

Supplementary Information

ATPase facilitates PlmCas12e-mediated gene editing through conformational modulation

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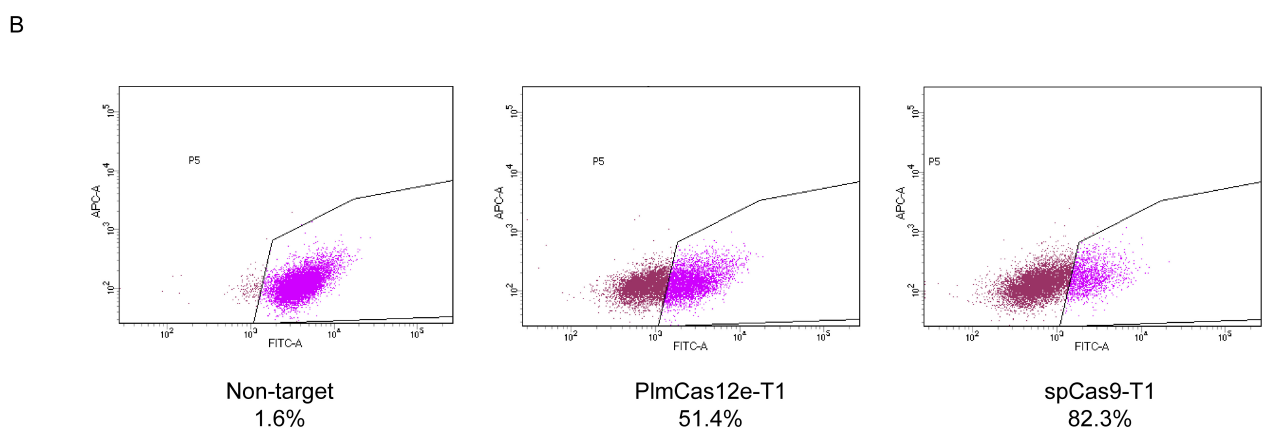
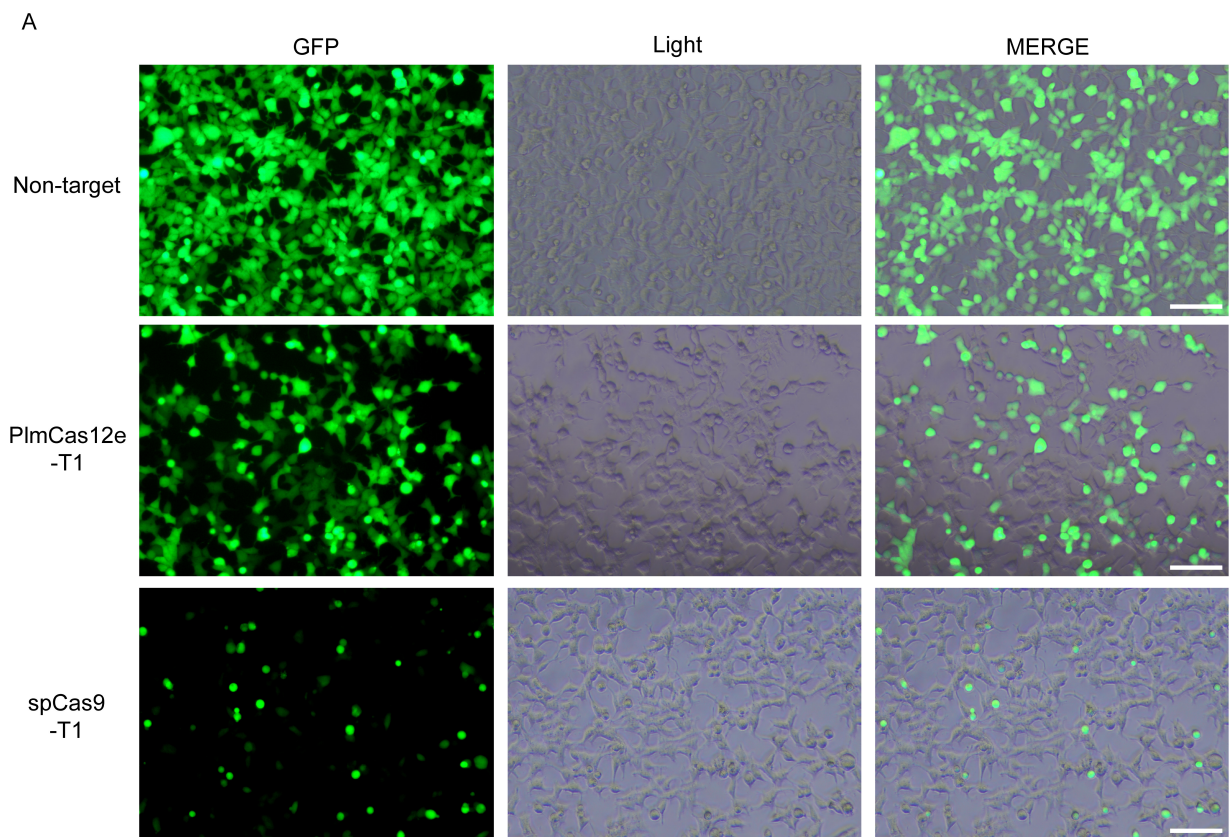


