

Supplementary Figures and Information of the Manuscript:

Structural Basis for DNA Break Sensing by Human MRE11-RAD50-NBS1 and its Regulation by Telomeric Factor TRF2

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Christophe Jung¹, Karl-Peter Hopfner^{1,*}

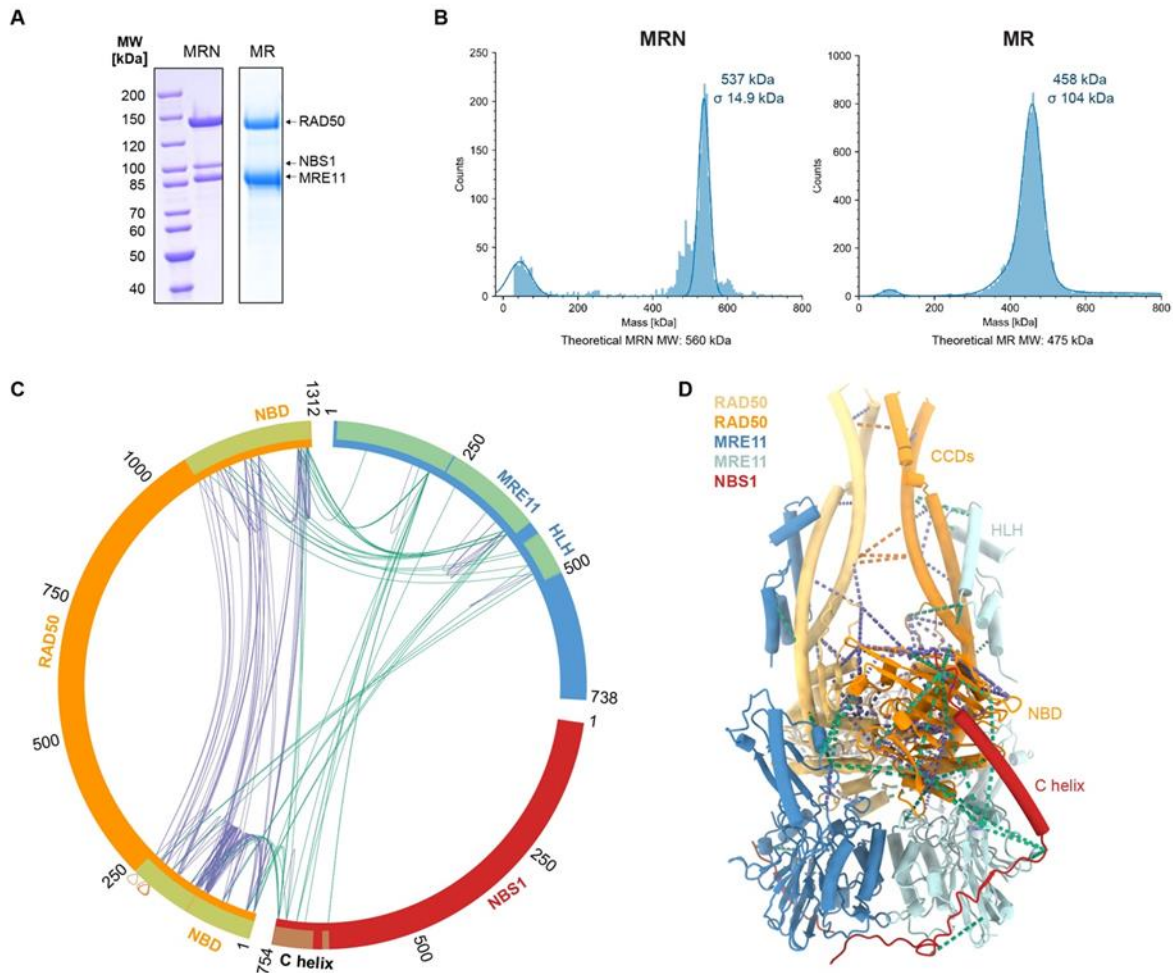
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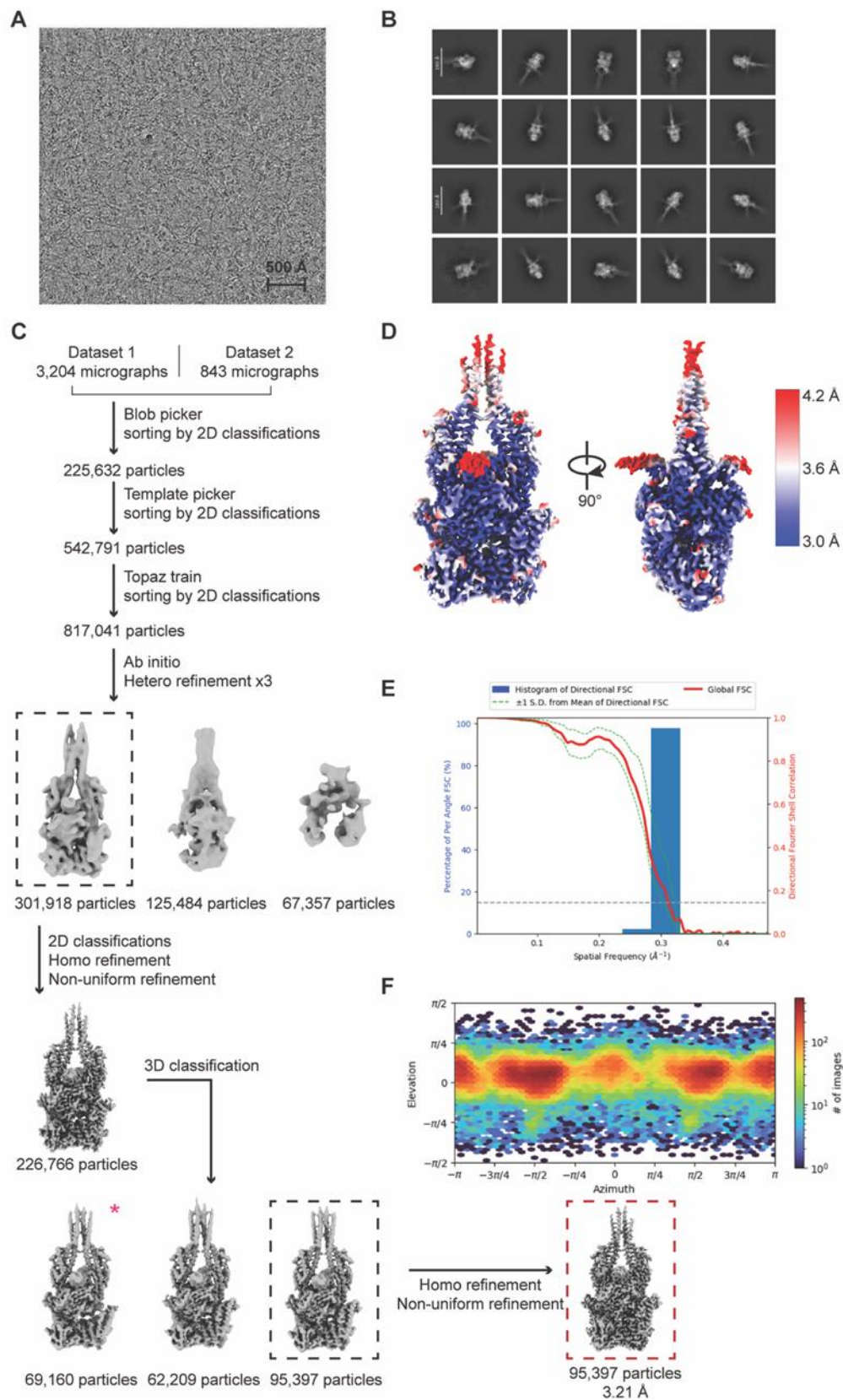
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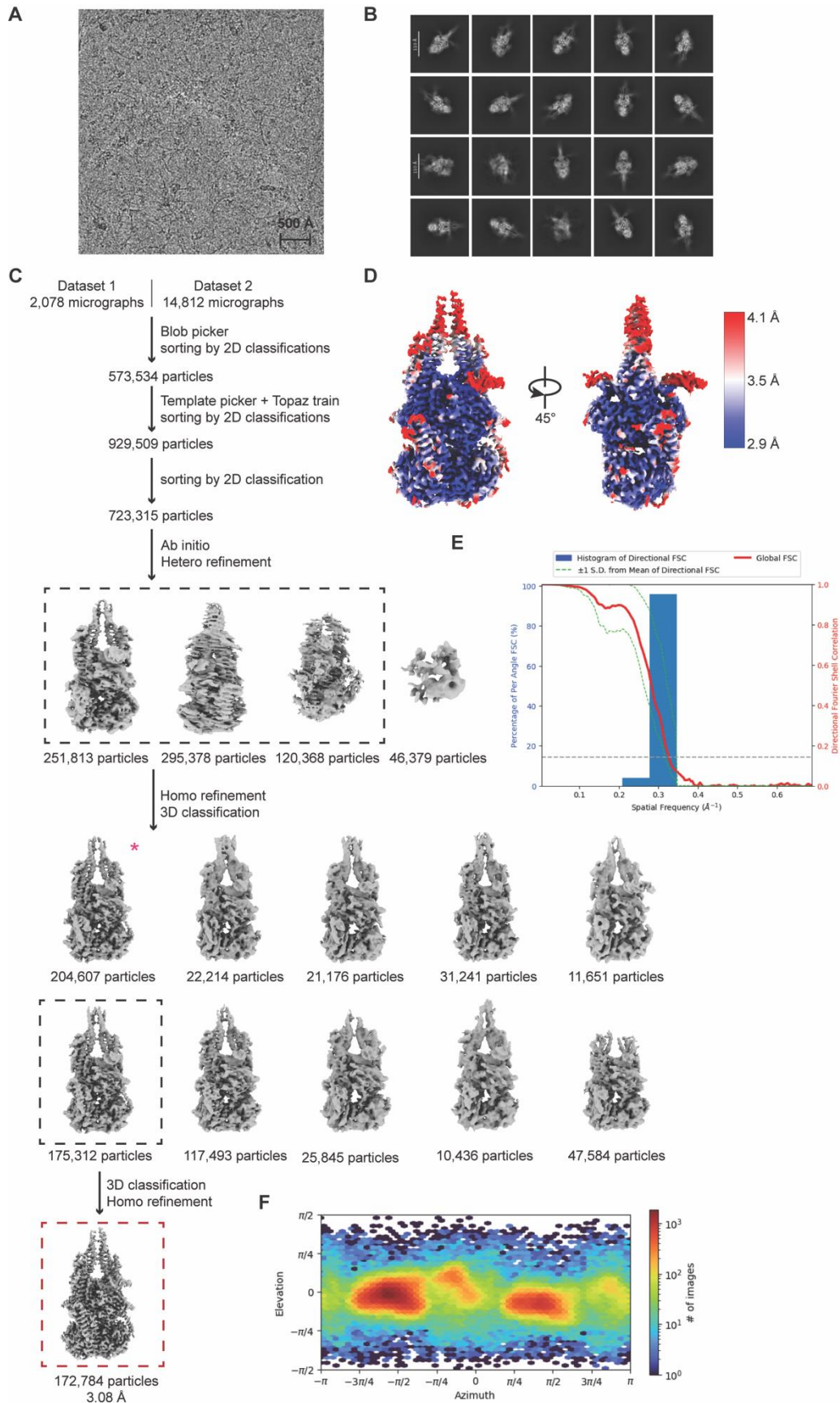
Supplementary Figures



