Molecular Cell

Supplemental information

Allosteric control of Type I-A CRISPR-Cas3 complexes and establishment as effective nucleic acid detection and human genome editing tools

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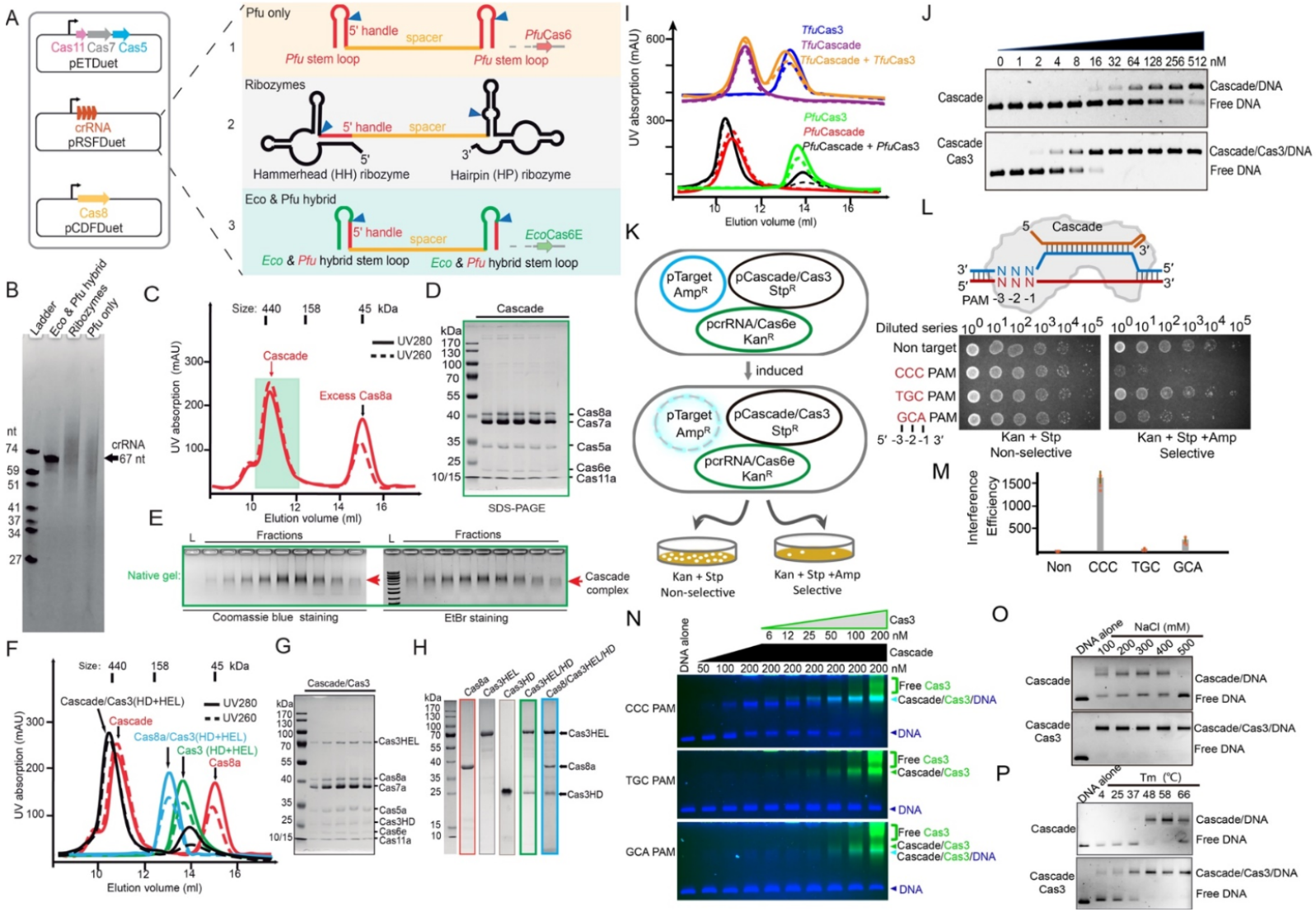
Supplemental Figures S1-S10
Supplemental Tables S1-S3


A
Cas11 Cas7 Cas5
pETDuet
crRNA
pRSFDuet
Cas8
pCDFDuet
1
Pfu only
5' handle
spacer
PfuCas6
Pfu stem loop
Pfu stem loop
2
Ribozymes
5' handle
spacer
5'
3'
Hammerhead (HH) ribozyme
Hairpin (HP) ribozyme
3
Eco & Pfu hybrid
5' handle
spacer
EcoCas6E
Eco & Pfu hybrid stem loop
Eco & Pfu hybrid stem loop
B
Ladder
Eco & Pfu hybrid
Ribozymes
Pfu only
nt
74
59
51
41
37
34
27
crRNA
67 nt
C
Size: 440 158 45 kDa
UV280
UV260
Cascade
Excess Cas8a
UV absorption (mAU)
300
200
100
10 12 14 16
Elution volume (ml)
D
Cascade
kDa
170
130
100
70
55
40
35
25
10/15
Cas8a
Cas7a
Cas5a
Cas6e
Cas11a
SDS-PAGE
E
L
Fractions
L
Fractions
Native gel:
Cascade complex
Coomassie blue staining
EtBr staining
F
Size: 440 158 45 kDa
Cascade/Cas3(HD+HEL)
Cascade
Cas8a/Cas3(HD+HEL)
Cas3 (HD+HEL)
Cas8a
UV280
UV260
UV absorption (mAU)
300
200
100
10 12 14 16
Elution volume (ml)
G
Cascade/Cas3
kDa
170
130
100
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Cas3HEL
Cas8a
Cas7a
Cas5a
Cas3HD
Cas6e
Cas11a
H
Cas8a
Cas3HEL
Cas3HD
Cas3HEL/HD
Cas8a/Cas3HEL/HD
kDa
170
130
100
70
55
40
35
25
15
10
Cas3HEL
Cas8a
Cas3HD
I
UV absorption (mAU)
600
300
TfuCas3
TfuCascade
TfuCascade + TfuCas3
PfuCas3
PfuCascade
PfuCascade + PfuCas3
10 12 14 16
Elution volume (ml)
J
0 1 2 4 8 16 32 64 128 256 512 nM
Cascade
Cascade/DNA
Free DNA
Cascade Cas3
Cascade/Cas3/DNA
Free DNA
K
pTarget Amp^R
pCascade/Cas3 Stp^R
pcrRNA/Cas6e Kan^R
induced
pTarget Amp^R
pCascade/Cas3 Stp^R
pcrRNA/Cas6e Kan^R
Kan + Stp
Non-selective
Kan + Stp +Amp
Selective
L
5'
Cascade
3'
3'
N N N
N N N
5'
5'
3'
PAM -3 -2 -1
Diluted series 10^0 10^1 10^2 10^3 10^4 10^5
10^0 10^1 10^2 10^3 10^4 10^5
Non target
CCC PAM
TGC PAM
GCA PAM
5' -3 -2 -1 3'
Kan + Stp
Non-selective
Kan + Stp +Amp
Selective
M
Interference Efficiency
1500
1000
500
Non
CCC
TGC
GCA
N
DNA alone
Cas3
6 12 25 50 100 200 nM
Cascade
50 100 200 200 200 200 200 200 200 nM
CCC PAM
TGC PAM
GCA PAM
Free Cas3
Cascade/Cas3/DNA
DNA
Free Cas3
Cascade/Cas3
DNA
Free Cas3
Cascade/Cas3
Cascade/Cas3/DNA
DNA
O
DNA alone
NaCl (mM)
100 200 300 400 500
Cascade
Cascade/DNA
Free DNA
Cascade Cas3
Cascade/Cas3/DNA
Free DNA
P
DNA alone
Tm (°C)
4 25 37 48 58 66
Cascade
Cascade/DNA
Free DNA
Cascade Cas3
Cascade/Cas3/DNA
Free DNA

Figure S1. Reconstitution and biochemical characterization of I-A *Pfu* Cascade-Cas3 effector complex, related to Figure 1.