Figure S1 Representative examples of GFP reporter system. A, Representative fluorescence microscopy images showing CRISPR-Cas-mediated GFP knockout in HEK293T cells. Scale bar, 100 μ m. B, Representative flow cytometry plots quantifying editing efficiency for PlmCas12e and SpCas9.

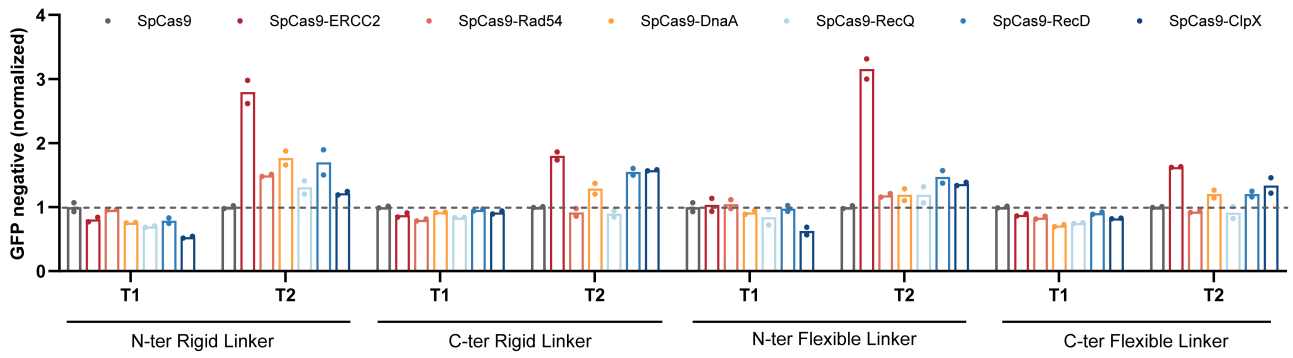


Figure S2 Initial editing efficiency screening of SpCas9-ATPase variants. Editing efficiency of SpCas9 fused to six ATPases at the N- or C-terminus via flexible or rigid linkers, targeting two EGFP sites. Data are presented as the mean from two biological replicates.

