot showing the protein level of Flag-tagged AsCas12f variants in HEK293T cells. **b**, SDS-PAGE analysis of wild-type AsCas12f and enAsCas12f proteins used for *in vitro* DNA cleavage experiments. **c**, **d**, Gel electrophoresis monitoring *in vitro* DNA cleavage by wild-type AsCas12f and enAsCas12f over time courses at 37 ℃ (**c**) and 50 ℃ (**d**). Expected DNA sizes are noted on the right of the gel images.



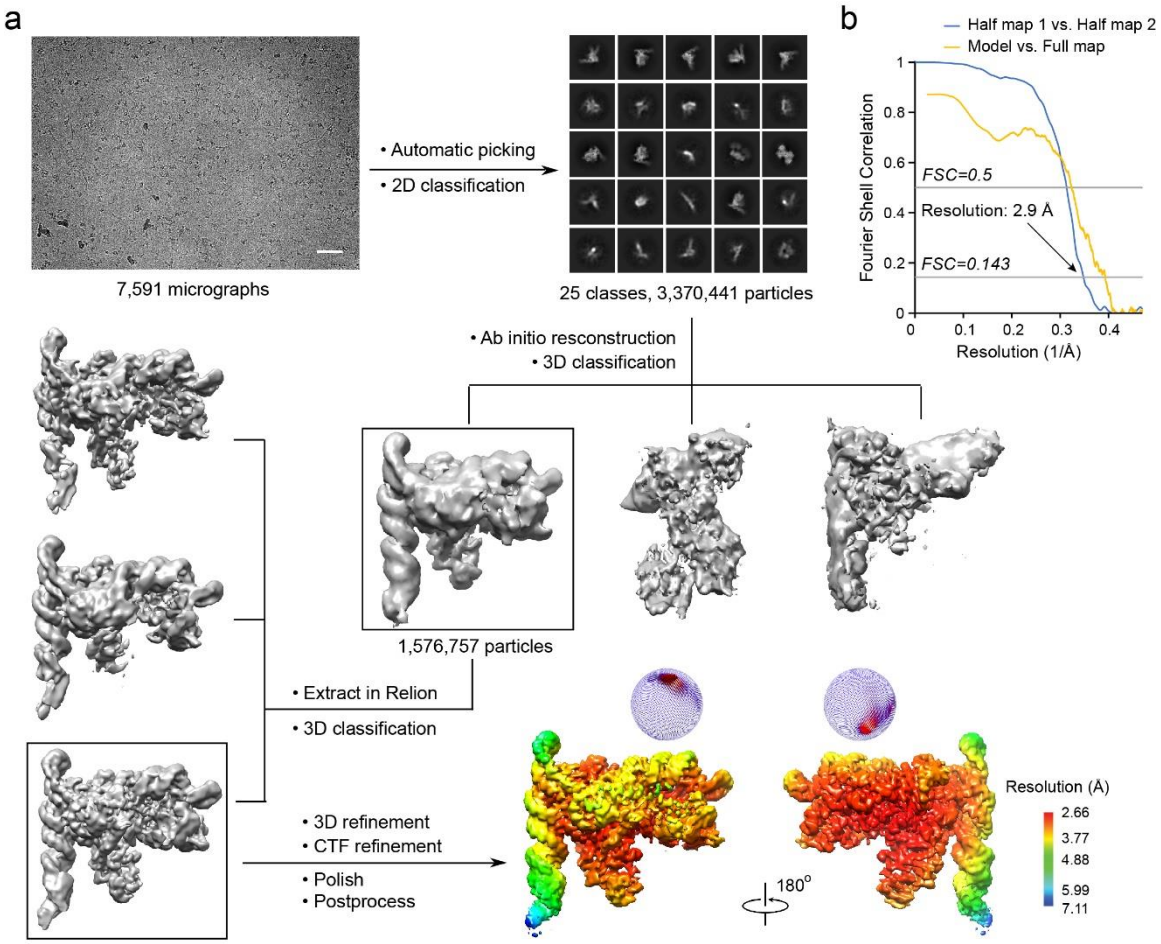
Supplementary Fig. 6 | Representative deep sequencing data processed by CRISPEResso2 showing DNA-editing patterns generated by wild-type AsCas12f and enAsCas12f. a, Metaplots showing the positions of insertions, deletions, and substitutions from samples edited by wild-type AsCas12f (left) and enAsCas12f (right). **b**, Frequencies of insertions, deletions, and substitutions observed near the PAM sequence from samples edited by wild-type AsCas12f (top) and enAsCas12f (bottom). **c**, Raw sequencing reads from samples edited by wild-type AsCas12f (top) and enAsCas12f (bottom). Sequence variants with ≥ 1% of total reads are shown.



