

## **Supplementary Information**

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

	DrmAB-ADP (PDB 7S9V, EMD-24938)	DrmAB-ADP- DNA (PDB 7S9W, EMD-24939)	DrmAB
<b>Data collection and processing</b>			
Magnification	22,500		
Voltage (kV)	300 kV		
Electron exposure (e-/Å <sup>2</sup> )	80		
Defocus range (μm)	-1.5 to -2.5		
Pixel size (Å)	1.1		
Symmetry imposed	C1		
Initial particle images (no.)	4,669,932	1,540,419	
Final particle images (no.)	120,119	121,764	148,062
Map resolution (Å) at 0.143 FSC threshold	3.31	3.41	3.84
Map resolution range (Å)	3.1 to 4.3	3.3 to 4.5	3.6-6.8
<b>Refinement</b>			
Initial model used (PDB code)	<i>De novo</i>		
Model resolution (Å)	3.6	3.8	
FSC threshold	0.5		
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-136.8	-151.1	
Model composition			
Non-hydrogen atoms	27680	28125	
Protein residues	1808	1751	
Nucleotides	0	7	
Ligands	1	1	
Mean <i>B</i> factors (Å <sup>2</sup> )			
Protein	62.08	106.66	
Nucleotides	N/A	116.37	
R.m.s. deviations			
Bond lengths (Å)	0.002	0.002	
Bond angles (°)	0.617	0.631	
Validation			
MolProbity score	1.52	1.53	
Clashscore	3.27	3.34	
Poor rotamers (%)	0	0	
Ramachandran plot			
Favored (%)	93.93	93.90	
Allowed (%)	6.01	6.1	
Disallowed (%)	0.6	0	

**Supplementary Table 2 | DNA oligos used for *in vitro* assays in this study.**

Oligo name	Sequence (5'-3')
DNA stem loop	TTTTTTCATCGATGAGCACTGCTATTCCCTAGCAGTGCTCATCGATGATTTCATCGATGAGCGGGTTT
Methylated DNA stem loop*	TTTTTTCATmCGATGAGCACTGCTATTCCCTAGCAGTGCTCATmCGATGATTTCATmCGATGAGCGGGTTT
Fluorescent DNA	Cy5-ATTTGACAGCCCACATGGCTTGATGAGTGGCGCACTCGCCAGCCTGAGCATGGCGAAAACTCCTCCAGTCTGCT
Unlabeled complement DNA	AGCAGACTGGAGGAGTTTCGCCATGCTCAGGCTGGCGAGTGCGCCACTCATCAAGCCATGTGGGCTGTCAAAT
5' block	GCCATGTGGGCTGTCAAAT
3' block	AGCAGACTGGAGGAGTTTC

mC corresponds to 5-methyl-Cytosine.

