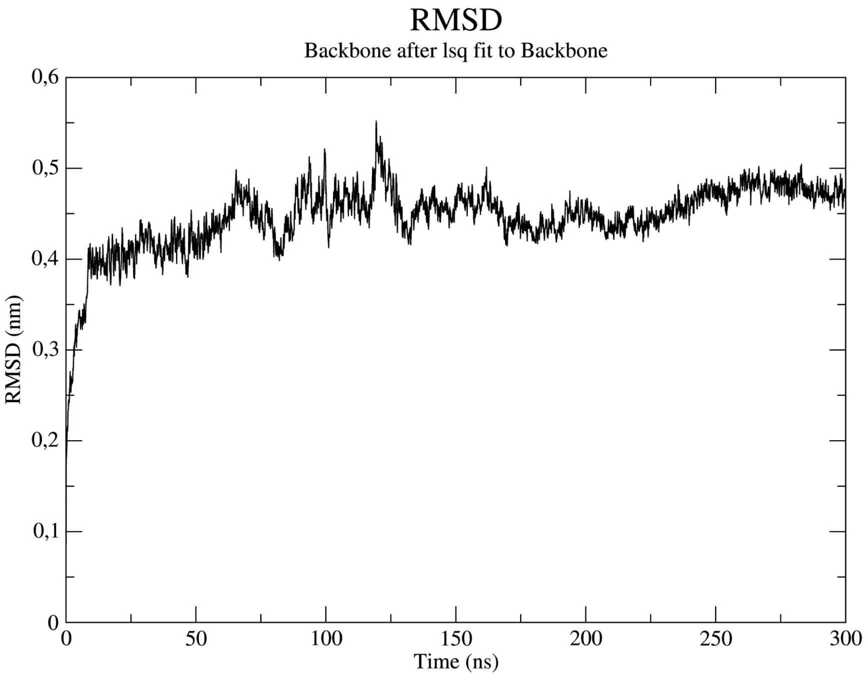
Supplementary Figure S1

A



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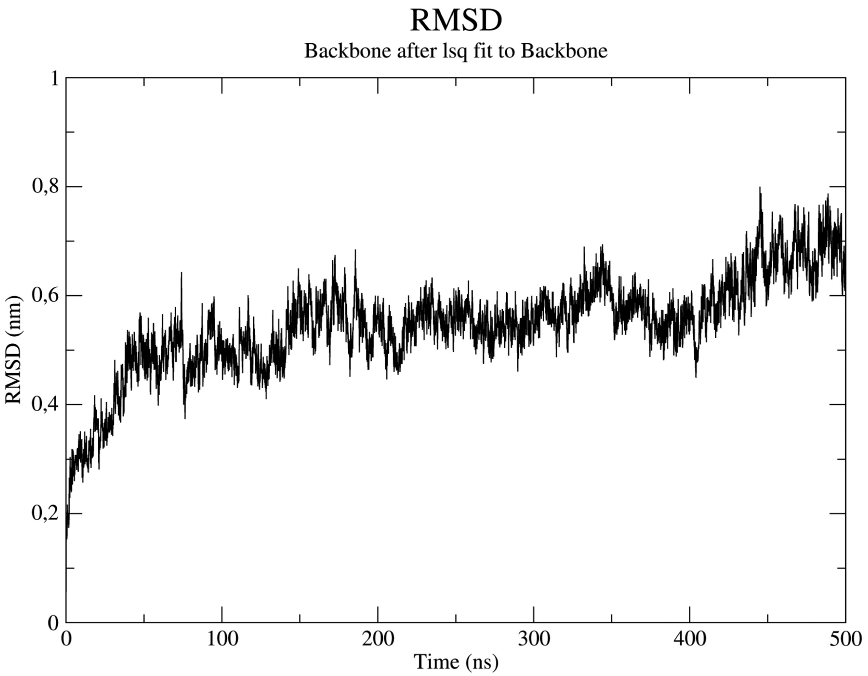
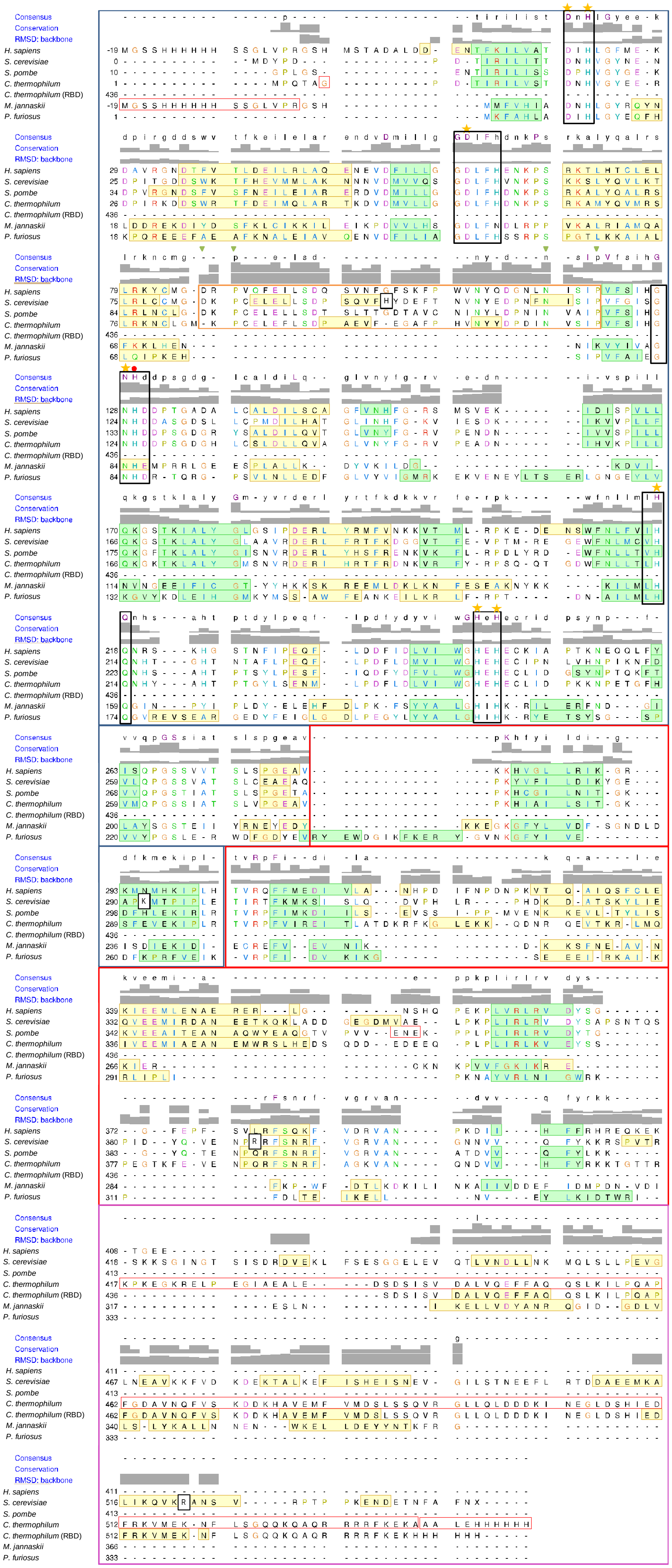
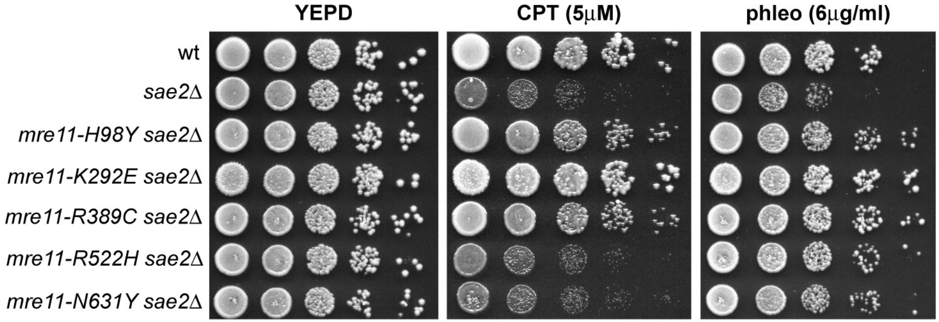


