Supplemental materials

Cryo-EM structure of Mcm2-7 double hexamer on DNA suggests a lagging strand DNA extrusion model

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The supplemental materials contain one table, ten figures, and four movie files.

Supplemental movie 1. The modeled atomic structure in cartoon view superimposed on the cryo-EM 3D map rendered in semi-transparent surface view.

Supplemental movie 2. Morphing between the structures of the apo double-hexamer (PDB 3JA8) and double-hexamer-dsDNA.

Supplemental movie 3. Morphing between the structures of the apo double-hexamer (PDB 3JA8) and double-hexamer-dsDNA, focusing on conformational changes of the DNA-binding PS1 and H2I loops. The two conserved residues (KA) are shown as gray spheres.

Supplemental movie 4. Morphing between the Mcm2-7 hexamer structure in the double hexamer bound to dsDNA and the Mcm2-7 hexamer found in the CMG structure on forked ssDNA-dsDNA (PDB ID 5U8S). The structures were aligned based on the C-tier motor rings.

Supplemental Table 1. Cryo-EM data collection and refinement statistics

	DH-dsDNA (EMD-9400, PDB 5BK4)
Data collection	
EM equipment	Titan Krios
Voltage (kV)	300
Detector	Gatan K2
Pixel Size (Å)	1.088
Electron Dose (e^{-} Å^{-2})	60
Underfocus range (μm)	1.5 – 2.5
3D reconstruction	
Software	RELION 2.1
Number of particles used	58,772
Resolution (Å)	3.9
Map-sharpening B factor (Å^2)	-168
Atomic model composition	
Peptide chains	12
Protein residues	7548
dsDNA (base pair)	60
ADP	8
Refinement statistics	
R.m.s deviation	
Bond length	0.005
Bond angle	0.981
Ramachandran plot	
Preferred (%)	89.70
Allowed (%)	9.97
Outlier (%)	0.33
Validation	
MolProbity score	1.95
Good rotamer (%)	96.42
Clashscore, all atoms	5.75

