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Supplemental Information

Nej1 Interacts with Mre11 to Regulate Tethering and Dna2 Binding at DNA Double-Strand Breaks

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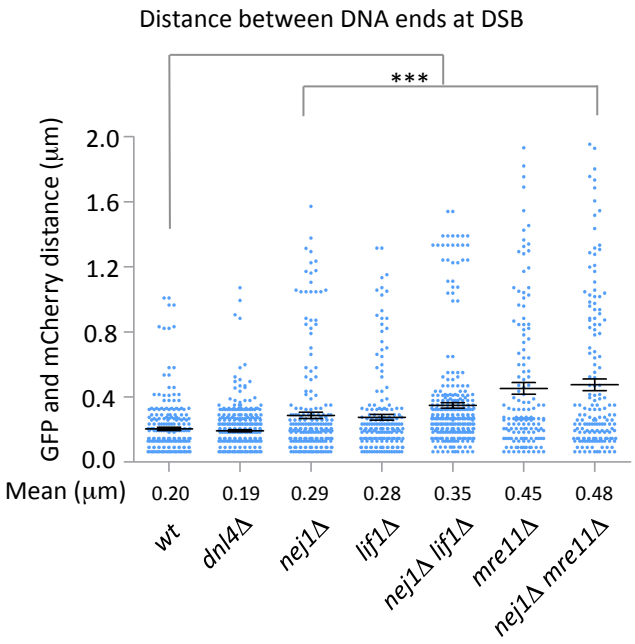
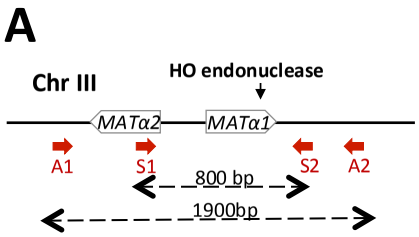
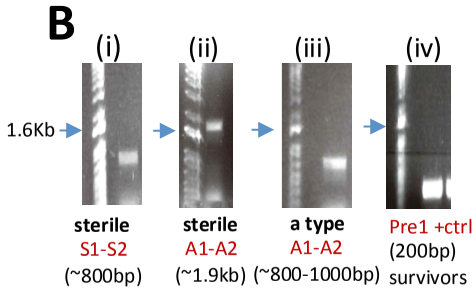


Figure S1 : Scatter data plot showing the tethering of DSB ends in various mutants. Related to Figure 2. The distance between the GFP and mCherry foci was measured in wild type (JC- 4066), dnl4Δ (KD-1106), *nej1*Δ (JC-4364), *lif1*Δ (KD-1069), *nej1*Δ *lif1*Δ (KD-1075), *mre11*Δ (KD-925) and *nej1*Δ *mre11*Δ (KD-1108).

This work was performed in the Dubrana Lab. For HO endonuclease induction, strains were grown on rich medium containing 3% glycerol, 2% lactic acid and 0.05% glucose prior adding 2% galactose for 2h and imaging. Live cell images were acquired using a wide-field microscope based on an inverted microscope (Leica DMI-6000B) equipped with Adaptive Focus Control to eliminate Z drift, a 100x/1.4 NA immersion objective with a Prior NanoScanZ Nanopositioning Piezo Z Stage System, a CMOS camera (ORCA-Flash4.0; Hamamatsu) and a solid state light source (SpectraX, Lumencore). The system is piloted by MetaMorph software (Molecular Device).

For GFP-mCherry two-color images, 25 focal steps of 0.2µm were acquired sequentially for GFP and mRFP with an exposure time of 50ms using solid state 475 and 575 nm diodes and appropriate filters (GFP-mRFP filter; excitation: double BP, 450–490/550–590 nm and dichroic double BP 500–550/600–665 nm; Chroma Technology Corp.). Distance measurement were performed on 2D maximal projection of three-dimensional data sets using Volocity software (PerkinElmer).

