

CasX enzymes comprise a distinct family of RNA-guided genome editors

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Supplementary Data for

CRISPR-CasX is an RNA-dominated enzyme active for human genome editing

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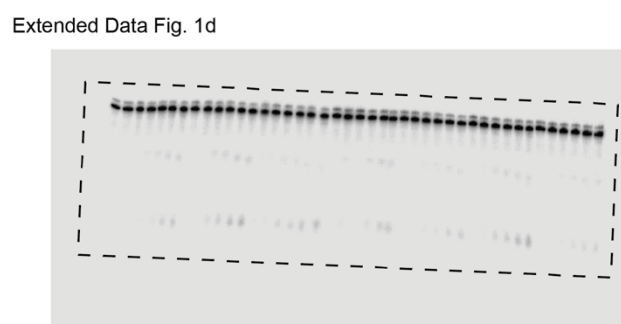
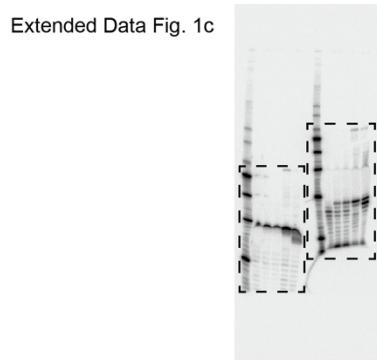
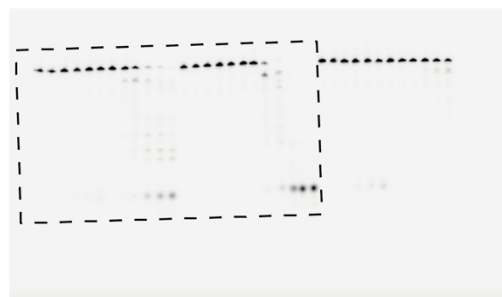
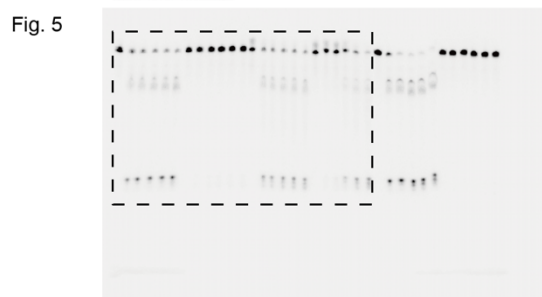
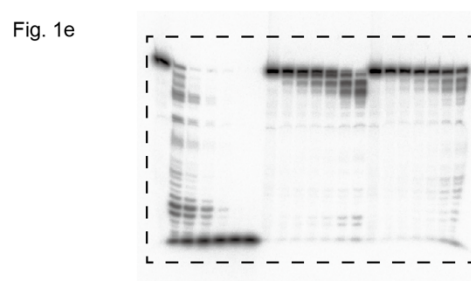
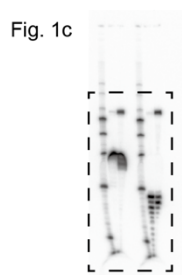
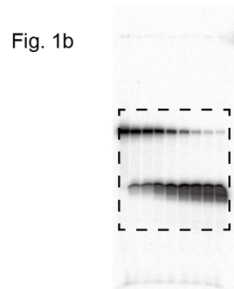
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Extended Data Fig. 1g

