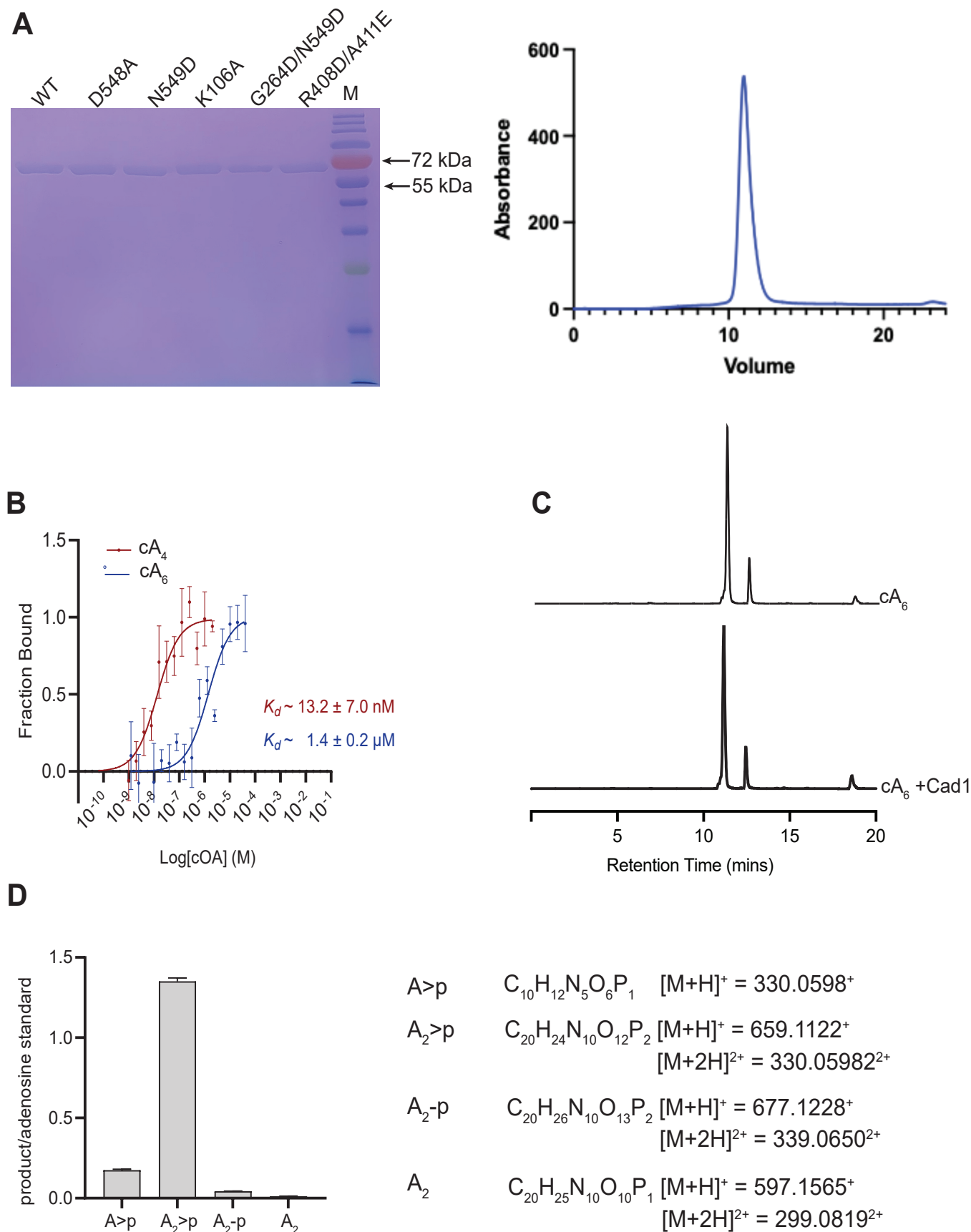


Appendix for
The twist-and-squeeze activation of CARF-Fused Adenosine Deaminase by Cyclic
Oligoadenylates

