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Supplemental Information

Both Interaction Surfaces within Cohesin's Hinge Domain Are Essential for Its Stable Chromosomal Association

Ajay Mishra, Bin Hu, Alexander Kurze, Frédéric Beckouët, Ana-Maria Farcas, Sarah E. Dixon, Yuki Katou, Syma Khalid, Katsuhiko Shirahige, and Kim Nasmyth

Mutation at the hinge interface	Phenotype	Deletion rescued/ number of tetrads
smc1L635K;L639K	Slow growth (synthetic lethal with Δ*mad2*)	7/18
smc1I571K	functional	11/12
smc1L564K	functional	14/16
smc3E570K	functional	10/10
smc3F590R	lethal	0/18
smc3L672R	functional	10/12

Figure S1. Mutations in Cohesin's Hinge Domain and Their Phenotype, Related to Figure 1

The table shows the mutations (other than the ones in Figure 1C) at the hinge interface and their phenotypes. The last column in the table represents the ability of ectopic copies of wild-type *SMC1*/*SMC3* or mutant *smc1*/*smc3*, integrated into strains heterozygous for Δ*smc1*/Δ*smc3*, to rescue the deletion by tetrad dissection analysis. Ideally, one rescue per tetrad is expected if the ectopic gene can complement the deletion of endogenous gene.

Figure S2.

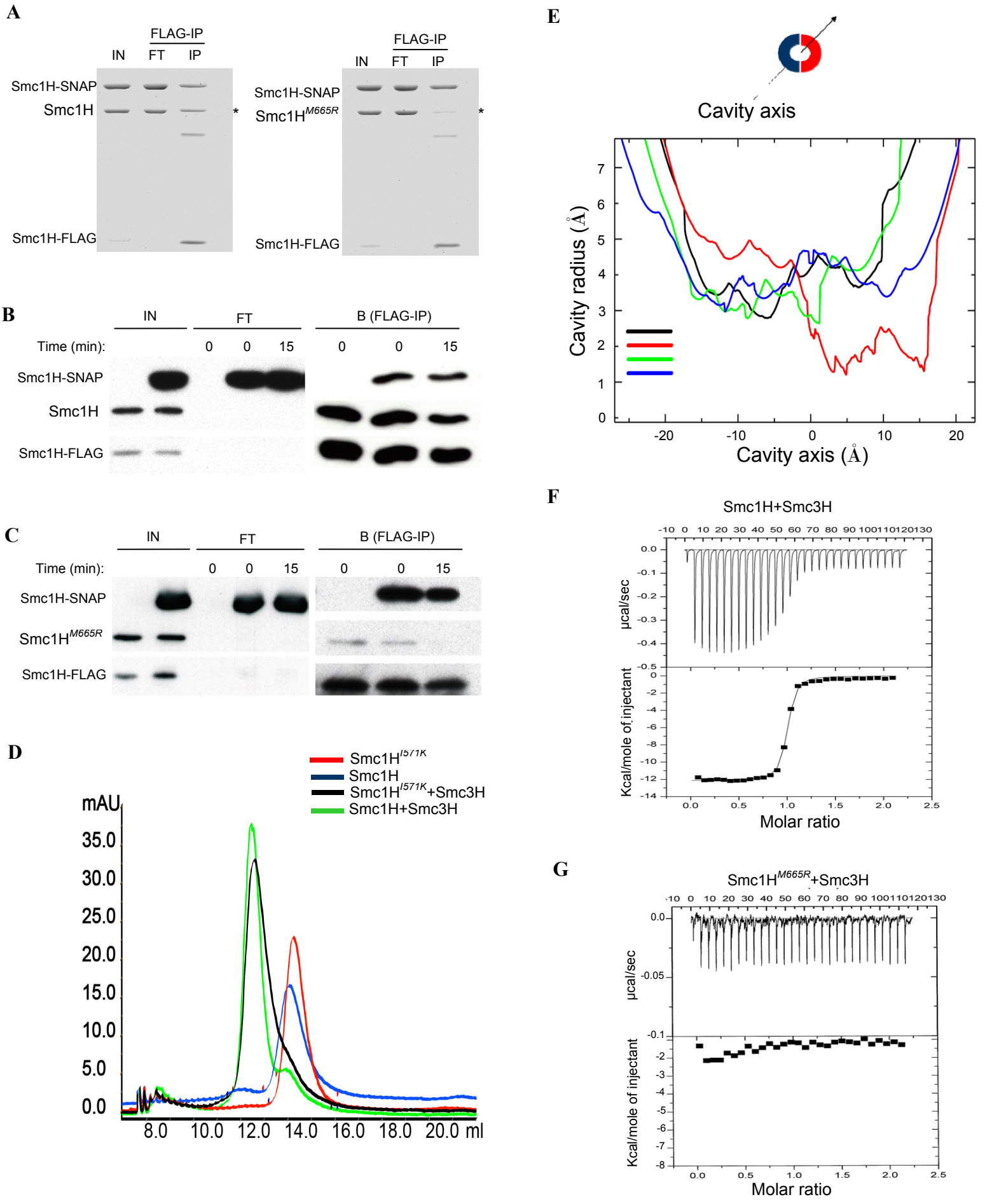
A
FLAG-IP
IN
FT
IP
Smc1H-SNAP
Smc1H
Smc1H-FLAG
Smc1H-SNAP
Smc1H^M665R
Smc1H-FLAG
B
IN
FT
B (FLAG-IP)
Time (min):
0
0
15
0
0
15
Smc1H-SNAP
Smc1H
Smc1H-FLAG
C
IN
FT
B (FLAG-IP)
Time (min):
0
0
15
0
0
15
Smc1H-SNAP
Smc1H^M665R
Smc1H-FLAG
D
Smc1H^I571K
Smc1H
Smc1H^I571K+Smc3H
Smc1H+Smc3H
mAU
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0 ml
E
Cavity axis
Cavity radius (Å)
Cavity axis (Å)
F
Smc1H+Smc3H
µcal/sec
Kcal/mole of injectant
Molar ratio
G
Smc1H^M665R+Smc3H
µcal/sec
Kcal/mole of injectant
Molar ratio

