

Supplemental Information (Fine et al.)

Table S1. Yeast Strains

Strain	Genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0</i>	(BRACHMANN <i>et al.</i> 1998)
BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0</i>	(BRACHMANN <i>et al.</i> 1998)
FY834	<i>MATα leu2Δ1 lys2Δ202 trp1Δ63 his3Δ200 ura3-52</i>	(Winston <i>et al.</i> 1995)
ML1	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167</i>	(LI <i>et al.</i> 2010)
ML150	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 MCD1-13xMyc:kanMX4</i>	(LI <i>et al.</i> 2010)
UCC5179	<i>MATa ade2::hisG his3 leu2 lys2 ura3Δ0 trpΔ63 HOΔ::SCW11 pr-Cre-EBD78-natMX loxP-UBC9-loxP-LEU2 loxP-CDC20-loxP-hphMX</i>	(LINDSTROM AND GOTTSCHLING 2009)
UCC5181	<i>MATα ade2::hisG his3 leu2 met15Δ::ADE2 ura3Δ0 trpΔ63 HOΔ::SCW11 pr-Cre-EBD78-natMX loxP-UBC9-loxP-LEU2 loxP-CDC20-loxP-hphMX</i>	(LINDSTROM AND GOTTSCHLING 2009)
UCC5185	UCC5179/UCC5181	(LINDSTROM AND GOTTSCHLING 2009)
YPH278	<i>MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 CFIII [pCEN3.L.YPH278, SUP11, URA3]</i>	(SPENCER <i>et al.</i> 1990)
RF3	UCC5179; <i>MCD1-13xMyc-kanMX4</i>	This Study
RF4	UCC5181; <i>MCD1-13xMyc-kanMX4</i>	This Study
RF10	RF3/RF4; <i>MCD1-13xMyc-kanMX4/MCD1-13xMyc-kanMX4</i>	This Study
RF30	UCC5181; <i>SCC2-13xMyc-kanMX4</i>	This Study
RF31	UCC5179; <i>SCC2-13xMyc-kanMX4</i>	This Study
RF32	YPH278; <i>sir2Δ::natMX4</i>	This Study
RF33	YPH278; <i>hst1Δ::natMX4</i>	This Study
RF34	RF30/RF31; <i>SCC2-13xMyc-kanMX4/SCC2-13xMyc-kanMX4</i>	This Study
3349-1B	<i>LacO [DK] NAT::lys4 trp1-1 bar1 [pHIS3, GFP-LacI, HIS3]::his3-11,15 leu2-3,112 ura3-52 GAL⁺</i>	(GUACCI <i>et al.</i> 1997)

