Supplemental Materials

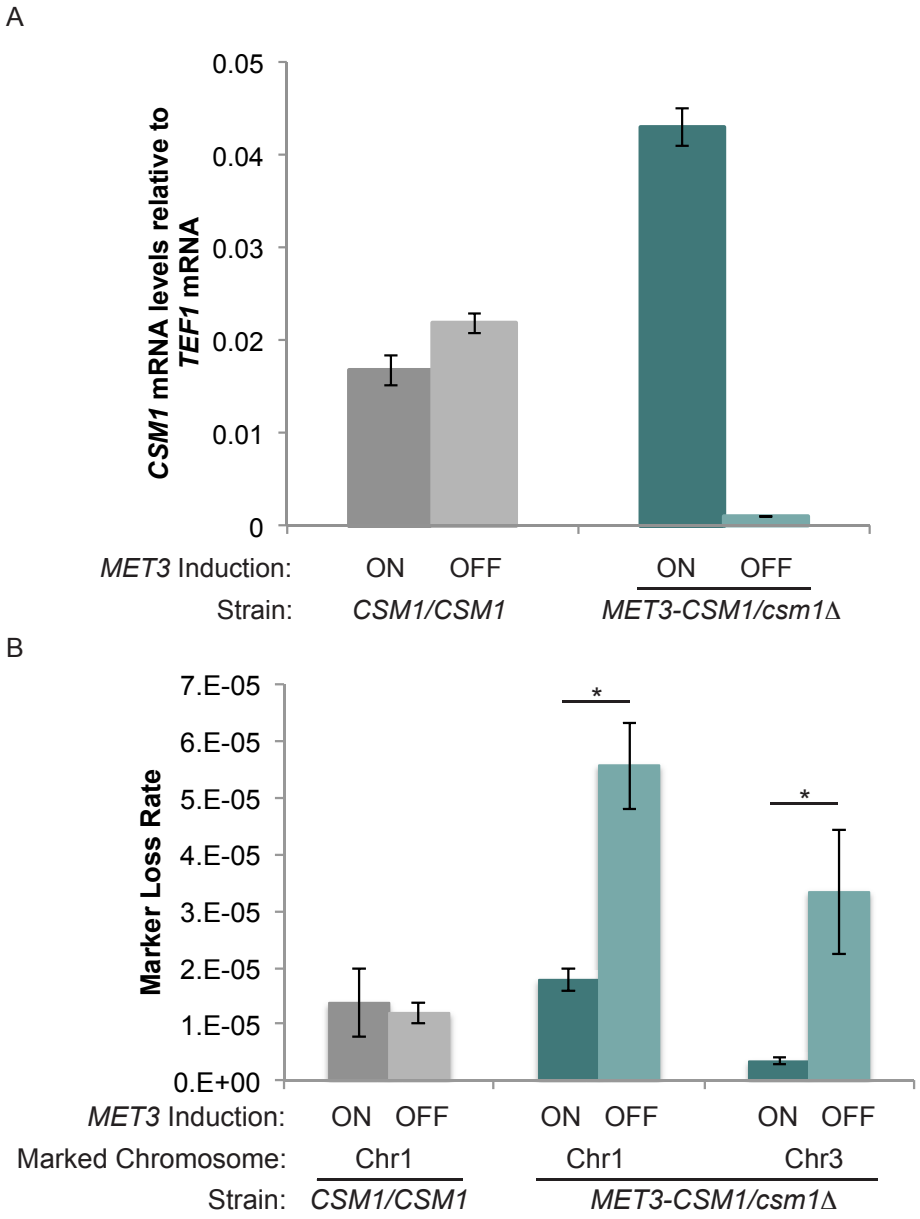
Molecular Biology of the Cell

Burrack et al.