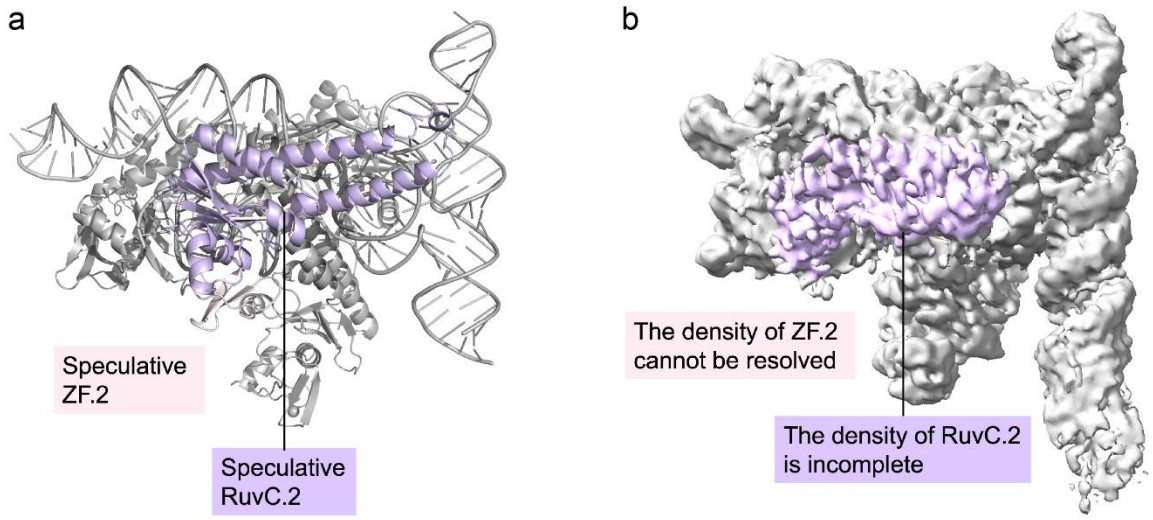
Supplementary Fig. 7 | Performance of engineered AsCas12f across a wide range of target sites with different PAM sequences. a-c, Frequencies of indels delivered by denoted AsCas12f and UnCas12f systems at sites of TTTG (**a**), TTTA (**b**), and MTTG (**c**) PAM sequences (M = A/C). n = 9 target sites for (**a**), n = 4 target sites for (**b**) and (**c**). Error bars represent mean ±SD. **d**, Fold changes of indel frequencies generated by engineered AsCas12f variants compared to wild-type AsCas12f. **e**, Box-and-whisker plot showing fold change mediated by engineered AsCas12f variants. All data points (n = 17 target sites) were plotted, with the centerline showing the median and the whiskers showing the minimum to the maximum. The boundaries of the boxes indicate the 25th and 75th percentiles. **f**, Fold changes of indel frequencies generated by CasMINI compared to wild-type UnCas12f paired with ge4.1.



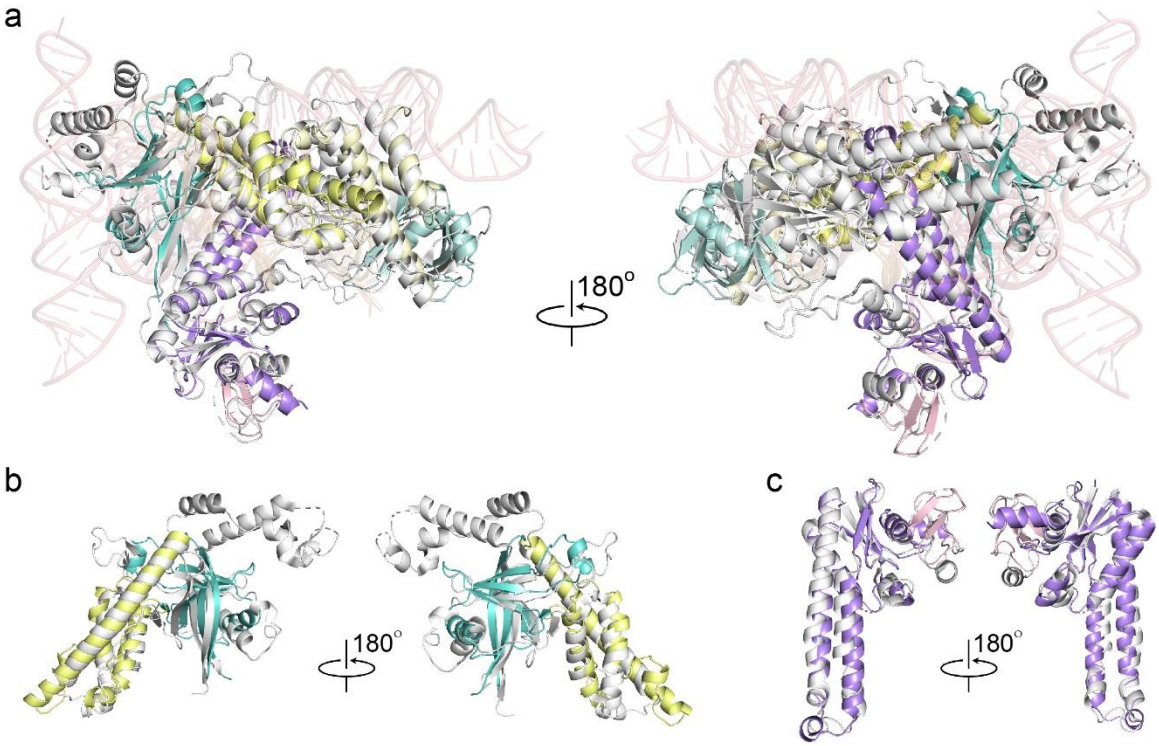
Supplementary Fig. 8 | Comparison between enAsCas12f, AsCas12a, and SpCas9. a, Indel frequencies mediated by enAsCas12f and AsCas12a in HEK293T cells. **b**, Indel frequencies mediated by