(A) Co-expression scheme and schematic explanation of three strategies to promote efficient pre-crRNA procession in complex reconstitution. **(B)** RNA quality evaluated by denaturing-PAGE in each of the three strategies described in A. **(C)** Elution profile of the successfully purified *Pfu* Cascade on size-exclusion chromatography (SEC). **(D)** SDS-PAGE analysis of the SEC peak fractions. **(E)** Native-PAGE analysis of the SEC peak fractions stained with Coomassie blue and EtBr for protein and nucleic acid components. **(F)** Comparison of the SEC profiles of the reconstituted *Pfu* Cascade-Cas3, Cascade, Cas8a-Cas3, Cas3, and Cas8a complexes and components. **(G)** Representative SDS-PAGE analysis of the SEC peak fractions containing the *Pfu* Cascade-Cas3 complex. **(H)** SDS-PAGE analysis of the purified Cas8a, Cas3 (HD+HEL), Cas3 HEL, Cas HD, and Cas8a-Cas3 (HD+HEL). (**I)** Comparison of the SEC profiles of *Pfu*- and *Tfu*- Cascade+Cas3, Cascade, and Cas3. *Pfu*-Cascade complexes with Cas3 in the absence of the target DNA, whereas *Tfu*-Cascade fails to do so. **(J)** Native-agarose EMSA showing *Pfu*Cascade-Cas3 has much tighter affinity for the target DNA than *Pfu* Cascade alone. **(K)** Schematic diagram explaining the *in vivo* interference setup. **(L)** Evaluation of the PAM code used by *Pfu* Cascade-Cas3 using dilution-spotting assay. **(M)** Quantification of the interference efficiency mediated by three different PAMs using the assay format depicted scheme in K. **(N)** Native-agarose EMSA confirming that CCC PAM promotes tight binding by *Pfu* Cascade-Cas3, presumably through R-loop formation. FAM-labeled dsDNA is rendered in blue color, Cy3-labeled Cas3 in green, Cascade not colored. **(O), (P)** Native-agarose EMSA revealing that *Pfu* Cascade-Cas3/DNA interaction remains stable in the presence of 0.5 M NaCl **(O)** or at 66 ˚C **(P)**, respectively. *Pfu* Cascade/DNA interaction is less stable at each category.

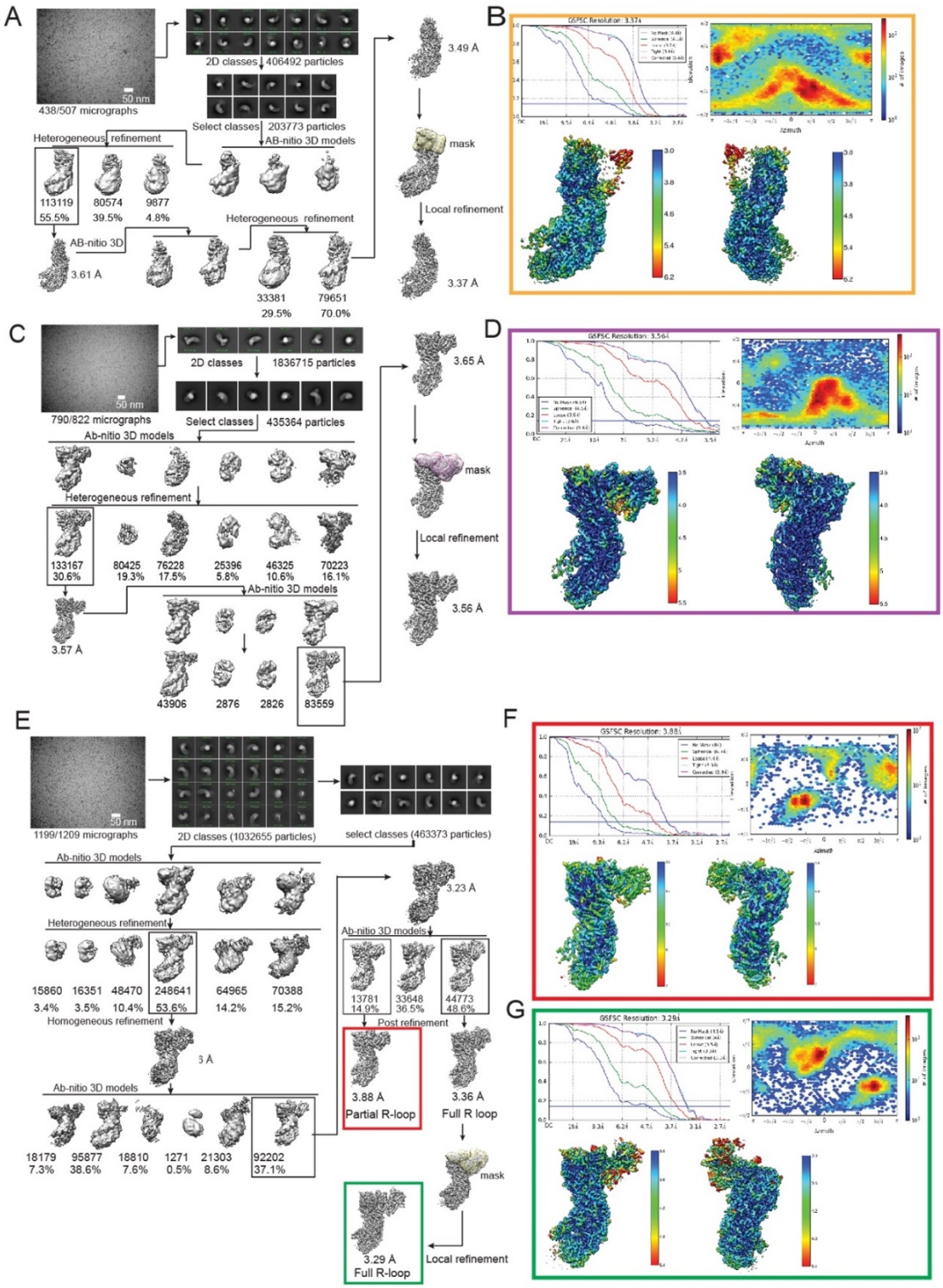

Figure S2. Flow-chart of the cryo-EM single particle reconstructions of *Pfu* Cascade-Cas3 in four different functional states, related to Figure 2.

(A), (B) Workflow of the cryo-EM image processing and 3D reconstruction for the *apo Pfu* Cascade complex. **(C), (D)** Workflow of the cryo-EM image processing and 3D reconstruction for the *Pfu* Cascade-Cas3 complex. **(E), (F), (G)** Workflow of the cryo-EM image processing of the *Pfu* Cascade-Cas3/dsDNA complex, which produced two high-resolution 3D reconstructions, *Pfu* Cascade-Cas3/partial R-loop **(F)** and *Pfu*

Cascade-Cas3/full R-loop **(G)**.

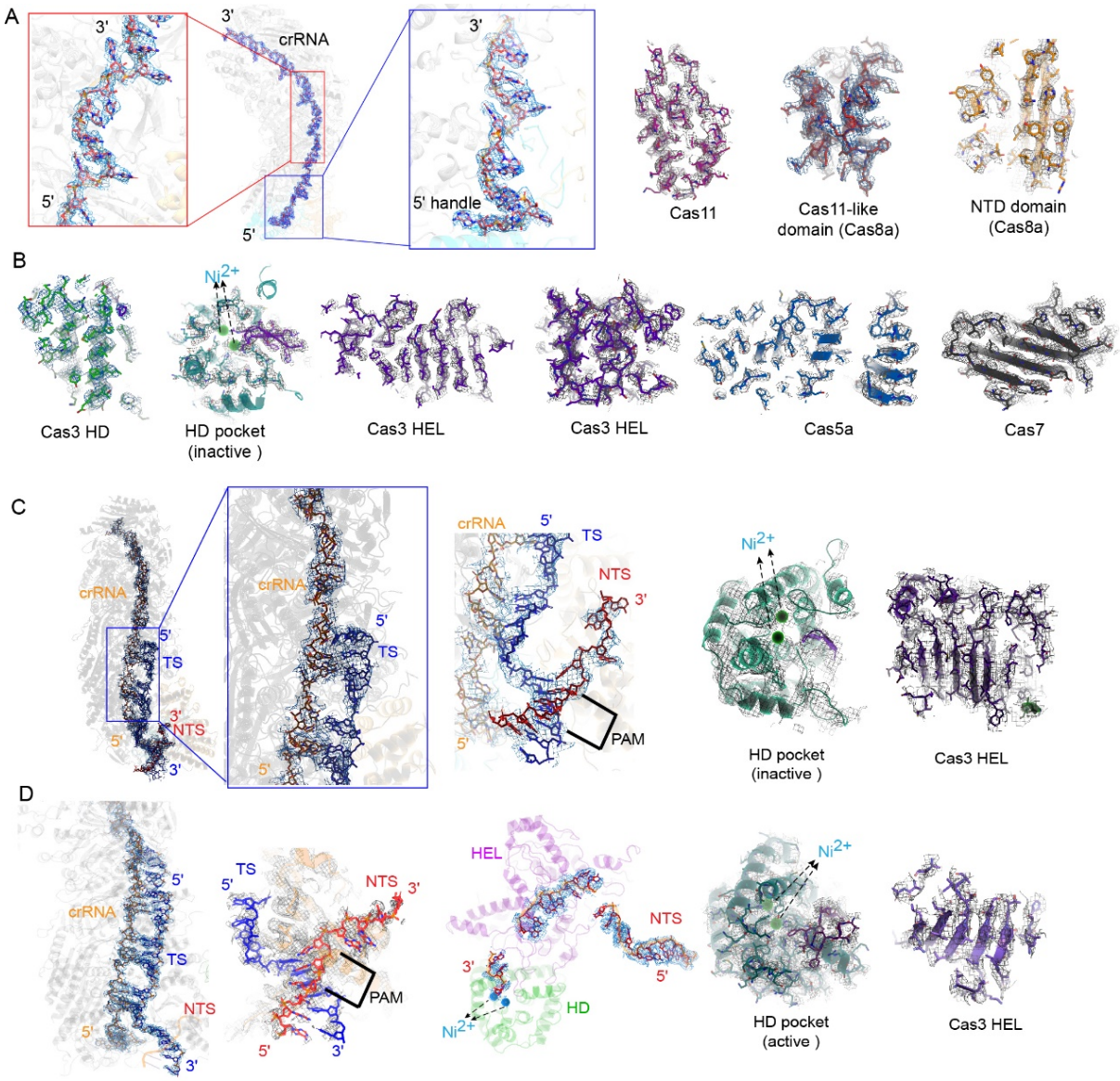

Figure S3. Flow-chart of the cryo-EM single particle reconstructions of *Pfu* Cascade-Cas3 in four different functional states, related to Figure 2.