**Supplementary Table 3 | DNA oligos used for cloning in this study.**

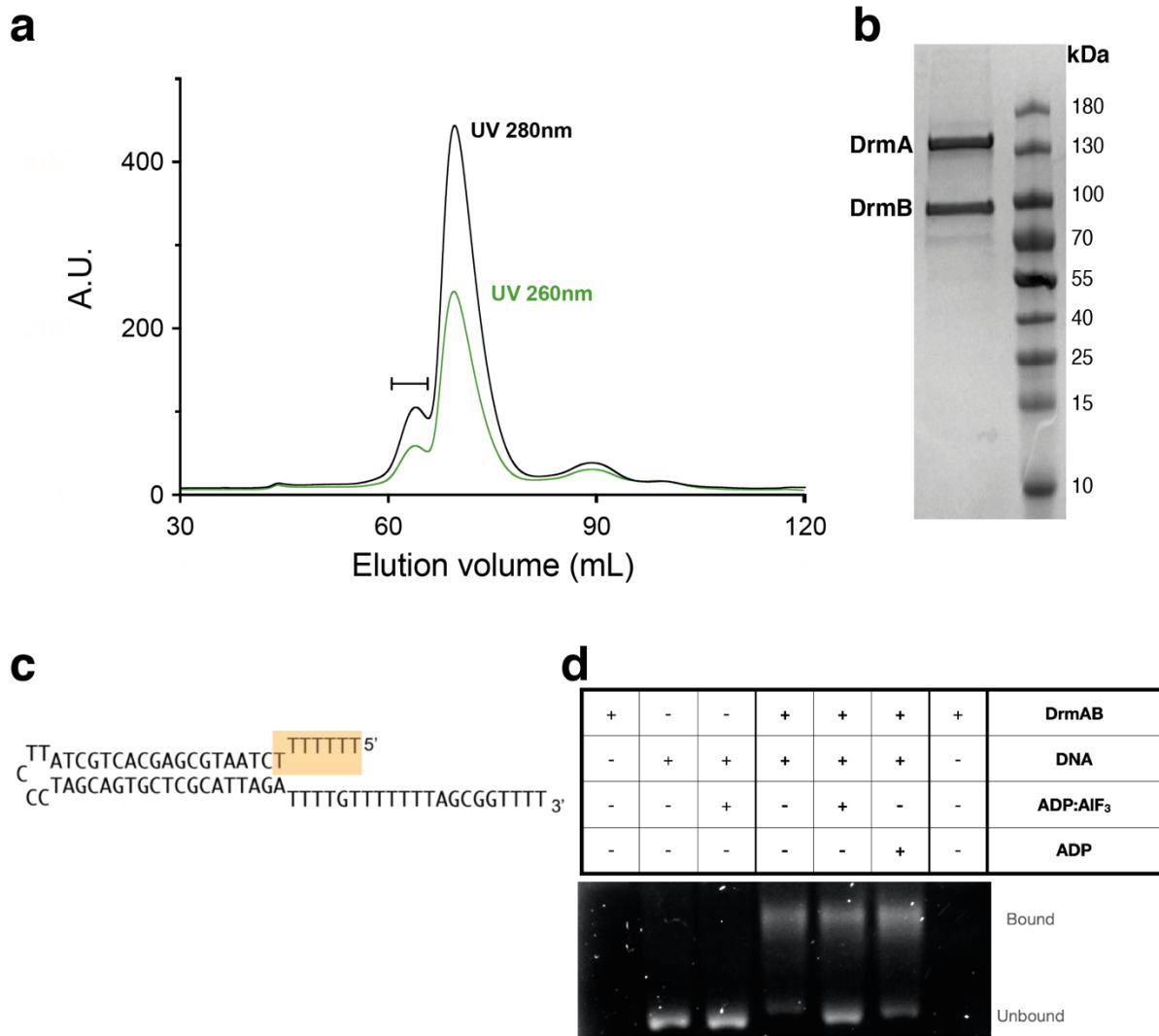
Oligo name	Sequence (5' - 3')	Used for: (in fw/rv couples)	fw/rv
BN1750	TACTCCAATCCAATGCAATGATCAT CAATAACAAAATCCAG	Clone <i>drmB</i> into plasmid 13SS using LIC cloning	fw
BN1751	TTATCCACTCCAATGTTATTATTAAA AACCTTAAAAAATGCAGC	Clone <i>drmB</i> into plasmid 13SS using LIC cloning	rv
BN1060	TATATATACATATGACTGATAACAACA AATCTAG	Clone <i>drmA</i> into plasmid pACYC-Duet using RE cloning-NdeI	fw
BN1061	ATATATATCTCGAGTCAATCCTCGTC TTTCGTG	Clone <i>drmA</i> into plasmid pACYC-Duet using RE cloning-XbaI	rv
BN172	AGATCTGCCATATGTATATCTCCTTC	Amplify pACYC-Duet backbone for cloning of <i>drmA</i> insert	rv
BN2290	GATCGCTGACGTCGGTACCCTCGAG TC	Amplify pACYC-Duet backbone for cloning of <i>drmA</i> insert	fw
BN1064	TATATATACATATGATGGATGAACTC TTAGATGC	Clone <i>drmC</i> into plasmid pET-Duet using RE cloning-NdeI	fw
BN1065	ATATATATCTCGAGTTAAATCAGACT AATGATATTGTG	Clone <i>drmC</i> into plasmid pET-Duet using RE cloning-XbaI	rv
BN172	AGATCTGCCATATGTATATCTCCTTC	Amplify pET-Duet backbone for cloning of <i>drmC</i> insert	rv
BN2290	GATCGCTGACGTCGGTACCCTCGAG TC	Amplify pET-Duet backbone for cloning of <i>drmC</i> insert	fw