Supplementary Fig. 1: MR(N) complex and chemical cross-linking coupled with mass spectrometry. **A**, Representative SDS-PAGE analysis of purified MR/MRN complexes after gel filtration. **B**, Mass photometry histograms of the MR/MRN complexes (50 nM). **C**, XiVIEW-generated circular crosslinking map of the MRE11, RAD50, NBS1 subunits. The False Discovery Rate (FDR) was set to 1% and the crosslinking distance <30 Å. The PDB annotated regions in each subunit are highlighted in light green. Purple and green lines denote self and heteromeric crosslinks, respectively. **D**, Crosslinks mapped onto the MRN-DNA structure. DNA is not shown for better visual representation. Crosslinking-mass spectrometry data are available via ProteomeXchange with identifier PXD067324. Source data for **A** and **C** are provided as a Source Data file.

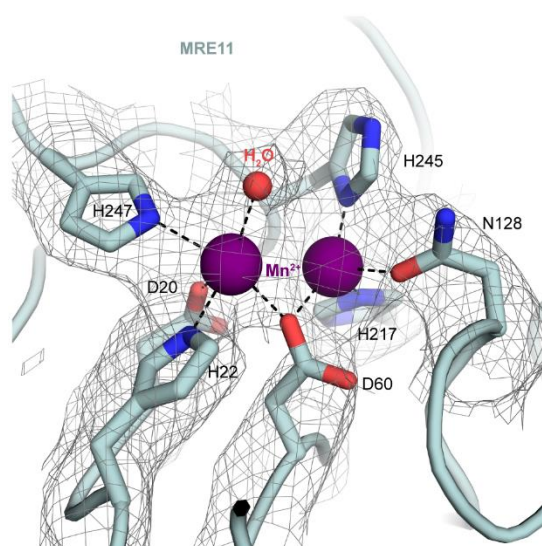


Supplementary Fig. 2: Cryo-EM data analysis of MR-DNA. **A**, Representative micrograph of MR-DNA among 4047 collected movies. **B**, Representative 2D classes of the particles used for the final MR-DNA reconstruction. **C**, Cryo-EM data processing workflow of MR-DNA using cryoSPARC¹. Pink asterisk denotes the class with MRE11 detached from RAD50^{NBD}. **D**, Local resolution visualisation of MR-DNA calculated in cryoSPARC. Blue indicates higher resolution, red indicates lower resolution. **E**, Histogram of directional Fourier shell correlation (FSC)² (blue) and global FSC curve (red) of the final MR-DNA reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 3.21Å. **F**, Angular distribution of the particles used for final MR-DNA reconstruction.

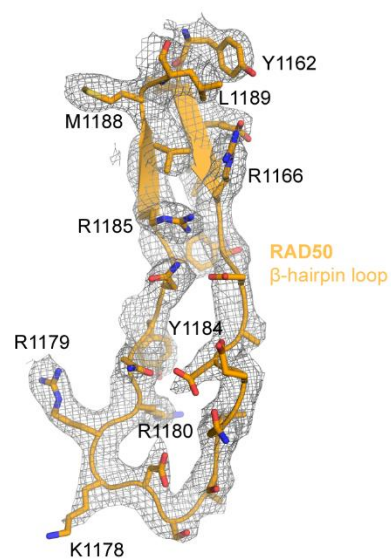


Supplementary Fig. 3: Cryo-EM data analysis of MRN-DNA. **A**, Representative micrograph of MRN-DNA among 16,890 collected movies. **B**, Representative 2D classes of the particles used for the final MRN-DNA reconstruction. **C**, Cryo-EM data processing workflow of MRN-DNA using cryoSPARC¹. Pink asterisk denotes the class with MRE11 detached from RAD50^{NBD}. **D**, Local resolution visualisation of MRN-DNA calculated in cryoSPARC. Blue indicates higher resolution, red indicates lower resolution. **E**, Histogram of directional FSC² (blue) and global FSC curve (red) of the final MRN-DNA reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 3.08Å. **F**, Angular distribution of the particles used for final MRN-DNA reconstruction.