Supplemental Table S1. *C. albicans* strains used in this study

Strain Number	Strain Details	Reference
YJB2348	*Candida albicans* SC5314	(Fonzi and Irwin, 1993)
YJB7617	YJB2348; *ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	(Wilson *et al.*, 1999)
YJB9221	*Saccharomyces cerevisiae* BY4743 (*his3Δ1/his3Δ1 leu2Δ0/leu20 ura3Δ0/ura3Δ0 lys2Δ0/+ met15Δ0/+*)	(Brachmann *et al.*, 1998)
YJB10038	YJB2348; *ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	(Noble and Johnson, 2005)
YJB10744	YJB7617, *ERG13-GFP-URA3/ERG13*	This work
YJB11700	YJB7617, *ade2Δ/ADE2 gal1Δ::HIS1/GAL1 bud3Δ::URA3/BUD3*	This work
YJB12291	YJB10038, *csm1Δ::UAU/csm1Δ::URA3*	This work
YJB12292	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/CSM1*	This work
YJB12344	YJB10038, *NAT1-MET3p-CSM1/csm1Δ::HIS1*	This work
YJB12380	YJB10038, *cse4::PCK1p-CSE4(URA3)/CSE4 NAT1-MET3p-CSM1/csm1Δ::HIS1*	This work
YJB12465	YJB7617, *SMC4-HA-NAT1/SMC4*	This work
YJB12550	YJB10038, *csm1Δ::UAU/csm1Δ::URA3*	This work
YJB12624	YJB10038, *csm1Δ::UAU/csm1Δ::URA3 SMC4-HA-NAT1/SMC4*	This work
YJB12715	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/csm1Δ::URA3*	This work
YJB12716	YJB10038, *gal1Δ::NAT1/GAL1 csm1Δ::UAU/csm1Δ::URA3*	This work
YJB12796	YJB10038, *csm1Δ::UAU/csm1Δ::URA3 SMC4-HA-NAT1/SMC4*	This work
YJB12797	YJB10038, *csm1Δ::UAU/csm1Δ::URA3 SMC4-HA-NAT1/SMC4*	This work
YJB12830	YJB7617, *NOP1-M Cherry-NAT1/NOP1 CSM1-GFP-URA3/CSM1-GFP-HIS1*	This work
YJB12928	YJB7617, *CSM1-GFP-URA3/CSM1*	This work
YJB12942	YJB7617, *MTW1-M Cherry-NAT1/MTW1 CSM1-GFP-URA3/CSM1-GFP-HIS1*	This work
YJB12990	YJB9221, *ChrX(119548..119797)::URA3*	This work
YJB12991	YJB9221, *ChrX(119548..119797)::URA3*	This work
YJB12992	YJB9221, *ChrXII(554014..554154)::URA3*	This work
YJB12993	YJB9221, *ChrXII(554014..554154)::URA3*	This work
YJB12994	YJB9221, *csm1Δ::KanMX/csm1Δ::KanMX*	*S. cerevisiae* diploid deletion collection
YJB12995	YJB9221, *csm1Δ::KanMX/csm1Δ::KanMX ChrX(119548..119797)::URA3*	This work
YJB12996	YJB9221, *csm1Δ::KanMX/csm1Δ::KanMX ChrX(119548..119797)::URA3*	This work
YJB12997	YJB9221, *csm1Δ::KanMX/csm1Δ::KanMX*	This work

