

Supplementary Information for:

Structure guided functional analysis of the *S. cerevisiae* Mre11 complex

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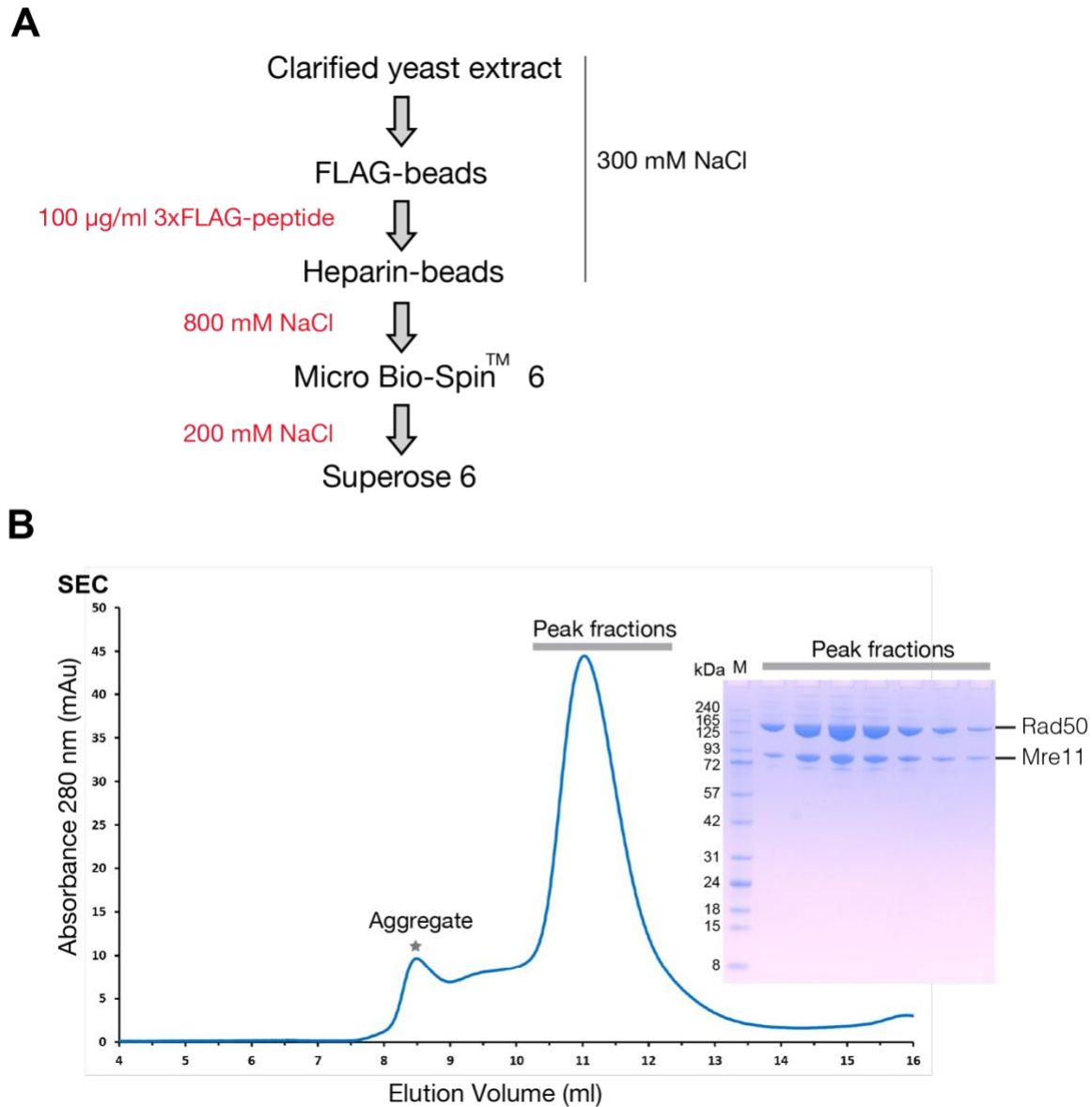
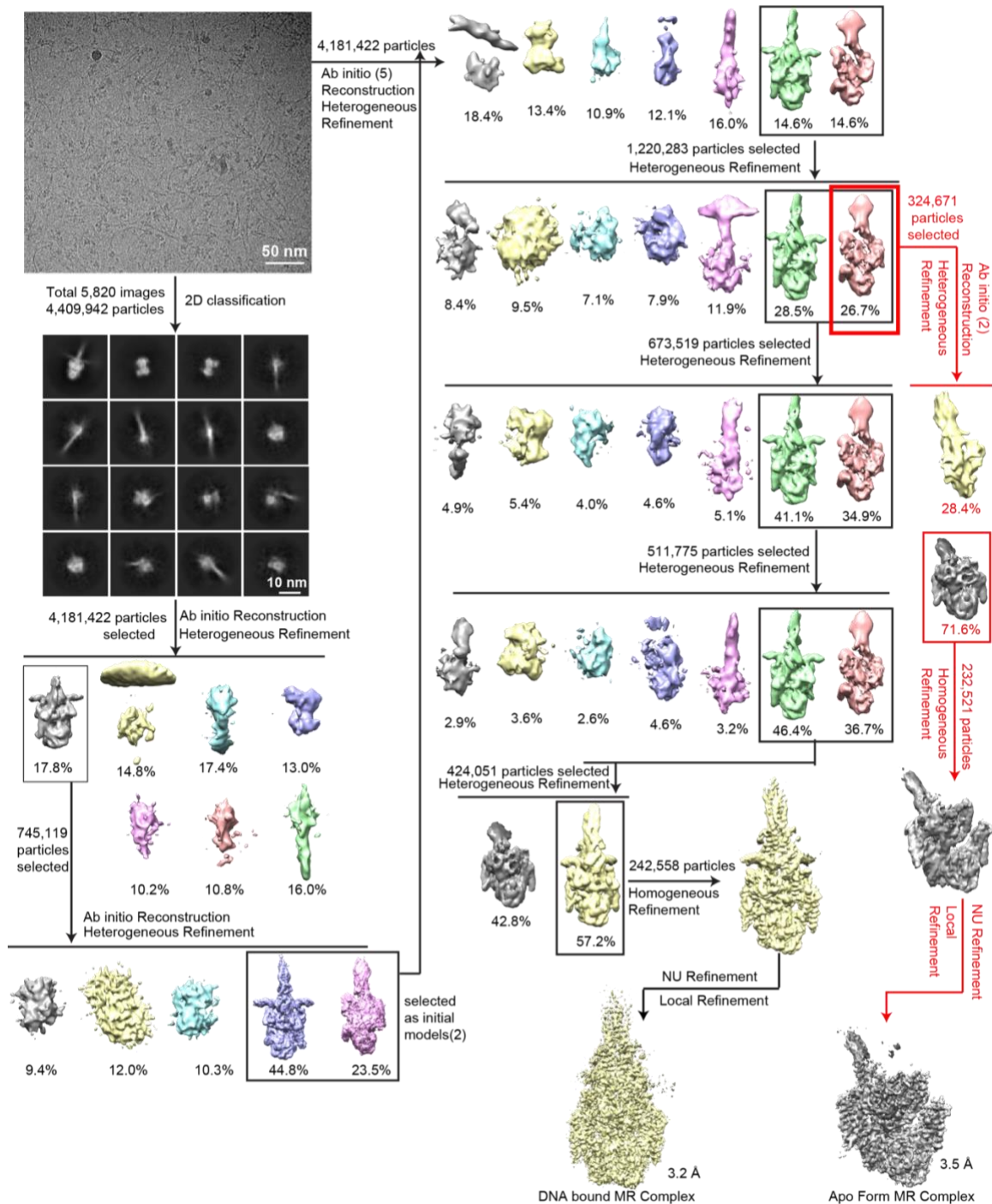
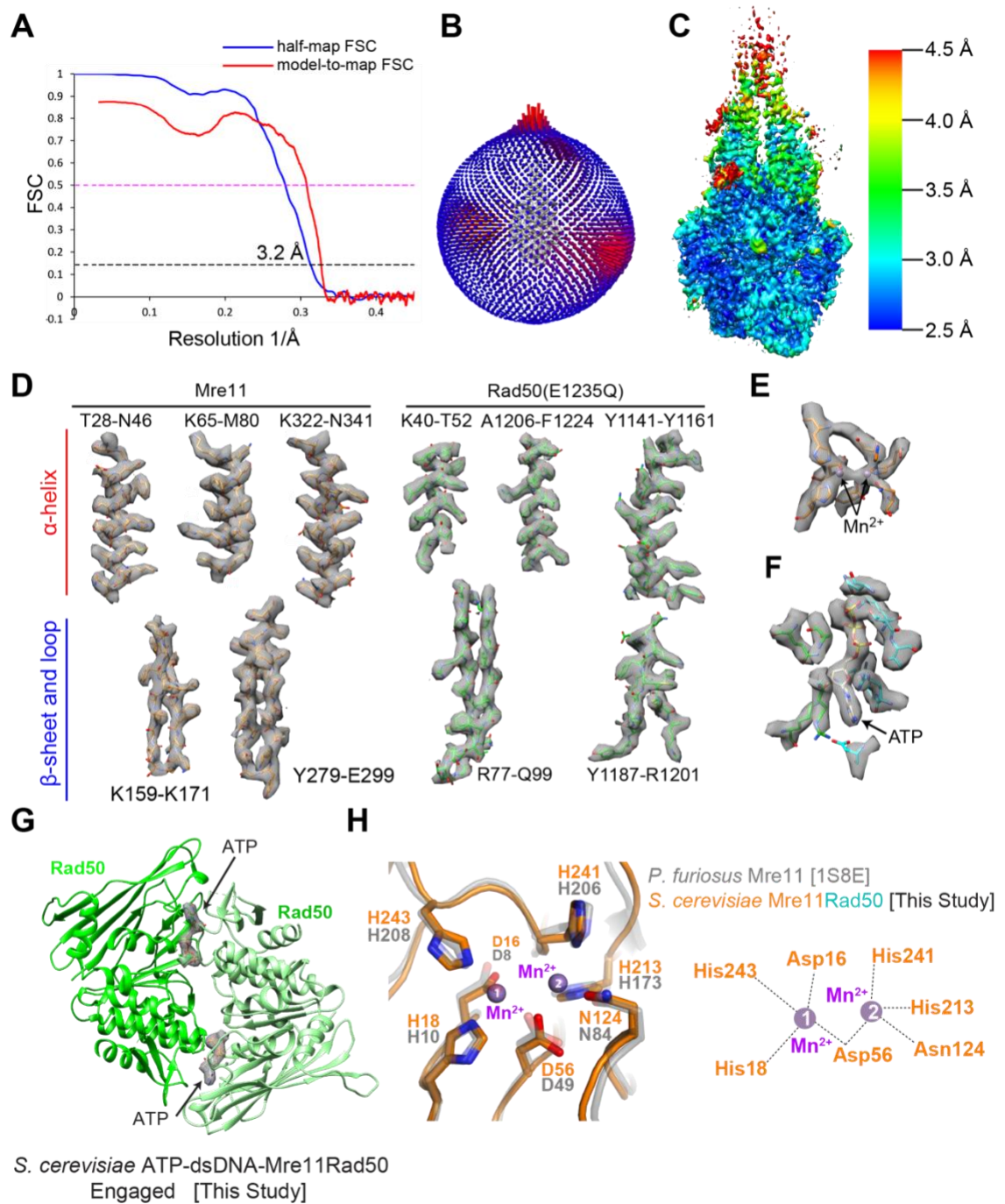
Figure S1**Figure S1. Preparation and purification of *S. cerevisiae* MR-DNA complex.****(A)** Flow chart of purification steps of endogenous Mre11-Rad50 (MR) complex.**(B)** Sizing column (Sepharose 6 Increase) elution profile of MR and Coomassie stained gel picture showing Mre11 and Rad50 subunits in the peak fraction used for generation of the cryo-EM sample with added dsDNA, ATP, and Mg/Mn cations. Buffer: 200 mM NaCl, 50 mM HEPES, 2 mM DTT, pH 7.5, 2% glycerol, 0.025% CHAPSO detergent. Molecular weight markers are given on the left of the Coomassie stained SDS-PAGE.