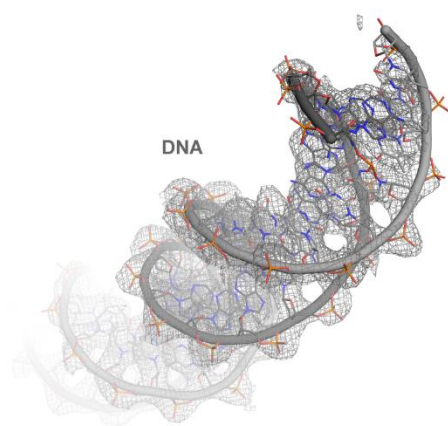
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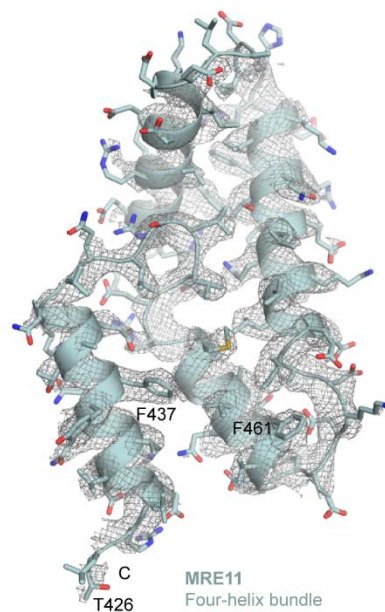
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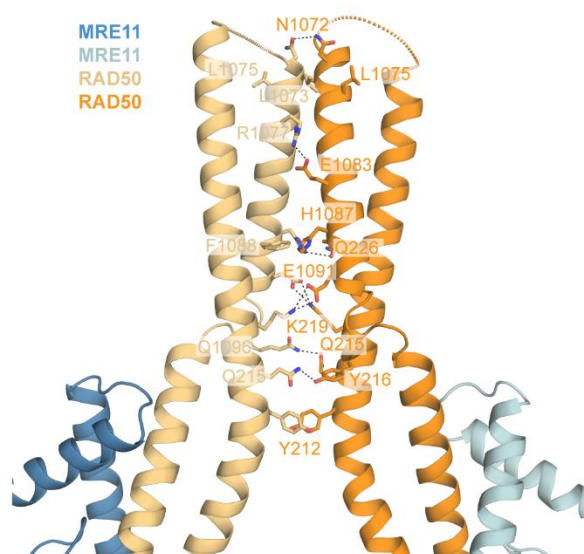
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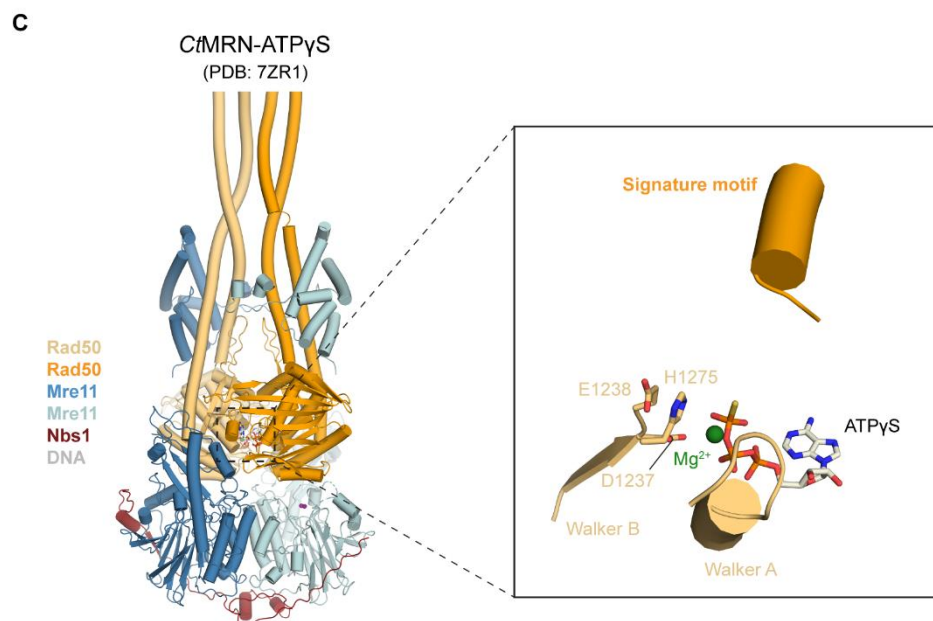
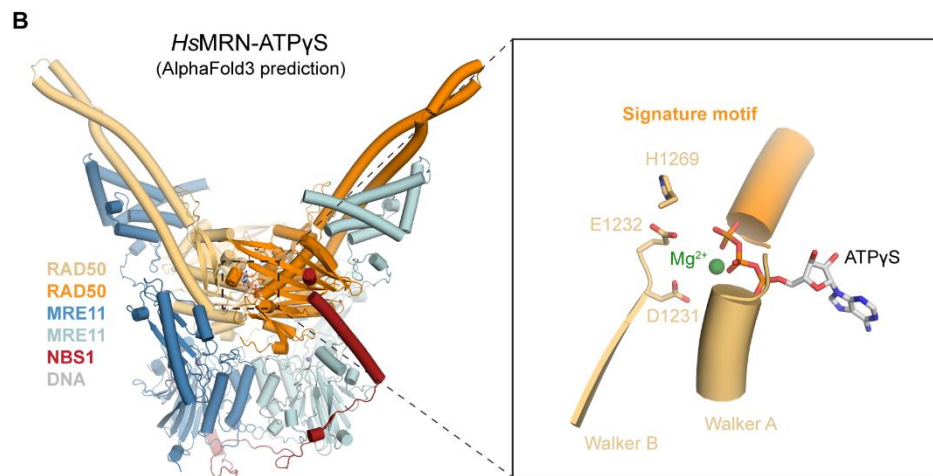
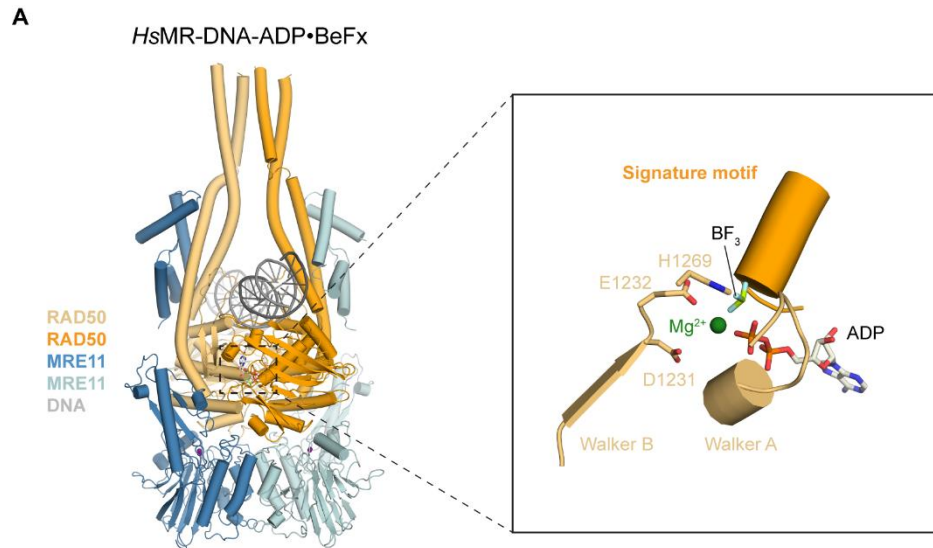
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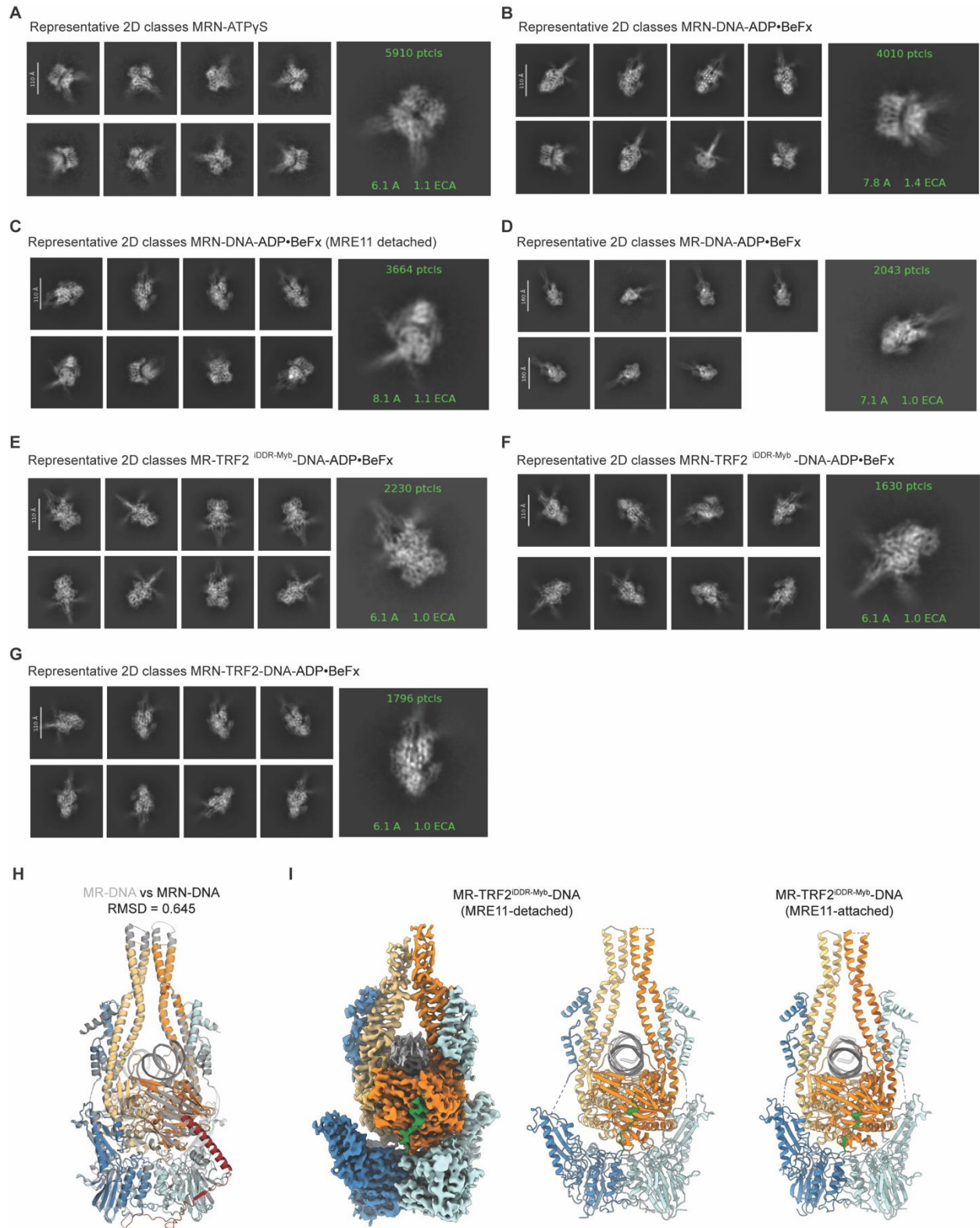
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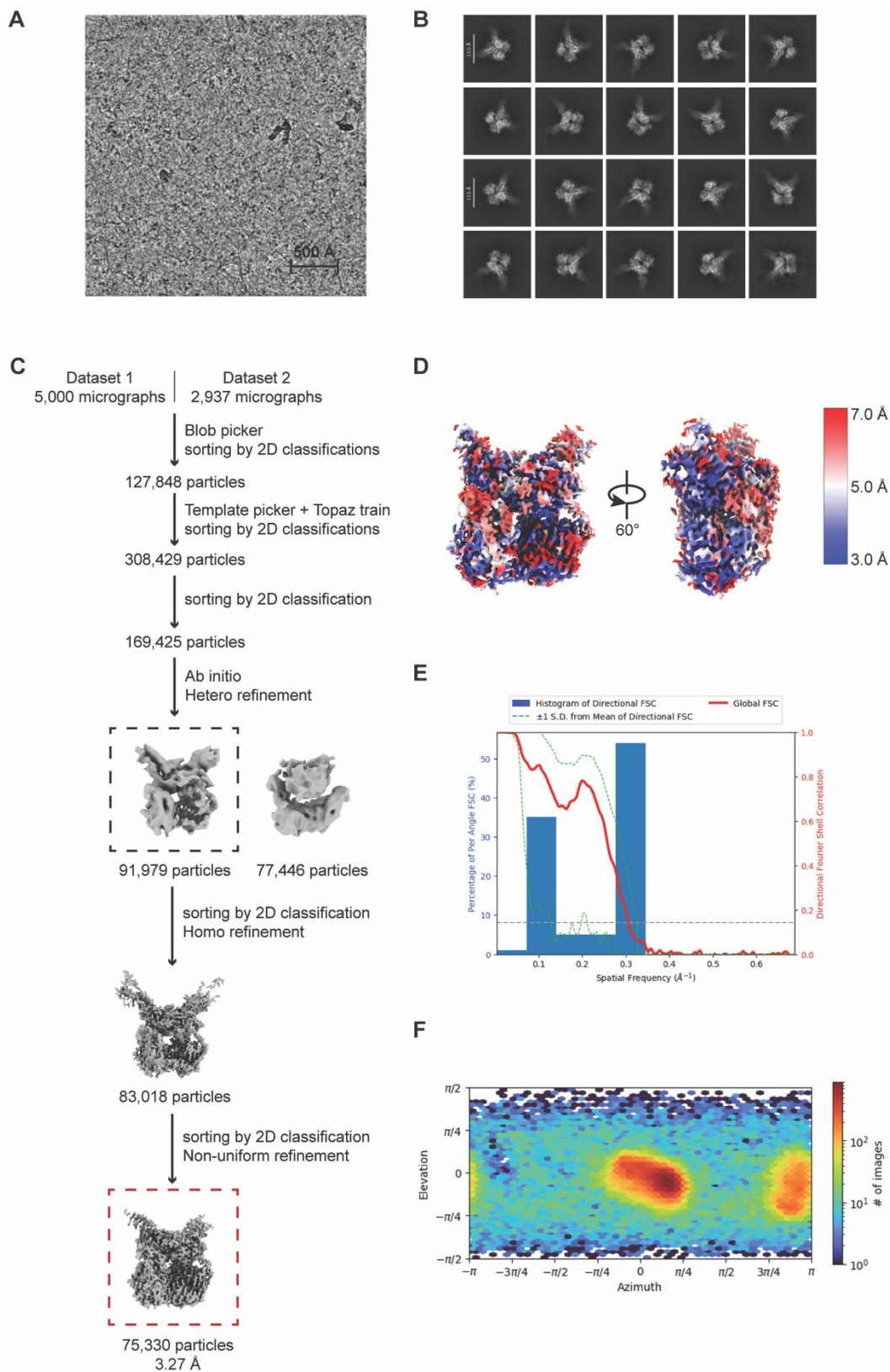
Supplementary Fig. 4. Quality of cryo-EM reconstructions. A-E, Densities for various parts of MR-TRF2^{iDDR-Myb}-DNA complex as indicated. Interface of RAD50 CCDs with interacting residues shown as sticks and labeled.