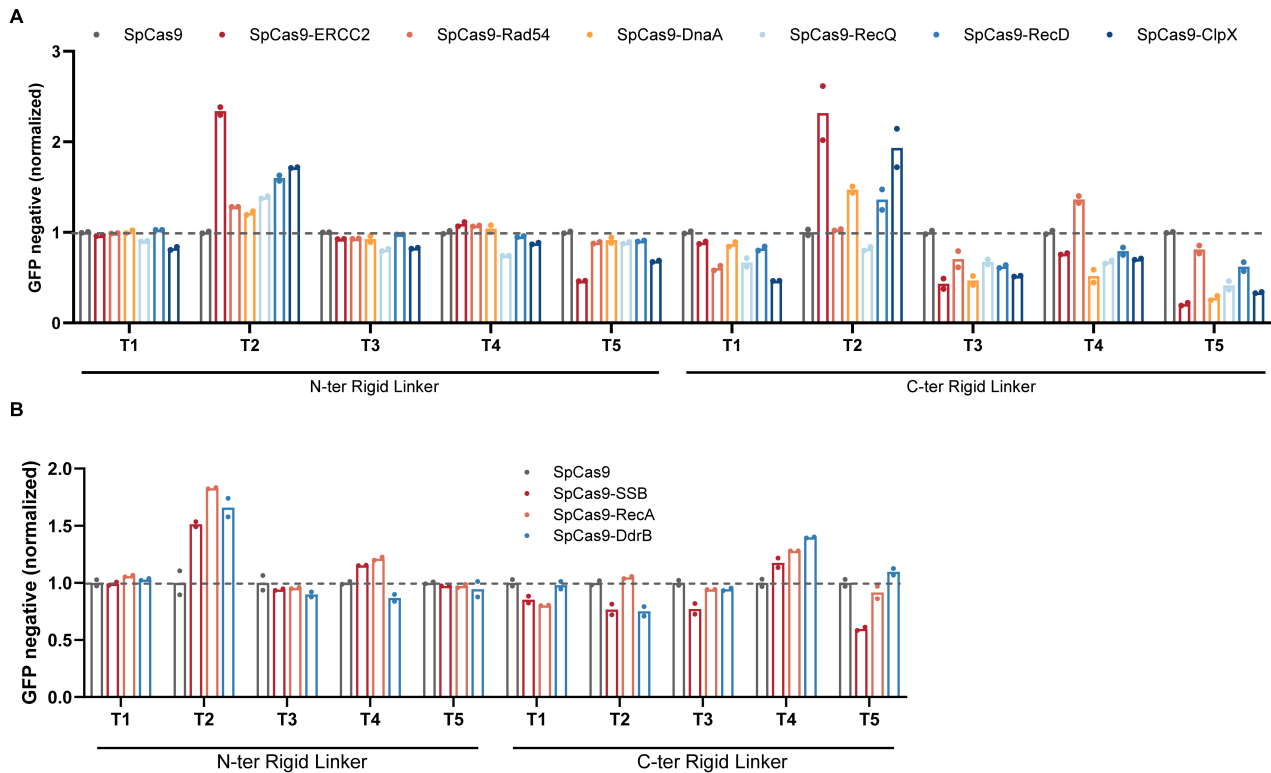


Figure S3 Further editing efficiency validation of spCas9 variants using the GFP reporter assay. A, Normalized frequency of GFP-negative cells for six ATPase variants fused to SpCas9 via rigid linkers, targeting five EGFP loci. B, Normalized frequency of GFP-negative cells for three small ATPase variants fused to SpCas9 via rigid linkers, targeting five EGFP loci. Data are presented as the mean from two biological replicates.

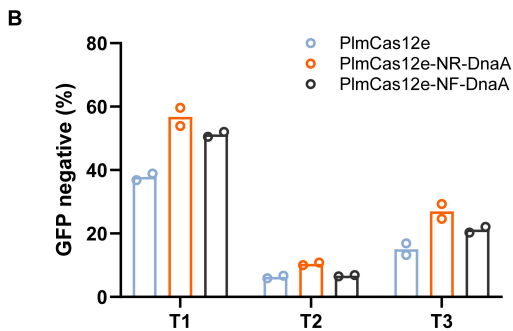
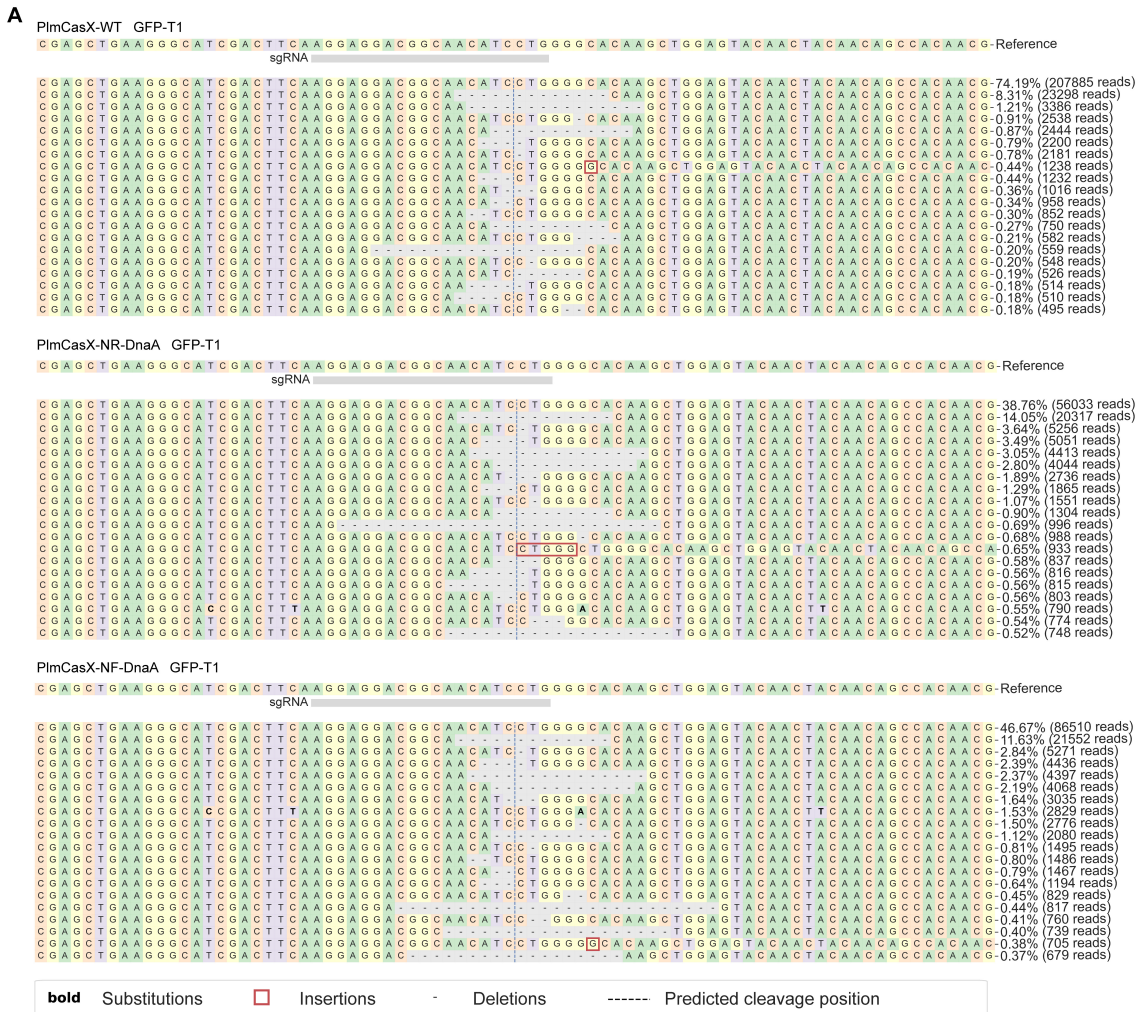