Figure S2

**Figure S2. Cryo-EM reconstruction of the MR-dsDNA and apo-MR complex.**

Workflow of cryo-EM image processing of the MR-dsDNA complex and an apo-MR complex, from one cryo-EM dataset. The apo-MR complex (3.5 Å resolution) is largely similar the DNA-bound structure, but only one coiled coil proximal to the globular domain is traceable.

Figure S3

**Figure S3. Cryo-EM structure determination of apo-MR and MR-dsDNA complex.**

(A) Global Fourier Shell Correlation (FSC) curve of MR-dsDNA complex. The curve for two half datasets is in blue and that for the refined model versus cryo-EM map is in red. The overall cryo-EM resolution is 3.2 Å with FSC set at 0.143.

(B) Angular distribution plot of final 3D EM map for MR-dsDNA complex.

(C) Final 3D reconstruction map of the MR-dsDNA complex colored according to local resolution.

(D-F) Sample maps and models of Mre11 and Rad50 subunits (panel D), of Mre11 nuclease catalytic pocket with Mn^{2+} ions (panel E), and of Rad50 ATP-binding pocket (panel F) in the MR-dsDNA complex. Sample maps and models are shown as surfaces and sticks respectively in the 3.2 Å resolution structure of the MR-dsDNA complex.

