nature portfolio

https://doi.org/10.1038/s41586-021-03206-x

Supplementary information

The Card1 nuclease provides defence during type III CRISPR immunity

In the format provided by the authors and unedited

Supplementary Methods Table 1

Data collection and refinement statistics.

	SeMET Cad1	Apo Cad1 (PDB 6WXW)	cA_4-Cad1 (PDB 6WXX)	cA_6-Cad1 (PDB 6WXY)	cA_4-Cad1 (D294N) (PDB 6XL1)
Data collection					
Wavelength (Å)	0.9792	0.9792	0.9792	0.9792	0.9792
Space group	*C*121	*C*121	*P*12$_1$1	*C*121	*P*4$_1$2$_1$2
Cell dimensions					
a, b, c (Å)	181.0, 42.6, 125.8	172.2, 124.7, 42.6	85.1, 121.2, 103.7	172.9, 124.8, 42.4	111.9, 111.9, 172.2
α, β, γ (°)	90, 109.4, 90	90, 96.9, 90	90, 113.1, 90	90, 96.8, 90	90, 90, 90
Resolution (Å)	118.7-2.61 (2.72-2.61)	86.45-2.32 (2.41-2.32)	28.34-3.0 (3.107-3.0)	85.85-2.10 (2.18-2.10)	39.58-1.95 (2.02-1.95)
R-merge	0.093 (1.231)	0.078 (0.762)	0.143 (1.215)	0.056 (0.630)	0.080(0.661)
R-pim	0.055(0.715)	0.045(0.450)	0.082 (0.726)	0.033(0.368)	0.021(0.174)
I/σI	10.3 (1.0)	12.9 (1.6)	8.06 (1.06)	15.5 (2.0)	23.41 (4.08)
Completeness (%)	98.7 (96.3)	98.8 (96.3)	98.7 (98.5)	98.4 (92.1)	99.9 (99.9)
Redundancy	3.7 (3.3)	3.9 (3.8)	3.9 (3.7)	3.9 (3.8)	15.0 (15.2)
CC1/2	0.997 (0.474)	0.997 (0.624)	0.994 (0.457)	0.998 (0.705)	0.998 (0.936)
Unique Reflections	27,896 (3,290)	38,511 (3,738)	38,486 (3,808)	51,025 (4,689)	80,132 (7,872)
Refinement					
R_{work}/R_{free} *(%)*	--	20.5/24.4	20.4/23.7	20.6/24.6	17.8/20.2
Reflections in refinement	--	38,406 (3,736)	38,366 (3,807)	50,768 (4,688)	80,121 (7,871)
No. of non-hydrogen atoms					
Proteins	--	6,184	12,368	6,184	6,771
cAn	--	--	176	132	88
ligands	--	0	4	0	19
Water	--	138	0	218	552
Protein residues	--	744	1488	744	746
B-factors (Å^2)					
Proteins	--	60.0	78.1	52.3	39.8
cAn	--	--	51.7	77.8	22.7
ligands	--	--	123.5	--	55.39
Water	--	52.2	--	49.3	39.76
R.m.s. deviations					
Bond lengths (Å)	--	0.007	0.006	0.008	0.008

Bond angles (°)	--	0.82	0.80	0.95	0.84
Ramachandran plots					
Favored (%)	--	96.9	97.7	96.8	97.7
Allowed (%)	--	3.0	2.3	3.2	2.3
Outliers (%)	--	0.1	0	0	0
Rotamer outliers (%)	--	0.15	2.11	0.73	1.74
Clashscore	--	6.66	8.96	5.52	5.11
Statistics for the highest-resolution shell are shown in parenthesis.					

Supplementary Methods Table 2

Oligonucleotides used in this study.

