

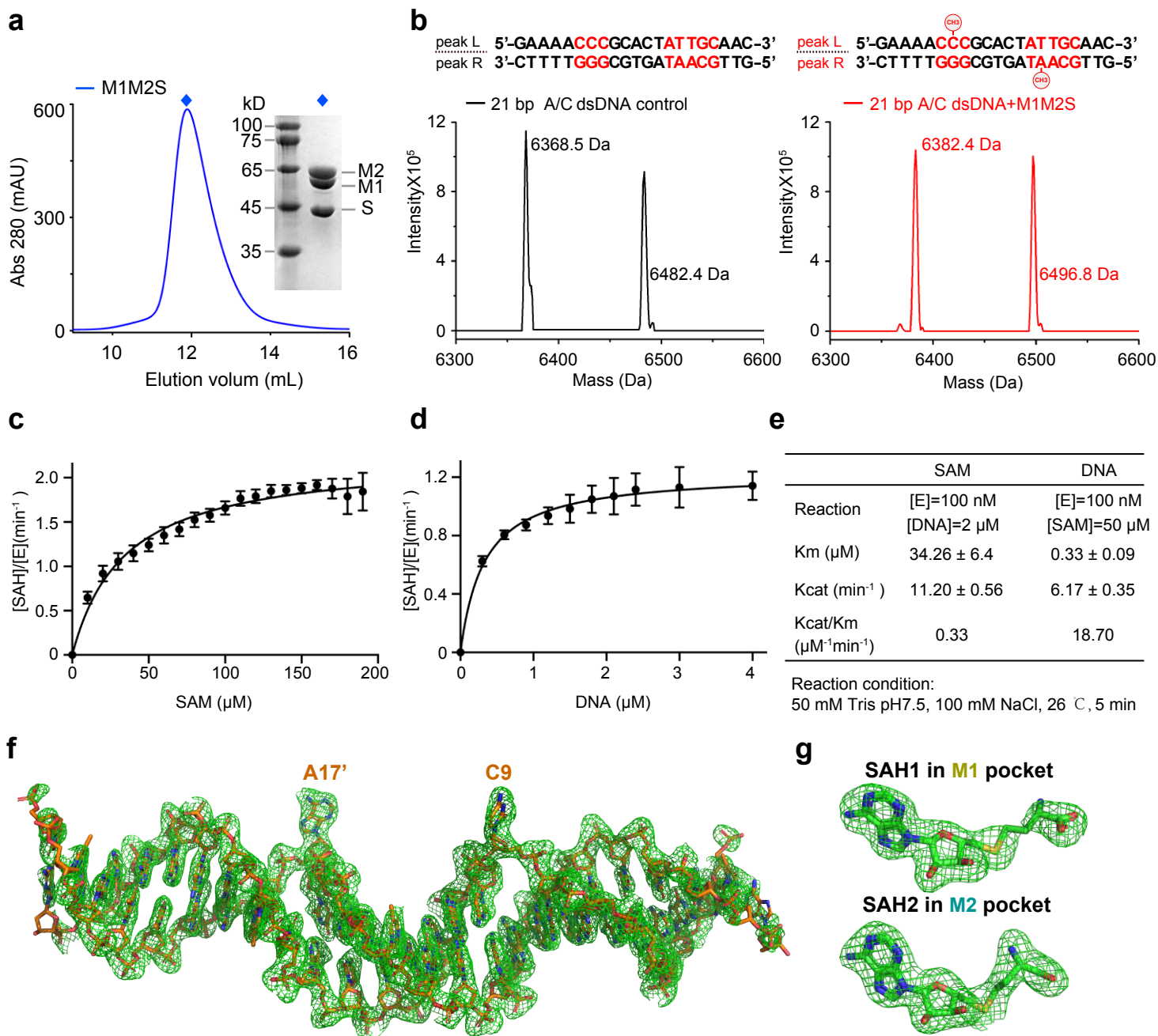
Supplementary Information

Molecular insights into DNA recognition and methylation by non-canonical type I restriction-modification systems

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Supplementary Figures 1-11 and Legends

Supplementary Tables 1-3



Supplementary Fig. 1 | Enzymatic activity of PacII MTase and electron density of DNA and SAH molecules.

a, Purification of PacII_M1M2S complex. Size exclusion chromatography on Superdex 200 10/300 column and SDS-PAGE result of the peak fraction (n=3).

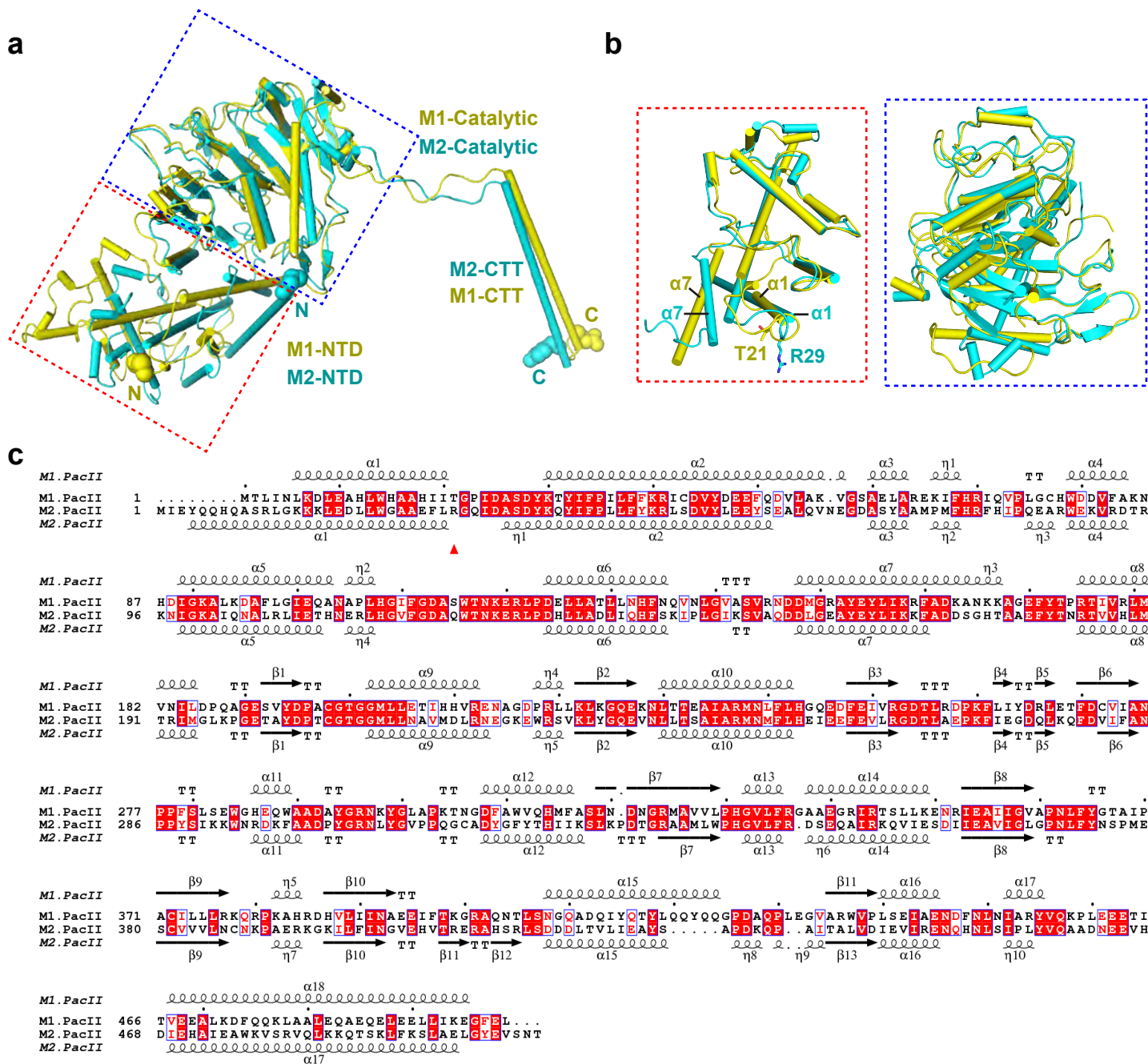
b, Mass spectrometry analysis of the substrate DNA with (red) or without (black) wild-type enzyme (n=3).

c, d, The production of SAH was measured via a luminescence assay, and curves of [SAH]/[E] dependent on substrate concentration of methyl donor SAM (**c**) or DNA (**d**) was fitted according to the Michaelis-Menten equation (n= 3). Source data are provided as a Source Data file.

e, Summary of PacII MTase kinetic parameters. The mean $\pm$ SD represents three independent determinations (n=3).

f, g, mFo-Fc omit map (3 σ) of DNA (**f**) and SAH molecules (**g**), the target base-flipped adenine and cytosine were visible.

