Supplemental Materials

Molecular Biology of the Cell

Lawrimore et al.

Figure S1. Phleomycin and Zeocin sensitivity. Ten-fold dilutions series on YPD and 0.1 µg/mL, 1 µg/mL, and 5 µg/mL Phleomycin and 1 µg/mL and 5ug/mL Zeocin. All strains are in the KS406 background.

Figure S2. NucleaseCutting Efficiency.
We monitored cutting efficiency using quantitative PCR to measure the decrease in intensity of a fragment upon introduction of a DNA double strand break. For I-SceI, the cut site lies in the LYS2 gene (see Figure 1). (B) KS406 (blue) contains I-SceI and the I-SceI cut site, KS414 (orange) is derived from KS406 and is deleted for *mre11*. *mre11* is part of the RMX DNA repair complex and required for double strand break repair (Lobachev *et al.*, 2004). KS453 (grey) does not contain I-SceI. (D) The uncut LYS2 fragment (2.1 kb) is shown in samples grown on I-SceI repression conditions (yeast extract, peptone, lactate YPL). I-SceI was induced (galactose, gal) for 3 and 6 hours. The experiments were run in triplicate and fragment intensity (fluorescence units) corrected for input (E). There is an 85% reduction in the fragment containing the LYS2 by 6 hours following I-SceI induction. This value is consistent with recent reports (Seeber *et al.*, 2016). For HO, the cut site lies to the left of CEN3 (A). Fragment containing the HO cut site was reduced to 40% of uncut values (C). (F) Efficiency of cutting in nocodazole treated cells. I-SceI was induced for 3 and 6 hours in the presence(grey) or absence(blue) of nocodazole (20 µg/ml) in KS406 that contains I-SceI and the I-SceI cut site. The experiments were run in triplicate and fragment intensity corrected for input (LEU2). Data expressed as a ratio of the two fragments was plotted as a function of time on galactose. There is an 89% reduction in the LYS2 fragment 6 hours following I-SceI induction in the presence or absence of nocodazole.

Figure S3. Images of pericentric cohesin in untreated, Phleomycin, sDSB, and Zeocin treated cells. GAL HO induced DSB at MAT (4h galactose), 3 µg/mL Phleomycin for 30 min and 250 µg/mL Zeocin for 2 hours. Scale bar, 1 µm.

Figure S4. Expansion of Pericentric cohesin is dependent of the DNA checkpoint pathway and histone phosphorylation. (A) Cohesin sagittal width in a *rad9Δ*. Neither phleomycin treatment (KBY6054) nor induction of a single DSB (KBY6056)caused significant expansion of cohesin (Student's T-test, $p > 0.05$). *rad9Δ* untreated (n=33), +phleomycin (n=79), *rad9Δ* GALHO no induction (n=43), *rad9Δ* GALHO + induction (n=48). (B) Cohesin sagittal width in two histone H2A non-phosphorylatable mutants, S129A(Mec1/Tel1 kinase) (KBY6062) and S121A (Bub1 kinase)(KBY8789). Phleomycin treatment did not cause significant expansion of cohesin in either mutant (Student's T-test, $p > 0.05$). S129A untreated (n= 52), +phleomycin (n=72), S121A untreated (n=23), S121A + phleomycin (n=46). Error bars are standard deviation.

Figure S5. Population images of Rad52-GPF foci in WT, esc1Δ and Phleomycin treated cells. Scale bar, 1 µm.

Figure S6. Comparison of MSD curves for 3 second vs 30 second intervals for 10 kb array of lacO/LacI-GFP 6.8 kb from CEN XV (KBY8065). Error bars are standard devation.

Figure S7. Mean squared displacements of mid-beads of polymer simulations. The ensemble mean squared displacement of the middle bead in each of the four chains (A-D) for simulations where the centromere and telomere is fixed in space (blue), the centromere is free to move (orange), and the telomere is free to move (gray). Each condition was run 10 times. Error bars are SEM.

Strain	Sample Size	Relevant Genotype/Drug Treatment	Treatment Media	I-SceI Active	Ensemble Averaged Radius of Confinement	Standard Error of the Mean Radii of Confinement
KS406	25	WT	Glucose	No	697	28
KS406	61	Nocodazole	Glucose	No	771	27
KS406	68	Phleomycin	Glucose	No	828	30
KS406	23	Zeocin	Glucose	No	781	55
KS453	36	No Gal1/10 I-SceI	Galactose	NA	745	43
KS406	48	sDSB	Galactose	Yes	918	81
KBY8065	43	WT	Glucose	NA	372	12
KBY8065	53	Phleomycin	Glucose	NA	402	21
KS421	39	esc1Δ	Glucose	No	810	46
KS418	17	yku70Δ	Glucose	No	857	90
KS419	47	yku70Δ esc1Δ	Glucose	No	809	54
KS406	20	Nocodazole+sDSB	Galactose	Yes	588	49
KS406	22	Latrunculin A + sDSB	Galactose	Yes	745	48
KBY6091	24	kar9Δ+ sDSB	Galactose	Yes	697	44
KBY6093	22	dhc1Δ+sDSB	Galactose	Yes	835	49