enAsCas12f and SpCas9 in HEK293T cells. To satisfy different PAM requirements, SpCas9 sgRNAs for *PDCD1*, *TP53-1*, and *VEGFA-1* were designed to recognize sites proximal to the ones targeted by AsCas12f sgRNAs. **c**, Schematic construct design for CRISPRa mediated by different Cas proteins. **d**, Gene activation effects by different CRISPRa constructs in HEK293T cells. Transcription activation was measured by the relative RNA level of *HBG*, *IL1RN*, and *HBB* normalized to *GAPDH*. Fold changes are normalized to RNA levels in cells transfected with a non-target sgRNA.



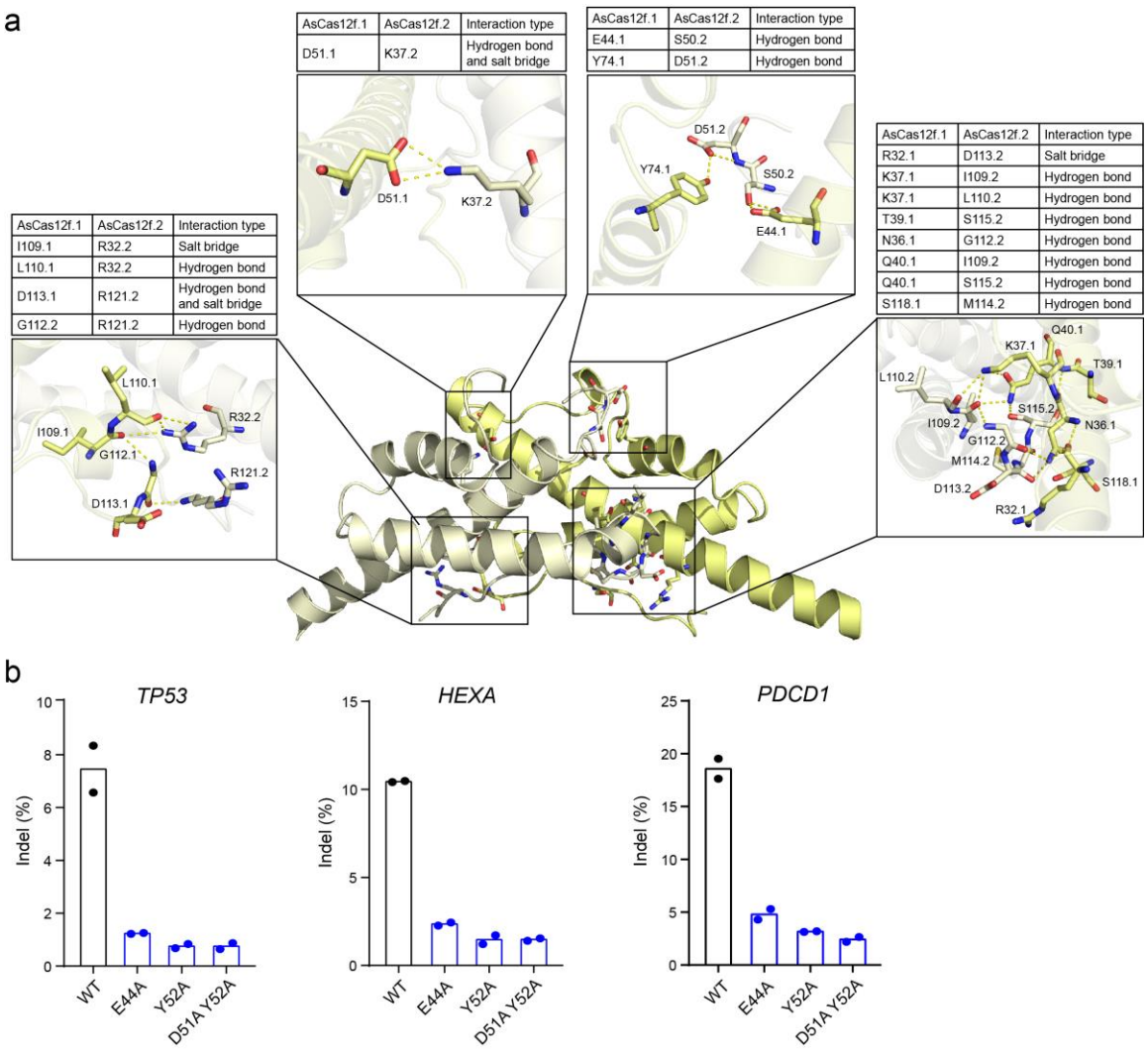
Supplementary Fig. 9 | Single-particle cryo-EM analysis of the AsCas12f-gRNA-DNA complex. a, Data processing workflow. A representative micrograph from 7,591 micrographs is shown along with a 50 nm scale bar. After the first heterogeneous refinement in CryoSPARC, the particles were exported to RELION for further processing. **b**, Fourier shell correlation curves of the half map 1 versus the half map 2 (blue), and the refined model versus the full map (yellow). The overall resolution of the reconstruction was estimated by the Fourier shell correlation (FSC) = 0.143 criterion.