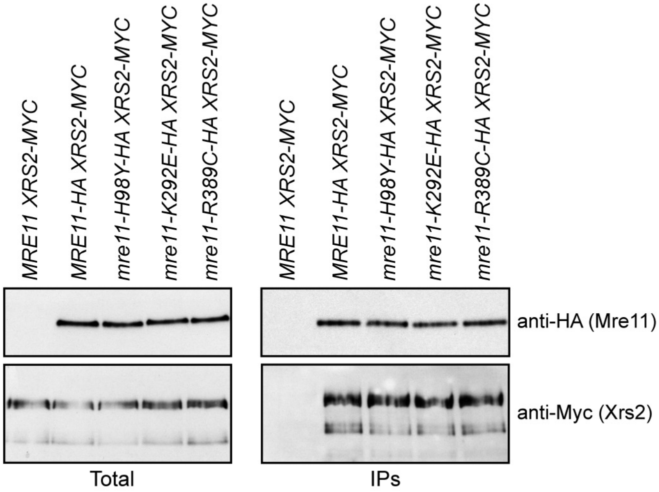
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



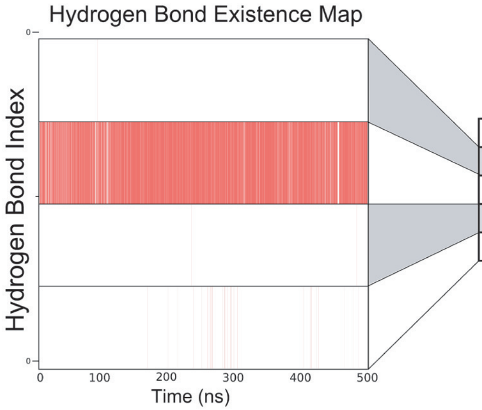
Supplementary Figure S5A

H98-A Vs chain A

Hydrogen bonds

☐ none

🟥 present



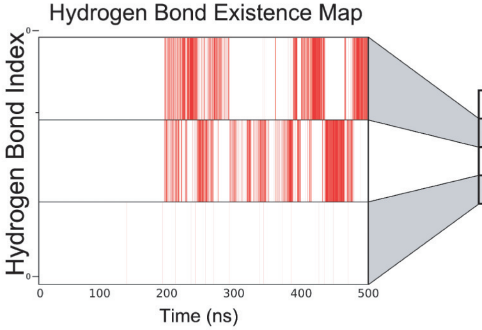
Donor	Hydrogen	Acceptor
HIS98ND1	HIS98HD1	PHE102N
HIS98ND1	HIS98HD1	TYR99O
HIS98ND1	HIS98HD1	TYR99N
HIS98ND1	HIS98HD1	HIS98O

H98-B Vs chain B

Hydrogen bonds

☐ none

🟥 present



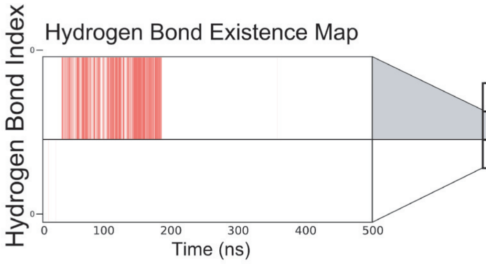
Donor	Hydrogen	Acceptor
HIS98NE2	HIS98HE2	GLU101OE2
HIS98NE2	HIS98HE2	GLU101OE1
HIS98N	HIS98H	GLU88OE2