	ChrXII(554014..554154)::URA3	
YJB12998	YJB9221, *csm1Δ::KanMX/csm1Δ::KanMX ChrXII(554014..554154)::URA3*	This work
YJB13002	YJB10038, *csm1Δ::HIS1/NAT1-MET3p-CSM1 cse4::PCK1p-CSE4(URA3)/CSE4 gal1Δ::ARG4/GAL1*	This work
YJB13008	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/csm1Δ::URA3 gal1Δ::NAT1/GAL1*	This work
YJB13009	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/csm1Δ::URA3 gal1Δ::NAT1/GAL1*	This work
YJB13010	YJB10038, *csm1Δ::UAU/CSM1 gal1Δ::HIS/GAL1 NAT1-MET3p-SMC4/SMC4*	This work
YJB13024	YJB10038*, ORF19.1963::TetR-GFP-Nat::ORF19.1961/ ORF19.1963,ORF19.1961 TetO-HIS1::CEN7*	This work
YJB13040	YJB10038, *csm1Δ::UAU/csm1Δ::URA3 gal1Δ::HIS/GAL1 NAT1-MET3p-SMC4/SMC4*	This work
YJB13077	YJB10038, *ORF19.1963::TetR-GFP-Nat::ORF19.1961/ ORF19.1963,ORF19.1961 TetO-HIS1::CEN7 csm1Δ::UAU/csm1Δ::URA3*	This work
YJB13142	YJB11700, *NAT1-MET3p-SMC4/SMC4*	This work
YJB13146	YJB10744, *NAT1-MET3p-SMC4/SMC4*	This work
YJB13149	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/csm1Δ::URA3 MTW1-GFP-NAT1/MTW1*	This work
YJB13150	YJB10038, *cse4::PCK1p-CSE4(HIS1)/CSE4 csm1Δ::UAU/csm1Δ::URA3 TUB1-GFP-NAT1/TUB1*	This work



Supplemental Figure S1. Conditional repression of CSM1 increases chromosome loss rates.
(A) Control (gray) and *MET3p-CSM1/csm1Δ* (teal) strains were grown under activating conditions (SDC-Met-Cys, *CSM1* ON) or repressing conditions (SDC+Met+Cys, *CSM1* OFF) for 6 h. mRNA levels of *CSM1* relative to the reference gene *TEF1* were measured by qRT-PCR. Data shown are mean ± SEM of 2 biological replicates. (B) Fluctuation analysis of loss of *GAL1* (Chr1) or *URA3* (Chr3 – near *CSE4* locus) in control and *MET3p-CSM1/csm1 Δ* strains during growth under activating conditions (SDC-Met-Cys, *CSM1* ON) or repressing conditions (SDC+Met+Cys, *CSM1* OFF). Loss of *GAL1* or *URA3* was quantified by plating cells on non-selective media and on media containing 2-DOG to select for loss of *GAL1* or 5-FOA to select for loss of *URA3*. Colony counts were used to calculate the rate of loss per cell division. Results are the mean ± SEM of the rates calculated from at least 3 experiments, each with 8 cultures per condition. Significance between activating and repressing conditions was determined by a two-tailed unpaired t-test. * $p<0.05$.