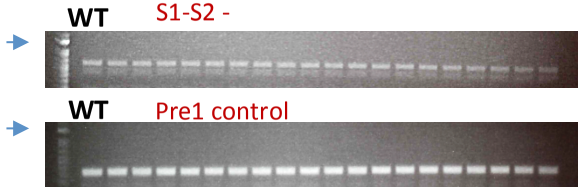


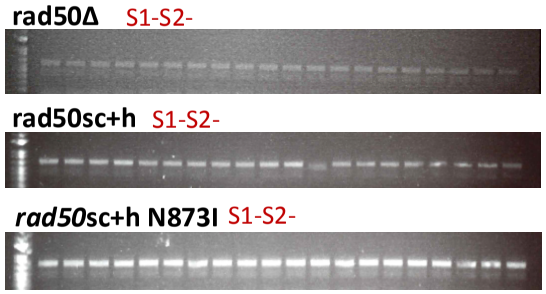


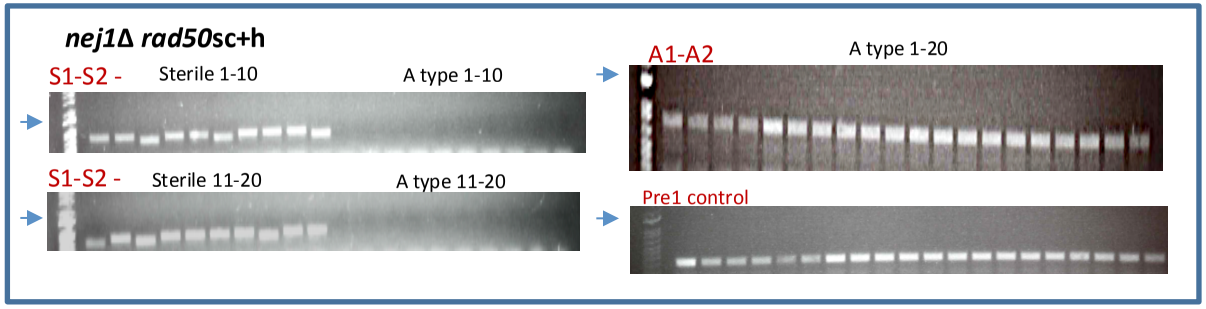
C 20 sterile survivors for each genotype

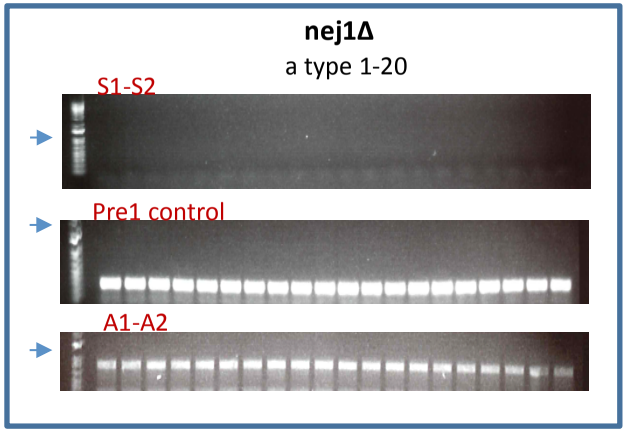
Genotype		events
WT		+CA(9), ΔACA(5), ΔA(3), +ACA(3)
rad50Δ		ΔC(6), ΔACA(7), ΔA(3), ΔCA(4)
*rad50*sc+h		ΔC(5), ΔACA(6), ΔA(3), ΔAC(2), +CA(4)
*rad50*sc+h N873I		ΔACA(5), ΔA(3), ΔAC(2), +CA(4), +ACA(4), ΔGCA(2)
*nej1*Δ *rad50*sc+h		ΔACA(5), ΔA(2), ΔC(5), ΔAC(1), Δ107bp (7)
*nej1*Δ *rad50*sc+h N873I		ΔGCA(3), ΔACAG(1), ΔA(2), ΔC(5), Δ107bp (9)

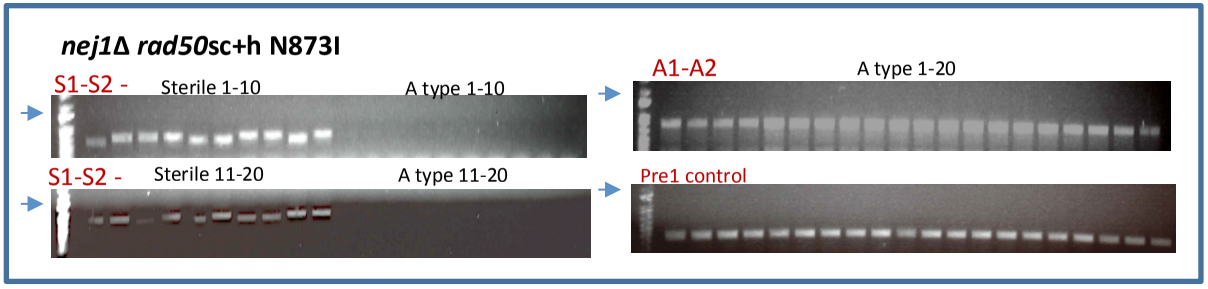
events	Site of event at DSB
+CA	CGCAACACAGTATA
ΔACA	CGCAACAGTATA
ΔA	CGCAACAGTATA
+ACA	CGCAACAACAGTATA
ΔC	CGCAACAGTATA
ΔCA	CGCAACAGTATA
ΔAC	CGCAACAGTATA
ΔGCA	CGCAACAGTATA
ΔACAG	CGCAACAGTATA
Δ107bp	CAG..... CGCAACAGTATAATTT











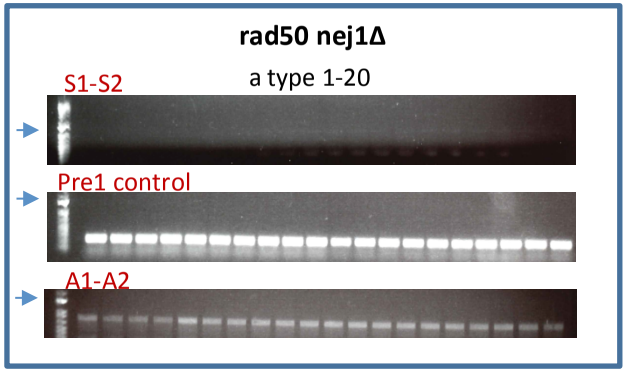


Figure S2 : Survivors were verified by PCR and DNA sequencing. Related to Figure 3. (A) Schematic representation of DSB site in Chromosome III and with primer sets (S1-S2 and A1-A2) used to verify the mutations in sterile and a-type survivors. (B) PCR products using various primer sets - (i) ~800bp PCR product from sterile-type survivors using S1-S2, (ii) ~1900bp PCR product from sterile-type survivors using A1-A2, (iii) ~1000bp PCR product from a-type survivors using A1-A2, (iv) 200bp PCR product from survivors using Pre1 primers. (C.) Sterile survivors were sequenced for WT (JC-727), *rad50*Δ (JC-3313), *rad50*sc+h (JC-4424), *rad50*sc+h N873I (JC-4561), *nej1*Δ rad50sc+h (JC-4476) and *nej1*Δ rad50sc+h N873I (JC-4597). Survivors that were a type were verified to have large genomic deletions of ~800-1100bp by DNA sequencing the PCR product amplified with A1-A2.

