

Supplementary Materials for **Impaired cohesion and homologous recombination during replicative aging in budding yeast**

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Fig. S1

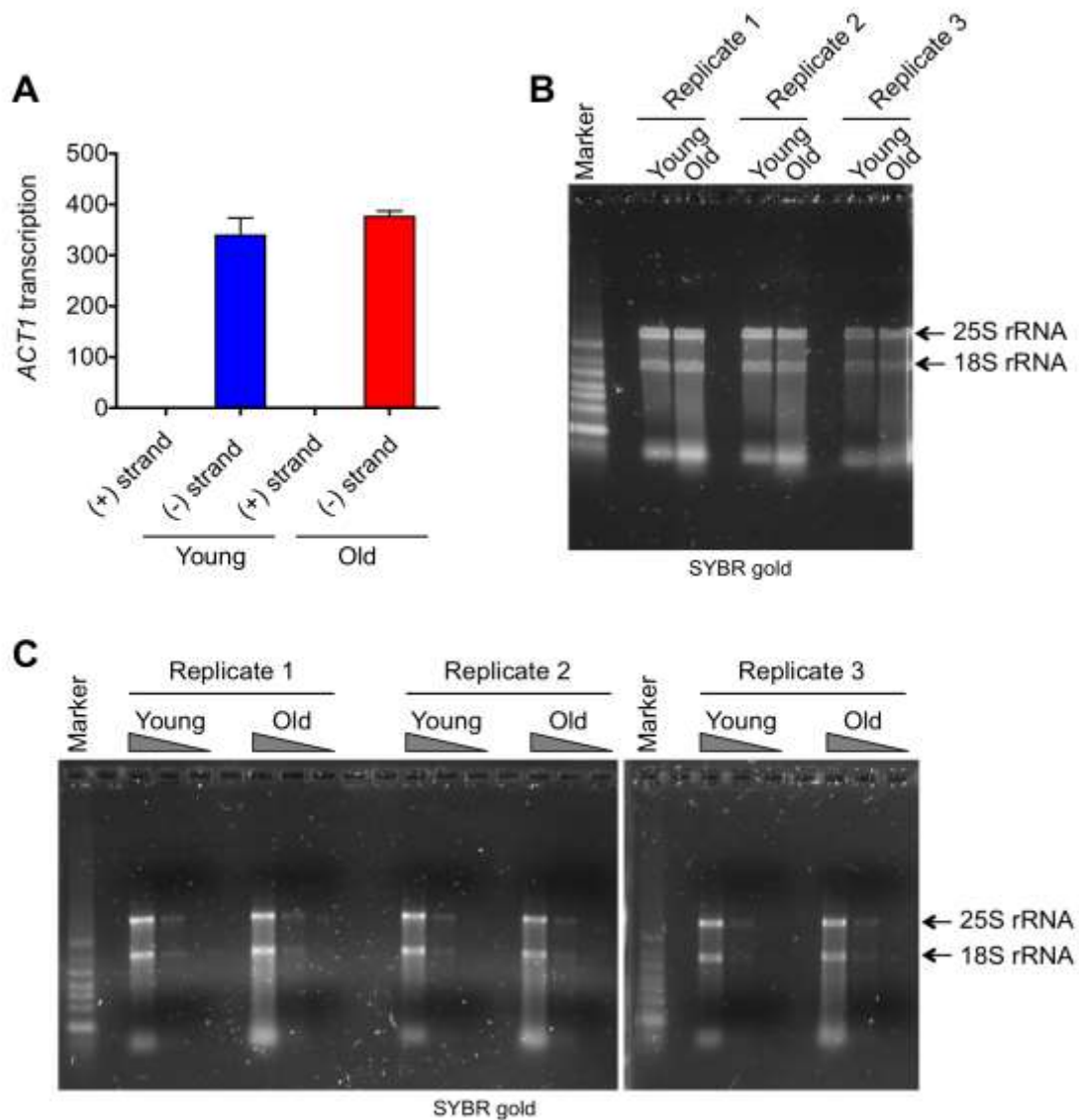
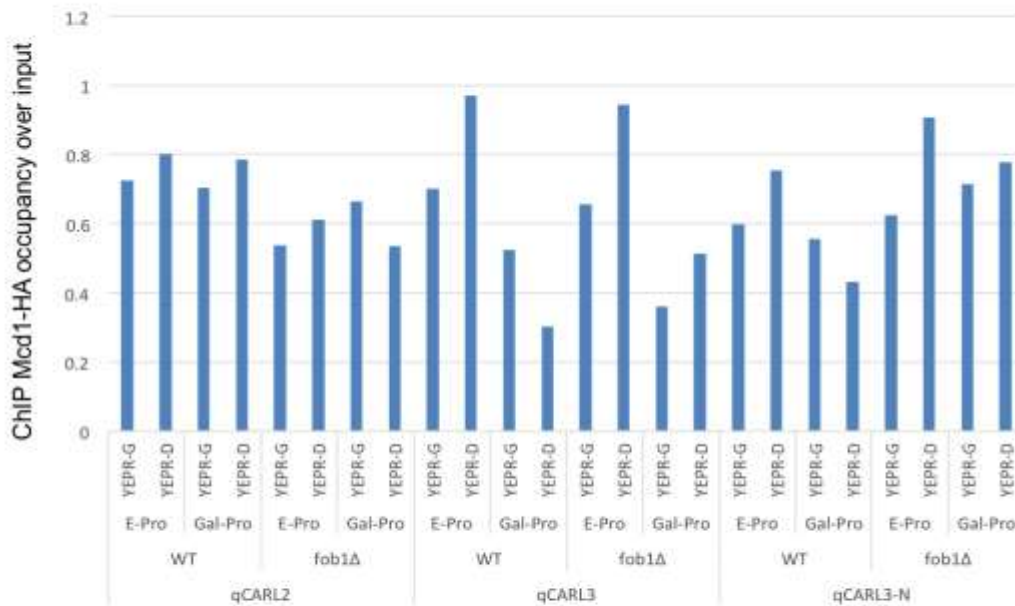