(G) “Engaged” ATP-bound (ATP density map shown in mesh) head segments of Rad50 in the structure of MR-dsDNA complex (this study).

(H) Left panel depicts the ribbon representation superposition of amino acids coordinated to a pair of Mn^{2+} divalent cations in the nuclease catalytic pocket of Mre11 in the cryo-EM structure of the MR-dsDNA complex (in orange; this study) and in the X-ray structure of *P. furiosus* Mre11 (in silver; PDB: 1S8E). Right panel shows a schematic representation of the coordination of the pair of Mn^{2+} cations in the MR-dsDNA complex.

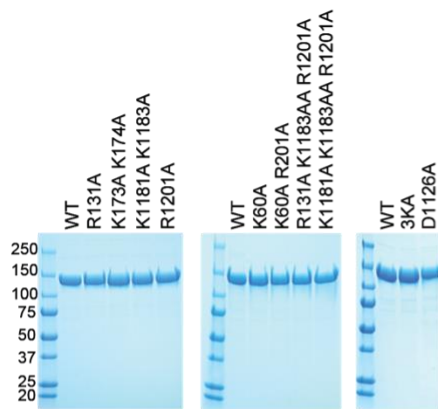
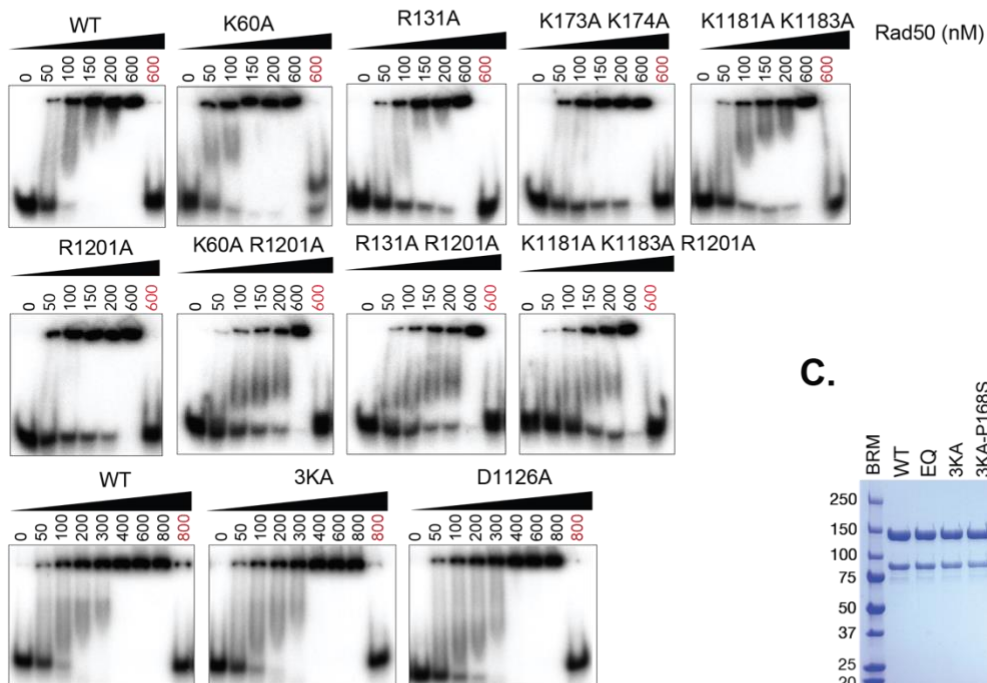
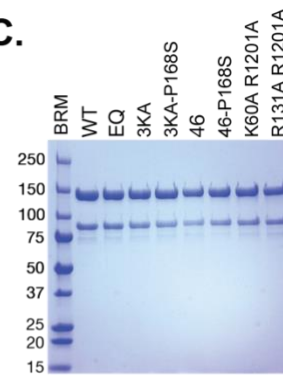
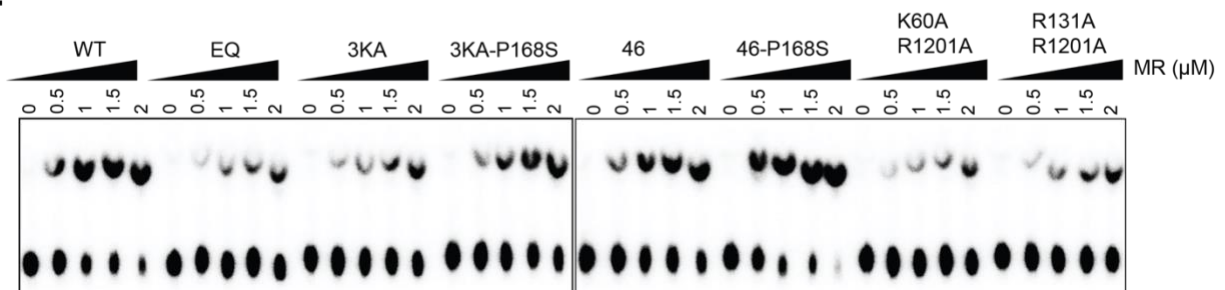
Figure S4
A.

B.

C.

D.


Figure S4. Rad50-dsDNA binding and MR-ATPase activity of Rad50 WT and mutant proteins.

(A) Rad50 WT and mutant purified proteins. 4 µg of Rad50 proteins were run on a 4-20% SDS-PAGE stained with Coomassie Brilliant Blue. Molecular weight markers in kDa are indicated on the left.

(B) Rad50-DNA binding was assessed by electrophoretic mobility shift assay (EMSA). 5 nM radiolabeled 83-mer duplex DNA was incubated in the presence of 2 mM cold ATP, 5 mM MgCl₂ with increasing amounts of Rad50 proteins (0–600 nM) on ice for 20 min prior to loading on a 5% native Polyacrylamide gel. To ensure ATP-dependent binding, 600 nM Rad50 was also incubated in binding buffer without ATP (last lane depicted in red label). At least three independent experiments were quantified, and binding efficiencies were calculated as the ratio of bound fraction to total signal per lane.

(C) 2 µg of purified WT and Rad50-mutant MR complexes were run on a 4-20% SDS-Page stained with Coomassie Brilliant Blue. Molecular weight markers in kDa are indicated on the left.

(D) Rad50-ATPase activity of WT and Rad50-mutant MR complexes. Increasing amounts of MR complexes (0-2 µM) were incubated with $\gamma^{32}\text{P}$ -ATP in ATPase buffer in the presence of both dsDNA and ssDNA for 90 min. ATP hydrolysis was measured by thin-layer chromatography. The percentage of ATP hydrolysis was calculated as ratio of released ^{32}P to total radioactivity per lane following quantification with ImageGauge software (GE). Error bars denote standard deviation from at least three experiments.