Agarose gel pictures showing the PCR products obtained from sterile and a-type survivors using S1-S2, A1-A2 and Pre1 primer set, in the strains WT (JC-727), *rad50*Δ (JC-3313), *rad50*sc +h (JC-4424), r*ad50*sc+h N873I (JC-4561), *nej1*Δ (JC-1342), *nej1*Δ *rad50*Δ (JC-3314), *nej1*Δ *rad50*sc+h (JC-4476) and *nej1*Δ rad50sc+h N873I (JC-4597). The Pre1 primer set was used with all strains but not all are included here due to spacing.

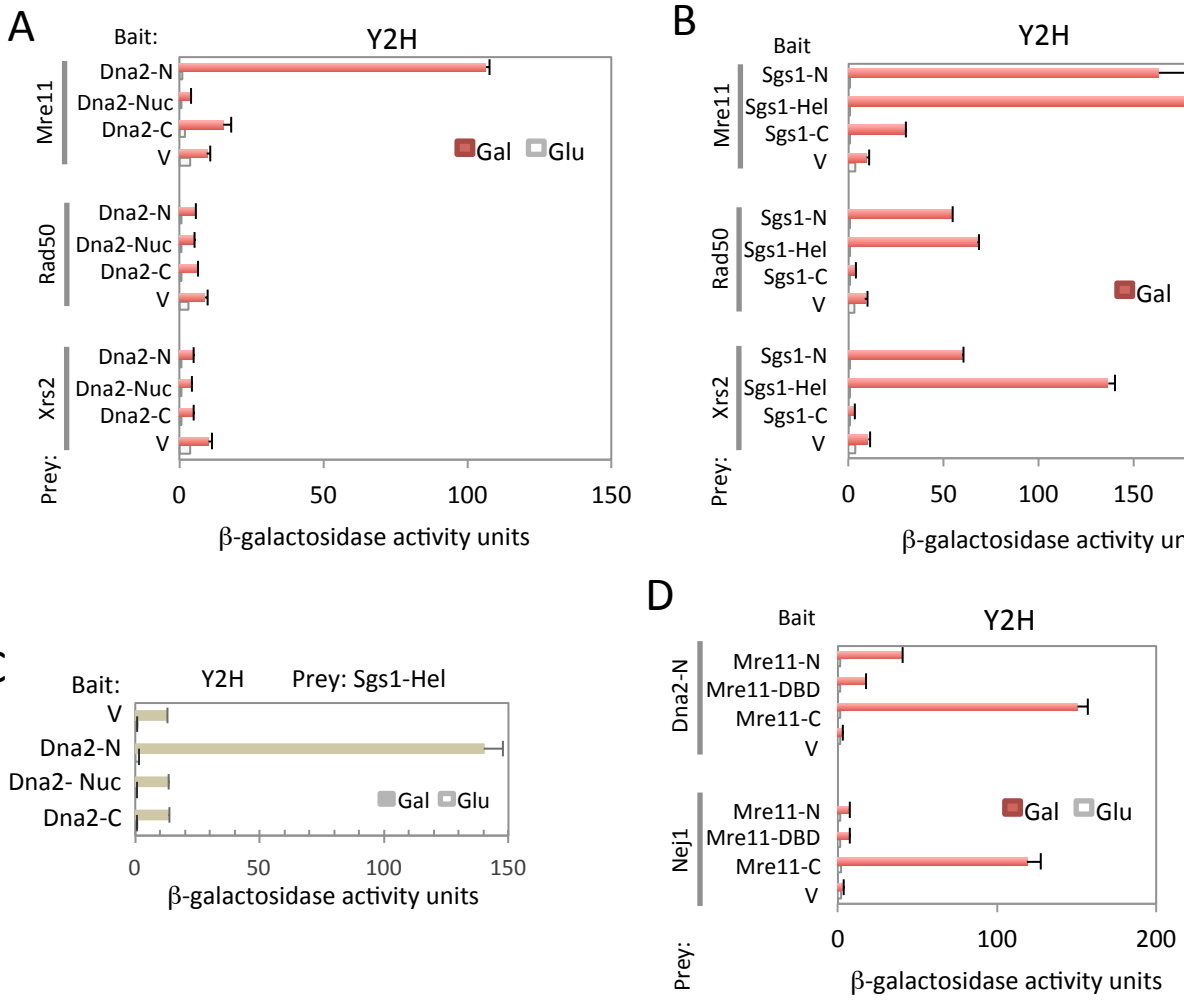


Figure S3: Interaction of MRX with Sgs1 and Dna2. Related to Figure 4. (A and B) Y2H analysis, between regions of Dna2 and Sgs1 fused to lexA-DBD and Mre11, Rad50 and Xrs2 fused to HA-AD, was performed using a quantitative β-galactosidase assay. (C) Y2H analysis, between Helicase domain of Sgs1 and regions of Dna2, was performed using a quantitative β-galactosidase assay. (D) Y2H analysis, between N-terminal region of Dna2 or Nej1 and regions of Mre11, was performed using a quantitative β-galactosidase assay.