(A), (B) Workflow of the cryo-EM image processing and 3D reconstruction for the *apo Pfu* Cascade complex. **(C), (D)** Workflow of the cryo-EM image processing and 3D reconstruction for the *Pfu* Cascade-Cas3 complex. **(E), (F), (G)** Workflow of the cryo-EM image processing of the *Pfu* Cascade-Cas3/dsDNA complex, which produced two high-resolution 3D reconstructions, *Pfu* Cascade-Cas3/partial R-loop **(F)** and *Pfu* Cascade-Cas3/full R-loop **(G)**.

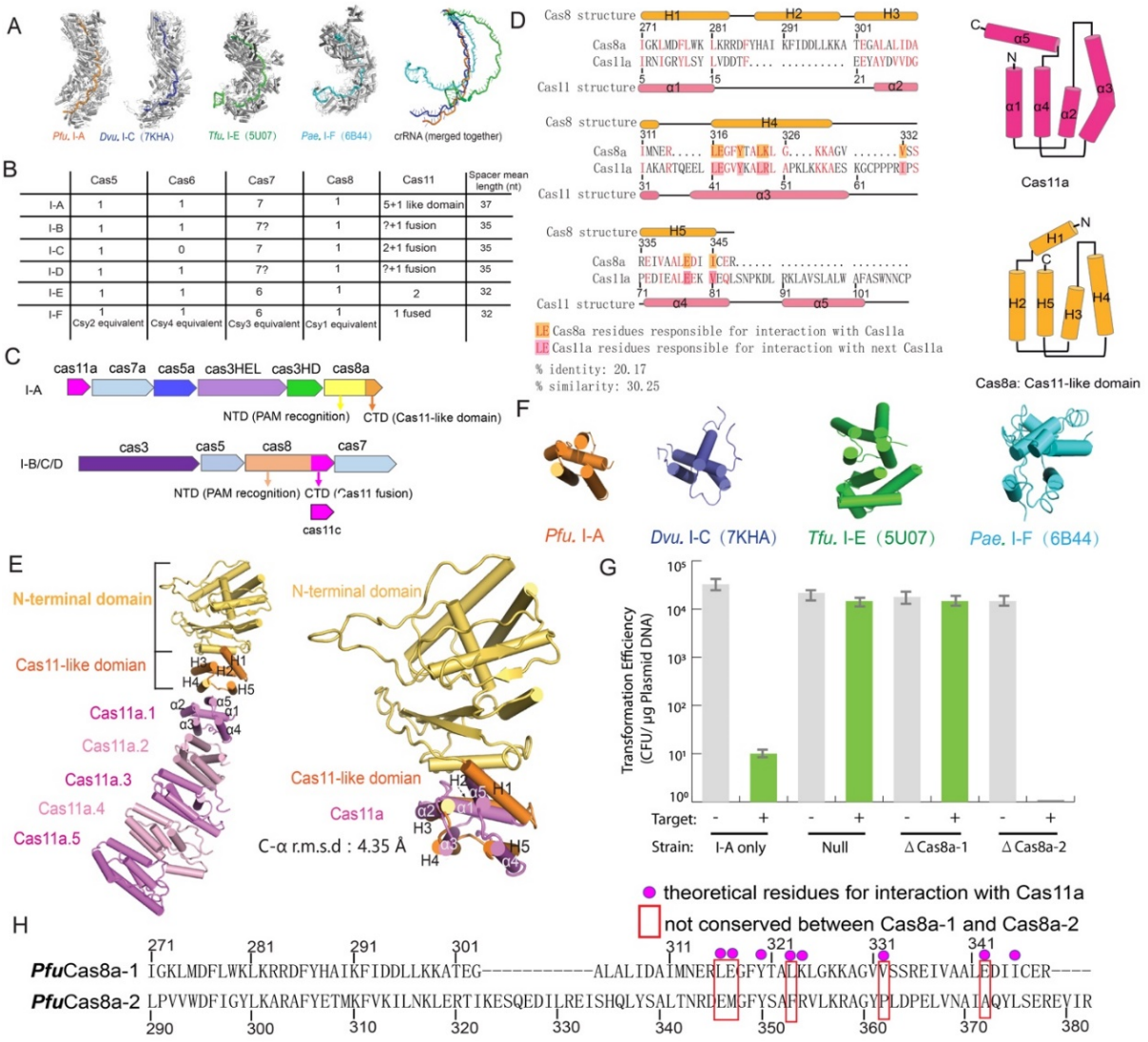

Figure S4. Structure-function analysis of *Pfu* Cascade and *Pfu* Cascade-Cas3, related to Figures 3.

(A) Comparison of the overall shape and backbone curvature among representative I-A, I-C, I-E, and I-F Cascades. PDB accession codes are denoted. I-A *Pfu* Cascade resembles I-C *Dvu* Cascade in overall shape and curvature. **(B)** Tabulating the differences in the subunit stoichiometry and crRNA length among subtypes of Cascades. **(C).** I-A and I-C *cas* operon differences. I-C *cas11* is encoded by a hidden ORF inside *cas8c*. I-A *cas11* is separately encoded and *cas8a* further encodes a Cas11-like domain. **(D)** Left: Sequence and structure similarity between Cas8a CTD (the Cas11-like domain) and Cas11. Interface residues mediating the Cas11-Cas11 interaction are highly conserved in the Cas11-like domain. Right: comparison of the 2D topographic structure between Cas11-like domain of Cas8a and Cas11. **(E)** Arrangement of the *Pfu* Cascade "inner belly", assembled from Cas8a and multiple copies of Cas11. Cas11 and Cas11-like domain in Cas8a are structural homologs, with a Cα r.m.s.d. of 4.35 Å. **(F)** I-A *Pfu* Cas11 is structurally homologous to the I-C Cas11c (PDB: 7KHA), I-E Cas11e (PDB: 5U07), and I-F Cas11 (Csy1 fused) (PDB: 6B44) (G) Cas8a variants knock out interference assay. *P. furiosus* strains with only the I-A effector module (I-A only), deletion of all effector modules (Null), or with only the I-A module and deletion of

either Cas8a-1 or Cas8a-2 (ΔCas81-1 or ΔCas8a-2 respectively) were challenged with either a plasmid containing a miniature CRISPR array consisting of three spacers targeting three essential genes on the *P. furiosus* chromosome (Target: +) or the same plasmid lacking these spacers (Target: -). **(H)** Partial sequence alignment between Cas8a-1, which assembles into *Pfu* Cascade and used in this structural study, and its paralog in *Pfu* I-A *cas* operon Cas8a-2. Interface residues to Cas11 in Cas8a-1 are not conserved in Cas8a-2, which explains why Cas8a-2 does not assemble into *Pfu* Cascade and not functional for interference.