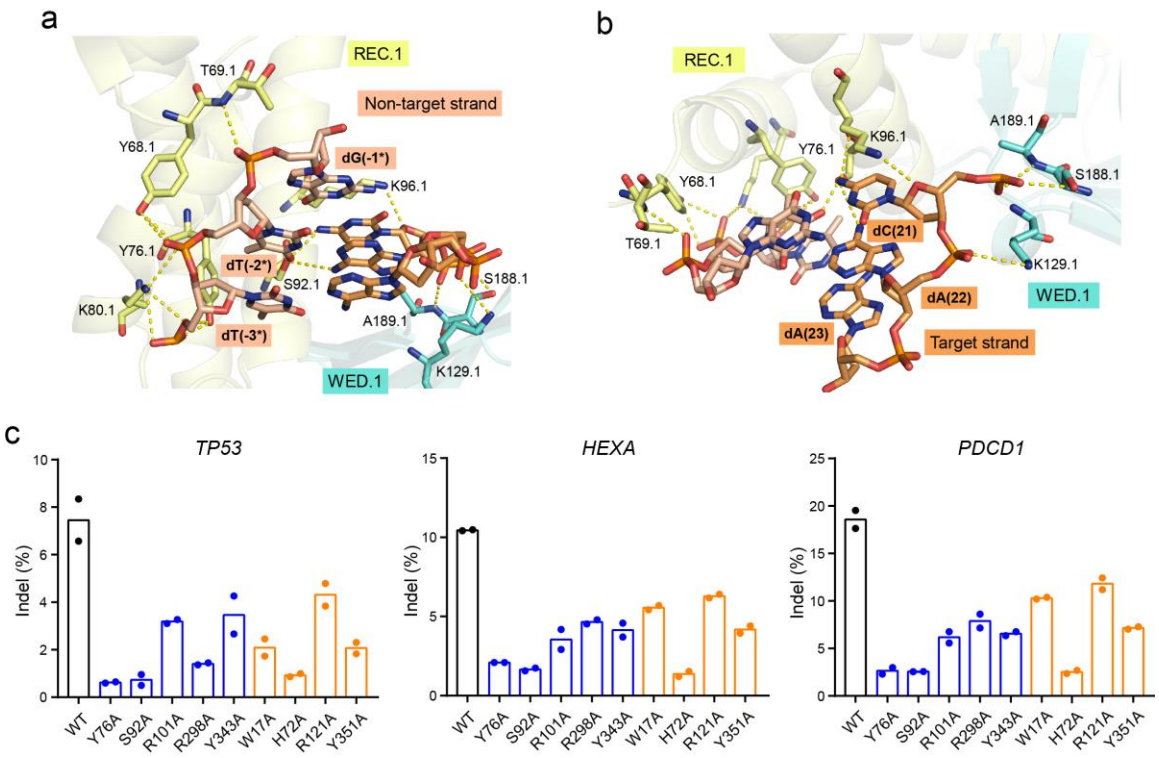
Supplementary Fig. 10 | The C-lobe of AsCas12f.2 was not fully resolved. a, The AsCas12f-sgRNA-DNA structure with speculative RuvC.2 and ZF.2 domains. **b**, Unsharpened AsCas12f-sgRNA-DNA cryo-EM map (contoured at a level of 0.01) with incomplete RuvC.2 domain density colored in light purple.