H98-B Vs chain A

Hydrogen bonds

☐ none

🟥 present

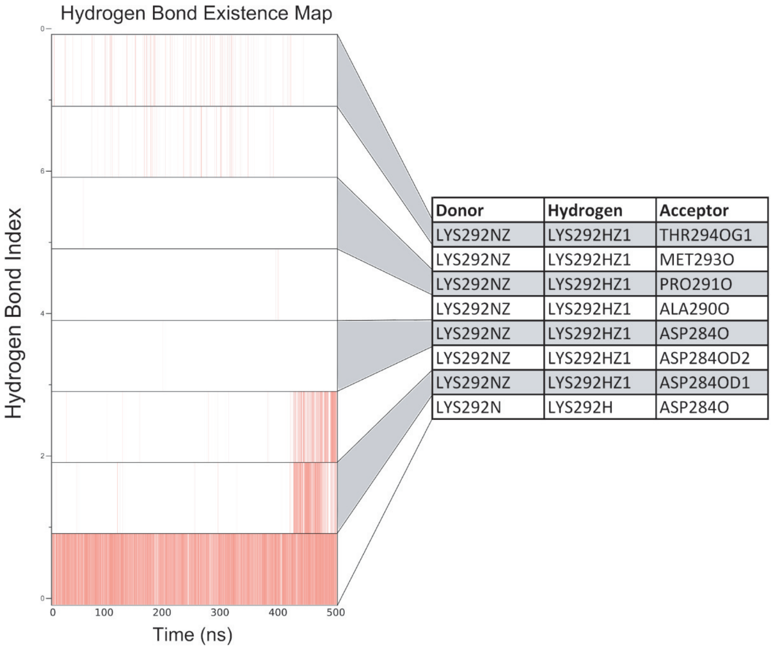


Donor	Hydrogen	Acceptor
HIS98NE2	HIS98HE2	LEU134O
HIS98NE2	HIS98HE2	LEU133O

Supplementary Figure S5B

K292-A Vs chain A

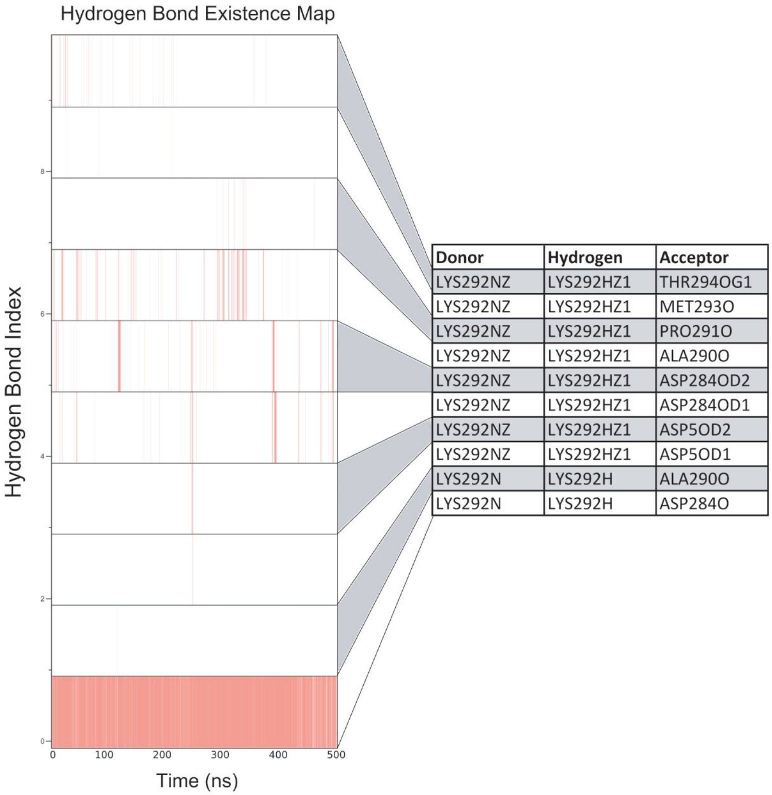
Hydrogen bonds
☐ none
🟥 present



Donor	Hydrogen	Acceptor
LYS292NZ	LYS292HZ1	THR294OG1
LYS292NZ	LYS292HZ1	MET293O
LYS292NZ	LYS292HZ1	PRO291O
LYS292NZ	LYS292HZ1	ALA290O
LYS292NZ	LYS292HZ1	ASP284O
LYS292NZ	LYS292HZ1	ASP284OD2
LYS292NZ	LYS292HZ1	ASP284OD1
LYS292N	LYS292H	ASP284O