fig. S1. No change in rRNA levels during aging. (A) Levels of *ACT1* strand-specific transcription from same number of young and old cells. Average of two independent replicates are plotted. (B) No significant difference in Pol I transcriptional activity is observed during aging, as observed from the levels of 25S and 18S rRNA. Results from three independent replicates are shown. (C) Ten-fold serial dilutions of the same samples used in B.

Fig. S2

A



B

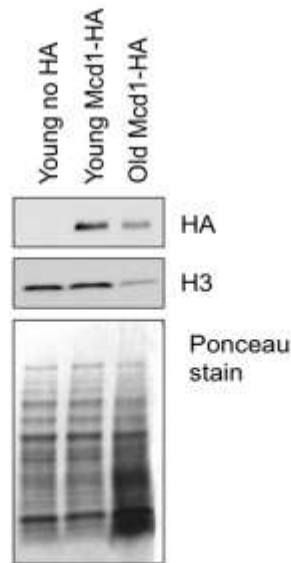


fig. S2. Cohesin occupancy is not affected by *NTS1* transcription, but cohesins are reduced during aging. (A) Mcd1 levels are not changed drastically by FOB1 deletion in the E-pro and Gal-pro strains used in Fig. 3A. (B) Mcd1 levels go down during aging, when normalized to DNA. Western blot analysis to measure protein levels of HA-tagged Mcd1 in young and old cells, with untagged strain as an additional control. Samples are loaded according to the same DNA equivalent.