Name	Sequence	Purpose
GG424	CATATTGCCTGATGAAGTGAATAG	Cloning
GG425	CTATTCACTTCATCAGGCAATATG	Cloning
JTR476	GAACAGTGTCTAACAACTGCAATTCACTAAATGCTGTAA	Cloning
JTR477	GATCTTACAGCATTTAGTGAATTGCAGTTGTTAGACACT	Cloning
JTR592	TTTTGTGTGTTGCGGCTCCTATTCTCCCGACTTTGGTACC	Protospacer target transcript Northern probe
JTR595	GCGGGAACCAATCATCAAATTTAAACTTCATTGCATAATC	*repF* Northern probe
JTR600	CTTTAGTTAATGGTAATTCTAACTCAGCTGCTTTTTGACG	*def* Northern probe
JTR606	GTGTTCGGCATGGGAACAGGTGTGACCTCC	5S rRNA Northern probe
JTR632	ATGATAAATAAAATTACAGTAGAGTTAGACTTGC	Cloning
JTR633	TATAGCACCTCATTATTTAACTCTTGAAAAC	Cloning
JTR638	CAAGAGTTAAATAATGAGGTGCTATAATGAAAGAGACTATTTTGGTTAACTTGG	Cloning
JTR639	CTAACTCTACTGTAATTTTATTTATCATATGTATATCTCCTTCTCATAATTGTGTACCGTCTTTAATGTC	Cloning
JTR678	GTTACACGTGATAGCATGTGCTAGTTTCGTCGATGGAAACG	Cloning
JTR679	GACGAAACTAGCACATGCTATCACGTGTAACTTATTGTCTTTGTC	Cloning
JTR859	GATCCGGCTGCTAACAAAGC	Cloning
JTR860	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAG	Cloning
JTR861	GTTTAACTTTAAGAAGGAGATATACATATGAAAGAGACTATTTTGGTTAACTTGG	Cloning
JTR862	CGGGCTTTGTTAGCAGCCGGATCTTAGTGATGGTGATGGTGATGTCCTG	Cloning
JTR951	CAACAAAAGTATTTAAACTTTCTTTTAAGACTC	Cloning
JTR952	GAAAGTTTAAATACTTTTGTTGCCAATGTCATTCACCTACTTAATTTTAAGATTTG	Cloning
JTR972	AACAATTCCTAATGTACAATTTATCAAGTGG	Cloning
JTR973	GATAAATTGTACATTAGGAATTGTTGCCTCACTAACCAAGTTAACCAAAATAGTC	Cloning
JTR974	GAGCAAACAATTCCTAATGTACAATTTATC	Cloning
JTR975	GTACATTAGGAATTGTTTGCTCTGCAACCAAGTTAACCAAAATAGTCTCTTTC	Cloning
JTR976	ATGGAACAGAAAGAAAAATCATTGTTC	Cloning
JTR977	CAATGATTTTTCTTTCTGTTCCATTGCCTTAGTACTCACTAACAATATCTTCATTGG	Cloning

JTR978	GAACAGAAAGAAAAATCATTGTTCATCAAG	Cloning
JTR979	GAACAATGATTTTTCTTTCTGTTCTGCCTCCTTAGTACTCACTAACAATATCTTCATTGG	Cloning
JTR980	AGGTAAAGAATTGCAGGAGTTATACC	Cloning
JTR981	GTATAACTCCTGCAATTCTTTACCTGCAGGTTGGTAGAATATCTCTGTGTTAGG	Cloning
JTR982	GTAAAGCAACACTTGTATACGAAAAG	Cloning
JTR983	CTTTTCGTATACAAGTGTTGCTTTACTGCTAATCCGAACTTACTCTTGATGATCG	Cloning
JTR984	ATGTAAGAGTTTCGTCGATGGAAACG	Cloning
JTR985	CCATCGACGAAACTCTTACATGCTATCACGTGTAACTTATTGTCTTTGTCC	Cloning
JTR986	AATGTAAGAGTTTCGTCGATGG	Cloning
JTR987	CCATCGACGAAACTCTTACATTGTATCACGTGTAACTTATTGTCTTTGTCC	Cloning
JTR993	CAACCTATAGGTAAAGAATTGCAGG	Cloning
JTR994	CAATTCTTTACCTATAGGTTGTGCGAATATCTCTGTGTTAGGTTTGTTATTAAAGAAATC	Cloning
JTR995	GTTATCTACTTGGACAAAGACAATAAGTTAC	Cloning
JTR996	GTCTTTGTCCAAGTAGATAACTGCCAACTCGTTTTTGTCATTTCCC	Cloning
JTR1052	TAATACGACTCACTATAGGGTATAGTTTAACAACGTTTGCAGCTTGTGGAC	*msaB* Northern probe
W614	GGTTATACTAAAAGTCGTTTGTTGG	Cloning
PM797	AAACCATCCTCAAGACTTATTAAGTCAATTAGTTG	Cloning
PM798	AAAACAACTAATTGACTTAATAAGTCTTGAGGATG	Cloning
poly-rU	/56-FAM/rUrUrUrUrUrUrUrUrUrUrUrUrUrU/3IABkFQ/	Card1 ssRNase specificity fluorescence assay
poly-rA	/56-FAM/rArArArArArArArArArArArArArArA/3IABkFQ/	Card1 ssRNase specificity fluorescence assay
poly-rC	/56-FAM/rCrCrCrCrCrCrCrCrCrCrCrCrCrC/3IABkFQ/	Card1 ssRNase specificity fluorescence assay
W852	CCAACAAACGACTTTTAGTATAACC	Cloning
W1169	CCGATTAAAAATAAAGCTGCACCGCCTGAATATATAGCAGTAATTTG	Cloning
W1170	TATTCAGGCGGTGCAGCTTTATTTTTAATCGGTGCATGGGATG	Cloning
soJTR1	/5Cy3/TGATATTAATAACACTATAGACCACCGCCC	Cy3 30-nt DNA for RNase reaction. Used with soJTR2