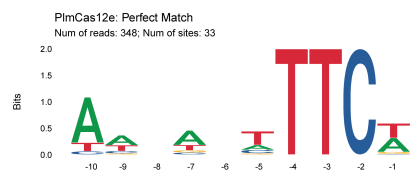
Figure S4 Further editing efficiency validation of PlmCas12e-DnaA variants. A, Representative NGS analysis of indels at the GFP-T1 locus, analyzed by CRISPResso2. B, Independent validation of editing efficiency using the GFP reporter system across three targets. Data are presented as the mean from two biological replicates.

A

PlmCas12e_Rep1RC

Reads	Range	Type	Gene
ACCAATTTTCA	chr3:52549926-52549955	Intron	PBRM1
TTTTGTTTCA	chr1:45751854-45751883	Upstream	IPP
AACACTTTTCT	chr11:3813664-3813693	Exon	PGAP2
AAAGATTTCCT	chr8:55767646-55767675	Intron	TMEM68
AAAGCTTTTCT	chr14:73637599-73637628	Intergenic	ACOT6-DNAL1
AAAAGCTTTC	chr16:58554950-58554979	Intron	CNOT1
ACTGCTTTCCT	chr7:27072469-27072497	Intergenic	SFAP2-HOXA1
ATCAACTTCG	chr1:18256267-18256296	Intron	DEF
ATAAACTTCA	chr3:133658172-133658201	Intron	TOPBP1
ATAATTTTCT	chr20:31626937-31626966	Intergenic	MIR3193-COX4I2
AATATTTC	chr15:85096155-85096184	Intron	PDE8A
ATAAATTTCT	chr12:132884795-132884824	Intron	CHFR
CAGCCTTTCT	chr10:102777845-102777874	Exon	WBP1L
AATTTATTCA	chr2:745644929-74564558	Intron	MIAF
AAAATGTTCA	chr19:33659543-33659572	Exon	CHST8
TACTCATTCA	chr3:57254143-57254172	Exon	APPL1