Figure S5

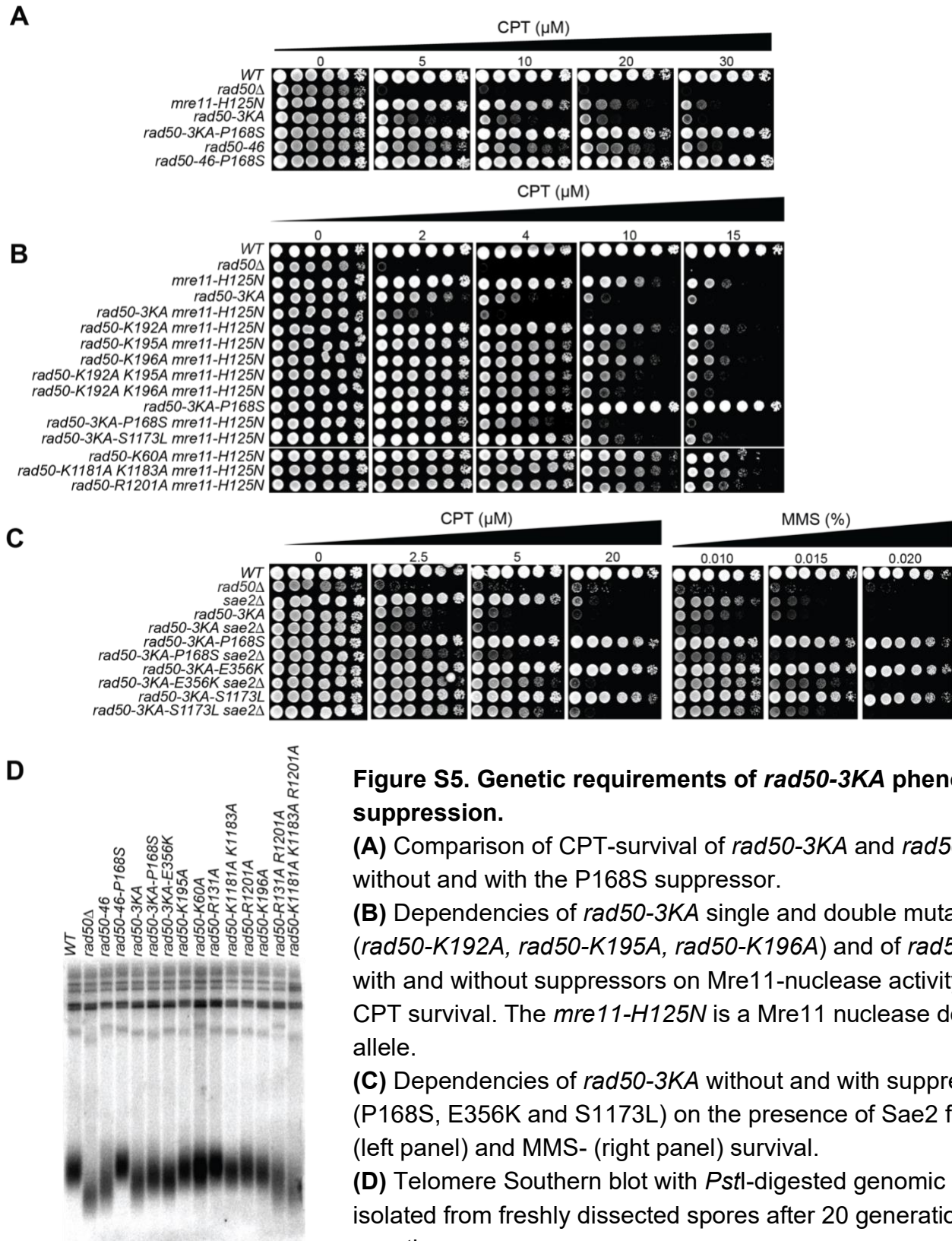


Figure S6

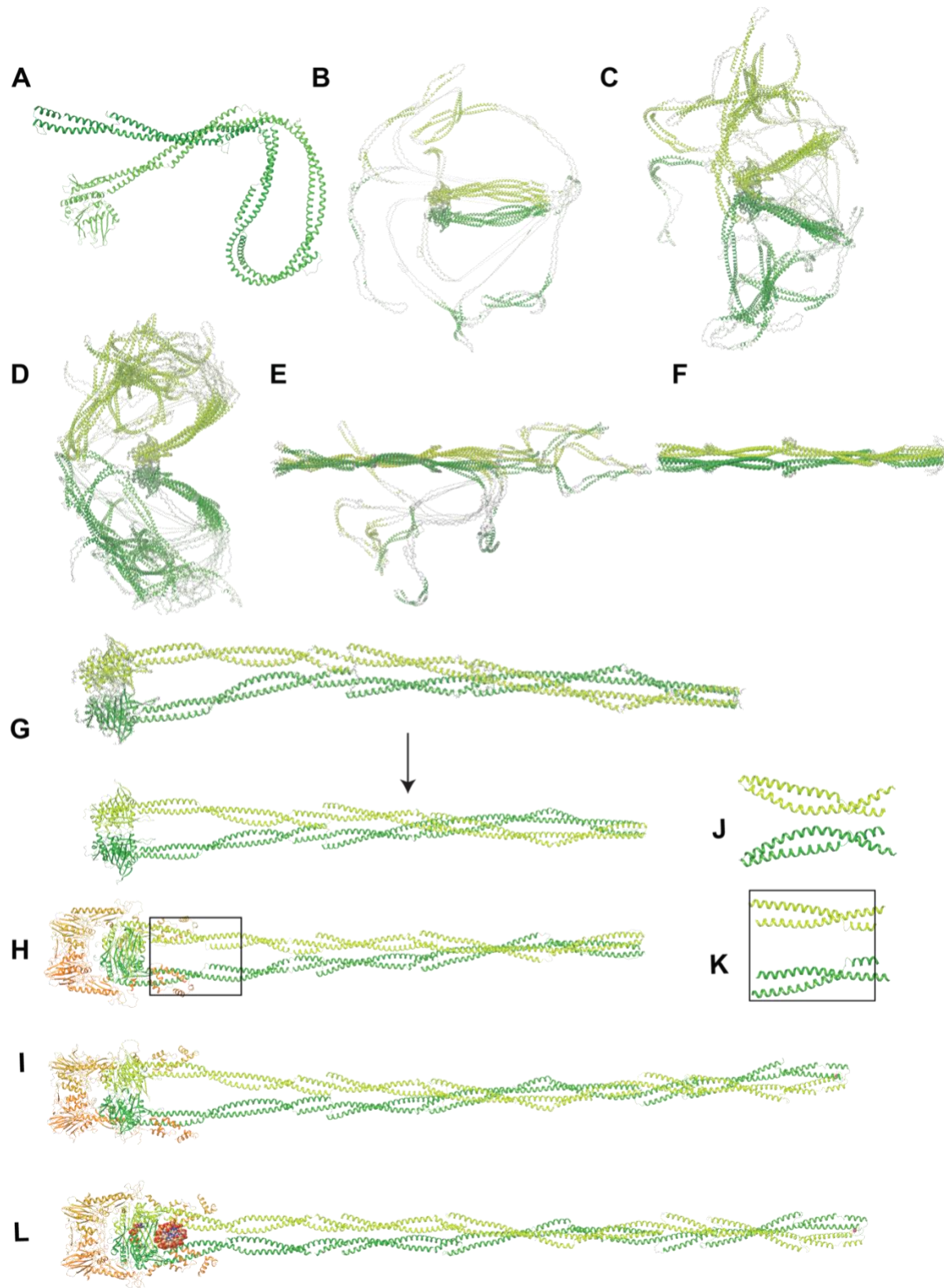


Figure S6. Sequential steps of modeling the dsDNA bound Mre11-Rad50 complex of *S. cerevisiae* with full-length Rad50 coiled coils.

(A) Monomeric Rad50 computed by AF-2.

(B, C and D) Three classes of dimeric full-length Rad50 models (panels B, C and D) computed by AF-2 and superpositioned by C α atoms of Rad50 head domains.

(E) Dimeric coiled coil models for the region A181 to M1139 computed by AF-2 and superpositioned by C α atoms of the region with a good convergency.

(F) Tetrameric coiled coil models computed by AF-2 for the regions I250 to Q526 and N824 to T1069 and merged as two dimers, colored light and dark green, which are defined by hydrophobic interactions between the constituent parts.

(G) The symmetrization step of the dimeric Rad50 construct obtained by combining well-converged fragments shown in panels B, E, and F computed by AF-2.