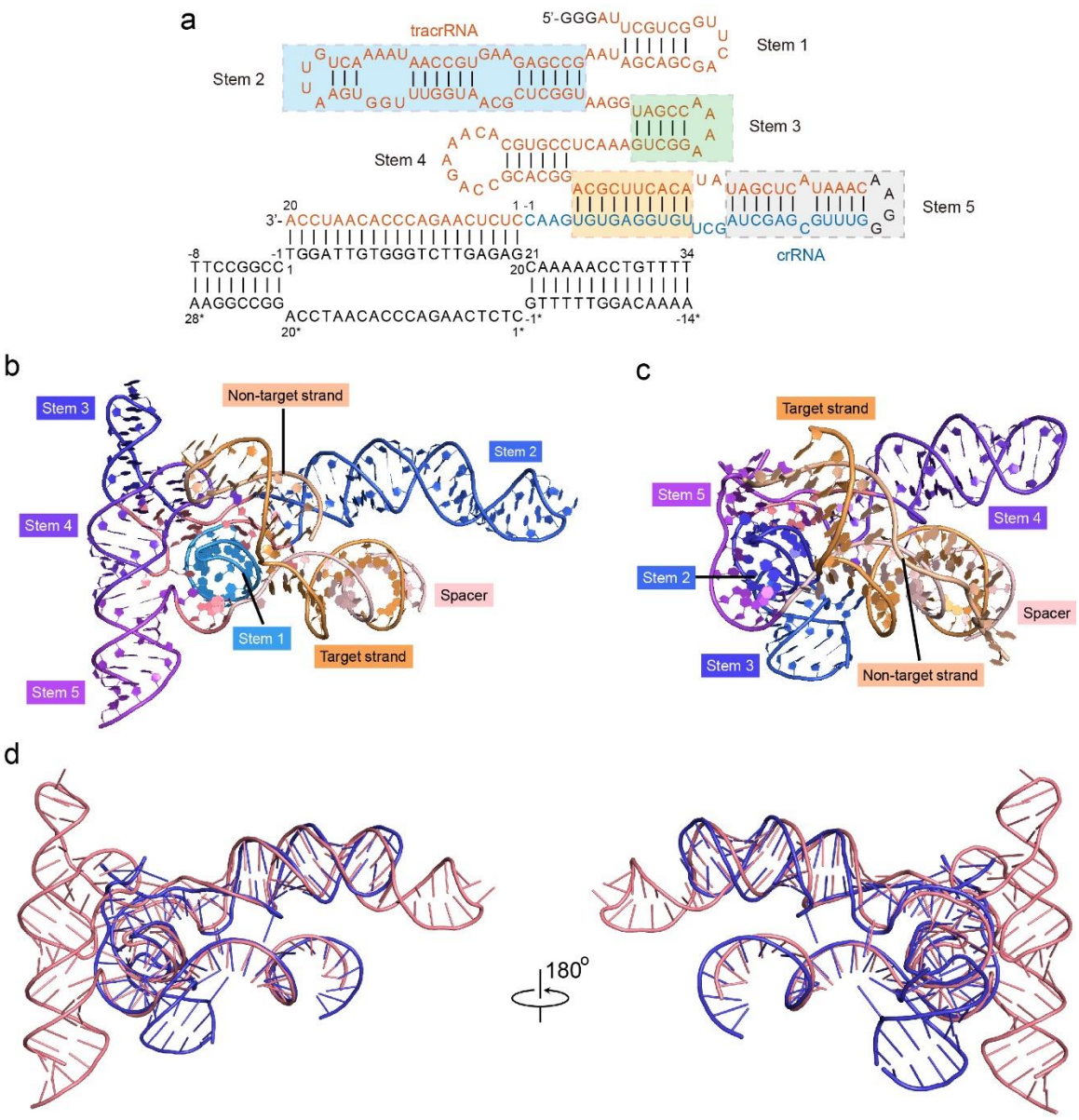
Supplementary Fig. 11 | Comparison of the AsCas12f the UnCas12f complexes. a, Superimposition of two complexes based on the protein part. UnCas12f is colored grey. The C-lobe of UnCas12f.2 (321 - 529) was hidden for comparison. RMSD = 6.117Å (all atom). **b**, The N-lobe of AsCas12f.1 superimposed with the N-lobe of UnCas12f.1 (grey). RMSD = 6.117 Å (all-atom). **c**, The C-lobe of AsCas12f.1 superimposed with the C-lobe of UnCas12f.1 (grey). RMSD = 2.344 Å (all-atom).