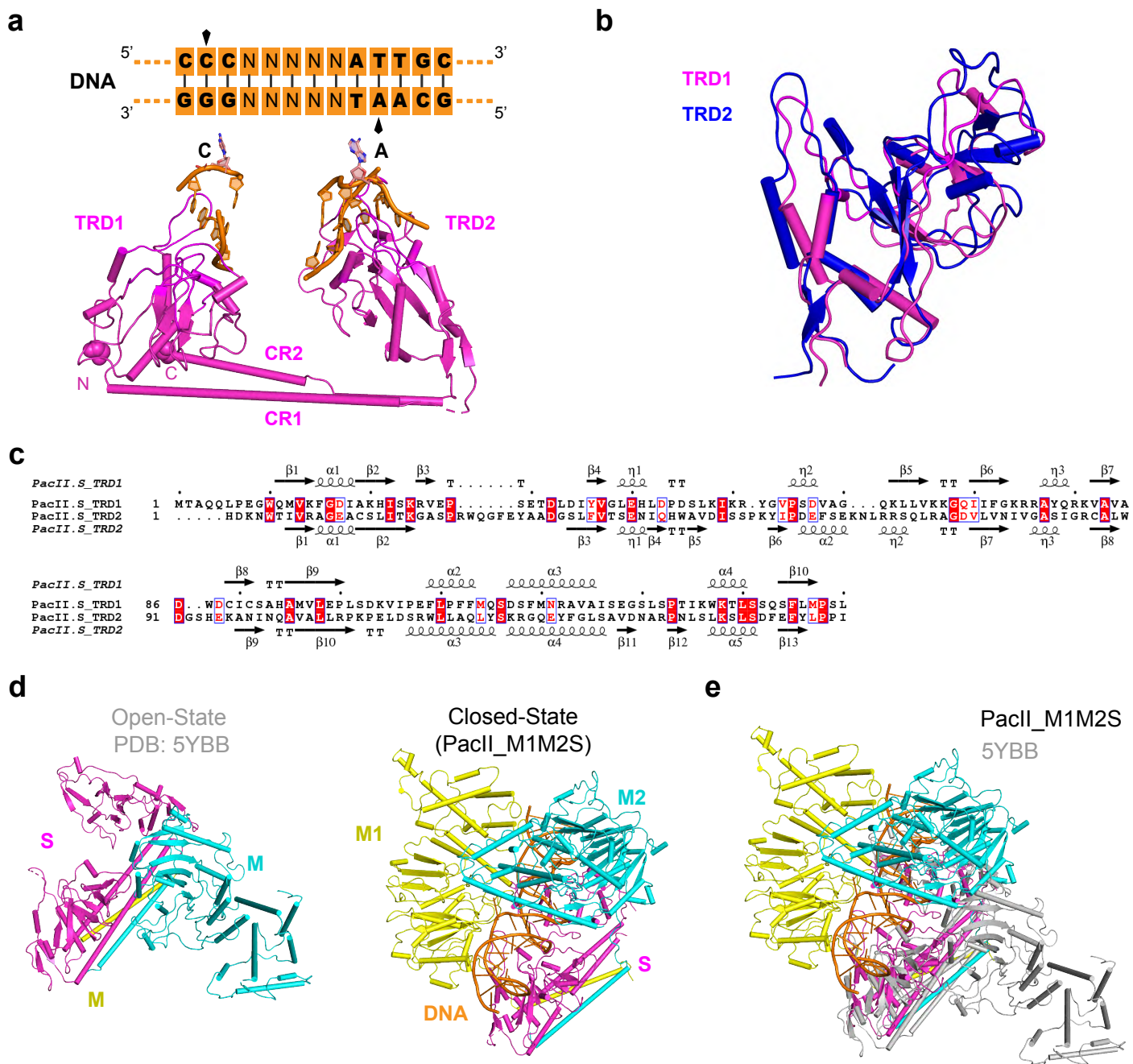
Experiments in **a, c, d** were repeated independently three times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 2 | Structure comparison and sequence alignment of M1 and M2 subunits.

a, b, Overall structure (**a**) and individual domains (**b**) superimposition of M1 and M2 subunits.

c, Sequence alignment of full-length M1 and M2 subunits with secondary structure shown in up and down, respectively.



Supplementary Fig. 3 | Comparison of two TRDs of S subunit, as well as open and closed type I MTases.

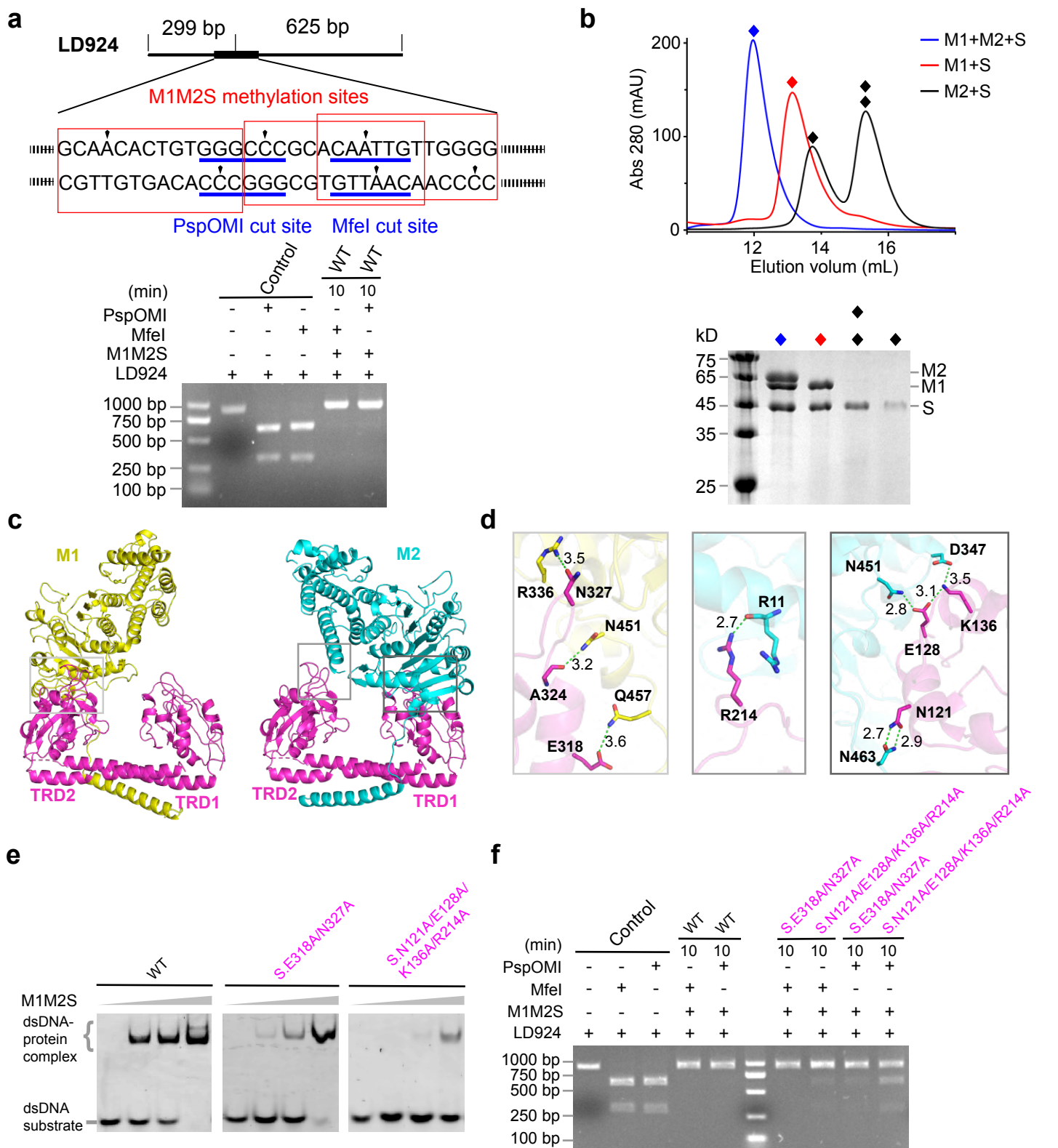
a, Structure of S subunit bound with bipartite specific DNA motifs.

b, Structural superimposition of TRD1 (magenta) and TRD2 (blue).

c, Sequence alignment of TRD1 and TRD2 with secondary structure shown in up and down, respectively.

d, Structures of the classical open-state type I M_2S (PDB: 5YBB) and the novel closed-state *PacII_M1M2S*-DNA-SAH. S subunits are shown in the same orientations in both structures.

e, Superimposition between the open-state and closed-state structures using the S subunits as references. *PacII_M1M2S* was color-coded as in (d) and the classical open-state type I M_2S (PDB: 5YBB) was shown in gray.



