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Supplemental information

Structural snapshots of R-loop formation

by a type I-C CRISPR Cascade

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Figure S1. Related to Figures 1-5. Purification of the type I-C Cascade Cas8c mutant Cascade, AcrIF2 and AcrIC4. A,B. Size-exclusion chromatograph and SDS-Page analysis of WT, Cas8c-Q212A, and Cas8c-N72A Cascade. C-E. Size-exclusion chromatograph (C) and SDS-Page analysis of AcrIF2 and AcrIC4.



Figure S2. Related to Figure 1. Cryo-EM data analysis. A. Gold standard Fourier Shell Correlation (FSC) curves for type I-C Cascade bound to dsDNA complex. B. Map-to-

model FSC for type I-C Cascade bound to dsDNA complex. **C.** Euler plot for type I-C Cascade bound to dsDNA complex **D**. Gold standard Fourier Shell Correlation (FSC) curves for type I-C Cascade partial R-loop complex. **E** Map-to-model FSC for type I-C Cascade partial R-loop complex **F.** Euler plot for type I-C Cascade partial R-loop. **G**. Gold standard Fourier Shell Correlation (FSC) curves for unbound type I-C Cascade. **H.** Map-to-model FSC for type I-C Cascade bound to unbound type I-C Cascade. **J**. Gold standard Fourier Shell Correlation (FSC) curves for type I-C Cascade bound to AcrIF2. **K.** Map-to-model FSC for type I-C Cascade for type I-C Cascade bound to AcrIF2. **K.** Map-to-model FSC for type I-C Cascade for type I-C Cascade bound to AcrIF2. **M**. Gold standard Fourier Shell Correlation (FSC) curves for type I-C Cascade bound to AcrIF2. **N**. Map-to-model FSC for type I-C Cascade bound to AcrIC4. **N**. Map-to-model FSC for type I-C Cascade bound to AcrIC4. **O**. Euler plot for type I-C Cascade bound to AcrIC4.



Figure S3. Local resolution analysis. Related to Figures 1-5. A-E. Sharpened maps colored according to local resolution. F. Cryo-EM density of partial R-loop structure.



Figure S4. Related to Figures 1-3. Representative cryo-EM densities. A. NTS. B. NTS and TS reannealing at the top of the R-loop. C. NTS, TS, and crRNA densities and

corresponding model **D.** NTS aromatic clamps and positively charged backbone stabilizing residues. **E.** PAM-recognition site **F.** Cas8c N-term.



Figure S5. Related to Figure 2. Conservation of NTS aromatic clamps across type I Cascades. A. Aromatic and positively charged NTS stabilizing residues, and residues involved in PAM recognition (inset) are highly conserved across type I Cascades. B.

Multiple aromatic residues positioned along the putative NTS path (green) of type I-E Cascade [S1].



Figure S6. Related to Figures 4-5. Differences in positioning of Cas8 PAM blocking anti-CRISPRS. AcrIF2 inhibits both Cas8c and Cas8f through the same interface and at the same PAM site as AcrIF10, but at different orientations [S2, S3].