Figure S2. Effect of the Mutations in the Hinge on Smc1/3 Hinge Dimerization In Vitro and Dimensions of the Cavity Region, Related to Figure 2

(A) Smc1 M665R hinge fails to compete against wild-type Smc1 hinge: Competition assay was performed by mixing both Smc1-SNAP and Smc3-FLAG hinges either with MBP-Smc1-His6 (*left panel*) or MBP-Smc1 M665R-His6 hinge (*right panel*) proteins in a 1:1:1 molar ratio followed by immunoprecipitation with anti-FLAG beads. The protein complexes were eluted from beads with 100 mg/ml 3xFLAG peptide. *IN = input, FL = flow through, B = bound.* * marks the antibody band.

(B and C) Time course competition assay: Similar experiment, as in A, was performed with a time course to test the competence of either wild-type Smc1 hinge domain (B) or mutant Smc1 M665R hinge (C) against wild-type Smc1-SNAP (added in 750 fold excess of other proteins), to bind with Smc3 hinge domain.

IN = input, FL = flow through, B = bound.

(D) Functional mutation does not affect Smc1/3 hinge dimerization: An overlay of the chromatograms for Smc1(I571K)H (a functional mutant), Smc3H, Smc1(I571K)H+Smc3H and wild-type Smc1H+Smc3H as a control experiment for figure 2B.

(E) Dimensions of the cavity region in wild-type and mutant hinge domain simulations: The inner surface of the cavity was probed using the HOLE program [S3] to calculate its dimensions. The dimensions of the wild-type Smc1, Smc1*M665R* and Smc1*I571K* hinge domains are similar, with the narrowest point being a radius of ~ 2.7 Å in Smc1*M665R*. This is in stark contrast to Smc1*F584R* which has a radius of <1.15 Å in some regions, this is too narrow even for the passage of a single file of water molecules.

(F and G) Measurement of the affinity between Smc1 and Smc3 hinge domains using ITC: Wild-type Smc3 hinge (100 μM) was titrated into wild-type Smc1 hinge (10 μM) in 29 injections (5 μl per injection). Buffer used for ITC had 20mM HEPES (pH 7.5), 95mM NaCl and 2mM β-mercaptoethanol. All the titrations were done at 20 degrees. Upper panel in the figure shows the raw ITC data. Lower panel shows the points obtained after integrating each peak and curve fitting based on one site binding model. The parameters calculated were Stoichiometry (N) ~ 1, ΔH=-12.19kcal/mole and $K_d = 24 \pm 8$ nM.