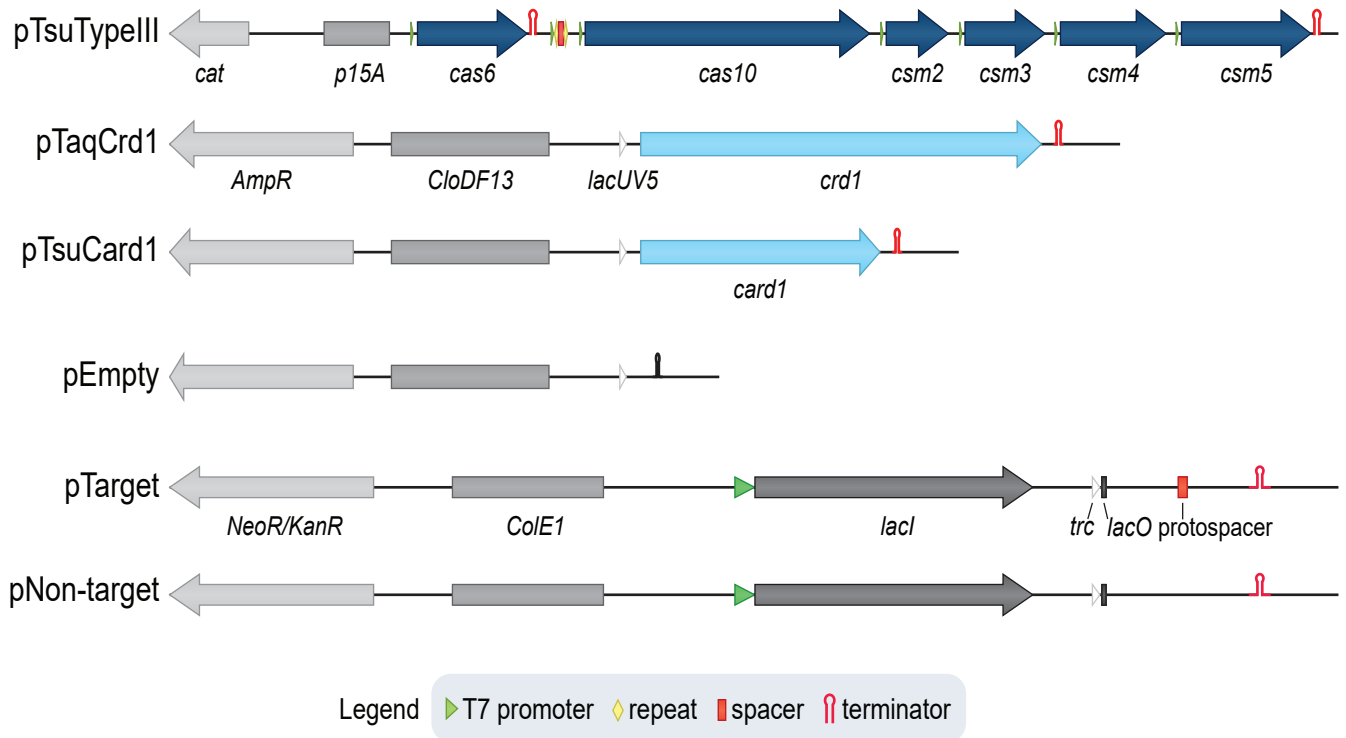
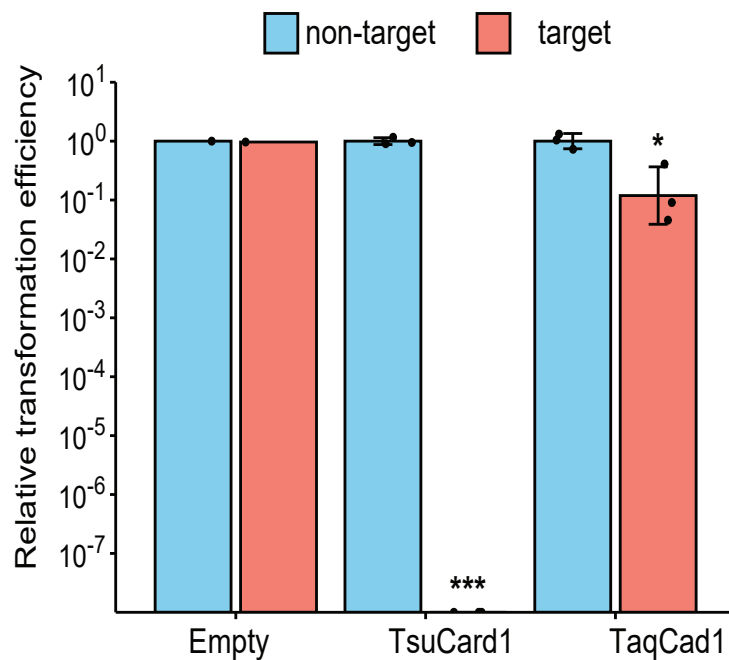
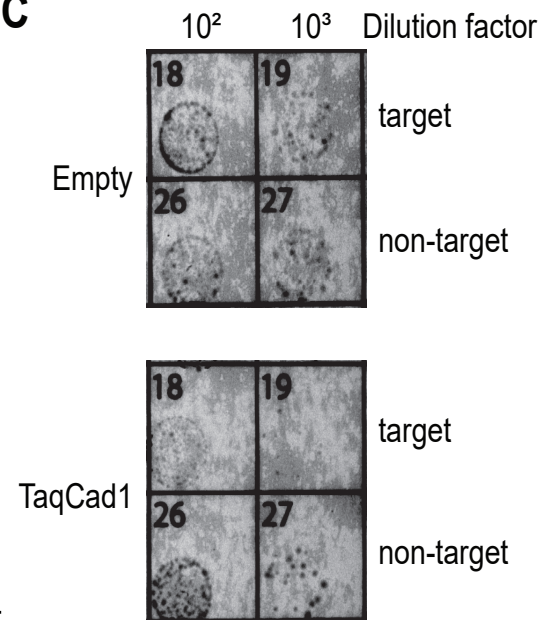
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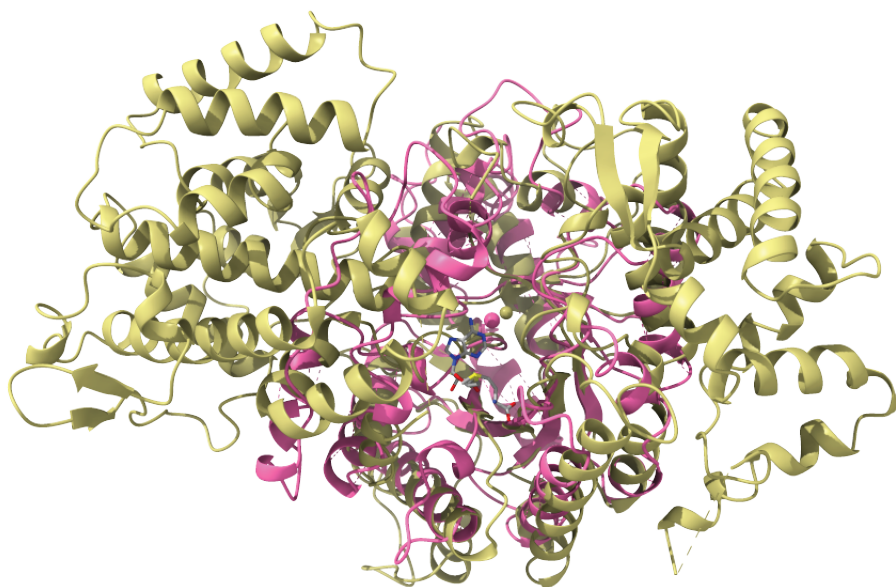
Appendix Figure S1. Protein purification and functional characterizations of the CARF domain of *Taq*Cad1.

