Supplementary Methods

Strain constructions

For experiments shown in Figures 1B and 2A, strains were *rad5* deletions in the DF5 background (1) harboring either *wt* or mutant versions of the *RAD5* gene under control of its own promoter on an integrative plasmid as previously described (2). Strains that carried an inducible *HO* gene were based on JKM179 (3), a kind gift from Jim Haber. All 9myc-epitopes were introduced directly in the respective genomic locus as previously described (4). Strains used for pull-down of His-tagged Pol30p (5) and two-hybrid analysis (4) have been described. All other experiments were performed in the BY4741/BY4742 background available through the EUROSCARF collection (6). All single deletions were *MATα* cells bearing *KanMX4* constructs directly from the collection. Combinations of deletions were constructed sequentially by mating and tetrad dissection. Relevant genotypes were identified by replica plating, PCR and phenotypic analysis. The *rad5(GAA)* mutant was constructed in this strain background by a two-step replacement of the *wt* gene: after integration of plasmid YIp211-P_{RAD5}-*rad5(GAA)* into the *RAD5* locus plasmid loss was induced by selection on 5-fluoroorotic acid, and clones that had retained the mutant copy of the gene were identified by their UV sensitivity and the presence of a diagnostic restriction site associated with the mutant sequence. The same strategy was followed for introduction of *pol30(K164R)* into the *POL30* locus.

ChIP Analysis

Cells were treated with 1% formaldehyde for 15 min at room temperature for crosslinking. The reaction was terminated by the addition of 2.5 M glycine solution to a final concentration of 125 mM, followed by washing with PBS. Total cell extracts were prepared by glass beads lysis in 50 mM HEPES/KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1%

SDS supplemented with benzamidine (5 mM) and Complete™ protease inhibitor (Roche). Chromatin was sheared by sonification (3 pulses of 10 seconds each with 30 sec intervals) into 600 to 1000 bp fragments. The extract was centrifuged for 30 min at 11000 g, and the supernatant was used for immunoprecipitation. Non-specific adsorption of DNA was prevented by incubating the extract with Sepharose CL-4B for 30 min at 4°C followed by centrifugation at 8000 rpm for 5 min. The supernatant was incubated with 1 μg rabbit anti-myc polyclonal antibody (Santa Cruz Biotechnology) for 3 h at 4°C. Protein G Sepharose (10 µl) was added, and the mixture incubated for a further 1 h. The beads were washed for 20 min each at 4°C with wash buffer 1 (lysis buffer supplemented with 500 mM NaCl), wash buffer 2 (10 mM Tris/HCl, pH 8,0, 0,25 M LiCl, 0,5% NP-40, 0,5% sodium deoxycholate, 1 mM EDTA), and TE buffer. Immune complexes were eluted from the beads with 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1% SDS. Crosslinks were resolved by incubating overnight at 65°C in TE with 1% SDS, followed by phenol extraction, and the DNA was ethanol-precipitated and resuspended in TE buffer.

Supplementary References

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Supplementary Table. Yeast strains used in this study

Strain	Genotype	Source/Reference
DF5 background		
DF5	*his3-Δ200 leu2-3,112 lys2-801 trp1-1(am) ura3-52*	(1)
wt	*DF5 rad5::HIS3 LEU2::YIp128-RAD5*	(2)
rad5	*DF5 rad5::HIS3 LEU2::YIplac128*	(2)
ubc13	*DF5 rad5::HIS3 ubc13::HIS3 LEU2::YIp128-RAD5*	(2)
rad5(I916A)	*DF5 rad5::HIS3 LEU2::YIp128-rad5(I916A)*	(2)
rad5(GAA)	*DF5 rad5::HIS3 LEU2::YIp128-rad5(KT538/539AA)*	this study
ubc13 rad5(GAA)	*DF5 rad5::HIS3 ubc13::HIS3 LEU2::YIp128-rad5(KT538/539AA)*	this study
HP30 wt	*DF5 pol30::URA3 LEU2::YIp128-His$_6$-POL30*	(5)
HP30 rad5	*DF5 rad5::HIS3 pol30::URA3 LEU2::YIp128-His$_6$-POL30*	(5)
HP30 rad5(GAA)	*DF5 rad5(KT538/539AA) pol30::URA3 LEU2::YIp128-His$_6$-POL30*	this study
EUROSCARF background		
wt (BY4742)	*Matα, leu2Δ0, lys2Δ0, ura3Δ0, his3Δ0*	(6)
rad5	*BY4742 rad5::KanMX*	(6)
rad18	*BY4742 rad18::KanMX*	(6)
rad51	*BY4742 rad51::KanMX*	(6)
rad52	*BY4742 rad52::KanMX*	(6)
yku70	*BY4742 yku70::KanMX*	(6)
mre11	*BY4742 mre11::KanMX*	(6)
rad50	*BY4742 rad50::KanMX*	(6)
xrs2	*BY4742 xrs2::KanMX*	(6)
ubc13	*BY4742 ubc13::KanMX*	(6)
pol30(K164R)	*BY4742 pol30(K164R)*	this study
rad52 yku70	*BY4742 rad52::KanMX yku70::KanMX*	this study
mre11 rad52	*BY4742 mre11::KanMX rad52::KanMX*	this study
mre11 yku70	*BY4742 mre11::KanMX yku70::KanMX*	this study
rad5 rad18	*BY4742 rad5::KanMX rad18::KanMX*	this study
rad5 rad51	*BY4742 rad5::KanMX rad51::KanMX*	this study
rad5 rad52	*BY4742 rad5::KanMX rad52::KanMX*	this study
rad5 yku70	*BY4742 rad5::KanMX yku70::KanMX*	this study
rad5 rad52 yku70	*BY4742 rad5::KanMX yku70::KanMX*	this study
rad5 mre11	*BY4742 rad5::KanMX mre11::KanMX*	this study
rad5 rad50	*BY4742 rad5::KanMX rad50::KanMX*	this study
rad5 xrs2	*BY4742 rad5::KanMX xrs2::KanMX*	this study

rad5(GAA)	*BY4742 rad5(KT538/539AA)*	this study
rad5(GAA) rad52	*BY4742 rad5(KT538/539AA) rad52::KanMX*	this study
rad5(GAA) yku70	*BY4742 rad5(KT538/539AA) yku70::KanMX*	this study
rad5(GAA) rad52 yku70	*BY4742 rad5(KT538/539AA) rad52::KanMX yku70::KanMX*	this study
rad5(GAA) mre11	*BY4742 rad5(KT538/539AA) mre11::KanMX*	this study
rad18 rad51	*BY4742 rad18::KanMX rad51::KanMX*	this study
rad18 rad52	*BY4742 rad18::KanMX rad52::KanMX*	this study
rad18 mre11	*BY4742 rad18::KanMX mre11::KanMX*	this study
rad18 rad50	*BY4742 rad18::KanMX rad50::KanMX*	this study
rad18 yku70	*BY4742 rad18::KanMX yku70::KanMX*	this study
JKM179 background		
JKM179	*Matα Δho Δhml::ADE1 Δhmr::ADE1 ade1-110 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL10:HO*	(3)
YKU70-9myc	*JKM179 YKU70-9myc::klTRP1*	this study
RAD52-9myc	*JKM179 RAD52-9myc::klTRP1*	this study
RAD5-9myc	*JKM179 RAD5-9myc::klTRP1*	this study
RAD18-9myc	*JKM179 RAD18-9myc::klTRP1*	this study