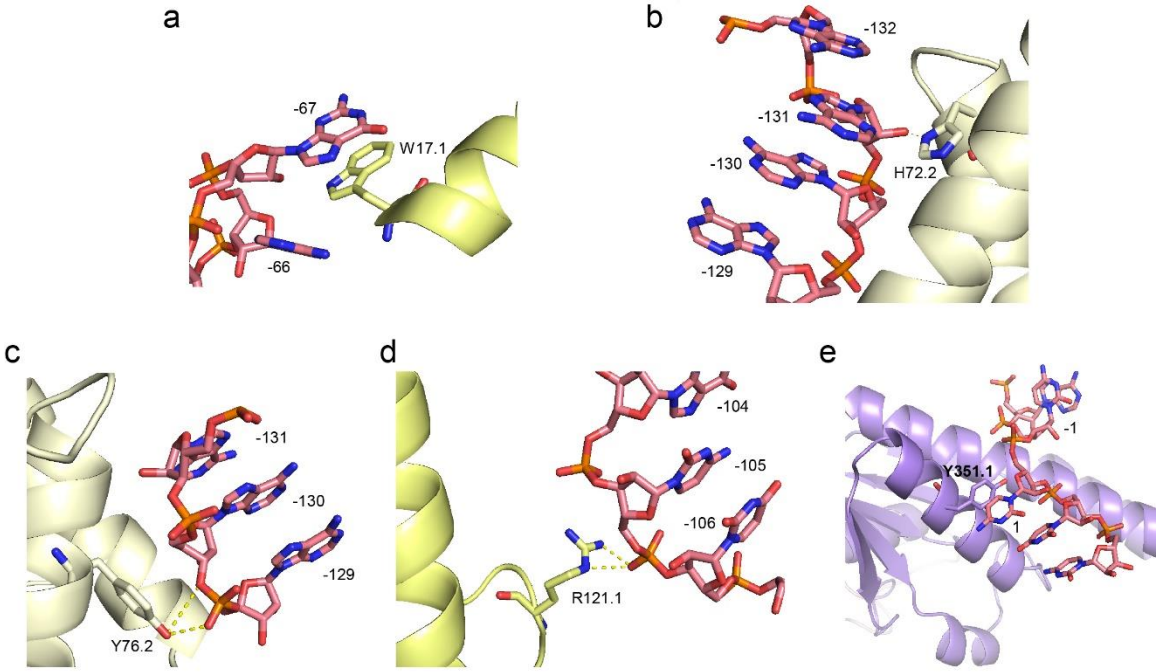
Supplementary Fig. 12 | Dimerization is essential for the activity of AsCas12f. a, Zoom-in views of the dimer interfaces. **b**, Indel frequencies generated by wild-type AsCas12f and AsCas12f variants bearing mutations that disrupt the dimer interfaces.



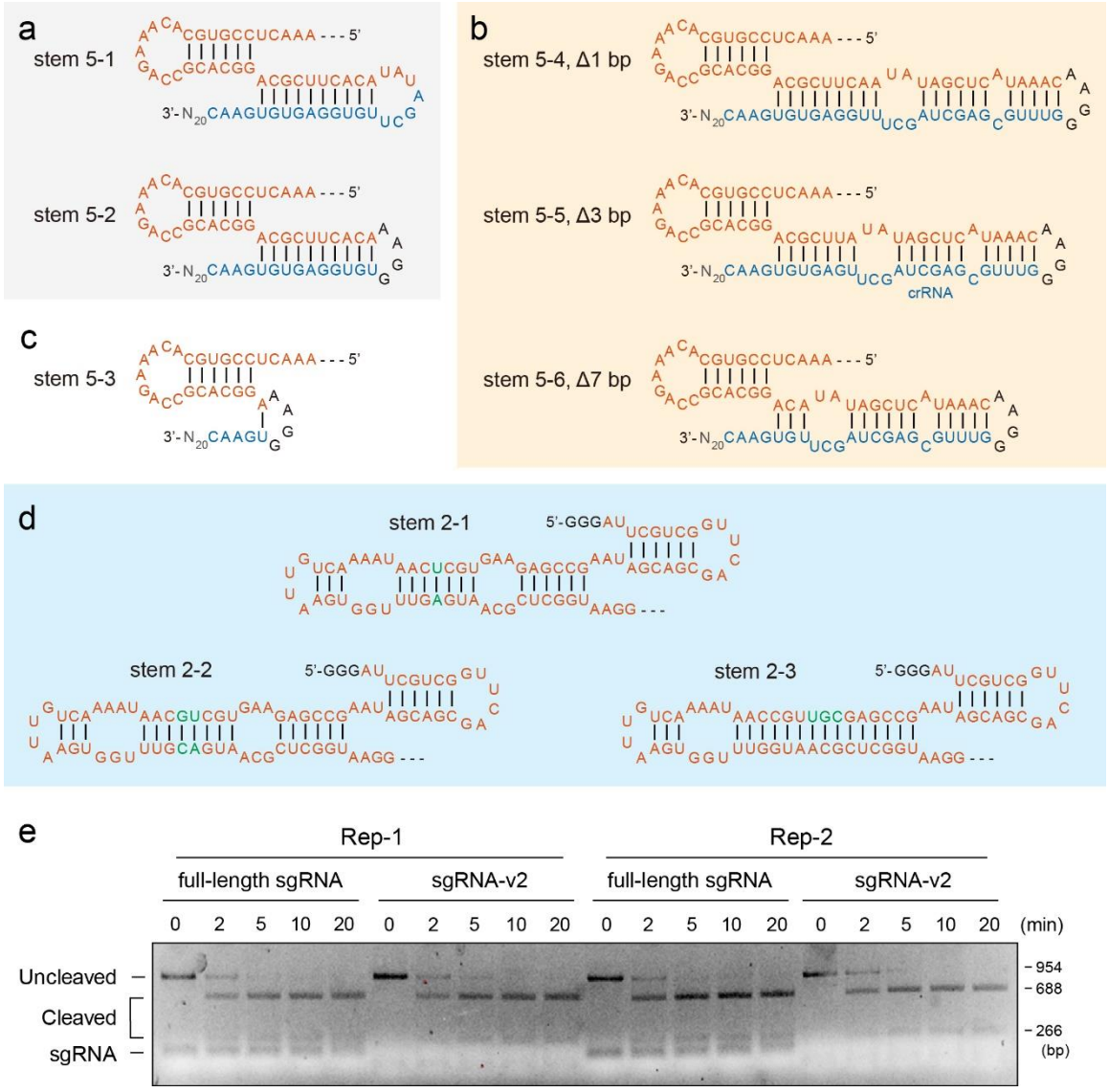
Supplementary Fig. 13 | PAM and sgRNA recognition by AsCas12f. a, **b**, Recognition of the non-target strand (**a**) and the target strand (**b**). Numberings of sgRNA and DNA are shown in Supplementary Figure 12. **c**, Indel frequencies generated by wild-type AsCas12f and AsCas12f variants bearing mutations that disrupt interactions between the protein and DNA (blue) or sgRNA (yellow).



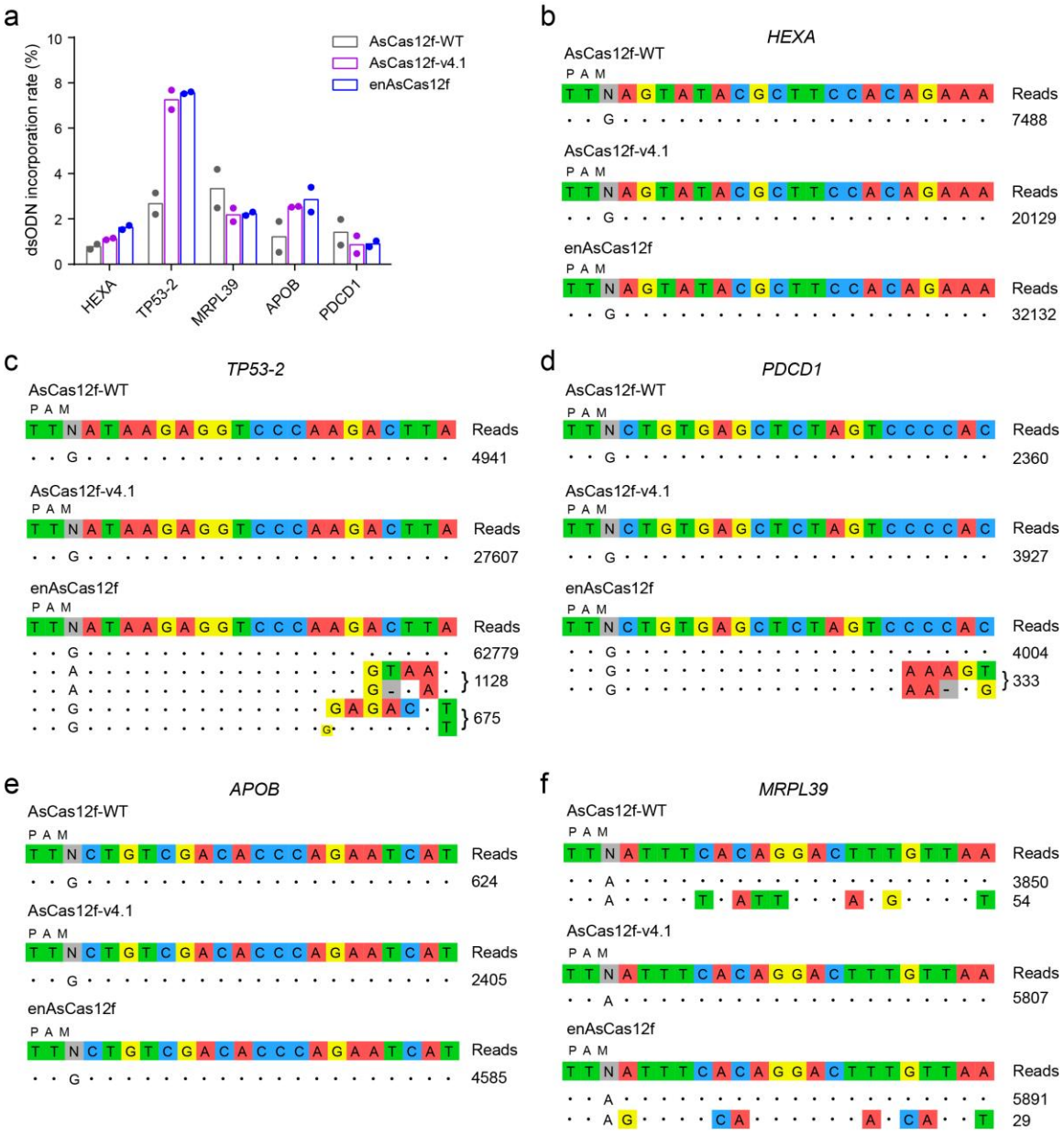
Supplementary Fig. 14 | Comparison of AsCas12f and UnCas12f gRNAs. a, Secondary structure scheme of the wild-type AsCas12f sgRNA and regions interacting with the target DNA. **b-c**, Structure of the sgRNA and the target dsDNA in AsCas12f-sgRNA-DNA (**b**) and UnCas12f-sgRNA-DNA (**c**) complexes. **d,** Superimposition of UnCas12f sgRNA (violet) and AsCas12f sgRNA (red).



