

Supplemental Information

The non-homologous end-joining factor Nej1 inhibits resection mediated by Dna2-Sgs1 at DNA double strand breaks

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Supplemental Figure S1. Chromatin immunoprecipitation (ChIP) in non-tagged controls.

Supplemental Figure S2. Resection in *exo1Δ rad50Δ* and in combination with other mutations.

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Supplemental Table S2: Strains used in this study

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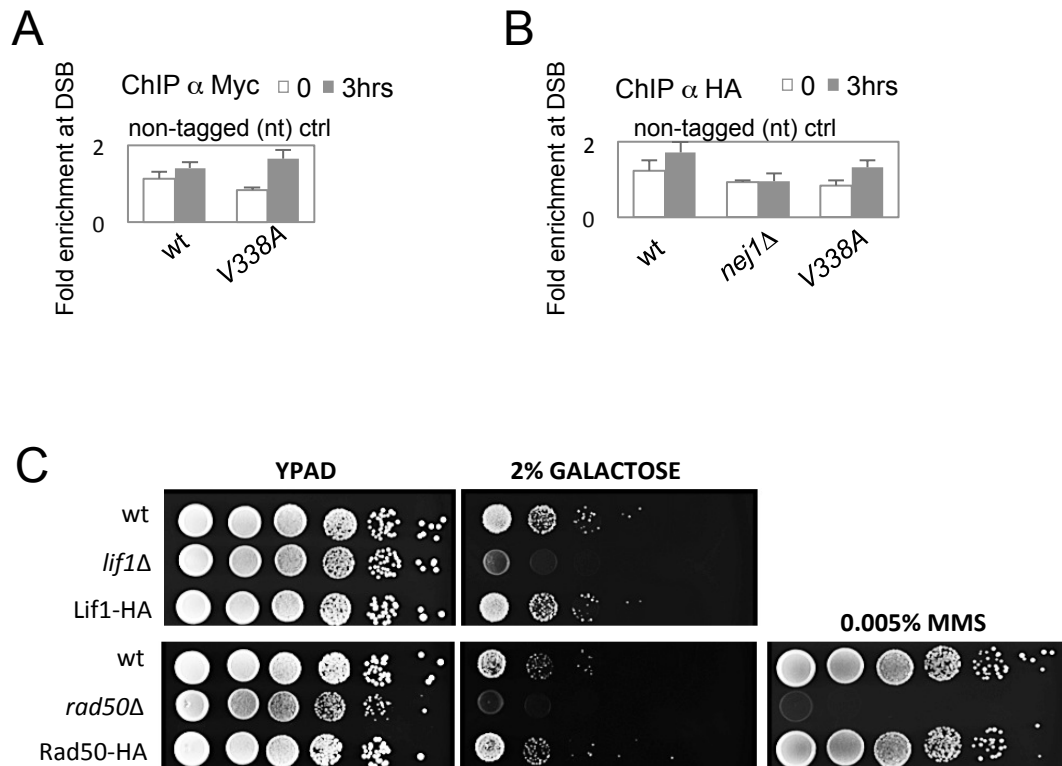


Figure S1. Chromatin immunoprecipitation (ChIP) in non-tagged controls. (A-B) ChIP with α -Myc and α -HA was performed on non-tagged control wild type (JC727), *nej1* Δ (JC1342) and *nej1*-V338A (JC2659) cells in parallel with the ChIP experiments described in Figs. 1G-H and Fig. 2G. The inclusion of the Myc-epitope tag was previously shown to not compromise Nej1 functionality (16) and (C) Drop assays with wild type (JC727), *lif1* Δ (JC1343), Lif1-3HA (JC2665), *rad50* Δ (JC3313) and Rad50-6HA (JC3306) show that the HA- epitope tagged fusions do not compromise sensitivity to genotoxic stress as the survival of the fusion are similar to wild type.