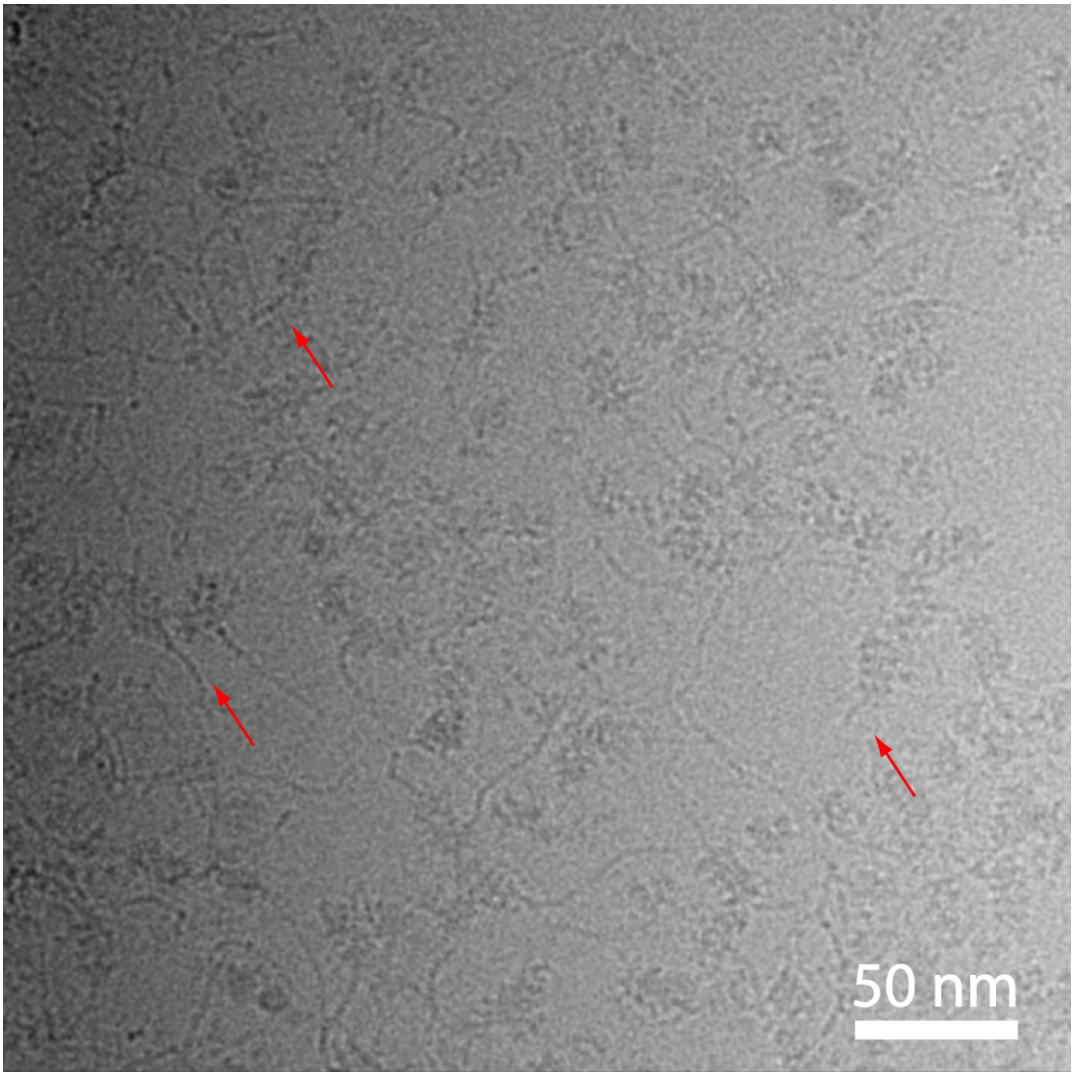


Fig. S1. Raw electron micrograph of double-hexamer-dsDNA particles embedded in vitreous ice. This is an enlarged view of Fig. 1B to better show the dsDNA emerging from many of the double-hexamer particles.