(A) Left, protein purity of the wild-type (WT) and the mutants is assessed on an SDS-PAGE gel stained by Coomassie brilliant blue. Right, an example size exclusion chromatography elution profile of the wild-type protein monitored by 260 nm absorbance. **(B)** Microscale thermophoresis (MST) profiles of titrating *Taq*Cad1 with cA₄ (red) or cA₆ (black). The binding dissociation constant is estimated by fitting the binding isotherms to a one-site binding model. **(C)** High Performance Liquid Chromatography (HPLC) analysis of cA₆ molecules incubated with (lower) or without (upper) *Taq*Cad1. **(D)** Mass spectrometry analysis of the ring nuclease products of *Taq*Cad1 upon incubating with cA₄. The chemical formula and mass to charge ratios of each of the detected products are listed to the right. Error bars represent the standard deviation of the mean. Data represent the mean $\pm$ standard deviation of the mean ($n = 3$ independent experiments).

A**B****C**

Appendix Figure S3. In vivo plasmid challenge assays to investigate the phenotype *TaqCad1* activation.

(A) Transformation efficiencies (relative to non-target control) of target and non-target plasmids in *E. coli* co-expressing *TaqCad1* and *cA₄*-producing *Treponema succinifaciens* type III CRISPR-Cas complex. Error bars represent the standard deviation of the mean. Statistical significance was calculated using Welch's t-test (n = 3 biological replicates). * p = 3.6E-2, *** p = 9.7E-6. **(B)** Comparison of colony sizes obtained from the assay after overnight growth. **(C)** Growth of transformants in liquid culture, measured as the optical density at 600 nm (OD₆₀₀). Data represent the mean ± standard deviation of the mean (n = 3 biological replicates).