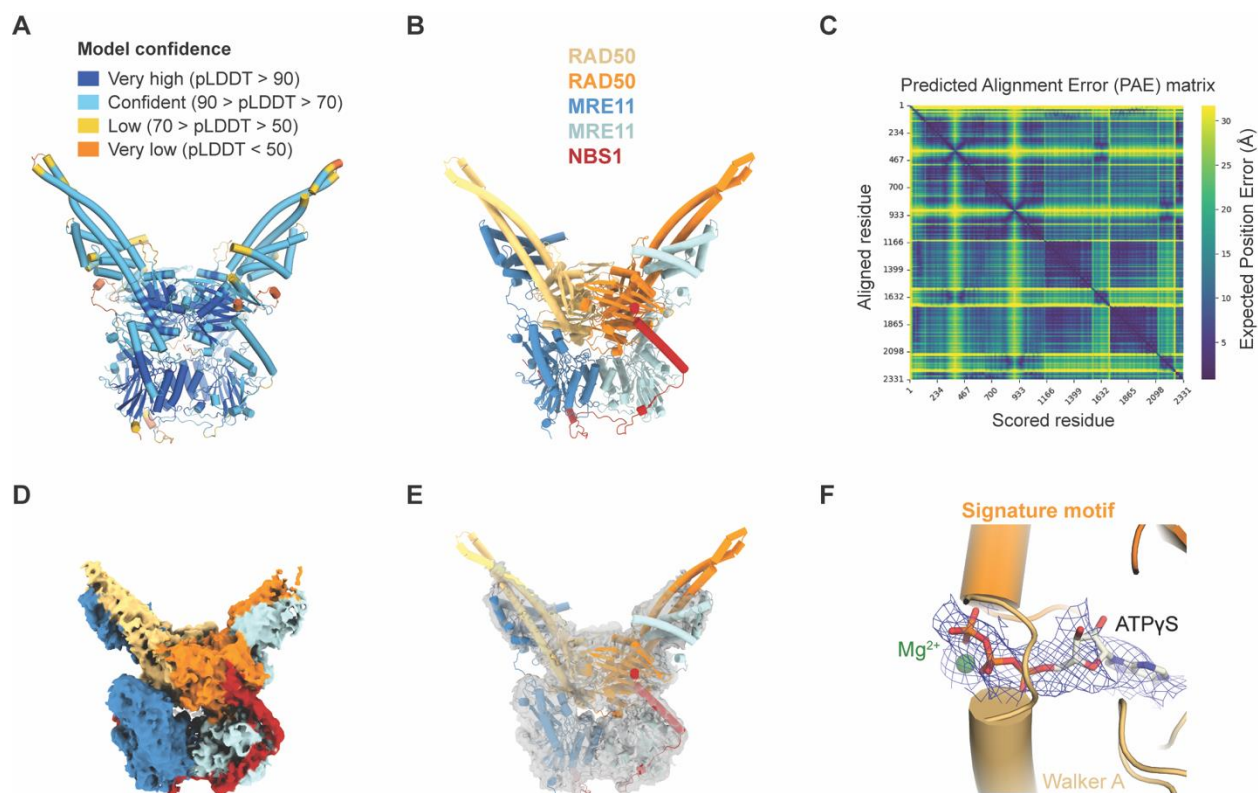
Supplementary Fig. 5. Structural comparison of nucleotide binding pockets in different MR(N) structures. A-C, Side-by-side comparison of MR(N) structures, emphasizing the unique structural arrangement of the signature motif surrounding RAD50's nucleotide-binding site in **A**, *Hs*MR-DNA-ADP•BeF_x (current study), **B**, *Hs*MRN-ATPyS (current study) and **C**, *Ct*MRN-ATPyS (PDB: 7ZR1). Corresponding subunits were colored consistently. *Hs*: *Homo sapiens*, *Ct*: *Chaetomium thermophilum*.