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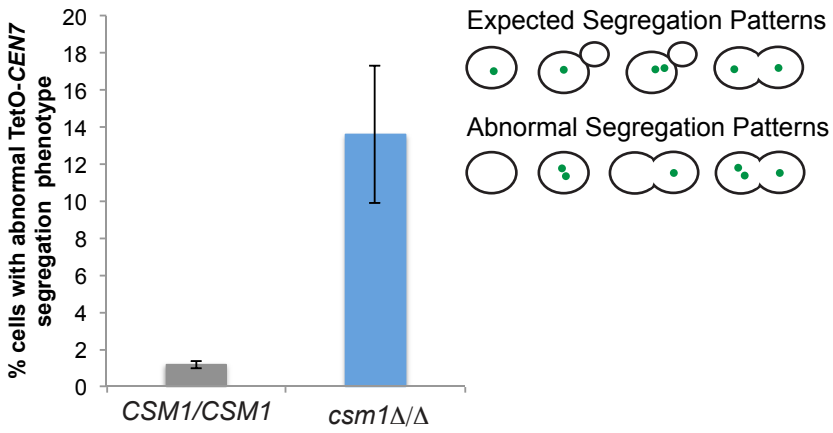
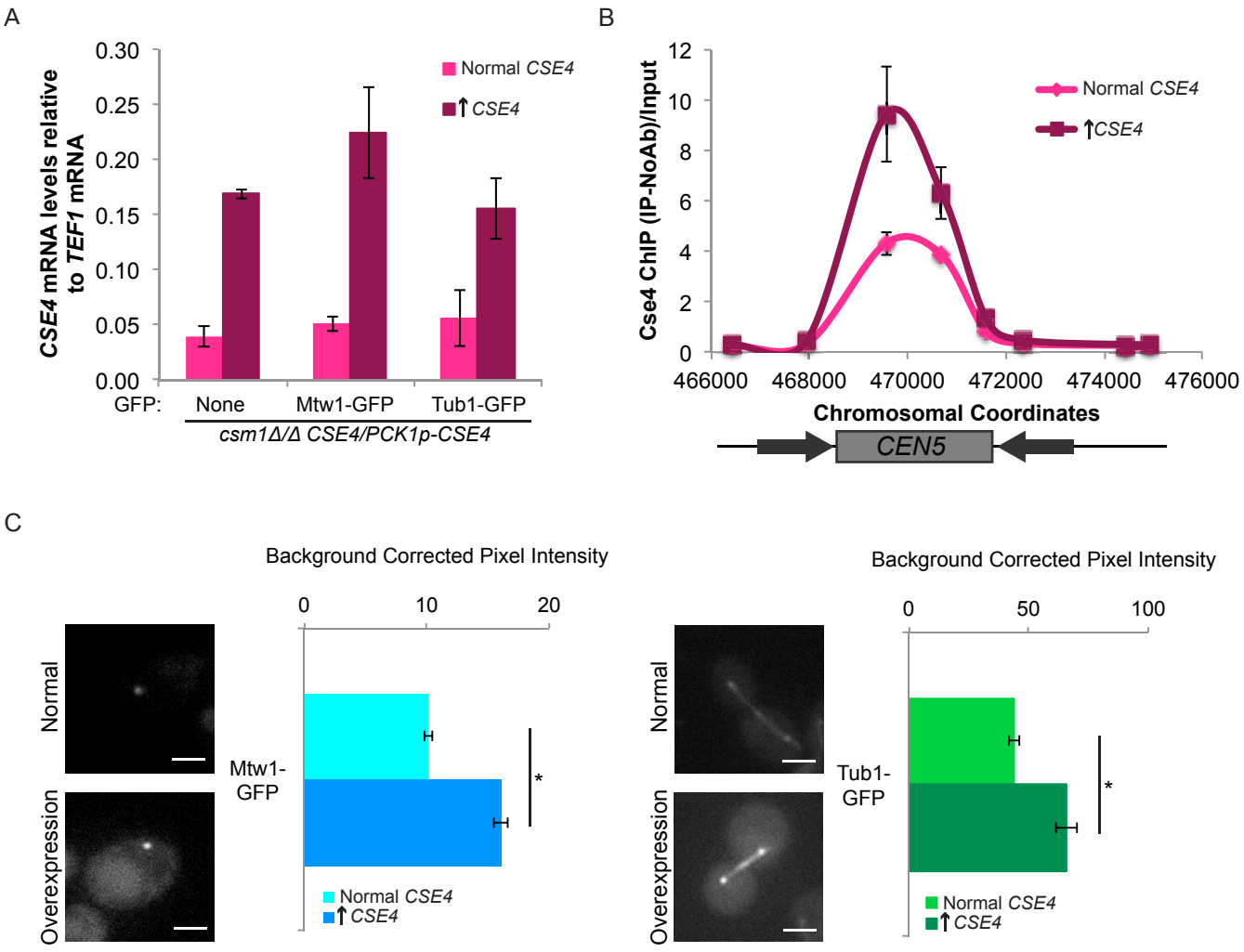


Supplemental Figure S2. Monopolin deletion increases abnormal chromosome segregation. (A) Control strains and *csm1Δ/Δ* TetO-*CEN7-GFP* strains were grown in were grown in SDC-glucose for 4 h. Cells imaged at 1000X total magnification with a GFP filter set. The percentage of cells with abnormal segregation patterns was determined (right panels show expected and abnormal segregation patterns of TetO-*CEN7*). Results are the mean ± SEM of 2 biological replicates with at least 200 cells per replicate.