Fig. S3

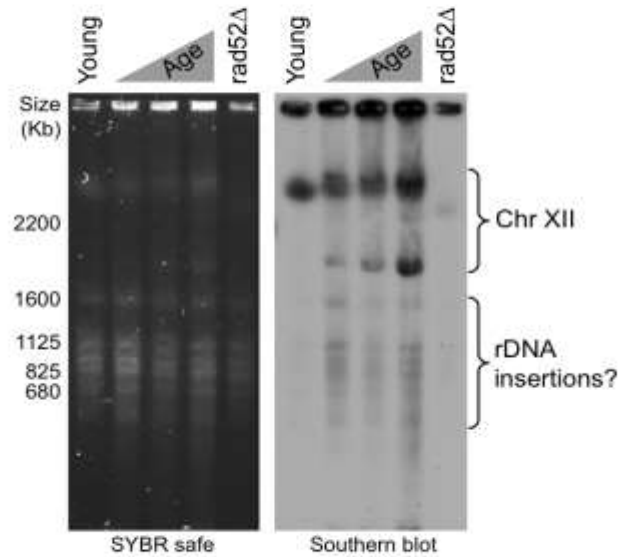


fig. S3. Appearance of two major chromosomal bands containing rDNA during aging. Similar analysis as Fig. 4A, but in this case analysis is from intact yeast chromosomes collected at the indicated time points during aging. Left panel indicates SYBR safe staining, while the right panel indicates Southern hybridization using a chr XII (rDNA) specific probe. Fragmentation of DNA increases with the age progression, which may also be indicative of rDNA integration at other genomic sites.

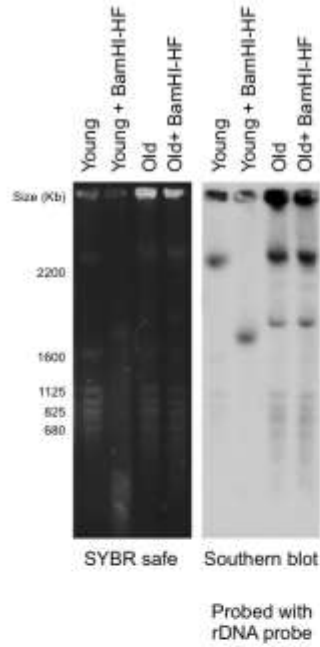
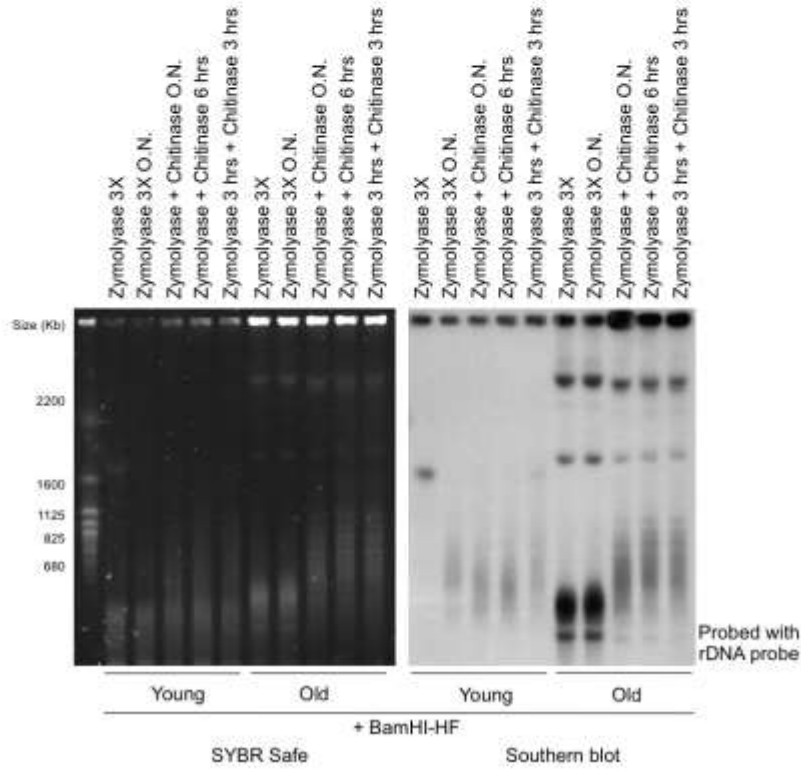
A**Fig. S4****B**

fig. S4. The two major chromosomal bands containing rDNA appear to comprise mainly rDNA repeats in old cells. (A) Chromosomal DNA preparation followed by PFGE analysis to determine rDNA repeat numbers in young and old cells. Same numbers of young and old WT yeast cells were collected and processed in agarose molds. Restriction digestion was performed with BamHI enzyme, while the cells were embedded within agarose molds to liberate the entire rDNA locus, as BamHI does not cut within rDNA locus, followed by Southern hybridization using a rDNA specific probe. Left panel indicates SYBR safe staining, while the right panel indicates Southern hybridization using rDNA probe. (B). Similar analysis as A, but using multiple other treatment conditions as indicated in the figure to ensure efficient cell wall lysis in old cells and proper restriction digestion by BamHI. Yeast cell wall lysis using Zymolyase alone or in combination with Chitinase was performed for 3 hours where time is not mentioned, performed overnight (O.N.), or as indicated in the figure. 3X means 3 times more Zymolyase was used for those conditions.