3312-7A	<i>mcd1-1 LacO-NAT::lys4 trp1-1 bar1 [pHIS3,GFP-LacI, HIS3]::his3-11,15 leu2-3,112 ura2-52 GAL⁺</i>	(GUACCI <i>et al.</i> 1997)
3460-2A	<i>LacO [DK]-NAT: 10kb CENIV trp1-1 bar1 [pHIS3, GFP-LacI, HIS3]::his3-11,15 leu2-3,112 ura2-52 GAL⁺</i>	(GUACCI <i>et al.</i> 1997)
RF43	YPH278; <i>hst1Δ::natMX4, sir2Δ::kanMX4</i>	This Study
RF77	UCC5181; <i>SMC1-13xMyc-kanMX4</i>	This Study
JH5275b	<i>MATalphaΔ::hphMX hoΔ hml1Δ::ADE1 hmrΔ::ADE1 ade1-110 leu2,3-112::MCD1-LEU2 lys5 trp1::hisG ura3-52 ade3::GAL10:HO met17::trna-SUP53-G418 mcd1Δ::natMX</i>	(HEIDINGER-PAULI <i>et al.</i> 2010)
JH5276b	<i>MATalphaΔ::hphMX hoΔ hml1Δ::ADE1 hmrΔ::ADE1 ade1-110 leu2,3-112::mcd1L12STOP-LEU2 lys5 trp1::hisG ura3-52 ade3::GAL10:HO met17::trna-SUP53-G418 mcd1Δ::natMX</i>	(HEIDINGER-PAULI <i>et al.</i> 2010)
RF89	UCC5179; <i>SMC1-13xMyc-kanMX4</i>	This Study
RF90	RF77/RF89; <i>SMC1-13xMyc-kanMX4/SMC1-13xMyc-kanMX4</i>	This Study
RF113	JH5275b; <i>ura3-52::[pRS306, Empty, URA3]</i>	This Study
RF114	JH5276b; <i>ura3-52::[pRS306, Empty, URA3]</i>	This Study
RF118	JH5275b; <i>ura3-52::[pJSB186, SIR2, URA3]</i>	This Study
RF119	JH5276b; <i>ura3-52::[pJSB186, SIR2, URA3]</i>	This Study
RF127	YPH278; <i>sir2Δ::kanMX4</i>	This Study
RF131	YPH278; <i>hst1Δ::natMX4, sir2Δ::kanMX4 fob1Δ::hphMX4</i>	This Study
RF132	YPH278; <i>sir2Δ::kanMX4 hmrΔ::hphMX4</i>	This Study
RF134	YPH278; <i>hst1Δ::natMX4, sir2Δ::kanMX4 hmrΔ::hphMX4</i>	This Study
RF135	YPH278; <i>hst1Δ::natMX4, hmrΔ::hphMX4</i>	This Study
RF137	YPH278; <i>hmrΔ::hphMX4</i>	This Study
RF138	YPH278; <i>hst1Δ::natMX4, fob1Δ::hphMX4</i>	This Study
RF146	JH5276B; <i>[pCM252, pTET::Empty, TRP1]</i>	This Study
RF147	JH5275B; <i>[pCM252, pTET::Empty, TRP1]</i>	This Study
RF148	YPH278; <i>fob1Δ::hphMX4</i>	This Study
RF149	YPH278; <i>fob1Δ::hphMX4 sir2Δ::kanMX4</i>	This Study
RF176	UCC5181; <i>LRS4-13xMyc::kanMX4</i>	This Study
RF179	JH5275B; <i>[pRF4, pTET::MCD1, TRP1]</i>	This Study
RF180	JH5276B; <i>[pRF4, pTET::MCD1, TRP1]</i>	This Study
RF190	UCC5181; <i>SIR4-13xMyc-kanMX4</i>	This Study
RF204	UCC5181; <i>MATa NET1-13xMyc::kanMX4 met15^l</i>	This Study
RF206	UCC5179; <i>LRS4-13xMyc-kanMX4</i>	This Study
RF207	RF196/RF206; <i>LRS4-13xMyc-kanMX4/LRS4-13xMyc-kanMX4</i>	This Study
W303AR	<i>RDN::ADE2 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i>	(KAEBERLE <i>et al.</i> 1999)
DSY1034	<i>RDN::ADE2 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 sir2Δ::TRP1</i>	(KAEBERLE <i>et al.</i> 1999)
RF212	UCC5181; <i>NET1-13xMyc-kanMX4 met15::ADE2</i>	This Study
RF213	RF204/RF212; <i>NET1-13xMyc-kanMX4/NET1-13xMyc-kanMX4 met15::ADE2/met15^l</i>	This Study

RF217	YPH278; <i>MATa fob1Δ::hphMX4 sir2Δ::kanMX</i>	This Study
RF218	YPH278; <i>leu2Δ1::[pRF10, pTET::Empty, LEU2]</i>	This Study
RF219	YPH278; <i>leu2Δ1::[pRF11, pTET::MCD1, LEU2]</i>	This Study
RF222	YPH278; <i>sir2Δ::kanMX leu2Δ1::[pRF10, pTET::Empty, LEU2]</i>	This Study
RF223	YPH278; <i>sir2Δ::kanMX leu2Δ1::[pRF11, pTET::MCD1, LEU2]</i>	This Study
RF224	YPH278; <i>sir2Δ::kanMX hst1Δ::natMX4 leu2Δ1::[pRF10, pTET::Empty, LEU2]</i>	This Study
RF225	YPH278; <i>sir2Δ::kanMX hst1Δ::natMX4 leu2Δ1::[pRF11, pTET::MCD1, LEU2]</i>	This Study
RF226	W303AR; <i>leu2Δ1::[pRF10, pTET::Empty, LEU2]</i>	This Study
RF227	W303AR; <i>leu2Δ1::[pRF11, pTET::MCD1, LEU2]</i>	This Study
RF228	DSY1034; <i>[pRF10, pTET::Empty, LEU2]::leu2Δ1</i>	This Study
RF229	DSY1034; <i>[pRF11, pTET::MCD1, LEU2]::leu2Δ1</i>	This Study
RF232	YPH278; <i>sir2Δ::kanMX hst1Δ::natMX4 hmrΔ::hphMX fob1Δ::hphMX</i>	This Study
RF242	UCC5179; <i>SIR4-13xMyc::kanMX4, lys2¹ met15::ADE2</i>	This Study
RF243	RF190/RF242; <i>SIR4-13xMyc::kanMX4/ SIR4-13xMyc::kanMX4 lys2¹/LYS2 met15::ADE2/met15::ADE2</i>	This Study
RF258	3460-2A; <i>SPB1-dsRED-kanMX4</i>	This Study
RF278	3460-2A; <i>SPB1-dsRED-kanMX4 fob1Δ::hphMX4</i>	
RF288	FY834; <i>[pRF11, pTET::MCD1, LEU2]::leu2Δ1</i>	This Study
RF289	FY834; <i>[pRF10, pTET::Empty, LEU2]::leu2Δ1</i>	This Study
RF290	3460-2A; <i>SPB1-dsRED-kanMX4 sir2Δ::hphMX4</i>	

¹Allele status unknown

Table S2. Oligonucleotides used in the study.