Supplementary Fig. 6. MR/MRN 2D classes. **A**, Representative 2D classes obtained for MRN with ATP γ S. **B**, Representative 2D classes obtained for MRN with DNA and ADP•BeF $_x$. The complex displayed a heterogeneity with open and closed CCDs, as well as asymmetrically bound MRE11 dimers. **C-G**, Representative 2D classes of MRE11 dimer detached as a rigid body from one RAD50^{NBD}. **H**, Structural superimposition of MR-DNA with MRN-DNA complex. MR-DNA structure was colored in grey for simplicity, while MRN-DNA structure was colored consistently in the manuscript. **I**, Overview of the MR-TRF2^{iDDR-Myb}-DNA complex structure with one partially detached MRE11 subunit (left two panels) and the side-by-side comparison to the fully attached MRE11 complex structure (rightmost panel). High resolution cryo-EM map of the partially detached MRE11 complex structure was displayed on the leftmost panel.

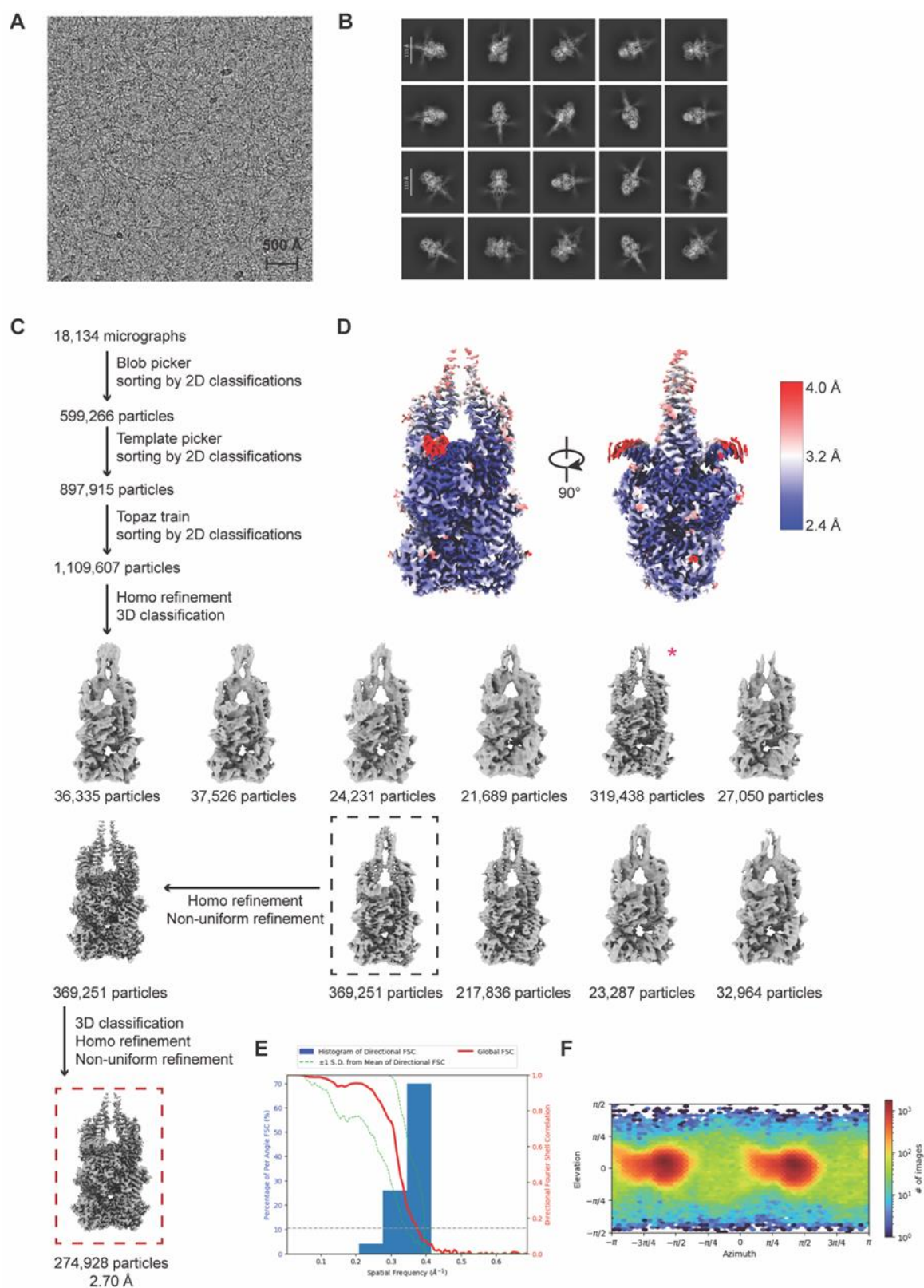


Supplementary Fig. 7: Cryo-EM data analysis of MRN-ATPyS. **A**, Representative micrograph of MRN-ATPyS among 7,937 collected movies. **B**, Representative 2D classes of the particles used for the final MRN-ATPyS reconstruction. **C**, Cryo-EM data processing workflow of MRN-ATPyS using cryoSPARC¹. **D**, Local resolution visualization of MRN-ATPyS calculated in cryoSPARC. Blue indicates higher resolution, while red indicates lower resolution. **E**, Histogram of directional Fourier shell correlation (FSC)² (blue) and global FSC curve (red) of the final MRN-ATPyS reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 3.27 Å. **F**, Angular distribution of the particles used for final MRN-ATPyS reconstruction.