Fig. S5

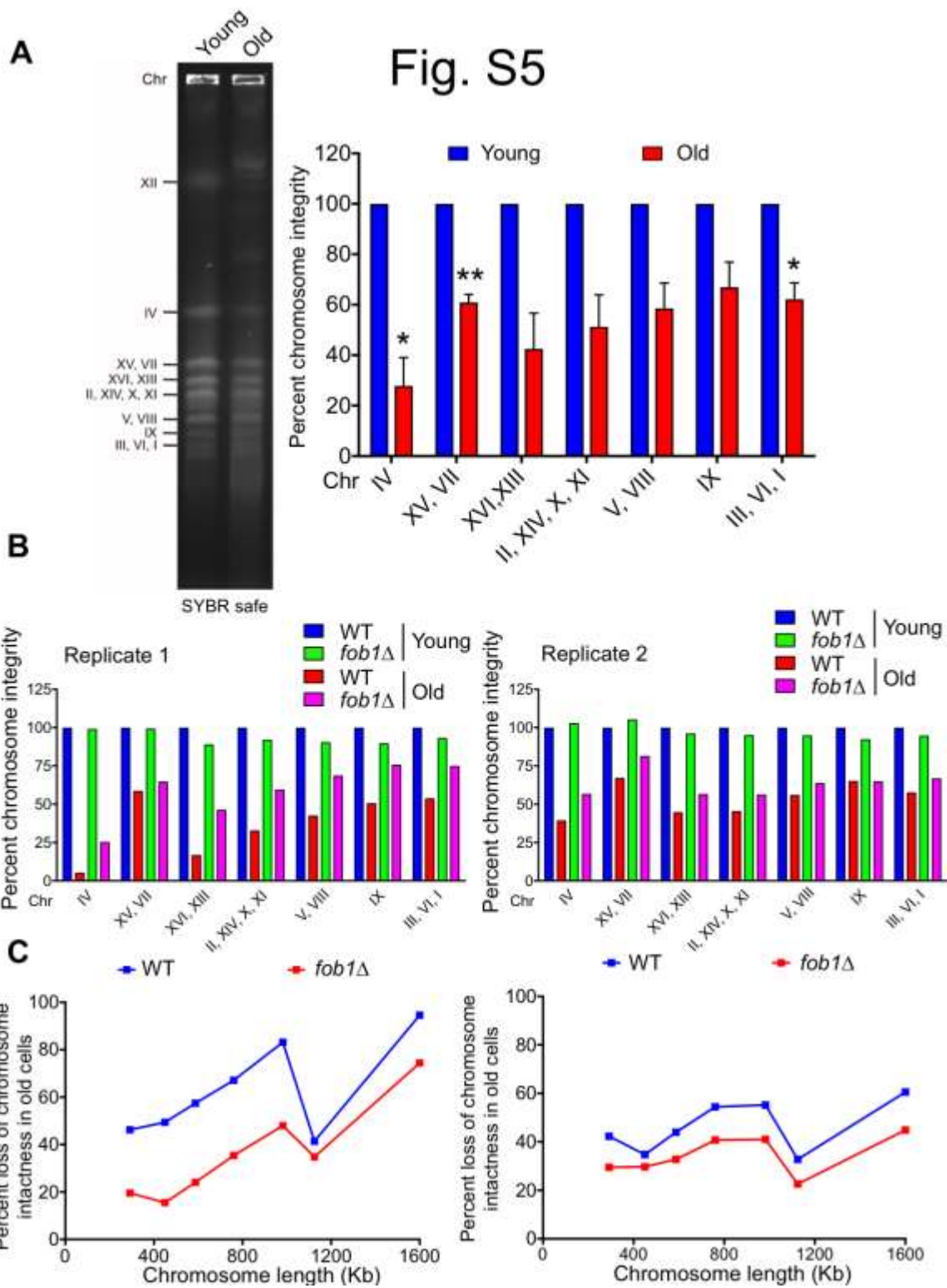


fig. S5. Increased chromosomal instability proportional to chromosome length and rDNA instability is observed during aging in WT cells. (A) SYBR safe staining is shown on the left and quantitation on the right from the agarose gel image using AlphaView software. (B) Increased chromosomal instability is directly proportional to rDNA instability..Quantification of chromosomal band intensities from SYBR safe stained gels of chromosomes from young and old WT and *fov1Δ* yeast strains, obtained utilizing Alphaview software. Two individual replicates are shown here. (C) Percent loss of intactness of chromosomes from the above replicates (scatter plot).