soJTR2	/5Cy5/rUrGrArUrArUrUrArArUrArArCrArCrUrArUrArGrArCrCrArCrCrGrCrCrC	Cy5 30-nt RNA for RNase reaction. Used with soJTR2
soJTR3	/5Cy5/GCGTTGGTAAGATTCAGGATAAAATTGTAGCTGGGTGCAAAATAGCAACT	Cy5 50-nt DNA for RNase reaction. Used with soJTR4
soJTR4	/5Cy3/rGrCrGrUrUrGrGrUrArArGrArUrUrCrArGrGrArUrArArArArUrUrGrUrArGrCrUrGrGrGrUrGrCrArArArArUrArGrCrArArCrU	Cy3 50-nt RNA for RNase reaction. Used with soJTR3
60-nt RNA Cy3	/5Cy3/rGrGrCrArCrArCrCrCrGrCrArGrGrGrArGrGrArGrCrCrArArArGrCrArCrArCrGrUrCrCrArUrCrArUrUrCrCrGrUrUrGrCrCrArCrArGrCrArGrArArGrCrCrC	Cy-3 labelled 60nt RNA for use in Card1 RNase assays

Supplementary Methods Table 3

Plasmids used in this study

Plasmid name	Plasmid contents	Made in this study?
pGG-BsaI-R	Type III with no spacer	No[23]
pJTR170	Type III, Φ12γ3 ORF27 spacer, Csm6	Yes
pJTR172	Type III, Φ12γ3 ORF27 spacer, dCsm6	Yes
pJTR330	Cad1-His_6 on pET23 overexpression	Yes
pJTR378	dCad1-His_6 on pET23 overexpression	Yes
pJTR393	Type III, no targeting spacer, Cad1	Yes
pJTR394	Type III, anti-pTarget spacer, $Cas10^{HD}$, $Cas10^{Palm}$, Cad1	Yes
pJTR395	Type III, anti-pTarget spacer, Cad1	Yes
pJTR396	Type III, anti-pTarget spacer, $Cas10^{HD}$, Cad1	Yes
pJTR400	Type III, Φ12γ3 ORF9 spacer, Cad1	Yes
pJTR401	Type III, Φ12γ3 ORF9 spacer, $Cas10^{HD}$, Cad1	Yes
pJTR402	Type III, Φ12γ3 ORF27 spacer, Cad1	Yes
pJTR403	Type III, Φ12γ3 ORF27 spacer, $Cas10^{HD}$, Cad1	Yes
pJTR405	Type III, anti-pTarget spacer, Cad1	Yes
pJTR406	Type III, anti-pTarget spacer, $Cas10^{HD}$, dCad1	Yes
pJTR424	Type III, Φ12γ3 ORF27 spacer, $Cad1^{Q13A}$	Yes
pJTR426	Type III, Φ12γ3 ORF27 spacer, $Cad1^{E41A}$	Yes
pJTR427	Type III, Φ12γ3 ORF27 spacer, $Cad1^{M42A}$	Yes
pJTR428	Type III, Φ12γ3 ORF27 spacer, $Cad1^{I125A}$	Yes
pJTR429	Type III, Φ12γ3 ORF27 spacer, $Cad1^{Y340A}$	Yes
pJTR431	Type III, Φ12γ3 ORF27 spacer, $Cad1^{E308Q}$	Yes
pJTR434	Type III, Φ12γ3 ORF27 spacer, $Cad1^{S11A}$	Yes
PJTR435	Type III, Φ12γ3 ORF27 spacer, $Cad1^{E308A}$	Yes
pJTR436	Type III, Φ12γ3 ORF27 spacer, $Cad1^{Y122}$	Yes
pJTR437	Type III, Φ12γ3 ORF27 spacer, $Cad1^{D294A}$	Yes
pJTR439	Type III, Φ12γ3 ORF9 spacer, dCad1	Yes
pJTR441	Type III, Φ12γ3 ORF27 spacer, dCad1	Yes
pJTR443	Type III, ΦNM1γ6 gp14 spacer, Cad1	Yes
pJTR444	Type III, anti-pTarget spacer, dCad1	Yes
pJTR446	Type III, anti-pTarget spacer, $Cas10^{Palm}$, dCad1	Yes
pPM169	Cas9 targeting Φ12γ3	Yes
pTarget	aTc-inducible promoter in front of a protospacer	No[5]
pWJ246	Type III, ΦNM1γ6 gp14 spacer, dCsm6 (-Cad1)	No[18]