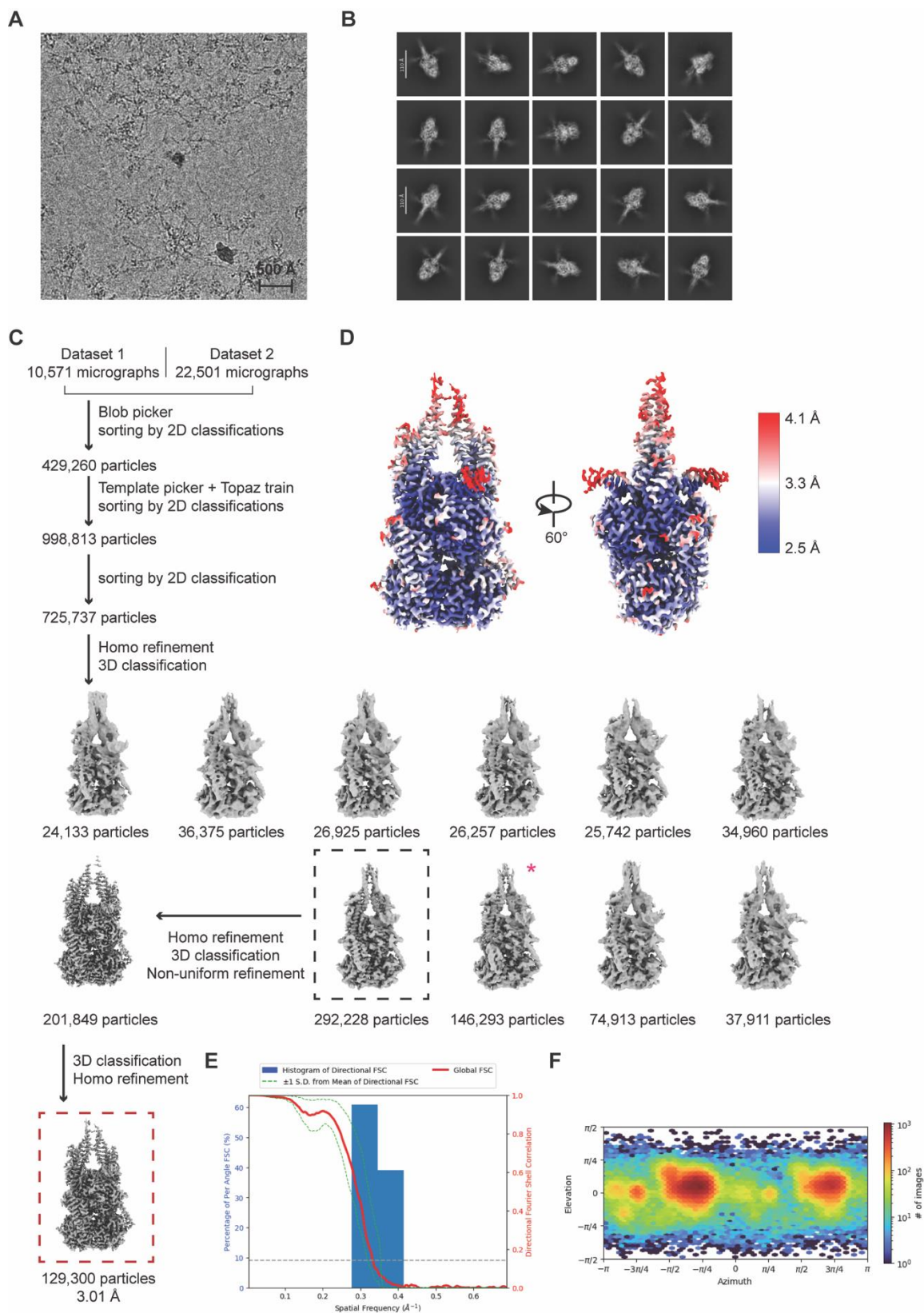


Supplementary Fig. 8: AlphaFold3 prediction of MRN-ATP fitting into map of MRN-ATPyS.

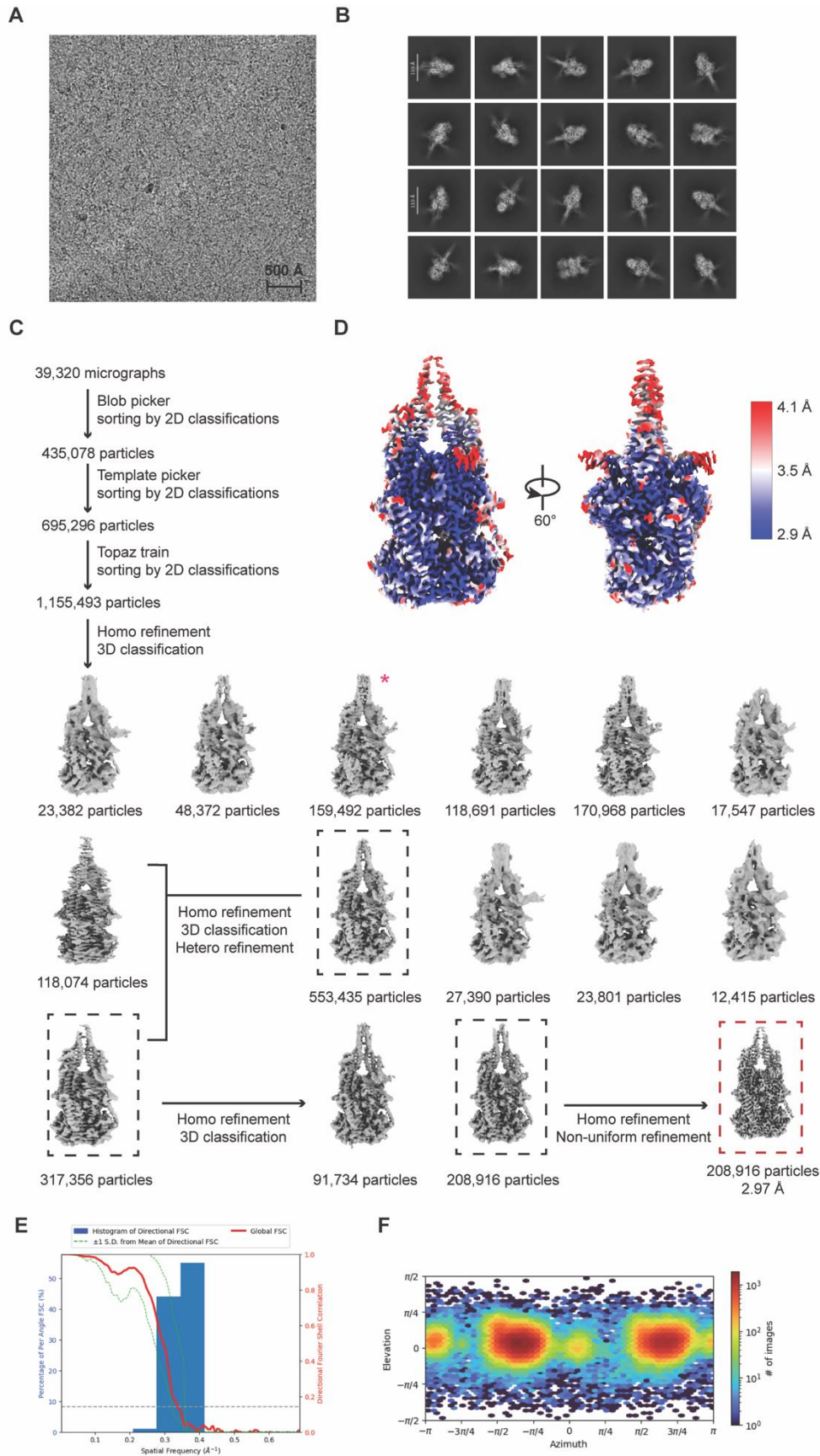
A, AlphaFold3-predicted model of MRN-ATP colored based on pLDDT values. **B**, The same prediction colored by chain. **C**, Predicted Alignment Error (PAE) matrix of the model; darker color represents higher confidence. **D**, The cryo-EM map of MRN-ATPyS at 3.27 Å. **E**, Rigid body fitting of the AlphaFold3-predicted MRN-ATP model into the refined 3D construction map using ChimeraX³. **F**, Densities for ATP γ S and magnesium ion in the nucleotide binding pocket of RAD50.