Fig. S6

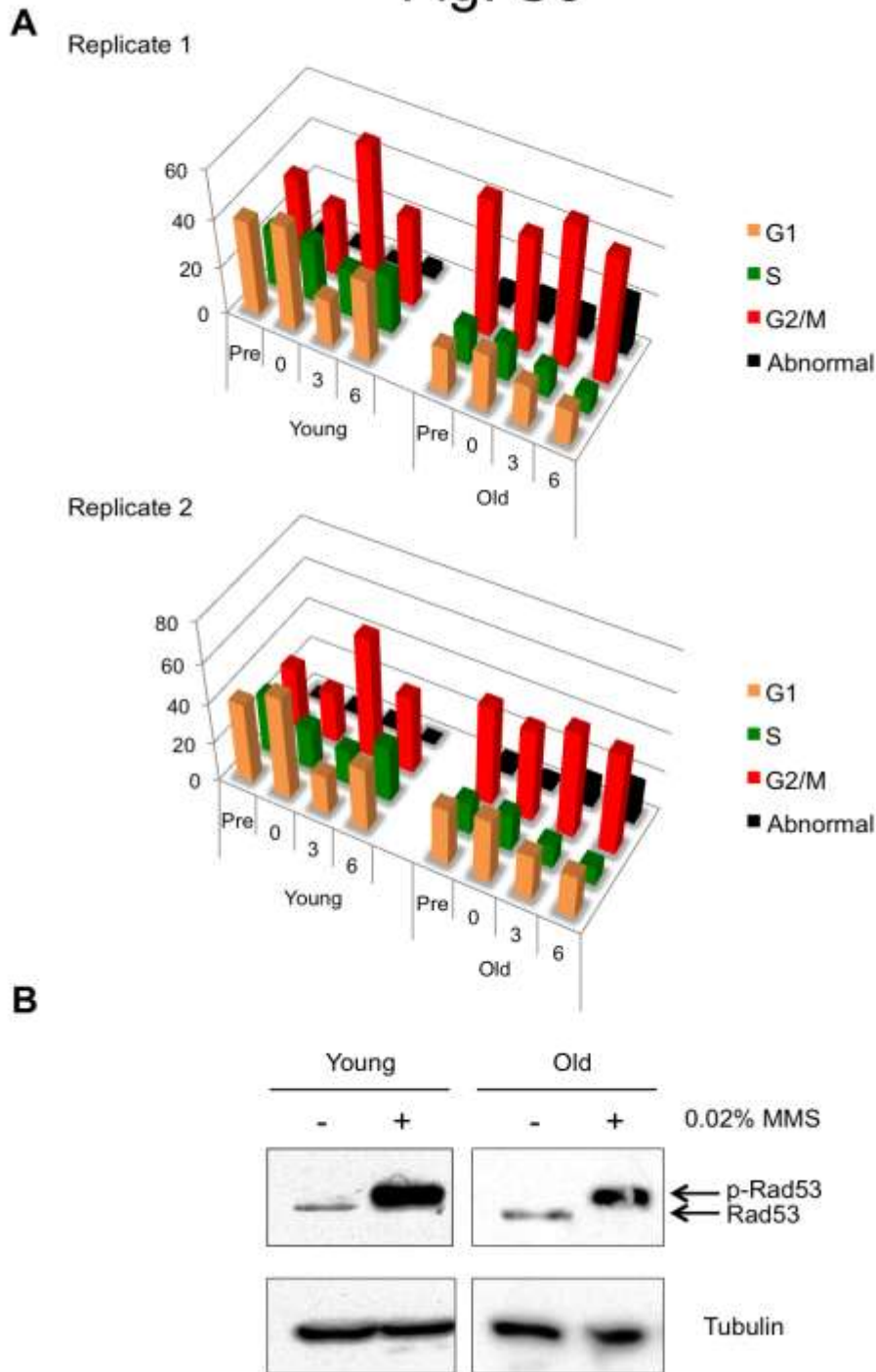


fig. S6. The DNA damage checkpoint is intact in old cells. (A) Budding index of young and old yeast cells collected during the DNA repair time-course. Two independent replicates are shown. (B) Western blot analysis to detect Rad53 phosphorylation (p-Rad53) levels in young and old cells following addition of MMS. Loading of samples was normalized according to the Tubulin levels.

Fig. S7

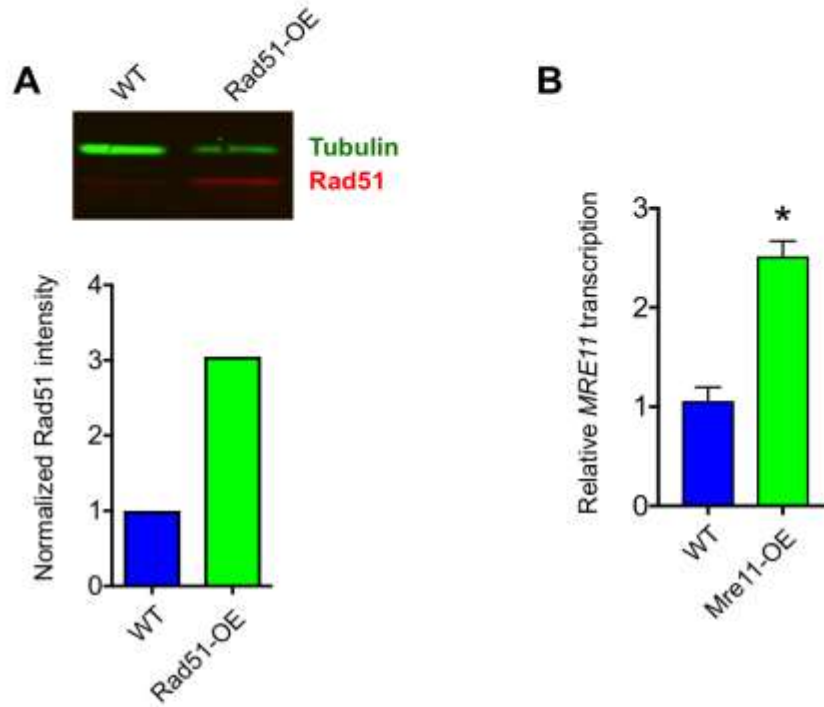