Table S1. Radii of confinement

	Relevant Genotype	**Full Genotype**
KS406	WT arm locus 240kb from CEN2	MATα, ade5-1, trp1-289, ura3Δ, leu2-3, 112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd
KS453	No Gal1/10 I-SceI	MATα, ade5-1, trp1-289, ura3Δ, leu2-3, 112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd
KBY8065	WT arm locus 8.8kb from centromere	MATa ade2-1 his3-11 trp1-1 ura3-1 leu2-3,112 can1 LacINLSGFP:HIS3 lacO::URA3 (1.8kb from CEN15) Spc29RFP::Hb
KS421	esc1	MATα, ade5-1, trp1-289, ura3Δ, leu2-3,112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd, esc1Δ::TRP1
KS418	yku70	MATα, ade5-1, trp1-289, ura3Δ, leu2-3,112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd, yku70Δ::KanMX
KS419	yku70 esc1	MATα, ade5-1, trp1-289, ura3Δ, leu2-3,112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd, yku70Δ::KanMX, esc1Δ::TRP1
KBY6093	dhc1	MATα, ade5-1, trp1-289, ura3 Δ, leu2-3,112, lys2::insI-Sce1, lacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP:Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd, dhc1Δ::KAN
KBY6091	kar9	MATα, ade5-1, trp1-289, ura3 Δ, leu2-3,112, lys2::insI-Sce1, lacO array next to RAD16 promoter, tetO array next to LYS2 promote, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP:Nat, ade1::URAptetR-CFP:Hyg, Spc29-RFP::Bsd, kar9Δ::KAN
KBY6047	Ame1-GFP	MATα, ade5-1, trp1-289, ura3Δ, leu2-3, 112, lys2::insI-Sce1, LacO array next to RAD16 promoter, tetO array next to LYS2 promoter, arg4::hisG Gal1/10 I-Sce1, thr1::HISpLacI-GFP::Nat, ade1::URAptetR-CFP::Hyg, Spc29-RFP::Bsd, Ame1-GFP::Kan
WLY8912	WT	MATα, trp1Δ-63, ura3-52, his3 Δ200, lys2-8Δ1, Smc3-GFP::URA3, Spc29-RFP::Hb
KBY6050	GALHO	hoΔ, hmlΔ::ADE1, MAT α, hmrΔ::ADE1, ade1-100, leu2-3,112, lys5, trp::hisG, ura3-52, ade3::GAL::HO, Spc29-RFP::Hb, Smc3-GFP::URA3
KBY6054	rad9	MATα, trp1Δ63, ura3-52, his3Δ200, lys2-8Δ1, Smc3-GFP::URA3, Spc 29-RFP::Hb, rad9Δ::TRP1
KBY6056	rad9	MATα, hoΔ, hmlΔ::ADE1hmrΔ::ADE1, ade1-100, leu2-3,112, lys5, trp::hisG, ura3-52, ade3::GAL::HO, Spc29-RFP::Hb, Smc3-GFP::URA3, rad9Δ::TRP1
KBY6062	H2AS129A	MATα, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, ura3-52,1, leu2Δ1, lys2Δ1, lys2-128Δ, his3Δ200, trp1Δ63, HTA1-S129A-HTB1(HIS3 CEN plasmid), Spc29-RFP::Hb, Smc3-GFP::URA3
KBY8786	H2AS121A	MATα, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his200, trp163, lys2-188, ura3-52, leu21, HTA1-S121A-HTB1(HIS3 CEN plasmid), Smc3-GFP::URA3, Spc29-RFP::Hb
KBY6100	Tel3L-GFP	MATa, ade1, met14, ura3-52, leu2-3,112, lys2delta::LacI-GFP-NLS-NATr, his3-11,15, TEL3::LacO::LEU2 (pAFS102), Nup49-RFP::KAN
KBY6102	Tel3L-GFP esc1	MATa, ade1, met14, ura3-52, leu2-3,112, lys2delta::LacI-GFP-NLS-NATr, his3-11,15, TEL3::LacO::LEU2 (pAFS102),Nup49-RFP::KAN, esc1Δ::Hb
KBY6105	Tel3L-GFP yku70	MATa ade1 met14 ura3-52, leu2-3,112, lys2delta::LacI-GFP-NLS-NATr, his3-11,15, TEL3::LacO::LEU2 (pAFS102), Nup49-RFP::KAN, kan::HIS3, yku70Δ::KAN

