

Supplementary files

Molecular basis for DNA cleavage by the mini-RNA-guided endonuclease Cas δ -1

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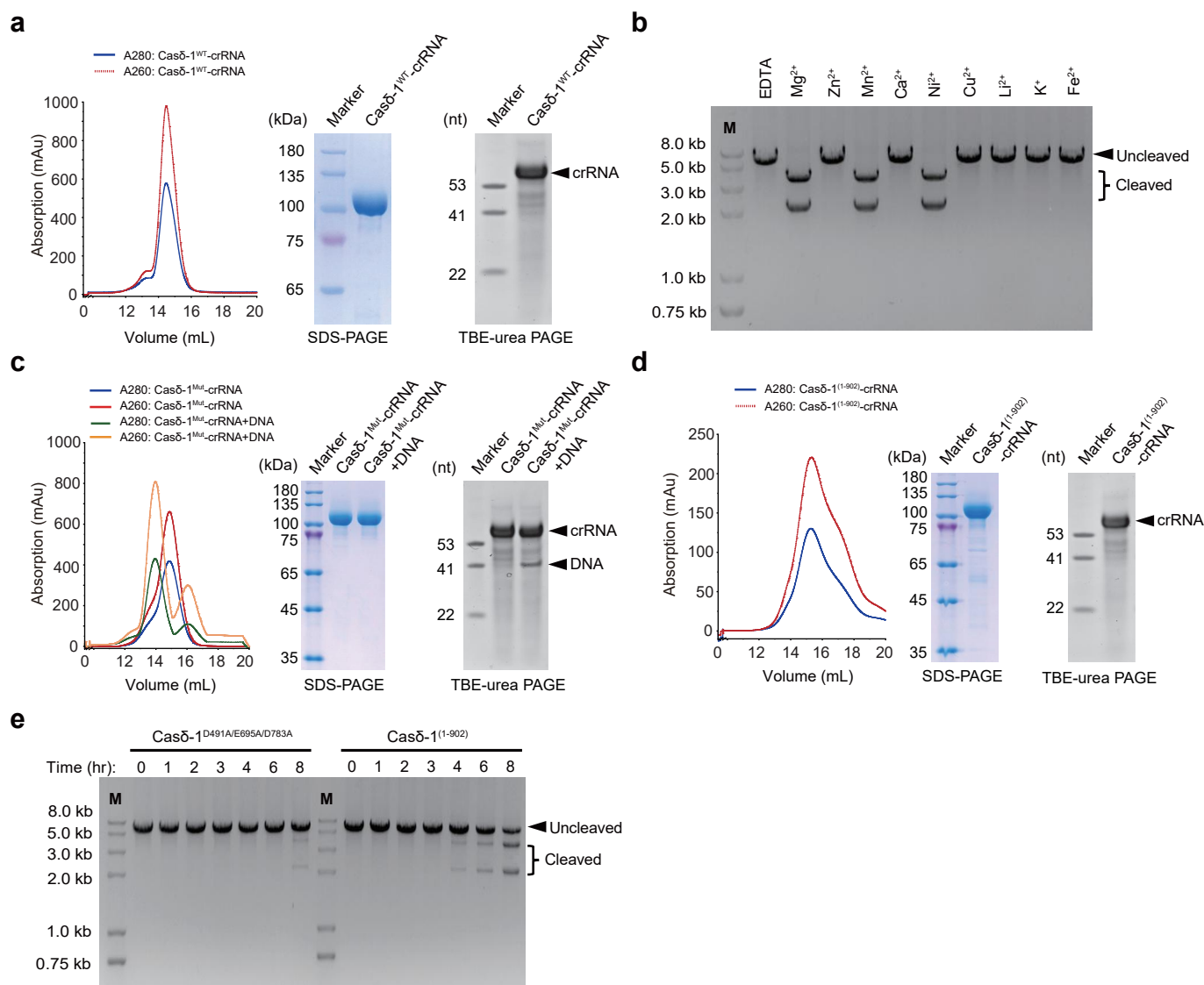
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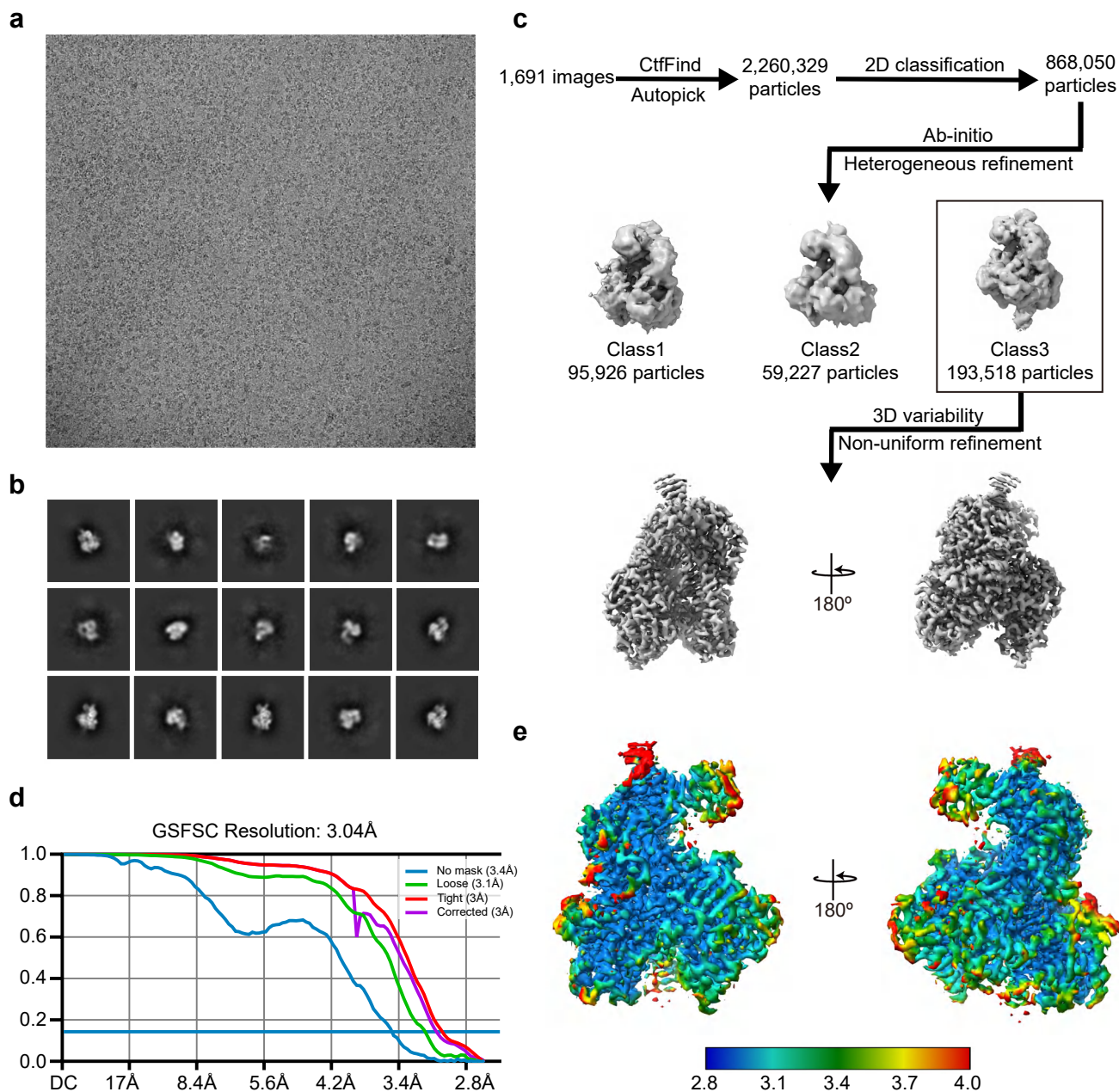
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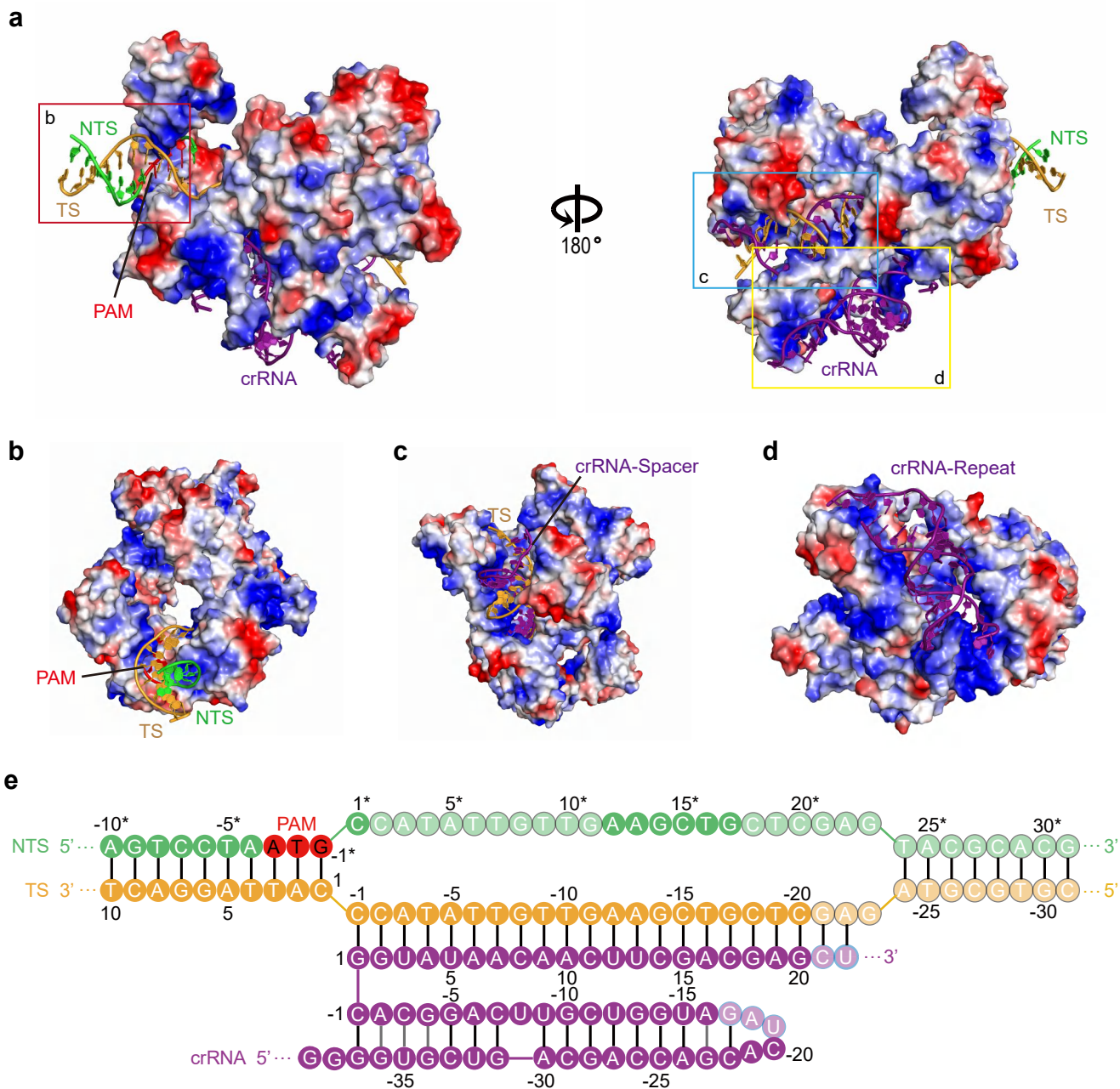
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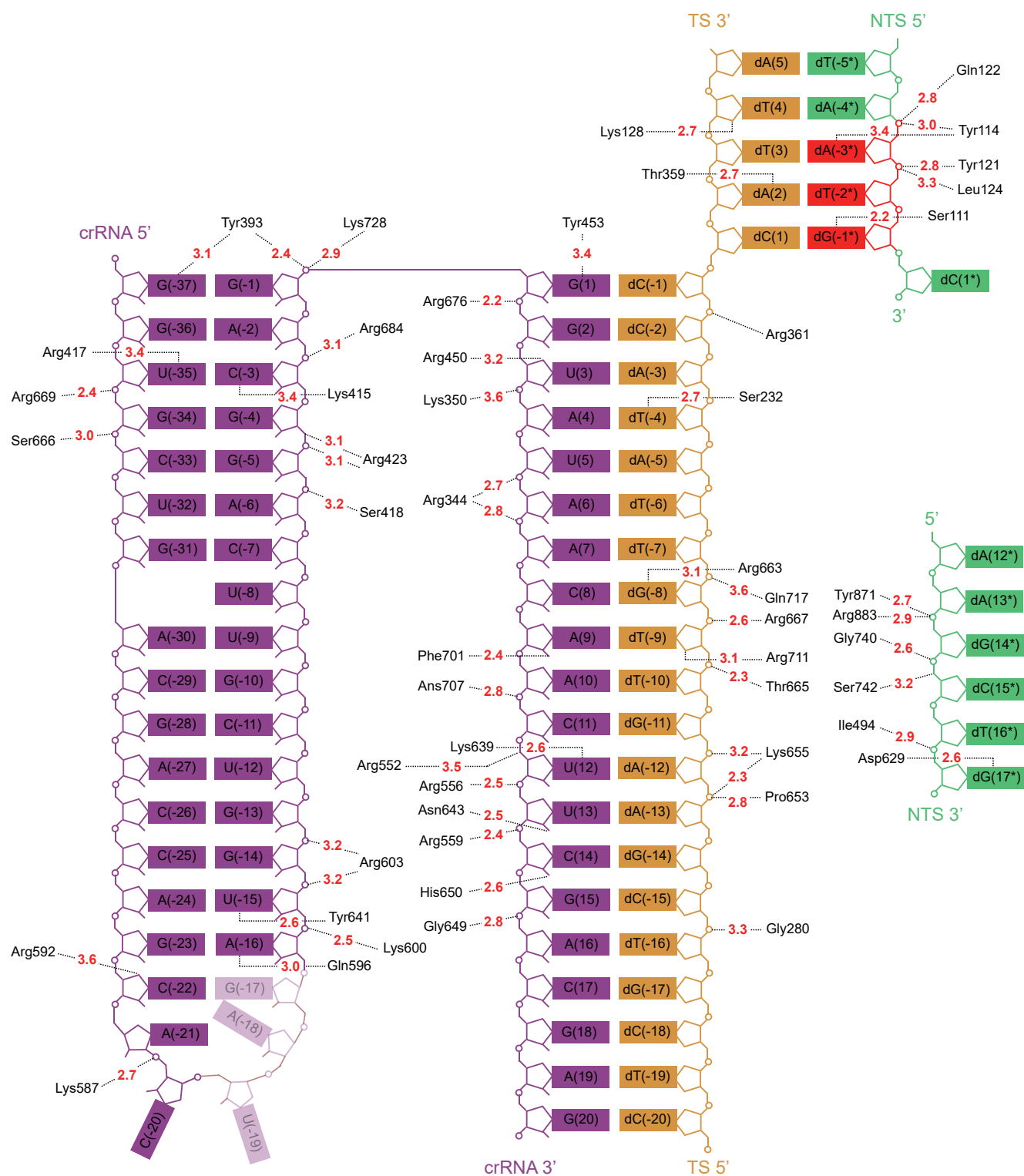
Supplementary Fig. 1 | Biochemical purification, complex assembly and DNA cleavage profiling of Cas δ -1 effector. **a**, Purification and identification