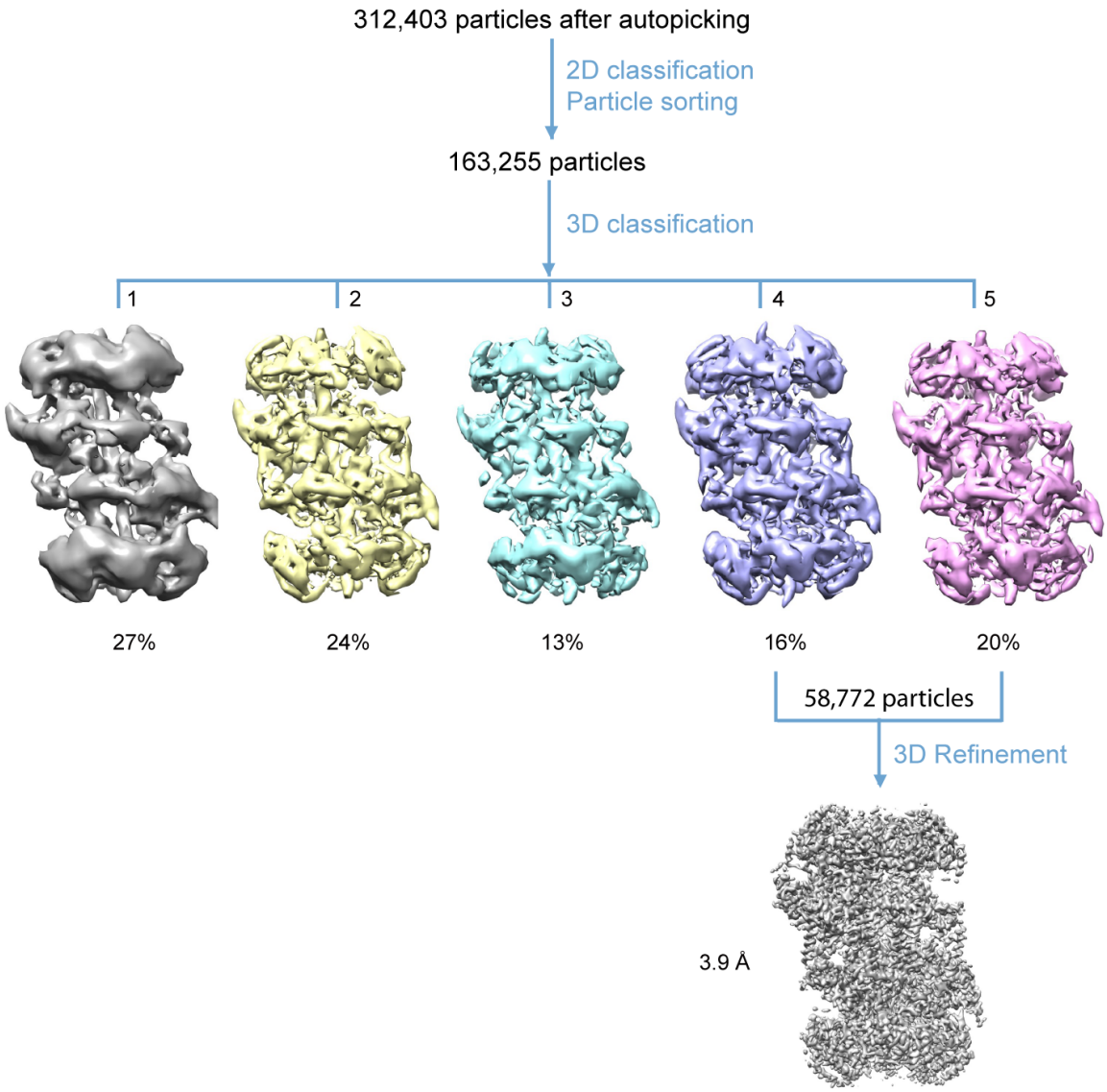


Fig. S2. 3D classification procedure of the cryo-EM images of the *S. cerevisiae* Mcm2-7 double-hexamer loaded *in vitro* on *ARS1*-containing DNA.

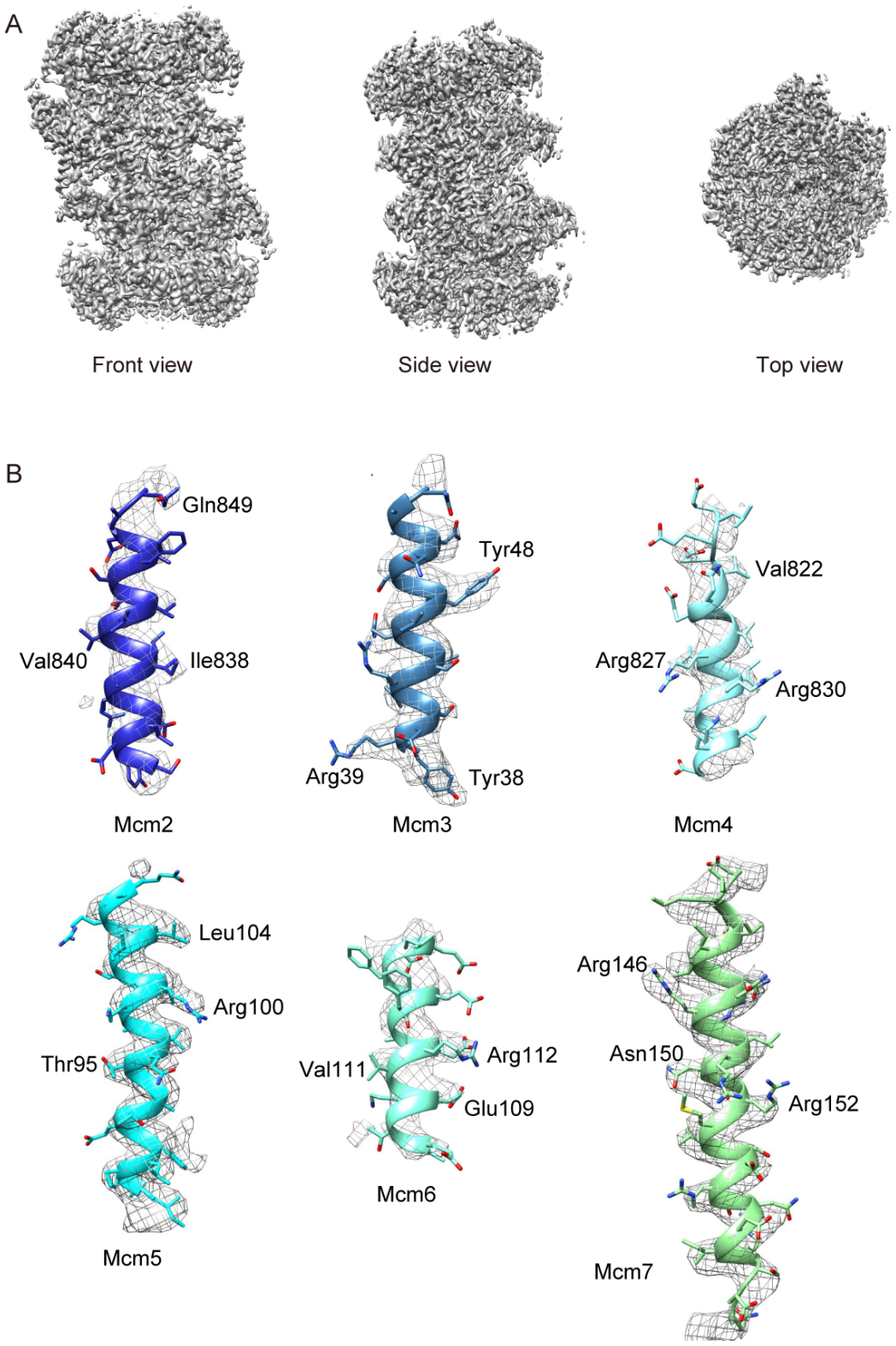


Fig. S3. 3D map of the double-hexamer-dsDNA complex. (A) Surface rendered cryo-EM 3D map of double-hexamer-dsDNA in three orthogonal views. (B) Electron density in mesh view of one α-helix selected from each Mcm subunit, superimposed with the atomic model in cartoon and stick views.