Supplementary Fig. 4 | Assembly and asymmetric interactions of PacII_M1M2S complex.

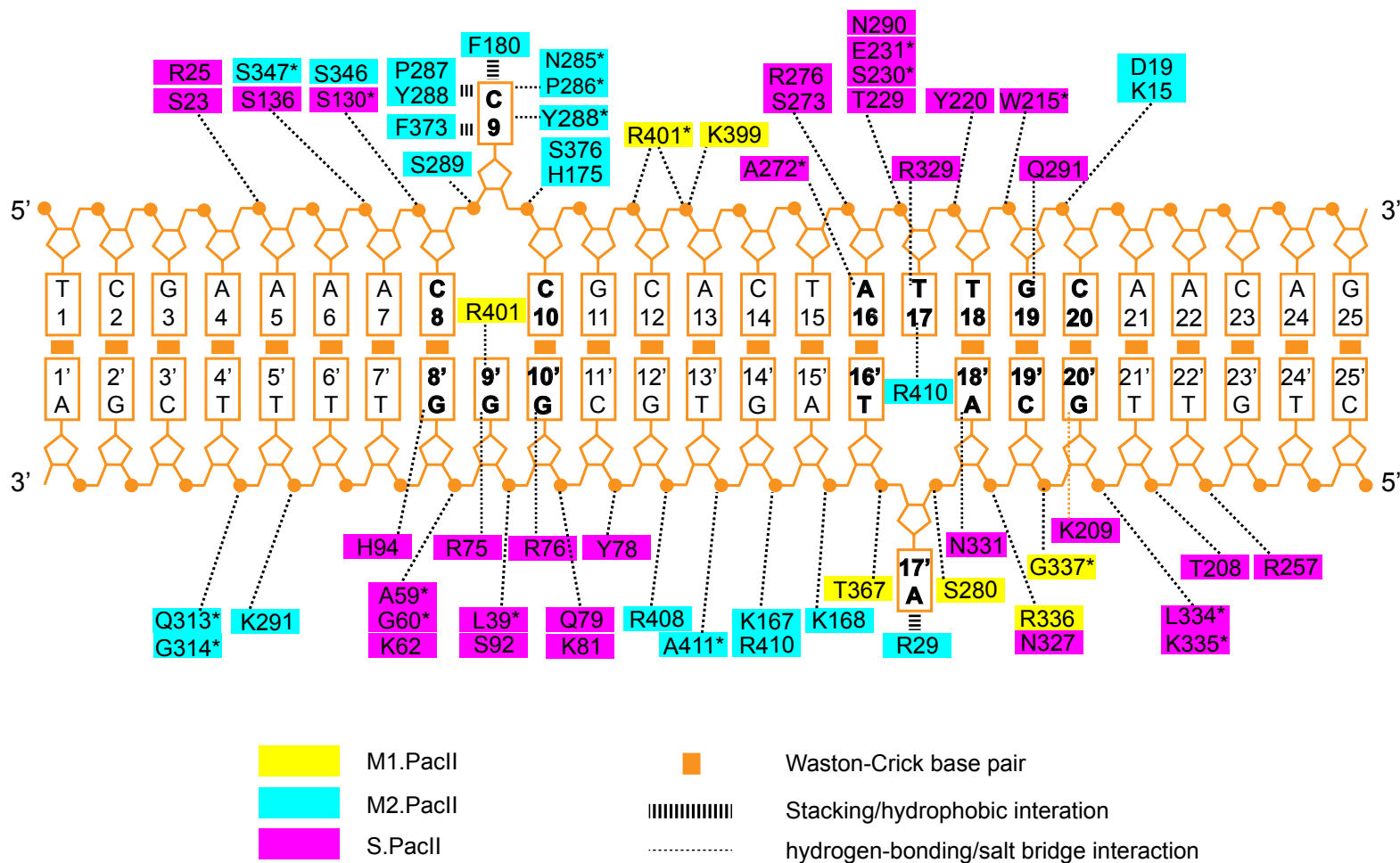
a, A linear DNA (termed as LD924) containing three PacII recognition sites (up) and the cleavage assay by PspOMI/MfeI-digestion (down) (n=3).

b, Size exclusion chromatography (up) and SDS-PAGE results (down) of co-expressed samples including M1M2S, M1S, M2S. The S subunit alone exhibited a dynamic equilibrium of monomer and dimer (n=3).

c, d, Overall view (**c**) and detailed interactions (**d**) of M1-TRD2 and M2-TRD2/TRD1 interfaces. Green dashed lines indicate hydrogen bonds.

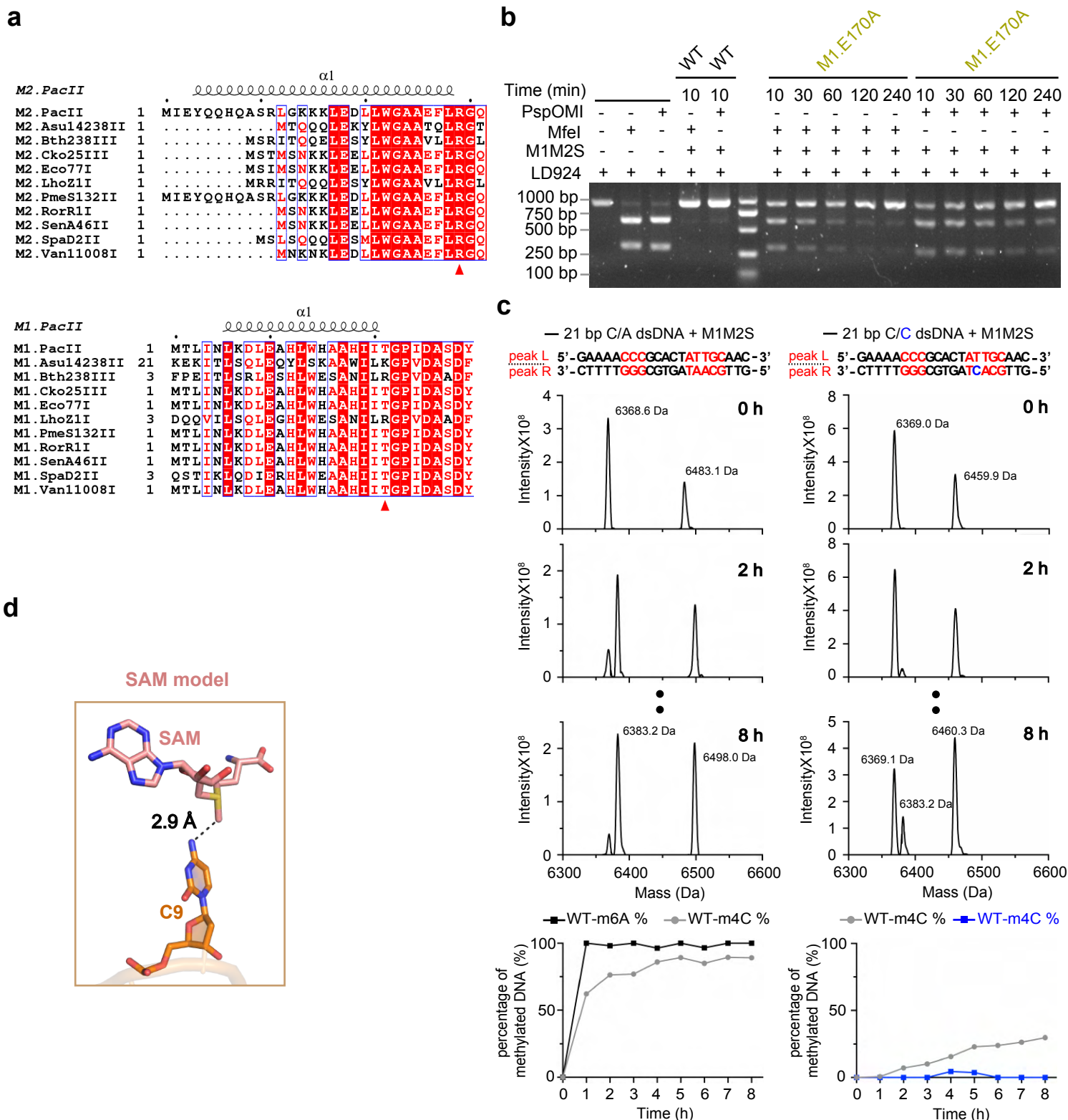
e, f, Electrophoretic mobility shift analysis (**e**) and PspOMI/MfeI-digestion analysis (**f**) of mutations within M1-TRD2 and M2-TRD2/TRD1 interfaces (n=3).