of the Cas δ -1^{WT}-crRNA complex via gel filtration chromatography. The Cas δ -1^{WT}-crRNA complex was fractionated using a Superdex 200 Increase 10/300 column, and the peak fractions were analyzed by SDS-PAGE and TBE-Urea-PAGE gels. **b**, Effects of metal ions on the cleavage activity of Cas δ -1. Mg²⁺, Mn²⁺, and Ni²⁺ metal ions support Cas δ -1 catalytic activity, while Zn²⁺, Ca²⁺, Cu²⁺, Li²⁺, K⁺ and Fe²⁺ do not. Depletion of the cation by EDTA abrogates phosphodiester hydrolysis. **c**, Purification and identification of the Cas δ -1^{Mut}-crRNA-target DNA complex via gel filtration chromatography. Cas δ -1^{Mut} represents Cas δ -1^{D491A/E695A/D783A}-crRNA. The Superdex 200 Increase 10/300 column gel filtration chromatography reveals the interaction between Cas δ -1 crRNA and target DNA, and the peak fractions were analyzed by SDS-PAGE and TBE-Urea-PAGE gels. **d**, Purification and identification of the Cas δ -1⁽¹⁻⁹⁰²⁾-crRNA complex via gel filtration chromatography. The Cas δ -1⁽¹⁻⁹⁰²⁾-crRNA complex was fractionated using a Superdex 200 Increase 10/300 column, and the peak fractions were analyzed by SDS-PAGE and TBE-Urea-PAGE gels. **e**, Time course of the cleavage reaction by Cas δ -1^{D491A/E695A/D783A} and Cas δ -1⁽¹⁻⁹⁰²⁾.