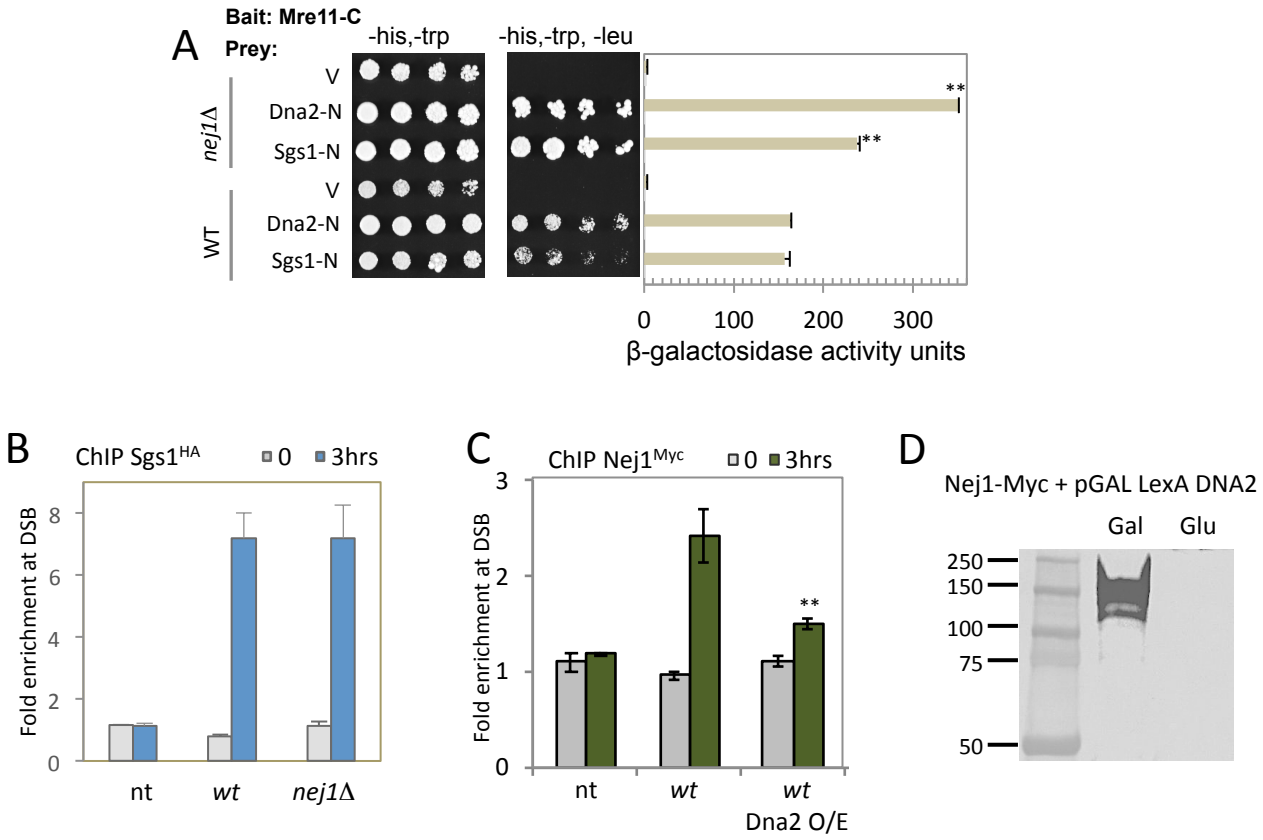


Figure S4: Interplay between Nej1 with Dna2-Sgs1 for interaction with Mre11-C and recruitment to the DSB. **Related to Figure 4**. (A) Y2H analysis, between Sgs1-N or Dna2-N and Mre11-C, was performed in wild type cells and in isogenic cells with *nej1*Δ using a quantitative β-galactosidase assay and a drop assay on drop-out (-His, -Trp, -Leu) selective media plates. (B) Enrichment of $Sgs1^{HA}$ at DSB, at 0 and 3 hour time point, in wild type (JC-4135) and *nej1*Δ (JC-4136) were determined at 0.6kb from DSB. (C) Enrichment of $Nej1^{Myc}$ at DSB, at 0 and 3 hour time point, in wild type (JC-4135) + Y2H plasmid J-965 with or without full length with Dna2 O/E under galactose induction. (D) Cells were grown overnight in –URA with 2% raffinose. Next day, cells were transferred into –URA media with either 2% GLU or 2% GAL for 3 hrs at 30°C for induction of HO and O/E of Dna2. The lower enrichment for Nej1 WT here compared to Fig.1A is like due to growth in selective media.