KBY6106	Tel3L-GFP yku70 esc1	MATa ade1 met14 ura3-52 leu2-3,112 lys2delta::lacI-GFP-NLS-NATr his3-11,15 TEL3::lacO::LEU2 (pAFS102 BsaBI), Nup49-RFP:KAN, kan Δ::HIS3, yku70Δ::KAN, esc1Δ::URA3
KBY6301	Tel3L-GFP kar9	MATa ade1 met14 ura3-52 leu2-3,112 lys2delta::lacI-GFP-NLS-NATr his3-11,15, TEL3::lacO::LEU2 (pAFS102 BsaBI), Nup49-RFP::KAN, kan Δ::HIS3, kar9Δ::KAN
KBY6107	Tel3L-GFP dhc1	MATa ade1 met14 ura3-52 leu2-3,112 lys2delta::lacI-GFP-NLS-NATr his3-11,15 TEL3::lacO::LEU2 (pAFS102 BsaBI) Nup49-RFP::KAN (isolate 15) kan Δ::HIS3 dhc1 Δ::KAN
KBY3584	Rad52-GFP	MATa ade2 ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys 2-8Δ1 Rad52-GFP::Kan Spc29-CFP::HygB
KBY6302	Rad52-GFP esc1	MATa ade2 ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys 2-8Δ1 Rad52-GFP::Kan Spc29-CFP::HygB esc1Δ::TRP1
KBY8745	Ame1-GFP	MATa ade2 ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys 2-8Δ1 Spc29-RFP::Hg, Ame1-GFP::Kan
KBY8227	CEN3 adjacent HO cut site	MATahmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG' ura3-52 ade3::GAL::HO CEN3HOcs::HPH, pFD025 (URA3+) inserted right of CEN3 (NAT-target site inserted 3.1kb downstream of CEN3) pSR12 (lacO/lexA::Leu2 inserted at NAT) His3p::LacI-GFP:NAT Spc29CFP::Kan
SGD10.2	GALCEN3	Mat a CEN3(3.8)-GFP[10kb] ade2, ura3, leu2, trp1, his3, can1-100 Cen linked LacO is URA3 tagged, URA3Δ::Nat, GALCEN3::URA3, Spc29-RFP::Hb