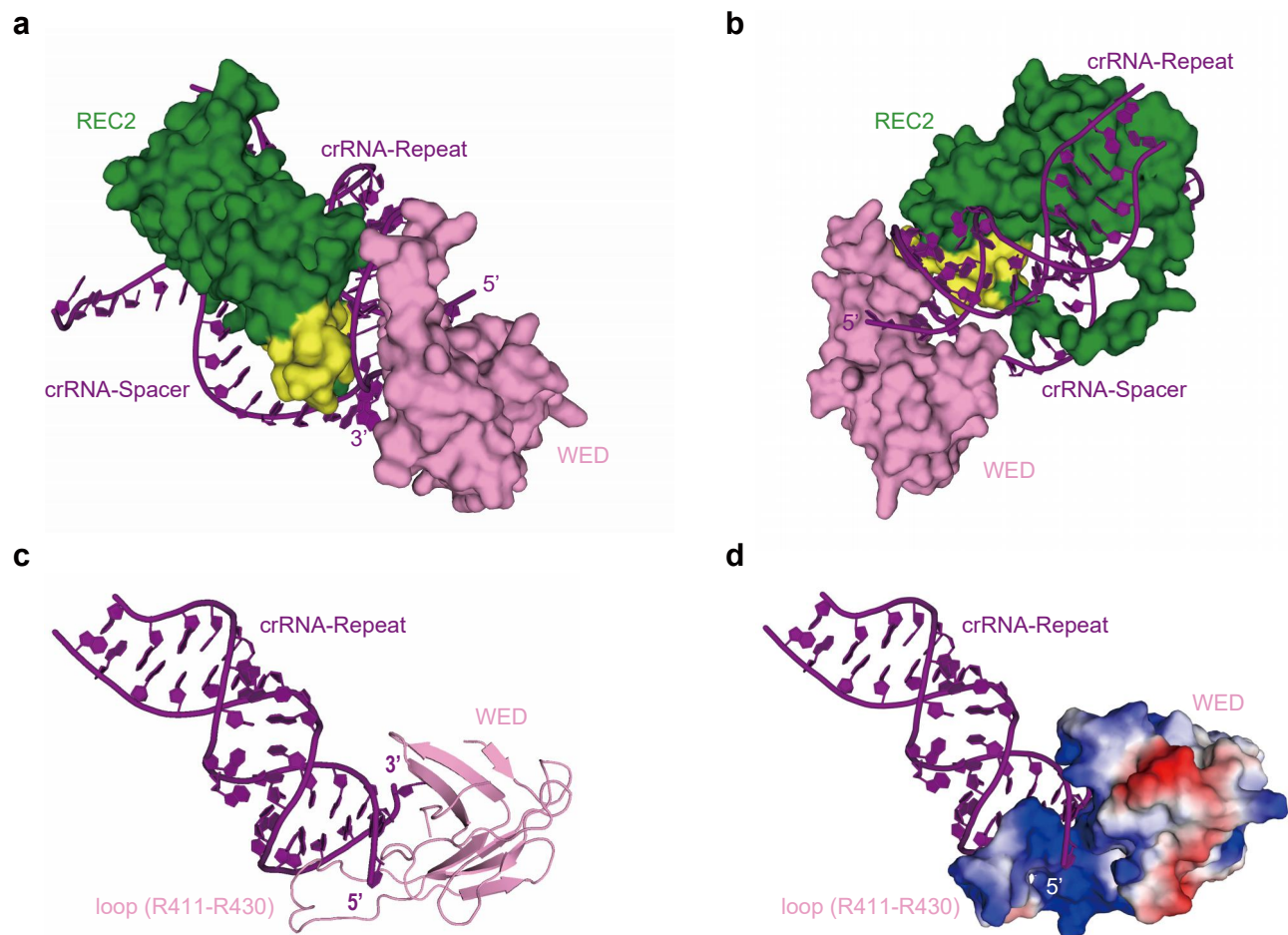
Supplementary Fig. 2 | Single-particle cryo-EM analysis of the Cas δ -1-crRNA-target DNA ternary complex. **a**, Representative cryo-EM micrograph, recorded on a Talos Arctica 200 KV FEG with a K2 camera. **b**, Representative 2D class average images. **c**, Single-particle cryo-EM image processing workflow. **d**, Fourier shell correlation (FSC) curve for the 3D reconstruction. **e**, Cryo-EM density map according to the local resolution.