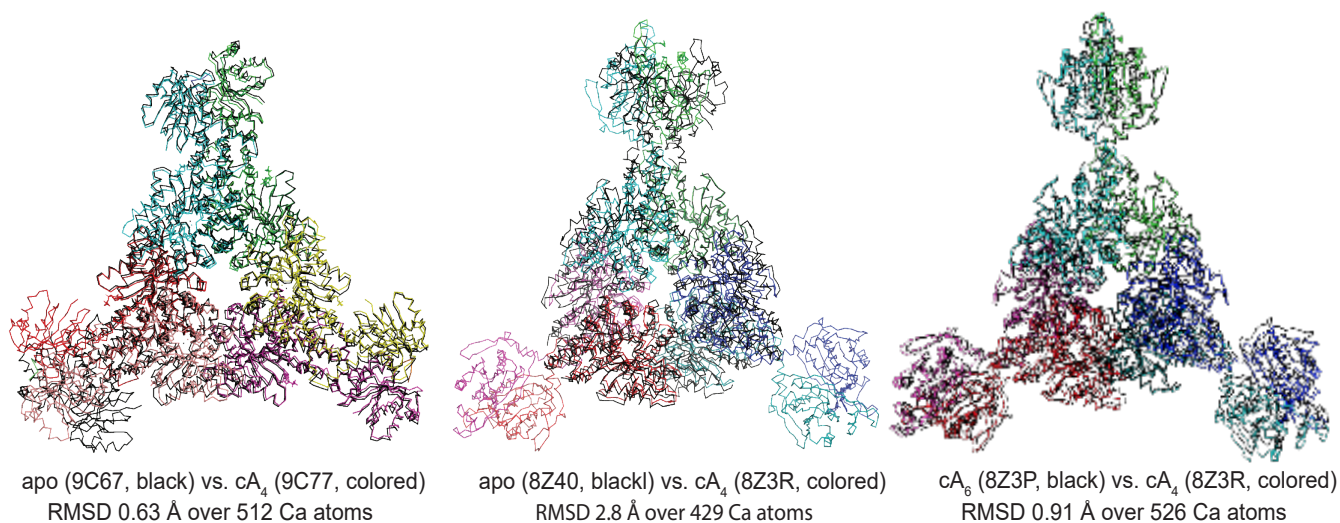
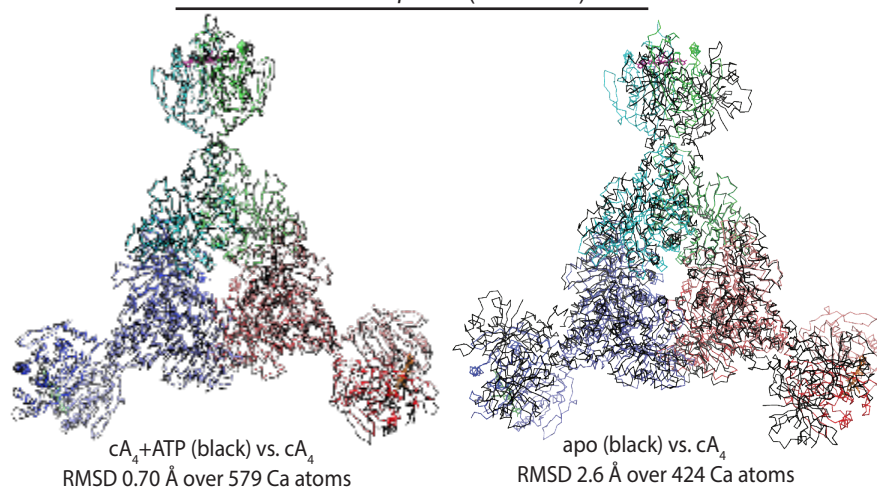
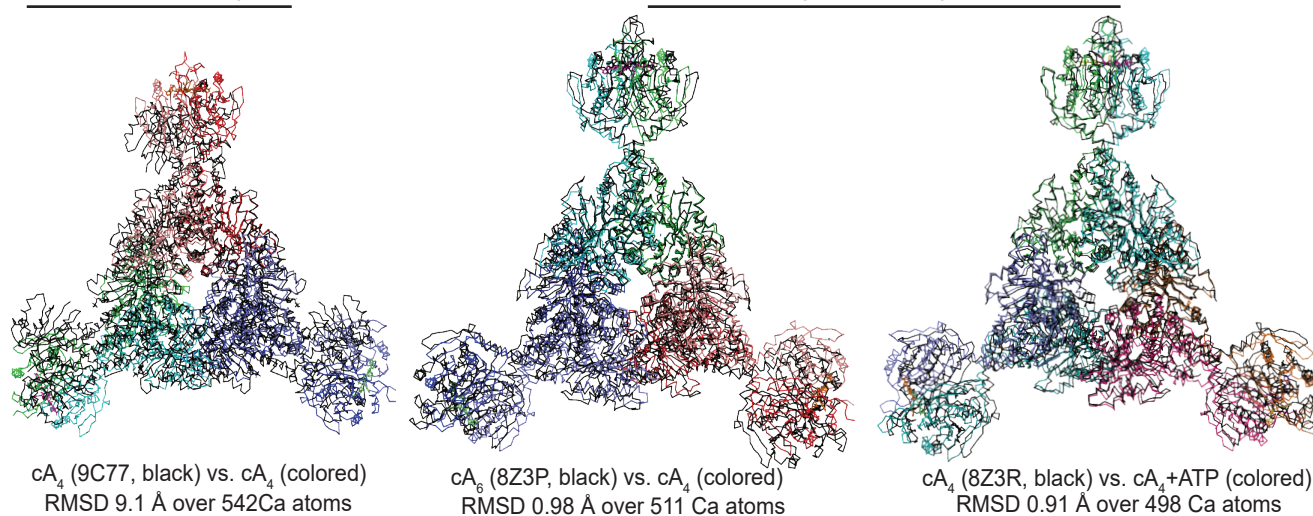
RADAR-RrB deaminase (8FNV)
***TaqCad1* ADA**