Experiments in **a, b, e, f** were repeated independently three times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 5 | Schematic of intermolecular interactions between DNA and PacII_M1M2S heterotrimer complex.

Residues from M1, M2, and S subunits are colored in yellow, cyan, and magenta, respectively (* means the mainchain of indicated residue). The bipartite specific DNA sequences recognized by S subunit are colored in bold black. Hydrogen bonds and salt bridges are shown in slight dotted lines. Stacking and hydrophobic interactions are shown in bold dotted lines. Watson-Crick base pairs are shown as orange squares.



Supplementary Fig. 6 | Asymmetric methylation analysis of M1 and M2 subunits.

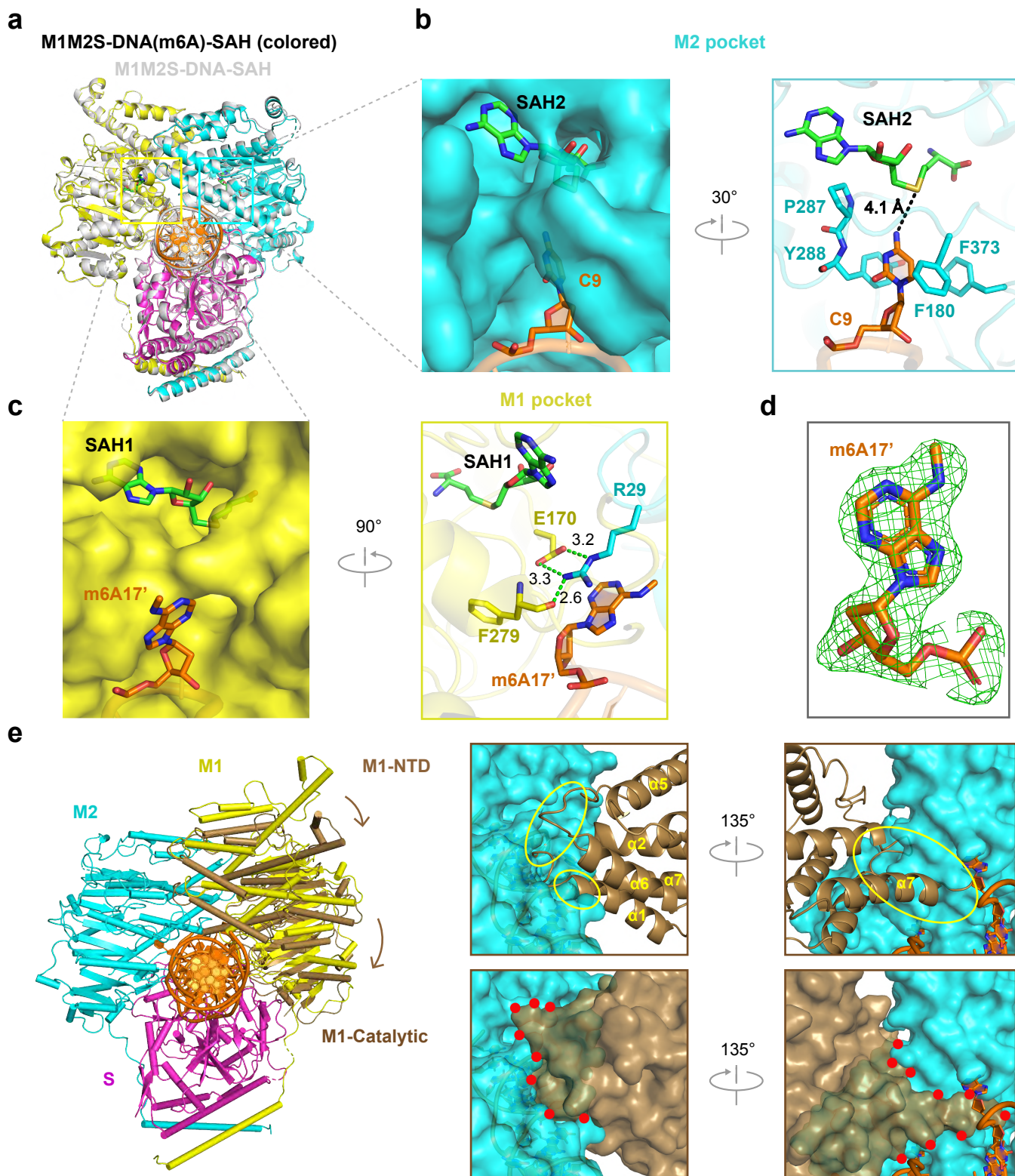
a, Sequence alignment of helix $\alpha 1$ from candidate M1 and M2 subunits, respectively. The positions of M1_T21 and M2_R29 were highlighted by red triangles.

b, PspOMI/MfeI-digestion analysis of mutant M1_E170A (n=3).

c, Mass spectrometry analysis of original and mutant DNA (A17'C) substrates at different time points (0 to 8 h), proportions of m6A and m4C methylated DNA were counted and plotted, respectively.

d, The N4 atom of target cytosine (C9) within M2's catalytic pocket contacted with the modeled SAM molecule (2.9 Å, black dashed line).

Experiment in **b** was repeated independently three times with similar results. Source data are provided as a Source Data file.



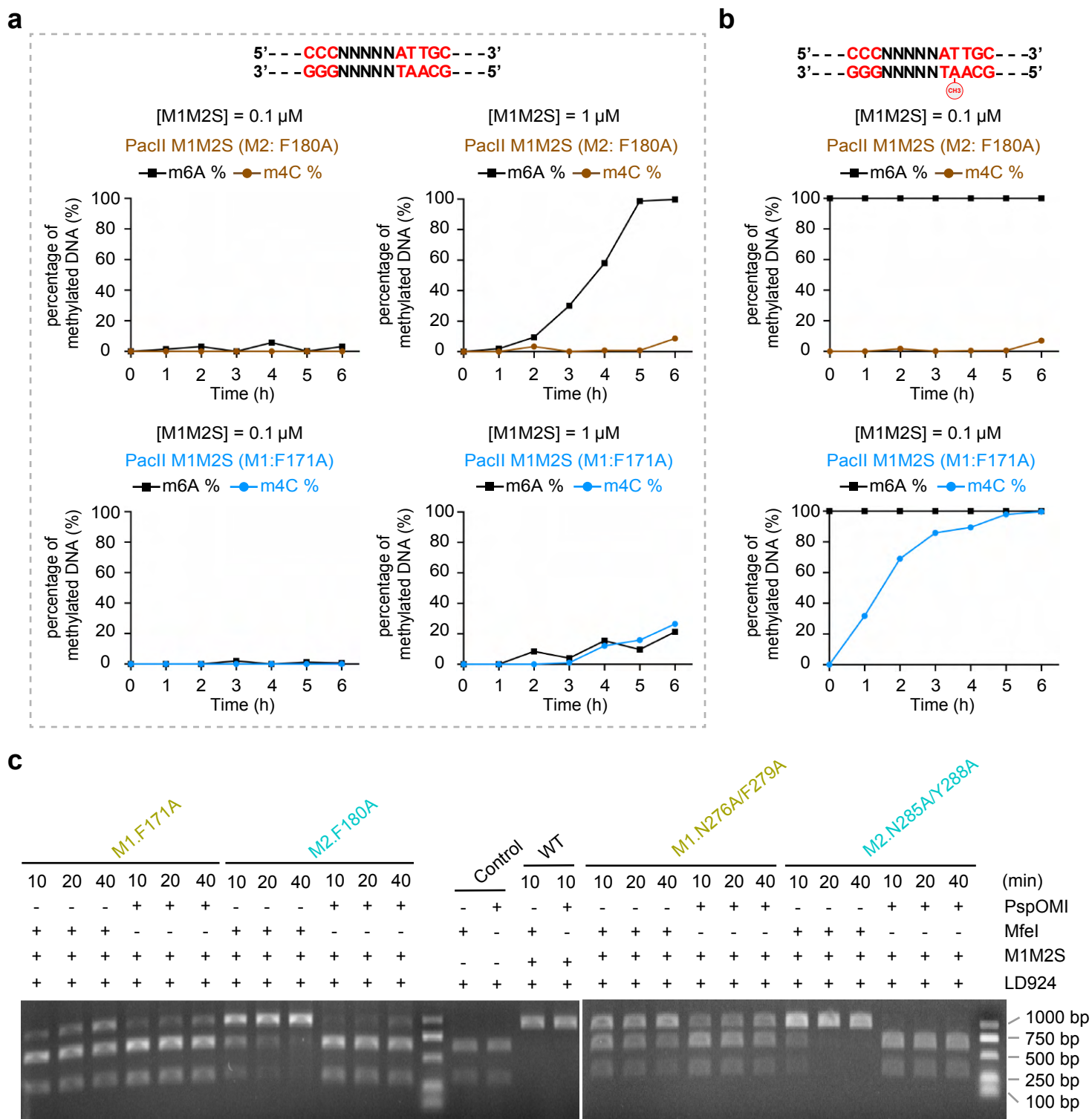
Supplementary Fig. 7 | Catalytic pockets of M1M2S-DNA(m6A)-SAH complex, and lying-down structural model of M1 subunit.