Data collection and processingAcril-2Acril-2Acril-2Acril-2Voltage (kV)200300300200200Electron exposure (e– (Å2)40.5808040.540.5Defocus range (µm)1.5 to 2.51.2 to 2.21.2 to 2.21.5 to 2.51.5 to 2.5Pixel size (Å)0.941.11.10.940.94Symmetry imposedC1C1C1C1C1Initial particle images (no.)2 million1.2 million1.2 million160,716128,780Tinal particle images (no.)73,22096,964174,00421,62521,651Map resolution (Å) FSC threshold2.842.862.803.13.1Map resolution range (Å)N/AN/AN/AN/AN/AModel resolution nage (Å)2.83.13.13.23.4Sign ensolution (Å) FSC threshold2.83.13.13.23.4Noel resolution (Å) (Å2)2.83.13.13.23.4Sign ensolution (Å) FSC threshold2.83.13.13.23.4Model resolution fange (Å)N/AN/AN/AN/AN/AModel resolution range (Å)N/AN/AN/AN/AN/AN/AN/AN/AN/AN/AN/ANon-tydrogen atoms Protein residues (Å2)N/AN/AN/A12.55630822912Ligands48135<		Type I-C apo model unbound	Type I-C Cascade full R-loop	Type I-C Cascade partial R-loop	Type I-C Cascade bound to	Type I-C Cascade bound to
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Pixel size (Å)	0.94	1.1	1.1	0.94	0.94
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Map resolution (Å) 2.84 2.86 2.80 3.1 3.1 FSC threshold 0.143 0.143 0.143 0.143 0.143 0.143 Map resolution range (Å) N/A N/A N/A N/A N/A Refinement - - - - - Initial pdb model used 7KHA 7KHA 7KHA 7KHA 7KHA 7KHA 5UZ9 - Model resolution (Å) 2.8 3.1 3.1 3.2 3.4 - FSC threshold 0.5 0.5 0.5 0.5 0.5 - <t< td=""><td>Final particle images (no.)</td><td>73,220</td><td>96,964</td><td>174,004</td><td>21,625</td><td>21,651</td></t<>	Final particle images (no.)	73,220	96,964	174,004	21,625	21,651
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Map resolution range (A) N/A N/A N/A N/A N/A N/A Refinement	FSC threshold	0.143	0.143	0.143	0.143	0.143
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Model resolution (Å) FSC threshold2.8 0.53.1 0.53.1 	Initial pdb model used	/KHA	/КНА	/KHA	7KHA 5UZ9	/КНА
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	Nucleotide	00.0082.89/	12.60/223.93	00.00/133.42	25.94/152.35	46.22/141.66
		20.00	/00.70	700.00	100.22	102.21
R.M.S. deviations	R.m.s. deviations					
Bond lengths (Å) .0005 .003 .003 0.009 0.003	Bond lengths (Å)	.0005	.003	.003	0.009	0.003
Bond angles (°) .706 .623 .682 0.921 0.646	Bond angles (°)	.706	.623	.682	0.921	0.646
Validation	Validation					
MolProbity score 1.68 1.56 1.71 1.61 1.75	MolProbity score	1.68	1.56	1.71	1.61	1.75
Clashscore 4.92 4.06 6.01 6.73 9.49	Clashscore	4.92	4.06	6.01	6.73	9.49
Poor rotamers (%) 1.71 1.69 1.25 0.27 0.00	Poor rotamers (%)	1.71	1.69	1.25	0.27	0.00
Hamachandran plot Image: second sec	Ramachandran plot	00.00	00.00	05.54	00.07	00.00
Favored (%) 96.33 96.82 95.54 96.37 96.30 Allowed (%) 0.07 0.40 4.40 0.00 0.00	Favored (%)	96.33	96.82	95.54	96.37	96.30
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Table S1. Related to Figure 1. Model Statistics for the type I-C Cascade structures.

Plasmid	Description	Reference
#81185	D. <i>vulgaris</i> type I-C Cascade for purification and cryo-EM studies	Addgene
#81186	D. vulgaris type I-C crRNA for co-expression with Cascade	Addgene
#89234	AcrIF2 for purification and cryo-EM studies	Addgene
pIF199	Backbone used for AcrIF2 and AcrIC4 for <i>in vivo</i> interference assay	Dillard et al [S4]
pIF412	Backbone used for dsDNA target for <i>in vivo</i> interference assay	Dillard et al [S4]
pIF385	Backbone used for Cascade-Cas3 for in vivo interference	Dillard et al [S4]
#48313	Backbone vector used for AcrIC4 for in vivo interference	Addgene
pRO01	Type I-C Cascade Cas8c mutant Q212A for purification and EMSA	This study
pRO02	Type I-C Cascade Cas8c mutant N72A for purification and EMSA	This study
pRO03	AcrIC4 for purification and cryo-EM studies	This study
#196399	pCDF-Duet1 vector with target sequence and 5' PAM for <i>in vivo</i> interference	Addgene
		This study
#196400	pBAD vector with IC cascade and cas3 for <i>in vivo</i>	Addgene
	Interference	This study
#196402	pET28b-Duet1 vector with Acr IF2 for <i>in vivo</i> interference	Addgene
		This study
#196403	pET28b-Duet1 vector with Acr-IC4 for in vivo interference	Addgene
		This study
pDR009	pBAD vector with IC Cascade and Cas3 with Cas8c N72A mutation for <i>in vivo</i> interference assay	This study
pDR010	pBAD vector with IC Cascade and Cas3 with Cas8c Q212A mutation for <i>in vivo</i> interference assay	This study
pDR011	pBAD vector with IC Cascade and Cas3 with Cas8c Aromatic mutants for <i>in vivo</i> interference assay	This study
pDR012	pBAD vector with IC Cascade and Cas3 with Cas8c Positive mutants for <i>in vivo</i> interference assay	This study
pDR013	pBAD vector with IC Cascade and Cas3 with Cas8c R363A/K94A/K92A mutants for <i>in vivo</i> interference assay	This study

Table S2. Related to Figure 1. Plasmids used in this study.