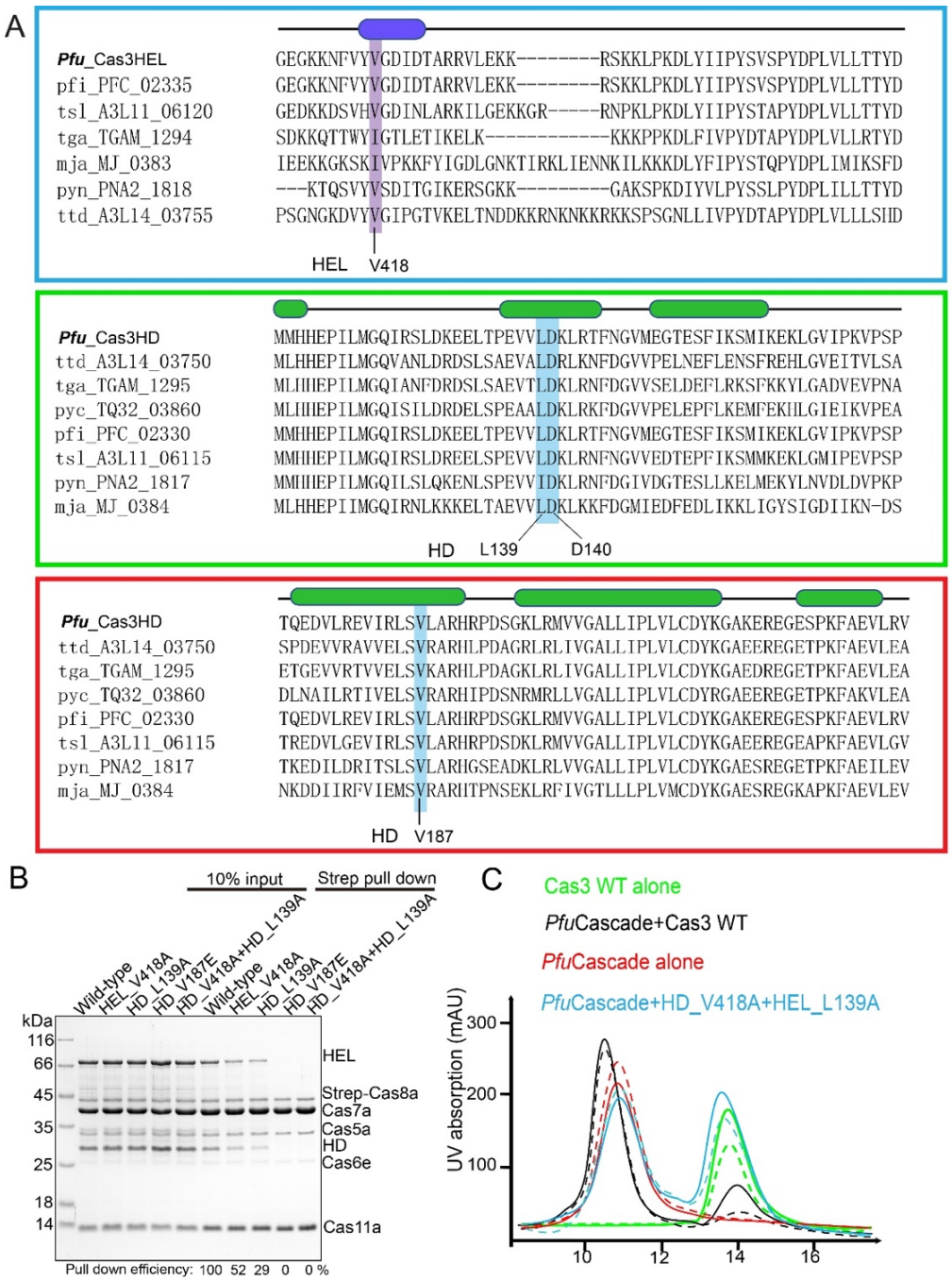

Figure S5, Structure-function analysis of Pfu Cascade and Pfu Cascade-Cas3, related to Figures 3.

(A) Sequence alignment of the Cascade-Cas3 interface residues among representative I-A homologs. Highly conserved interface residues were identified for mutagenesis validation. **(B)** Strep-tagged Cas8a pull-down assay results analyzed by SDS-PAGE. Left five lanes are input controls containing Strep-tagged wildtype and interface mutant *Pfu* Cascade samples, mixed with equimolar amount of Cas3 (HD+HEL). Right five lanes are the samples after Strep-tag pull-down. Note that the HD and HEL subunits of Cas3 are significantly less or completely absent from the mutant lanes.**(C)** SEC profile showing that *Pfu* Cas3 bearing V418A and L139A mutations no longer complexes with *Pfu* Cascade.

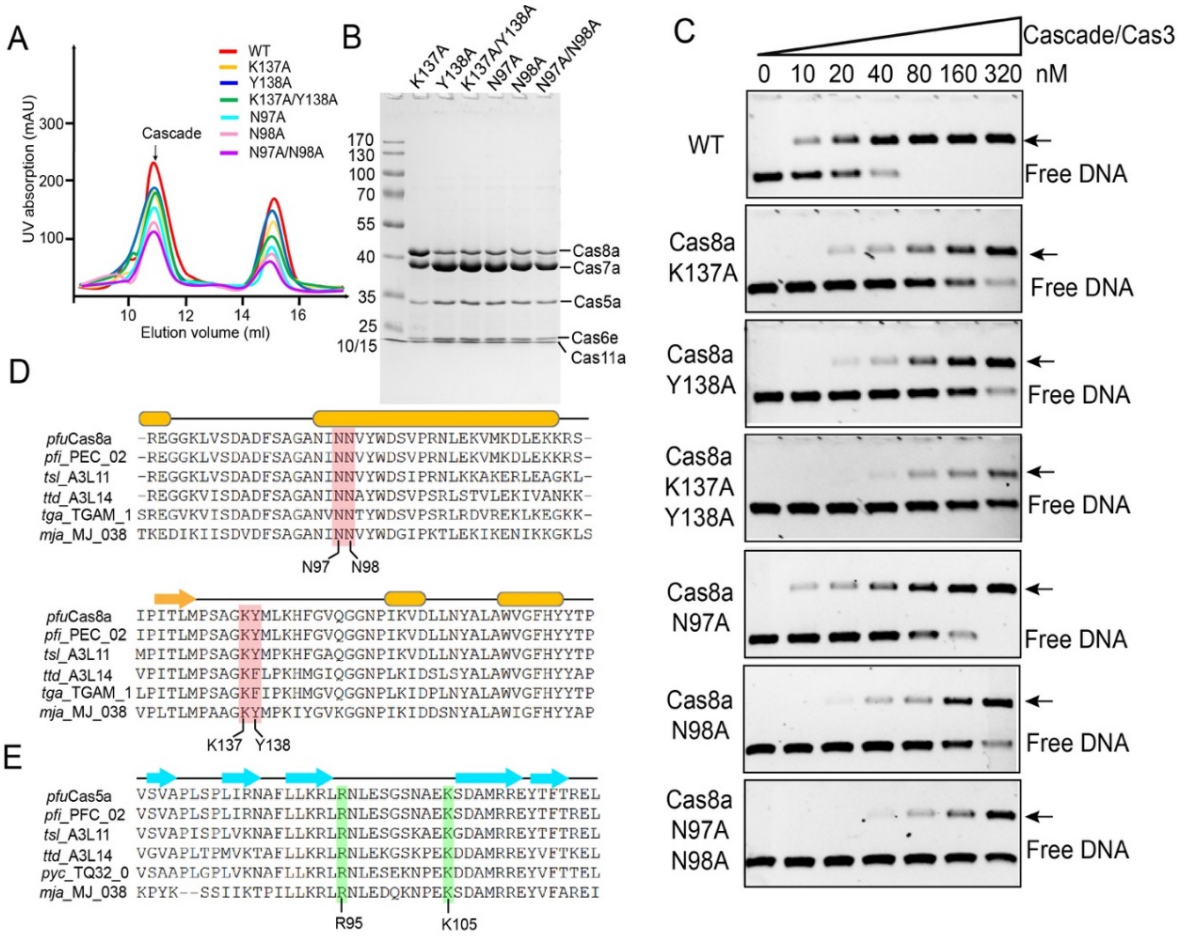

Figure S6. Structure-function validation of the PAM recognition mechanism by *Pfu* Cascade-Cas3, related to Figure 4.

(A) SEC profiles of various *Pfu* Cascade samples bearing PAM-recognition residue mutations. **(B)** SDS-PAGE analysis of the integrity of the PAM recognition mutants purified in (A). **(C)** Native-agarose EMSA quantifying the extent of DNA-binding reduction caused by each PAM-recognition mutation. **(D)** Sequence alignment showing that the PAM-recognition residues are highly conserved among I-A Cas8a homologs. **(E)** Same sequence alignment among Cas5a homologs. Again, PAM-recognition residues are highly conserved.