Table S2. Strains and Genotypes

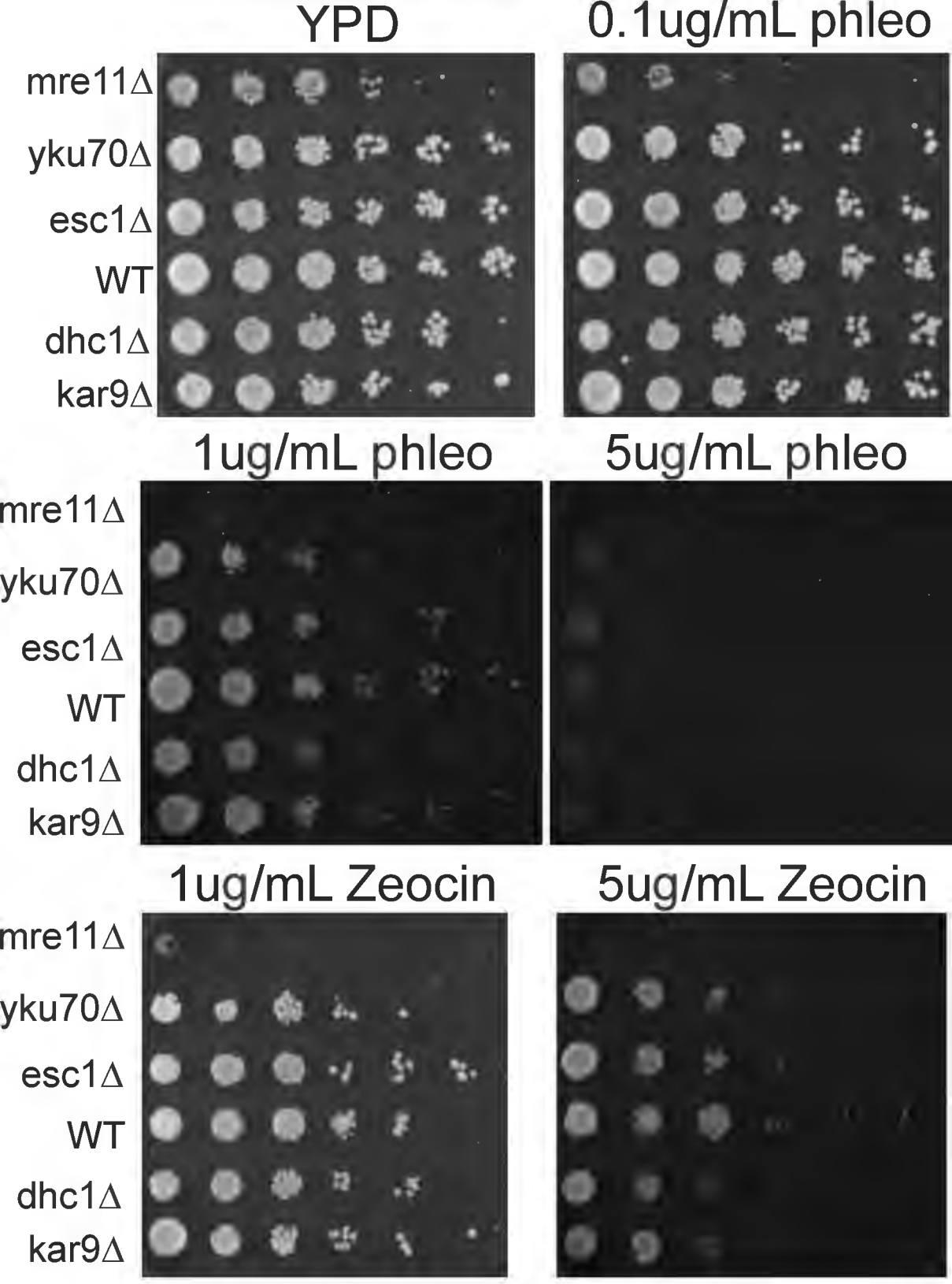

YPD
0.1ug/mL phleo
mre11Δ
yku70Δ
esc1Δ
WT
dhc1Δ
kar9Δ
1ug/mL phleo
5ug/mL phleo
mre11Δ
yku70Δ
esc1Δ
WT
dhc1Δ
kar9Δ
1ug/mL Zeocin
5ug/mL Zeocin
mre11Δ
yku70Δ
esc1Δ
WT
dhc1Δ
kar9Δ