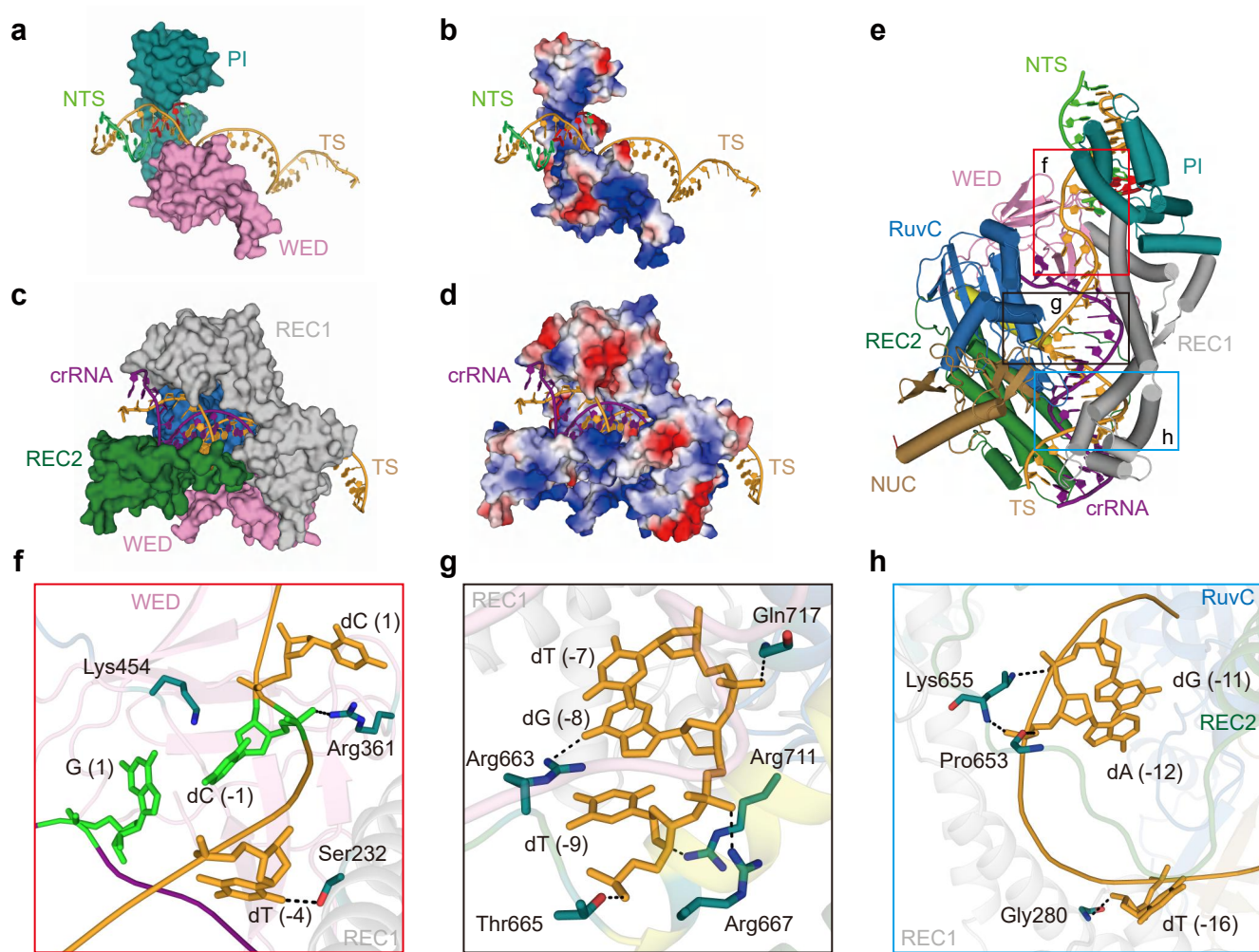
Supplementary Fig. 3 | Positively charged grooves accommodate crRNA-target DNA. **a**, Electrostatic surface potential of the Cas δ -1-crRNA-target DNA complex. **b-d**, Three positively charged grooves formed by Cas δ -1 accommodate the TS-NTS duplex (**b**), the TS-crRNA heteroduplex (**c**) and the crRNA repeat (**d**). The crRNA is colored in purple, as well as the TS and the NTS are colored in brightorange and green, respectively. Specially, the PAM is colored in red. **e**, The schematic of the crRNA and target DNA sequences. Transparent circles denote the nucleotides that are not resolved in the density map.