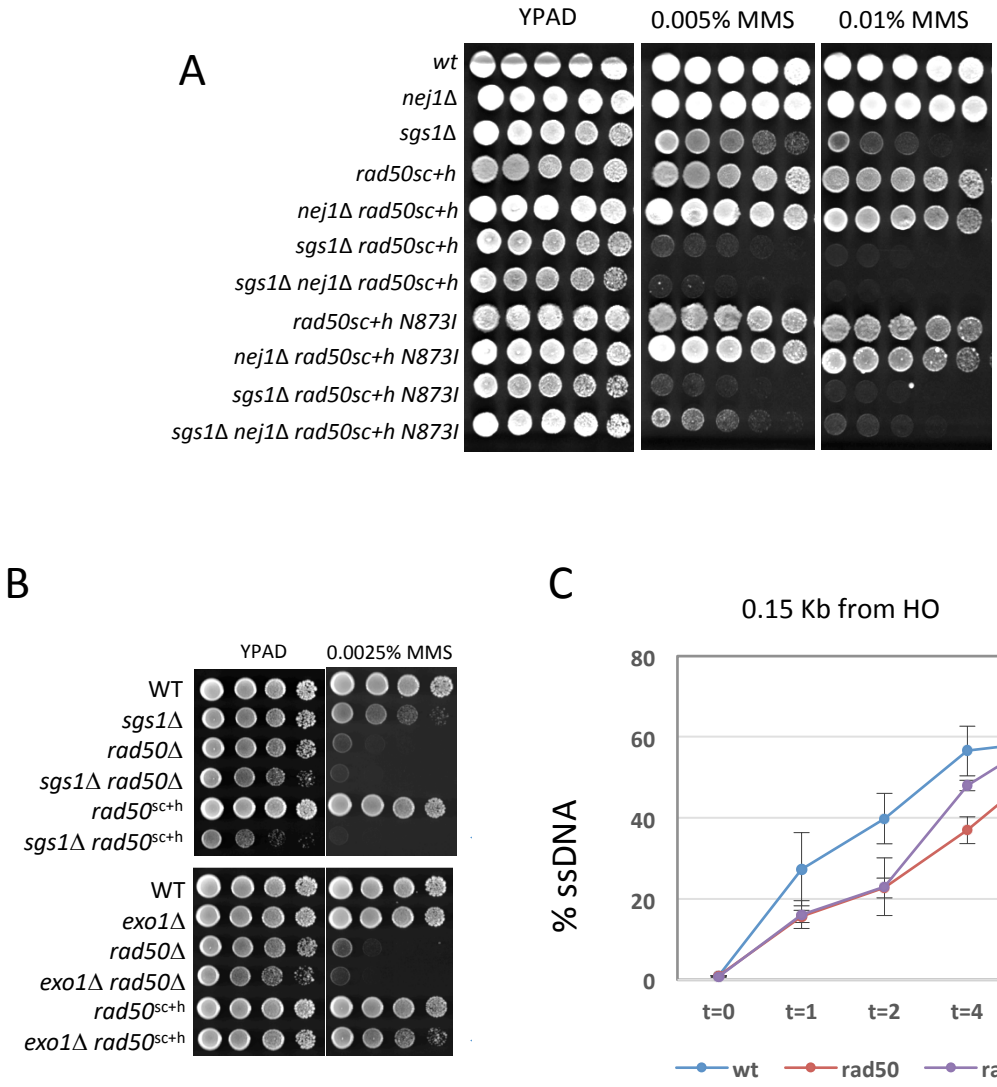


Figure S5. Genetic interactions and resection in *rad50*Δ and *rad50*sc+h. Related to Figure 6. (A) Five-fold serial dilutions were spotted on YPAD, YPAD + 0.005% MMS and YPAD + 0.01% MMS. Strains used include wild type (JC-727), *nej1Δ* (JC-1342), *sgs1Δ* (JC-3757), *rad50sc+h* (JC-4424), *nej1Δ rad50sc+h* (JC-4476), *sgs1Δ rad50sc+h* (JC-4478), *nej1Δ sgs1Δ rad50sc+h* (JC-4479), *rad50sc+h* N873I (JC-4561), *nej1Δ rad50sc+h* N873I (JC-4597), *sgs1Δ rad50sc+h* N873I (JC-4607) and *nej1Δ sgs1Δ rad50sc+h* N873I (JC-4605).

(B) Five-fold serial dilutions of the following strains were spotted on YPAD and YPAD + 0.0025% MMS. Strains used include wild type (JC-727), *sgs1Δ* (JC-3757), *rad50Δ* (JC-3313), *sgs1Δ rad50Δ* (JC-3760), *rad50sc+h* (JC-4424) and *rad50sc+h sgs1Δ* (JC-4478) *exo1Δ* (JC-3767), *rad50Δ exo1Δ* (JC-3769), *rad50sc+h exo1Δ* (JC-4519).

(C) Resection of DNA 0.15kb away from the HO DSB, as measured by %ssDNA, 0 to 8 hrs post DSB induction in asynchronous cells in wild type (JC-3585), *rad50Δ* (JC-3882) and *rad50sc+h* (JC-4458). Error bars represent the standard error of three replicates.

