nature portfolio

https://doi.org/10.1038/s41579-022-00739-4

Supplementary information

Structural biology of CRISPR–Cas immunity and genome editing enzymes

In the format provided by the authors and unedited

Supplementary Table 1: CRISPR integrase protein complexes

Name / protein domains	Type/subtype	Species	State / bound substrate	PDB
***Eco*Cas1—Cas2**	I-E	*Escherichia coli*	Apo	4P6I[1], 4QDL[2]
	I-E	*Escherichia coli*	Bound to protospacer DNA	5DS4[3]
	I-E	*Escherichia coli*	Bound to protospacer DNA +Mg^{2+}	5DS5[3]
	I-E	*Escherichia coli*	Bound to protospacer with splayed ends	5DS6[3]
	I-E	*Escherichia coli*	Bound to single-forked DNA	5DQU[4]
	I-E	*Escherichia coli*	Bound to dual-forked DNA with 23-bp duplex	5DLJ[4]
	I-E	*Escherichia coli*	Bound to dual-forked DNA with 22-bp duplex	5DQT[4]
	I-E	*Escherichia coli*	Bound to dual-PAM, dual-forked DNA with 23-bp duplex	5DQZ[4]
	I-E	*Escherichia coli*	Half-integration	5VVJ[5]
	I-E	*Escherichia coli*	Full-integration	5VVK[5]
	I-E	*Escherichia coli*	Full-site integration +Ni^{2+}	5VVL[5]
***Eco*Cas1—Cas2—IHF holo-complex**	I-E	*Escherichia coli*	Half-integration	5WFE[5]
***Efa*Cas1—Cas2**	II-A	*Enterococcus faecalis*	Bound to dual-forked DNA with 22-bp duplex	5XVN[6]
	II-A	*Enterococcus faecalis*	Half-integration	5XVO[6]
	II-A	*Enterococcus faecalis*	Full-integration	5XVP[6]
***Sth*Cas1—Cas2—Csn2**	II-A	*Streptococcus thermophilus*	Bound to ~30 bp duplex DNA, monomer complex	6QXF[7]
	II-A	*Streptococcus thermophilus*	Bound to ~30 bp duplex DNA, dimer complex	6QXT[7]
	II-A	*Streptococcus thermophilus*	Bound to ~30 bp duplex DNA, filament complex	6QY3[7]
***Synechocystis* Cas1—Cas2**	I-D	*Synechocystis sp. PCC6803*	Bound to dual-forked DNA with 22-bp duplex	7CR6[8]
	I-D	*Synechocystis sp. PCC6803*	Bound to dual-forked DNA with 26-bp duplex	7CR8[8]
***Pfu*Cas1—Cas2**	I-G	*Pyrococcus furiosus*	Apo	7EI1[9]
Cas4-Cas1—Cas2	I-G	*Geobacter sulfurreducens*	Bound to dual-PAM prespacer DNA	7MI4[10]
	I-G	*Geobacter sulfurreducens*	Bound to single-PAM prespacer DNA	7MI5, 7MID[10]
	I-G	*Geobacter sulfurreducens*	Half-integration, Cas4 still blocking the PAM side	7MIB[10]
	I-G	*Geobacter sulfurreducens*	Full-integration	7MI9[10]

***Thiomicrospira* Cas6-RT-Cas1—Cas2**	III	*Thiomicrospira sp.*	Apo* (complex preparation includes bound half-site integration substrates, though substrate density not observed)	7KFT, 7KFU[11]