Figure S1

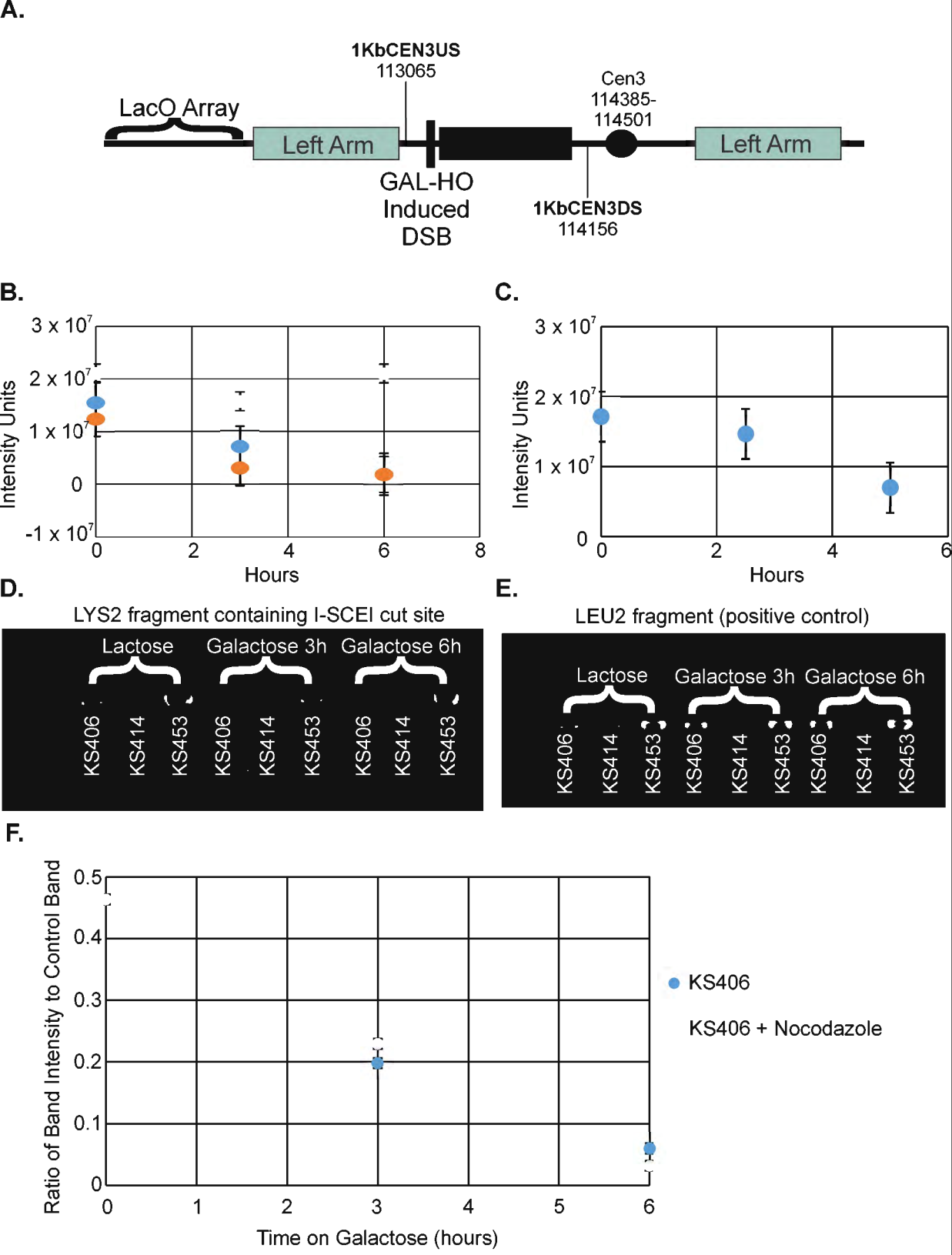

A.
1KbCEN3US
113065
Cen3
114385-
114501
LacO Array
Left Arm
Left Arm
GAL-HO
Induced
DSB
1KbCEN3DS
114156
B.
Intensity Units
3 x 10^7
2 x 10^7
1 x 10^7
0
-1 x 10^7
0
2
4
6
8
Hours
C.
Intensity Units
3 x 10^7
2 x 10^7
1 x 10^7
0
0
2
4
6
Hours
D.
LYS2 fragment containing I-SCEI cut site
Lactose
Galactose 3h
Galactose 6h
KS406
KS414
KS453
KS406
KS414
KS453
KS406
KS414
KS453
E.
LEU2 fragment (positive control)
Lactose
Galactose 3h
Galactose 6h
KS406
KS414
KS453
KS406
KS414
KS453
KS406
KS414
KS453
F.
Ratio of Band Intensity to Control Band
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Time on Galactose (hours)
KS406
KS406 + Nocodazole

Figure S2

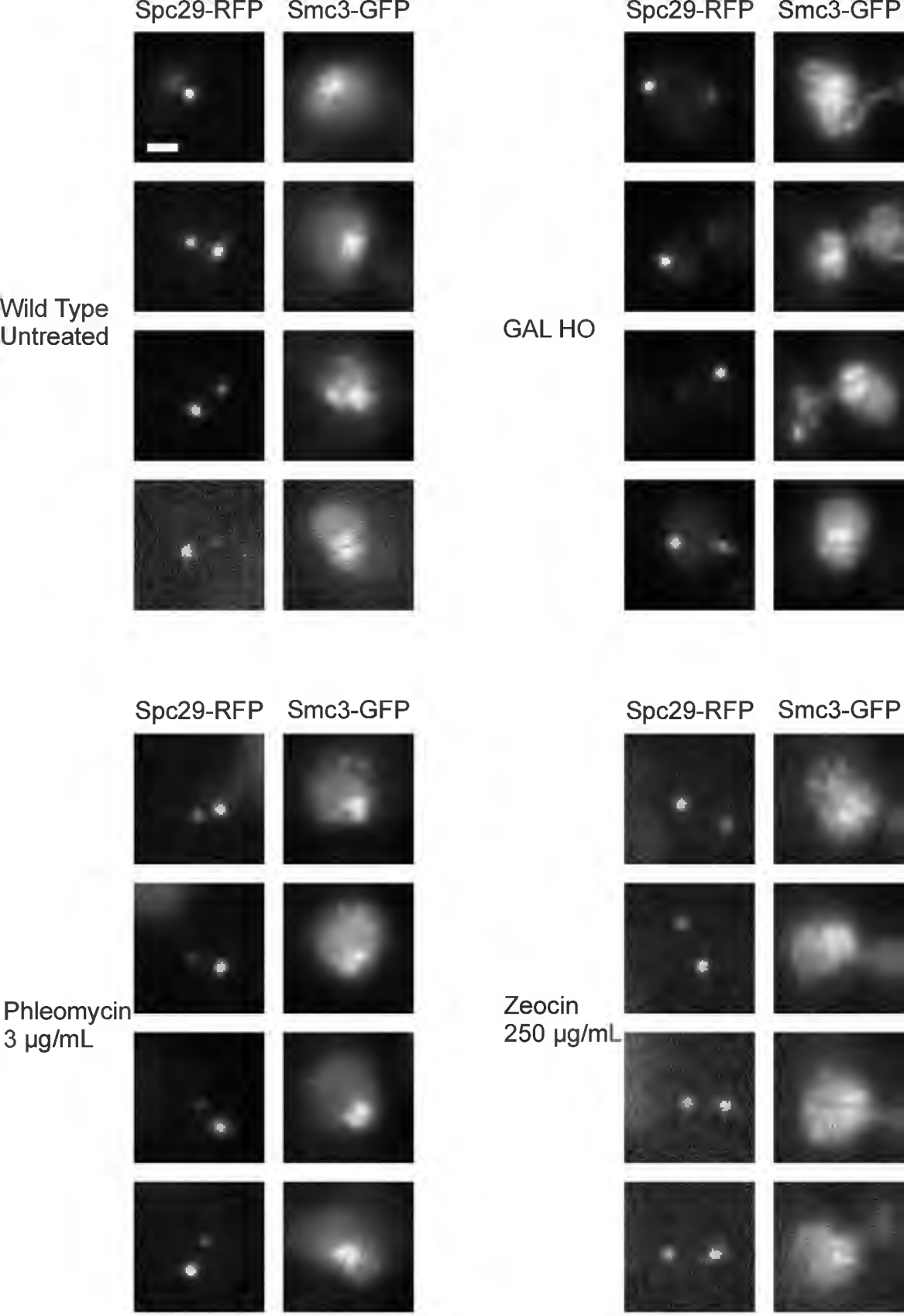
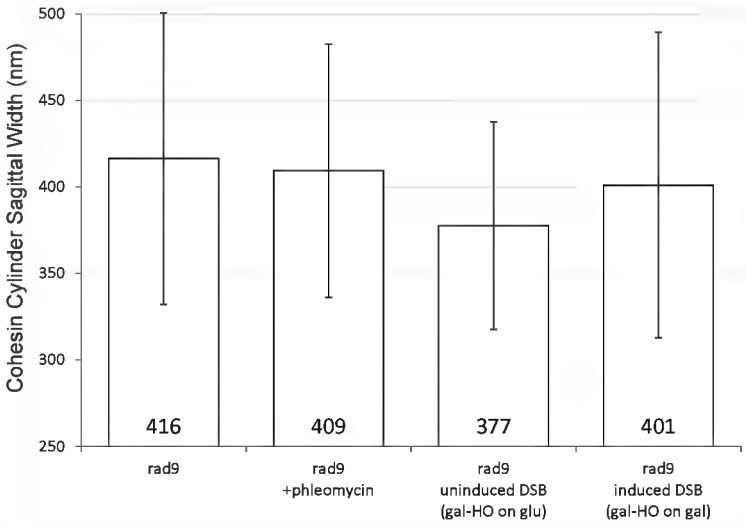