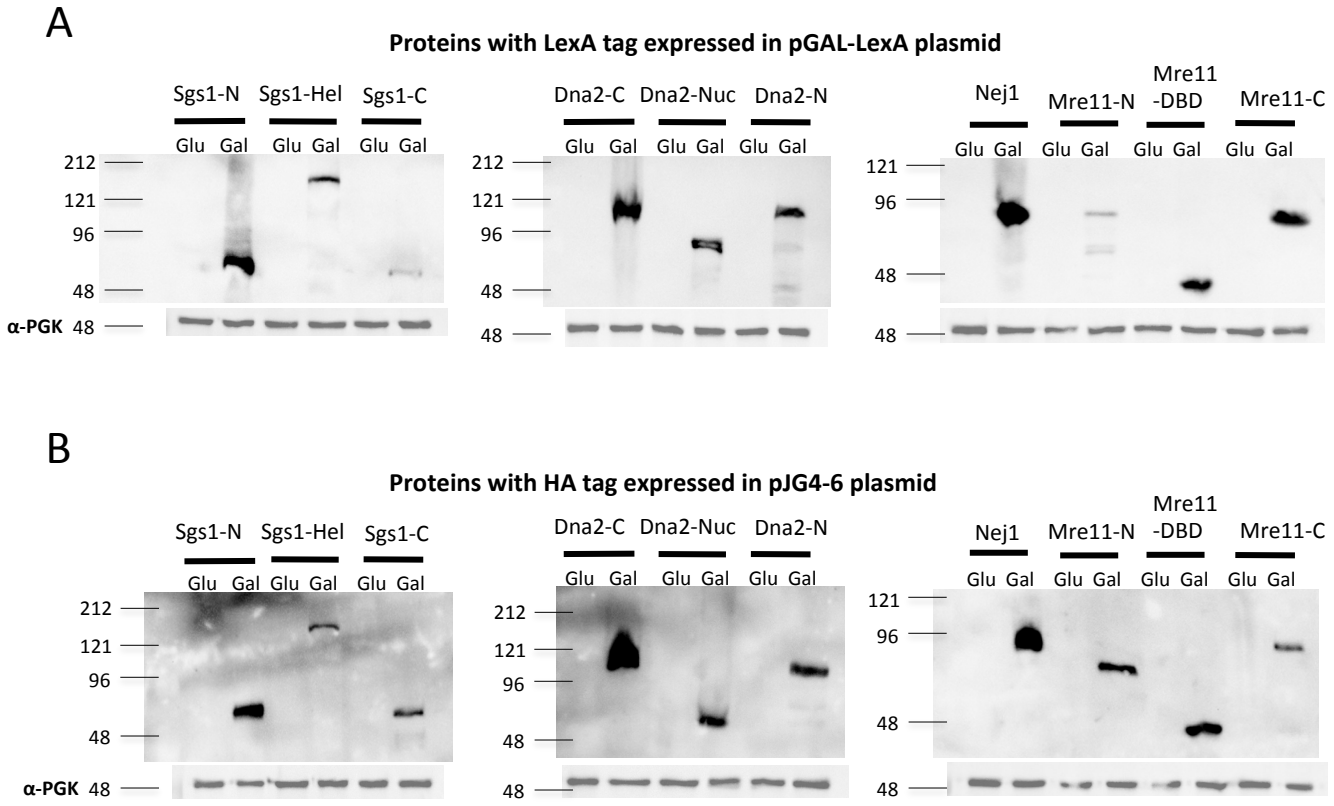


Figure S6. Expression of proteins upon galactose induction. Related to Figure1 and 4. (A) Western blots showing the expression of proteins fused to LexA tag upon galactose induction. (B) Western blots showing the expression of proteins fused to HA tag upon galactose induction. Polyglycerate kinase (PGK) was used as a loading control.

Table S1. Yeast strains used in this study. Related to Figures 1-6 and Figures S1-S6.

Strain	Genotype	Reference
JC-727	MAT α; *hml*::*ADE1 hmr*::*ADE1 ade3*::GAL-HO *ade1*-100 *leu*2-3, 112 *lys*5 *trp*1::*hisG ura3*-52	JKM179, [Lee et al. 1998]
JC-1280	MAT α; leu2::proLeu2-lexAop6 his3 ura3-52	This study
JC-1342	JC-727 with *nej1*Δ::*KanMX6*	MAV015, [Valencia et al. 2001]
JC-1687	JC-727 with *NEJ1-13MYC*::*TRP1*	Sorenson et al. 2017
JC-3306	JC-727 with *RAD50-6HA*::*TRP1*	This study
JC-3311	JC-1687 with *rad50*Δ::*NatRMX4*	This study
JC-3313	JC-727 with *rad50*Δ::*URA3*	Sorenson et al. 2017
JC-3314	JC-3313 with *nej1*Δ::*KanMX6*	Sorenson et al. 2017
JC-3319	JC-727 with *LIF1-6HA*::*TRP1*	This study
JC-3585	MAT a; *hml*::*ADE1 hmr*::*ADE1 ade3*::GAL-HO *ade1*-100 *leu*2-3, 112 *lys*5 *trp*1::*hisG ura3*-52	Sorenson et al. 2017
JC-3677	JC-1687 with *mre11*Δ::*KanMX6*	This study
JC-3688	JC-1280 with *rad50*Δ::*KanMX6*	This study
JC-3757	JC-727 with *sgs1*Δ::*NatRMX4*	This study
JC-3760	JC-3313 with *sgs1*Δ::*NatRMX4*	This study
JC-3767	JC-727 with *exo1*Δ::*NatRMX4*	This study
JC-3769	JC-3313 with *exo1*Δ::*NatRMX4*	This study
JC-3882	JC-3585 with *rad50*Δ::*URA3*	This study
JC-3884	JC-3585 with *nej1*Δ::*KanMX6*	This study
JC-3887	JC-3882 with *nej1*Δ::*KanMX6*	This study
JC-4066	JC-3585 with *ura3*::LacI-mCherry-*URA3*; *leu2*::TetR-GFP-*LEU2*; TAF2-LacOpFx-*TRP1*; 4.4kb MAT-TetO-*LEU2*.	This study
JC-4094	JC-4066 with *rad50*Δ::*NatRMX4*	This study
JC- 4117	JC-727 with *DNA2-6HA*::*TRP1*	This study
JC-4118	JC-4117 with *nej1*Δ::*KanMX6*	This study
JC-4135	JC-727 with *SGS1-6HA*::*TRP1*	This study
JC-4136	JC-4135 with *nej1*Δ::*KanMX6*	This study
JC-4138	JC-4135 with *rad50*Δ::*URA3*	This study
JC-4355	JC-4094 with *nej1*Δ::*KanMX6*	This study
JC-4364	JC-4066 with *nej1*Δ::*KanMX6*	This study
JC-4424	JC-727 with *rad50*sc+h::*HphMX4*	This study
JC-4425	JC-727 with *rad50*sc::*HphMX4*	This study
JC-4457	JC-4424 with *SGS1-6HA*::*TRP1*	This study
JC-4458	JC-3585 with *rad50*sc+h::*HphMX4*	This study
JC-4466	JC-4066 with *rad50*sc+h::*HphMX4*	This study
JC-4471	JC-4458 with *nej1*Δ::*KanMX6*	This study
JC-4476	JC-4424 with *nej1*Δ:: *KanMX6*	This study
JC-4478	JC-4424 with *sgs1*Δ::*NatRMX4*	This study
JC-4479	JC-4476 with *sgs1*Δ::*NatRMX4*	This study
JC-4496	JC-727 with *RAD50sc-6HA*::*TRP1*	This study
JC-4497	JC-727 with *RAD50sc+h-6HA*::*TRP1*	This study
JC-4502	JC-4117 with *sgs1*Δ::*NatRMX4*	This study