1. Nuñez, J. K. *et al.* Cas1–Cas2 complex formation mediates spacer acquisition during CRISPR–Cas adaptive immunity. *Nat. Struct. Mol. Biol.* **21**, 528–534 (2014).
2. Tamulaitiene, G. *et al.* Crystal structure of E.coli Cas1-Cas2 complex. (2014) doi:10.2210/pdb4qdl/pdb.
3. Nuñez, J. K., Harrington, L. B., Kranzusch, P. J., Engelman, A. N. & Doudna, J. A. Foreign DNA capture during CRISPR–Cas adaptive immunity. *Nature* vol. 527 535–538 (2015).
4. Wang, J. *et al.* Structural and mechanistic basis of PAM-dependent spacer acquisition in CRISPR-Cas systems. *Cell* **163**, 840–853 (2015).
5. Wright, A. V. *et al.* Structures of the CRISPR genome integration complex. *Science* **357**, 1113–1118 (2017).
6. Xiao, Y., Ng, S., Nam, K. H. & Ke, A. How type II CRISPR–Cas establish immunity through Cas1–Cas2-mediated spacer integration. *Nature* **550**, 137–141 (2017).
7. Wilkinson, M. *et al.* Structure of the DNA-bound spacer capture complex of a type II CRISPR-Cas system. *Mol. Cell* **75**, 90–101 (2019).
8. Wu, C. *et al.* Mechanisms of spacer acquisition by sequential assembly of the adaptation module in Synechocystis. *Nucleic Acids Res.* **49**, 2973–2984 (2021).
9. Tang, D. *et al.* A distinct structure of Cas1–Cas2 complex provides insights into the mechanism for the longer spacer acquisition in Pyrococcus furiosus. *Int. J. Biol. Macromol.* **183**, 379–386 (2021).
10. Hu, C. *et al.* Mechanism for Cas4-assisted directional spacer acquisition in CRISPR–Cas. *Nature* **598**, 515–520 (2021).
11. Wang, J. Y. *et al.* Structural coordination between active sites of a CRISPR reverse

transcriptase-integrase complex. *Nat. Commun.* **12**, 2571 (2021).

Supplementary Table 2: Cas9 proteins and domains *

Name	Species	State	PDB	RuvC alias	HNH alias	Rec1 alias	Rec2 alias	Rec3 alias	Wed/PI alias
Cas9a	*Streptococcus pyogenes*	Apo and Mn bound	4CMP, 4CMQ[1]	RuvC	HNH	α-helical lobe	α-helical lobe	α-helical lobe	TOPO/CTD
	Streptococcus pyogenes	Ternary (sgRNA/DNA)	4OO8[2]	RuvC	HNH	REC1	REC2	REC1	PI
	Streptococcus pyogenes	Ternary, PAM proximal mismatches (sgRNA,DNA)	4UN3, 4UN4, 4UN5[3]	RuvC	HNH	α-helical lobe	α-helical lobe	α-helical lobe	TOPO/CTD
	Streptococcus pyogenes	Binary (sgRNA)	4ZT0, 4Z90[4]	RuvC	HNH	Helical-I	Helical-II	Helical-III	CTD
	Staphylococcus aureus	Ternary (sgRNA, DNA)	5AXW, 5CZZ[5]	RuvC	HNH	REC	n.a.	REC	WED/TOPO/CTD
	Staphylococcus aureus	Ternary, Primed for DNA cleavage (sgRNA, DNA)	5F9R[6]	RuvC	HNH	Hel-I	Hel-II	Hel-III	CTD
	Streptococcus pyogenes	Ternary, 'Cleavage activated' at low resolution (sgRNA, DNA)	5Y36[7]	RuvC	HNH	REC1	REC2	REC3	WED/PI
	Streptococcus pyogenes	Ternary, pre-/post-catalytic and product (sgRNA, DNA +Mg^{2+})	6O0X, 6O0Y, 6O0Z[8]	RuvC	HNH	REC1	REC2	REC3	CTD
	Streptococcus thermophilus	Ternary (sgRNA, DNA)	6RJD[9]	RuvC	HNH	REC	REC	REC	WED/TOPO/CTD
	Streptococcus thermophilus	Ternary, catalytic (sgRNA, DNA)	6M0V, 6M0W, 6M0X[10]	RuvC	HNH	REC1	n.a.	REC2	WED/PI
	Streptococcus pyogenes	Ternary, PAM distal mismatches (sgRNA, DNA)	7S4U, 7S4V, 7S4X[11]	RuvC	HNH	REC1	REC2	REC3	CTD
Cas9b	*Franciscella tularesnsis subsp. novicida*	Ternary (sgRNA, DNA)	5B2O, 5B2P, 5B2Q[12]	RuvC	HNH	Rec1	Rec2	Rec3	WED/PI
Cas9c	*Actinomyces naeslundi str. Howell 279*	Apo	4OGC, 4OGE[1]	RuvC	HNH	α-helical lobe	α-helical lobe	α-helical lobe	β-hairpin/CTD
	Campylobacter jejuni	Ternary (sgRNA, DNA)	5X2G, 5X2H[13]	RuvC	HNH	REC1	n.a.	REC2	WED/PI
	Corynebacterium diphteriae	Ternary (sgRNA, DNA)	6JOO[14]	RuvC	HNH	REC1	n.a.	REC2	WED/PI
	Neisseria meningitidis	Binary (sgRNA)	6JDQ[15]	RuvC	HNH	REC1	n.a.	REC2	WED/PI
	Neisseria meningitidis	Ternary, Seed pairing, catalytic and post cleavage (sgRNA, DNA)	6JDV, 6JE3, 6JFU, 6KC7, 6KC8[15]	RuvC	HNH	REC1	n.a.	REC2	WED/PI