BN3096	CATACCCTATCTCATTGTTGAT	Create mutant DrmB-ΔDUF1998 in 13ss and in pCDF	fw
BN3095	TTATCATTACAGCATGGCATATCGGA TAC	Create mutant DrmB-ΔDUF1998 in 13ss and in pCDF	rv
BN3104	TTATCATTATCGCATTGACATAGGTA CAG	Create mutant <i>drmA</i> -ΔPA in pCDF	rv
BN3105	GAAGTTGAGTCTGGTGTAC	Create mutant <i>drmA</i> -ΔPA in pCDF	fw
BN3436	TTGGTGCCCAGTAGCCTC	Create mutant <i>drmA</i> -Δloop in pACYC and pCDF	rv
BN3437	TTCGGCTGCTTCACGTGG	Create mutant <i>drmA</i> -Δloop in pACYC and pCDF	fw
BN3377	CAACACGACTGGTAGCCTGAATATAC	Amplify DNA from pCDF-Duet with <i>drmA</i> -ΔPA and clone into pACYC-Duet	rv
BN3378	GGACACCAAATTATCAAGGACGATC	Amplify DNA from pCDF-Duet with <i>drmA</i> -ΔPA and clone into pACYC-Duet	fw
BN3375	GTATATTCAAGGCTACCAGTCGTGG	Amplify DNA from synthetic block to clone in 13SS and pCDF-Duet for <i>drmA</i> mutants R1294A, V1296G, V1296W	fw
BN3376	GATCGTCCTTGATAAATTGGTGTCC	Amplify DNA from synthetic block to clone in 13SS and pCDF-Duet for <i>drmA</i> mutants R1294A, V1296G, V1296W	rv
BN3377	CAACACGACTGGTAGCCTGAATATAC	Amplify vectors 13SS and pCDF-Duet DNA to insert amplified DNA of <i>drmA</i> mutants R1294A, V1296G, V1296W	rv
BN3378	GGACACCAAATTATCAAGGACGATC	Amplify vectors 13SS and pCDF-Duet DNA to insert amplified DNA <i>drmA</i> mutants R1294A, V1296G, V1296W	fw