a, Structural superimposition of M1M2S-DNA-SAH and M1M2S-DNA(m6A)-SAH complexes.

b, c, Surface views and detailed environments of the catalytic pockets within M2 (**b**) and M1 (**c**) subunits of M1M2S-DNA (m6A)-SAH complex.

d, mFo-Fc omit map (3σ) of target methylated adenine.

e, A modeled lying-down structure of M1 based on the current structure of M2 subunit, with the severe hindrance effects highlighted as yellow circles and red dots.



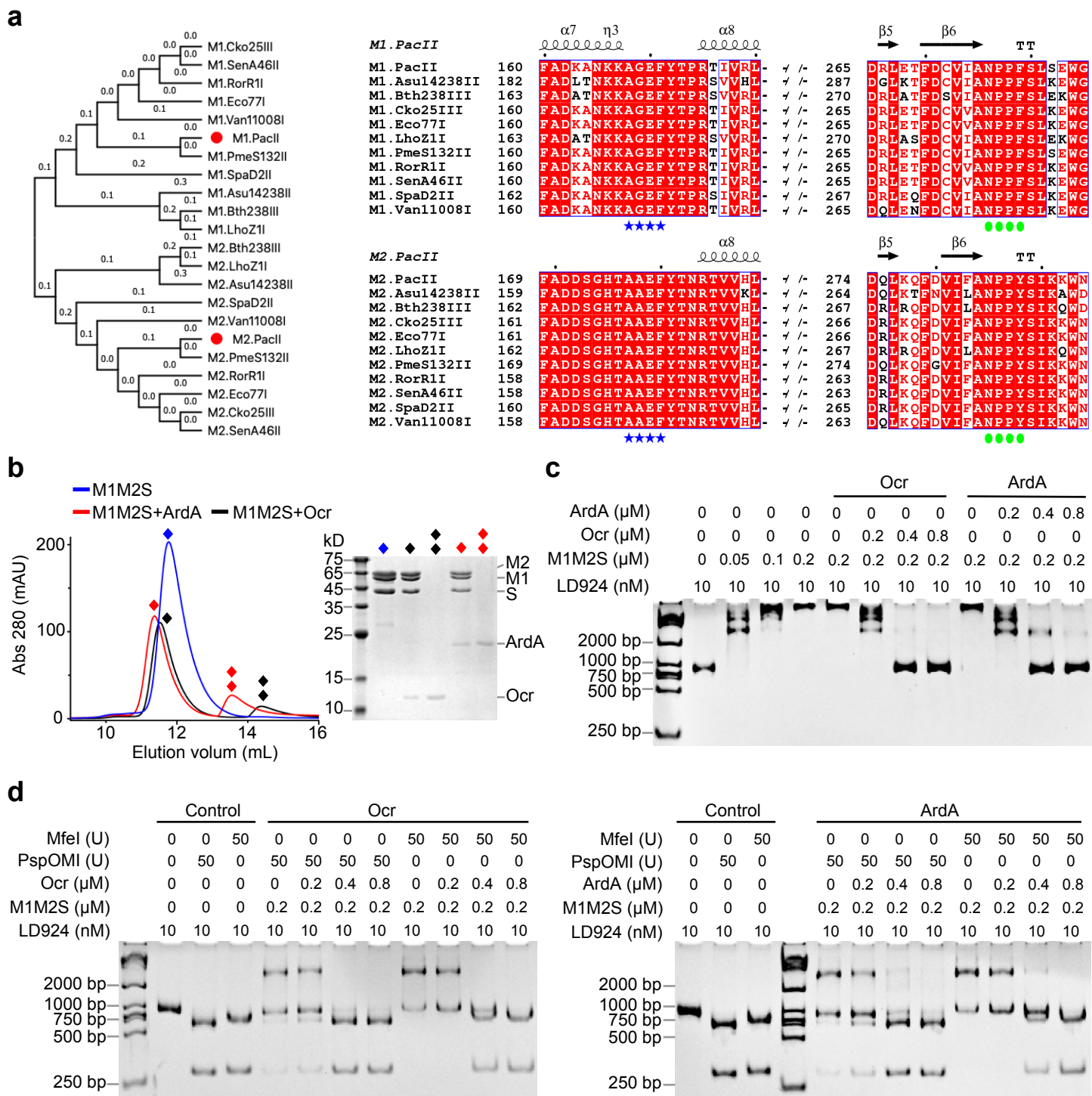
Supplementary Fig. 8 | Mass spectrometry and PspOMI/MfeI-digestion analyses of catalytic pocket mutations within M1 and M2 subunits.

a, Two sets mass spectrometry analyses (low or high enzyme concentration) of target DNA at different time points (0-6 h), proportions of m6A and m4C methylated DNA were counted and plotted, respectively.

b, Mass spectrometry analysis (low enzyme concentration) of m6A methylated DNA at different time points (0-6 h), proportions of m6A and m4C methylated DNA were counted and plotted, respectively.

c, PspOMI/MfeI-digestion analysis of mutations within the catalytic of M1 (F171A, N276A/F279A) and M2 (F180A, N285A/Y288A) subunits (n=3).

Experiment in **c** was repeated independently three times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 9 | Conservative catalytic pockets of M1 and M2 subunits, and Ocr/ArdA-mediated inhibition on PacII_M1M2S complex.