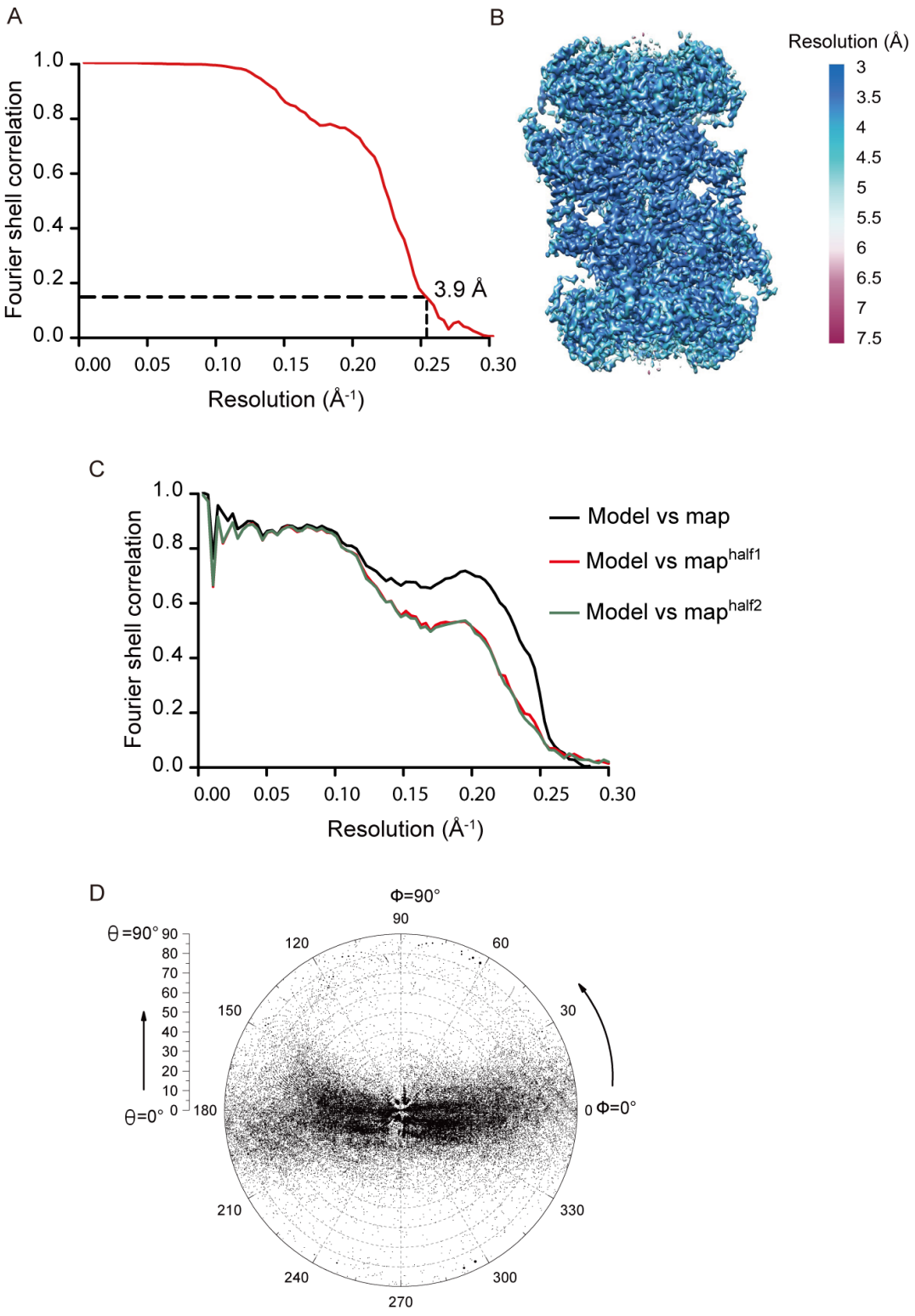


Fig. S4. Characterization of the 3D density map. (A) Gold-standard Fourier shell correction estimation of the resolution of the 3D map. (B) Color-coded local resolution map of the 3D map. (C) Correlation curves of the atomic model vs the final map as well as the two half maps. (D) Euler angle distribution of the raw particles used in the final reconstruction.