BN3379	GATGAACAGAACTTGAATGTGAGC	Amplify DNA from synthetic block to clone in 13SS and pCDF-Duet for <i>drmA</i> mutants R569D, Q572D, K610D, R658D, R649A, K803A, R810A	rv
BN3380	GATAATTAAATCAGGCGGTCGGATAG	Amplify DNA from synthetic block to clone in 13SS and pCDF-Duet for <i>drmA</i> mutants R569D, Q572D, K610D, R658D, R649A, K803A, R810A	fw
BN3381	GCTCACATTCAAGTTCTGTTCATC	Amplify vectors 13SS and pCDF-Duet DNA to insert amplified DNA <i>drmA</i> mutants R569D, Q572D, K610D, R658D, R649A, K803A, R810A	rv
BN3382	CTATCCGACCGCCTGATTTAATTATC	Amplify vectors 13SS and pCDF-Duet DNA to insert amplified DNA <i>drmA</i> mutants R569D, Q572D, K610D, R658D, R649A, K803A, R810A	fw

**Supplementary Table 4 | Plasmids used in this study.**

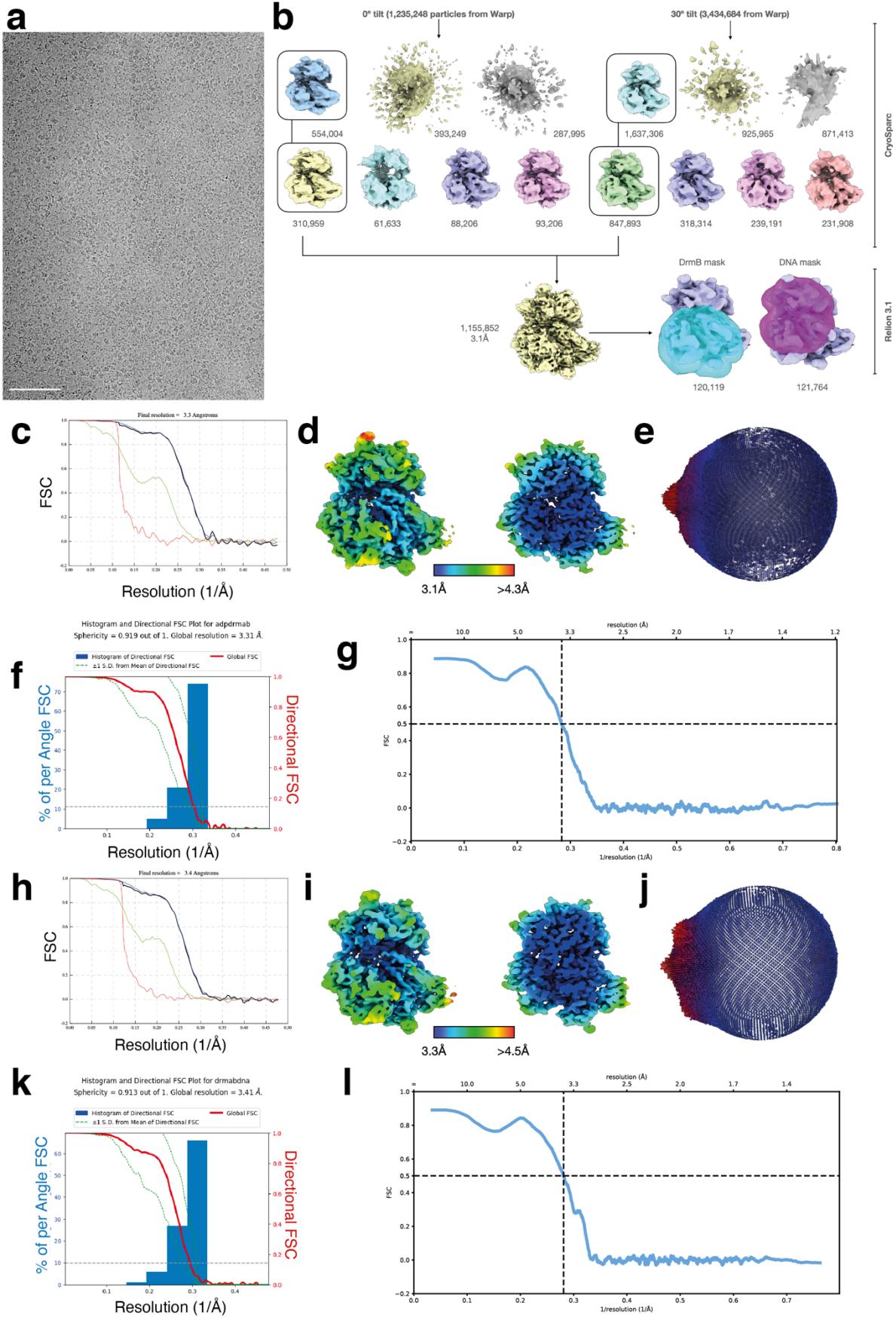
Plasmid	Description	Name in paper	Antibiotic resistance marker	Source
pTU515	13SS- <i>drmB</i> fused to His-tag		Spectinomycin	This paper
pTU518	13SS- <i>drmB</i> -ΔDUF1998 fused to His-tag		Spectinomycin	This paper
pTU516	pACYC-Duet- <i>drmA</i>		Chloramphenicol	This paper
pTU520	pACYC-Duet- <i>drmA</i> (ΔPA)		Chloramphenicol	This paper
pTU542	pACYC-Duet- <i>drmA</i> (Δloop)		Chloramphenicol	This paper
pTU522	pACYC-Duet- <i>drmA</i> (V1296G)		Chloramphenicol	This paper
pTU523	pACYC-Duet- <i>drmA</i> (V1296W)		Chloramphenicol	This paper
pTU524	pACYC-Duet- <i>drmA</i> (R569D)		Chloramphenicol	This paper
pTU525	pACYC-Duet- <i>drmA</i> (Q572D)		Chloramphenicol	This paper
pTU526	pACYC-Duet- <i>drmA</i> (K610D)		Chloramphenicol	This paper
pTU527	pACYC-Duet- <i>drmA</i> (R658D)		Chloramphenicol	This paper
pTU528	pACYC-Duet- <i>drmA</i> (R649A)		Chloramphenicol	This paper
pTU529	pACYC-Duet- <i>drmA</i> (K803A)		Chloramphenicol	This paper
pTU530	pACYC-Duet- <i>drmA</i> (R810A)		Chloramphenicol	This paper
pTU531	pACYC-Duet- <i>drmA</i> (R1294A)		Chloramphenicol	This paper
pTU517	pET-Duet- <i>drmC</i>		Ampicillin	Aparicio-Maldonado et al.
pTU495	pCDF-Duet- <i>drmABC</i>		Streptomycin	Aparicio-Maldonado et al.
pTU519	pCDF-Duet- <i>drmABC</i> with <i>drmB</i> -ΔDUF1998		Streptomycin	This paper
pTU521	pCDF-Duet- <i>drmABC</i> with <i>drmA</i> (ΔPA)		Streptomycin	This paper
pTU543	pCDF-Duet- <i>drmABC</i> with <i>drmA</i> (Δloop)		Streptomycin	This paper
pTU532	pCDF-Duet- <i>drmABC</i> with <i>drmA</i> (V1296G)		Streptomycin	This paper
pTU533	pCDF-Duet- <i>drmABC</i> with <i>drmA</i> (V1296W)		Streptomycin	This paper
pTU534	pCDF-Duet- <i>drmABC</i> with <i>drmA</i> (R569D)		Streptomycin	This paper