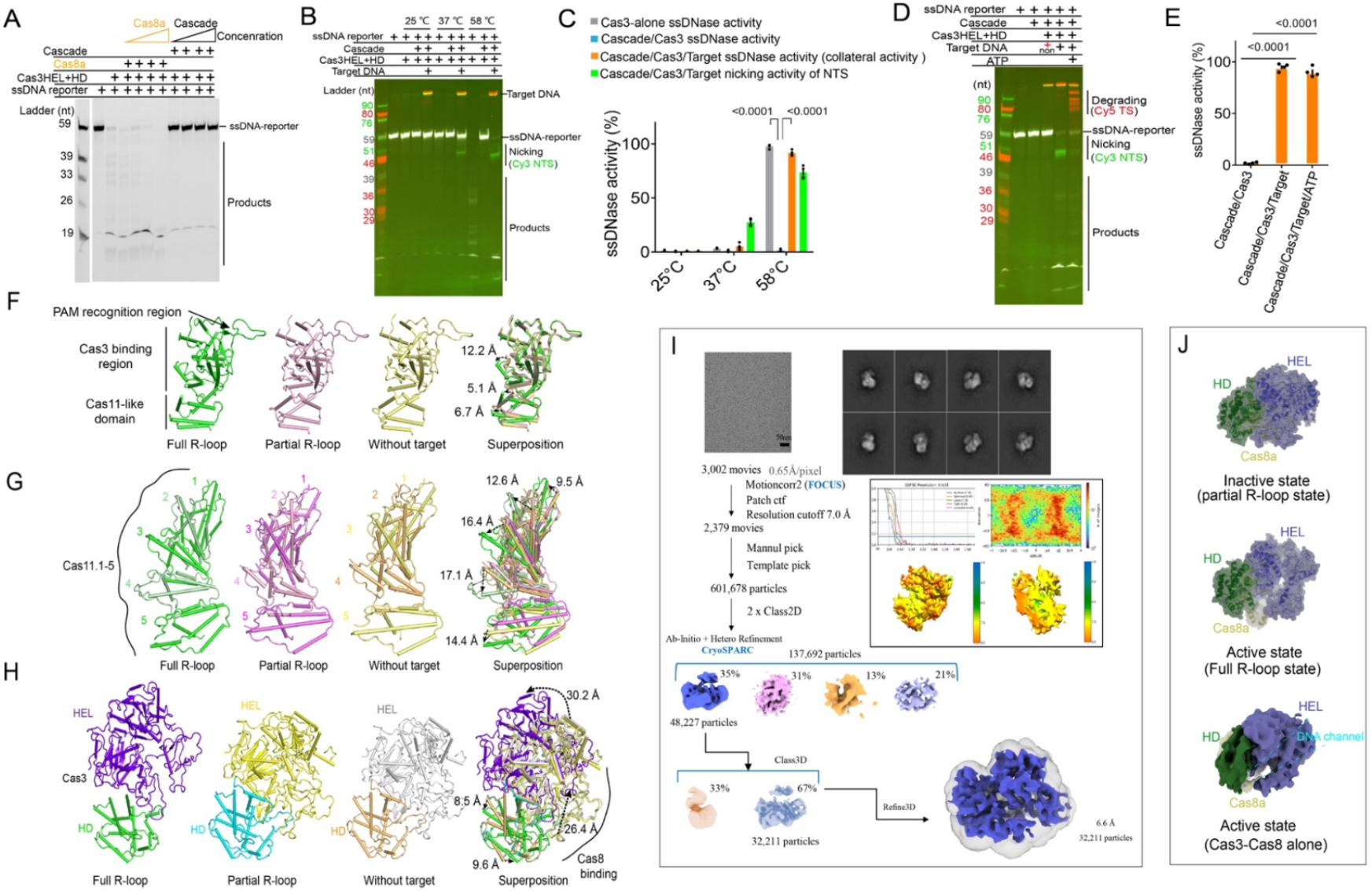

Figure S7. Thorough analysis of the RNA-guided and R-loop-dependent allosteric

activation mechanism inside *Pfu* Cascade-Cas3, related to Figure 5.
(A) Denaturing urea-PAGE showing that the HD-nuclease activity of *Pfu* Cas3 was robust by itself or in complex with Cas8a. It became undetectable upon complex formation with *Pfu* Cascade. The assay was performed using a 5'-FAM labeled ssDNA. **(B)** Temperature-dependent nuclease assay analyzed on urea-PAGE revealed that the autoinhibition was robust in various temperatures. At higher temperatures and in the presence of a cognate DNA target, both the target and the ssDNA reporter were robustly degraded by *Pfu* Cascade-Cas3. Coloring scheme is the same as in Figure 3C. **(C)** Quantification of the banding intensities in B. to reveal the nuclease activities changes in various conditions. **(D), (E)** Further urea-PAGE and band quantification analyses to compare the nuclease activity changes against cognate dsDNA substrate and the ssDNA reporter in the presence or absence of ATP. **(F)** Superposition of Cas8a before and after full R-loop formation revealing the hinge motion between Cas8a NTD and its C-terminal Cas11-like domain. **(G)** Superposition of Cas11.1-5 in different functional states revealing the twisting motion before and after full R-loop formation. **(H)** Superposition of Cas3 in different functional states revealing the global and local motions before and after full R-loop formation. **(I)** Workflow of the cryo-EM image processing and 3D reconstruction for the *Pfu* Cas3-Cas8a sub-complex. **(J)** The resulting 6.6 Å *Pfu* Cas3-Cas8a sub-complex reconstruction reveals that the HD-nuclease center adopts an open conformation, similar to the active state in the full R-loop *Pfu* Cascade-Case structure. This is consistent with *Pfu* Cas3-Cas8a possessing highly active nuclease activity.

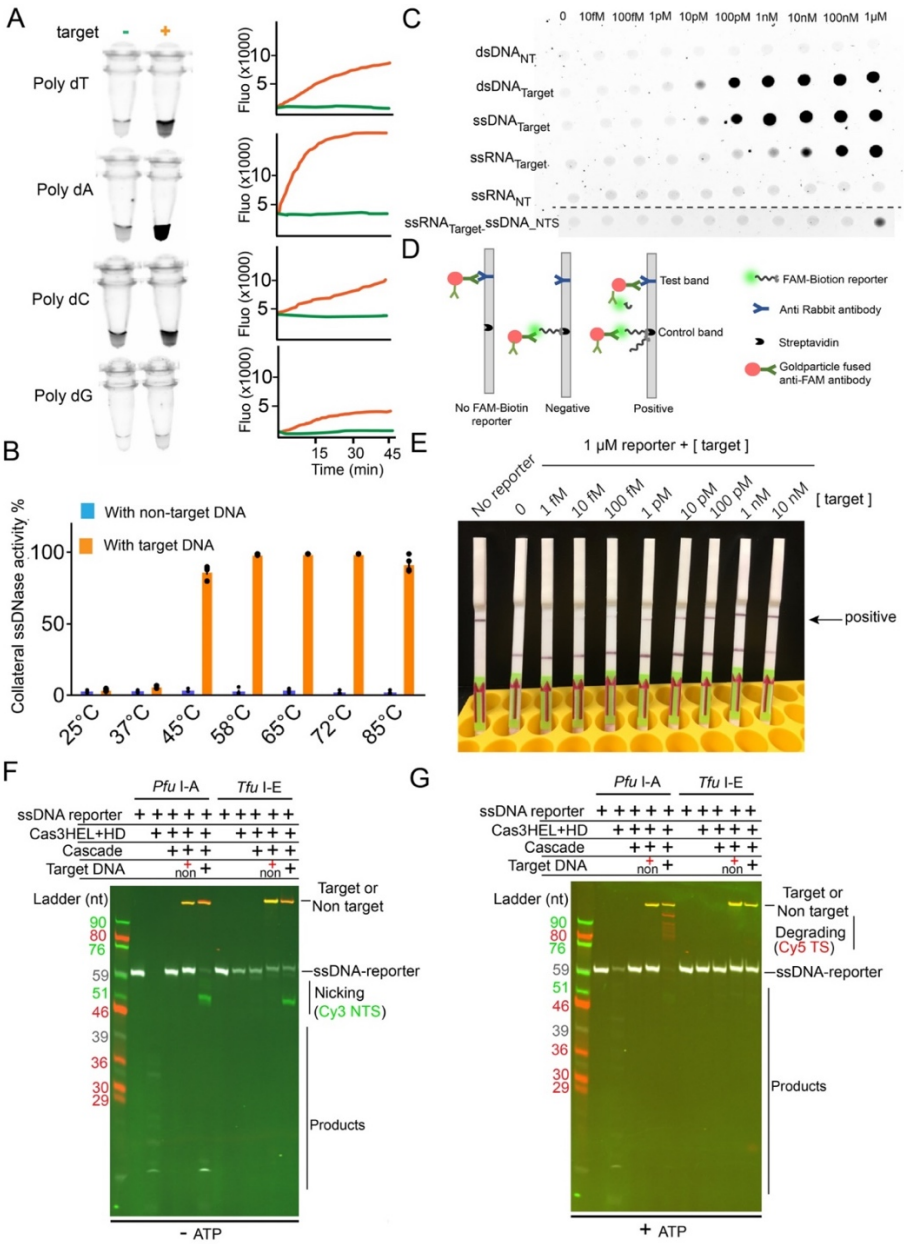

Figure S8. Additional data supporting the development of a nucleic acid detection platform from I-A *Pfu* Cascade-Cas3, related to Figure 6. (A)Nucleic acid detection assay with different poly-deoxynucleotide ssDNA-FQ reporters. Left: fluorescence changes in the test tube. Right: quantification of the fluorescence changes over time. **(B)** Temperature-dependency of the collateral damage activity by *Pfu* Cascade-Cas3 upon encountering a cognate DNA target. The system was highly active at 45 - 85˚C. **(C)** Piloting experiment to evaluate the detection limit against various nucleic acid targets. NT, Non-target; NTS, Non-target strand. **(D)** Schematic diagram explaining the binary reporting setup in the lateral flow strip assay. **(E)** The lateral strip assay reliably detected dsDNA at 100 fM concentration. **(F, G)** Nuclease activity changes inside I-A *Pfu* Cascade-Cas3 and I-E *Tfu* Cascade-Cas3, without or with ATP present, respectively. In the absence of ATP, *Tfu* Cascade-Cas3 suffered high background and small dynamic range issues. In the absence of ATP, *Tfu* Cascade-Cas3 suffered low sensitivity issue. Coloring scheme is the same as in Figure 3C.