Spc29-RFP
Smc3-GFP
Wild Type
Untreated
GAL HO
Phleomycin
3 µg/mL
Zeocin
250 µg/mL

Figure S3

A.


Cohesin Cylinder Sagittal Width (nm)
500
450
400
350
300
250
416
409
377
401
rad9
rad9 +phleomycin
rad9 uninduced DSB (gal-HO on glu)
rad9 induced DSB (gal-HO on gal)

B.

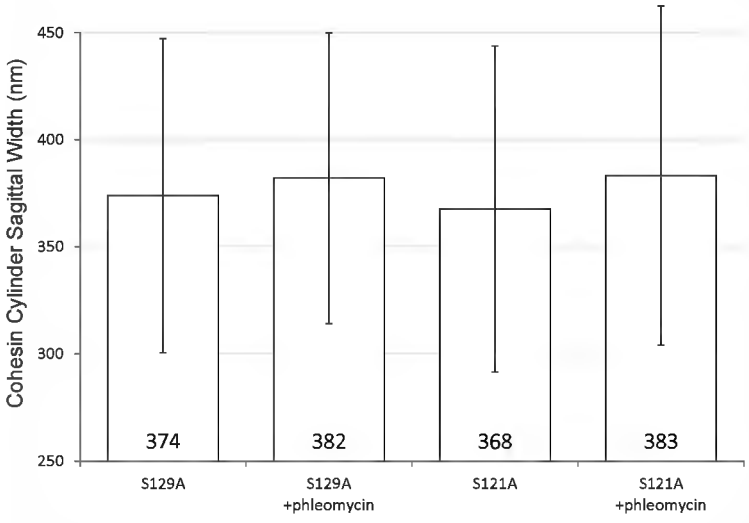

Cohesin Cylinder Sagittal Width (nm)
450
400
350
300
250
374
382
368
383
S129A
S129A +phleomycin
S121A
S121A +phleomycin