K292-B Vs chain B

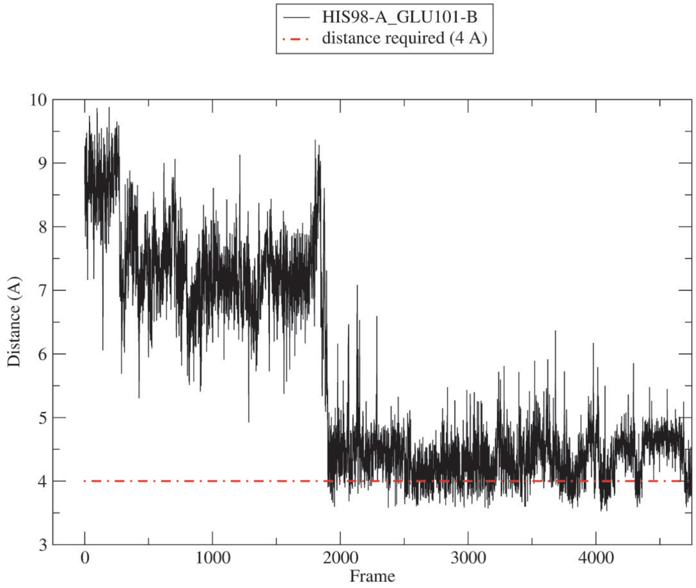
Hydrogen bonds
☐ none
🟥 present



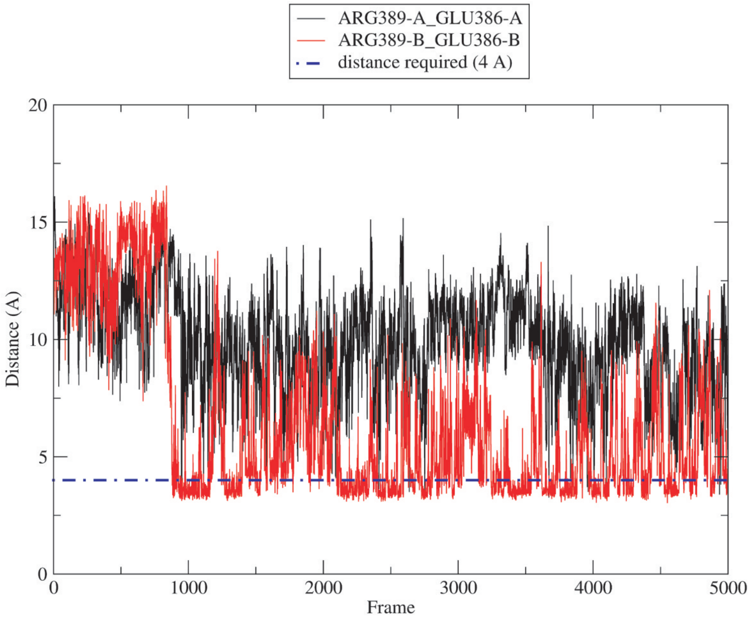
Donor	Hydrogen	Acceptor
LYS292NZ	LYS292HZ1	THR294OG1
LYS292NZ	LYS292HZ1	MET293O
LYS292NZ	LYS292HZ1	PRO291O
LYS292NZ	LYS292HZ1	ALA290O
LYS292NZ	LYS292HZ1	ASP284OD2
LYS292NZ	LYS292HZ1	ASP284OD1
LYS292NZ	LYS292HZ1	ASP5OD2
LYS292NZ	LYS292HZ1	ASP5OD1
LYS292N	LYS292H	ALA290O
LYS292N	LYS292H	ASP284O