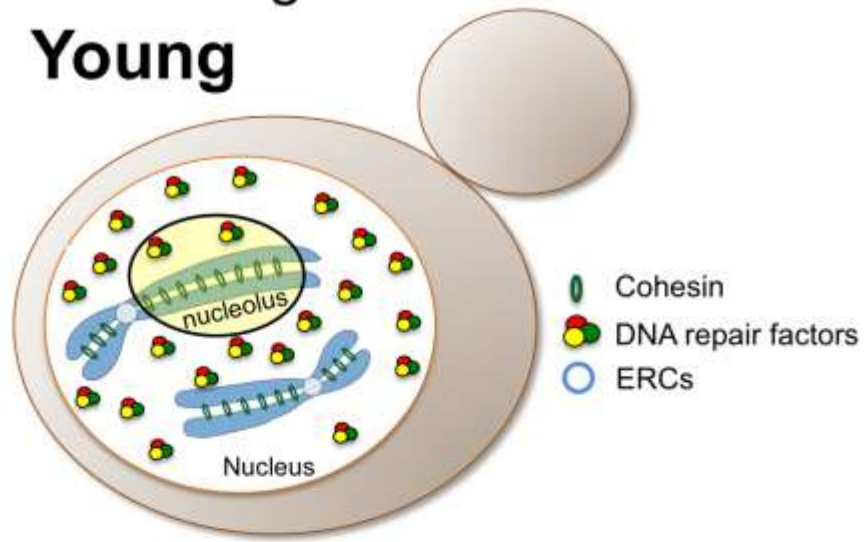


fig. S7. Rad51 and Mre11 overexpression. (A) Western blot of Rad51 protein level normalized to Tubulin protein level quantified on a LicoR Odyssey machine to confirm the over-expression of Rad51. (B) Relative levels of *MRE11* transcription to *ACT1* to confirm the over-expression of Mre11 in MEP strain background. Average and standard error of the mean of three independent replicated are plotted here.

