

Supplementary information

RNA targeting unleashes indiscriminate nuclease activity of CRISPR–Cas12a2

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RNA targeting unleashes indiscriminate nuclease activity of CRISPR-Cas12a2

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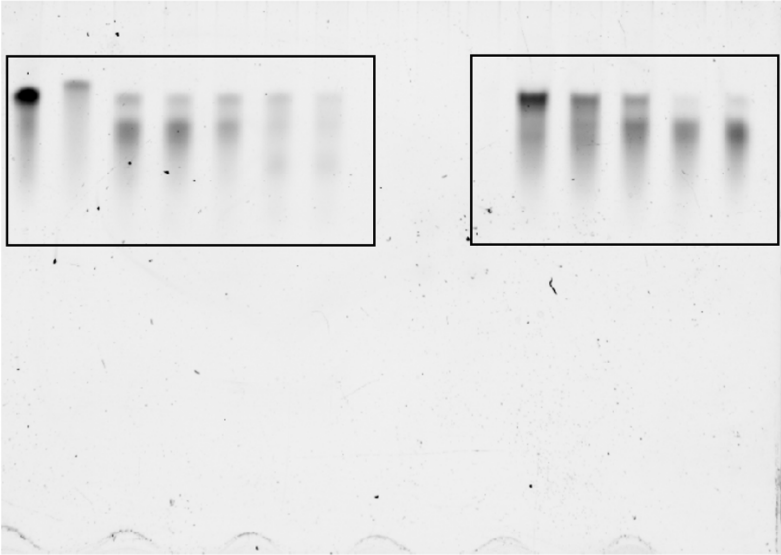
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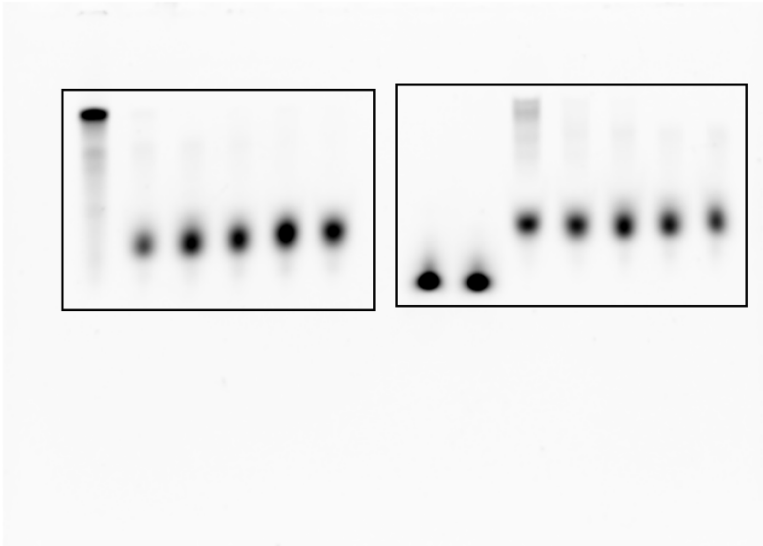
Supplementary Video 1 | Structural rearrangements in Cas12a2. Cas12a2 binary complex coloured as in **Fig. 1**. Morph to ternary complex, upon binding of target strand (orange). Rotation showing the crRNA:TS duplex running through the centre of the complex. RuvC active site, with collateral dsDNA bound (purple and green). Zoom-in on the two aromatic clamp pairs (non-cleaved strand and then cleaved strand).

Figure 1. Source data. Raw data for all gels.

Raw data for Fig. 2g.