pTU535	pCDF-Duet-drmABC with <i>drmA</i> (Q572D)		Streptomycin	This paper
pTU536	pCDF-Duet-drmABC with <i>drmA</i> (K610D)		Streptomycin	This paper
pTU537	pCDF-Duet-drmABC with <i>drmA</i> (R658D)		Streptomycin	This paper
pTU538	pCDF-Duet-drmABC with <i>drmA</i> (R649A)		Streptomycin	This paper
pTU539	pCDF-Duet-drmABC with <i>drmA</i> (K803A)		Streptomycin	This paper
pTU540	pCDF-Duet-drmABC with <i>drmA</i> (R810A)		Streptomycin	This paper
pTU541	pCDF-Duet-drmABC with <i>drmA</i> (R1294A)		Streptomycin	This paper



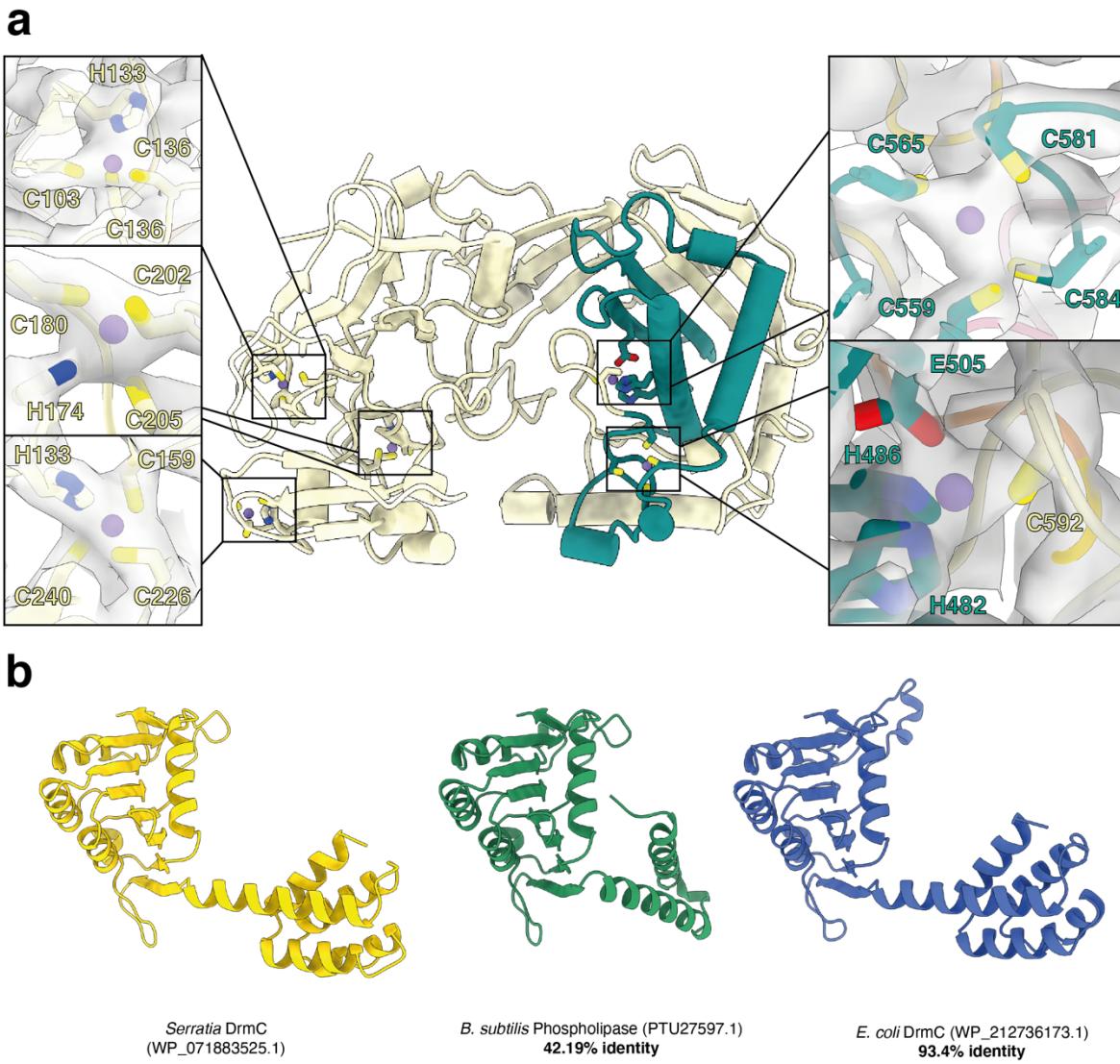
**Supplementary Fig. 1 | DNA binding by DrmAB**