Fig. S8

Young



Aging

Old

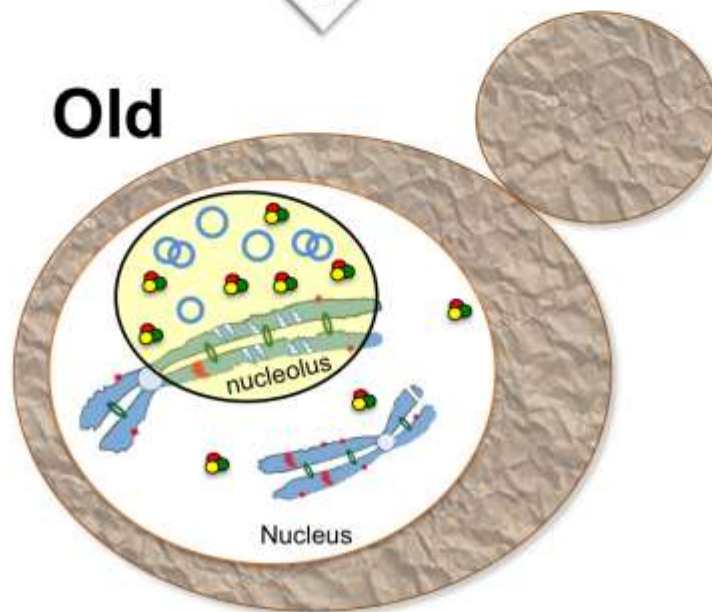


fig. S8. Model for rDNA instability and reduced HR causing global genomic instability to limit replicative life span. Schematic summarizing the findings of this work, as described in the text.

table S1. Yeast strains used in this study.

ZHY2	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX</i>	(5)
<i>rad52Δ</i>	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rad52Δ::KANMX</i>	(53)
SPY006	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX MCD1-6HA:his5+</i>	This study
SPY021	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX fob1Δ::KANMX4</i>	This study
YSI129	<i>MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 fob1Δ::LEU2 his3-11::GFP-LacI-HIS3 RDN1::LacO(50)-ADE2 (110 rDNA copies)</i>	(19)
YSI130	<i>MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 fob1Δ::LEU2 his3-11::GFP-LacI-HIS3 LacO(50)-ADE2:445kb ChXII (110 rDNA copies)</i>	(19)
SPY056	<i>MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 MCD1-6HA:his5+</i>	This study
SPY065	<i>MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 E-proΔ::GAL1/10-URA3 MCD1-6HA:his5+</i>	This study
SPY048	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX RAD54-3HA::KANMX6</i>	This study
SPY054	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX SAE2-3HA::KANMX6</i>	This study
SPY031	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX RAD51-HIS3</i>	This study
SPY074	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX MRE11-HIS3</i>	This study
SPY076	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX SMC1-TAP::HIS3</i>	This study
SPY080	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX SMC3-TAP::HIS3</i>	This study
SPY084	<i>MATalpha ade2::hisG his3 leu2 met15D::ADE2 trp1D63 ura3D0:: URA3 hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-HPMX SCC3-TAP::HIS3</i>	This study

table S2. Primers used in this study.