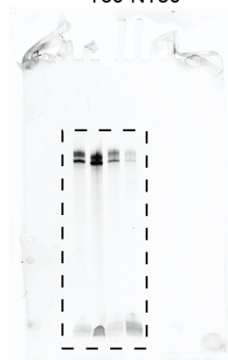
CasX binary complex



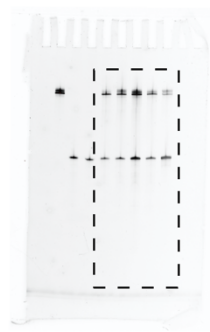
Extended Data Fig. 2a

CasX ternary complex

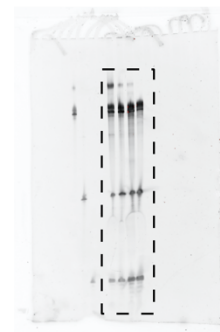
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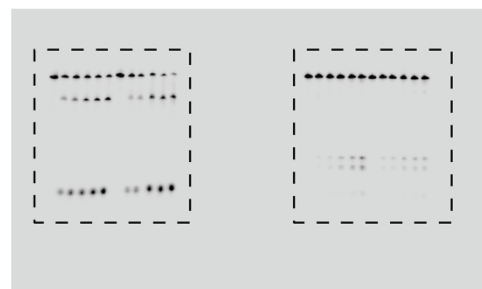
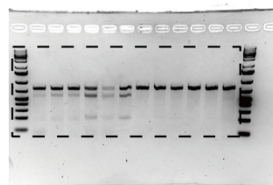
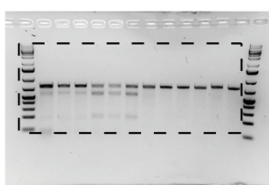
T45-NT45



T45-NT20



Extended Data Fig. 9b



Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics

	Apo-CasX (EMD-8987)	CasX-gRNA (EMD-8988)	CasX-gRNA-DNA(30bp) State I (EMD-8980) (PDB 6NY3)	CasX-gRNA-DNA(30bp) State II (EMD-8994) (PDB 6NY1)	CasX-gRNA-DNA(45bp) State I (EMD-8996) (PDB 6NY2)	CasX-gRNA-DNA(45bp) State II (EMD-8991)	CasX-gRNA-DNA (45T-20NT) State I (EMD-8989)	CasX-gRNA-DNA (45T-20NT) State II (EMD-8990)
Data collection and processing								
Magnification	105,000	105,000	135,000	135,000	135,000	135,000	135,000	135,000
Voltage (kV)	300	300	300	300	300	300	300	300
Electron exposure (e ⁻ /Å ²)	~46	~46	~46	~46	~46	~46	~46	~46
Defocus range (μm)	~0.5 (VPP)	1.2~3.5	0.5~2.0	0.5~2.0	0.5~2.0	0.5~2.0	0.5~2.0	0.5~2.0
Pixel size (Å)	0.9	1.15	0.9	0.9	0.9	0.9	0.9	0.9
Symmetry imposed	C1	C1	C1	C1	C1	C1	C1	C1
Initial particle images (no.)	556157	655926	1698815	1698815	1135443	1135443	801927	801927
Final particle images (no.)	48367	103621	258036	105395	149361	73566	60819	120190
Map resolution (Å)	20 at 0.143	7.5 at 0.143	3.7 at 0.143	4.2 at 0.143	3.2 at 0.143	5.2 at 0.143	4.5 at 0.143	4.4 at 0.143
FSC threshold								
Map resolution range (Å)	15~23	6~15	3~7	3.8~7	3~7	4.5~7	4~8	4~8
Refinement								
Initial model used (PDB code)			N/A	N/A	N/A			
Model resolution (Å)			3.93	4.37	3.28			
FSC threshold								
Model resolution range (Å)			3~7	3.8~7	3~7			
Map sharpening <i>B</i> factor (Å ²)			-178.2	-185.8	-114.5			
Model composition			10214 915	9911 904	10538 915			
Non-hydrogen atoms			164	154	180			
Protein residues								
Nucleotides								

Overall <i>B</i> factors (Å ²)	188	147	105
R.m.s. deviations	0.013	0.009	0.010
Bond lengths (Å)	1.260	1.203	1.114
Bond angles (°)			
Validation	2.01	2.30	2.07
MolProbity score	4.98	8.61	5.7
Clashscore	0.33%	0.33%	0.66%
Poor rotamers (%)			
Ramachandran plot	82.48%	80.99%	83.63%
Favored (%)	16.88%	18.62%	15.60%
Allowed (%)	0.64%	0.39%	0.77%
Disallowed (%)			