Supplemental Figure S3. Requirement for *CSM1* is not dependent on the number of kinetochore proteins and spindle microtubules. (A) The *csm1Δ/Δ CSE4/PCK1p-CSE4* strain was grown under repressing conditions (YPA-glucose – normal expression, magenta) and grown in activating conditions (YPA-succinate - overexpression, maroon) for 6 h. mRNA levels of *CSE4* relative to the reference gene *TEF1* were measured by qRT-PCR. Data shown are mean ± SEM of at least 2 biological replicates. (B) Anti-Cse4 ChIP analyzed with primers amplifying *CEN5* for *csm1Δ/Δ CSE4/PCK1p-CSE4* strains grown in repressing conditions (YPA-glucose - normal expression, magenta) and grown in activating conditions (YPA-succinate - overexpression, maroon) for 6 h. Data shown are mean ± SEM of 2 biological replicates.(C) Overexpression of *CSE4* increases copy number of Mtw1-GFP (representative kinetochore protein, left panels) and Tub1-GFP (microtubule, right panels) per centromere. *csm1Δ/Δ CSE4/PCK1p-CSE4* strains with Mtw1-GFP or Tub1-GFP were grown in repressing conditions (SDC-glucose - normal expression) and grown in activating conditions (SDC-succinate- overexpression) for 6 h. Cells were imaged at 1000X total magnification with a GFP filter set. GFP fluorescence was quantified by selecting the kinetochore region in each cell and measuring total pixel intensity in the region, corrected for background fluorescence. Data shown are mean ± SEM for at least 50 cells/experiment for 3 biological replicates. Differences between normal expression of *CSE4* and overexpression of *CSE4* for each strain were found to be statistically significant (<0.0001) using two-tailed unpaired Student's t-tests (*). Scale bar = 2μm.

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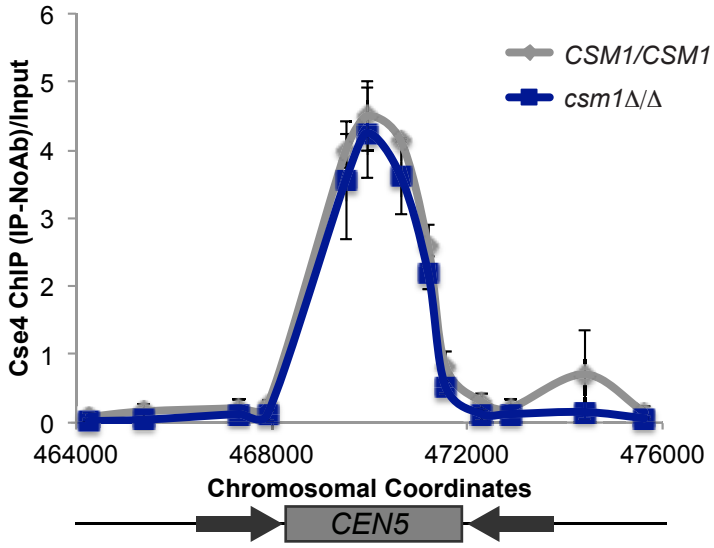

Supplemental Figure S4. Monopolin deletion does not alter Cse4 incorporation into centromeric chromatin. (A) Anti-Cse4 ChIP analyzed with primers amplifying *CEN5* for *SMC4-HA* (gray) and *SMC4-HA csm1Δ/Δ* (dark blue) strains grown in YPA-glucose for 4 h. Data shown are mean ± SEM of 2 biological replicates.