**a**, Size-exclusion chromatogram of DrmAB complex. DrmB was tagged and used to pull down co-expressed DrmAB complex. Line denotes DrmAB species analyzed in panel B. **b**, SDS PAGE analysis of DrmAB complex purified by size-exclusion chromatography. DrmAB assembles with a 1:1 DrmA:DrmB stoichiometry. **c**, Secondary structure diagram of DNA stem-loop used for structural analyses (Table 2). 5' region visible in cryo-EM reconstruction is denoted by orange box. **d**, Native gel shift assay used to determine sample preparation conditions for cryo-EM. 4  $\mu$ M DNA hairpin (Table 2) was heat annealed and incubated with 10  $\mu$ M DrmAB in the absence or presence of 1 mM ADP or ADP:AlF<sub>3</sub>. Bound and free DNA were separated using EMSA.



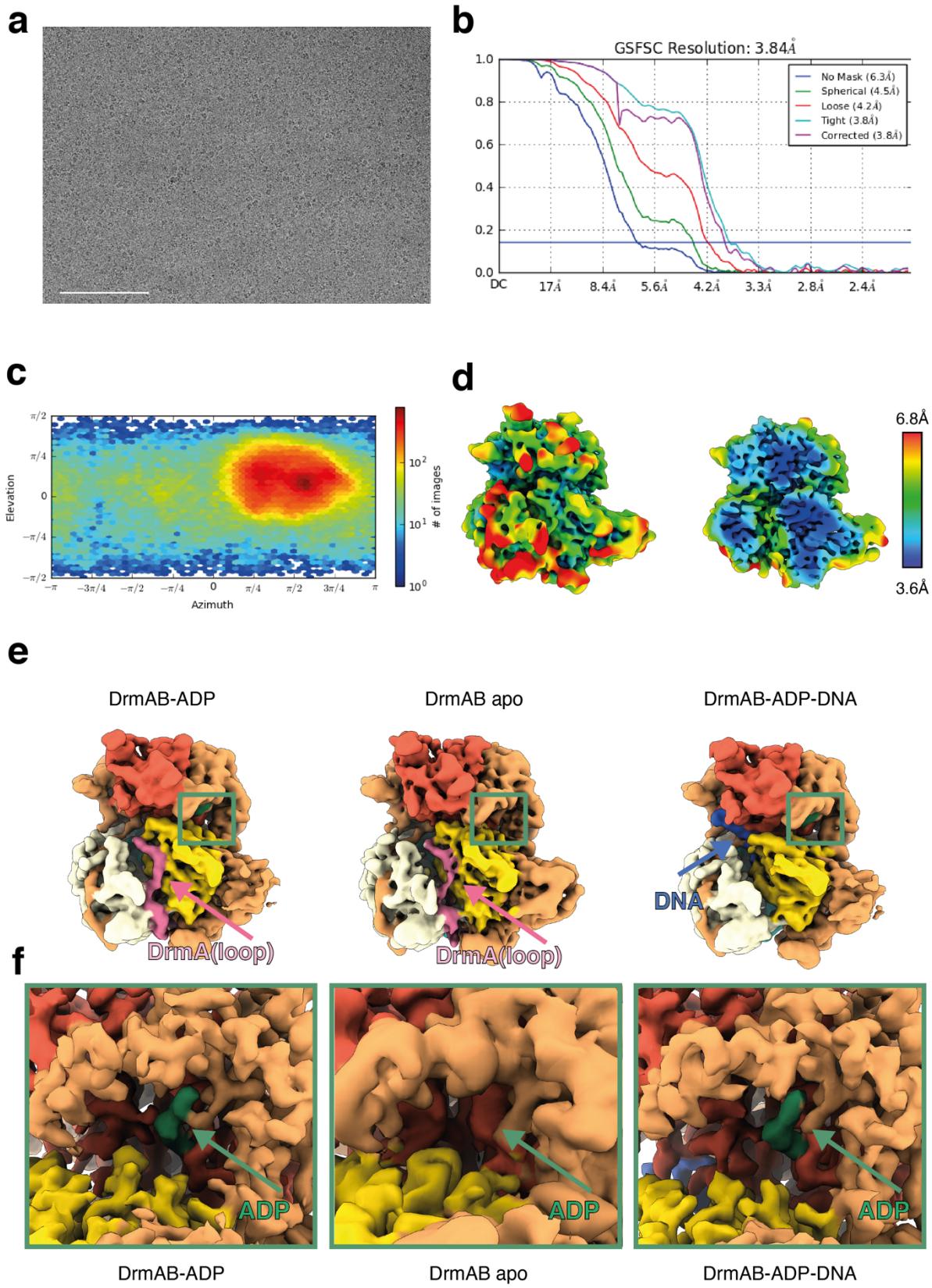
## Supplementary Fig. 2 | Cryo-EM data processing of DrmAB-ADP-DNA dataset