	Acidothermus cellulolyticus	Ternary (crRNA, DNA)	6WBR, 6WC0[16]	RuvC	HNH	REC1	n.a.	REC2	WED/TOPO/PI
Cas9d	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

* w/o engineered variants and Acr bound structures

References

1. Jinek, M. *et al.* Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science* **343**, 1247997 (2014).
2. Nishimasu, H. *et al.* Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* **156**, 935–949 (2014).
3. Anders, C., Niewoehner, O., Duerst, A. & Jinek, M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature* **513**, 569–573 (2014).
4. Jiang, F., Zhou, K., Ma, L., Gressel, S. & Doudna, J. A. A Cas9–guide RNA complex preorganized for target DNA recognition. *Science* **348**, 1477–1481 (2015).
5. Nishimasu, H. *et al.* Crystal structure of Staphylococcus aureus Cas9. *Cell* **162**, 1113–1126 (2015).
6. Jiang, F. *et al.* Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. *Science* **351**, 867–871 (2016).
7. Huai, C. *et al.* Structural insights into DNA cleavage activation of CRISPR-Cas9 system. *Nat. Commun.* **8**, 1375 (2017).
8. Zhu, X. *et al.* Cryo-EM structures reveal coordinated domain motions that govern DNA cleavage by Cas9. *Nat. Struct. Mol. Biol.* **26**, 679–685 (2019).
9. Fuchsbauer, O. *et al.* Cas9 allosteric inhibition by the anti-CRISPR protein AcrIIA6. *Mol. Cell* **76**, 922–937 (2019).
10. Zhang, Y. *et al.* Catalytic-state structure and engineering of Streptococcus thermophilus Cas9. *Nature Catalysis* **3**, 813–823 (2020).

11. Bravo, J. P. K. *et al.* Structural basis for mismatch surveillance by CRISPR–Cas9. *Nature* 1–5 (2022).
12. Hirano, H. *et al.* Structure and engineering of Francisella novicida Cas9. *Cell* **164**, 950–961 (2016).
13. Yamada, M. *et al.* Crystal structure of the minimal Cas9 from Campylobacter jejuni reveals the molecular diversity in the CRISPR-Cas9 systems. *Mol. Cell* **65**, 1109–1121 (2017).
14. Hirano, S. *et al.* Structural basis for the promiscuous PAM recognition by Corynebacterium diphtheriae Cas9. *Nat. Commun.* **10**, 1968 (2019).
15. Sun, W. *et al.* Structures of Neisseria meningitidis Cas9 complexes in catalytically poised and anti-CRISPR-inhibited states. *Mol. Cell* **76**, 938–952 (2019).
16. Das, A. *et al.* The molecular basis for recognition of 5’-NNNCC-3' PAM and its methylation state by Acidothermus cellulolyticus Cas9. *Nat. Commun.* **11**, 6346 (2020).