Supplementary Table 2. DNA/RNA sequences and plasmids used in this study

Experiment	Name	Sequence/comments
Preparation of the first (30-30) ternary complex for cryoEM	TS-EM1	TTTGATTTTCTGCTGCAGGATGAAATCCCG
Preparation of the first (30-30) ternary complex for cryoEM	NTS-EM1	CGGGATTTCATCCTGCAGCATCCCCGACCC
Preparation of the second (20-45) ternary complex for cryoEM	TS-EM2	CATGTATCGTTATACTTTGATTTTCTGCTGCAGGATGAAATCCG
Preparation of the second (20-45) ternary complex for cryoEM	NTS-EM2	CGGGATTTCATCCTGCAGCA
Preparation of the third (45-45) ternary complex for cryoEM	TS-EM3	CATGTATCGTTATACTTTGATTTTCTGCTGCAGGATGAAATCCG
Preparation of the third (45-45) ternary complex for cryoEM	NTS-EM3	CGGGATTTCATCCTGCAGCATCCCCGACCCGTATAACGATACATG
sgRNA used for EM complex preparations, and most of cleavage assays	sgRNA-main	ggCGCGUUUAUCCAUUACUUUGGAGCCAGUCCAGCGACUAUGUCGUAUGGACGAAGCGCUUAUUUAUCGGAGAGAAACGAUAAGUAAAACGCAUCAAAAGUCCUGCAGCAGAAAAUCAAA
DNA cut site determination	NTS	GCCCCGCGGATTCATCCTGCAGCAGAAAATCAAAAGACAATGAATATTTCCGGCGC
DNA cut site determination	TS	GCGCCGAAATATTCATTGTCCTTGATTTTCTGCTGCAGGATGAAATCCCGCGGGC
sgRNA used for cut site determination (older variant of sgRNA)	sgRNA-1	ggACAUCUGGCGCGUUUAUCCAUUACUUUGGAGCCAGUCCAGCGACUAUGUCGUAUGGACGAAGCGCUUAUUUAUCGGAAGagaaCCGAUAAGUAAAACGCAUCAAAAGUCCUGCAGCAGA

		AAAUCAAA
trans- cleavage assay (comparison of different CRISPR effectors)	M13 phage ssDNA	M13mp18 ssDNA (New England Biolabs)
trans- cleavage (comparison of wild type CasX and CasX- delta101-191)	random-ssDNA50nt	GTTTATTTACTTTAGTCACTCCAGGATTCCAATAGATATTTAC TTGAAG
Activator for CasX trans- cleavage	TS50	CGCTAGCTACGTTTGATTTTCTGCTGCAGGATGAAATCCCGT AATCGCGC
Completely complementary target 50bp	TS50	CGCTAGCTACGTTTGATTTTCTGCTGCAGGATGAAATCCCGT AATCGCGC
Completely complementary target 50bp	NTS50	GCGCGATTACGGGATTTCATCCTGCAGCAGAAAAATCAAA CGTAGCTAGCG
Bubbled target 50bp	BUB-50TS	CGCTAGCTACGTTTGATTTTCTGCTGCAGGATGAAATCCCGT AATCGCGC
Bubbled target 50bp	BUB-50NTS	GCGCGATTACGGGATTTCATCCTGCAGCATCCCCGACCCCGT AGCTAGCG
Unwound target 60	TS-u60	GCTAGCTACGTTTGATTTTCTGCTGCAGGATGAAATCCCGTA ATCGCGCATATCTAGCTG
Unwound target 60	NTS-u60	CAGCTAGATATGCGCGATTACGGGATTTCAGGACGTCGTCT TTAGTTTGCATCGATCG
Single target strand for cleavage assays	JT-60	TATCTAGCTGGCTAGCTACGTTTGATTTTCTGCTGCAGGATG AAATCCCGTAATCGCGCA
primer for Gibson assembly of CasX- delta101-191 and CasX- delta823-928	NO-040218-Tev-for	CTGTACTTCCAATCCAATGCAATGGAGAAGCGT
primer for Gibson assembly of CasX- delta101-191	NO-040218-Del1-rev	CGTGGATGGAATAAAAGTCAAGAGCggagccgccCTGTGCAAAC TTACACATCAAACCTA
primer for Gibson assembly of CasX- delta101-191	NO-040218-Del1-for	TAGGTTTGATGTGTAAGTTTGCACAGggcggtccGCTCTTGACT TTTATTCCATCCACG
primer for Gibson assembly of CasX- delta101-191 and CasX- delta823-928	NO-040218-End-rev	TCCACTTCCAATGTTATCAAGCATTCGGCTTCCAG
primer for Gibson assembly of CasX- delta101-191 and CasX- delta823-928	NO-040218-End-for	CTGGAAGCCGAATGCTTGATAACATTGGAAGTGGA