(H) A product of the symmetrization step of the Rad50-Mre11 dimer assembled based on the cryo-EM structure of the MR-DNA complex without the HTH domain; the boxed region depicts the position where DNA is bound in the cryo-EM structure.

(I) Symmetric Mre11-Rad50 model with full-length Rad50 and Zn hook; the HTH domain of Mre11 interacting with coiled coils of Rad50 is continuously connected with the capping domain via two loops built by Schrödinger software to connect D430-E439 helix positioned by docking computations.

(J, K) Coiled-coil curvature around bound DNA in the cryo-EM structure (panel J) and the model with the full-length Rad50 dimer (panel K), boxed in panels K and H.

(L) Complete model of Mre11-Rad50-dsDNA with adjusted coiled coils around the bound dsDNA.

Figure S7

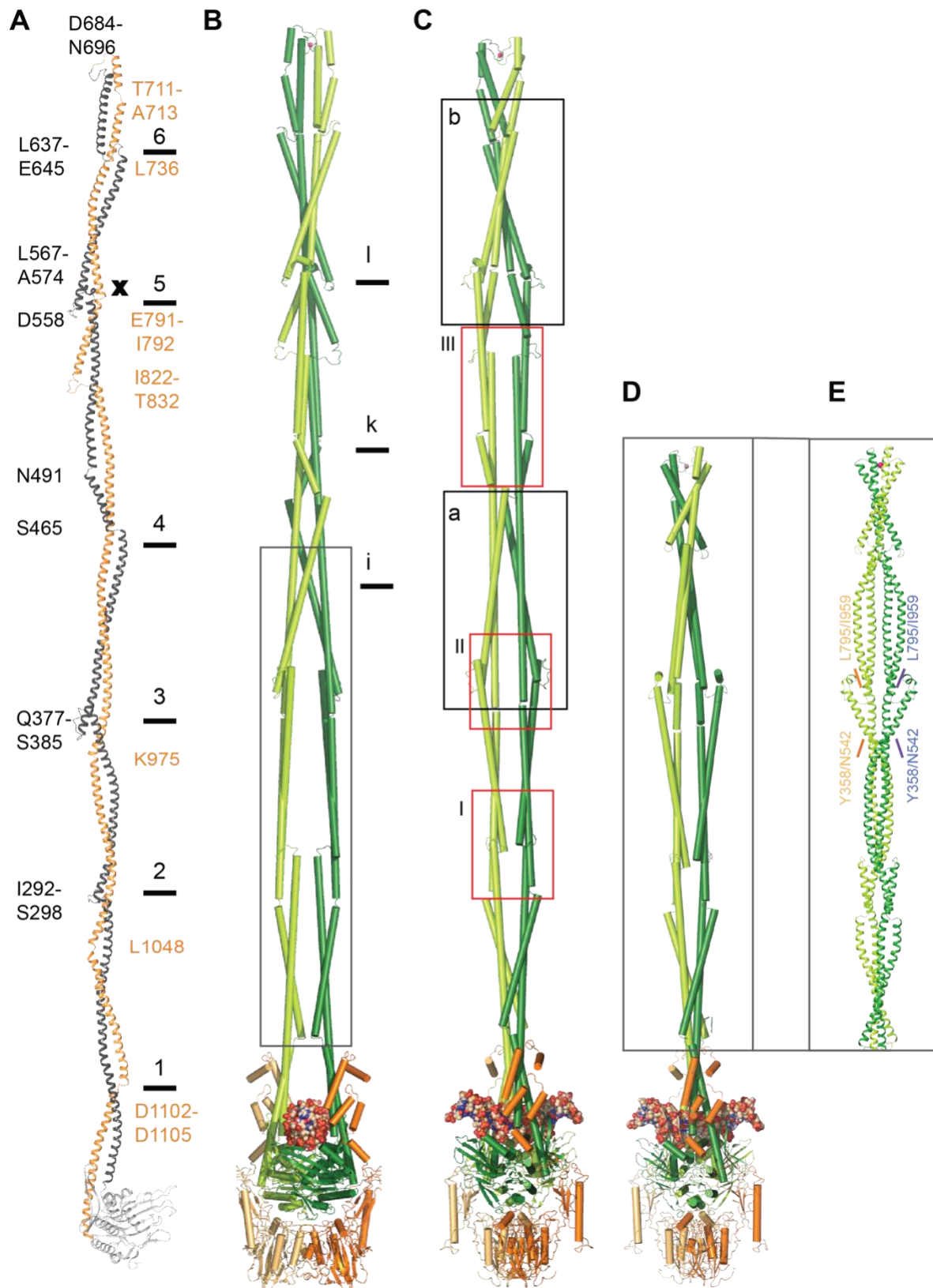


Figure S7. Topology and coiled coil interactions in the dsDNA-bound full-length and coiled-coil truncated MR complexes.