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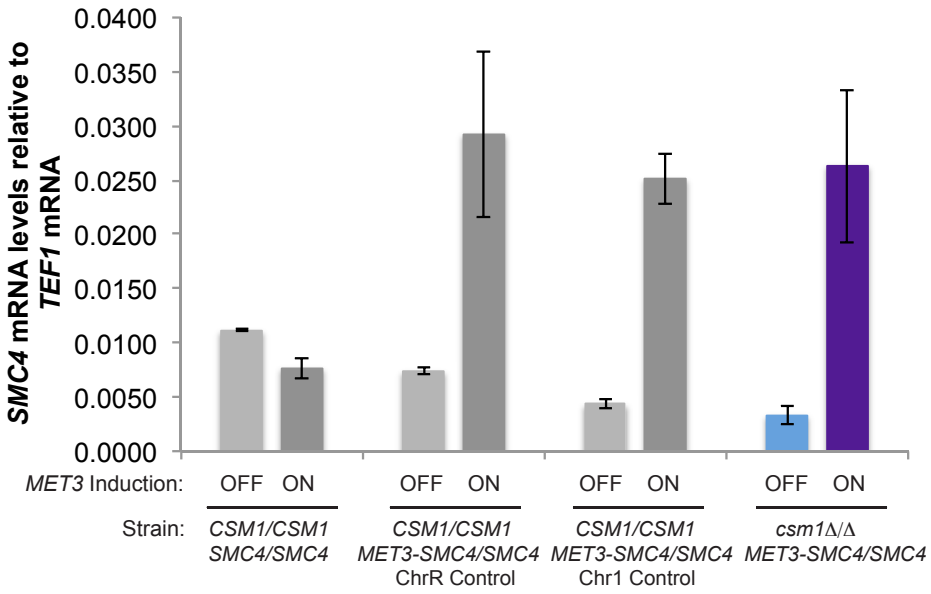

Supplemental Figure S5. Overexpression of *SMC4* is induced with a conditional *MET3* promoter. (A) Control *SMC4/SMC4* and *MET3p-SMC4/SMC4* (gray) and *csm1Δ/Δ MET3p-SMC4/SMC4* strains (blue/purple) were grown in repressing conditions (SDC+Met+Cys - normal expression in *MET3p-SMC4* strain indicated in blue) and growth in activating conditions (SDC-Met-Cys – overexpression in *MET3p-SMC4* strain indicated in purple) for 6 h. mRNA levels of *SMC4* relative to the reference gene *TEF1* were measured by qRT-PCR. Data shown are mean ± SEM of at least 2 biological replicates.

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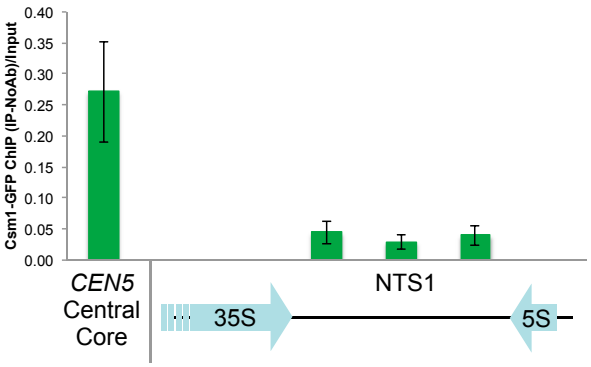

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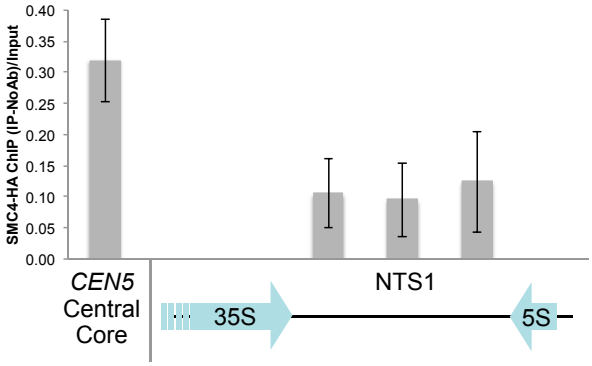

Supplemental Figure S6. Monopolin and condensin bind to the NTS1 region of rDNA. (A) Anti-GFP ChIP from a *CSM1-GFP* strain released from stationary phase for into YPA-glucose for 15 min (G1) analyzed with primers amplifying *CEN5* and the NTS1 region of the rDNA repeats. Data shown are mean ± SEM of 4 biological replicates. (B) Anti-HA ChIP from an *SMC4-HA* strain released from stationary phase for into YPA-glucose for 15 min (G1) analyzed with primers amplifying *CEN5* and the NTS1 region of the rDNA repeats. Data shown are mean ± SEM of 3 biological replicates.