Primers	Purpose	Sequence
NTS2-1_A	cDNA synthesis of NTS2 (+) strand	5'- GGT AGG TCG AAA CAG AAC ATG AAA GTT GG -3'
NTS2-1_B	cDNA synthesis of NTS2 (-) strand	5'- GCT ACT CTC ATG GTC TCA ATA CTG CC -3'
NTS1-2_A	cDNA synthesis of NTS1 (+) strand	5'- CAG AGC GGC AAA CAT GAG TGC TTG TAT AAG -3'
NTS1-2_B	cDNA synthesis of NTS1 (-) strand	5'- CGT AGT ACA TCT TAC AAC TCC GCA TAC C -3'
ACT1_RV	cDNA synthesis of ACT1 (Crick strand)	5'- CGG ACA ATT TCT CTT TCA GCA GTG GTG G -3'
qNTS2_1_FW	qPCR analysis of NTS2 region	5'- GAG GTA GTT TCA AGG TGA CAG G -3'
qNTS2_1_RV		5'- CTC TCA TGG TCT CAA TAC TGC C -3'
qNTS1_2_FW	qPCR analysis of NTS1 region	5'- GAG GCT ACT GGG AAG AAG AAA GAG -3'
qNTS1_2_RV		5'- CAT CTT ACA ACT CCG CAT ACC G -3'
qACT1_1_FW	qPCR analysis of ACT1 control	5'- GTG ATG GTG TTA CTC ACG TC -3'
qACT1_1_RV		5'- GTA GTC AGT CAA ATC TCT ACC GGC -3'
rDNA_probe2_FW	Primers to design probe for Southern blot against rDNA (Chr XII)	5'- CAG GTT ATG AAG ATA TGG TGC AA -3'
rDNA_probe2_RV		5'- AAA ATG GCC TAT CGG AAT ACA -3'
Chr2_1.FW	Primers to design probe for Southern blot against Chr II	5'- GTC TAT ATC GAC GGT AGC CCA CAC TTG C -3'
Chr2_1.RV		5'- CTT GTC GCC CTG TTT CAC CAC ATC G -3'
qCARL2_.FW	ChIP primers to check Mcd1 occupancy at different regions of Chr XII	5'- CCT GCA TTG TCC TCA TTT AGT GCT CAG G -3'
qCARL2_.RV		5'- TTT GGC AAC GAG ATA GTT GTG CCC -3'
qCARL3_.FW		5'- ATG CCA CCT ACC GAC CAA CTT TCA -3'
qCARL3_.RV		5'- AGA GGT GTT ATG GGT GGA GGA CAA -3'
qCARL3-N_.FW		5'- TCC ACT TTC AAC CGT CCC TCC AAA -3'
qCARL3-N_.RV		5'- AGA ATG TCG GCG GCA GTA TTG AGA -3'

qCARC1_positive.F W	ChIP primers to check Mcd1 occupancy at centromeric regions of Chr III (+ve control)	5'- CAG ACG ATA AGT TGA GTA GCG G -3'
qCARC1_positive.R V		5'- GTC CAC TAG GAG ACT CTT GAA C -3'
qCARC_negative.F W	ChIP primers to check Mcd1 occupancy at genomic regions of Chr III lacking Mcd1 binding (-ve control)	5'-CAG TGC TAT CGG ATC TAG GAA G -3'
qCARC_negative.R V		5'- GTA TTT GAC GTC TCC GCT TTC C -3'
DSB probe.FW	Primers to design probe for Southern blot against DSB probe	5'- GCC ATT TAC AAA AAC ATA ACG -3'
DSB probe.RV		5'- GGG CCT AGT TTA GAG AGA AGT-3'
Probe C.FW	Primers to design probe for Southern blot against Control probe	5'- ACA GAT GTG CCG CCC CAG CCA AAC TCC -3'
Probe C.RV		5'- CCT GGA TAT GGA TTC TTC ACG GTA ACG -3'
Chr1_left.FW	Primers to design probe for Southern blot against Chr I	5'- GAT TGC CTC TTT TGG GAG GTC TGG -3'
Chr1_left.RV		5'- CCA TCA GGC TCA GAT GAA TCA TGG GCC -3'
ChrX_right.FW	Primers to design probe for Southern blot against Chr X	5'- CAC CAC ACC CTG CTT ATT AAA GC -3'
ChrX_right.RV		5'- GAC AGA GGA CTT GTG TGA CG -3'
ChrIV_left.FW	Primers to design probe for Southern blot against Chr IV	5'- CAT TGG GAC AGG TAC TAG ATG G -3'
ChrIV_left.RV		5'- CTA TCT GTC TCT GCT CAG TGT GG -3'
ChrXII_left.FW	Primers to design probe for Southern blot against left arm of Chr XII	5'-GTA GGT ACA CTT AAC CCC GAG C -3'
ChrXII_left.RV		5'- GTA TAT GAT CTC GTT GCA AGG GTG CG -3'