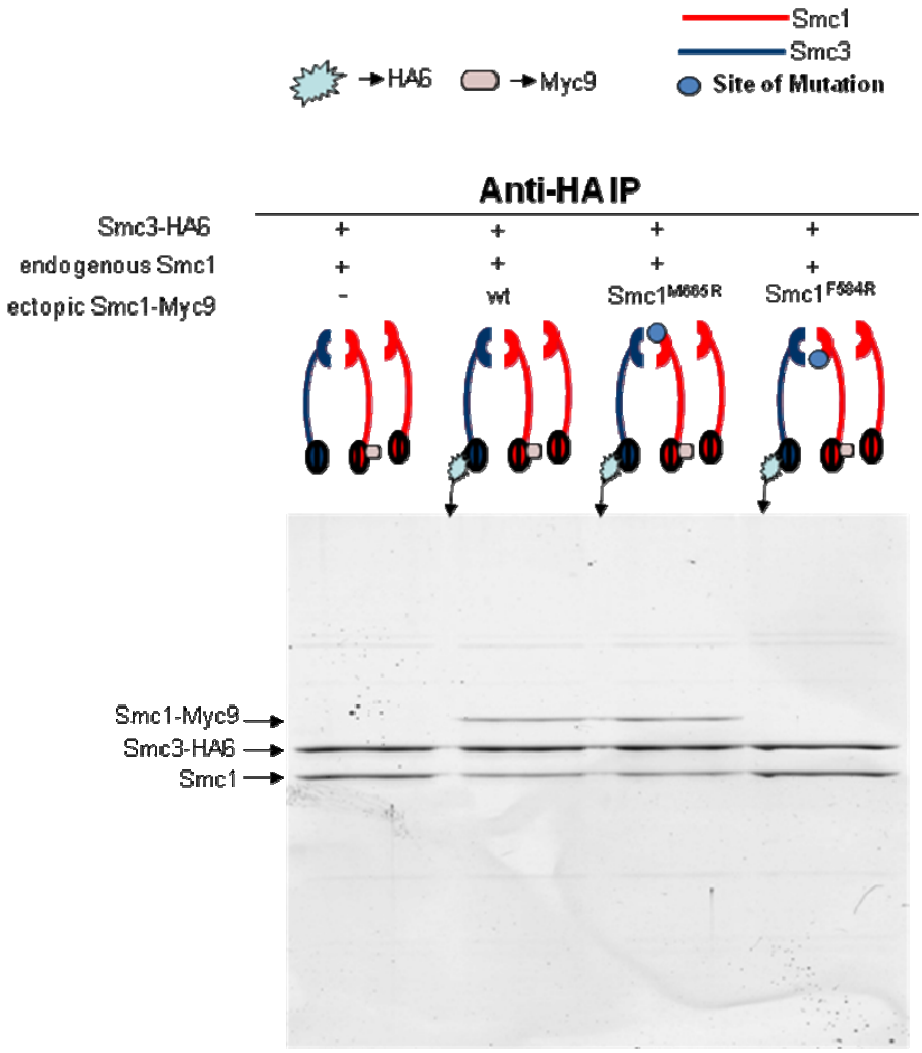

Figure S3. Smc1-Myc9 and Smc1 M665R-Myc9 Compete Efficiently with Endogenous Smc1 for Binding to Endogenous Smc3-HA6 in Yeast, Related to Figure 3

Yeast strains K12639 (*SMC3-HA6::HIS3, MATa);* K16578 (*SMC3-HA6::HIS3, SMC1-Myc9::URA3, MATa*); K16684 (*SMC3-HA6::HIS3, smc1(M665R)-Myc9::URA3, MATa*) and K16686 (*SMC3-HA6::HIS3, smc1(F584R)-Myc9::URA3, MATa*) were grown to exponential phase, lysed and immunoprecipitated with anti-HA-beads. Beads were washed and boiled in 2xSDS-running buffer. Proteins were analysed by SDS-PAGE and visualized by silver staining.

Figure S4.