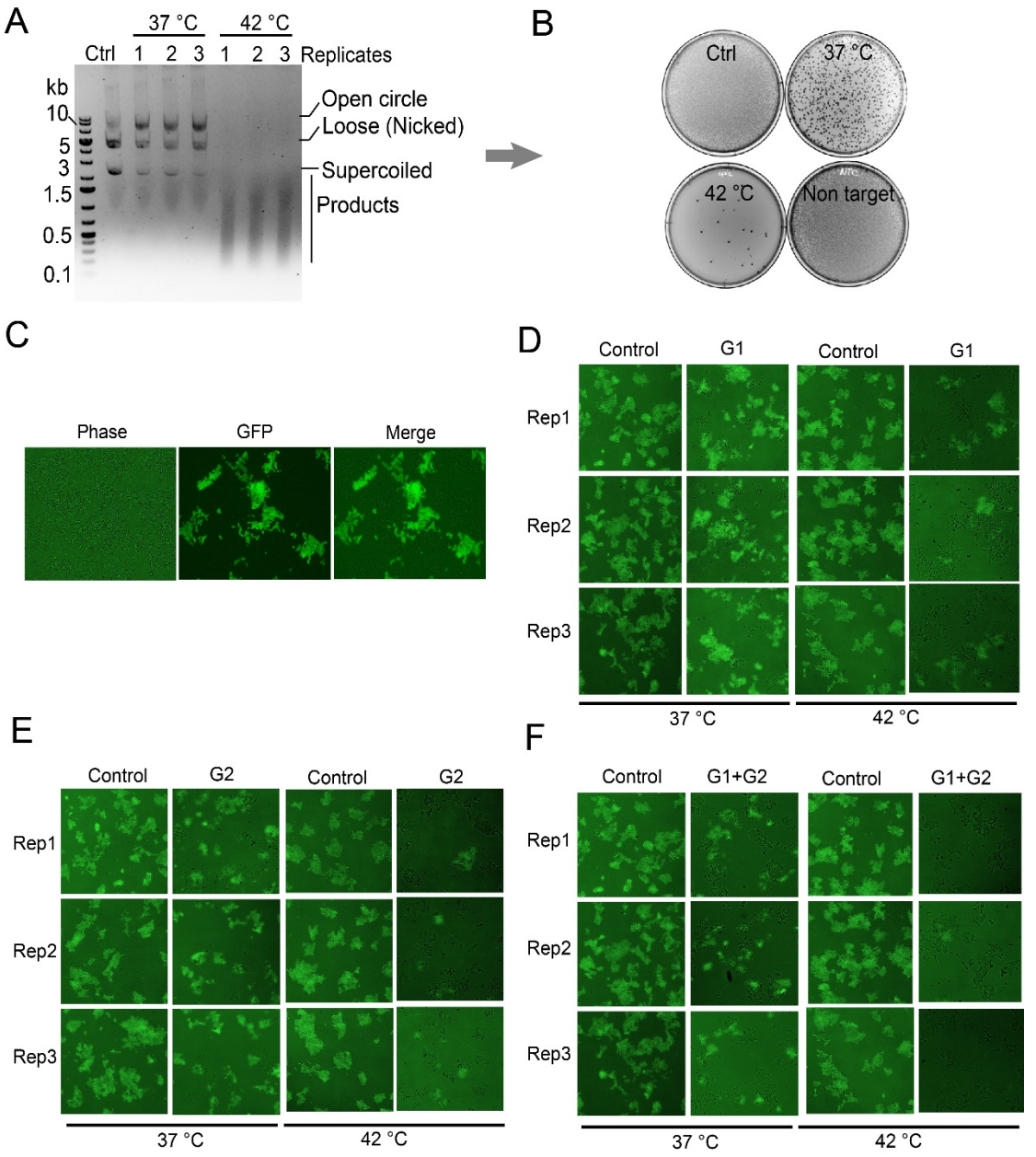

Figure S9, *In vitro* and *in vivo* data supporting the bi-directional deletion activity in *Pfu* Cascade-Cas3, related to Figure 7.

(A) The cleavage activity increases dramatically from 37˚C to 42˚C. **(B)** Transformation after cleavage assay in **(B)** and phenol extraction. **(C**) The representative images to show phase and GFP channel of cell culture. **(D, E)** Representative views of Guide 1 **(D),** Guide 2 **(E)** mediated GFP knock-out experiment in HAP1-GFP cells, in triplicates. **(F)** Representative views of Guide 2 mediated GFP knock-out experiment in HAP1-GFP cells, in triplicates. **(F)** Representative views of Guide 1 and Guide 2 combined GFP knock-out experiment in HAP1-GFP cells, in triplicates.

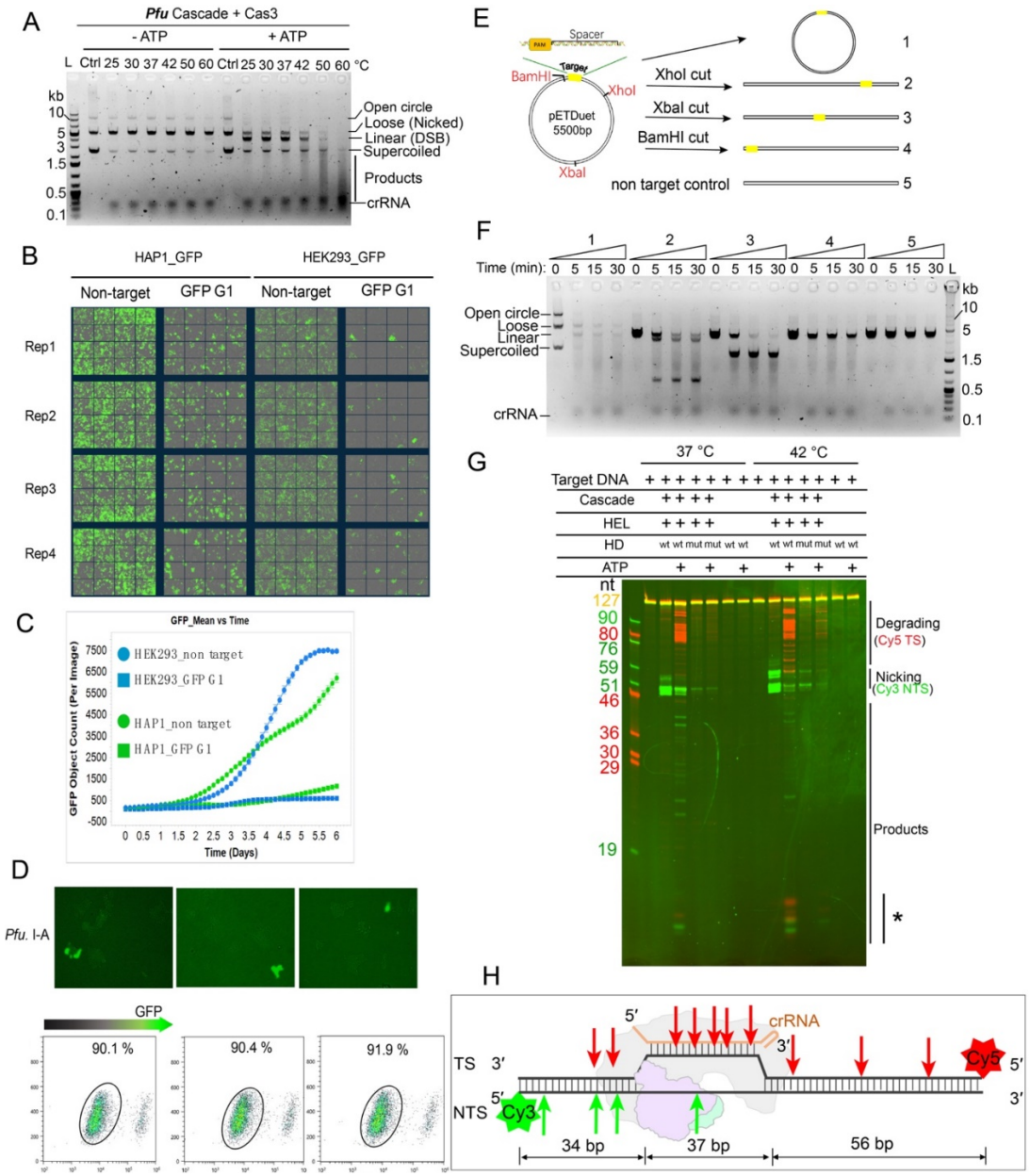

Figure S10. Additional data supporting the bi-directional deletion activity in *Pfu* Cascade-Cas3, *in vitro* and *in vivo*, related to Figure 7.