Fig. S5. The *S. cerevisiae* Mcm5 contains a unique sequence inserted into the N-tier Zn finger domain. (A) ZFI is present only in Mcm5, but not in any other subunits of the yeast Mcm2-7. Sequence alignment was performed by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). (B) The Mcm5-ZFI is predicted to be disordered by the online server JPred4 (http://www.compbio.dundee.ac.uk/jpred/). "E": β-stand, "-": loop. (C) Alignment of the Mcm5 sequences of yeast, fly, and human by the online server MultAlin (http://multalin.toulouse.inra.fr/multalin/). The yellow square box marks the Mcm5 ZFI region.

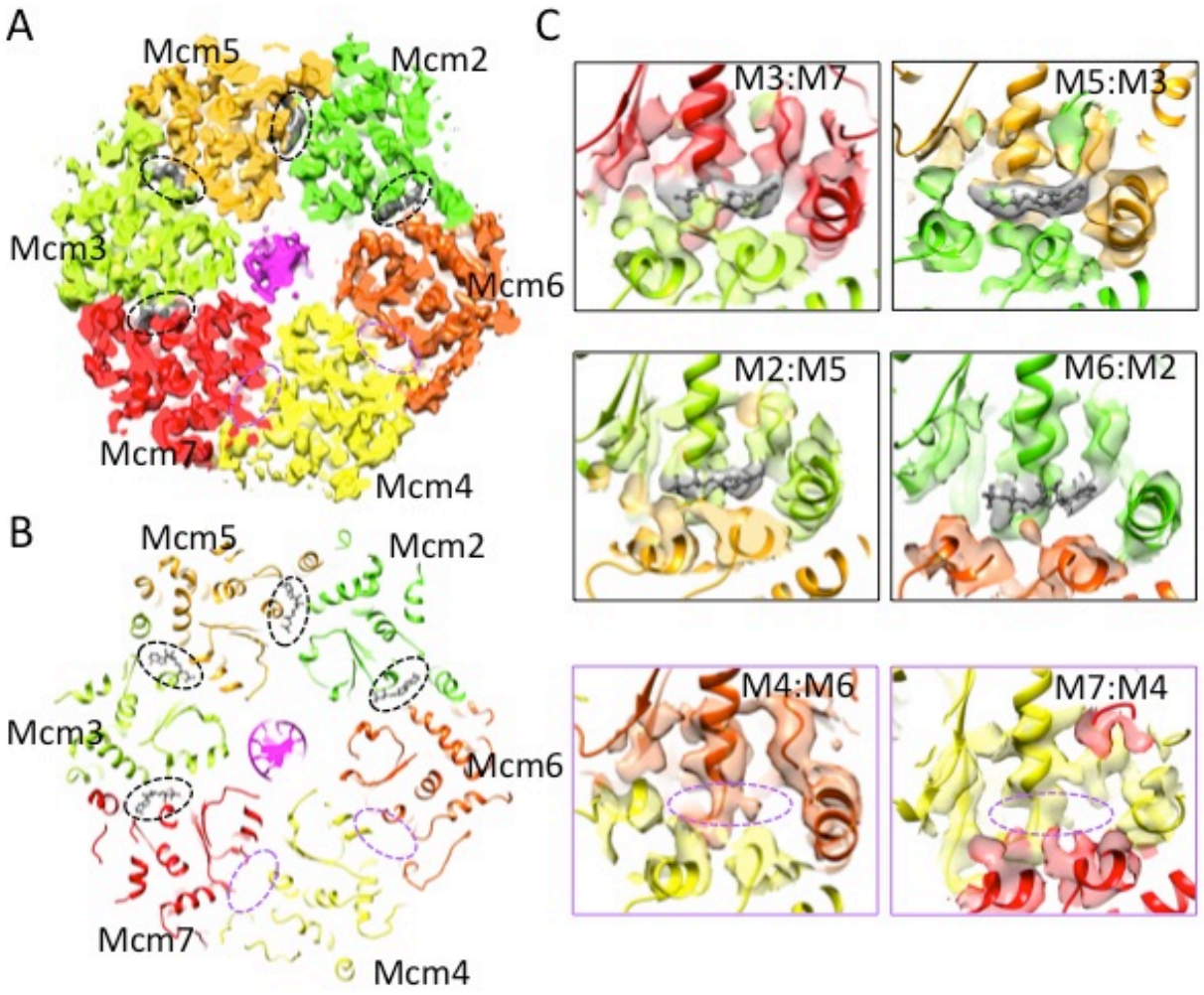


Fig. S6. ATP site occupancy in the double-hexamer-dsDNA structure. (A) An axial section of the 3D density map at the nucleotide sites showing the presence of the nucleotide densities at four out of six sites. The occupied sites are marked by dashed ovals, and the empty sites by dashed pink ovals. (B) A section of the double-hexamer-dsDNA structure at the same axial position as in panel (A). (C) Zoomed views of the six nucleotide binding sites. Note the nucleotide density at the Mcm6:Mcm2 interface is weaker than other three occupied sites.