(A) Topography of α -helices and intervening loops on monomeric folded back antiparallel coiled coil of Rad50. The N-terminal part (from the head domain to the Zn hook domain) is colored black and the C-terminal domain (from the Zn hook domain to the head domain) is colored orange. The numbers in corresponding colors indicate the positions of intervening loops. At the six superhelical left-handed crossings, a stretch of a continuous α -helix in the antiparallel coiled coil coincides with the intervening loop connecting α -helical stretches of the coiled coil partner running in the opposite direction (segments numbered 1 through 6). Except for a short D558-F565 helix running perpendicular to the helical axis, marked by an 'x' at crossing 5, all other helices form a braid-like interaction with their partners.

(B) Three partial non-intertwining superhelical crossings between two constituent monomeric Rad50 coiled coils: *j*, right-handed, *k*, left-handed, *l*, right-handed.

(C) Two blocks of nearly repetitive pattern in helical progression are enclosed in boxes labeled *a* and *b*. Note the switch in the helical handedness in these blocks and the parallel progression of the intervening section between them. Hub segments corresponding to contacts between identical helices of two monomeric coiled coils running in parallel, at which all four constitutive helices engage, are shown as red boxes labeled I, II and III.

(D) A truncated version of Rad50 coiled coils obtained by replacing the block labeled 'a' in Figure S7C with the block labeled 'b' in Figure S7C.

(E) Details of secondary structure highlighting the smoothness of Y358/N542 and L795/I959 connections in the truncated version of coiled coils.

Figure S8

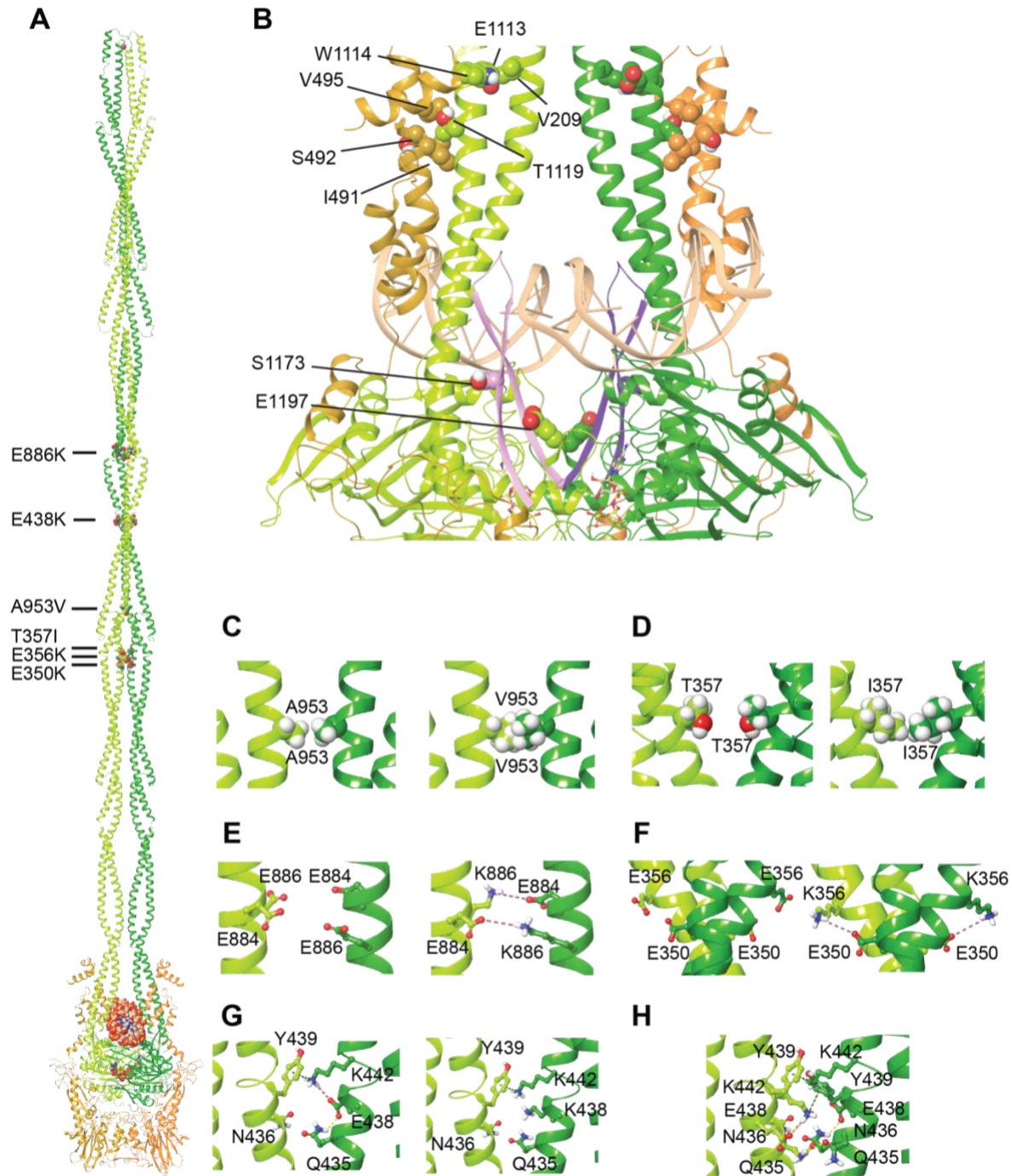


Figure S8. *rad50-3KA* coiled coil suppressors are localized in the parallel interaction interface of two antiparallel coiled coils and affect the coiled coil structure.

(A, B) Complete model of MR (panel A) and fragment centered on the top segment of the head domain and the head proximal coiled coils (panel B). Location of *rad50-3KA* coiled coil suppressors proximal to the globular domain (mid-range). Several mid-range