Supplementary Methods Table 4

Cloning strategies for plasmids used in this study

Name	Cloning strategy
pJTR170	Cleavage of plasmid pGG-BsaI-R with BsaI-HF, then ligation of the linearized plasmid with the annealed oligo pair JTR476 and JTR477
pJTR172	PCR amplification of pJTR170 with W852 and GG425, and of pWJ241 with GG424 and W614, followed by Gibson assembly of the two PCR products
pJTR330	PCR amplification of pPS3 with JTR859 and JTR860, and with pJTR325 by JTR861 and JTR862, followed by Gibson assembly of the two PCR products
pJTR378	PCR amplification of pJTR330 by JTR861 and JTR679, and with JTR860 and JTR678
pJTR393	PCR amplification of pGG-BsaI-R with W852 and GG425, and of pRF7 with GG424 and W614, followed by Gibson assembly of the two PCR products
pJTR394	PCR amplification of pJTR121 with W852 and GG425, and of pRF7 with GG424 and W614, followed by Gibson assembly of the two PCR products
pJTR395	PCR amplification of pWJ191 by JTR633 and JTR632, and of pJTR224 by JTR638 and JTR639, followed by Gibson assembly of the two PCR products
pJTR396	PCR amplification of pJTR125 with JTR632 and JTR633, and of pJTR224 with JTR638 and JTR639, followed by Gibson assembly of the two PCR products
pJTR400	PCR amplification of pJTR169 with W852 and GG424, and of pJTR395 with W614 and GG424, followed by Gibson assembly of the two PCR products
pJTR401	PCR amplification of pJTR169 with W852 and GG424, and of pJTR396 with W614 and GG424, followed by Gibson assembly of the two PCR products
pJTR402	PCR amplification of pJTR170 with W852 and GG425, and of pJTR395 with GG424 and W614, followed by Gibson assembly of the two PCR products
pJTR403	PCR amplification of pJTR170 with W852 and GG425, and of pJTR396 with GG424 and W614, followed by Gibson assembly of the two PCR products
pJTR405	PCR amplification of pJTR395 with JTR678 and W614, and with JTR679 and W852, followed by Gibson assembly of the two PCR products
pJTR406	PCR amplification of pJTR396 with JTR678 and W614, and with JTR679 and W852, followed by Gibson assembly of the two PCR products
pJTR424	PCR amplification of pJTR402 with JTR972 and W614, and with JTR973 and W852, followed by Gibson assembly of the two PCR products
pJTR426	PCR amplification of pJTR402 with JTR976 and W614, and with JTR977 and W852, followed by Gibson assembly of the two PCR products
pJTR427	PCR amplification of pJTR402 with JTR978 and W614, and with JTR979 and W852, followed by Gibson assembly of the two PCR products
pJTR428	PCR amplification of pJTR402 with JTR980 and W614, and with JTR981 and W852, followed by Gibson assembly of the two PCR products
pJTR429	PCR amplification of pJTR402 with JTR982 and W614, and with JTR983 and W852, followed by Gibson assembly of the two PCR products
pJTR431	PCR amplification of pJTR402 with JTR986 and W614, and with JTR987 and W852, followed by Gibson assembly of the two PCR products
pJTR434	PCR amplification of pJTR402 with JTR974 and W614, and with JTR975 and W852, followed by Gibson assembly of the two PCR products
pJTR435	PCR amplification of pJTR402 with JTR984 and W614, and with JTR985 and W852, followed by Gibson assembly of the two PCR products

pJTR436	PCR amplification of pJTR402 with JTR993 and W614, and with JTR994 and W852, followed by Gibson assembly of the two PCR products
pJTR437	PCR amplification of pJTR402 with JTR995 and W614, and with JTR996 and W852, followed by Gibson assembly of the two PCR products
pJTR439	PCR amplification of pJTR400 with W852 and JTR679, and with W614 and JTR678, followed by Gibson assembly of the two PCR products
pJTR441	PCR amplification of pJTR402 with W852 and JTR679, and with W614 and JTR678, followed by Gibson assembly of the two PCR products
pJTR443	PCR amplification of pJTR288 with W852 and JTR952, and with W614 and JTR951, followed by Gibson assembly of the two PCR products
pJTR444	PCR amplification of pJTR109 with W852 and GG425, and of pJTR441 with W614 and GG424, followed by Gibson assembly of the two PCR products
pJTR446	PCR amplification of pJTR395 with W852 and W1169, and with W614 and W1170, followed by Gibson assembly of the two PCR products
pPM169	Annealing of oligos PM797 and PM798, digestion of plasmid pDB114 with BsaI, and ligation of the annealed oligo pair into the plasmid