Supplementary Table 3: Cas12 proteins and domains *

Name	Alias	gRNA	Guide processing	*Cis* target	*Trans* target	Species	State	PDB	RuvC alias	OBD alias	Rec1 alias	Rec2 alias	Nuc alias	PI alias	Additional domain(s)
Cas12a	Cpf1	crRNA	Autoprocessing by OBD	dsDNA	ssDNA	*Lachnospiraceae bacterium*	Binary (crRNA)	5ID6 [1]	RuvC	OBD	Helical I	Helical II	UK	LHD	n.a.
						Acidaminococcus sp. Bv3L6	Ternary (crRNA, dsDNA)	5B43 [2]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Acidaminococcus sp. Bv3L6	Ternary (crRNA, dsDNA)	5KK5 [3]	RuvC	OBD	Helical-I	Helical II	Nuc	LHD	n.a.
						Francisella tularensis	Ternary (pre-crRNA, dsDNA)	5NFV [4]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Francisella tularensis	Binary (crRNA)	5NG6 [4]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Francisella tularensis	Ternary, postcatalytic (crRNA, dsDNA)	5MGA [5]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Lachnospiraceae bacterium	Ternary, alternative PAMs (crRNA, dsDNA)	5XUZ, 5XUS, 5XUU, 5XUT [6]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Francisella tularensis	Ternary, transition states (crRNA, dsDNA)	6GTC, 6GTD, 6GTE, 6GTF, 6GTG [7]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Francisella tularensis	Ternary (crRNA, dsDNA)	6I1K [8]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Francisella tularensis	Ternary (crRNA, ssDNA)	6I1L [8]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
Cas12b	C2c1	crRNA/ tracrRNA	unknown	dsDNA	ssDNA	*Alicyclobacillus acidoterrestris*	Ternary, target strand in RuvC (sgRNA, dsDNA)	5U30 [9]	RuvC	OBD	Helical-I	Helical-II	Nuc	n.a.	n.a.
						Alicyclobacillus acidoterrestris	Ternary, *trans*-substrate in RuvC (sgRNA,	5U31[9]	RuvC	OBD	Helical-I	Helical-II	Nuc	n.a.	n.a.

							dsDNA)								
						Alicyclobacillus acidoterrestris	Ternary, non-target strand in RuvC (sgRNA, dsDNA)	5U33 [9]	RuvC	OBD	Helical-I	Helical-II	Nuc	n.a.	n.a.
						Alicyclobacillus acidoterrestris	Binary (sgRNA)	5U34 [9]	RuvC	OBD	Helical-I	Helical-II	Nuc	n.a.	n.a.
						Alicyclobacillus acidoterrestris	Binary (sgRNA)	5WQE [10]	RuvC	Wed	Rec1	Rec2	Nuc	n.a	n.a.
						Alicyclobacillus acidoterrestris	Ternary (sgRNA, dsDNA)	5WTI [10]	RuvC	Wed	Rec1	Rec2	Nuc	n.a	n.a.
Cas12c	C2c3	crRNA/ tracrRNA	Autoprocessing by RuvC	dsDNA (CRISPR interference, no DNAse activity in some Cas12c)	n.a.	metagenome	Binary (sgRNA)	7V93 [11]	RuvC	Wed	Rec1	Rec2	TNB	n.a.	n.a.
						metagenome	Ternary (sgRNA, dsDNA)	7V94 [11]	RuvC	Wed	Rec1	Rec2	TNB	PI	n.a
Cas12d	CasY	crRNA/ scoutRNA	Autoprocessing, unknown active site	dsDNA	ssDNA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Cas12e	CasX	crRNA/ tracrRNA	unknown	dsDNA	ssDNA	Deltaproteobacteria bacterium	Ternary (sgRNA, short dsDNA)	6NY3 [12]	RuvC	OBD	Helical I	Helical II	TSL	n.a.	NTSB
						Deltaproteobacteria bacterium	Ternary (sgRNA, short dsDNA)	6NY1 [12]	RuvC	OBD	Helical I	Helical II	TSL	n.a.	NTSB
						Deltaproteobacteria bacterium	Ternary (sgRNA, dsDNA)	6NY2 [12]	RuvC	OBD	Helical I	Helical II	TSL	n.a.	NTSB
						Planctomycetes	Ternary, Diverse states (engineered sgRNA, dsDNA)	7WAY, 7WAZ, 7WB0, 7WBI [13]	RuvC	OBD	Helical I	Helical II	TSL	n.a.	NTSB
Cas12f	Cas14, Casα, CasZ, C2c4, C2c8,	crRNA/ tracrRNA	unknown	dsDNA	ssDNA	Uncultured archaeon, synthetic construct	Ternary (sgRNA, dsDNA)	7C7L [14]	RuvC	Wed	Rec	Part of the RuvC	TNB	n.a.	ZF (OBD insertion)