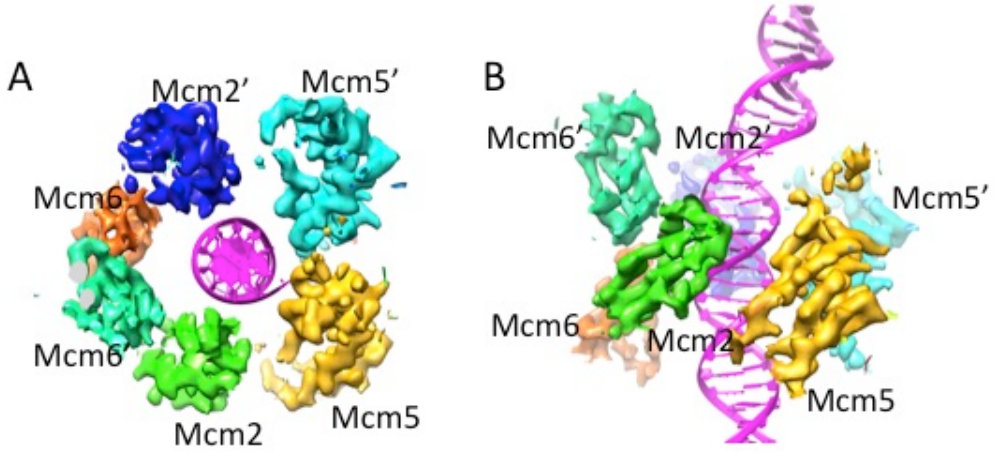
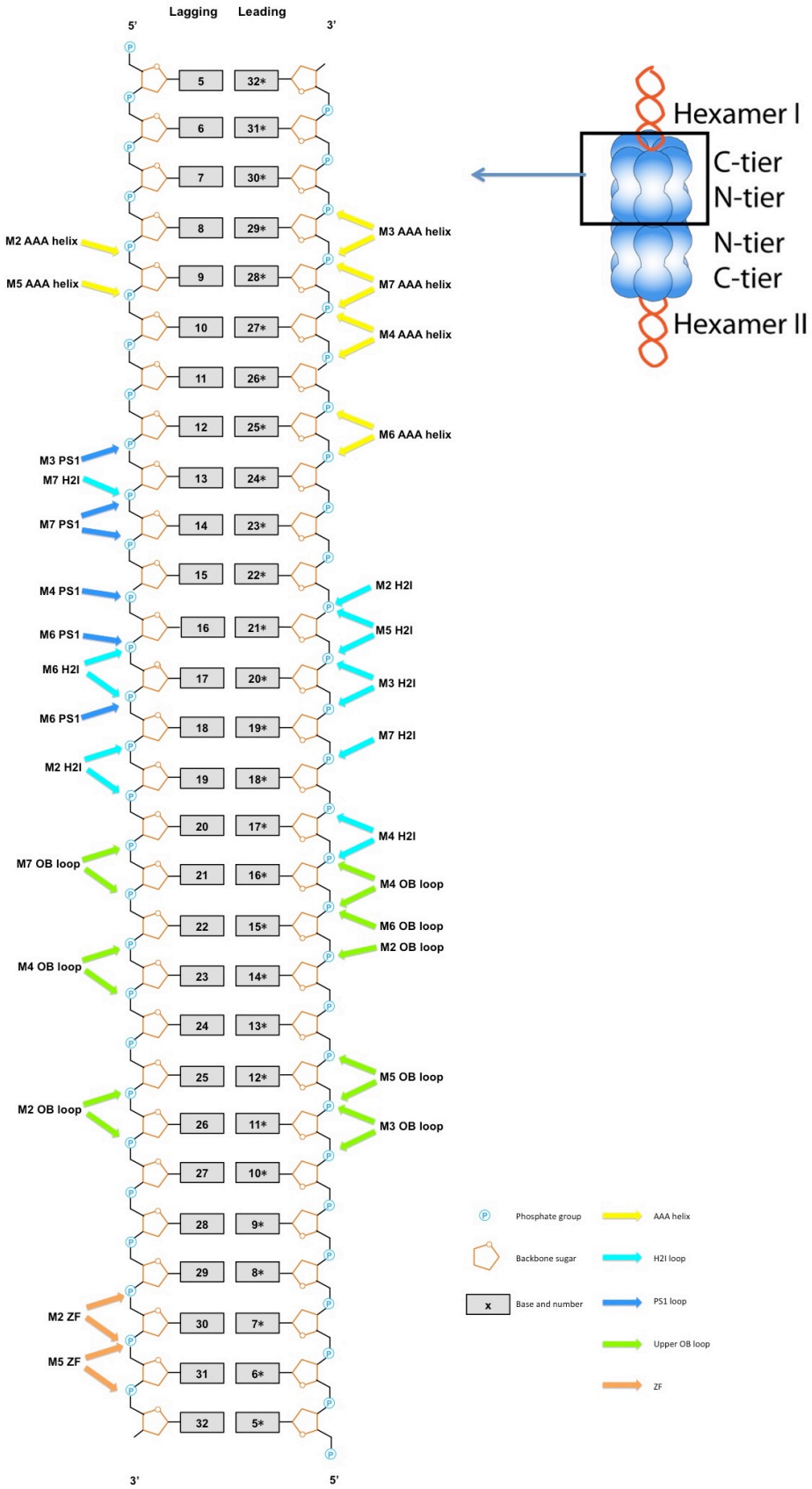