Supplementary Figure S5C

H98 Salt bridge

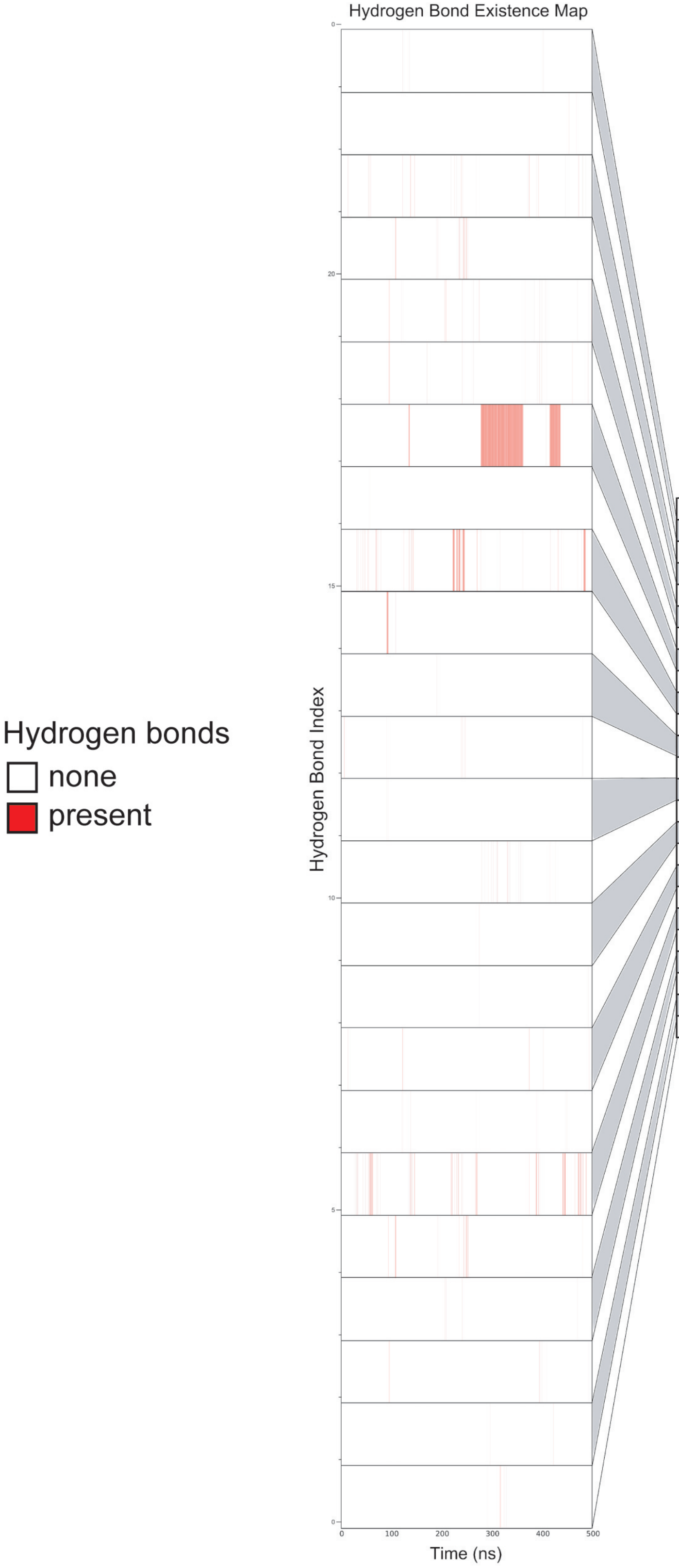


R389 Salt bridge



Supplementary Figure S5D

R389-A Vs chain A



Donor	Hydrogen	Acceptor
ARG389NH2	ARG389HH21	ASN403O
ARG389NH2	ARG389HH21	ASN403ND2
ARG389NH2	ARG389HH21	ASN403OD1
ARG389NH2	ARG389HH21	ASN393OD1
ARG389NH2	ARG389HH21	GLU386OE2
ARG389NH2	ARG389HH21	GLU386OE1
ARG389NH1	ARG389HH11	ASN403O
ARG389NH1	ARG389HH11	ASN403ND2
ARG389NH1	ARG389HH11	ASN403OD1
ARG389NH1	ARG389HH11	GLY402O
ARG389NH1	ARG389HH11	ASN393ND2
ARG389NH1	ARG389HH11	ASN393OD1
ARG389NH1	ARG389HH11	ARG389O
ARG389NH1	ARG389HH11	PRO388O
ARG389NH1	ARG389HH11	GLU386OE2
ARG389NH1	ARG389HH11	GLU386OE1
ARG389NE	ARG389HE	ASN403O
ARG389NE	ARG389HE	ASN403ND2
ARG389NE	ARG389HE	ASN403OD1
ARG389NE	ARG389HE	ASN393OD1
ARG389NE	ARG389HE	GLU386OE2
ARG389NE	ARG389HE	GLU386OE1
ARG389N	ARG389H	ASN387O
ARG389N	ARG389H	GLU386O

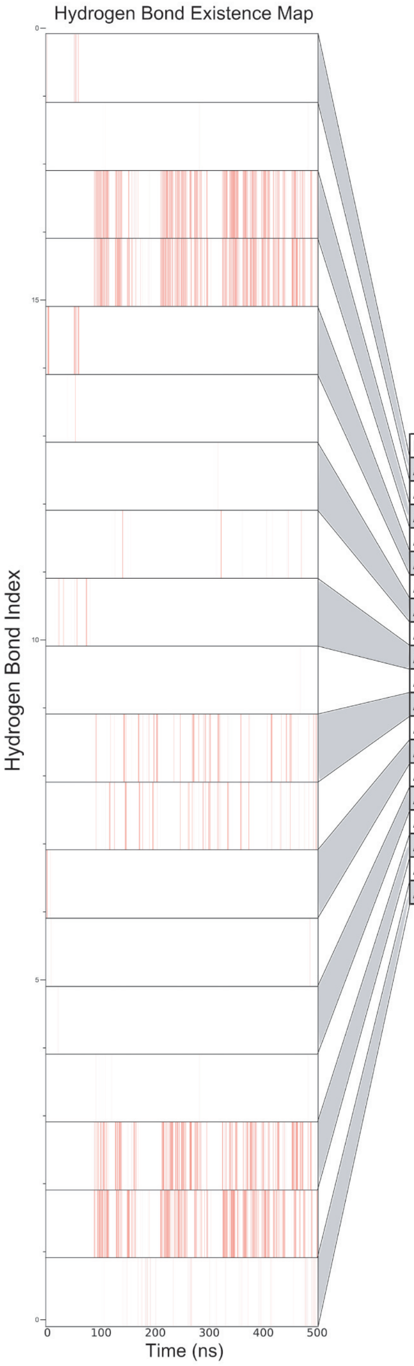
Supplementary Figure S5E

R389-B Vs chain B

Hydrogen bonds

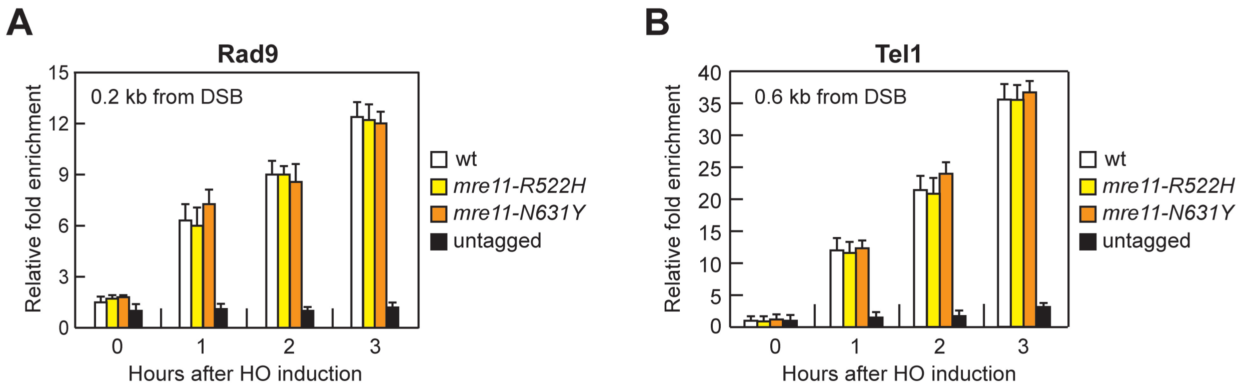
☐ none

🟥 present



Donor	Hydrogen	Acceptor
ARG389NH2	ARG389HH21	ASN403OD1
ARG389NH2	ARG389HH21	GLU386O
ARG389NH2	ARG389HH21	GLU386OE2
ARG389NH2	ARG389HH21	GLU386OE1
ARG389NH1	ARG389HH11	ASN403OD1
ARG389NH1	ARG389HH11	SER392OG
ARG389NH1	ARG389HH11	ARG390NH2
ARG389NH1	ARG389HH11	ARG389O
ARG389NH1	ARG389HH11	PRO388O
ARG389NH1	ARG389HH11	GLU386O
ARG389NH1	ARG389HH11	GLU386OE2
ARG389NH1	ARG389HH11	GLU386OE1
ARG389NE	ARG389HE	ASN403OD1
ARG389NE	ARG389HE	ARG389O
ARG389NE	ARG389HE	PRO388O
ARG389NE	ARG389HE	GLU386O
ARG389NE	ARG389HE	GLU386OE2
ARG389NE	ARG389HE	GLU386OE1
ARG389N	ARG389H	GLU386O