murine adenosine deaminase (2ADA)
***TaqCad1* ADA**

Appendix Figure S4. Structural comparison of *TaqCad1* with ADA.

Top, Comparison of the *TaqCad1* ADA domain (pink) and the RrB subunit of the RADAR anti-phage system (yellow).
 Bottom, Comparison of the *TaqCad1* ADA domain (pink) and a murine adenosine deaminase (yellow). PDB codes are indicated.

A*BabCad1* (Baca et al. 2024)*LngCAAD* (Li et al. 2025)**B***TaqCad1* (This work)**C***BabCad1* vs *TaqCad1**LngCad1* vs *TaqCad1***Appendix Figure S5. Structural comparison of *TaqCad1* with otherCad1 structures.**

Root mean square (RMSD) and the aligned Cα atoms are listed under each pair of compared structures. PDB codes for the previously published Cad1 structures are listed. “This work” refers to the structures presented in this work. “cA₄” refers to the cA₄-bound structure. “cA₄+ATP” refers to the structure from the enzyme incubated with cA₄ and ATP. **(A)** Left, comparison of *BabCad1* apo and cA₄-bound (cA₄) structures. Right, comparison of *LngCAAD* structures. **(B)** Comparison of *TaqCad1* structures. **(C)** Comparison of *TaqCad1* structures to those of *BabCad1* and *LngCAAD*.

Appendix Table S1. Statistics of cryo-EM data collection and processing

Data collection & processing	TaqCad1-cA₄	ApoTaqCad1	TaqCad1-cA₄-ATP
Microscope	Titan Krios G3i	Titan Krios G3i	Titan Krios G3i
Detector	Gatan K3	Gatan K3	Gatan K3
Voltage	300 kV	300 kV	300 kV
Collecting mode	Counted super-resolution	Counted super-resolution	Counted super-resolution
Total dose (e ⁻ /Å ²)	60	60	50
Defocus range (μm)	(-0.8) - (-2.5)	(-0.8) - (-2.5)	(-1.0) - (-2.2)
Nominal magnification	81K	81K	105K
Frames collected per exposure	60	60	60
Frame-alignment software	MotionCor2-1.5.0	MotionCor2-1.5.0	MotionCor2-1.5.0
CTF estimation software	Gctf	Gctf	Gctf
Raw images collected	8597	4186	7862
2D classification software	cryoSPARC(v4.1.1)	cryoSPARC(v4.1.1)	cryoSPARC(v4.1.1)
3D classification software	Relion5.0	Relion5.0	Relion5.0
Final reconstruction software	cryoSPARC(v4.1.1)	cryoSPARC(v4.1.1)	cryoSPARC(v4.1.1)
Applied symmetry	C1	C1	C1
Resolution method	FSC 0.143 cutoff	FSC 0.143 cutoff	FSC 0.143 cutoff
Local resolution software	Cryosparc(v4.1.1) Local Resolution Estimation	Cryosparc(v4.1.1) Local Resolution Estimation	Cryosparc(v4.1.1) Local Resolution Estimation
Map and model visualization software	Pymol/Chimera/Chimera-X, Coot	Pymol/Chimera/Chimera-X, Coot	Pymol/Chimera/Chimera-X, Coot