distance mutants (E1197K, S1173L, T1119I and V209I) are involved in suppression of CPT sensitivity of *rad50-3KA* in the MR-DNA complex (Figure 5A). Rad50 mutants E1197K and S1173L are located in the β -hairpin (T1168-K1193) 'paddle' segment (Figure S8B), which communicates the structural dynamics between the Rad50 coiled coils and head domains. Rad50 head-proximal coiled coil mutant T1119I affects the interaction with Mre11 HTH residues I491, S492 and V495 (Figure S8B). Rad50 head-proximal coiled coil mutant V209I, is also located close to interaction with the Mre11 HTH domain and affects the intra-monomer coiled coil interaction with Rad50 W1111 and E1113 residues (Figure S8B). **(C-H)** All long-range distance coiled coil *rad50-3KA* suppressor mutants (A953V, T357I, E886K, E350K, E356K and E438K) are located at the contact points between full-length coiled coils of two monomeric Rad50 molecules in our reconstructed full-length complex and affect the strength of their mutual interactions (panels C-G). Of these six long-range distance mutants involved in suppression of CPT sensitivity of *rad50-3KA*, A953V (panel C) and T357I (Figure panel D) are expected to enhance the interaction between the coiled coils of two Rad50 monomers through the formation of a hydrophobic cluster for both mutants. Amongst the four charge reversal (glutamate to lysine) mutants, E886K (panel E), E356K (panel F) and E350K are shown in our full-length reconstruction to alter the unfavorable interaction of negatively charged side chains between the two monomers of Rad50 coiled coils via formation of favorable direct electrostatic interactions through salt bridge formation. The E438K (panels G and H) charge reversal mutant affects the intermolecular interaction indirectly, weakening the intramolecular α -helical segment from Q435 to K442, which additionally results in a decrease of the probability of intermolecular Y439 to K442 π -cation interaction. A953V enhances the inter-monomeric interaction of coiled coils via increased hydrophobicity (panel C). T357I enhances the inter-monomeric interaction of coiled coils via increased hydrophobicity (panel D). E886K mutation enhances the inter-monomeric interaction of coiled coils via a salt bridge formed between E884 and K886 (panel E). E356K mutation enhances the inter-monomeric interaction of coiled coils via a salt bridge formed between E350 and K356 (panel F). E438K weakens the inter-monomeric interaction of coiled coils because of the increased flexibility of K442, negatively affecting the cation- π interaction between K442 and Y439. Shown for a single pair of alpha-helices (panel G). The effect of the long range (Figure S8) E438K mutant is demonstrated for two pairs of α -helices located at the same point in Rad50 coiled coils (panel H).

Table S1. Cryo-EM data collection, processing, and validation statistics

Sample	dsDNA bound MR(E/Q) complex EMD-44558 PDB: 9BI4	Apo MR(E/Q) complex EMD-44559 PDB: 9BI5
Data collection		
Microscope	Titan Krios	Titan Krios
Detector	Gatan K3	Gatan K3
Automation software	Leginon	Leginon
Nominal magnification	22,500	22,500
Voltage (kV)	300 kV	300 kV
Total dose (e ⁻ /Å ²)	51.22	51.22
Number of frames collected	50	50
Defocus range (μm)	-1.0 to -2.5	-1.0 to -2.5
Pixel size (Å)	1.083	1.083
Collected Micrographs	5,826	5,826
Selected Micrographs	5,820	5,820
Reconstruction		
Initially autopicked particles	4,409,942	4,409,942
Particles used for classification	4,181,422	4,181,422
Particles in the final map	242,558	232,521
Symmetry	C1	C1
Resolution		
FSC 0.5 (unmasked/masked, Å)	3.4/3.3	4.1/4.0
FSC 0.143 (unmasked/masked, Å)	3.2/3.2	3.5/3.5
Map sharpening B factor (Å ²)	-107.2	-106.6
Model composition		
Protein residues	1881	1565
Nucleotides	44	
Nonhydrogen atoms	15869	12413
Validation		
MolProbity	2.63	2.96
Clash score	11.38	18.46
Map Correlation Coefficient	0.78	0.64
R.m.s. deviations		
Bond lengths (Å)	0.008	0.006
Bond angles (°)	1.212	1.104
Ramachandran plots		
Favored (%)	94.14	90.68
Allowed (%)	5.43	9.00
Outliers (%)	0.43	0.32
Rotamer outliers (%)	7.5	7.6

Table S2. The distances between pairs of catalytically competent atom pairs in the WT and mutant MR complexes at the beginning and end of the MD simulation.

MR variant	Pairs of atoms	Distances, Å		
		At 1 nsec	At 30 nsec	Difference
WT	E1235 CO ⁻ / Mg ²⁺	4.7	4.3	-0.4
	E1235 CO ⁻ / K40 NZ	4.1	3.8	-0.3
	D1234 CO ⁻ / Mg ²⁺	4.0	4.4	0.4
	Q158 CO ⁻ / Mg ²⁺	2.0	1.9	-0.1
	H1272 NE2/ Mg ²⁺	6.0	6.2	0.2
	Sum of differences			-0.2
3KA	E1235 CO ⁻ / Mg ²⁺	6.5	6.2	-0.3
	E1235 CO ⁻ / K40 NZ	6.4	6.5	0.1
	D1234 CO ⁻ / Mg ²⁺	4.6	4.3	0.3
	Q158 CO ⁻ / Mg ²⁺	2.0	7.1	5.1
	H1272 NE2/ Mg ²⁺	6.4	6.1	-0.3
	Sum of differences			4.9
3KA+P168S	E1235 CO ⁻ / Mg ²⁺	6.3	4.3	-2.0
	E1235 CO ⁻ / K40 NZ	6.5	4.0	-2.5
	D1234 CO ⁻ / Mg ²⁺	4.2	4.7	0.5
	Q158 CO ⁻ / Mg ²⁺	2.1	2.0	-0.1
	H1272 NE2/ Mg ²⁺	6.2	6.1	-0.1
	Sum of differences			-6.2

^a For Glu and Asp acidic residues, distances are measured from the negatively charged oxygen of the carboxylate group as computed by the SCRODINGER program.