(A) RNA-guided plasmid cleavage by *Pfu* Cascade-Cas3, at different temperatures and +/- ATP. *Pfu* Cascade-Cas3 mainly nicks plasmids in the absence of ATP, but switches to a processive degradation behavior in the presence of ATP. **(B)** Comparison of GFP knock-out experiments in HAP1-GFP and HEK291-GFP cells, in quadruplicates. **(C)** Real-time GFP signal monitoring after delivery of *Pfu* I-A Cascade-Cas3 in HEK293 and Hap1 cell lines. **(D)** Different concentration combination of Cascade:Cas3 for I-A system. **(E)** Schematics of the substrates used in the DNA degrading assay. The circular plasmid was linearized by different restriction enzymes to place PAM into different locations. **(F)** Deletion polarity was defined by observing the relative stability of the two cleavage product bands. *Pfu* I-A Cascade-Cas3 was found here to degrade both the PAM-proximal and -distal dsDNA. **(G)** Mapping of the *Pfu* I-A Cascade-Cas3 cleavage sites on TS and NTS, using a dual-labeled DNA substrate, at 37 or 42˚C, +/- ATP, +/- HD active site mutation. **(H)** Illustration of the cleavage pattern in

(G).

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics. (Related to Figure 2)

Data collection	**Cascade alone**	**Cascade-Cas3**	**Partial R-loop**	**Full R-loop**	**Cas3-Cas8a alone**
PDB code	**7TR6**	**7TR8**	**7TR9**	**7TRA**	**--**
EMDB code	**26081**	**26082**	**26083**	**26084**	**26097**
Magnification(nominal)	67k	67k	67k	67k	60k
Voltage (Kv)	200	200	200	200	300
Electron exposure(e-/Å²)	50	50	50	50	60
Defocus range(um)	1.-2.5	1-2.5	1-2.5	1-2.5	0.8-2.5
Raw Pixel size(Å)	0.615	0.615	0.615	0.615	0.65
symmetry	C1	C1	C1	C1	C1
Micrographs	507	822	1,209		3,002
Initial particle images(No.)	203,773	435,364	463,373		601,678
Final particle images(No.)	79,651	83,559	13,781	44,773	32,211
Map resolution(Å)	3.4	3.6	3.9	3.3	6.6
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	20-3.0	20-3.4	20-3.6	20-3.0	20-6.0
Refinement					
Initial model used	De novo	De novo	De novo	De novo	–
Map sharpening B factor(Å²)	-100	-100	-100	-100	–
Model composition					
Nonhydrogen atoms	27,450	33,583	34,173	34,874	–
Protein residues /Nucleotide	3,383/45	4,192/45	4,192/75	4,174/113	–
Ligands	0	2	2	2	–
Water	0	0	0	0	–
B factors (Å²)					
protein	50.7/500.3/147.2	66.8/214.3/112.0	116.5/475.4/215.0	55.9/316.4/113.8	–
Nucleotide	69.5/244.7/114.6	82.6/146.5/97.0	152.7/343.2/232.6	64.3/301.4/112.9	–
Ligand	–	160.1/160.2/160.2	138.8/376.8/292.7	176.4/178.8/177.6	–
Water	–	–	–	–	–
R.m.s.d deviations					
Bond lengths (Å)	0.003(0)	0.002(0)	0.003(0)	0.003(0)	–
bond angles(°)	0.574(4)	0.549(9)	0.643(10)	0.607(7)	–
Validation					
MolProbity score	2.07	2.06	2.20	2.19	–
Clashscore	13.36	11.68	15.77	15.97	–
Poor rotamers(%)	0.00	0.00	0.00	0.00	–
Ramachandran plot					
Favored(%)	93.33	92.13	91.60	92.10	–

Allowed(%)	6.64	7.48	8.11	7.66	–
Disallowed(%)	0.03	0.39	0.29	0.24	–

Supplementary Table 2. Plasmids used in this work. (Related to Figure 1 and Figure 7)

Name in this study	Insert	Derived from vector	Marker	purpose
pCDFuet-Cascade/Cas3	Native operon	pETDeut	streptomycin	Interference
pCRISPR-1-ribozymes	CRISPR array	pRSFDuet	Kanamycin	purification
pCRISPR-1-PfuCas6a	CRISPR array	pRSFDuet	Kanamycin	purification
pCRISPR-1-EcoCas6e	CRISPR array	pRSFDuet	Kanamycin	purification
pETDuet-Target DNA	Target DNA	pRSFDuet	Ampicillin	Interference
pCRISPR-GFP_G1-EcoCas6e	CRISPR array	pRSFDuet	Kanamycin	purification
pCRISPR-GFP_G2-EcoCas6e	CRISPR array	pRSFDuet	Kanamycin	purification
pRSFDuet-G1 target DNA	Target DNA	pRSFDuet	Kanamycin	Cleavage assay
pETDuet-Cas11-Cas7-Cas5-His tag	Cas11-Cas7-Cas5	pETDuet	Ampicillin	purification
pCDFDuet-Strep tag-Cas8	Cas8	pCDFDuet	streptomycin	purification
pRSFDuet-Cas3HD	Cas3HD	pRSFDuet	Kanamycin	purification
pRSFDuet-Cas3HEL	Cas3HEL	pRSFDuet	Kanamycin	purification
pRSFDuet-Cas3HEL-NLS tag	Cas3HEL	pRSFDuet	Kanamycin	purification
pRSFDuet-Cas3HD-NLS tag	Cas3HD	pRSFDuet	Kanamycin	purification
pCDFDuet-Strep tag-NLS tag-Cas8	Cas8	pCDFDuet	streptomycin	purification
pETDuet-Cas11-Cas7-NLS tag-Cas5-His	Cas11-Cas7-Cas5	pETDuet	Ampicillin	purification
pET19-Strep tag-NLS tag-TfuCasA-NLS	CasA	Mol cell, 2018	Ampicillin	purification
pCDFDuet-TfuCasB-CasC-NLS-CasD-CasE	CasB-E	Mol cell, 2018	streptomycin	purification
pET19-TfuCas3-NLS-Strep tag	Cas3	Mol cell, 2018	Ampicillin	purification
pJET-I-A G1 deletion library	deletion library	pJET	Kanamycin	Sequencing
pETDuet-Cas11-Cas7-Cas5-His tag	Cas11-Cas7-Cas5	pETDuet	Ampicillin	purification
pCDFDuet-Target DNA	Target DNA	pRSFDuet	streptomycin	Interference

Supplementary Table 3. Primers and protein sequence used in this work. (Related to Figure 1 and Figure 7)