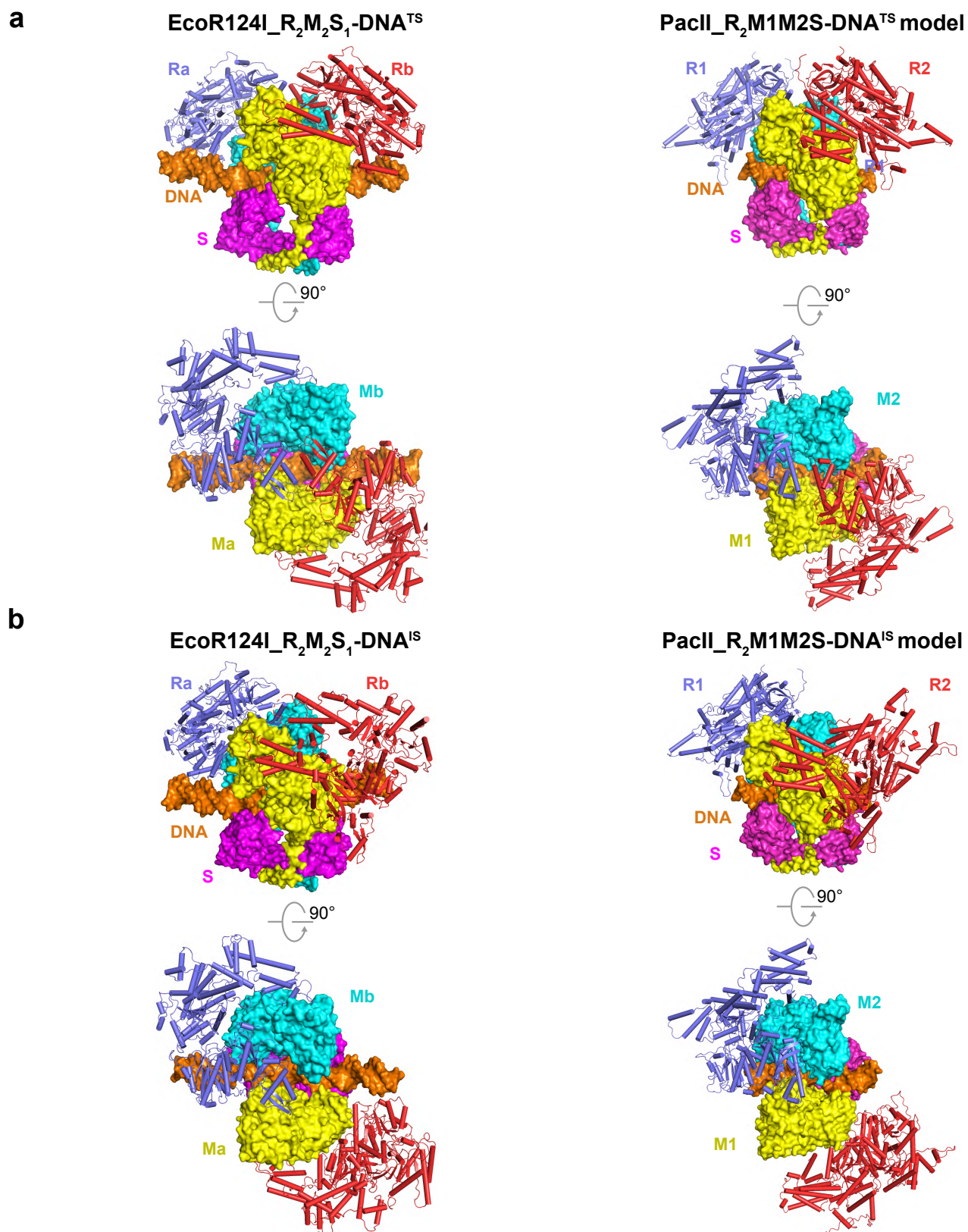
a, Phylogenetic tree (numbers represent standardized distance scores) and sequence alignment of m6A/m4C Type I methyltransferase of different species. The conserved regions were highlight in blue stars (M1: aa 168-171; M2: aa 177-180) and green dots (M1: aa 276-279; M2: aa 285-288).

b, Elution profiles of size exclusion chromatography and SDS-PAGE results for indicated samples (n=3).

c, Electrophoretic mobility shift analysis show that both Ocr and ArdA competed for the binding of PacII_M1M2S to DNA (n=3).

d, PspOMI/MfeI-digestion analysis show that both Ocr and ArdA inhibited the methylation activity of PacII_M1M2S (n=3).

Experiments in **b**, **c**, **d** were repeated independently three times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 10 | Potential models of PacII holoenzyme in two conformational states based on EcoR 124I.

a, Two views of the translocation state (TS) for EcoR124I_{R₂M₂S₁} (left) and the predicted PacII_{R₂M1M2S} holoenzyme (right).

b, Two views of the intermediate state (IS) for EcoR124I_{R₂M₂S₁} (left) and the predicted PacII_{R₂M1M2S} holoenzyme (right).