Primer	Target	Sequence
JS369	<i>fob1Δ</i> _Det_2793	GACGACAATACCGCTGGCTCCCGT
JS646	<i>SIR4</i> -Myc_Fw	GGAAAAAGATTTTCAAGTGAATAAGGAGATAAAACCGT ATCGGATCCCCGGGTTAATTAA
JS647	<i>SIR4</i> -Myc_Rv	GGTACACTTCGTTACTGGTCTTTTGTAGAATGATAAAAA GGAATTCGAGCTCGTTTAAAC
JS900	<i>fob1Δ</i> _hphMX_Fw	GGAGAACAATTTAACGATTGTGTGAGTAATTTGTGCTCC GGCGCGAAGCAAAAATTACGGC
JS901	<i>fob1Δ</i> _hphMX_Rv	CACCTATGACTCCTCCTTTCATTCTATCCTACATATTCGG CGTTAGTATCGAATCGACAGC
JS1100	NTS1_Fw	TGTTAGTGCAGGAAAGCGGG
JS1101	NTS1_Rv	CTACACCCTCGTTTAGTTGC
JS1146	<i>ACT1</i> RT-qPCR_Fw	ATGTGTAAAGCCGGTTTTGCC
JS1147	<i>ACT1</i> RT-qPCR_Rv	TGGGAAGACAGCACGAGGAG
JS2129	<i>MCD1</i> -Myc_Fw	AGACGCCAAACCTGCACTATTTGAAAGGTTTATCAATG CTCGGATCCCCGGGTTAATTAA
JS2130	<i>MCD1</i> -Myc_Rv	TATTGGGTCCACCAAGAAATCCCCTCGGCGTAACTAGG TTGAATTCGAGCTCGTTTAAAC
JS2131	<i>SCC2</i> -Myc_Fw	CAAGCTTCTTACATATTTTAGAAAACACGTGAAGGATA CGCGGATCCCCGGGTTAATTAA
JS2132	<i>SCC2</i> -Myc_Rv	AATGATTATTAATACTATGTATATTTTAAGTGCAATATA TGAATTCGAGCTCGTTTAAAC
JS2153	<i>sir2Δ</i> _natMX_Fw	CATTCAAACCATTTTTCCCTCATCGGCACATTAAAGCTG GCCGCGCGAAGCAAAAATTACGGC
JS2154	<i>sir2Δ</i> _natMX_Rv	TATTAATTTGGCACTTTTAAATTATTAAATTGCCTTCTAC CGGCGTTAGTATCGAATCGACAGC
JS2155	<i>hst1Δ</i> _natMX_Fw	CTCTTCTTTTTTGTGTTTTTGTGAGAAAAAAAATCTA A CCGGCGCGAAGCAAAAATTACGGC
JS2156	<i>hst1Δ</i> _natMX_Rv	TGCAATAGCAGCGGTATACTTATTTTTACTCCCCCTTCT G CGGCGTTAGTATCGAATCGACAGC
JS2167	<i>PDC1</i> _Fw	GCCGACAGTCTGTTGAATTGG
JS2168	<i>PDC1</i> _Rv	GAAGCGGACCCAGACTTAAGC
JS2300	<i>TELXV</i> _Rv	TGGGTAAATGGCAAAGGGTA
JS2301	<i>TELXV</i> _Fw	GCACCCACATCATTATGCAC
JS2478	<i>natMX</i> _C_Readout	CCTGGACACCGCCCTGTA
JS2480	<i>hst1Δ</i> _Det_JS2478	CGGACATTTTCATTCAAGAGG
JS2602	<i>CEN3</i> _Fw	AGCGCCAAACAATATGGAAA
JS2603	<i>CEN3</i> _Rv	TGAGCAAAACTTCCACCACT
JS2606	10kb right of <i>CEN3</i> _Fw	CCTGCGTCACACATGAGAAA
JS2607	10kb right of <i>CEN3</i> _Rv	TCACAGTTTACCCGGAGGTC
JS2608	<i>CEN4</i> _Fw	ATTGCTTGCAAAAGGTCACA
JS2609	<i>CEN4</i> _Rv	GCCGCTCCTAGGTAGTGCTT