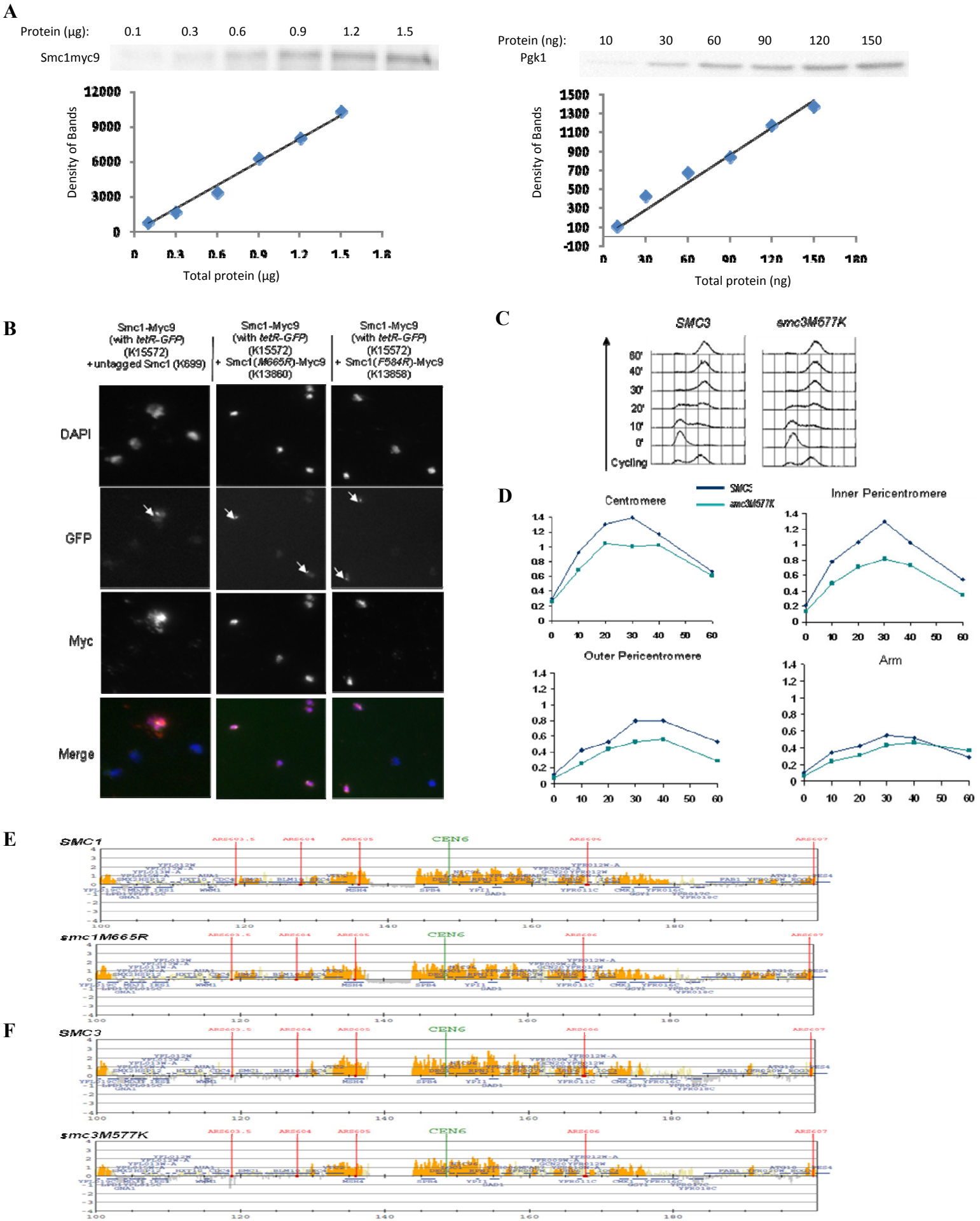
A
Protein (µg): 0.1 0.3 0.6 0.9 1.2 1.5
Smc1myc9
Density of Bands
Total protein (µg)
Protein (ng): 10 30 60 90 120 150
Pgk1
Density of Bands
Total protein (ng)
B
Smc1-Myc9 (with tetR-GFP) (K15572) +untagged Smc1 (K699)
Smc1-Myc9 (with tetR-GFP) (K15572) + Smc1(M665R)-Myc9 (K13860)
Smc1-Myc9 (with tetR-GFP) (K15572) + Smc1(F584R)-Myc9 (K13858)
DAPI
GFP
Myc
Merge
C
SMC3
smc3M577K
60'
40'
30'
20'
10'
0'
Cycling
D
SMC
smc3M577K
Centromere
Inner Pericentromere
Outer Pericentromere
Arm
E
SMC1
smc1M665R
F
SMC3
smc3M577K
CEN6