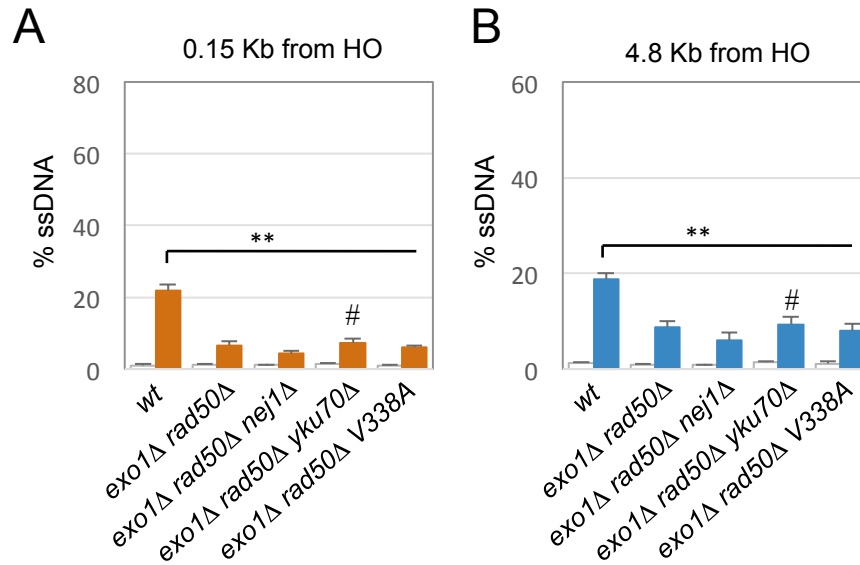


Figure S2. Resection in *exo1Δ rad50Δ* and in combination with other mutations. Resection of DNA 0.15kb and 4.8kb away from the HO DSB, as measured by % ssDNA at 0 and 6 hrs. post DSB induction in G1 cells in (A-B) wild type (JC3585), *exo1Δ rad50Δ* (JC3881), *exo1Δ rad50Δ nej1Δ* (JC3889), *exo1Δ rad50Δ yku70Δ* (JC3880) and *exo1Δ rad50Δ nej1-V338A* (JC3901). Error bars represent standard error of three replicates. Significance was determined using 1-tailed, unpaired Student's t test where mutants were compared with wild type cells for changes in resection levels ($P < 0.01$ **). # indicates statistical difference compared to *rad50Δ yku70Δ* at $P < 0.01$.