JS2734	<i>SMC1</i> -Myc_Fw	GTCGAAGATCATAACTTTGGACTTGAGCAATTACGCAG AACGGATCCCCGGGTAAATTAA
JS2735	<i>SMC1</i> -Myc_Rv	TAGTTATTTGACGGGTATAGCAGAGGTTGGTTTCATAG AGAATTCGAGCTCGTTTAAAC
JS2757	<i>CEN11</i> _Fw	CCTAATACCTCAATGGTCCAATAC
JS2758	<i>CEN11</i> _Rv	TGCTTTGATTTATACAGAGGAAGC
JS2763	<i>STE2</i> _Fw	TTCGTGACCTTCGGTATAAGG
JS2764	<i>STE2</i> _Rv	CGTAAAAGCAAAGGTGGTTTCT
JS2789	<i>LRS4</i> -Myc_Fw	TGAAGAATTGAGTAATAATTAAATGTTGACGAGTTTGTA CGGATCCCCGGGTAAATTAA
JS2790	<i>LRS4</i> -Myc_Rv	AAAAGAGAGAGGGAAGGGCAGGGACGTAGATAGCTGT TACGAATTCGAGCTCGTTTAAAC
JS2793	<i>hphMX_N</i> _Readout	CTTCCGGAATCGGGAGCGCG
JS2813	<i>hmrΔ</i> _hphMX_Fw	CGGGCTCATTCTTTCTTTCTTTGCCAGAGGCTCACCGCCG GCGCGAAGCAAAAATTACGGC
JS2814	<i>hmrΔ</i> _hphMX_Rv	ACGAATTTATTTAGATCTCATACGTTTATTTATGAACGG CGTTAGTATCGAATCGACAGC
JS2819	<i>hmrΔ</i> _Det_JS2793	TGAGAATAAGCGCAGGTACTCCTGGT
JS2864	<i>MCD1</i> _ORF_ <i>NotI</i> _Fw	GAATTTGCGGCCGCATGGTTACAGAAAATCCTCAACGT CTTAC
JS2865	<i>MCD1</i> _ORF_ <i>PstI</i> _Rv	GAATTTCTGCAGCGGTAGAGGTGTGGTCAATAAGAG
JS2842	15kb right of <i>CEN4</i> _Fw	TCAGTGCATCCATCGTCTTC
JS2843	15kb right of <i>CEN4</i> _Rv	TTGAGCTTCTAGCGACAGCA
JS2844	<i>MCD1</i> RT-qPCR_Fw	ACAGAAAGAGAAGTTTTGGTCACC
JS2949	<i>MCD1</i> RT-qPCR_Rv	TGAAGTATCCCAGGGAGCAG
JS3028	200kb right of <i>CEN4</i> _Fw	TTTTGGCACAAAGGCAATG
JS3029	200kb right of <i>CEN4</i> _Rv	TGAATCCATTTGTTTGTTAATAGTTT
JS3034	<i>CEN6</i> _Fw	TTGAAGACTATATTTCTTTTCATCACG
JS3035	<i>CEN6</i> _Rv	AACTTTCAACCTATTTTACATCTTCG
JS3036	<i>CEN14</i> _Fw	GCAATTAATTGAGTTGTTGTGAAATGG
JS3037	<i>CEN14</i> _Rv	CGCAGATATCCTTAAATACCAAA

Table S3. Detailed bud scar and western blot quantitation data.

Microscopy			Western Blot	
Factor	Bud Scars	St. Error	Qty.	St. Error
Mcd1_young	0.833333	0.272845	1	0
Mcd1_old	6.400877	0.445052	0.146018	0.119223
Mcd1_CR_young	0.216667	0.044096	1	0
Mcd1_CR_old	4.793333	0.716038	0.252445	0.08759
Sir2_young	0.975362	0.114328	1	0
Sir2_old	5.573099	0.777897	0.228414	0.186502
Scc2_young	0.54386	0.072505	1	0
Scc2_old	6.416667	0.469338	0.436795	0.083468
Smc1_young	0.637009	0.235205	1	0
Smc1_old	4.951629	0.226633	0.188713	0.154083
Hst1_young	0.508696	0.158696	1	0
Hst1_old	4.919553	0.450378	0.37225	0.111099
Net1_young	0.25	0.028868	1	0
Net1_old	5.328571	0.289469	0	0
Lrs4_young	0.066667	0.033333	1	0
Lrs4_old	5.049206	0.262414	0.077961	0.063655
Sir3_young	0.483333	0.208833	1	0
Sir3_old	4.995238	0.145238	1.229025	0.244495
Sir4_young	0.825	0.375	1	0
Sir4_old	6.0375	0.9875	0.138803	0.098148