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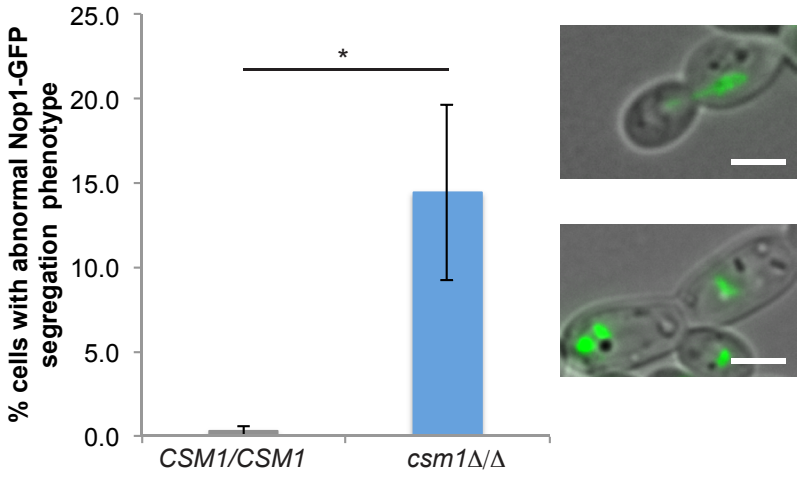

Supplemental Figure S7. Monopolin deletion increases abnormal nucleolar segregation. (A) Control strains and *csm1Δ/Δ NOP1-GFP* strains were grown in were grown in SDC-glucose for 4 h. Cells imaged at 1000X total magnification with a GFP filter set. The percentage of cells with abnormal nucleolar segregation was determined (right panels show representative abnormal cells). Results are the mean ± SEM of 3 biological replicates. Significance was determined by a two-tailed paired t-test. * p=0.05. Scale bar = 5μm.