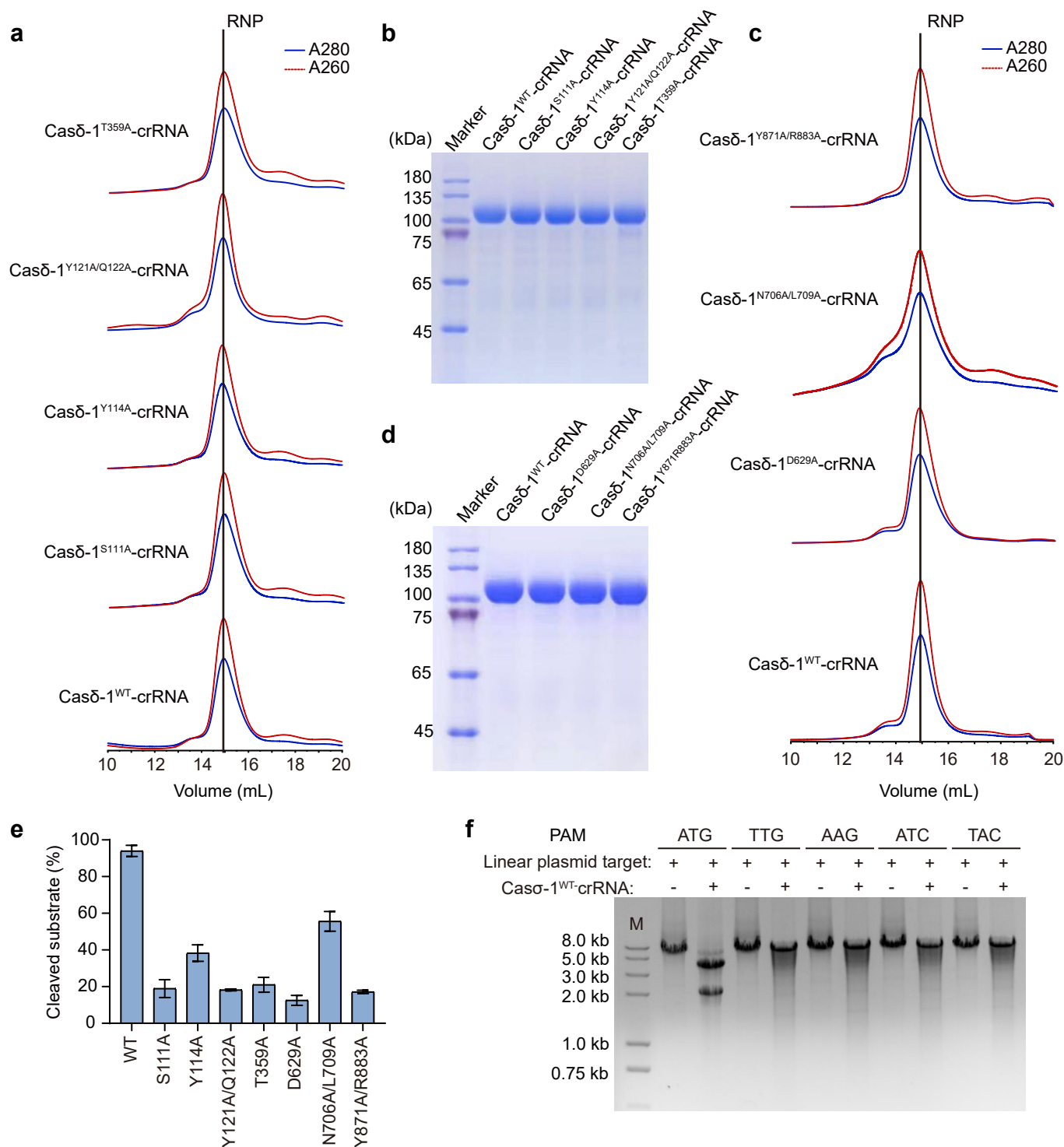
Supplementary Fig. 4 | Schematic of nucleic-acid recognition. Bond lengths are indicated by red numbers. Transparent panes denote the nucleotides that are not resolved in the density map.