	C2c9, C2c10					Unidentified	Binary (sgRNA)	7L48 [15]	RuvC	Wed	Rec1	Rec2	Nuc	n.a.	ZF (OBD insertion)
						Unidentified	Ternary (sgRNA,dsDNA)	7L49 [15]	RuvC	Wed	Rec1	Rec2	Nuc	n.a.	ZF (OBD insertion)
Cas12g	n.a.	crRNA/ tracrRNA	unknown	ssRNA	ssRNA, ssDNA	*Lachnospiraceae bacterium* ND2006	Binary (sgRNA)	6XMF [16]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a
						Lachnospiraceae bacterium ND2006	Ternary (sgRNA,ssRNA)	6XMG [16]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a
Cas12h	n.a.	crRNA	unknown	dsDNA	ssDNA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Cas12i	n.a.	crRNA	Autoprocessing by OBD	dsDNA	ssDNA	Unidentified synthetic construct	Binary (crRNA)	6LTP [17]	RuvC	Wed	Helical-I and Helical-II	Helical-III	Nuc	PI	n.a.
						Unidentified synthetic construct	Ternary, short crRNA spacer (crRNA, dsDNA)	6LU0 [17]	RuvC	Wed	Helical-I and Helical-II	Helical-III	Nuc	PI	n.a.
						Unidentified synthetic construct	Ternary, trans-catalytic, single cofactor Mg^{2+} (crRNA, dsDNA, Mg^{2+})	6LTR [17]	RuvC	Wed	Helical-I and Helical-II	Helical-III	Nuc	PI	n.a.
						Unidentified synthetic construct	Ternary, trans-catalytic, double cofactor Mg^{2+} (crRNA, dsDNA, Mg^{2+})	6LTU [17]	RuvC	Wed	Helical-I and Helical-II	Helical-III	Nuc	PI	n.a.
						Lachnospiraceae bacterium ND2006	Binary (crRNA)	6W62 [18]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Lachnospiraceae bacterium ND2006	Ternary, I1 state, (crRNA, dsDNA)	6W64 [18]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Lachnospiraceae bacterium ND2006	Ternary, cat. inactive state (crRNA, dsDNA)	6W5C [18]	RuvC	Wed	Rec1	Rec2	Nuc	PI	n.a.
						Lachnospiraceae bacterium ND2006	Binary (crRNA)	7D8C [19]	RuvC	Wed	Helical-I	Helical-II	Nuc	PI	n.a.
						Lachnospiraceae	Ternary,	7EU9 [19]	RuvC	Wed	Helical-I	Helical-II	Nuc	PI	n.a.

						bacterium ND2006	pre-catalytic state, SeMet variant (crRNA, dsDNA)								
						Lachnospiraceae bacterium ND2006	Ternary, pre-catalytic state (crRNA, dsDNA)	7D2L [19]	RuvC	Wed	Helical-I	Helical-II	Nuc	PI	n.a.
						Lachnospiraceae bacterium ND2006	Ternary, post-catalytic state (crRNA, dsDNA)	7D3J [19]	RuvC	Wed	Helical-I	Helical-II	Nuc	PI	n.a.
Cas12j	CasΦ	crRNA	Autoprocessing by RuvC	dsDNA	ssDNA	Biggiephage	Binary (crRNA)	7M5O [20]	RuvC	OBD	RecI	RecII	ZR	PI	n.a.
						Biggiephage	Ternary, pre-catalytic state (crRNA, dsDNA)	7LYS [20]	RuvC	OBD	RecI	RecII	ZR	PI	n.a.
						Biggiephage	Ternary, *cis*-catalytic state (crRNA, dsDNA, Mg^{2+})	7LYT [20]	RuvC	OBD	RecI	RecII	ZR	PI	n.a.
						Biggiephage	Ternary, post-catalytic state	7ODF [21]	RuvC	RBD	TPID	STP and BH	Part of RuvC	NPID	n.a.
Cas12k	C2c5	crRNA/ tracrRNA	unknown	Nuclease inactive	Nuclease inactive	*Scytonema hofmannii*	Binary (sgRNA)	7N3O [22]	RuvC (inactive)	Wed	Rec1	Rec2	n.a.	PI	n.a.
						Scytonema hofmannii	Ternary (sgRNA, dsDNA)	7N3P [22]	RuvC (inactive)	Wed	Rec1	Rec2	n.a.	PI	n.a.
						Scytonema hofmannii	Ternary (sgRNA, dsDNA)	7PLA [23]	RuvC (inactive)	Wed	Rec	Bridge	n.a.	PI	n.a.