D

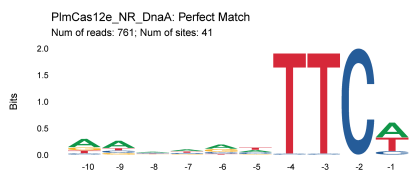


B

PlmCas12e_NR_DnaA_Rep1RC

Reads	Range	Type	Gene
GGACAATTTC	chr19:17935863-17935892	Exon	CCDC124
ACATAATTTC	chr3:23388715-23388744	Exon	UBE2F2
CCTGCTTTCCT	chr8:125487855-125487884	Intergenic	TRIB1-LINC00861
GACAATGTTCT	chr3:47673932-47673961	Intron	SMARCC1
AATACTTTTC	chr11:34426531-34426560	Intergenic	ATBR2-CAT
TAGCACTTTC	chr4:151168713-151168742	Intron	SH3D19
TACAAGTTTC	chr15:72622985-72623014	Intron	LINC02259
AAAAGCTTTC	chr16:58554950-58554979	Intron	CNOT1
AAAAACTTTC	chr7:70257598-70257627	Exon	AUTS2
AAATAATTTC	chr2:231236505-231236534	Exon	ARMC9
ATAATTTTCT	chr21:17795753-17795782	Upstream	C21orf91-OT1
AACAAGTTTC	chr17:49366394-49366423	Upstream	ZNF652
ATAACTTTCT	chr15:26039613-26039642	Intron	LINC02346
ACTAAGTTTC	chr6:89004344-89004373	Intergenic	RNU1T-PNRC1
CAGCCTTTCCT	chr10:102777845-102777874	Exon	WBP1L
AAAAAATTTC	chr17:61883462-61883491	Intron	INTS2

E



C

PlmCas12e_NF_DnaA_Rep1RC

Reads	Range	Type	Gene
ATAAACTTTC	chr2:133658172-133658201	Intron	TOPBP1
ACATAATTTC	chr8:69461011-69461040	Intron	APBA1
AACATGTTCT	chrX:100748739-100748768	Intergenic	SYTL4-CSTF2
ACCAATTTTC	chr3:52549926-52549955	Intron	PBRM1
AAAAGCTTTC	chr16:58554950-58554979	Intron	CNOT1
ATTTATTTCCT	chr4:39215053-39215082	Exon	WDR19
TTGTTGTTCT	chr22:37422415-37422444	Intron	ELFN2
AAAAGATTTC	chr6:41852898-41852927	Intron	USF49
ATAATTTTCT	chr21:17795753-17795782	Upstream	C21orf91-OT1
TACAAGTTTC	chr15:72622985-72623014	Intron	LINC02259
CACAATTTTC	chr10:102221776-102221807	Intergenic	NOLC1-ELOVL3
TCCTTTTTCCT	chr6:121309742-121309771	Intron	TBC1D32
TGGAACATTCCT	chr6:110625445-110625474	Intron	CDK19
TCTACTTTCCT	chr5:137626712-137626741	Intron	KLHL3
TATTTATTCT	chr1:143755644-143755673	Downstream	LOC105375800
GATAGATTCT	chr9:35173704-35173733	Exon	UNC13B

F

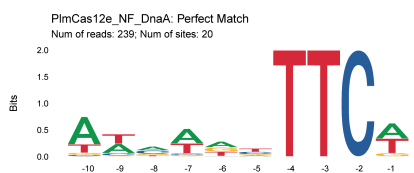


Figure S5 GenomePAM analysis of PlmCas12e variants. A-C, Top-ranked perfect match reads from the GenomePAM assay using the Rep-1RC spacer in HEK293T cells. D-F, Summary of PAM motifs derived from associated perfect match reads, indicating that DnaA fusion does not alter the native PAM preference of PlmCas12e.