M1. PacII	1	MTLINLKDLEAHLWHAHHIT	GPIDASDYKTYIFPILFFKRICDVYDEEFQ				
M2. PacII	1	MIEYQQHQASRLGKKKLEDLLWGAAEF	GGQIDASDYKQYIFPLLFYKRLSDVYLEEYS				
M. EcoAI	1	MSISSVIKSLQDIMRKDA	GGVDGDAHGLGQLSLWLLFLKIFDAQE	EA			
M. EcoKI	1	MNNNDLVAKLWKLCNNLR	GGGVSYQYVNLASLWLLFLKIFDAQE	EA			
M. EcoRI24I	1	MKMTSQQRAELHRQIQIANDV	GGGSVDGWDFKQYVNLALFLYRFIS	ENFSSYI			
M. Kpn143III	1	MTEFEKQ.....KLGKILWAIADKL	GGGSVDGWDFKQYVNLALFLYRFIS	ENFSSYI			
M. Lam395I	1	MSPQIEAQNLSLQGRLLWNIA	GGKMNADDFRDYCLGFVFLYKYLSE	KFVAYANKILAE			
M. Pae0110II	1	MSISSTIKSIQDIMRKDV	VDGDAQERIGQLVWMLFLKIFDRE	QE			
M. Rba05I	1	MTPITQADINKAAWASACD	VVDPSIYKDVVLTMLFLKYLSDVVKD	DHHT			
M. StySBLI	1	MAKAPTCKAKAKKGFEDTLWD	SVESSEYKHVVLSLFLKFISSDKFE	ARRK			
M. XbaIII	1	MSKTDIAQDITINAAVWGACD	GTVDPSVYKDVVLTMLFLKYLSDVVKD	DHHT			
▲							
M1. PacII	52	DVLAKVGSAAELAREKIFHRIQVPLGCHWDDVFAKNH	DIG.....KALKDAFLGTEQANAP	LHGI			
M2. PacII	60	EALQVNEGSDASYAAMPFMRHFIHQIPQEARWEKVRDTRKN	IG.....KALQNALRLTEHNER	LHGV			
M. EcoAI	46	LELEQDNQYQPIPIQRYLWRSWAANAQGITGDSLLLEFV	NDD.....LFPALKNLTAPIDKNPRG	YVVKQAFSD			
M. EcoKI	45	QEAAYLPEGYRWDDDLKSRIG	QEQ.....LQFYRKMLVHLGEDDDKK	LVQAVFHN			
M. EcoRI24I	62	YAKLDDSVITDDIKDDAIKTKGYFIYPSQLFCNVAANKANTN	DRNLNADLNSIFVAIESAYGYSPSEADIKGL	FAD			
M. Kpn143III	72	LWYESNYEDVPEFEKQMRKRVHYVIEPQYLWGNIAEMARKQ	DDELLRTLQKGFKVIEESF	ASTFRGLFSE			
M. Lam395I	70	AEHPAYQDITVDVAKEDSIRTLGYFLPPTDLFHTMAERIA	NDFPKGAGTGFILEDLAATLRN	QSTLTGDSADDFSLFED			
M. Pae0110II	46	WELLDDAYRSPICESCRWRTWAADPEGITGDELKNFI	DN.....LFPQLQNLHEYS	YVVRGVFED			
M. Rba05I	52	RYTTDHPNAPDLVAALMAQEAFLVPGKASFDLLYARRHEP	GLGERIDKALHAIEANIT	KLRDVFQD			
M. StySBLI	58	QMEDEGGQDFTLEMEVFYQDDNIFLPEEARWSFIKQNAQ	QDD.....IAVRIDTALSTIEKRNP	LKGLALFQD			
M. XbaIII	54	DYKTKHGNKPGGLIEELLKSERFVLPHSANFYTYLYDQRRH	NGNERIDTALHAIEDANLG	KLRDVFQD			
★ ★ ★ ★							
M1. PacII	114	ASWTNKER.....LPDEL	ATLLNHNFNQVNLGVASVRNDD	MGRAYEYIKRFAADKANKKAGEFYTPRTIVRLMVNII			
M2. PacII	123	AQWTNKER.....LPDHL	ADLIHFHSKIPLGKSVQDD	FAADDSGLFAEFYTPRTIVRLMTRIM			
M. EcoAI	113	AYN.....YMKNGTL	LRQVKNLNEIDFTSASERHLF	GDIIYEQIKLQDSAGNAEFYTPRTIVRLMTRIM			
M. EcoKI	93	VSTT.....ITEPKQI	TALVSNMDSLDVWNGAHGKSR	DDFGDIIYEQIKLQDSAGNAEFYTPRTIVRLMTRIM			
M. EcoRI24I	136	FDTTSNRLNGITVVKDKNAR	LAUVLKGVEG.....LKLGDVNEHQIDFL	GDAYEFLISNVAANAGSKSGEFYTPRPHVSKLIIAQLA			
M. Kpn143III	143	INLADSKLGKTYIERNAR	LCKIIAEIAG.....GLVQFSTDS	DTLGDAYEYLLIGQFAAGSGKKAGEFYTPRORISDIISAIV			
M. Lam395I	150	LDLGGSKLGNATAKKNEL	IGKVITELDK.....LSFNLSSEASSDIL	GDAYEYLLIGQFAAGSGKKAGEFYTPRORISDIISAIV			
M. Pae0110II	112	AYN.....YMKSGQL	IRQVINKIQEG.....VDFNKQAERHAF	GDMEYQLLRDLQNAQNAEFYTPRPRVTEFMRVMV			
M. Rba05I	119	ISFNSTRNLGAED.QKNDI	LRFLLEDFAKPAIDLRPSRGTDLII	GGAYEYLSRFAATAGKKAGEFYTPPAEVSILMAQLV			
M. StySBLI	125	NYFSRQNLGT.....KKLAS	LIDTIDNIETLAHETDEVALSKEDLV	GRVYEFLLGKFAATEGKSGEFYTPKCVSDITLTEM			
M. XbaIII	121	ISFNANKLGEEQ.QKNDL	LRHLLLEDFAKPAIDLRPSRIGQDLII	GNAYEYLTKNFASSSGKKAGEFYTPPEVSALMARLM			
★ ★ ★ ★							
M1. PacII	186	DPQ.....AG	ESVYDPA	CGTGGMLLETIHVRENAGDPRILK	LKGGQENLTTETAIARMNIFLHG		
M2. PacII	195	GLK.....PG	ESTAYDPT	CGTGGMLLNAVMDLVNNEGKWSRVK	LYGGQENLTTETAIARMNIFLHG		
M. EcoAI	179	DPK.....LG	ESIMDPA	CGTGGMLLACAFDHVKNKYKSVADHQT	LQQQIHGGQENLTTETAIARMNIFLHG		
M. EcoKI	164	KPQ.....PRE	VVDPAAGTAG	FLLEADRYVKSQTNLDLDDGDTQDFQI	HRAFI		
M. EcoRI24I	214	MHG.....QTHVN	KIYDPAAGSG	SLLLQAKKKQFNDHIEEG	FF		
M. Kpn143III	219	TLDSQEPATGRKSHLSV	TFVCHVCS	SLLLLVRRLLMGPHGIGK	IHNHTTYN		
M. Lam395I	227	TT.....HKL	KLKNV	DPACGSG	SLLLLVRRLLMGPHGIGK	IHNHTTYN	
M. Pae0110II	179	DPK.....LD	EKKVMD	DPACGSG	SLLLLVRRLLMGPHGIGK	IHNHTTYN	
M. Rba05I	198	DPQ.....PG	DDICDPT	CGSAS	LLMKCARLIRDRHNSRYHA	LF	
M. StySBLI	202	EPF.....QK	IYDPT	CGSAS	LLMKCARLIRDRHNSRYHA	LF	
M. XbaIII	200	DPQ.....PG	DEIDPT	CGSAS	LLMKCARLIRERTGTGKYA	LY	
★ ★ ★ ★							
M1. PacII	245	Q.....EDF	EIVRG	DTLRLDP	KFLIYDRLETF	DCVIANPFFSLSEWGH	EQWAADAYGRNKYGLAPKTNQDFAWVQ
M2. PacII	254	I.....EEF	EVLRG	DTLRLDP	KFLIYDRLETF	DCVIANPFFSLSEWGH	EQWAADAYGRNKYGLAPKTNQDFAWVQ
M. EcoAI	244	IE.....VPV	QIRHD	NLTNKP	LSSWD	EQLDV	IVTNPFFGGTEEDG
M. EcoKI	234	IEGNLDHGGAI	IRLGN	LTGSD	GENLPKAHI	IVATNPFFGSAAGTN	ITRTFVH
M. EcoRI24I	272	IN.....DKF	DIKLG	NLTNLT	EPHFREKPPF	IVANPFFSVKWI	GSDPTLINDERFAPAGVLA
M. Kpn143III	284	VKD.....SEF	DIKLG	NLTNLT	EPHFREKPPF	IVANPFFSVKWI	GSDPTLINDERFAPAGVLA
M. Lam395I	282	VHY.....ADF	EIIQED	DTLRLDP	KFLIYDRLETF	DCVIANPFFSLSEWGH	EQWAADAYGRNKYGLAPKTNQDFAWVQ
M. Pae0110II	244	IE.....VP	SQIKHD	NLTNLT	EPHFREKPPF	IVANPFFSVKWI	GSDPTLINDERFAPAGVLA
M. Rba05I	256	E.....ENH	QVWGD	PTIRNP	KLRTDRLDRLH	FDVIVANPFFSLEKGV	ESAENDRFGFRRLGLP
M. StySBLI	260	LS.....ANL	GERPA	DTIRNP	KLRTDRLDRLH	FDVIVANPFFSLEKGV	ESAENDRFGFRRLGLP
M. XbaIII	258	E.....DNH	RIE	WGD	PTIRNP	KLRTDRLDRLH	FDVIVANPFFSLEKGV
● ● ● ●							
M1. PacII	314	HMFASLN	DNRGRMAVVLPH	HGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M2. PacII	323	HIISKLP	KDPTGRAAMLW	PHGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. EcoAI	303	QLIVEVLAKN	GAAAVVLPH	GVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. EcoKI	295	HIETLHPG	GAAAVVLPH	GVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. EcoRI24I	342	HALNYLSAK	GAAAVVLPH	GVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. Kpn143III	358	HGFHFLKQD	GMAIILPH	HGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. Lam395I	349	HMFFHLEDD	GMAIILPH	HGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. Pae0110II	305	VLIMHLLK	QDGRAAVVLPH	GVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. Rba05I	326	HMVETL	KKPRTGRMAVVLPH	HGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
M. StySBLI	326	HMLSKL	SANGTAGFVL	ANGSMSSNTSG	EAETRAQMIEND	LIDCMIALP	GQLEFFGTQIIPVCLWFMTSKAADPAKGYRN
M. XbaIII	327	HMIATM	KPSRGRMAVVLPH	HGVLF	RGAAEGRTSLLKEN	RIEATIGVAPNLE	YGTAIIPACIILLRKRQRPKARHHDVLI
● ● ● ●							
M1. PacII	391	INAAEIEFTK	GRAQNTLS	NGQA	DIYQTYLQ	YQQGPDAQ	LEGVARWVPLSETAEND
M2. PacII	400	INGVEHVIT	TERAHSRLS	DDDL	TVLIEAX	APDKOP	AITALVDTEVIRENQ
M. EcoAI	382	PYPAGVKN	YSKTKPMKF	EEFO	AEIIDW	EADGFASR	VENEQAWKVSIDVIARN
M. EcoKI	372	TDVVWVY	DLRTNMP	PSFG.....KRT	PFTDEHL	PFPERVYGED	PHGLSPRTEGSEFNAEETEVADSE
M. EcoRI24I	416	IDASLEL	FKKETNN	ILIT.....DAHE	QIMQVFA	SKEDVAHL	AKSVAFTVVDND
M. Kpn143III	432	INAAEIEFK	EGKRQSQ	LLRTD	EMTDGGIGH	EKIIDTYR	YRKEEPYSR
M. Lam395I	425	IDASNEH	FEKVKTQ	NRLL.....AEH	KIADTYN	DWKDIEK	YSHAASLEDIRAND
M. Pae0110II	384	QYPAGYK	YSKTKPMRI	EEFA	VEAEAWG	EADGFAAR	VENEFAWKVSIDELRARN
M. Rba05I	401	IDASRE	FEAGTNQ	NTLT.....PANL	DRITATYR	ARESVDK	YAYRATSLADVEGND
M. StySBLI	404	RQGETL	FIDARNLGT	MIN	RTTKELT	ADDIATIDTY	HAWRSTPEELVERVKRGDSQLAQYEDQAGFCKVATIAIKAND
M. XbaIII	402	IDASRQY	QDGKNQNL	LR.....ESD	LQGRILDTVQ	ARQSV	DKYAYLASPAEIAAGND
● ● ● ●							
M1. PacII	448	FNLNIIAR	YVQKPLEEE	TIT	VEEALKD	FQQKLA	ALEQAEQELEELLIKEGFEL
M2. PacII	450	HNLSIFL	YVQAADNEE	VHD	IEHAIEA	WKVSRV	QLKKQTSKFLFKSLAELGYEVS
M. EcoAI	437	FNLDIKN	PHQAETV	SHD	PDELLAQ	YAKQQA	IQTLRNQLRDIIGAALSVKEV
M. EcoKI	434	ENKNTD	QHILATSRWRK	KFSREWIR	TAKSDSLDISWLKDKS	SDADSLPEP	DVIAAAEAMGELVQALSLELDALMRLEGASDEA
M. EcoRI24I	466	YNLSVSS	YVEAKDNRE	IID	IAELNAE	LKT	TVSKIDQLRKDIDAIVAEIEGCEVQ
M. Kpn143III	491	FNLNII	SRVYSTVQAEE	EID	DLAAVHT	LLVVEK	QIGAKERYNQYLAELGL
M. Lam395I	475	YNLNII	PRVYDTFEAD	EID	DLNAVAQ	TRELGE	VAKDNTIAGFCKELGIDAPV
M. Pae0110II	439	WNLDCKN	PHVGEQ	SHD	FEELLRN	YAQLQGE	IGELRAQLKAMLAELERQAF
M. Rba05I	451	FNLNII	PRVYDTFEAD	QID	DLMAVRE	RMELKD	EMEALEARMAGYLVLEGYGA
M. StySBLI	483	SVLTPGR	YVGAEEQEDDG	VAF	ETKMR	ELSQT	LFAQMKQAEELDN
M. XbaIII	452	YNLNII	PRVYDTFEAQ	EID	DLMAVRR	REQLKA	ELARLEEQMGAGYLVLEGYGA
● ● ● ●							
M1. PacII	504	T.....					
M2. PacII	504	T.....					
M. EcoAI	489	N.....					
M. EcoKI	514	DLQRQLLE	EAFGGVKE				
M. EcoRI24I	520	K.....					
M. Kpn143III	529	TLSEG	NK				
M. Lam395I	529	TLSEG	NK				
M. Pae0110II	529	TLSEG	NK				
M. Rba05I	529	TLSEG	NK				
M. StySBLI	529	TLSEG	NK				
M. XbaIII	529	TLSEG	NK				