JC-4503	JC-4117 with *rad50*Δ::*URA3*	This study
JC-4515	JC-727 with *XRS2-6HA*::*TRP1*	This study
JC-4516	JC-4515 with *rad50*Δ::*URA3*	This study
JC-4517	JC-4515 with *rad50*sc::*HphMX4*	This study
JC-4518	JC-4515 with *rad50*sc+h::*HphMX4*	This study
JC-4519	JC-4424 with *exo1*Δ::*NatRMX4*	This study
JC-4526	JC-1687 with *rad50*sc+h::*HphMX4*	This study
JC-4528	JC-1687 with *sgs1*Δ:: *NatRMX4*	This study
JC-4531	JC-4117 with *rad50*sc+h::*HphMX4*	This study
JC-4533	JC-4466 with *nej1*Δ:: *KanMX6*	This study
JC-4556	JC-1280 with *nej1*Δ::*KanMX6*	This study
JC-4559	JC-4066 with *rad50sch-N873I::HphMX4*	This study
JC-4561	JC-727 with *rad50sch-N873I::HphMX4*	This study
JC-4563	JC-4561 with *NEJ1-13MYC::TRP1*	This study
JC-4564	JC-4561 with *DNA2-6HA::TRP1*	This study
JC-4565	JC-4561 with *SGS1-6HA::TRP1*	This study
JC-4566	JC-3319 with *rad50sch-N873I::HphMX4*	This study
JC-4567	JC-3585 with *rad50sch-N873I::HphMX4*	This study
JC-4569	JC-4567 with *nej1*Δ::*KanMX6*	This study
JC-4572	JC-4515 with *rad50sch-N873I*::*HphMX4*	This study
JC-4579	JC-3319 with *rad50*sc+h::*HphMX4*	This study
JC-4580	JC-4364 with *rad50sch-N873I::HphMX4*	This study
JC-4597	JC-4561 with *nej1*Δ::*KanMX6*	This study
JC-4605	JC-4597 with *sgs1*Δ:: *NatRMX4*	This study
JC-4607	JC-4561 with *sgs1*Δ:: *NatRMX4*	This study
KD-925	JC-4066 with *mre11*Δ::*HphMX4*	This study
KD-1069	JC-4066 with *lif1*Δ::*KanMX6*	This study
KD-1073	JC-4066 with *nej1*Δ::*KanMX6*	This study
KD-1075	KD-1073 with *lif1*Δ::*KanMX6*	This study
KD-1106	JC-4066 with *dnl4*Δ::*KanMX6*	This study
KD-1108	KD-1073 with *mre11Δ::HphMX4*	This study

Table S2. Plasmids used in this study. Related to Figures 1 and 4, and Figures S3-S4 and S6.