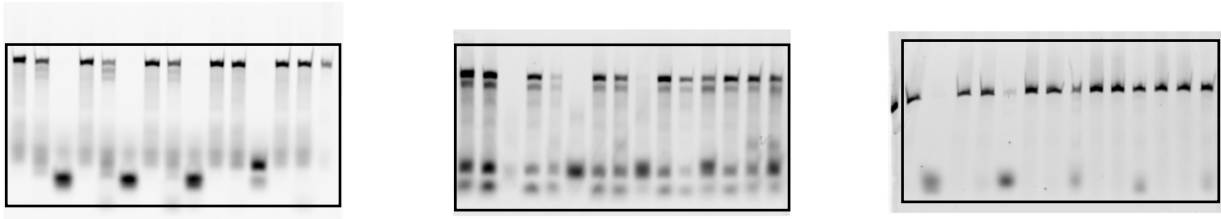


Sybr gold staining



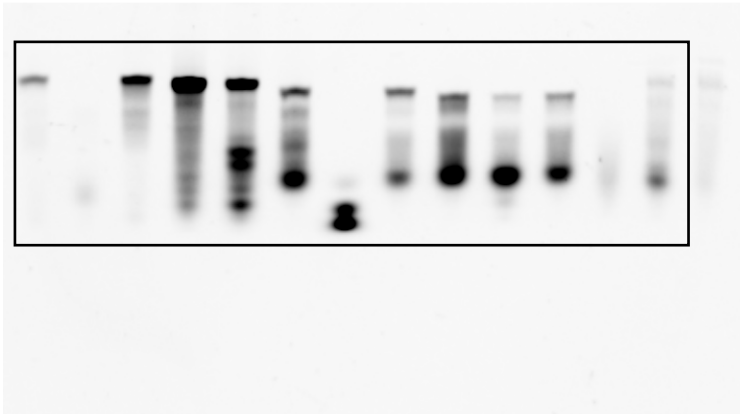
Fluorescence from FAM label

Raw data for Fig. 3h.

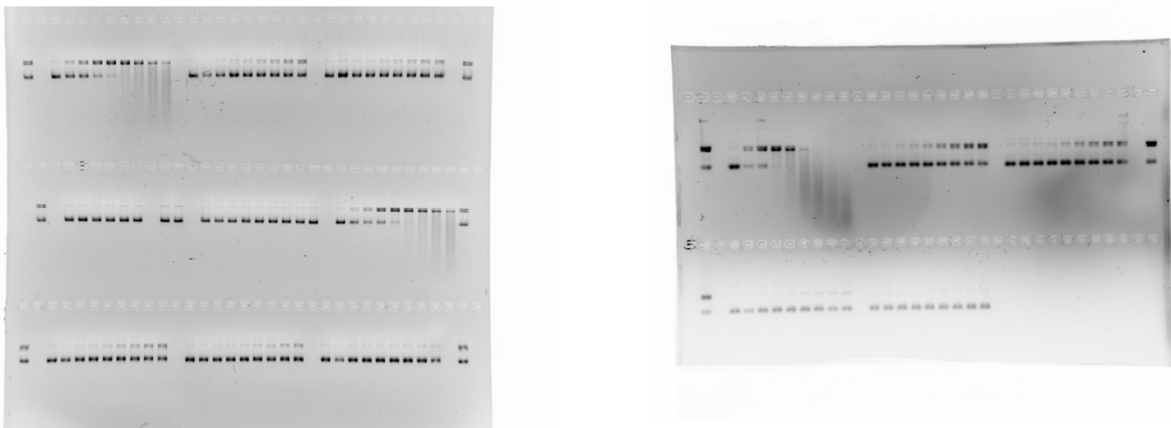


Left to right. ssDNA, ssRNA, dsDNA.

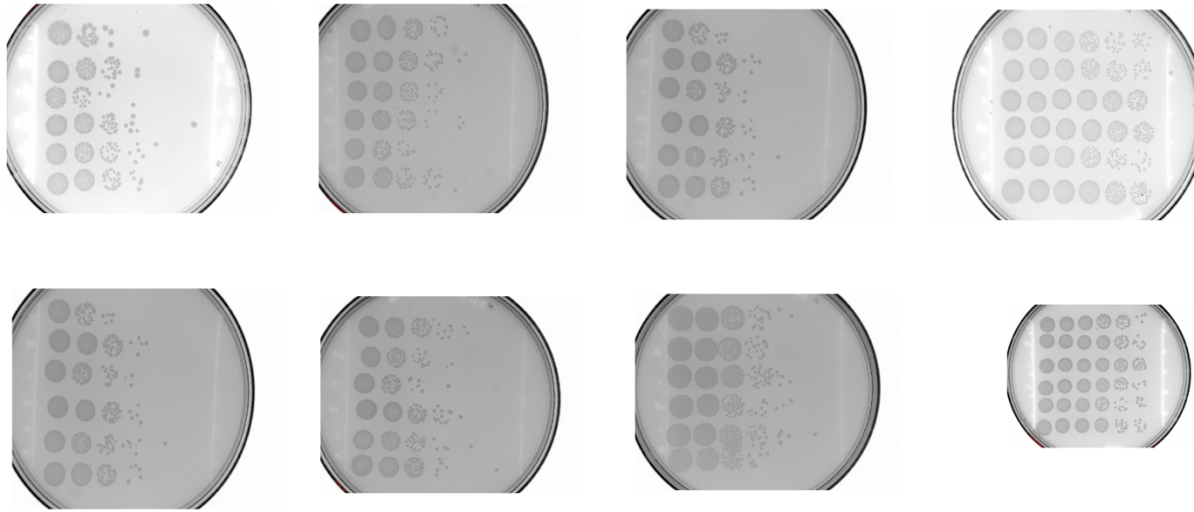
Raw data for Extended Data Fig. 4e.



Raw data for Extended Fig. 8b.



Raw data for Extended Data Fig. 9.