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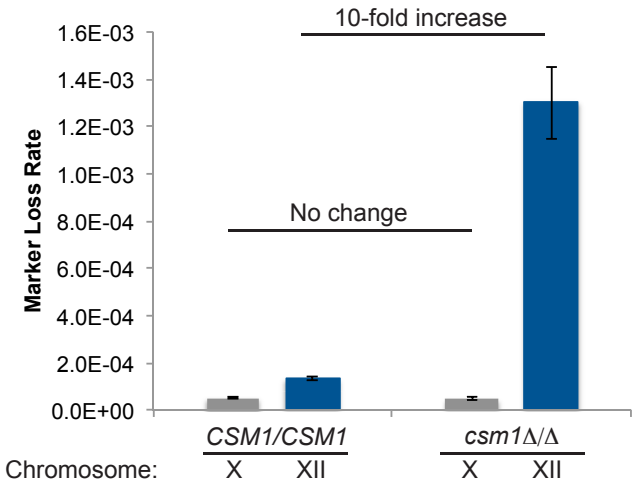
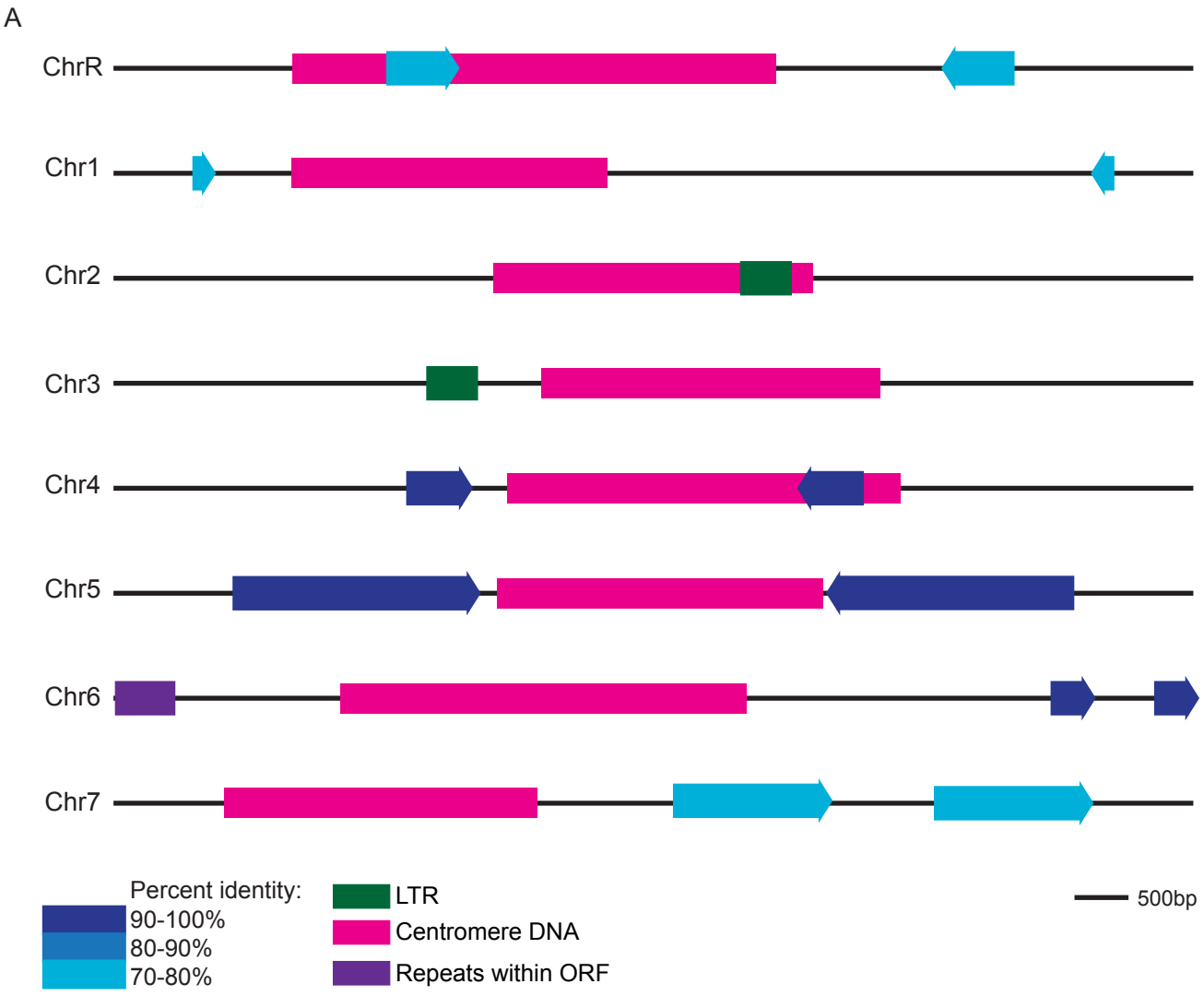


Supplemental Figure S8. Monopolin is required for rDNA segregation in *S. cerevisiae*. (A) Fluctuation analysis of loss of *URA3* inserted in a Chromosome X intergenic region or in a Chromosome XII intergenic region in control and *csm1Δ/Δ* diploid *S. cerevisiae* strains. Loss of *URA3* was quantified by plating cells on non-selective media to obtain total numbers of cells and on media containing 5-FOA to select for loss of *URA3*. Colony counts were used to calculate the rate of loss per cell division. Results are the mean ± SEM of the rates calculated from at least 3 experiments, each with 8 cultures per condition.



Supplemental Figure S9. Repeat structures found near *C. albicans* centromeres. (A) Centromere regions as annotated in the Candida Genome Database are indicated in pink. Tandem and inverted repeats are in shades of blue with degree of homology indicated on the blue color bar scale (see scale). Long terminal repeats (LTRs) are shown in green. The 3' end of the *ALS2* gene adjacent to *CEN6* containing many tandem repeats indicated in purple.