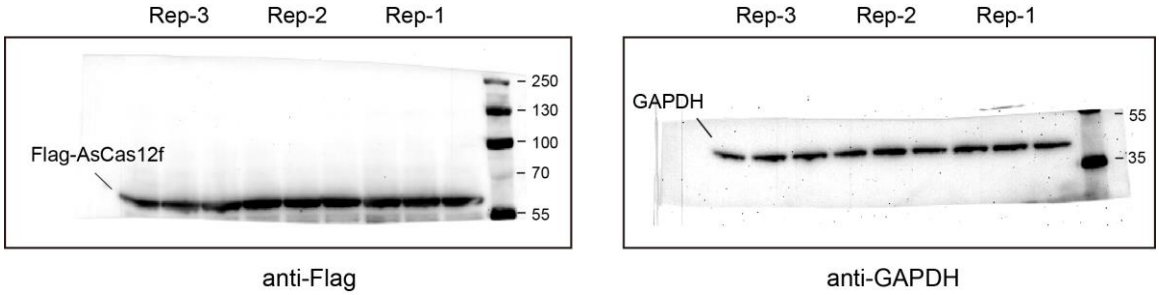
Supplementary Fig. 15 | AsCas12f-gRNA interactions. a, W17.1 forms a π - π interaction with G(-67) of the gRNA. **b**, H72.2 forms a hydrogen bond with A(-131) at O^2 position. **c**, Y76.2 forms a hydrogen bond with the phosphate backbone of A(-129) of the gRNA. **d**, R121.1 forms a hydrogen bond with the phosphate backbone of C(-105) of the gRNA. **e**, Y351.1 forms a π - π interaction with C(1) of the gRNA.



Supplementary Fig. 16 | Schematics showing modifications on the AsCas12f gRNA. **a-c**, Truncations of stem 5 (colored in the grey and yellow boxes in Supplementary Fig. 12a). **d**, Modifications of stem 3 (colored in the blue box in Supplementary Fig. 12a). **e**, Gel electrophoresis monitoring *in vitro* DNA cleavage over time courses using full-length sgRNA and sgRNA-v2. The assay was conducted using enAsCas12f at 37 ℃. Expected DNA sizes are noted on the right of the gel images.



Supplementary Fig. 17 | Genome-wide specificity of wild-type and engineered AsCas12f variants. a, On-target GUIDE-seq tag integration efficiency measured by targeted amplicon sequencing. **b-f**, Off-target editing sites for wild-type AsCas12f, AsCas12f-v4.1, and enAsCas12f with gRNAs targeting *HEXA* (**b**), *TP53-2* (**c**), *PDCD1* (**d**), *APOB* (**e**), and *MRPL39* (**f**) loci, reported by GUIDE-seq in HEK293T cells. Mismatch positions are highlighted in colors. GUIDE-seq experiments were performed in duplicates, with the read counts of one replicate shown to the right of the corresponding sequences. Results from the other replicate are shown in Fig. 5. Full-length sgRNAs were applied in all GUIDE-seq experiments. In (f), the two off-target integrations detected in AsCas12f-WT and enAsCas12f samples are regarded as false positives because they are not detected in the other replicate nor other conditions for the same target.



Supplementary Fig. 18 | Uncropped scans for blots shown in Supplementary Fig. 5a.