Figure S4

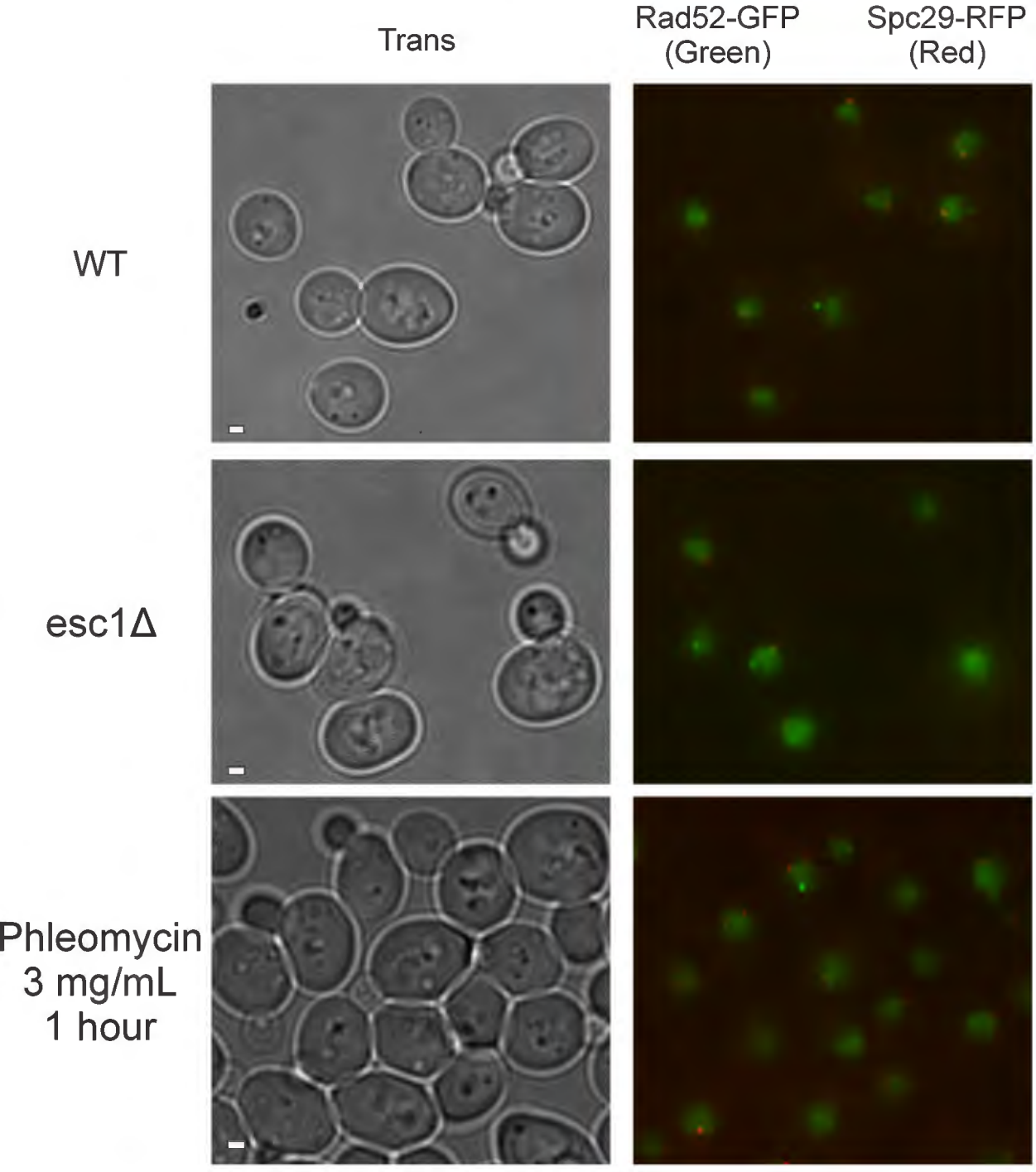

Trans
Rad52-GFP
(Green)
Spc29-RFP
(Red)
WT
esc1Δ
Phleomycin
3 mg/mL
1 hour

Figure S5

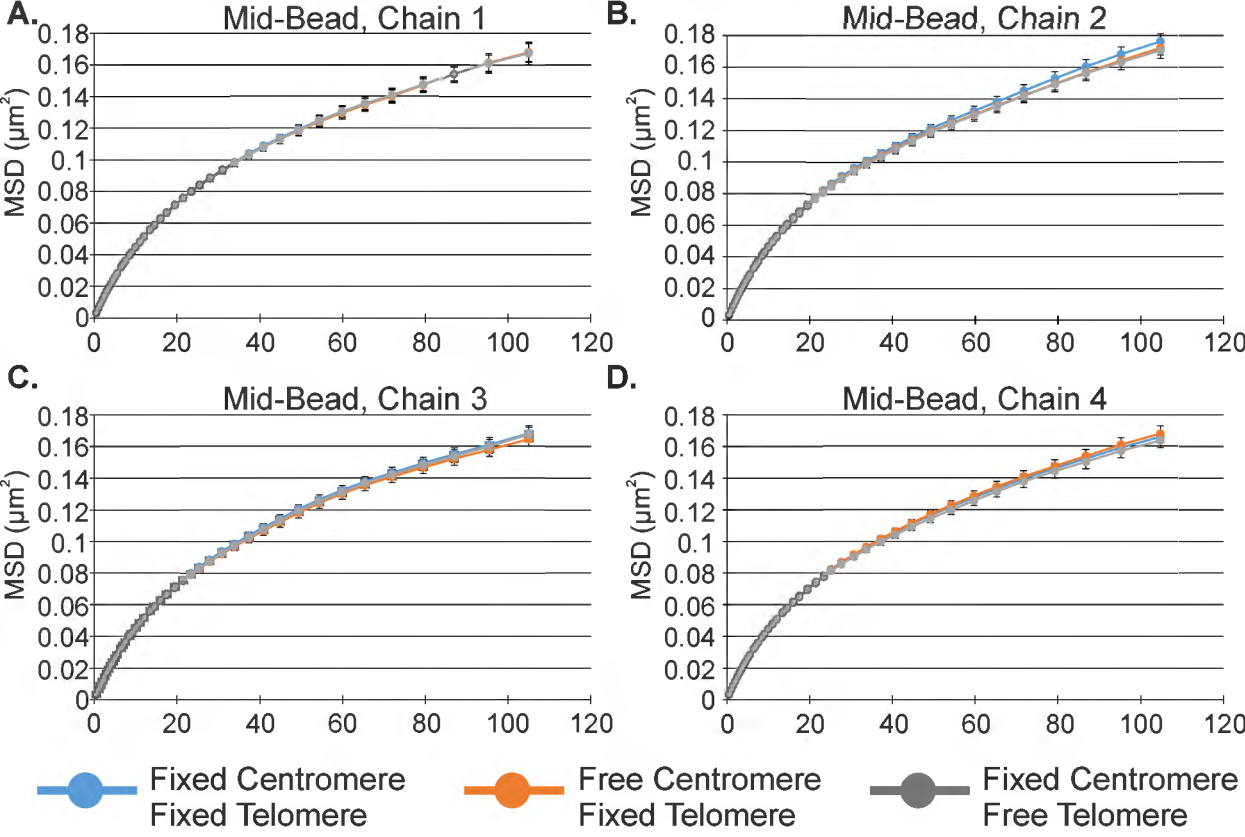

A.
Mid-Bead, Chain 1
B.
Mid-Bead, Chain 2
C.
Mid-Bead, Chain 3
D.