Supplementary Figure S6



Supplementary Figure S7

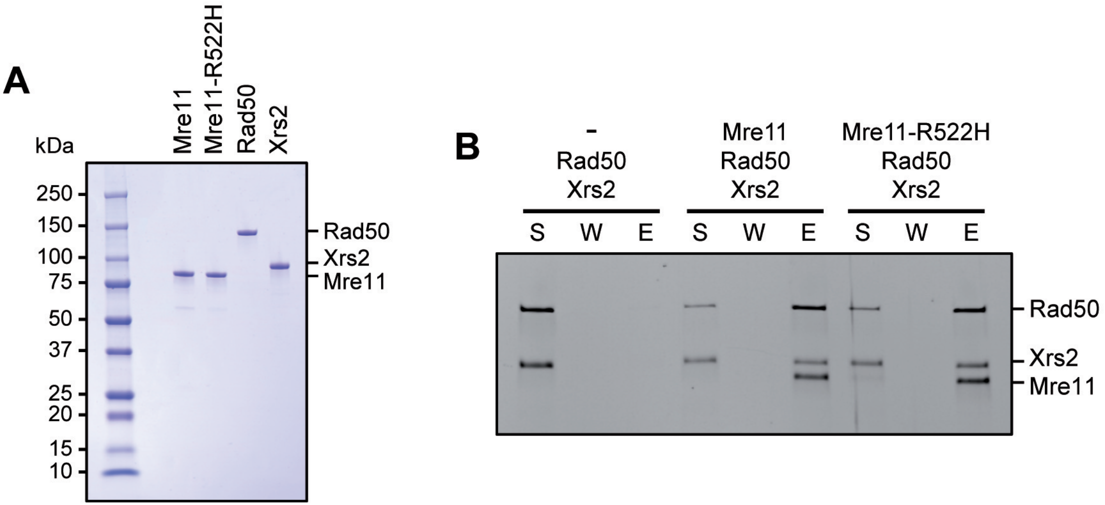


Table S1. Strains used in this study.

Strain	**Relevant genotype**	**Source**
W303	*MAT*a/α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535*	
YLL3658.3	*W303 MATa MRE11-HPH*	This study
YLL1070.1	*W303 MATa sae2Δ::HIS*	This study
YLL1069.3	*W303 MATa sae2Δ::KANMX*	1
YLL3743.8	*W303 MATa MRE11-H98Y-HPHMX sae2Δ::HIS3*	This study
YLL3760.1	*W303 MATa MRE11-K292E-HPHMX sae2Δ::KANMX*	This study
YLL3735.4	*W303 MATa MRE11-R389C-HPHMX sae2Δ::KANMX*	This study
YLL3766.1	*W303 MATa MRE11-T426I-HPHMX sae2Δ::KANMX*	This study
YLL3736.2	*W303 MATa MRE11-R522H-HPHMX sae2Δ::KANMX*	This study
YLL3737.1	*W303 MATa MRE11-N631Y-HPHMX sae2Δ::KANMX*	This study
YLL3738.1	*W303 MATa MRE11-R651X-HPHMX sae2Δ::KANMX*	This study
DMP6605/3C	*W303 MATa MRE11-H98Y-HPHMX*	This study
DMP6413/3D	*W303 MATa MRE11-K292E-HPHMX*	This study
DMP6562/2A	*W303 MATa MRE11-R389C-HPHMX*	This study
DMP6700/1A	*W303 MATa MRE11-T426I-HPHMX*	This study
DMP6441/1D	*W303 MATa MRE11-R522H-HPHMX*	This study
DMP6399/9C	*W303 MATa MRE11-N631Y-HPHMX*	This study
DMP6400/4C	*W303 MATa MRE11-R651X-HPHMX*	This study
DMP6041/6B	*W303 MATa mre11-H125N::URA3 sae2Δ::KANMX*	This study
YLL4019.1	*W303 MATa mre11- H98Y, H125N::URA3 sae2Δ::KANMX*	This study
YLL4020.2	*W303 MATa mre11-H125N, K292E::URA3 sae2Δ::KANMX*	This study
YLL4021.1	*W303 MATa mre11-H125N, R389C::URA3 sae2Δ::KANMX*	This study
DMP6082/6D	*W303 MATα sae2Δ::KANMX exo1Δ::LEU2*	This study
DMP6659/6C	*W303 MATa MRE11-H98Y-HPHMX sae2Δ::KANMX exo1Δ::LEU2*	This study
DMP6660/8A	*W303 MATa MRE11-K292E-HPHMX sae2Δ::KANMX exo1Δ::LEU2*	This study
DMP6661/6A	*W303 MATa MRE11-R389C-HPHMX sae2Δ::KANMX exo1Δ::LEU2*	This study
DMP6110/10A	*W303 MATa sae2Δ::KANMX dna2-1*	This study
DMP6605/1C	*W303 MATa sae2Δ::KANMX MRE11-H98Y-HPHMX dna2-1*	This study
DMP6658/10D	*W303 MATα sae2Δ::KANMX MRE11-K292E-HPHMX dna2-1*	This study
DMP6606/8A	*W303 MATα sae2Δ::KANMX MRE11-R389C-HPHMX dna2-1*	This study
DMP5066/2A	*W303 MATα sgs1Δ::TRP1*	This study
JKM139	*MAT*a *hmlΔ::ADE1, hmrΔ::ADE1, ade1-100, lys5, leu2-3,112, trp1::hisG ura3-52, ho, ade3::GAL-HO site*	2
YLL3188.3	*JKM139 MATa MRE11-3HA::URA3*	This study
YLL3879.2	*JKM139 MATa MRE11-H98Y-3HA::URA3*	This study
YLL3868.1	*JKM139 MATa MRE11-K292E-3HA::URA3*	This study
YLL3880.6	*JKM139 MATa MRE11-R389C-3HA::URA3*	This study
YLL4022.1	*JKM139 MATa MRE11-T426I-3HA::URA3*	This study