Primer	Sequence (5' to 3')	Reference
roO1	ggctGTTGCCTCGATAGTGTCCTTTAACAACAC	This study
Cas8c Q212A F		
roO2	gctgcACCGCGAACGCCGTCTCG	This study
Cas8c Q212A R		
roO2	gggcATCAAACCGAACTTCCTG	This study
Cas8c N72A F		
roO3	gccccCTTCTTTTCCGCGATAGG	This study
Cas8c N72A R		
roO4 AcrIC4 F	TACTTCCAATCCAATGCAATGGACAATAAGATTACTCCT GCT	This study
roO5 AcrIC4 R	TTATCCACTTCCAATGTTATTAAGTTTCATCTCCACGCC A	This study
TS for cryo-EM	AGCAGACTGGAGGAGTTTTCGCCATGCTCAGGCTGGC	Hochstrasser et al
experiments	G AGTGCGCCACTCATCAAGCCATGTGGGCTGTCAAAAT	[55]
NTS for cryo-EM	AGCAGACTGGAGGAGTTTTCGCCATGCTCAGGCTGGC	Hochstrasser et al
EMSA	AGTGCGCCACTCATCAAGCCATGTGGGCTGTCAAAAT	[00]
TS for EMSA	5' FAM- AGCAGACTGGAGGAGTTTTCGCCATGCTCAGGCTGGC	Hochstrasser et al [S5]
	AGTGCGCCACTCATCAAGCCATGTGGGCTGTCAAAAT	
dr001 pCDF-Duet1 vector with target sequence F	TTCGCCATGCTCAGGCTGGCGAGTGCGCCACTCATCA ACCTGTAGAAATAA TTTTGTTTAACTTTAAT	This study
dr002 pCDF-Duet1 vector with target sequence R	GGAATTGTTATCCGCTCAC	This study
iso020 pBAD vector with IC cascade-Cas3 F	GTTCGAAAAGTAAGAATTCGAAGCTTGG	This study
iso021 pBAD vector with IC cascade-Cas3 R	GCTGCAGATCTCGAGCTCGG	This study
iso063 pBAD vector with IC cascade-Cas3 F	CCATGGATCCGAGCTCGAGATCTGCAGCATGACACAT GGGGCTG	This study
iso064	CCAAGCTTCGAATTCTTACTTTTCGAACCTACAGCATCT CTTTGACCTC	This study

Table S3. Related to Figures 1. Oligonucleotides used in this study.

pBAD vector with IC cascade-Cas3 R		
dr007 pET28b-Duet1 vector with Acr IF2 F	GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCAT GATGATCGCGCAACAGCATAA	This study
dr008 pET28b-Duet1 vector with Acr IF2 R	TACAGTATCCTTATGCTGTTGCGCGATCATCATGGTATA TCTCCTTCTTAAAGTTAAACAA	This study
dr009 pET28b-Duet1 vector with Acr IF2 F	GAACGCCTGCTGGAGTCCGTAGAGGAGGAGCACCACC ACCACCACCA	This study
dr010 pET28b-Duet1 vector with Acr IF2 R	CTTTGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTG GTGCTCCTCCTCTACGGACTCCA	This study
dr011 pET28b-Duet1 vector with Acr IF2 F	TGAGATCCGGCTGCTAAC	This study
dr012 pET28b-Duet1 vector with Acr IF2 R	CTCCTCCTCTACGGACTCC	This study
dr013 pET28b-Duet1 vector with Acr-IC4 F	TTTTGTTTAACTTTAAGAAGGAGATATACCATGGATAAC AAAATCACACCTGCGGAC	This study
dr014 pET28b-Duet1 vector with Acr-IC4 R	GGTGTGATTTTGTTATCCATGGTATATCTCCTTCTTAAA GTTAAACAAAATTATTTCTAG	This study
dr015 pET28b-Duet1 vector with Acr-IC4 F	AAATATATTGAATGGCGTGGCGATGAAACGTGAGATCC GGCTGCTAA	This study
dr016 pET28b-Duet1 vector with Acr-IC4 R	CTTTCGGGCTTTGTTAGCAGCCGGATCTCACGTTTCAT CGCCACGCCA	This study
dr017 pBAD vector with IC cascade-Cas3 F	GTAGAGGTCAAAGAGATGCTGTAGGTTCGAATGGCAAA CTTGGCTGCTACTTTCGCTGAA	This study
dr018 pBAD vector with IC cascade-Cas3 R	GATGATGGTCGACGGCGCTATTGCTAACCAAACCTTCT GGCTGCCAAACTTC	This study
dr019 pBAD vector with IC cascade-Cas3 F	GAAGTTTGGCAGCCAGAAGGTTTGGTTAGCAATAGCG CCGTCGACCATCATC	This study