Table S3. Yeast strains used in this study

Strain ID	Genotype
JPY7899	<i>rad50ΔKanMX Ycp50-RAD50</i>
JPY7900	<i>rad50ΔKanMX Ycp50 empty</i>
JPY8459	<i>rad50ΔKanMX Ycp50-rad50-D1167A</i>
JPY8460	<i>rad50ΔKanMX Ycp50-rad50-E1243A</i>
JPY8461	<i>rad50ΔKanMX Ycp50-rad50-D1167A E1243A</i>
JPY8464	<i>rad50ΔKanMX Ycp50-rad50-E1155A D1167A E1243A (rad50-MRI-3A)</i>
JPY7128	<i>mre11ΔLEU2 pRS316-empty</i>
JPY8347	<i>mre11ΔLEU2 pRS316-MRE11</i>
JPY8348	<i>mre11ΔLEU2 pRS316-mre11-K410A</i>
JPY8467	<i>mre11ΔLEU2 pRS316-mre11-R412A</i>
JPY8481	<i>mre11ΔLEU2 pRS316-mre11-K322A N387A R389A R390A R412A (mre11-MRI-5A1)</i>
JPY8487	<i>mre11ΔLEU2 pRS316-mre11-K322A N387A R389A R390A K410A (mre11-MRI-5A2)</i>
JPY8484	<i>mre11ΔLEU2 pRS316-mre11-K322A N387A R389A R390A K410A R412A (mre11-MRI-6A)</i>
JPY8373	<i>rad50ΔKanMX Ycp50-rad50-D1126A</i>
JPY8411	<i>rad50ΔKanMX Ycp50-rad50-D1126N</i>
JPY8413	<i>rad50ΔKanMX Ycp50-rad50-D1126E</i>
JPY8356	<i>rad50ΔKanMX Ycp50-rad50-W1157A</i>
JPY8390	<i>rad50ΔKanMX Ycp50-rad50-W1157Y</i>
JPY8483	<i>rad50ΔKanMX Ycp50-rad50-R13A</i>
JPY8474	<i>rad50ΔKanMX Ycp50-rad50-N36A</i>
JPY8381	<i>rad50ΔKanMX Ycp50-rad50-E159A</i>
JPY8379	<i>rad50ΔKanMX Ycp50-rad50-K1209A</i>
JPY8490	<i>rad50ΔKanMX Ycp50-rad50-K60A</i>
JPY8114	<i>rad50ΔKanMX Ycp50-rad50-103A K104A</i>
JPY8496	<i>rad50ΔKanMX Ycp50-rad50-T111A</i>
JPY8317	<i>rad50ΔKanMX Ycp50-rad50-R131A</i>
JPY8466	<i>rad50ΔKanMX Ycp50-rad50-S169A</i>
JPY8318	<i>rad50ΔKanMX Ycp50-rad50-K173A K174A</i>
JPY8319	<i>rad50ΔKanMX Ycp50-rad50-K186A</i>
JPY8321	<i>rad50ΔKanMX Ycp50-rad50-K1181A K1183A</i>
JPY8491	<i>rad50ΔKanMX Ycp50-rad50-R1201A</i>
JPY8328	<i>rad50ΔKanMX Ycp50-rad50-K192A</i>
JPY8329	<i>rad50ΔKanMX Ycp50-rad50-K195A</i>
JPY8345	<i>rad50ΔKanMX Ycp50-rad50-K196A</i>
JPY8330	<i>rad50ΔKanMX Ycp50-rad50-K192A K195A</i>
JPY8333	<i>rad50ΔKanMX Ycp50-rad50-K192A K196A</i>
JPY8320	<i>rad50ΔKanMX Ycp50-rad50-K192A K195A K196A (rad50-3KA)</i>
JPY8502	<i>rad50ΔKanMX Ycp50-rad50-K60A R1201A</i>
JPY8503	<i>rad50ΔKanMX Ycp50-rad50-103A K104A R1201A</i>
JPY8504	<i>rad50ΔKanMX Ycp50-rad50-T111A R1201A</i>
JPY8505	<i>rad50ΔKanMX Ycp50-rad50-R131A R1201A</i>
JPY8506	<i>rad50ΔKanMX Ycp50-rad50-S169A R1201A</i>
JPY8507	<i>rad50ΔKanMX Ycp50-rad50-K173A K174A R1201A</i>
JPY8508	<i>rad50ΔKanMX Ycp50-rad50-K186A R1201A</i>
JPY8509	<i>rad50ΔKanMX Ycp50-rad50-K192A R1201A</i>
JPY8510	<i>rad50ΔKanMX Ycp50-rad50-K195A R1201A</i>
JPY8511	<i>rad50ΔKanMX Ycp50-rad50-K196A R1201A</i>
JPY8512	<i>rad50ΔKanMX Ycp50-rad50-K1181A K1183A R1201A</i>
JPY8444	<i>rad50ΔKanMX Ycp50-rad50-3KA P57A</i>
JPY8450	<i>rad50ΔKanMX Ycp50-rad50-3KA M92I</i>
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JPY8440	<i>rad50ΔKanMX Ycp50-rad50-3KA P168S</i>
JPY8442	<i>rad50ΔKanMX Ycp50-rad50-3KA V209I</i>
JPY8443	<i>rad50ΔKanMX Ycp50-rad50-3KA E350K</i>
JPY8447	<i>rad50ΔKanMX Ycp50-rad50-3KA E356K</i>
JPY8449	<i>rad50ΔKanMX Ycp50-rad50-3KA T357K</i>
JPY8445	<i>rad50ΔKanMX Ycp50-rad50-3KA E438K</i>
JPY8446	<i>rad50ΔKanMX Ycp50-rad50-3KA E886K</i>
JPY8454	<i>rad50ΔKanMX Ycp50-rad50-3KA A953V</i>
JPY8438	<i>rad50ΔKanMX Ycp50-rad50-3KA T1119I</i>
JPY8448	<i>rad50ΔKanMX Ycp50-rad50-3KA S1173L</i>
JPY8439	<i>rad50ΔKanMX Ycp50-rad50-3KA E1197K</i>
JPY8456	<i>rad50ΔKanMX Ycp50-rad50-3KA R1201K</i>
JPY8455	<i>rad50ΔKanMX Ycp50-rad50-3KA A1206T</i>
JPY8457	<i>rad50ΔKanMX Ycp50-rad50-3KA A1249T</i>
JPY8523	<i>pGBD-RAD50 pGAD-MRE11</i>
JPY8521	<i>pGBD-empty pGAD-MRE11</i>
JPY8524	<i>pGBD-RAD50 pGAD-mre11-MRI-6A</i>
JPY8525	<i>pGBD-RAD50 pGAD-mre11-E38K</i>
JPY8527	<i>pGBD-MRE11 pGAD-MRE11</i>
JPY8528	<i>pGBD-mre11-MRI-6A pGAD-mre11-MRI-6A</i>
JPY8529	<i>pGBD-mre11-38K pGAD-mre11-38K</i>
JPY8531	<i>pGBD-XRS2 pGAD-MRE11</i>
JPY8532	<i>pGBD-XRS2 pGAD-mre11-MRI-6A</i>
JPY8533	<i>pGBD-XRS2 pGAD-mre11-38K</i>
JPY5596	<i>HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoM IV)-URA3 (SK1)</i>
JPY5597	<i>dmc1Δ/ΔHYG HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoM IV)-URA3 (SK1)</i>
JPY5598	<i>rad50-46/46::HYG HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoM IV)-URA3 (SK1)</i>
JPY8561	<i>rad50-3KA/3KA::HYG HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoM IV)-URA3 (SK1)</i>
JPY8561	<i>rad50-3KA-P168S/3KA-P168S::HYG HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoM IV)-URA3 (SK1)</i>
JPY8551	<i>rad50ΔKanMX mec1ΔNAT sml1ΔHYG. Ycp50-RAD50</i>
JPY7550	<i>rad50ΔKanMX mec1ΔNAT sml1ΔHYG. Ycp50-rad50-46</i>
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JPY2137	<i>rad50Δ:: ura3- mec1::HYG sml1Δ::HIS3 FLAG-RAD53::LEU2 sae2Δ::TRP1</i>
JPY8567	<i>JPY2137 + Ycp50-RAD50</i>
JPY8569	<i>JPY2137 + Ycp50-rad50-46-P168S</i>
JPY8568	<i>JPY2137 + Ycp50-rad50-3KA</i>
JPY8570	<i>JPY2137 + Ycp50-rad50-3KA-P168S</i>
JPY8571	<i>JPY2137 + Ycp50-rad50-3KA-E356K</i>