**a**, Representative cryo-electron micrographs of DrmAB:ADP:DNA. Scale bar – 100 nm. **b**, Data processing workflow detailing how tilted and untilted particle stacks were combined to a consensus reconstruction. While further classification of said reconstruction yielded a 2.8Å-resolution map, the quality of density corresponding to both ssDNA and DrmB N-terminal half were poor. To ameliorate this, focused 3D classification using either a DrmB mask or a DNA mask was used. **c**, Gold-standard Fourier shell correlation (FSC) curve for DrmAB:ADP. **d**, EM density map of DrmAB:ADP color-coded according to local resolution. **e**, Angular distribution plot calculated in Relion of DrmAB:ADP. **f**, Directional 3D FSC for DrmAB:ADP calculated by 3DFSC<sup>44</sup>. **g**, Map-to-model FSC for DrmAB:ADP. **h**, Gold-standard Fourier shell correlation (FSC) curve for DrmAB:ADP:DNA. **i**, EM density map of DrmAB:ADP:DNA color-coded according to local resolution. **j**, Angular distribution plot calculated in Relion of DrmAB:ADP:DNA. **k**, Directional 3D FSC for DrmAB:ADP:DNA calculated by 3DFSC<sup>44</sup>. **l**, Map-to-model FSC for DrmAB:ADP:DNA.



### Supplementary Fig. 3 | Structural analysis of DrmB and DrmC

**a**, Multiple putative metal coordination sites within DrmB, with representative cryo-EM densities. **b**, Structural models of DrmC from *Serratia* (yellow), a *B. subtilis* PLD-nuclease protein homologue of DrmC (green) and an *E. coli* PLD-nuclease protein homologue of DrmC (blue). The DISARM operon in this study originated in *Serratia*, and *in vivo* assays were performed in *E. coli*. *B. subtilis* was the model organism used for *in vivo* assays in a previous DISARM study<sup>7</sup>. Structural models were generated using AlphaFold2<sup>45</sup>.



**Supplementary Fig. 4 | Structural features of DrmAB complex and TL**

**a**, Representative cryo-electron micrograph of DrmAB. Scale bar – 100 nm. **b**, Gold-standard FSC curves for DrmAB. **c**, Angular distribution plot for DrmAB calculated in cryoSPARC. **d**, EM density map of DrmAB color-coded according to local resolution. **e**, Presence of TL EM density features within DrmAB:ADP, DrmAB, and DrmAB:ADP:DNA maps. TL is pink, and DNA is blue. Green box corresponds to ATP binding site. **f**, ATP-binding site within DrmA RecA domains. DrmAB apo complex contains no nucleotide density, but still contains TL density.