Figure S4. Chromatin Association of Hinge Interface Mutants, Related to Figure 4

(A) Yeast strains K14133 (*MATa, SMC1-Myc9::URA3, SMC3-HA3::LEU2*) was grown to exponential phase and lysed. The indicated amounts of proteins were separated by SDS-PAGE and the protein levels of the endogenous Pgk1 and Smc1-myc was determined by quantitative western blot. The density of band was plotted against the amount of total protein.

(B) Cycling cultures of yeast cells expressing either Smc1(*M665R*)-Myc9 (K13860: s*mc1(M665R)-Myc9::URA3*) or Smc1(*F584R*)-Myc9 (K13858: s*mc1(F584R)-Myc9::URA3*) were mixed with cells expressing wild-type Smc1-Myc9 (K15572: *SMC1-Myc9::URA3, 1.4kb left of CEN5:: tetO2X112 ::HIS3, his3::HIS3tetR-GFP*) for carrying out chromosome spreads. Yeast cells with wild-type Smc1 are marked with tetR-GFP (as indicated by an arrow) to differentiate from mutant cells. Chromosome spreads were immunostained with anti-Myc antibody (to detect the presence of Smc1) and with DAPI to visualize DNA.

(C) FACS analysis shows the cell cycle progression of either wild-type or mutant strains, used in Chromatin Immunoprecipitation, which were arrested in G1 with α-factor and released at permissive temperature (25^{o}C).

(D) Quantitative measurement of Cohesin's association with DNA at known cohesin binding loci. Yeast cells were arrested with α-factor in G1 and released at 25^{o}C. Samples were collected every 10 minutes post release. Chromatin was immunoprecipitated by virtue of HA3-tagged wild-type Smc3 (K13560: *SMC3-HA3::LEU2*) or mutant Smc3 M577K (K14043: *smc3(M577K)-HA3::LEU2*) and quantified by qPCR. Cohesin binding is analyzed at the core centromere and inner pericentromere, outer pericentromere and an arm region located 2.5kb, 15kb and 25kb away from the centromere respectively.

(E and F) Crude extracts prepared from unsynchronized yeast strains (E) K11850 (*SMC1-Myc9*), K13860 (*Smc1(M665R)-Myc9*) and (F) K13560 (*SMC3-HA3*) and K13043 (*Smc3(M577K)-HA3*) were used for ChIP on Chip analyses. Each yellow bar represents the binding ratio of loci showing significant enrichment in ChIP fraction. Gray bars represent the loci that are not enriched in ChIP fraction. The scale of the vertical axis is log2. Positions of the centromere (CEN6) and all previously mapped autonomously replicating sequences (ARSs) are shown. The horizontal axis represents the length of chromosome VI in kilobases.