primer for Gibson assembly of CasX-delta101-191 and CasX-delta823-928	NO-040218-Tev-rev	ACGCTTCTCCATTGCATTGGATTGGAAGTACAG
Gibson assembly of CasX-delta823-928	NO-040218-Del2-wt-rev	GTTTCATCAGCATGAACTTCATGGCCggagccgccAGTTTtagAGG TGTACTGAGCAAGAG
Gibson assembly of CasX-delta823-928	NO-040218-Del2-wt-for	CTCTTGCTCAGTACACCTCTAAAACTggcggctccGGCCATGAAG TTCATGCTGATGAAC
CasX GFP targeting CRISPRi guide	CasX GFP Guide 1	CTGGAGTTGTCCCAATTCTT
CasX GFP targeting CRISPRi guide	CasX GFP Guide 2	GTCCGTGGAGAGGGTGAAGG
CasX GFP targeting CRISPRi guide	CasX GFP Guide 3	TTAAAGAGGAGAAAGGTACC
CasX GFP targeting CRISPRi guide	CasX GFP Guide 4	GGGTGGTGAATTTGACAGCT
CasX GFP targeting CRISPRi guide	CasX GFP Guide 5	AAATTCGCCACAACGTTGAA
CasX GFP targeting CRISPRi guide	CasX GFP Guide 6	ACAAGAATTGGGACAACTCC
CasX GFP targeting CRISPRi guide	CasX GFP Guide 7	CCCTCTCCACGGACAGAAAA
CasX GFP targeting CRISPRi guide	CasX GFP Guide 8	CCTTTGCTCATGGTACCTTT
CasX GFP targeting CRISPRi guide	CasX GFP Guide 9	TTCAAAATTCGCCACAACGT
CasX VS Cas9 human GFP targeting	CasX-g1	CCGGGGTGGTGCCCATCCTG
CasX VS Cas9 human GFP targeting	CasX-g2	GCGTGTCCGGCGAGGGCGAG
CasX VS Cas9 human GFP targeting	CasX-g3	GGGTCAGCTTGCCGTAGGTG
CasX VS Cas9 human GFP targeting	CasX-g4	TCTGCACCACCGGCAAGCTG
CasX VS Cas9 human GFP targeting	CasX-g5	GCCGCTACCCCGACCACATG
CasX VS Cas9 human GFP targeting	CasX-g6	GGCATGGCGGACTTGAAGAA
CasX VS Cas9 human GFP targeting	CasX-g7	CCTCGGCGCGGGTCTTGTAG
CasX VS Cas9 human GFP targeting	CasX-g8	AGGGCGACACCCTGGTGAAC
CasX VS Cas9 human GFP targeting	CasX-g9	GCTCGATGCGGTTACACAGG
CasX VS Cas9 human GFP targeting	CasX-g10	AGGAGGACGGCAACATCCTG
CasX VS Cas9 human GFP targeting	Cas9-g1	CACCGGGGTGGTGCCCATCC
CasX VS Cas9 human GFP targeting	Cas9-g2	CAGCGTGTCCGGCGAGGGCG
CasX VS Cas9 human GFP targeting	Cas9-g3	CAGGGTCAGCTTGCCGTAGG
CasX VS Cas9 human GFP targeting	Cas9-g4	CCGGCAAGCTGCCCGTGCCC

GFP targeting		
CasX VS Cas9 human GFP targeting	Cas9-g5	GTCGTGCTGCTTCATGTGGT
CasX VS Cas9 human GFP targeting	Cas9-g6	GACGTAGCCTTCGGGCATGG
CasX VS Cas9 human GFP targeting	Cas9-g7	CAACTACAAGACCCGCGCCG
CasX VS Cas9 human GFP targeting	Cas9-g8	GAAGTTCGAGGGCGACACCC
CasX VS Cas9 human GFP targeting	Cas9-g9	CAGCTCGATGCGGTTACCA
CasX VS Cas9 human GFP targeting	Cas9-g10	CAAGGAGGACGGCAACATCC
Plasmid	Contains	Comments
2CT-10-DpBCasX	His-MBP-TEV-DpbCasX, expression vector	This study
2CT-10-DpBCasX-D672A-E769A-D935A	His-MBP-TEV-DpbCasX- D672A-E769A-D935A, expression vector	This study
2CT-10-DpBCasX-delta101-191	His-MBP-TEV-DpbCasX-delta101-191, expression vector	This study
2CT-10-DpbCasX-delta823-928	His-MBP-TEV-DpbCasX-delta823-928, expression vector	This study
pBLO 55.5	TetR-DpbCasX D672A-E769A-D935A -CM	This study
pBLO 55.5	TetR-DpbCasX-CM	This study
pBLO 2.X	J23119-CasX guide-Amp	This study
PBLO 62.4.1	U6-CasX guide-CMV-CasX-Puro	This study