Supplementary Fig. 11 | Sequence alignment of PacII_M1, PacII_M2, and different M subunits from classical m6A/m6A Type I R-M systems.

The conserved arginine residue, AGEF/AAEF motif and NPPF/NPPY motif were highlighted in red triangle, blue stars, and green dots, respectively.

Supplementary Table 1. Data collection and refinement statistics.

Crystal	M1M2S-DNA-SAH (7VRU)	M1M2S-DNA(m6A)-SAH (7VS4)
Beam line	SSRF-18U	SSRF-18U
Wavelength (Å)	0.979	0.979
Resolution range (Å)	41.24-2.40 (2.48-2.40)	49.17-2.55 (2.64-2.55)
Space group	P 2 ₁ 2 ₁ 2 ₁	P 2 ₁ 2 ₁ 2 ₁
Unit cell		
a, b, c (Å)	109.8 122.7 131.4	109.1 121.7 129.8
α, β, γ (°)	90 90 90	90 90 90
Total reflections	138658 (13395)	88024 (5361)
Unique reflections	69399 (6698)	56313 (5126)
Completeness (%)	99.29 (97.19)	98.84 (91.10)
Mean I/sigma(I)	17.54 (2.22)	18.54 (2.71)
R-merge	0.086 (0.905)	0.137 (1.048)
R-pim	0.031 (0.247)	0.023 (0.319)
CC1/2	0.998 (0.856)	1 (0.911)
CC*	1 (0.96)	1 (0.977)
Reflections used in refinement	69307 (6698)	56298 (5126)
Reflections used for R-free	3511 (331)	2773 (249)
R-work	0.189 (0.253)	0.184 (0.224)
R-free	0.234 (0.309)	0.240 (0.290)
Number of non-hydrogen atoms	12230	12392
macromolecules	11942	11964
ligands	SAH	SAH
solvent	288	428
Protein residues	1362	1365
RMS (bonds)	0.009	0.008
RMS (angles)	1.04	1.03
Ramachandran favored (%)	96.66	96.82
Ramachandran allowed (%)	3.34	3.18
Ramachandran outliers (%)	0.00	0.00

Statistics for the highest-resolution shell are shown in parentheses.

Supplementary Table 2. Novel m4C/m6A Type I R-M systems used for sequence alignments.

No.	organism	enzyme	Specificity of S subunit	Base Methylation of M subunits
1	<i>Pseudomonas alcaligenes</i>	PacII_M1M2S	5'... C <u>C</u> CNNNNNNR <u>T</u> TGY ... 3'	M1-m6A/M2-m4C
2	<i>Aequorivita sublithicola</i>	Asu14238II_M1M2S	5'... G <u>C</u> CNNNNNNN <u>T</u> CC ... 3'	M1-m6A/M2-m4C
3	<i>Burkholderia thailandensis</i> FDAARGOS_238	Bth238III_M1M2S	5'... C <u>C</u> CNNNNNNC <u>T</u> GG ... 3'	M1-m6A/M2-m4C
4	<i>Citrobacter koseri</i> AR_0025	Cko25III_M1M2S	5'... C <u>C</u> CNNNNNNR <u>T</u> CG ... 3'	M1-m6A/M2-m4C
5	<i>Escherichia coli</i> AR_0077	Eco77I_M1M2S	5'... C <u>C</u> CNNNNNNC <u>T</u> C ... 3'	M1-m6A/M2-m4C
6	<i>Laribacter hongkongensis</i> HLGZ1	LhoZ1I_M1M2S	5'... G <u>C</u> CNNNNNNC <u>T</u> CC ... 3'	M1-m6A/M2-m4C
7	<i>Pseudomonas mendocina</i> S13.2	PmeS132II_M1M2S	5'... C <u>C</u> CNNNNNNN <u>T</u> GCG ... 3'	M1-m6A/M2-m4C
8	<i>Raoultella terrigena</i> R1Gly	RorR1I_M1M2S	5'... C <u>C</u> CNNNNNNN <u>T</u> GAA ... 3'	M1-m6A/M2-m4C
9	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agona 460004 2-1	SenA46II_M1M2S	5'... C <u>C</u> CNNNNNNR <u>T</u> AG ... 3'	M1-m6A/M2-m4C
10	<i>Salinispira pacifica</i> L21-RPul-D2	SpaD2II_M1M2S	5'... G <u>C</u> CNNNNNNN <u>T</u> CG ... 3'	M1-m6A/M2-m4C
11	<i>Vibrio anguillarum</i> CNEVA NB11008	Van11008I_M1M2S	5'... G <u>C</u> CNNNNNNN <u>T</u> GCT ... 3'	M1-m6A/M2-m4C

Supplementary Table 3. Classical Type I R-M systems used for sequence alignments.

No.	organism	enzyme	Specificity of S subunit	Base methylation of M subunits
1	<i>Arenibacter algicola</i> SMS7	AaISMS7III_M ₂ S	5'... GAGNNNNNNNGT _I G ... 3'	M-m6A/M-m6A
2	<i>Escherichia coli</i> 15T-	EcoAI_M ₂ S	5'... GAGNNNNNNNGT _I CA ... 3'	M-m6A/M-m6A
3	<i>Escherichia coli</i> K-12 substr. MG1655	EcoKI_M ₂ S	5'... AACNNNNNNNGT _I GC ... 3'	M-m6A/M-m6A
4	<i>Escherichia coli</i> (R124)	EcoR124I_M ₂ S	5'... GAANNNNNNR _I TCG ... 3'	M-m6A/M-m6A
5	<i>Klebsiella pneumoniae</i> AR_0143	Kpn143III_M ₂ S	5'... ACGNNNNNGT _I TG ... 3'	M-m6A/M-m6A
6	<i>Lelliottia amnigena</i> FDAARGOS_395	Lam395I_M ₂ S	5'... CAAGNNNNNGGT _I ... 3'	M-m6A/M-m6A
7	<i>Pseudomonas aeruginosa</i> AR_0110	Pae0110II_M ₂ S	5'... CACNNNNNNNR _I TGT ... 3'	M-m6A/M-m6A
8	<i>Rhodobaca barguzinensis</i> alga05	Rba05I_M ₂ S	5'... TGGANNNNNNNNT _I TC ... 3'	M-m6A/M-m6A
9	<i>Salmonella enterica</i> serovar blegdam	StySBLI_M ₂ S	5'... GGYANNNNNNTCG ... 3'	M-m6A/M-m6A
10	<i>Xanthomonas badrii</i>	XbaIII_M ₂ S	5'... CCAGNNNNNGGT _I ... 3'	M-m6A/M-m6A