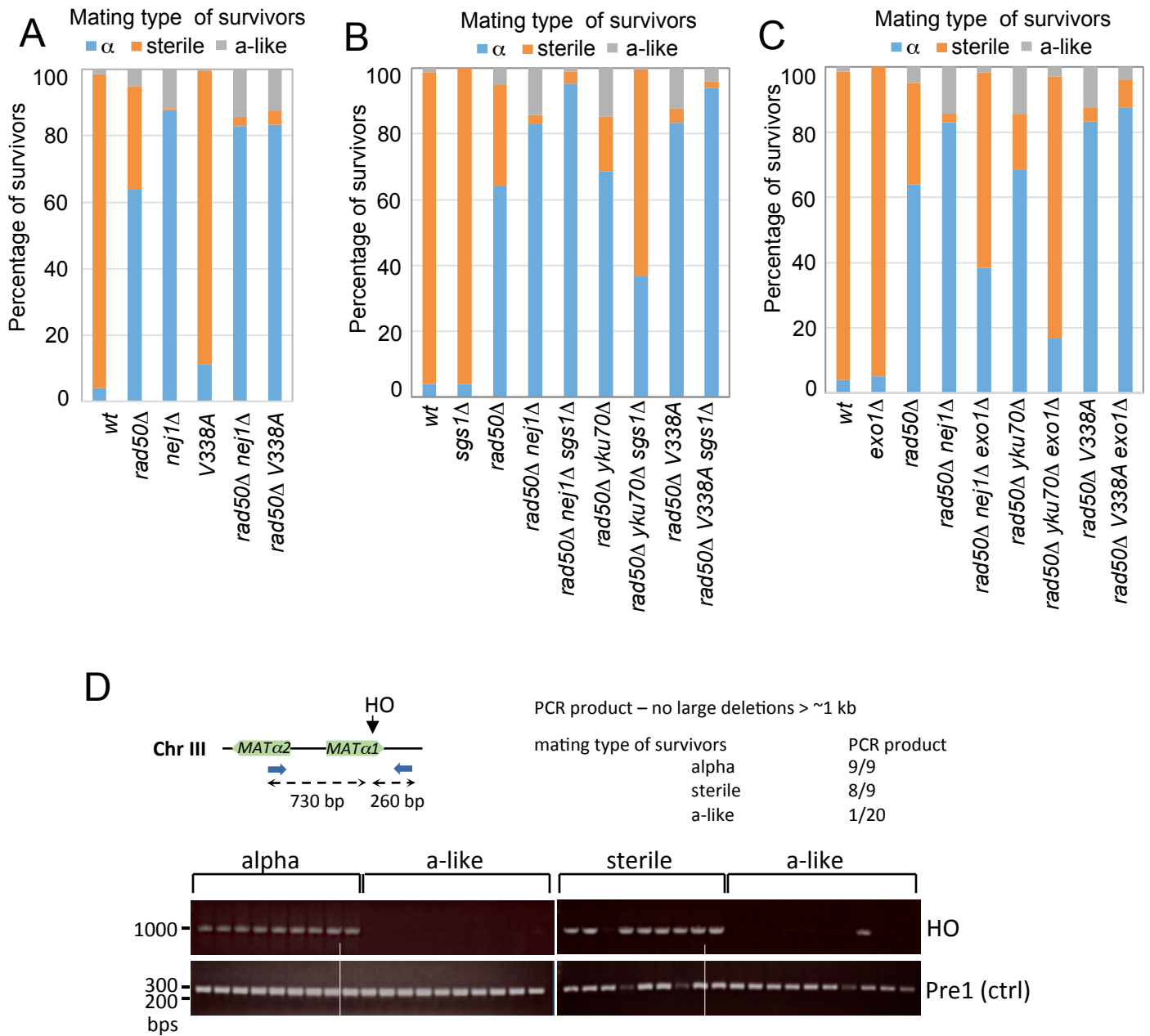


Figure S3. Mating types of all survivors in Fig 5D-F. (A-C) Mating type analysis of survivors of chronic DSB induction assays as in Fig 5D-F. Mating type of survivors was determined and interpreted as follows: α survivors, (mutated HO endonuclease), sterile survivors (small insertions and deletions) and “a-like” survivors (>700 bps deletion) in (A) wild type (JC727), *lif1* Δ (JC1343), *nej1* Δ (JC1342), *nej1*-V338A (JC2659), *yku70* Δ (JC3848), *rad50* Δ (JC3313), *nej1* Δ *rad50* Δ (JC3314) and *nej1*-V338A *rad50* Δ (JC3833). (B) *sgs1* Δ (JC3757), *rad50* Δ *nej1* Δ *sgs1* Δ (JC3761), *rad50* Δ *yku70* Δ (JC3835), *rad50* Δ *yku70* Δ *sgs1* Δ (JC3840) and *rad50* Δ *nej1*-V338A *sgs1* Δ (JC3846). (C) *exo1* Δ (JC3767), *rad50* Δ (JC3313), *rad50* Δ *nej1* Δ *exo1* Δ (JC3770), *rad50* Δ *yku70* Δ *exo1* Δ (JC3841), and *rad50* Δ *nej1*-V338A *exo1* Δ (JC3774). (D) PCR with deletion validation (DV) primers to detect the presence or loss of gDNA surrounding the HO cut site following the repair of a chronic DSB in *nej1*-V338A (JC2659) alpha, sterile and a-like survivors, with Pre1 serving as a control. Loss of PCR product signifies the deletion of a large region of gDNA on either side of the HO DSB during repair.