Supplemental References

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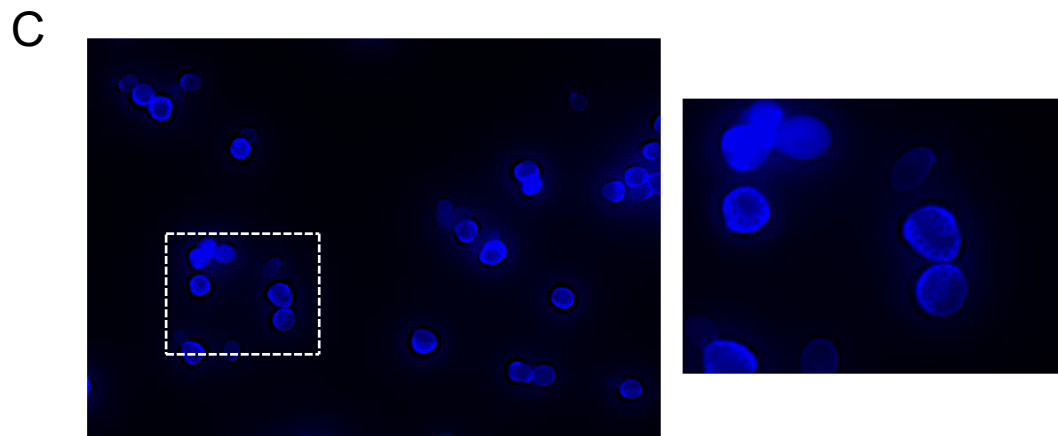
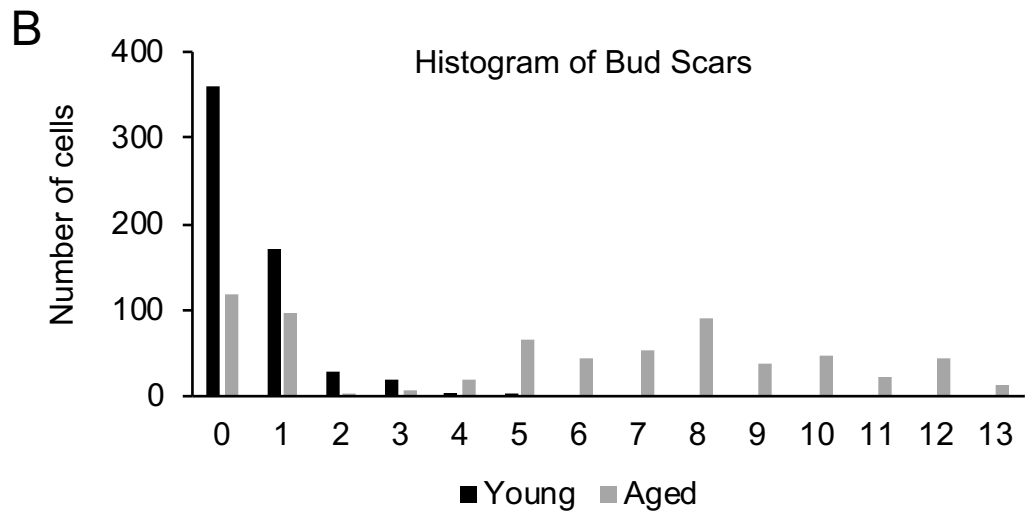
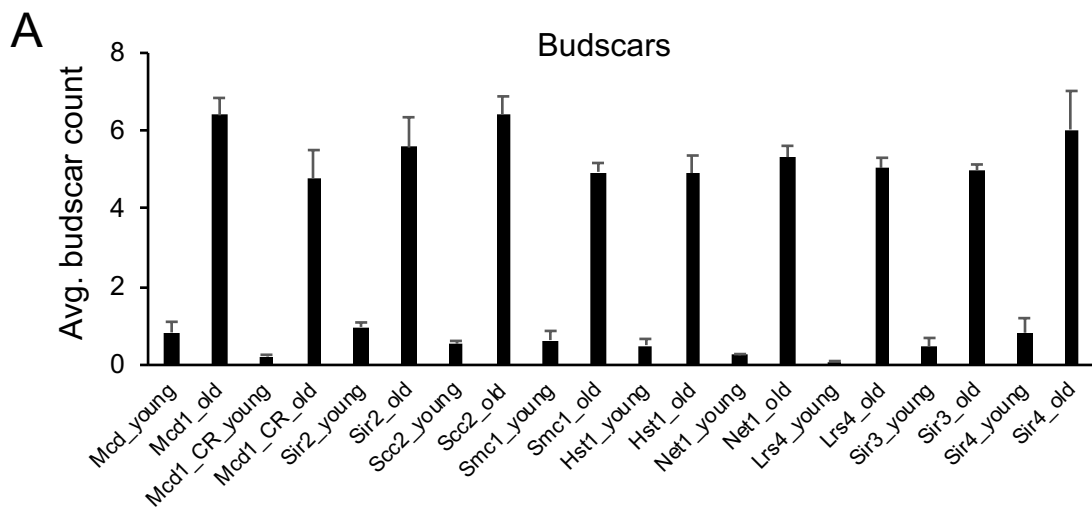


Figure S1. Detailed western blot and bud scar count quantification. (A) Bar graph showing average bud scars from young and aged cell populations that were used for western blotting. (B) Histogram of bud scars from young and aged populations used in western blot experiments. (n=584 and 658) (C) Representative image of an enriched aged cell population from the third Lrs4-13xMyc biological replicate. Inset image allows counting of individual bud scars.