YLL3841.2	*JKM139 MATa MRE11-R522H-3HA::URA3*	This study
YLL3070.1	*JKM139 MATa MRE11-N631Y-3HA::URA3*	This study
DMP6488/10B	*JKM139 MATa MRE11-R651X-3HA::URA3*	This study
YLL1523.3	*JKM139 MATa sae2Δ::KANMX*	1
DMP6574/9C	*JKM139 MATa MRE11-H98Y-HPHMX*	This study
YLL3869.1	*JKM139 MATa MRE11-H98Y-HPHMX sae2Δ::KANMX*	This study
DMP6414/1C	*JKM139 MATa MRE11-K292E-HPHMX*	This study
YLL3768.11	*JKM139 MATa MRE11-K292E-HPHMX sae2Δ::KANMX*	This study
DMP6556/1A	*JKM139 MATa MRE11-R389C-HPHMX*	This study
YLL3739.1	*JKM139 MATa MRE11-R389C-HPHMX sae2Δ::KANMX*	This study
DMP6401/9B	*JKM139 MATa MRE11-R522H-HPHMX*	This study
YLL3740.4	*JKM139 MATa MRE11-R522H-HPHMX sae2Δ::KANMX*	This study
DMP6415/9D	*JKM139 MATa MRE11-N631Y-HPHMX*	This study
DMP6515/8C	*JKM139 MATa MRE11-N631Y-HPHMX sae2Δ::KANMX*	This study
DMP6023/5A	*JKM139 MATa SGS1-3HA::URA3*	1
DMP6239/8C	*JKM139 MATa SGS1-3HA::URA3 sae2Δ::KANMX*	This study
DMP6688/2C	*JKM139 MATa SGS1-3HA::URA3 MRE11-H98Y-HPHMX*	This study
DMP6647/2D	*JKM139 MATa SGS1-3HA::URA3 MRE11-K292E-HPHMX*	This study
DMP6689/4C	*JKM139 MATa SGS1-3HA::URA3 MRE11-R389C-HPHMX*	This study
DMP6688/4A	*JKM139 MATa SGS1-3HA::URA3 MRE11-H98Y-HPHMX sae2Δ::KANMX*	This study
DMP6647/7C	*JKM139 MATa SGS1-3HA::URA3 MRE11-K292E-HPHMX sae2Δ::KANMX*	This study
DMP6648/5D	*JKM139 MATa SGS1-3HA::URA3 MRE11-R389C-HPHMX sae2Δ::KANMX*	This study
YLL3421.2	*JKM139 MATa RAD9-3HA::TRP1*	This study
DMP6574/8A	*JKM139 MATa RAD9-3HA::TRP1 MRE11-H98Y-HPHMX*	This study
DMP6555/6B	*JKM139 MATa RAD9-3HA::TRP1 MRE11-K292E-HPHMX*	This study
DMP6557/6C	*JKM139 MATa RAD9-3HA::TRP1 MRE11-R389C-HPHMX*	This study
DMP6766/1A	*JKM139 MATa RAD9-3HA::TRP1 MRE11-H98Y-HPHMX sae2Δ::KANMX*	This study
DMP6767/2A	*JKM139 MATa RAD9-3HA::TRP1 MRE11-K292E-HPHMX sae2Δ::KANMX*	This study
DMP6768/3A	*JKM139 MATa RAD9-3HA::TRP1 MRE11-R389C-HPHMX sae2Δ::KANMX*	This study
DMP5816/1A	*JKM139 MATa RAD9-3HA::TRP1 sae2Δ::KANMX*	1
YLL3222.6	*JKM139 MATa TEL1-3HA::NATMX*	1
DMP6435/1A	*JKM139 MATa TEL1-3HA::NATMX sae2Δ::KANMX*	This study
DMP6628/11C	*JKM139 MATa TEL1-3HA::NATMX MRE11-H98Y-HPHMX*	This study
DMP6627/2D	*JKM139 MATa TEL1-3HA::NATMX MRE11-K292E-HPHMX*	This study
DMP6626/1C	*JKM139 MATa TEL1-3HA::NATMX MRE11-R389C-HPHMX*	This study
DMP6628/1B	*JKM139 MATa TEL1-3HA::NATMX MRE11-H98Y-HPHMX sae2Δ::KANMX*	This study
DMP6627/10D	*JKM139 MATa TEL1-3HA::NATMX MRE11-K292E-HPHMX sae2Δ::KANMX*	This study