Fig. S7. DNA-double-hexamer interaction at the interface region. (A-B) Top and side views of the middle interface region of the double-hexamer. Six ZnF domains of Mcm2, Mcm2', Mcm5, Mcm5', Mcm6, and Mcm6' surround the dsDNA.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M4 AAA helix
M6 AAA helix
M3 PS1
M7 H2I
M7 PS1
M4 PS1
M6 PS1
M6 H2I
M6 PS1
M2 H2I
M7 OB loop
M4 OB loop
M2 ZF
M5 ZF
M2 H2I
M5 H2I
M3 H2I
M7 H2I
M4 H2I
M4 OB loop
M6 OB loop
M2 OB loop
M5 OB loop
M3 OB loop
3'
5'
Hexamer I
C-tier
N-tier
N-tier
C-tier
Hexamer II
Phosphate group
Backbone sugar
Base and number
AAA helix
H2I loop
PS1 loop
Upper OB loop
ZF

Fig. S8. A sketch of detailed DNA-protein interactions in the double-hexamer-dsDNA structure. Interactions are identified by the online server COCOMAPS (https://www.molnac.unisa.it/BioTools/cocomaps/).

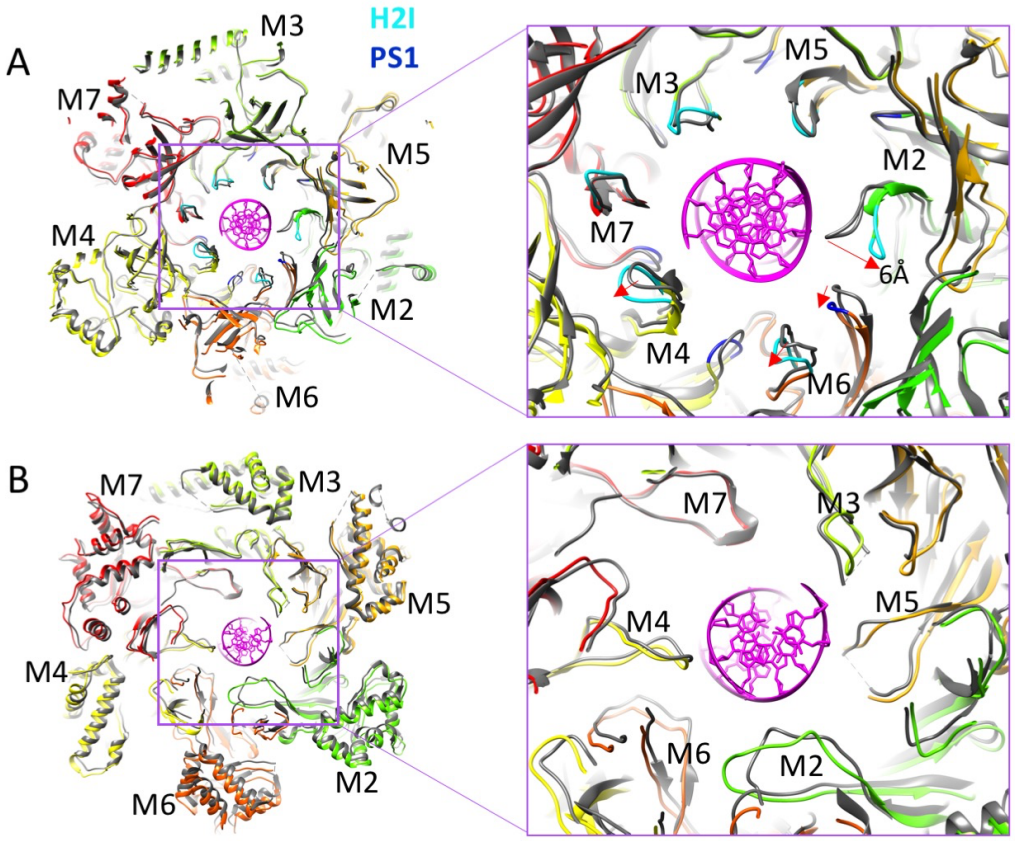

Fig. S9. Structural alignment of the apo double-hexamer and double-hexamer-dsDNA. (A) Alignment at the DNA-interaction PS1 and H2I regions in the C-tier AAA+ domains, revealing outward movement of the PS1 loops to accommodate the dsDNA inside the central channel. The moved distances are shown by red arrows. (B) Alignment at the N-tier upper OB insertion loop region. The structure of apo double-hexamer is in grey. Alignment was based on all atoms.

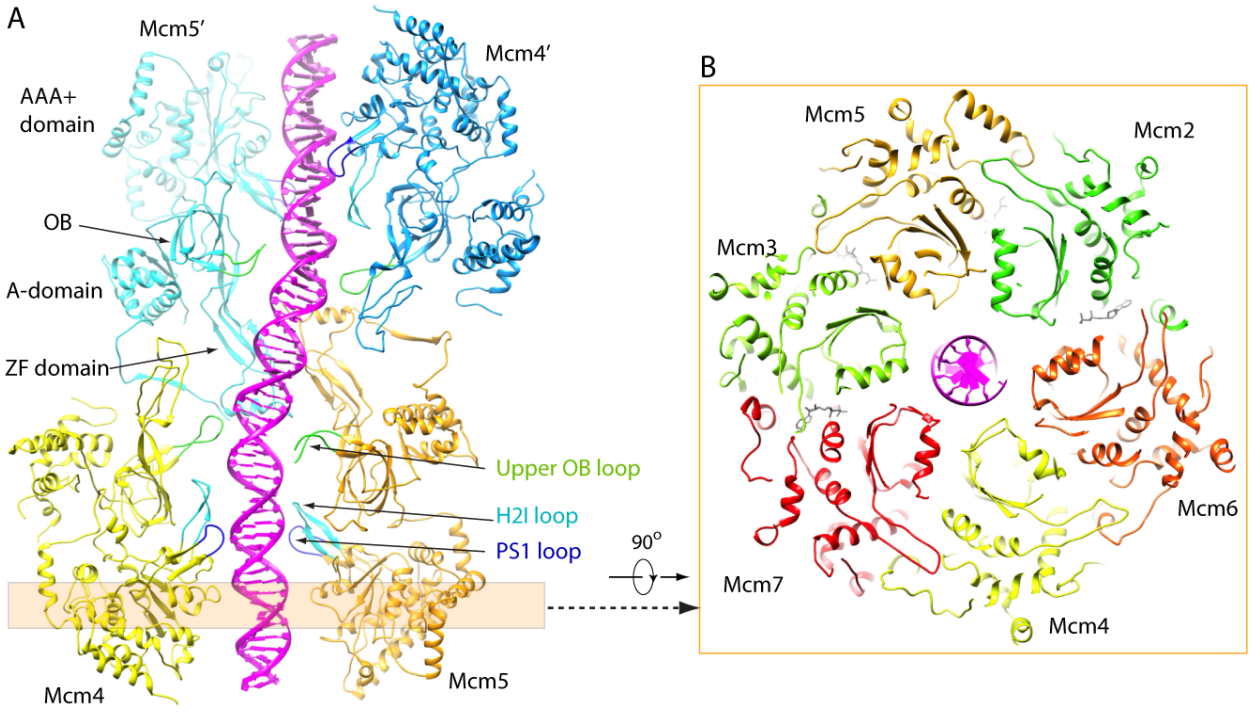

Fig. S10. The DNA interacting α-helix in the AAA+ domain of Mcm2-7 hexamer, in addition to the H2I and PS1 loops. (A) A cut-open view of the double-hexamer-dsDNA, showing only two subunits in each hexamer (Mcm4 and Mcm5). Protein domains such ZnF, OB, and A-domains are labeled. The bottom orange strip highlights an α-helix in the AAA+ domain that interacts with dsDNA. (B) An axial view of the DNA-interacting helices in the six AAA+ domains of the Mcm2-7 hexamer.