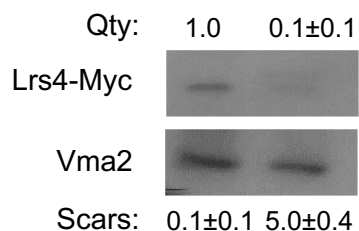


Figure S2. The Lrs4 subunit of cohibin/monopolin is depleted in aged yeast cells. Representative western blot of 13xMyc-tagged Lrs4. Vma2 is used as a loading control. Average bud scar counts are indicated at the bottom, and relative Lrs4-myc signal compared to the Vma2 loading control is indicated at the top (Qty).

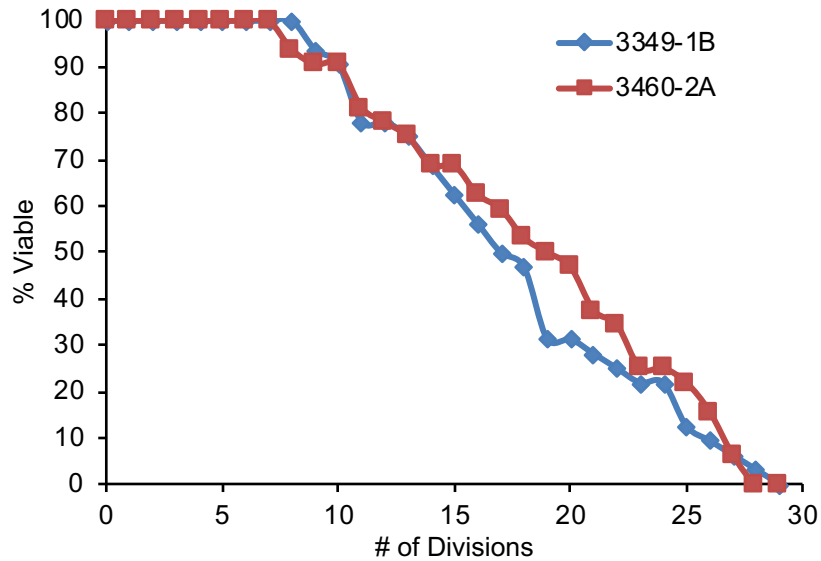


Figure S3. RLS of the cohesion visualization strains is normal and unaffected by position of the lacO array. Strain 3349-1B contains a lacO array at the *LYS4* locus on ChrIV and is used as a proxy for arm cohesion, while strain 3460-2A contains a lacO array on ChrIV 10 kb away from the *CEN4* locus that is used to monitor centromeric cohesion.

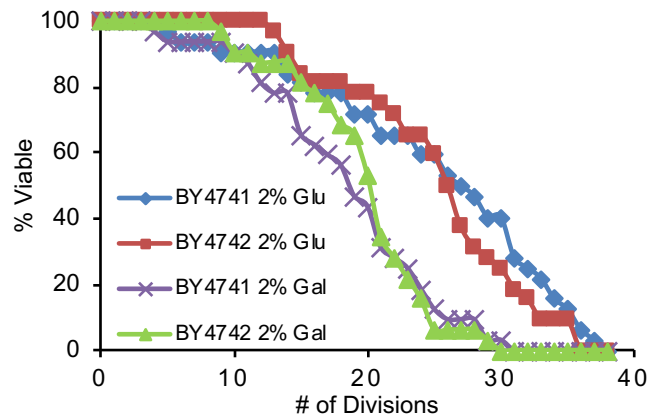


Figure S4. Galactose shortens yeast RLS in the common yeast BY4741/4742 strain background. (A) RLS of WT BY4741(*MATa*) and BY4742 (*MATa*) cells (n=32; mean rls: ◆24.3, ■24.2, ▲17.7, ×18.9) growing on YEP agar plates containing either 2% glucose or galactose as the carbon source.