* w/o engineered variants and Acr bound structures

References

1. Dong, D. *et al.* The crystal structure of Cpf1 in complex with CRISPR RNA. *Nature* **532**, 522–526 (2016).

2. Yamano, T. *et al.* Crystal Structure of Cpf1 in Complex with Guide RNA and Target DNA. *Cell* **165**, 949–962 (2016).

3. Gao, P., Yang, H., Rajashankar, K. R., Huang, Z. & Patel, D. J. Type V CRISPR-Cas Cpf1 endonuclease employs a unique mechanism for crRNA-mediated target DNA recognition. *Cell Res.* **26**, 901–913 (2016).
4. Swarts, D. C., van der Oost, J. & Jinek, M. Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a. *Mol. Cell* **66**, 221–233.e4 (2017).
5. Stella, S., Alcón, P. & Montoya, G. Structure of the Cpf1 endonuclease R-loop complex after target DNA cleavage. *Nature* **546**, 559–563 (2017).
6. Yamano, T. *et al.* Structural basis for the canonical and non-canonical PAM recognition by CRISPR-Cpf1. *Mol. Cell* **67**, 633–645 (2017).
7. Stella, S. *et al.* Conformational activation promotes CRISPR-Cas12a catalysis and resetting of the endonuclease activity. *Cell* **175**, 1856–1871.e21 (2018).
8. Swarts, D. C. & Jinek, M. Mechanistic insights into the cis- and trans-acting DNase activities of Cas12a. *Molecular Cell* **73**, 589–600 (2019).
9. Yang, H., Gao, P., Rajashankar, K. R. & Patel, D. J. PAM-dependent target DNA recognition and cleavage by C2c1 CRISPR-Cas dndonuclease. *Cell* **167**, 1814–1828 (2016).
10. Liu, L. *et al.* C2c1-sgRNA complex structure reveals RNA-guided DNA cleavage mechanism. *Mol. Cell* **65**, 310–322 (2017).
11. Kurihara, N. *et al.* Structure of the type V-C CRISPR-Cas effector enzyme. *Mol. Cell* **82**, 1–13 (2022).
12. Liu, J.-J. *et al.* CasX enzymes comprise a distinct family of RNA-guided genome editors. *Nature* **566**, 218–223 (2019).
13. Tsuchida, C. A. *et al.* Chimeric CRISPR-CasX enzymes and guide RNAs for improved genome editing activity. *Mol. Cell* **82**,

1199–1209 (2022).

14. Takeda, S. N. *et al.* Structure of the miniature type V-F CRISPR-Cas effector enzyme. *Mol. Cell* **81**, 558–570 (2021).
15. Xiao, R., Li, Z., Wang, S., Han, R. & Chang, L. Structural basis for substrate recognition and cleavage by the dimerization-dependent CRISPR–Cas12f nuclease. *Nucleic Acids Res.* **49**, 4120–4128 (2021).
16. Li, Z., Zhang, H., Xiao, R., Han, R. & Chang, L. Cryo-EM structure of the RNA-guided ribonuclease Cas12g. *Nat. Chem. Biol.* **17**, 387–393 (2021).
17. Huang, X. *et al.* Structural basis for two metal-ion catalysis of DNA cleavage by Cas12i2. *Nat. Commun.* **11**, 5241 (2020).
18. Zhang, H., Li, Z., Xiao, R. & Chang, L. Mechanisms for target recognition and cleavage by the Cas12i RNA-guided endonuclease. *Nat. Struct. Mol. Biol.* **27**, 1069–1076 (2020).
19. Zhang, B. *et al.* Mechanistic insights into the R-loop formation and cleavage in CRISPR-Cas12i1. *Nat. Commun.* **12**, 3476 (2021).
20. Pausch, P. *et al.* DNA interference states of the hypercompact CRISPR-CasΦ effector. *Nat. Struct. Mol. Biol.* **28**, 652–661 (2021).
21. Carabias, A. *et al.* Structure of the mini-RNA-guided endonuclease CRISPR-Cas12j3. *Nat. Commun.* **12**, 4476 (2021).
22. Xiao, R. *et al.* Structural basis of target DNA recognition by CRISPR-Cas12k for RNA-guided DNA transposition. *Mol. Cell* **81**, 4457–4466 (2021).
23. Querques, I., Schmitz, M., Oberli, S., Chanez, C. & Jinek, M. Target site selection and remodelling by type V CRISPR-transposon systems. *Nature* **599**, 497–502 (2021).