Appendix Table S2. Statistics of model refinement

	TaqCad1-cOA ₄ (sym expanded dimer composite)	TaqCad1-cOA ₄ (good hexamer)	Apo TaqCad1 (sym expanded dimer, composite)	ApoTaqCad1 (good hexamer)	TaqCad1-cOA ₄ - ATP (sym expanded dimer, composite)	TaqCad1-cOA ₄ - ATP (good hexamer)	TaqCad1-cOA ₄ - ATP (one dimer)	TaqCad1-cOA ₄ - ATP (two dimer)
CC (mask)	0.86	0.83	0.79	0.81	0.77	0.76	0.83	0.83
RMSD (bond lengths/bond angles)	0.003/0.597	0.003/0.578	0.006/0.841	0.027/1.628	0.004/0.561	0.003/0.531	0.002/0.551	0.002/0.489
# of particles for final reconstruction	4,288,906	409,445	938,192	15,816	859,261	149,171	165,680	257,900
Final resolution (Å)	2.29-2.36	2.36	2.55-2.87	3.08	2.45-2.54	3.17	2.86	2.68
Ramachandran plot								
Outliers	0.08	0.08	0.25	0.52	0.00	0.17	0.11	0.19
Allowed	2.00	3.46	4.29	4.45	2.08	4.35	3.56	3.54
Favored	97.91	96.46	95.47	95.03	97.92	95.48	96.33	96.27
C β Outliers (%)	0.0	0.0	0.0	0.61	0.0	0.0	0.0	0.0
ADP (B-factors)								
Iso/Aniso (#)	9542/0	28755/0	9530/0	28590/0	9662/0	28970/0	21982/0	25543/0
Protein	0.0/89.7/32.3	0.0/213.9/80.5	6.6/154.4/62.9	76.6/499.6/201.0	27.0/202.7/102.2	36.4/327.0/161.1	3.9/238.3/90.8	0.0/258.8/59.7
Nucleotide	0.0/22.5/12.7	0.0/29.6/11.6	---	---	22.2/66.3/47.4	32.8/96.7/65.5	25.7/66.6/43.6	20.9/60.0/38.4
Ligand	56.5/57.7/57.1	62.6/72.2/66.6	77.7/89.1/83.4	169.6/188.0/178.2	88.7/126.2/107.5	119.4/198.2/162.2	71.2/176.5/104.3	35.7/139.0/73.4
Water	---	---	---	---	43.6/80.4/68.39	67.6/187.3/126.7	30.1/93.6/66.1	12.2/49.6/31.5
(min/max/mean)								
MolProbity score	1.51	1.61	1.94	2.23	1.45	2.42	2.05	2.20
Clash score	9.12	6.75	13.01	25.0	7.89	41.9	20.38	28.94
Rotamer outlier (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FSC model (0/0.143/0.5)	--	2.4/2.5/2.6	--	3.1/3.1/3.4	--	3.0/3.1/3.5	2.80/2.80/3.1	2.6/2.7/2.9
Deposited EMDDB codes	47886 (consensus) 47887 (CARF focused) 47888 (ADA focused) 47890 (composite)	48405	70419 (consensus) 70423 (CARF focused) 70424 (ADA focused) 70417 (composite)	48116	70425 (consensus) 70426 (CARF focused) 70427 (ADA focused) 70422 (composite)	70428	70429	70430
Deposited PDB codes	9EBT	9MMW	9OF1	9EKA	9OFB	9OFC	9OFD	9OFE

Appendix Table S3. Oligos Used in This Study

Mutation	Oligos	Source
D548A	Forward: GAACACCGCTAACCTGGGTATTAGCCAG Reverse: CCAGGTTAGCGGTGTTTCAGGGTAACTGC	Eurofins
N549D	Forward: GAACACCGATGACCTGGGTATTAGCCAG Reverse: ACCCAGGTCATCGGTGTTTCAGGGTAAAC	Eurofins
K106A	Forward: GGCGGCTATGCAACCATTTCTGCAGCAATG Reverse: GAAATGGTTGCATAGCCGCCTGCCAGAC	Eurofins
A411E	Forward: GCGCGTCATTTAGAGTTAGCAATTACCGCAG Reverse: TTGCTAACTCTAAATGACGCGCAATACGAC	Eurofins
R408D	Forward: CGTAGTGATATTGCGCGTCATTTAGAGTTAG Reverse: GCGCAATATCACTACGATCGCCGCCTTCTTC	Eurofins
G264D	Forward: GTCATTTAGACGGTTTTTGCAACCCACGGCG Reverse: CAAAACCGTCCTAAATGACAATGCAGTTCAAC	Eurofins
M311D	Forward: GAGCGCTATGATCGTCTGGGCGATAATAACGGTAG Reverse: CCCAGACGATCATAGCGCTCTAAACCAATCGGTTC	Eurofins
G314F	Forward: TCGCTCTGTTCGATAATAACGGTAGCGCATTACTG Reverse: GTTATTATCGAACAGACGCATATAGCGCTCTAAAC	Eurofins
<i>Taq</i> Csx1 cloning	Forward: actttaataaggagatatataATGGAAACGATGCCAAGGCAAG Reverse: gcgcgccgagctcgaattcgTCACGCTTTGCCCTCCCC	Eurofins
RNA1 (RNA cleavage assay)	5'GGTTGGAAAGCCGGTTTTCTTTGATGTCACGGAACACGUUCUUUGAA CCAAGCUUCAAC 3'	IDT