Table S1. List of Strains

Strain #	Genotype
K699	*MATa,ade2-1,trp1-1,can1-100, leu2-3,112,his3-11,15,ura3,GAL,psi+*
K11850	*MATa,ura3::SMC1-Myc9::URA3,SMC1,SMC3*
K12639	*MATa,SMC3-HA6::HIS3,SMC1*
K13560	*MATa,leu2::SMC3-HA3::LEU2,SMC1,SMC3*
K13858	*MATa,ura3::smc1(F584R)-Myc9::URA3,SMC1,SMC3*
K13860	*MATa,ura3::smc1(M665R)-Myc9::URA3,SMC1,SMC3*
K14043	*MATa,leu2::smc3(M577K)-HA3::LEU2,SMC1,SMC3*
K14133	*MATa,ura3::SMC1-Myc9::URA3,leu2::SMC3-HA3::LEU2,SMC1,SMC3*
K14134	*MATa,ura3::smc1(F584R)-Myc9::URA3,leu2::SMC3-HA3::LEU2,SMC1,SMC3*
K14137	*MATa,ura3::smc1(M665R)-Myc9::URA3,leu2::SMC3-HA3::LEU2,SMC1,SMC3*
K15106	*MATa,ura3::SMC1-Myc9::URA3,leu2::SMC3-HA3::LEU2,SCC1-Pk9::KanMX, SMC1,SMC3*
K15108	*MATa,ura3::smc1(F584R)-Myc9::URA3,leu2::SMC3-HA3::LEU2,SCC1-Pk9::KanMX,SMC1,SMC3*
K15110	*MATa,ura3::SMC1-Myc9::URA3,leu2::smc3(M577K)-HA3::LEU2,SCC1-Pk9::KanMX,SMC1,SMC3*
K15794	*MATa,leu2::SMC3-HA3::LEU2,Δsmc3::HIS3,Δeco1::hphMX,Δrad61::NatMX,SMC1*
K16113	*Diploid,SMC3-GFP::KanMX,SMC3,SMC1/SMC1*
K16114	*Diploid,smc3(M577K)-GFP::KanMX,SMC3,SMC1/SMC1*
K16252	*Diploid,ura3::SMC1-GFP::URA3/ura3::SMC1-GFP::URA3, ADE2/ADE2,SMC1/SMC1,SMC3/SMC3*
K16253	*Diploid,ura3::Smc1(M665R)-GFP::URA3/ura3::Smc1(M665R)-GFP::URA3, ADE2/ADE2,SMC1/SMC1,SMC3/SMC3*
K16255	*MATa,ura3::smc1(M665R)-Myc9::URA3,leu2::smc3(M577K)-HA3::LEU2,SCC1-Pk9::KanMX,SMC1,SMC3*
K16335	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC1::kanMX-tetO2p-DHFRts-HA3-SMC1,ura3::SMC1-Myc9::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC3*
K16338	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC1::kanMX-tetO2p-DHFRts-HA3-SMC1,ura3::YIPlac211::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC3*
K16339	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC1::kanMX-tetO2p-DHFRts-HA3-SMC1,ura3::smc1(M665R)-Myc9::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC3*
K16341	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC3::kanMX-tetO2p-DHFRts-HA3-SMC3,ura3::YIPlac211::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC1*
K16342	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC3::kanMX-tetO2p-DHFRts-HA3-SMC3,ura3::SMC3-HA3::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC1*
K16343	*MATa,UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, SMC3::kanMX-tetO2p-DHFRts-HA3-SMC3,ura3::smc3(M577K)-HA3::URA3, TRP1ARS1CEN1(7.5kb minichromosome),SMC1*
K16345	*MATa,ura3::smc1(F584R)-Myc9::URA3,leu2::smc3(M577K)-HA3::LEU2,SCC1-Pk9::KanMX,SMC1,SMC3*
K16383	*MATa,ura3::smc1(M665R)-Myc9::URA3,leu2::SMC3-HA3::LEU2,SCC1-Pk9::KanMX,SMC1,SMC3*
K16428	*MATa,ura3::SMC1-GFP::URA3,MTW1::KanMX,SMC1,SMC3*
K16578	*MATa,ura3::SMC1-Myc9::URA3,SMC3-HA6::HIS3,SMC1*
K16611	*MATa,ura3::smc1(F584R)-GFP::URA3,MTW1::KanMX,SMC1,SMC3*
K16642	*MATa,ura3::smc1(M665R)-GFP::URA3,MTW1::KanMX,SMC1,SMC3*
K16684	*MATa,ura3::smc1(M665R)-Myc9::URA3,SMC3-HA6::HIS3,SMC1*
K16686	*MATa,ura3::smc1(F584R)-Myc9::URA3,SMC3-HA6::HIS3,SMC1*
K16891	*MATa,ura3::smc1(F584R)-GFP::URA3,leu2::SMC3-HA3::LEU2,MTW1::KanMX, SMC1,SMC3*
K16942	*MATa,ura3::SMC1-GFP::URA3,leu2::SMC3-HA3::LEU2,MTW1::KanMX, SMC1,SMC3*
K16944	*MATa,ura3::smc1(M665R)-GFP::URA3,leu2::SMC3-HA3::LEU2,MTW1::KanMX, SMC1,SMC3*