Table S1: % G1 arrested cells following alpha-factor treatment vs asynchronous cells

Strain	G1 arrested Cells (%G1)	asynchronous cells (%G1)
wt	89.42	39.34
<i>nej1</i> Δ	95.01	44.40
<i>nej1-V338A</i>	99.16	32.50
<i>yku70</i> Δ	93.13	35.14
<i>lif1</i> Δ	90.60	33.83
<i>exo1</i> Δ	98.46	28.86
<i>sgs1</i> Δ	100.0	37.52
<i>rad50</i> Δ	100.0	34.35
<i>nej1</i> Δ <i>exo1</i> Δ	93.96	26.08
<i>nej1</i> Δ <i>sgs1</i> Δ	97.61	34.19
<i>nej1</i> Δ <i>rad50</i> Δ	82.78	32.84
<i>nej1-V338A</i> <i>exo1</i> Δ	89.28	32.51
<i>nej1-V338A</i> <i>sgs1</i> Δ	87.47	39.47
<i>nej1-V338A</i> <i>rad50</i> Δ	95.19	35.02
<i>yku70</i> Δ <i>exo1</i> Δ	85.09	24.02
<i>yku70</i> Δ <i>sgs1</i> Δ	95.96	32.97
<i>yku70</i> Δ <i>rad50</i> Δ	97.94	30.10

Table S2: Strains used in this study

Strain	Genotype	Reference
JC727	MAT α ; <i>hml::ADE1 hmr::ADE1 ade3::GAL-HO ade1-100 leu2-3, 112 lys5 trp1::hisG ura3-52</i>	JKM179, [36]
JC1342	JC727 with <i>nej1Δ::KanMX6</i>	MAV015, [13]
JC1343	JC727 with <i>lif1Δ::KanMX6</i>	[13]
JC1687	JC727 with <i>NEJ1-13Myc::TRP1</i>	[16]
JC2659	JC727 with <i>nej1Δ::KanMX6; nej1-V338A::URA3</i>	[16]
JC2665	JC727 with <i>LIF1-3HA::TRP1</i>	This study
JC2884	JC2665 with <i>nej1Δ::KanMX6</i>	This study
JC3160	JC1342 with <i>nej1-V338A-13Myc::TRP1</i>	This study
JC3306	JC727 with <i>RAD50-3HA::TRP1</i>	This study
JC3307	JC3306 with <i>nej1Δ::KanMX6</i>	This study
JC3313	JC727 with <i>rad50Δ::URA3</i>	This study
JC3314	JC727 with <i>rad50Δ::URA3 nej1Δ::KanMX6</i>	This study
JC3315	JC727 with <i>rad50Δ::URA3 nej1Δ::KanMX6</i>	This study
JC3347	JC 3306 with <i>nej1Δ::KanMX6; nej1-V338A::URA3</i>	This study
JC3585	MAT α ; <i>hml::ADE1 hmr::ADE1 ade3::GAL-HO ade1 leu2-3, 112 lys5 trp1::hisG ura3-52</i>	This study
JC3632	JC3585 with <i>yku70Δ::KanMX6</i>	This study
JC3754	JC3585 with <i>sgs1Δ::NatRMX4</i>	This study
JC3755	JC3585 with <i>exo1Δ::NatRMX4</i>	This study
JC3757	JC727 with <i>sgs1Δ::NatRMX4</i>	This study
JC3761	JC3314 with <i>sgs1Δ::NatRMX4</i>	This study
JC3767	JC727 with <i>exo1Δ::NatRMX4</i>	This study
JC3770	JC3314 with <i>exo1Δ::NatRMX4</i>	This study
JC3828	JC2665 with <i>nej1Δ::KanMX6; nej1-V338A::URA3</i>	This study
JC3833	JC2659 with <i>rad50Δ::URA3</i>	This study
JC3835	JC3848 with <i>rad50Δ::URA3</i>	This study
JC3840	JC3835 with <i>sgs1Δ::NatRMX4</i>	This study
JC3841	JC3835 with <i>exo1Δ::NatRMX4</i>	This study
JC3846	JC3833 with <i>sgs1Δ::NatRMX4</i>	This study
JC3847	JC3833 with <i>exo1Δ::NatRMX4</i>	This study
JC3848	JC727 with <i>yku70Δ:: KanMX6</i>	This study
JC3850	JC3632 with <i>sgs1Δ::NatRMX4</i>	This study
JC3877	JC3632 with <i>exo1Δ::NatRMX4</i>	This study
JC3878	JC3632 with <i>rad50Δ::URA3</i>	This study
JC3880	JC3878 with <i>exo1Δ::NatRMX4</i>	This study
JC3881	JC3882 with <i>exo1Δ::NatRMX4</i>	This study
JC3882	JC3585 with <i>rad50Δ::URA3</i>	This study
JC3883	JC3882 with <i>sgs1Δ::NatRMX4</i>	This study
JC3884	JC3585 with <i>nej1Δ::KanMX6</i>	This study
JC3885	JC3884 with <i>sgs1Δ::NatRMX4</i>	This study
JC3886	JC3884 with <i>exo1Δ::NatRMX4</i>	This study
JC3887	JC3884 with <i>rad50Δ::URA3</i>	This study
JC3888	JC3887 with <i>sgs1Δ::NatRMX4</i>	This study
JC3889	JC3887 with <i>exo1Δ::NatRMX4</i>	This study
JC3896	JC3585 with <i>nej1Δ::KanMX6; nej1-V338A::URA3</i>	This study
JC3897	JC3896 with <i>rad50Δ::URA3</i>	This study
JC3898	JC3896 with <i>sgs1Δ::NatRMX4</i>	This study
JC3899	JC3896 with <i>exo1Δ::NatRMX4</i>	This study
JC3900	JC3897 with <i>sgs1Δ::NatRMX4</i>	This study
JC3901	JC3897 with <i>exo1Δ::NatRMX4</i>	This study
JC3906	JC3585 with <i>lif1Δ::KanMX6</i>	This study
JC3907	JC3906 with <i>rad50Δ::URA3</i>	This study
JC3928	JC3632 with <i>nej1Δ::KanMX6</i>	This study
JC4010	JC3585 with <i>mre11-3::URA3</i>	[18]
JC4047	JC4010 with <i>sgs1Δ::NatRMX4</i>	This study