Protein	Amino acid sequence
<i>TaqCad1</i> WT	MGHHHHHHGSSGMRIILCSVGTSWAVVPEAMQLLGSQGFDEVHVLTTASSKISPGVEQLLRYFEMHP GPRFSISRVDQFEDLRSEQDHMLFEEVLWRWLLQRAPQAAHRYICLAGGYKTISAAMQRAAALFGAC EVFHVLCPEPRFGPQGNREASTLEEVEQAIATNALRFVRLGPEPGWPQLRLLSAPSFPLESTLQGPVH WVRASDMRLRQHVEGVLEERSRHILAAWEGISELPIPALAAWPPSHLRWLHEPLDPVQDKAWVQALPK VELHCHLGGFATHGELLHKVRQEAA NPESLPPVRAIPLPPGWPIPEEPIGLERYMRLGDNNGSALLK DPGCLRAQCRLLYEALLADHVAYAEIRCSPANYASASRSPWVVLQEIRNHFQQAMEETPEDRRCHVN LLLTA TREEGGDRSRIARHLALAITAAEHWKNGCRVVGVDLAGFEDRTTRAAMFATDFEPVHRVGLA VT VHAGENDDVEGIWQAVFKLSARRLGHALHLSRSPDLLRVVAERGI AVELCPYANLQIKGFPLDEE QEGSETYPLRGYLAAGVAVTLNTDNLGISQASLTDNLLLTARLCPGITRLEV LKTQVF AAQAAFANQ AERKALWARLAQVPVPTDTEQKNGNDAKASHQPR-
<i>TaqCad1</i> (N549D)	MGHHHHHHGSSGMRIILCSVGTSWAVVPEAMQLLGSQGFDEVHVLTTASSKISPGVEQLLRYFEMHP GPRFSISRVDQFEDLRSEQDHMLFEEVLWRWLLQRAPQAAHRYICLAGGYKTISAAMQRAAALFGAC EVFHVLCPEPRFGPQGNREASTLEEVEQAIATNALRFVRLGPEPGWPQLRLLSAPSFPLESTLQGPVH WVRASDMRLRQHVEGVLEERSRHILAAWEGISELPIPALAAWPPSHLRWLHEPLDPVQDKAWVQALPK VELHCHLGGFATHGELLHKVRQEAA NPESLPPVRAIPLPPGWPIPEEPIGLERYMRLGDNNGSALLK DPGCLRAQCRLLYEALLADHVAYAEIRCSPANYASASRSPWVVLQEIRNHFQQAMEETPEDRRCHVN LLLTA TREEGGDRSRIARHLALAITAAEHWKNGCRVVGVDLAGFEDRTTRAAMFATDFEPVHRVGLA VT VHAGENDDVEGIWQAVFKLSARRLGHALHLSRSPDLLRVVAERGI AVELCPYANLQIKGFPLDEE QEGSETYPLRGYLAAGVAVTLNTDDLGISQASLTDNLLLTARLCPGITRLEV LKTQVF AAQAAFANQ AERKALWARLAQVPVPTDTEQKNGNDAKASHQPR
<i>TaqCad1</i> (D548A)	MGHHHHHHGSSGMRIILCSVGTSWAVVPEAMQLLGSQGFDEVHVLTTASSKISPGVEQLLRYFEMHP GPRFSISRVDQFEDLRSEQDHMLFEEVLWRWLLQRAPQAAHRYICLAGGYKTISAAMQRAAALFGAC EVFHVLCPEPRFGPQGNREASTLEEVEQAIATNALRFVRLGPEPGWPQLRLLSAPSFPLESTLQGPVH WVRASDMRLRQHVEGVLEERSRHILAAWEGISELPIPALAAWPPSHLRWLHEPLDPVQDKAWVQALPK VELHCHLGGFATHGELLHKVRQEAA NPESLPPVRAIPLPPGWPIPEEPIGLERYMRLGDNNGSALLK DPGCLRAQCRLLYEALLADHVAYAEIRCSPANYASASRSPWVVLQEIRNHFQQAMEETPEDRRCHVN LLLTA TREEGGDRSRIARHLALAITAAEHWKNGCRVVGVDLAGFEDRTTRAAMFATDFEPVHRVGLA VT VHAGENDDVEGIWQAVFKLSARRLGHALHLSRSPDLLRVVAERGI AVELCPYANLQIKGFPLDEE QEGSETYPLRGYLAAGVAVTLNTADLGISQASLTDNLLLTARLCPGITRLEV LKTQVF AAQAAFANQ AERKALWARLAQVPVPTDTEQKNGNDAKASHQPR