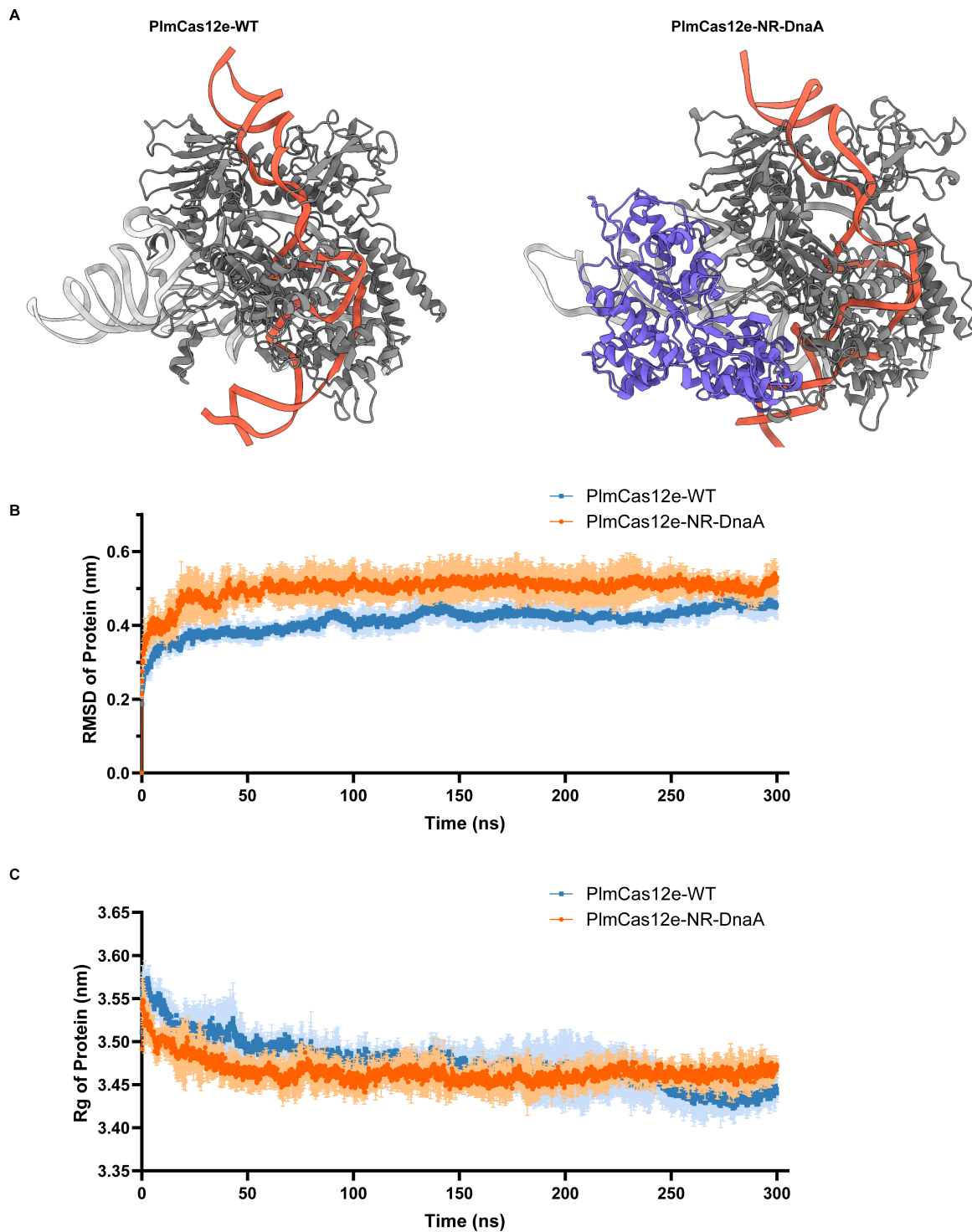


Figure S6 Molecular dynamics analysis of PlmCas12e variants. A, Representative snapshots of the PlmCas12e-WT and PlmCas12e-NR-DnaA ternary complexes extracted from the 300 ns simulation. Regions are colored as follows: Cas12e (dark gray), DnaA (purple), dsDNA (red), and sgRNA (light gray). B, Time-course RMSD of the Cas12e protein domain alone for PlmCas12e-WT and PlmCas12e-NR-DnaA. C, Time-course Rg of the Cas12e protein domain alone for PlmCas12e-WT and PlmCas12e-NR-DnaA. Data are presented as mean \pm SD from three independent replicates.

Table S1 Information and functions of selected ATPase.

ATPase	Organism	Length	Main function
Rad54	<i>Homo sapiens</i>	747 aa	DNA recombination
ERCC2	<i>Homo sapiens</i>	761 aa	DNA transcription
DnaA	<i>Escherichia coli</i>	467 aa	DNA replication
RecD	<i>Escherichia coli</i>	608 aa	DNA repair
RecQ	<i>Escherichia coli</i>	609 aa	DNA repair
ClpX	<i>Escherichia coli</i>	424 aa	Subunit of CLP proteinase
RecA	<i>Deinococcus radiodurans</i>	363 aa	DNA repair
DrSSB	<i>Deinococcus radiodurans</i>	301 aa	DNA replication, recombination
DdrB	<i>Deinococcus radiodurans</i>	188 aa	DNA repair, genome reconstitution

Table S2 Spacers for GFP disruption used in the study.

ID	Spacer
GFP Disruption Targets for spCas9	
T1	GGCATCGACTTCAAGGAGGA
T2	CAGAACACCCCCATCGGCGA
T3	GACGTAGCCTTCGGGCATGG
T4	GGGCACGGGCAGCTTGCCGG
T5	GATGCCGTTCTTCTGCTTGT
GFP Disruption Targets for PlmCas12e	
T1	AGGAGGACGGCAACATCCTG
T2	CCTCGGCGCGGGTCTTGTAG
T3	GGCATGGCGGACTTGAAGAA
T4	GGGTCAGCTTGCCGTAGGTG
T5	TCTGCACCACCGGCAAGCTG
T6	GCCGCTACCCCGACCACATG
T7	AGATCCGCCACAACATCGAG
T8	GCTGGTAGTGGTCGGCGAGC

Table S3 NGS Spacers and relative primers used in the study.