Mid-Bead, Chain 4
MSD (μm²)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
20
40
60
80
100
120
Fixed Centromere
Fixed Telomere
Free Centromere
Fixed Telomere
Fixed Centromere
Free Telomere

Figure S6

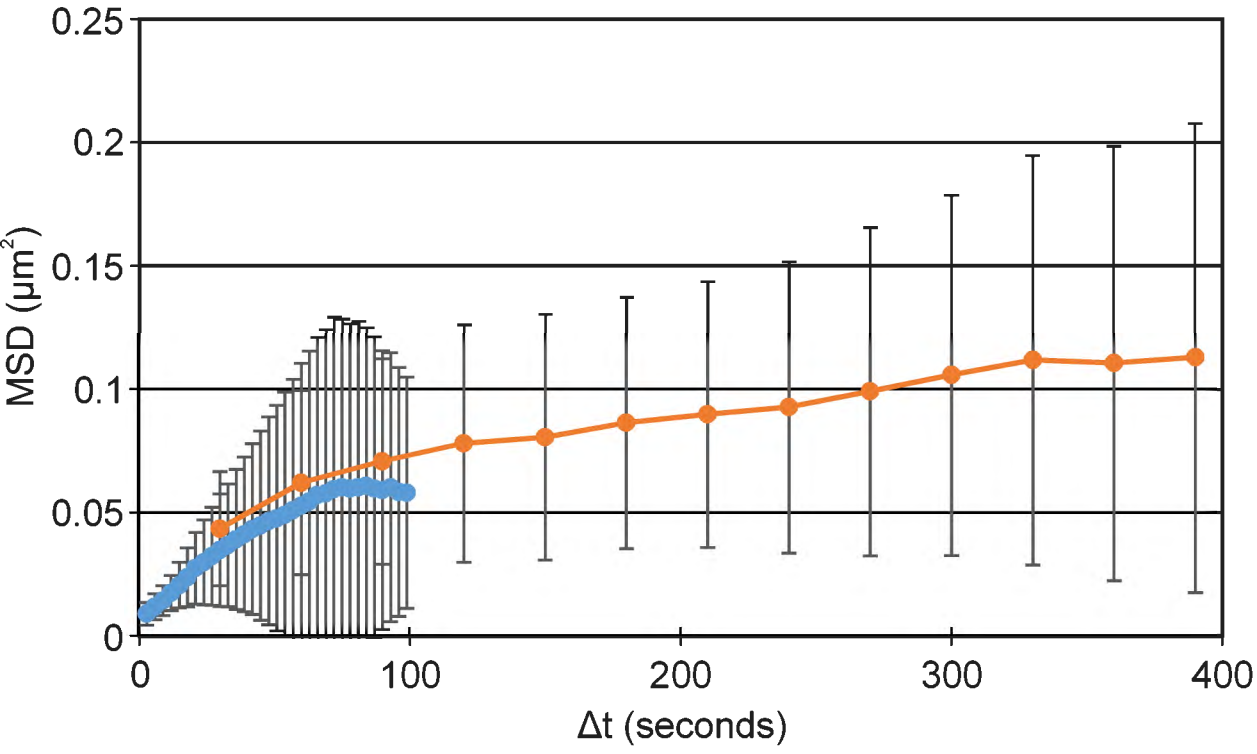


Figure S7