dr020	TTCAGCGAAAGTAGCAGCCAAGTTTGCCATTCGAACCT	This study
pBAD vector with	ACAGCATCTCTTTGACCTCTAC	
IC cascade-Cas3 R		
dr021	AGCCATGACCGCCATTGCC	This study
pBAD vector with		
IC cascade-Cas3 to		
anneal Cas8c		
gBlock insert F		
dr022	GCCCTCACCTCCGGTGAC	This study
pBAD vector with		
IC cascade-Cas3 to		
anneal Cas8c		
gBlock insert R		
dr023	CCCGTCGCGCACGTCACCGGAGGTGAGGGCATGATCC	This study
Cas8c gBlocks	TGCAGGCATTGCATGG	
insert F		
dr024	TCGTATCTGTTGGCAATGGCGGTCATGGCTAGTTCTCC	This study
Cas8c Aromatic	TTGTTCTTCTTGGTCGCAA	
gBlock insert R		
dr025	TCGTATCTGTTGGCAATGGCGGTCATGGCTAGTTCTCC	This study
Cas8c Positive	TTGTTCGCCGCG	
gBlock insert R		
dr026	TCGTATCTGTTGGCAATGGCGGTCATGGCTAGTTCTCC	This study
Cas8c	TTGTTCTTCTTGGTGAAAAGGG	
R363A/K92A/K94A		
gBlock insert R		

References

- [S1] Hayes, R.P., Xiao, Y., Ding, F., van Erp, P.B.G., Rajashankar, K., Bailey, S., Wiedenheft, B., and Ke, A. (2016). Structural basis for promiscuous PAM recognition in type I-E Cascade from E. coli. Nature *530*, 499–503. 10.1038/nature16995.
- [S2] Chowdhury, S., Carter, J., Rollins, M.F., Golden, S.M., Jackson, R.N., Hoffmann, C., Nosaka, L., Bondy-Denomy, J., Maxwell, K.L., Davidson, A.R., et al. (2017). Structure Reveals Mechanisms of Viral Suppressors that Intercept a CRISPR RNA-Guided Surveillance Complex. Cell *169*, 47-57.e11. 10.1016/j.cell.2017.03.012.
- [S3] Guo, T.W., Bartesaghi, A., Yang, H., Falconieri, V., Rao, P., Merk, A., Eng, E.T., Raczkowski, A.M., Fox, T., Earl, L.A., et al. (2017). Cryo-EM Structures Reveal Mechanism and Inhibition of DNA Targeting by a CRISPR-Cas Surveillance Complex. Cell 171, 414-426.e12. 10.1016/j.cell.2017.09.006.
- [S4] Dillard, K.E., Brown, M.W., Johnson, N.V., Xiao, Y., Dolan, A., Hernandez, E., Dahlhauser, S.D., Kim, Y., Myler, L.R., Anslyn, E.V., et al. (2018). Assembly and Translocation of a CRISPR-Cas Primed Acquisition Complex. Cell *175*, 934-946.e15. 10.1016/j.cell.2018.09.039.
- [S5] Hochstrasser, M.L., Taylor, D.W., Kornfeld, J.E., Nogales, E., and Doudna, J.A. (2016). DNA Targeting by a Minimal CRISPR RNA-Guided Cascade. Mol. Cell 63, 840–851. 10.1016/j.molcel.2016.07.027.