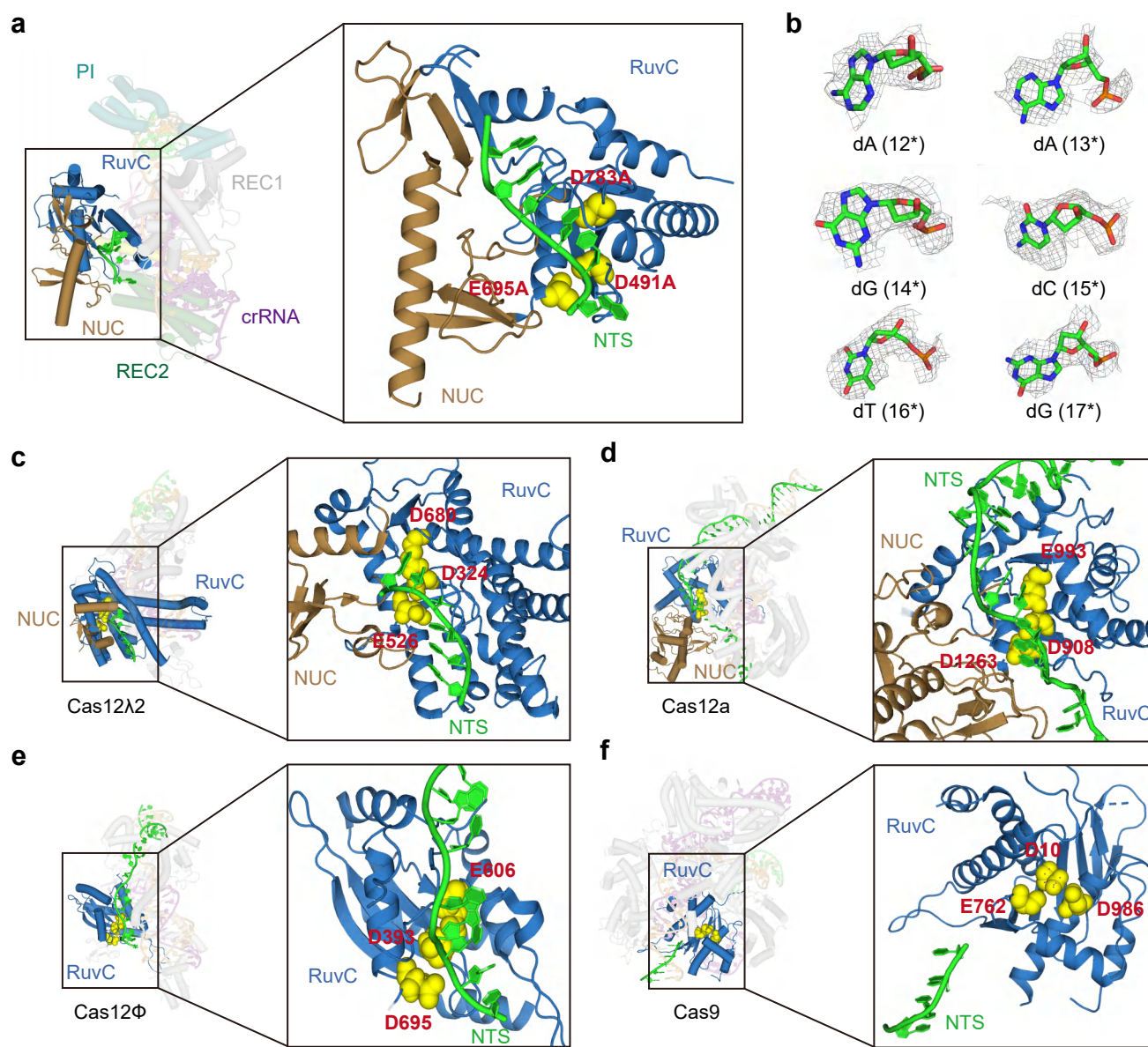
Supplementary Fig. 5 | Recognition of the major groove of the crRNA repeat. a,b, Recognition of 3'-end (a), and 5'-end (b) of crRNA repeat by REC2, BH and WED domains of Cas δ -1. **c,** The WED domain stabilizes the major groove of the crRNA repeat stem. **d,** A positively charged loop of the WED domain participates in stabilizing the crRNA repeat stem.