JC4048	JC4010 with <i>exoI</i> Δ:: <i>NatRMX4</i>	This study
JC4049	JC4010 with <i>nejI</i> Δ:: <i>KANMX6</i>	This study
JC4051	JC4049 with <i>sgsI</i> :: <i>NATMX4</i>	This study
JC4052	JC4049 with <i>exoI</i> :: <i>NATMX4</i>	This study
JC4179	JC4010 with <i>nejI</i> -V338A:: <i>KANMX6</i>	This study
JC4180	JC4079 with <i>sgsI</i> :: <i>NATMX4</i>	This study
JC4181	JC 4079 with <i>exoI</i> :: <i>NATMX4</i>	This study

Table S3: Primers and probes used in this study

Primer Name	Primer Sequence (5'-3')
HO2 Forward Primer	TTGCCCACTTCTAAGCTGATTC
HO2 Reverse Primer	GTACTTTTCTACATTGGGAAGCAATAAA
HO2 Probe	FAM-ATGATGTCTGGGTTTTGTTTGGGATGCA-TAMRA
HO6 Forward Primer	AATATGGGACTACTTCGCGCAACA
HO6 Reverse Primer	CGTCACCACGTA CTTCAGCATAA
HO6 Probe	FAM-CCTGGTTTTGGTTTTGTAGAGTGGTTGACGA-TAMRA
SMC2 Forward Primer	AATTGGATTTGGCTAAGCGTAATC
SMC2 Reverse Primer	CTCCAATGTCCCTCAAATTTCTT
SMC2 Probe	FAM-CGACGCGAATCCATCTTCCCAAATAATT-TAMRA
MAT1 Forward Primer	CCTGGTTTTGGTTTTGTAGAGTGG
MAT1 Reverse Primer	GAGCAAGACGATGGGGAGTTTC
MAT2 Forward Primer	ATTGCGACAAGGCTTCACCC
MAT2 Reverse Primer	CACATCACAGGTTTATTGGTTCC
Pre1 Forward Primer	CCCACAAGTCCTCTGATTTACATTCCG
Pre1 Reverse Primer	ATTCGATTGACAGGTGCTCCCTTTTC
DV Forward Primer	CTCATCTGTGATTTGTGGAT
DV Reverse Primer	GAGGCAAGTAGATAAGGGTA