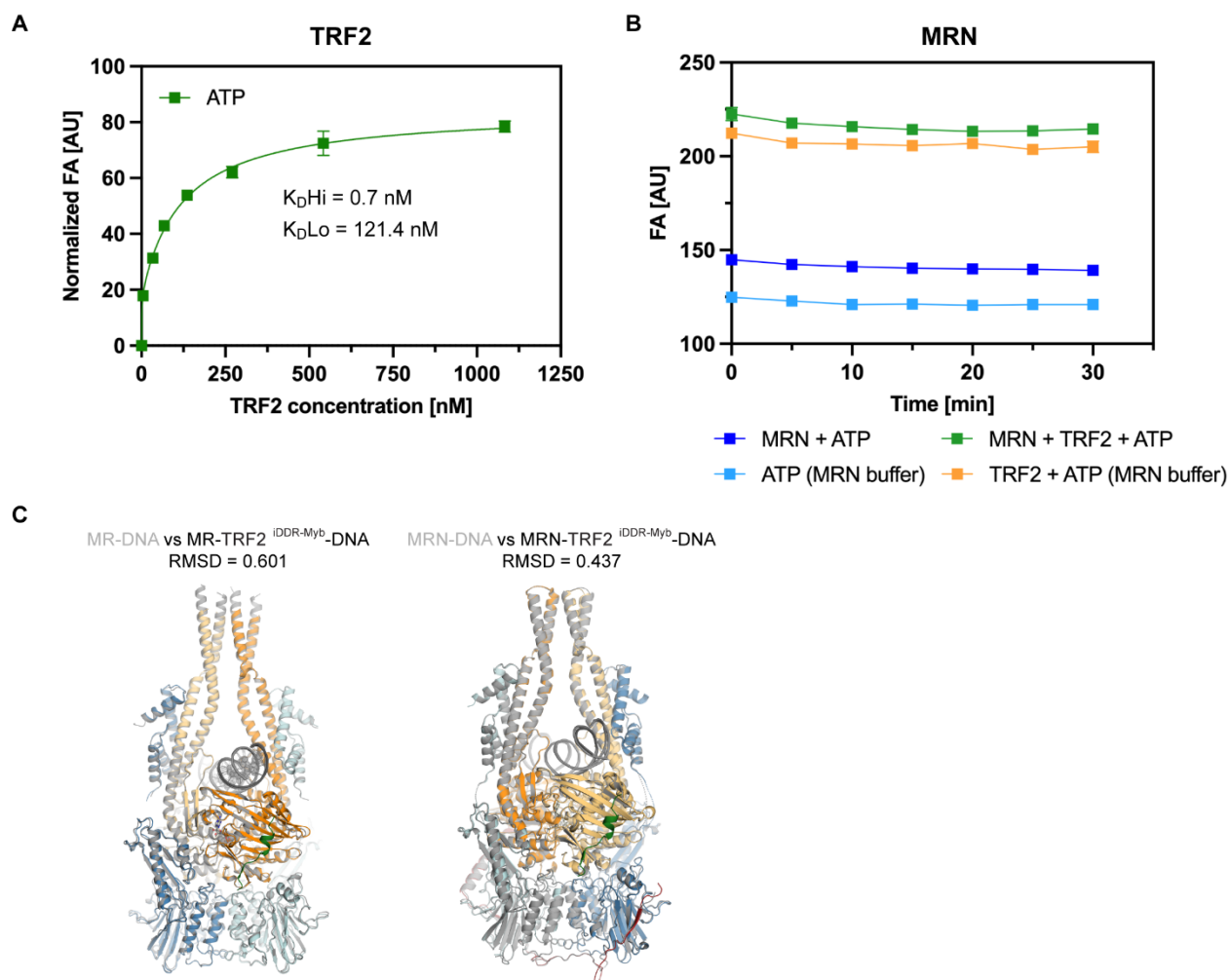
Supplementary Fig. 9: Cryo-EM data analysis of MR-TRF2^{iDDR-Myb}-DNA. **A**, Representative micrograph of MR-TRF2^{iDDR-Myb}-DNA among 18,134 collected movies. **B**, Representative 2D classes of the particles used for the final MR-TRF2^{iDDR-Myb}-DNA reconstruction. **C**, Cryo-EM data processing workflow of MR-TRF2^{iDDR-Myb}-DNA using cryoSPARC¹. Pink asterisk denotes a well-resolved class with MRE11 detached from RAD50^{NBD}. **D**, Local resolution visualisation of MR-TRF2^{iDDR-Myb}-DNA calculated in cryoSPARC. Blue indicates higher resolution, red indicates lower resolution. **E**, Histogram of directional Fourier shell correlation (FSC)² (blue) and global FSC curve (red) of the final MR-TRF2^{iDDR-Myb}-DNA reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 2.7Å. **F**, Angular distribution of the particles used for final MR-TRF2^{iDDR-Myb}-DNA reconstruction.



Supplementary Fig. 10: Cryo-EM data analysis of MRN-TRF2^{iDDR-Myb}-DNA. **A**, Representative micrograph of MRN-TRF2^{iDDR-Myb}-DNA among 33,072 collected movies. **B**, Representative 2D classes of the particles used for the final MRN-TRF2^{iDDR-Myb}-DNA reconstruction. **C**, Cryo-EM data processing workflow of MRN-TRF2^{iDDR-Myb}-DNA using cryoSPARC¹. Pink asterisk denotes a well-resolved class with MRE11 detached from RAD50^{NBD}. **D**, Local resolution visualisation of MRN-TRF2^{iDDR-Myb}-DNA calculated in cryoSPARC. Blue indicates higher resolution, red indicates lower resolution. **E**, Histogram of directional Fourier shell correlation (FSC)² (blue) and global FSC curve (red) of the final MRN-TRF2^{iDDR-Myb}-DNA reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 3.01Å. **F**, Angular distribution of the particles used for final MRN-TRF2^{iDDR-Myb}-DNA reconstruction.



Supplementary Fig. 11: Cryo-EM data analysis of MRN-TRF2-DNA. **A**, Representative micrograph of MRN-TRF2-DNA among 39,320 collected movies. **B**, Representative 2D classes of the particles used for the final MRN-TRF2-DNA reconstruction. **C**, Cryo-EM data processing workflow of MRN-TRF2-DNA using cryoSPARC¹. Pink asterisk denotes a well-resolved class with MRE11 detached from RAD50^{NBD}. **D**, Local resolution visualisation of MRN-TRF2-DNA calculated in cryoSPARC. Blue indicates higher resolution, red indicates lower resolution. **E**, Histogram of directional Fourier shell correlation (FSC)² (blue) and global FSC curve (red) of the final MRN-TRF2-DNA reconstruction. The spread of directional resolution values (green dashed lines) is defined as $\pm 1\sigma$. The grey dashed line shows the 0.143 cut-off criterion, indicating a nominal resolution of 2.97Å. **F**, Angular distribution of the particles used for final MRN-TRF2-DNA reconstruction.



Supplementary Fig. 12: Combined results of binding assays and structural comparison of MR(N)-DNA-(TRF2^{iDDR-Myb}) complexes. **A**, Fluorescence anisotropy (FA)-based assay assessing full length TRF2 binding to telomeric repeat containing 64-mer DNA template in the presence of ATP. Data are presented as mean values $\pm$ SD from three technical replicates. K_D value was derived by fitting the anisotropy data to two specific binding sites with Hill slope model in Prism (GraphPad). **B**, FA signals of each titration condition kept relatively stable over the measurement time period. A concentration of 300 nM MRN and/or 1.6 μ M TRF2 were used where indicated. Data are presented as mean values $\pm$ SD from three technical replicates. **C**, Structural superimposition of MR(N)-DNA with MR(N)-TRF2^{iDDR-Myb}-DNA complexes. MR(N)-DNA structures were colored in grey for simplicity, while MR(N)-TRF2^{iDDR-Myb}-DNA complex structures were colored consistently in the manuscript. Source data for **A** and **B** are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1: Chemicals and Reagents

Chemicals and Reagents	Source	Identifier
ATP (99%)	Sigma-Aldrich (Merck)	Cat# A3377 CAS 34369-07-8
ATP γ S	Sigma-Aldrich (Merck)	Cat# A1388 CAS
n-Octyl- β -D-Glucopyranosid (β -OG)	Carl Roth	Cat# CN23.3
BS3 (bis(sulfosuccinimidyl)suberate)	ThermoFisher	Cat #A39266
BeF ₂ (Beryllium fluoride)	Santa Cruz Biotechnology	Cat sc-263025 #CAS 7787-49-7
BSA (recombinant albumin)	New England BioLabs	Cat# B9200S
Chloramphenicol	Carl Roth	Cat# Chloramphenicol CAS 56-75-7
DTT	Carl Roth	Cat# 6908
EDTA	VWR International	Cat# 1084541000 CAS 6381-92-6
FLAG M2 affinity resin	Sigma-Aldrich (Merck)	Cat# A2220
3x FLAG peptide	TargetMol	Cat# TP1274 CAS 402750-12-3
Glycerol ROTIPURAN® $\geq 99,5$ %, p.a., water free	Carl Roth	Cat# 3783 CAS 56-81-5
HEPES PUFFERAN® MIN. 99,5% P.A.	Carl Roth	Cat# 9105 CAS 7365-45-9
Imidazole	Merck	Cat# 104716 CAS 288-32-4
IPTG (Isopropyl- β -D- thiogalactopyranoside)	Carl Roth	Cat# 2316.3 CAS 367-93-1
Kanamycin (Kanamycin sulphate)	Carl Roth	Cat# T832 CAS 25389-94-0
MgCl ₂ · 6H ₂ O	Sigma-Aldrich (Merck)	Cat# 1058321000 CAS 7791-18-6
MnCl ₂ · 4H ₂ O	Carl Roth	CAS 13446-34-9
NaCl	Merck	Cat# 1.06404.5000 CAS 7647-14-5