ID	Spacer	Primer-F	Primer-R
Endogenous Targets of PlmCas12e			
B2M-1	ATGTCGGATGGATG AAACCC	ACACTCTTTCCTACACGAC GCTCTTCCGATCTTCCCGAT ATTCCTCAGGTACTCCAAAG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTGCTGAA AGACAAGTCTGAATGCTCC
B2M-2	CACGGCAGGCATAC TCATCT	ACACTCTTTCCTACACGAC GCTCTTCCGATCTCAGACTT GTCTTTCAGCAAGGACTGG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTGACTAC TCATACACAACCTTCAGCAG C
FANCF-1	CTAATCCCGGAACT GGACCC	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGTTCTCC AGCAGGCGCAGAG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTCTGCGC CACATCCATCGGC
FANCF-2	GAAGGTCATAGTGC AAACGT	ACACTCTTTCCTACACGAC GCTCTTCCGATCTCTATTGT GCAACTCCTCCAGGG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTCTTTTGT CGCGCCCTCCAG

VEGFA-1	TGGATGTCTATCAG CGCAGC	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGAGCTGG GTGGAGAGAGGGG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTGGCTTG AAGATGTACTCGATCTCATC AG
VEGFA-2	TGATGATTCTGCCC TCCTCC	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGTGGCTG GGCCTGTGCAC	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTTTAGGC CATCCACCCATCCCCTG
VEGFA-3	AGATTATGCGGATC AAACCT	ACACTCTTTCCTACACGAC GCTCTTCCGATCTCTGGGTG CTGAGTGGCAGG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTACATCC TCACCTGCATTACATTTGT
Exogenous Targets of PlmCas12e			
GFP-T1	AGGAGGACGGCAA CATCCTG	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGTTCGAG GGCGACACCCTG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTGTTCAC CTTGATGCCGTTCTTCTG
GFP-T2	CCTCGGCGCGGGTC TTGTAG	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGCCGCTA CCCCGACCAC	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTCCCCAG GATGTTGCCGTCC
GFP-T3	GGCATGGCGGACTT GAAGAA	ACACTCTTTCCTACACGAC GCTCTTCCGATCTGAAGTTC ATCTGCACCACCGG	GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTTCGGCG CGGGTCTTGTAG

Table S4 Protein sequences used in the study.

Protein ID	Sequence
PlmCas12e	QEIKRINKIRRRLLVKDSNTKKAGKTGPMKTLVLRVMTPLRERLENLRKKPENIPQ PISNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVAQPAPKNIDQRK LIPVKDGNERTSSGFACSQCCQPLYVYKLEQVNDKGGKPHNTNYFGRCNVSEHERLI LLSPHKPEANDELVTYSLGKFGQRALDFYSIHVTRESNHPVKPLEQIGGNSCASGPV GKALSDACMGAVASFLTKYQDIILEHQKVIKKNEKRLANLKDIASANGLAFPKITL PPQPHTKEGIEAYNNVVAQIVIWNLNWQKLKIGRDEAKPLQRLKGFPSFPLVER QANEVDWWMVCNVKLINEKKEDGKVFQNLQLAGYKRQEALLPYLSSEEDRKK GKKFARYQFGDLLLHLEKKHGEDWGKVYDEAWERIDKKVEGLSKHIKLEEERS EDAQSKAALTDWLRAKASFVIEGLKEADKDEFRCCELKLQKWYGDLRGKPFQIAEA ENSILDISGFSKQYNCAFIWQKDGVKLNLYLIINYFKGGKLRFKKIKPEAFANRF YTVINKKSGEIVPMEVNFNFDPPNLIILPLAFGKRQGREFIWNDLLSLETGSLKLAN GRVIEKTLYNRRTRQDEPALFVALTFERREVLDSSENIKPMNLIGIDRGENIPAVIALT DPEGCPLSRFKDSLGNPHTILRIGESYKEKQRTIQAAKEVEQRRAGGYSRKYASKA KNLADDMVRNTARDLLYYAVTQDAMLIFENLSRGGFRQGRKFMAERQYTRME DWLTAKLAYEGLPSKTYLSKTLAQYTSKTCNSCGFTITSADYDRVLEKLKKTATG WMTTINGKELKVEGQITYYNRYKRQNVVKDLSVELDRLSEESVNNDISSWTKGRS GEALSLLKKRFSHPVQEKFVCLNCGFETHADEQAALNIARSWLFLRSQEYKKYQ TNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV

spCas9

DKKYSIGLDIGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGE
TAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLI
EGDLNPDNSVDKLFQVLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIA
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DQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
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KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGGKSKLKSVEKLLGITIMERSSEFEKN
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NFLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKV
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IHQSITGLYETRIDLSQLGGD