TaqCad1 (K106A) MGH HHHHHGSSGMRILLCSVGT SWAVVPEAMQLLGSQGFDEVHVLTTASSKISP GVEQLLRYFEMHP
GPRFSISRVDQFEDLRSEQDHMLFEEVLWRWLLQ RAPQAAHRYICLAGGYATISAAMQRAAALFGAC
EVFHVLC EPRFGPQGNREASTLEEVEQAIATNALRFVRLGPEPGWPQLRLLSAPSFPLESTLQGPVH
WVRASDMRLRQHVEGV LERSRHILA AEWEGISELP IPALAAWPPSHLRWLHEPLDPVQDKAWVQALPK
VELHCHLGGFATHGELLHKVRQE AANPESLPPVRAIPLPPGWPIPEEPIGLERYMRLGDNNGSALLK
DPGCLRAQCRLLYEALLADHVAYAEIRCS PANYASASRSPWVVLQEIRNHFQQAMEETPEDRRCHVN
LLL TATREEGGDRSRIARHLALAITAAEHWKNGCRVVGVDLAGFEDRTTRAAMFATDFEPVHRVGLA
VTVHAGENDDVEGIWQAVFKLSARRLGHALHLSRSPDLLRVVAERGI AVELCPYANLQIKGFPLDEE
QEGSETYPLRGYLAAGVAVTLNTDNLGISQASLTDNLLLTARLCPGITRLEV LKTQVFAAQAAFANQ
AERKALWARLAQVPVPTDTEQKNGNDAKASHQPR

**TaqCad1
(R408D,A411E)** MGH HHHHHGSSGMRILLCSVGT SWAVVPEAMQLLGSQGFDEVHVLTTASSKISP GVEQLLRYFEMHP
GPRFSISRVDQFEDLRSEQDHMLFEEVLWRWLLQ RAPQAAHRYICLAGGYKTISAAMQRAAALFGAC
EVFHVLC EPRFGPQGNREASTLEEVEQAIATNALRFVRLGPEPGWPQLRLLSAPSFPLESTLQGPVH
WVRASDMRLRQHVEGV LERSRHILA AEWEGISELP IPALAAWPPSHLRWLHEPLDPVQDKAWVQALPK
VELHCHLGGFATHGELLHKVRQE AANPESLPPVRAIPLPPGWPIPEEPIGLERYMRLGDNNGSALLK
DPGCLRAQCRLLYEALLADHVAYAEIRCS PANYASASRSPWVVLQEIRNHFQQAMEETPEDRRCHVN
LLL TATREEGGDRSDIARHLELAITAAEHWKNGCRVVGVDLAGFEDRTTRAAMFATDFEPVHRVGLA
VTVHAGENDDVEGIWQAVFKLSARRLGHALHLSRSPDLLRVVAERGI AVELCPYANLQIKGFPLDEE
QEGSETYPLRGYLAAGVAVTLNTDNLGISQASLTDNLLLTARLCPGITRLEV LKTQVFAAQAAFANQ
AERKALWARLAQVPVPTDTEQKNGNDAKASHQPR

TaqCsx1 MPRQAINLGERGPHLLLVLNLGLGQDKRGPLGYAETTYLMPDGSRYPTSLAGFALWKWLDATGRSPVA
VVFACTESAWNQKQEALRKHAAELGLEPSKISPPVILGLPQTLEDVWAMIEPMESRLRAYHDAAGPV
LHLDLTHAFRAIPLAHL LIALYFQERGLATVGVCYGVFQEGQAETPYIDL SHLLHLARWAQAVRSF
RERFDTTGLAGLLEQYEREARHAAVQSGMIPPEVRR LISARLAGPYFAAGLPLELGVHVGQTLGQ
TTRESLEKAATKLVA AQQSIVLELFDALHPLTPGQVAKKSAKASLVLDKQELARQMRL LKLWLQAGL
PERALLVLEVVINRLLLAANPESWLEHGIREQAETVLNDVFRPEEPPLSSPELKVLRSLWREITN
RRNPLAHAGMCKSEVDVGQLSEAVEELVEKLEALLELDEPWQELARQLSTSGEGKA-