DMP6628/3B	*JKM139 MATa TEL1-3HA::NATMX MRE11-R389C-HPHMX sae2Δ::KANMX*	This study
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DMP6463/3D	*JKM139 MATa TEL1-3HA::NATMX MRE11-R522H-HPHMX*	This study
DMP6645/5D	*JKM139 MATa TEL1-3HA::NATMX MRE11-N631Y-HPHMX*	This study
YJK40.6	*MATΔ hmlΔ hmrΔ can1 lys5 ade2 leu2 trp1 ura3 his3 ade3::GAL-HO VII::TRP1-HO LacI-GFP::URA3 LacO::LYS5 LacO::KanR*	3
YLL4008.1	*YJK40.6 MRE11-H98Y-HPHMX*	This study
YLL4001.1	*YJK40.6 MRE11-K292E-HPHMX*	This study
YLL4002.4	*YJK40.6 MRE11-R389C-HPHMX*	This study
YLL3811.1	*YJK40.6 MRE11-R522H-HPHMX*	This study
YLL3812.2	*YJK40.6 MRE11-N631Y-HPHMX*	This study
YLL1709.11	*YJK40.6 sae2Δ::NATMX*	4
YLL4023.1	*YJK40.6 MRE11-H98Y-HPHMX sae2Δ::NATMX*	This study
YLL4014.3	*YJK40.6 MRE11-K292E-HPHMX sae2Δ::NATMX*	This study
YLL4022.4	*YJK40.6 MRE11-R389C-HPHMX sae2Δ::NATMX*	This study
YLL3820.2	*YJK40.6 MRE11-R522H-HPHMX sae2Δ::NATMX*	This study
YLL3821.4	*YJK40.6 MRE11-N631Y-HPHMX sae2Δ::NATMX*	This study
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DMP6803	*JKM139 MATa/α MRE11-3HA::URA3/MRE11-3HA::URA3*	This study
DMP6734	*JKM139 MATa/α MRE11-18MYC::TRP1/MRE11-3HA::URA3*	This study
DMP6735	*JKM139 MATa/α MRE11-H98Y-18MYC::TRP1/MRE11-H98Y-3HA::URA3*	This study
tGI354	*ho hmlΔ::ADE1 MATa-inc hmrΔ::ADE1 ade1 leu2-3;112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HPHMX*	5
YLL3996.1	tGI354 *MRE11-R522H-KANMX*	This study
YLL3964.4	tGI354 *MRE11-R522H-KANMX sae2Δ::NATMX*	This study
YLL3914.2	tGI354 *sae2Δ::NATMX*	This study

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Backbone root-mean-square deviation (RMSD) profiles for ScRad50-Mre11 tetramer (**A**) and ScMre11 dimer (**B**). RMSD is calculated using as reference structure the starting point structure of the dynamic.

Supplementary Figure S2. Structural alignment of Mre11 orthologs from *Homo sapiens* (PDB ID:3T1I), *Schizosaccharomyces pombe* (PDB ID:4FCX), *Chaetomium thermophilum* (PDB ID:4KYE and 5DA9 for RBD region), *Methanococcus jannaschii* (PDB ID:3AV0) and *Pyrococcus furiosus* (PDB ID:1II7) with ScMre11 structural model obtained in this work. Secondary structures are indicated, wherever the structure is available (otherwise the sequence is marked by a red rectangle), as follows: yellow rectangles, alpha helix; green rectangles, beta sheets. Alignment residues coloring is according to ClustalW style. The consensus sequence and histograms representing residue conservation and backbone RMSD are indicated on top. Functional regions of the proteins are indicated by open boxes: blue box, endonuclease domain; red box, capping domain; orange box, latching loop; purple box, Rad50-binding domain. Black open boxes highlight endonuclease motifs. Stars are represented above the consensus for Mg^{2+} binding residues relevant for endonuclease activity; a red dot marks the catalytic His residue; green triangles indicate the residues identified for Xrs2/Nbs1 binding in SpMre11.

Supplementary Figure S3. Suppression in the JKM139 background. Exponentially growing cultures were serially diluted (1:10) and each dilution was spotted out onto YEPD plates with or without CPT or phleomycin at the indicated concentrations.

Supplementary Figure S4. Mre11-Xrs2 interaction. Protein extracts were analyzed by western blotting with anti-HA (Mre11), anti-Myc (Xrs2) antibodies either directly (Total) or after Mre11 immunoprecipitation (IPs) with anti-HA antibodies.

Supplementary Figure S5. H-bond and salt bridge analysis involving relevant residues during Mre11 dimer molecular dynamics. (**A, B, D, E**) Hydrogen bond existence maps of residues H98 (**A**), K292 (**B**) and R389 (**D, E**). Tables report the acceptor and donor groups for each hydrogen bond. The letters A and B indicate the two Mre11 monomers. (**C**) Salt bridges of H98 and R389 as a function of distance between oxygen atoms of acidic residues and the nitrogen atoms of basic residues. Salt bridge was considered formed if the distance between oxygen and nitrogen is within 4Å.

Supplementary Figure S6. Mre11-R522H and Mre11-N631Y do not affect Rad9 and Tel1 association to DSBs. (**A, B**) ChIP analysis. Exponentially growing YEPR cell cultures were transferred to YEPRG at time zero. Relative fold enrichment of the indicated fusion proteins at the indicated distances from the HO cleavage site was determined after ChIP with anti-HA antibody and subsequent qPCR analysis. Plotted values are the mean value with error bars denoting s.d. (n=3).

Supplementary Figure S7. Purification of Mre11-R522H and complex reconstitution. (**A**) Purified Mre11, Mre11-R522H, Rad50 and Xrs2 proteins were analyzed by SDS-PAGE and Coomassie blue staining. **(B**) 6xHis tagged Mre11 or Mre11-R522H (0.9 µg of each) was incubated with Rad50 (1.5 µg) and Xrs2 (1 µg) and protein complexes were captured by Ni-NTA agarose. The supernatant (S) containing unbound proteins, the wash (W), and the eluate (E) fractions were analyzed by SDS-PAGE and Oriole fluorescent gel staining.

SUPPLEMENTARY FILES

Supplementary File 1. PDB file of the structure of the most populated cluster for ScRad50-Mre11 tetramer.

Supplementary File 2. PDB file of the structure of the most populated cluster for ScMre11 dimer.