Top (left to right). E1063A, Y465A, Y1080A, Y465A/Y1080A
Bottom (left to right). Y1069A, F1092A, Y1069A/F1092A, Δ PI

Table 1. Strains, plasmids, and nucleic acid substrates.

Substrate	Abrv.	Sequence	FAM
Biochemistry			
crRNA	CR	5'- UAAAUUUCUACU AUUGUAGAUUGGAGCA ACACCUGAAGGAAGGCUUGA-3'	
Target Strand	TS	5'- UCUUUGCUGCCGCACUUGCUCAUCAAG CCU,UCCUU,CAGGUGUUGCUCAGAAA GGUGAGUUCUUCUUGUUGUU-3'	5'
Target Strand Δ PFS	TS Δ PFS	5'- UCUUUGCUGCCGCACUUGCUCAUCAAG CCUCCUUCAGGUGUUGCUCCA-3'	5'
Target Strand -5	TS-5	5'- UCCUUCAGGUGUUGCUCAGAAAAGGUG AGUUCUUCUUGUUGUU-3'	3'
Target Strand -10	TS-10	5'- CAGGUGUUGCUCAGAAAAGGUGAGUUC UUCUUGUUGUU-3'	3'
Target Strand -9	TS-9	5'- UCAGGUGUUGCUCAGAAAAGGUGAGUU CUUCUUGUUGUU-3'	3'
Target Strand -8	TS-8	5'- UUCAGGUGUUGCUCAGAAAAGGUGAGU UCUUCUUGUUGUU-3'	3'
Nontarget RNA	NT-RNA	5'- CAGAGUAAGUGACGCGCGGCGAGUGG CGCGCCACGUCGAAAUCUAGAGGCG- 3'	5'
Nontarget ssDNA	NT-ssDNA	5'- AACTGATATGACAATTGCGCGTAGCACG ACGACGATATGACACTTGCGCATAACGA CGACGATAACAATGAT-3'	5'
Nontarget dsDNA	NT-dsDNA	5'-AACTGATATGACAATTGCGCGTAGCA 3'-TTGACTATACTGTTAACGCGCATCGT	5'

		CGACGACGATATGACACTTGCGCATAAC GCTGCTGCTATACTGTGAACGCGTATTG	
		GACGACGATACAATGAT-3' CTGCTGCTATGTTACTA-5'	
Structure			
crRNA			None
Target Strand			None
Target Strand - flanks	TS-F		
PT Nontarget dsDNA	PT-dsDNA		None
Plasmids			
1408	pACYC_SuCas12a2_3xcrRNA		
1416	p__SuCas12a2		
dPI mutant 2290	pACYC_SuCas12a2(Δ a.a. 624 - 713)_3xcrRNA		
2286	pACYC_SuCas12a2(Y465A)_3xcrRNA		
2287	pACYC_SuCas12a2(Y1080A)_3xcrRNA		
2379	pACYC_SuCas12a2(Y465A, Y1080A)_3xcrRNA		

2247	pACYC_SuCas12a2(Y1069A)_3xcrRNA		
2288	pACYC_SuCas12a2(F1092A)_3xcrRNA		
2289	pACYC_SuCas12a2(Y1069A, F1092A)_3xcrRNA		
2270	pACYC_SuCas12a2(R1180A)_3xcrRNA		
2271	pACYC_SuCas12a2(D1184A)_3xcrRNA		
1412	pACYC_SuCas12a2(E1063A)_3xcrRNA		
Target(1094)	pET27b(+)_Target_TTTC		
Non-target(1095)	pET27b(+)_non-Target_TTTC		
Primers			
Cas12a2(Y465A)F		AGAAATCTTAgcTGGTAACACGATGTTC	
Cas12a2(Y465A)R		CAATTTGAAACTTATAAGAAGGATATAC	
Cas12a2(Y1080A)F		TGATGGCAGAgcCCTTCCGTCATAAC	
Cas12a2(Y1080A)R		ACTTCACAAGACGAGGATG	
Cas12a2(Y1069A)F		TGCACCTCCAgcGGCTCTTGAC	
Cas12a2(Y1069A)R		AAGTCCGGTTATGACGGA	
Cas12a2(F1092A)F		CTGTTCCCTTGgcATCTACATCCTC	
Cas12a2(F1092A)R		CAGAATCAGATGCTTGCAG	

Cas12a2(R1180A)F		AAATGTATACgcGGACAAGGATGACATCT TG	
Cas12a2(R1180A)R		GTTTTGCTACAGACGGGG	
Cas12a2(D1184A)F		GACAAGGATGcCATCTTGGTTTG	
Cas12a2(D1184A)R		CCTGTATACATTTGTTTTGC	
Cas12a2(Δ a.a.624 - 713)F	dPI	GCTTGTAAGGCTCTTAAC	
Cas12a2(Δ a.a.624 - 713)R	dPI	TCATATTTGCTGCAGAGC	