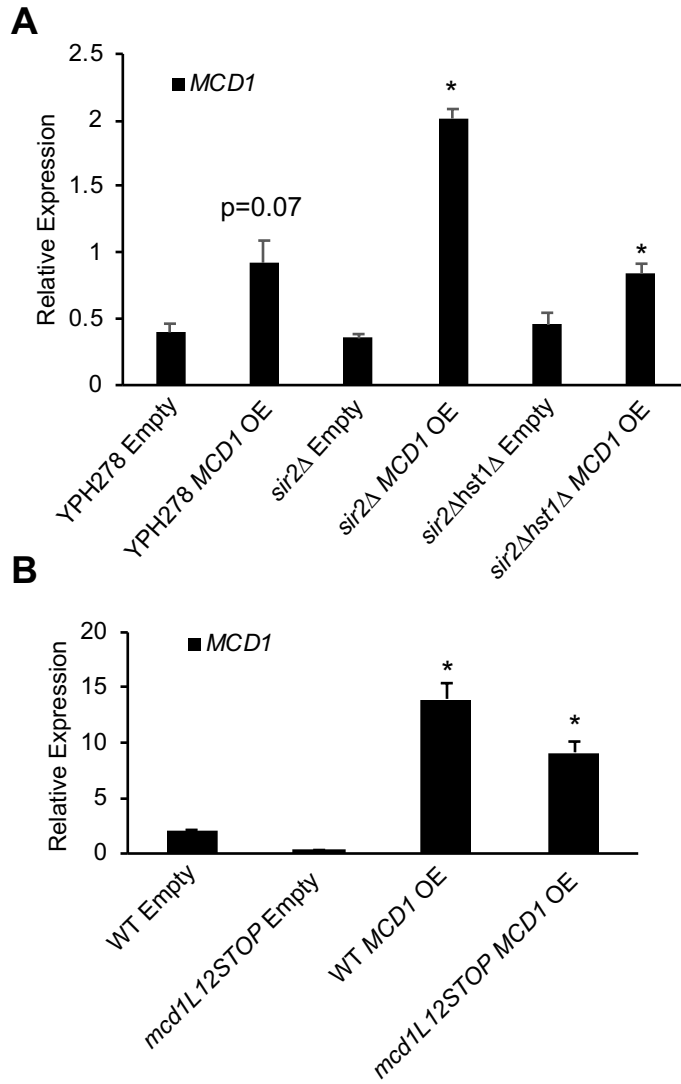


Figure S5. Doxycycline-induced *MCD1* overexpression in WT and *mcd1L12STOP* strains. (A) RT-qPCR of *MCD1* transcript levels relative to actin transcript levels in chromosome loss assay strains (YPH background) used to examine *SIR2* and *MCD1* epistasis in RLS. (B) RT-qPCR of *MCD1* transcript levels relative to actin transcript levels were quantified from the empty vector strains (RF146 and RF147) or the *MCD1* overexpression strains RF179 and RF180. Total RNA was isolated following 4 hours doxycycline induction during log-phase growth. (* $p < 0.05$, student's two-tailed t-test).

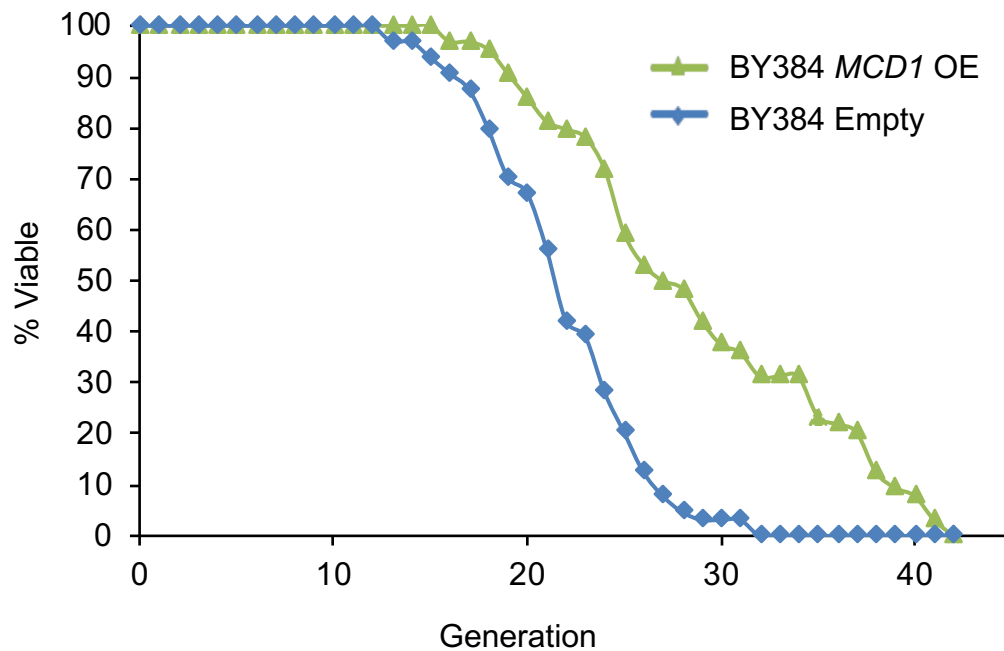


Figure S6. Doxycycline-induced *MCD1* overexpression in BY Background.