NADH	Carl Roth	Cat# AE12.1 CAS 606-68-8
NaF (sodium fluoride)	Sigma-Aldrich (Merck)	Cat# S7920 CAS 7681-49-4
Na ₄ P ₂ O ₇ (sodiumpyrophosphat)	Sigma-Aldrich (Merck)	Cat# P8010 CAS 7722-88-5
cOmplete EDTA-free Protease Inhibitor Cocktail	Roche (Merck)	Cat# 11836170001
SIGMAFAST Protease Inhibitor Cocktail Merck S8830 Tablet, EDTA free	Sigma-Aldrich (Merck)	Cat# S8830
PEI MAX® - Transfection Grade Linear Polyethylenimine Hydrochloride	Polysciences	Cat# 24765 CAS 49553-93-7
phosphoenolpyruvate (PEP)	BioChemica Panreac AppliChem ITW Reagents	Cat# A2271
Phenylmethylsulfonyl fluoride (PMSF)	Sigma-Aldrich (Merck)	Cat# 52332 CAS 329-98-6
PK/LDH (Pyruvate Kinase/Lactic Dehydrogenase)	Sigma-Aldrich (Merck)	Cat# P0294
PreScission Protrase	Cytiva	Cat# 27084301
Protino Ni-NTA	Machery-Nagel	Cat# 745400
TCEP (Tris-(2-carboxyethyl)-phosphin Hydrochlorid)	Carl Roth	Cat# HN95.3 CAS 51805-45-9
TRIS	Carl Roth	Cat# 4855 CAS 77-86-1
TURBO™ DNase (2 U/μL)	Thermo-Fisher Scientific	Cat# AM2238
Gibco™ Bacto™ Yeast Extract	Thermo-Fisher Scientific	Cat# 212720
Bacto™ Tryptone Gibco™	Thermo-Fisher Scientific	Cat# 211699

Supplementary Table 2: Expression plasmids

Plasmids	Origin	Purpose
pACEBac1_MRE11_3C_2xFlag_RAD50	This study	Expression in Hi5 cells
pACEBac1_MRE11_3C_2xFlag_RAD50_NBS1	This study	Expression in Hi5 cells
pACEMam1_MRE11 ^{H129N} _3C_2xFlag_RAD50_NBS1	Ref. ⁴	Expression in Expi293F
pACEMam1_MRE11_2xFlag_RAD50 ^{apex} _NBS1	Ref. ⁴	Expression in Expi293F
pBig1a zz TEV YBBR RAP1 ZZ TEV TRF2	Ahmet Yildiz, Addgene #185449, RRID:Addgene_185449	Subcloning into pFastBac-MBP
pFastBac-MBP-3C-TRF2-His ₁₀	This study	Expression in Hi5 cells
pET47b_His ₆ -SUMO_TRF2 ^{iDDR} (438-485)	This study	Expression in <i>E.coli</i> Rosetta
pET47b_His ₆ -SUMO_TRF2 ^{iDDR-Myb} (438-542)	This study	Expression in <i>E.coli</i> Rosetta

Supplementary Table 3: Recombinant proteins

Recombinant Proteins		Purpose
MRN WT (MRE11-RAD50-NBS1)	This study	EM, biochemistry
MR (MRE11-RAD50)	This study	EM, biochemistry
M ^{H129N} RN	Ref. ⁴	EM
MR ^{apex} N	Ref. ⁴	Crosslinking MS
TRF2 full-length	This study	EM
TRF2 (aa 438-485) (TRF2 ^{iDDR})	This study	purification, biochemistry
TRF2 (aa 438-542) (TRF2 ^{iDDR-Myb})	This study	EM, biochemistry

Supplementary Table 4: Deposited EM-Maps

Deposited Maps		Identifier
MR-DNA	This study	EMDB: 52959 (3.21 Å) and PDB: 9Q9H
M ^{H129N} RN-DNA	This study	EMDB: 52960 (3.08 Å) and PDB: 9Q9I
M ^{H129N} RN-ATPyS	This study	EMDB: 54397 (3.27 Å)
MR-TRF2 ^{iDDR-Myb} -DNA	This study	EMDB: 52962 (2.7 Å) and PDB: 9Q9K
MRN-TRF2 ^{iDDR-Myb} -DNA	This study	EMDB: 52961 (3.01 Å) and PDB: 9Q9J
MRN-TRF2-DNA	This study	EMDB: 52964 (2.97 Å) and PDB: 9Q9M

Supplementary Table 5: Oligonucleotides

Oligonucleotides		Sequence
50bp DNA fwd	This study, EM studies	TCTTTTTTTTTTTTTGTTCTTTT TTTTGATTCCGGTTTCTTTGA AATTTTT
50 bp DNA rev	This study, EM studies	AAAAATTTCAAAGAAACCGG AATCAAAAAAAAAAGAACAAAA AAAAAAAAAGA
80bp DNA fwd	Ref. ⁴	CGGGTAGTAGATGAGCGCA GGGACACCGAGGTCAAGTA CATTACCCTCTCATAGGAGG TGCGCTTTATCAGAAGCCAG AC
80bp DNA fwd	Ref. ⁴	GTCTGGCTTCTGATAAAGCG CACCTCCTATGAGAGGGTAA TGACTTGACCTCGGTGTCC CTGCGCTCA TCTACTACCCG
64 bp myb DNA (myb-DNA fwd 0 bp core + 34 bp telomeric repeat)	This study, EM studies	TCTAGGGTTAGGGTTAGATT AACGCTTCTGGAGAACTCA ACGAGCTCTAACCCTAACCC TAGA
64 bp myb DNA (myb-DNA rev 30 bp core + 34 bp telomeric repeat)	This study, EM studies	TCTAGGGTTAGGGTTAGAGC TCGTTGAGTTTCTCCAGAAG CGTTAATCTAACCCTAACCC TAGA
MRE11_H129N Forward primer	This study	CATCCACGGAAACA <u>A</u> CGAC GACCCAAC
MRE11_H129N Reverse primer	This study	GTTGGGTCGTCGT <u>I</u> GTTTCC GTGGATG

Supplementary Table 6: Commercial columns and consumables

Other		
SERVAGel™ TG PRiME™ 4 - 12 %	SERVA Electrophoresis GmbH	Cat# 43288.01
1 mL HiTrap® Heparin High Performance	Cytiva	Cat# 17040601
Superose® 6 Increase 10/300 GL	Cytiva	Cat# 29-0915-96
Superdex® 200 Increase 10/300 GL	Cytiva	Cat# 28-9909-44
Amicon Ultra-15 Centrifugal Filter Millipore Sigma 100 kDa MWCO	Sigma-Aldrich (Merck)	Cat# UFC910008
Filter units Millex® glass fibre, Millex®-AP 50	Merck-Millipore (Roth)	Cat# CY10.1
Glutathione Sepharose 4 Fast Flow column	Cytiva	Cat# 17513201
Greiner microplate, 384 well, flat bottom black non-binding	Greiner Bio-One	Cat# 781900
Quantifoil R2/1 + 2 nm C	Quantifoil	N/A

Supplementary Table 7: Software and algorithms

Software and algorithms		
MotionCor2 1.4.5	Ref. ⁵	https://msg.ucsf.edu/em/software/motioncor2.html
Phenix 1.20.1-4487	Ref. ⁶	https://www.phenix-online.org/
ChimeraX 1.6.1	Ref. ³	https://www.rbvi.ucsf.edu/chimera/
COOT 0.9.8.1	Ref. ⁷	https://www2.mrc-lmb.cam.ac.uk/Personal/pemsley/coot/
Prism 10.4.0	GraphPad	https://www.graphpad.com/
xiVIEW 1.0.18	Ref. ⁸	https://www.xiview.org/index.php
Custom-written script for FA analysis	Ref. ⁴	https://zenodo.org/record/7353848#Y38qNnaZNnl
CryoSPARC 4.6.2	Ref. ¹	https://cryosparc.com/
Topaz 4.0.2	Ref. ^{9,10}	https://emgweb.nysbc.org/topaz.ht
AlphaFold3	Ref. ¹¹	https://doi.org/10.1038/s41586-024-07487-w
PyMOL 2.5.5	PyMOL (The PyMOL Molecular Graphics System, v.2.5.5, Schrödinger, LLC)	https://www.pymol.org/
MaxQuant 2.1.3.0	Ref. ¹²	https://www.maxquant.org/
xiSEARCH 1.8.7	Ref. ¹³	https://www.rappsilberlab.org/software/xisearch/
EPU 3.5.1	ThermoFisher Scientific	
TEM User interface Titan 3.15.1	ThermoFisher Scientific	
Digital Micrograph 3.22.1461.0	Gatan, Inc.	

Supplementary Table 8: Experimental models: Cell lines and organisms

Cell lines	Purpose	Identifier
<i>Homo sapiens</i> Expi293F	Recombinant protein expression	Thermo Fisher (A14527)
<i>Escherichia coli</i> XL1 Blue	Cloning System, Propagation	N/A
<i>E. coli</i> DH10MultiBac	Bacmid generation	N/A
<i>Spodoptera frugiperda</i> (Sf21)	Virus production	Thermo Fisher (11497013)
<i>Trichoplusia ni</i> insect cells (Hi5)	Recombinant protein expression	Invitrogen (B85502)
<i>E.coli</i> BL21 Rosetta	Recombinant protein expression	N/A

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