Supplementary Fig. 6 | Recognition of the T-strand. **a,b**, Recognition of TS-NTS duplex by PI and WED domains of Cas δ -1. Proteins are shown as molecular surface (**a**) and electrostatic surface potential (**b**). **c,d**, Recognition of TS-crRNA heteroduplex by WED, REC1, REC2, and RuvC domains of Cas δ -1. Proteins are shown as molecular surface (**c**) and electrostatic surface potential (**d**). **e**, Recognition of TS-crRNA heteroduplex. Cas δ -1 protein is shown as cartoon. **f-h**, The detailed interactions of Cas δ -1 with nucleotides dC (-1) to dA (-5) (**f**), dT (-6) to dT (-10) (**g**), and dG (-11) to dC (-20) (**h**) within TS. The flipped bases dC (-1) and G (1) are colored in green.



Supplementary Fig. 7 | Purification and enzymatic activity analysis of DNA-interacting residues mutants. **a,b**, Purification and identification of the Casδ-1^{WT}-crRNA complex and its PAM-recognized key amino acid residue alanine substitution mutants. The complexes were fractionated using a Superdex 200 Increase 10/300 column (**a**), and the peak fractions were analyzed by SDS-PAGE gel (**b**). **c,d**, Purification and identification of the Cas δ-1^{WT}-crRNA complex and its catalytic site key amino acid residue alanine substitution mutants. The complexes were fractionated using a Superdex 200 Increase 10/300 column (**c**), and the peak fractions were analyzed by SDS-PAGE gel (**d**). **e**, Cleavage activity statistics following alanine substitution of key amino acid residues responsible for PAM recognition and NTS chain cleavage. Data are represented as mean ± SD from three independent experiments with similar results (n = 3). **f**, The detailed preference of Casδ-1 on substrates with different PAM sequences.



Supplementary Fig. 8 | Observation of the ssDNA substrate in the catalytic site in Cas5-1^{WT}-crRNA-DNA complex. **a**, The 6-nt ssDNA density bound observed within the groove between the RuvC and NUC domains. RuvC domain is colored in skyblue, NUC domain is colored in sand and NTS are colored in green. The active residues are colored in yellow and shown as sphere models. **b**, Electron densities for the 6-nt ssDNA density bound observed. Cryo-EM density maps contoured at 7 σ . **c**, The observation of a ssDNA substrate in the catalytic site in Cas12A2 (PDB: 9IZR), Cas12a (PDB: 8SFO), Cas12 Φ (PDB: 7LYT), or Cas9 (PDB: 6O0Y).

Data collection and processing

Magnification	105,000
Voltage (kV)	200
Electron exposure (e ⁻ /Å ²)	60
Defocus range (μm)	-1.0 ~ -3.0
Pixel size (Å)	1.32
Symmetry imposed	C1
Initial particle images (no.)	2,260,329
Final particle images (no.)	193,518
Map resolution (Å)	3.04
FSC threshold	0.413
Map resolution range (Å)	2.7-3.5

Refinement

Initial model used (PDB code)	None
Model resolution (Å)	3.2
FSC threshold	0.5
Model resolution range (Å)	-
Map sharpening <i>B</i> factor (Å ²)	-107.9
Model composition	
Non-hydrogen atoms	9007
Protein residues	854
Nucleotide residues	103
<i>B</i> factors (Å ²)	
Protein	14.22
Nucleotide	30.37
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.580
Validation	
MolProbity score	1.81
Clash score	10.27
Poor rotamers (%)	0.67
Ramachandran plot	
Favored (%)	96.00
Allowed (%)	4.00
Disallowed (%)	0

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

PDB code	rmsd (Å)	lali	nres	id%	Protein
9L12	3.1	797	870	26	Cas12h
8ZQC	3.8	694	930	16	Cas12X2
8XCA	4.2	583	668	16	Cas12j19
8AXA	4.3	311	465	14	Cas12k
6LU0	4.5	529	937	12	Cas12i2

Supplementary Table 2 | DALI search displaying the homology of Cas δ -1 with other Cas12 family