Plasmid number	Description
J-965	pGAL-lexA
J-1493	pJG4-6
J-359	pSH18-34 lexAGal1-lacZ
J-123	J-1493 with Nej1
J-125	J-965 with Nej1
J-183	J-1493 with Xrs2
J-196	J-1493 with Mre11
J-198	J-1493 with Rad50
J-454	J-1493 with Sgs1-(9-275) N-term
J-455	J-1493 with Sgs1-(290-1180) Helicase
J-572	J-1493 with Sgs1-(1120-1430) C-term
J-1043	J-965 with Sgs1-(9-275) N-term
J-1044	J-965 with Sgs1-(290-1180) Helicase
J-1045	J-965 with Sgs1-(1120-1430) C-term
J-1855	J-965 with Dna2-(1-440) N-term
J-1856	J-965 with Dna2-(441-920) Nuclease
J-1857	J-965 with Dna2-(921-1522) C-term
J-1858	J-1493 with Dna2-(1-440) N-term
J-1859	J-1493 with Dna2-(441-920) Nuclease
J-1860	J-1493 with Dna2-(921-1522) C-term
J-1868	J-965 with Mre11-(1-271) N-term
J-1869	J-1493 with Mre11-(1-271) N-term
J-1870	J-965 with Mre11-(272-422) DBD
J-1871	J-1493 with Mre11-(272-422) DBD
J-1872	J-965 with Mre11-(423-692) C-term
J-1873	J-1493 with Mre11-(423-692) C-term

Table S3. Primers and Probes used in this study. Related to STAR Methods

Primer Name	Primer Sequence (5'-3')
HO2 Forward Primer	TTGCCCACTTCTAAGCTGATTTC
HO2 Reverse Primer	GTACTTTTCTACATTGGGAAGCAATAAA
HO2 Probe	FAM-ATGATGTCTGGGTTTTGTTTGGGATGCA-TAMRA
HO1 Forward Primer	GTTCTCATGCTGTCGAGGATTTT
HO1 Reverse Primer	AGACGTCCTTCTACAACAATTCATAAGT
HO1 Probe	FAM-TTTGGGACGATATTGTCATTATAGGGCAGTGTG-TAMRA
HO6 Forward Primer	AATATGGGACTACTTCGCGCAACA
HO6 Reverse Primer	CGTCACCACGTACTTCAGCATAA
HO6 Probe	FAM-CCTGGTTTTGGTTTTGTAGAGTGGTTGACGA-TAMRA
SMC2 Forward Primer	AATTGGATTTGGCTAAGCGTAATC
SMC2 Reverse Primer	CTCCAATGTCCCTCAAAATTTCTT
SMC2 Probe	FAM-CGACGCGAATCCATCTTCCCAAATAATT-TAMRA
MAT1 Forward Primer	CCTGGTTTTGGTTTTGTAGAGTGG
MAT1 Reverse Primer	GAGCAAGACGATGGGGAGTTTC
MAT2 Forward Primer	ATTGCGACAAGGCTTCACCC
MAT2 Reverse Primer	CACATCACAGGTTTATTGGTTCC
Pre1 Forward Primer	CCCACAAGTCCTCTGATTTACATTCG
Pre1 Reverse Primer	ATTCGATTGACAGGTGCTCCCTTTTC
S1	TCTTGCCCACTTCTAAGCTG
S2	TCGAAAGATAAACAACCTCC
A1	GTCGTCTTGTTCAAGAAGGT
A2	AAGTCATGTGAACCGCATGG
Dna2_F1	GGAATTCATGCCCGGAACGCCACAGAAGAACAAGAGGTCTG
Dna2_R1320	CGGGATCCCTATATTTTTTGCGTTCCAATTTTTGG
Dna2_F1321	GGAATTCATGCTAGAGTGTATAGACGGCAAAGG
Dna2_R2760	CGGGATCCCTAGGGATTTATAAATTGTACACGACC
Dna2_F2761	GGAATTCATGGCTAAAATTGGTATCTCCGTAAAACG
Dna2_R4566	CGGGATCCTCAACTTTCATACTCTTGTAGAATTTCCTTTAT
Sgs1_F27	GGAATTCATGTTAAGAAGGGAGCACAAATGGTTAAAGG
Sgs1_R828	CGGGATCCCTAGTATTCCAAGGGGCTGGGCAGAATGC
Sgs1_F870	GGAATTCATGGCGACTACCGTCACTAAGGCATTAGC
Sgs1_R3540	CGGGATCCCTATTTCTTAGCATTGGGACCAACTTTCAC
Sgs1_F3360	GGAATTCATGAAAATTGTTCAGGCTAACCATGACAC
Sgs1_R4291	CGGGATCCCTATAGCAGACTTCTTGGACGACTTACTG
Nej1_F1	GGAATTCATGGATTCTGAGTTGAAAGGGCAGCAGC
Nej1_R1029	CGGGATCCCTATTAGTTTTTTATTCTCACCTTTCC
Mre11_F1	GGAATTCATGGACTATCCTGATCCAGACACAATAAG
Mre11_R813	CGGGATCCCTAACAAAGTGAAGTAGCTACAGATGAACCTGG
Mre11_F814	GGAATTCATGGAGGCTGAGGCACAACCCAAGTATGTC
Mre11_R1266	CGGGATCCCTAACCGGATTTTTTTGATCTAGTTACAGG
Mre11_F1267	GGAATTCATGATAAATGGAACAAGCATCAGTGATAGAGATG
Mre11_R2076	CGGGATCCCTATTTTCTTTTCTTAGCAAGGAGACTTCCAAG
Rad50_F1	GGAATTCATGAGCGCTATCTATAAATTATCTATTCAGG
Rad50_R3936	CGGGATCCCTATCAATAAGTGACTCTGTTAATATCGACC
Xrs2_F1	GGAATTCATGTGGGTAGTACGATACCAGAATACATTGG
Xrs2_R2562	CGGGATCCCTATTATCCTTTTCTTCTTTTGAACGTAAACTTCG