ERCC2

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Rad54

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DRLHCRRCVNSRQIRPPPDGSDCTSDLAGWNHCTDKWGLRDEVLQAAWDAASTA
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DnaA

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RecQ

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DDEE

RecD

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ClpX	TDKRKDGSGKLLYCSFCGKSQHEVRKLIAGPSVYICDECVDLCNDIREEIKEVAPH RERSALPTPHEIRNHLDDYVIGQEQAkkVLAVAVYNHYKRLRNGDTSNGVELGKS NILLIGPTGSGKTLLAETLARLLDVPFTMADATTLTEAGYVGEDVENIIQKLLQKCD YDVQKAQRGIVYIDEIDKISRKSDNPSITRDVSGEGVQQALLKLIEGTVA AVPPQGG RKHPQQEFLQVDTSKILFICGGAFAGLDKVISHRVETGSGIGFGATVKAKSDKASE GELLAQVEPEDLIKFLIPEFIGRLPVVATLNELSEEALIQLKEPKNALTKQYQALF NLEGVDLEFRDEALDAIAKKAMARKTGARGLRSIVEAALLDTMYDLPSMEDVEK VVIDESVIDGQSKPLLIYGKPEAQQASGE
SSB	ARGMNHVYLIGALARDPELRYTGNGMAVFEATVAGEDRVIGNDGRERNLPWYH RVSILGKPAEWQAERNLKGGD AVVVEGTLEYRQWEAPEGGKRS AVNVKALRME QLGTQPELIQDAGGGVRMSGAMNEVLVLGNVTRDPEIRYTPAGDAVLSLSIAVNE NYQDRQGQRQEKVHYIDATLWRDLAENMKELRKGD PVMIMGRLVNEGWTDKD GNKRNRSTRVEATRVEALARGAGNANSGYAAATPAAPRTQTASSAARPTSGGYQS QPSRAANTGSRSGGLDIDQGLDDFPPEEDLFP
RecA	SKDATKEISAPTDAKERSKAIETAMSQIEKAFGKGSIMKLG AESKLDVQVVSTGSL LDLALGVGGIPRGRITEIYGPESGGK TTLALAIVAQAQKAGGTCAFIDAEHALDPV YARALGVNTDELLVSQPDNGEQALEIMELLVRSGAIDVVVVSVAALTPRAEIEG DMGDSLPLGLQARLMSQALRKLTAILSKTGTA AIFINQVREKIGVMYGNPETTTGGR ALKFYASVRLDVRKIGQPTKVGND AVANTVKIKTVKNKVAAPFKEVELALVY GK GFDQLSDLVGLAADMDIHKAGSFYSYGDERIGQGKEKTIA YIAERPHEMEQEIRDR VMAAIRAGNAGEAPALAPAPAAPEAAEA
DdrB	LQIEFITDLGARVTVNVEHESRLLDVQRHYGRLGWTSGEIPSGGYQFPIENEADFD WSLIGARKWKSPEGEELVIHRGHAYRRRELEAVDSRKLKLPAAIKYSRGAKVSDP QHVREKADGDIEYVSLAIFRGGKRQERYAVPGGAAGNGQGRPAPQGGQPAQARPQ ATAARPAARPPVQPGQEEETPF

Table S5 SgRNA sequences used in the study.

ID	Sequence
PlmCas12e sgRNA-Scaffold	GGCGCTTTTATCTCATTACTTTGAGAGCCATCACCAGCGACTATGTCGTATGG GTAAAGCGCTTATTTATCGGAGAAACCGATAAATAAGAAGCATCAAAG gggtct tcgagaagaccc
spCas9 sgRNA-Scaffold	Ggggtcttcgagaagacca GTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAA TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT

Bold: DEST spacer for BbsI cleavage.

Table S6 dsODN sequences used in the study.

ID	Sequence
dsODN-Top	5'-Phos- ACTTCGGTACGCGTCGACGTACGGTCGACGAGTCGCGCGTACGACGATCGA*G*A*C
dsODN-Bot	5'-Phos- GTCTCGATCGTCGTACGCGCGACTCGTCGACCGTACGTCGACGCGTACCGA*A*G*T

Table S7 Scores of ipTM and pTM for the AlphaFold3 predicted structures.

Structure ID	ipTM	pTM
PlmCas12e-WT	0.8	0.83
PlmCas12e-NR-DnaA	0.77	0.68