Name	Sequence (5' – 3')	Description
CY032 CY033	ATATGGATCCATGGGGGGATGGATCAGAAATATTG, ATATCTCGAGTCAGCTCTCTGAAGAGTTTTTCGG	*Pfu*Cas11a-Cas7a-Cas5a PCR amplification
CY034 CY035	ATATGGATCC ATGAAATTTAACGAATTTAAAACACCT, ATATCTCGAGTTAACGTTCACATATTATATCCTCC	*Pfu*Cas8a PCR amplification
CY030 CY031	ATATGGATCCATGAGGTTTTTAATAAGACTAGTTCC, ATATCTCGAGTTATACTCCAGTTTTTAATTCCTCTC	*Pfu*Cas6a PCR amplification
CY077 CY078	GATTCAGGATCCATGTATCTCAGTAAAGTCATCATTG, GTAGTTCTCGAGTCACAGTGGAGCCAAAGATAGC	*Eco*Cas6e PCR amplification
CY042	GTTCCAATAAGACTACAAAAGAATTGAAAGAGTGCTTCCCCAAAC CCTTAACTGGTTGTAACAGTTGGTTCCAATAAGACTACAAAAG (pcrRNA)	*Pfu* repeat-spacer-repeat, For *pfu*Cas6a processing
CY043	CAACTTTCAATTGATGAGTCCGTGAGGACGAAACGGTACCCGGT ACCGTCATTGAAAGTTGAGTGCTTCCCCAAACCCTTAACTGGTTG TAACAGTTGAGAGTTCCCCGCGCCAGCGGGGATAAACCGTGGC AGTCCTGTTGAAAAACAGAGAAGCCAACCAGAGAAACACACGTT GTGGTATATT ACCTGGTAAGCTATCTAA, (pcrRNA)	HH ribozyme-*Pfu* 5' handle-spacer-HP ribozyme
CY072	GAGTTCCCCGCGCCAGCGGGGATTGAAAGAGTGCTTCCCCAAA CCCTTAACTGGTTGTAACAGTTGGAGTTCCCCGCGCCAGCGGGG , (pcrRNA)	*Eco* repeat (recognition and cleavage part) – *Pfu* 5' handle- spacer- Eco repeat, For EcoCas6e processing
CY056 CY057	GCCGTACCTCTGAATCACGAAGGGGTTTGGGAATTGACCAACAT TGTCAACGGGAGTTACTCGCG (TS), CGCGAGTAACTCCCAGTGCTTCCCCAAACCCTTAACTGGTTGTAA CAGTTGTTCAGAGGTACGGC (NTS)	Target DNA, CCC PAM - spacer
CY216 CY217	GCCGTACCTCTGAATCACGAAGGGGTTTGGGAATTGACCAACAT TGTCAACGCAAGTTACTCGCG (TS), CGCGAGTAACTTGCAGTGCTTCCCCAAACCCTTAACTGGTTGTAA CAGTTGTTCAGAGGTACGGC (NTS)	Target DNA, TGC PAM - spacer
CY218 CY219	GCCGTACCTCTGAATCACGAAGGGGTTTGGGAATTGACCAACAT TGTCAACTGCAGTTACTCGCG (TS), CGCGAGTAACTGCAAGTGCTTCCCCAAACCCTTAACTGGTTGTAA CAGTTGTTCAGAGGTACGGC (NTS)	Target DNA, GCA PAM - spacer
CY179 CY037	ATATAT GGATCC AGTTGTAAGGCATTCCAAGGA, GATATGTCGACTCACTTCATCATTTCAACTCTAAGA	*Pfu* Cas3 HD PCR amplification
CY180 CY036	ATATAT GTCGAC TCATACTCTCACCCCCCAGT, ATATGGATCCATGGATACCGAAAAACTCTTCAGAG	*Pfu* Cas3 HEL PCR amplification
CY057	/56-FAM/-CGCGAGTAACTCCCTTGTAGTATGCGGTCCTTGCGGCTGAGAGC ACTTCAGAGGTACGGC	F-ssDNA reporter

CY579 CY581	CCCAGAGCAGGGCCTTAGGGAAG, GAAAGATGGCCGCTTCCCG	1 kb Forward and reverse primers to map genome deletions.
	CAGACCCAGGAGTCCAGGC, GCTGGGACCACCTTATATTCCC	2 kb Forward and reverse primer for mapping the genome editing.
CY584 CY585	AGGGATCCTGTGTGGCCATC, TGCGATGTCCGGAGAGGATGG	2 kb Forward and reverse primer for mapping the genome editing.
ssDNA_FQ	/56-FAM/-AAAAAAAA/3BHQ1	ssDNA-FQ reporter
ssDNA_F_Biotin	/56FAM/-AGGGAAACCTTTGGGCCCAATTCCGG/3Biotin/	ssDNA-F-Biotin reporter
Cas8a sequence	MGSSWSHPQFEKGGGSGGGSGGSAWSHPQFEKMSDSEVNQEAKPEVKPEVHREQIGGSKFNEFKTPQIDPIFDLYVAYGYVESLIRGGAKEATLIPHGASYLIQTDVSNEEFRHGLVDALSEMLSLHIALARHSPREGGKLVSDADFSAGANINNVYWDSVPRNLEKVMKDLEKKRSVKGTATIPITLMPSAGKYMLKHFGVQGGNPIKVDLLNYALAWVGFHYYTPYIKYAKGDTTWIHIYQIAPVEEVDMISILSLKDLKMHLPHYYESNLDFLINRRLALLYHLLHSESLGALELFTEKEFVIHSYTLERSGNNQAIRSFEEEEIGKLMDFLWKLKRRDFYHAIKFIDDLLKKATEGALALIDAIMNERLEGFYTALKLGKKAGVVSSREIVAALEDIICER.	Twin-strep tag-linker-Cas8a
Cas11a sequence	MGGWIRNIGRYLSYLVDDTFEEYAYDVVDGIAKARTQEELLEGVYKALRLAPKLKKKAESKGCPPPRIPSPEDIEALEEKVEQLSNPKDLRKLAVSLALWAFASWNNCPKKGKGTEGGVE	no tag
Cas7a sequence	MMYVRISGRIRLNAHSLNAQGGGGTNYIEITKTKVTVRTENGWTVVEVPAITGNMLKHWHFVGFVDYFKTTPYGVNLTERALRYNGTRFGQGETTATKANGATVQLNDEATIIKELADADVHGFLAPKTGRRRVSLVKASFILPTEDFIKEVEGERLITAIKHNRVDVDEKGAIGSSKEGTAQMLFSREYATGLYGFSIVLDLGLVGIPQGLPVKFEENQPRPNIVIDPNERKARIESALKALIPMLSGYIGANLARSFPVFKVEELVAIASEGPIPALVHGFYEDYIEANRSIIKNARALGFNIEVFTYNVDLGEDIEATKVSSVEELVANLVKMVGGKE	no tag
Cas5a sequence	MDILLVCLRFPFFSVAKRSYQVRTSFLLPPPSALKGALAKGLILLKPEKYASSSLDEAALKAIKEIESKLVDIKAVSVAPLSPLIRNAFLLKRLRNLESGSNAEKSDAMRREYTFTRELLVAYIFKNLTQEEKNLYLKAAMLIDVIGDTESLATPVWASFVKPEDKKAPLAFSAPYTEIYSLLSSKIQAKGKIRMYIEKMRVSPEYSKTKGPQEEIFYLPIEERRYKRIVYYARTIYPPEVEKALTVDGEVLGIWIPKNSSESGSHHHHHHH	no tag
E. coli Cas6e for crRNA processing	MYLSKVIIARAWSRDLYQLHQGLWHLFPNRPDAARDFLFHVEKRNTPEGCHVLLQSAQMPVSTAVATVIKTKQVEFQLQVGVPLYFRLRANPIKTILDNQKRLDSKGNIKRCRVPLIKEAEQIAWLQRKLGNAARVEDVHPISERPQYFSGDGKSGKIQTVCFEGVLTINDAPALIDLVQQGIGPAKSMGCGLLSLAPL	no tag
Cas3_HEL	MGSSHHHHHHSQLEVLFQGPLDTEKLFRELTGFEPYDYQLRAWEKI	His tag-3C site-Cas3HEL

	REIMNNGGKVIIEVPTAGGKTETAVMPFFAGIYNNNWPVARLVYVLP TRSLVEKQAERLRNLVYKLLQLKGKSKEEAEKLARELVVVEYGLEKT HAFLGWVVVTTWDAFLYGLAAHRTVGNRFTFPAGAIAQSLVIFDEV QMYQDESMYMPRLLSLVVGILEEANVPLVIMSATIPSKLREMIAGDT EVITVDKNDKNKPSRGNVKVRLVEGDITDVLNDIKKILKNGKKVLVVR NTVRKAVETYQVLKKKLNDTLANPSDALLIHSRFTIGDRREKERALD SARLIVATQVVEAGLDLPNVGLVVTDIAPLDALIQRIGRCARRPGEEG EGIILIPVENCIEHEKIVRGLSELMEKIGEDTVVFATVTSTNEYDRVVEI HYGEGKKNFVYVGDIDTARRVLEKKRSKKLPKDLYIIPYSVSPYDPLV LLTTYDELSKIGEYLADTTKARKALDRVYKFHYENNIVPKEFASAYIYF KELKLFSAPPEYELRSRPELYVLLYPMNIEKNERVEDKVIDNLETARII RISYSVKEWKKSDVVIGRLMKEWDKNAEKWVWKVRKSFKIDPYEIY VIDAKYYNSELGFITNLSDTNSHTDSDSKVRTRNSEHSSKKNRSKG KKGQTSLENWGVRV	
Cas3_HD	MGSSHHHHHHSQLEVLFQGPLGSSCKAFQGQTLREHIEAMLAAWE IVKNKYIPSIIRVMKTVGVKFTEEDADKFMKTLIILHDVGKCSEVYQK HLSNNEPLRGFRHELVSAYYAYNILKDMFKDETIAFIGALVVMMHHE PILMGQIRSLDKEELTPEVVLDKLRTFNGVMEGTESFIKSMIKEKLGV IPKVPSPTQEDVLREVIRLSVLARHRPDSGKLRMVVGALLIPLVLCDY KGAKEREGESPKFAEVLRVEMMK	His tag-3C site- Cas3HD