Replicative lifespan viability assay in which an integrated inducible tetracycline promoter overexpressed either the *MCD1* gene or an empty vector (mean RLS = ▲ 28.0 ♦ 21.0).

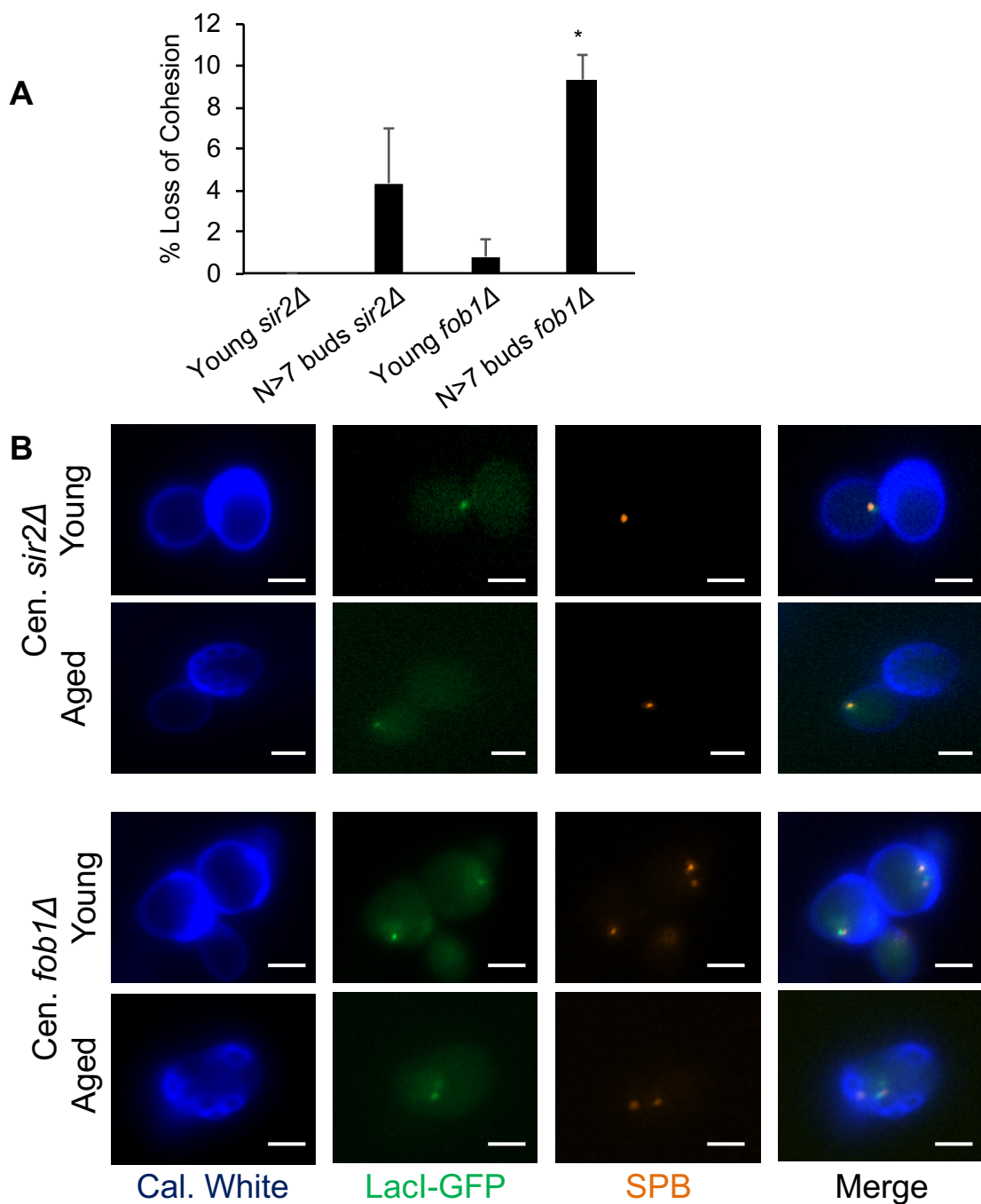


Figure S7. rDNA stability does not affect sister chromatid cohesion. (A) Quantification of cells assayed in (B) for loss (2-GFP Dots) of chromatid cohesion. (n=60 cells) (B) Representative screen shots of young (log-phase) or aged yeast cells monitoring sister chromatid cohesion 10kb proximal to *CENIV* in *sir2Δ* and *fob1Δ* mutants.