Supplemental Experimental Procedures

Purification of Smc1 and Smc3 Hinge Domains

Smc1H (S503-E681) and Smc3H (T495-L705) fused to a 6xHis tag at the C-terminus were amplified by PCR from the vector K4468 and cloned into pMAL vector to get the translational fusion of Maltose Binding Protein (MBP) domain at the N-terminus of the hinge domain. The expression of Smc1H and Smc3H in *E.coli* strain BL21(DE3)-RIPL, growing at 25^{o}C, was induced with 1mM IPTG when OD_{600} of the culture was 0.6. Induction was done for 12 hours at 20^{o}C. Cells were harvested and resuspended in lysis buffer (50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 1mM PMSF and complete protease inhibitor mix (Roche)) and lysed by French press (Constant System, UK) at 17 Kpsi. The supernatant was incubated with Talon Superflow beads (Clontech) for 2 hours at 4^{o}C. Beads were washed with lysis buffer containing 10 mM imidazole (Sigma). Proteins were eluted in lysis buffer with 250mM imidazole and loaded onto a Superdex 200 26/600 size exclusion chromatography column (GE Healthcare) equilibrated with a buffer consisting of 20 mM HEPES (pH 7.5), 95mM NaCl and 2mM β-ME. Peak fractions were collected and concentrated using Vivaspin columns (Sartorius Stedim biotech).

Analytical Gel Filtration for Binding Analysis

Superdex 200 10/300 GL gel filtration columns (GE Healthcare) were used to investigate the binding between Smc1H and Smc3H. Before gel filtration, proteins were incubated at room temperature for 30 minutes. 200µl of 10µM Smc1H and Smc3H were loaded separately and together in three different runs. Fractions collected for each run were run on 7.5% SDS-PAGE and the proteins were visualized by Coomassie staining.

Isothermal Titration Calorimetry (ITC)

All ITC experiments were carried out at 20^{o}C with MicroCal VP-ITC (MicroCal). Both the cell and the injection syringe were thoroughly washed with distilled water and degassed ITC buffer (10mM HEPES, pH8; 95mM NaCl; 3mM β-mercaptoethanol) prior to use. Smc1H was filled in the cell at 10µM concentration and Smc3H in the injection at 100µM concentration. Stirring speed was set at 270 rpm and the feedback gain was set at high mode. In each titration experiment 29 injections were carried out with 5µl of Smc3H per injection into 1.8 ml of Smc1H. Since the first injection tends to deliver inaccurate data, only 2µl was injected in this step. As a control Smc3H was titrated with the ITC buffer.

ITC Data Analysis

ITC data was analyzed using 'Origin' (MicroCal) software. The recorded data for the first injection of every experiment was discarded. The value obtained for the control experiment was subtracted from the corresponding value of the main experiment. Binding isotherms were fitted according to the 'one binding site model'.

Coimmunoprecipitation Assays

Spheroplasts of yeast cells were prepared by zymolase and lysed in the lysis buffer (100mM KCl, 15mM $MgCl_2$, 0.025% TritonX, 1mM DTT and 1mM PMSF). Protein concentration of the crude extract was measured by Bradford method. 5mg of the protein was incubated with anti-HA affinity matrix for 4 hours at 4^{o}C. After washing 5 times with lysis buffer the matrix was boiled in 2X Lamelli buffer. Samples were run on a 7.5% SDS-PAGE. For western analysis anti-HA,

anti-Myc, anti-Pk and anti-Swi6 antibodies were used to probe for Smc3, Smc1, Scc1 and Swi6 respectively.

Chromatin Immunoprecipitation—Quantitative PCR

ChIP-qPCR was performed as described [18] with 2μg of antibodies (9E11 for Myc and 12CA5 for HA). qPCR was run using Corbett Rotorgene cycler. Primers pairs used for chromosome VI were - *DEG1* (Centromere) 5'GCGGCCTTAAGTTCGTAGTG-3' and 5'-AAGTGCCGGAAATTGTCTTG-3'; *SPB4* (Pericentromere) 5'-GACGAAAGAACGGAAACTCG-3' and 5'- CCTTGGATAGCTTTGCTGGA-3'; *CMK1* (Inner Arm) 5'-ACGGTTCAGTTCCTCCATTG-3' and 5'- TGCAAAAGCTTTGCTGGTTA-3'. These chosen genomic loci were based on previous ChIP on Chip results [S4]. ChIP on Chip was executed as described previously [S5].

Quantitative Western Blot

Yeast cells were grown to exponential phase and lysed. To determine the protein levels of Smc1myc9 and the endogenous Pgk1, 1 or 0.1 μg of total protein was loaded into a SDS-PAGE gel. The resolved proteins were transferred onto a PVDF membrane (Millipore) and blotted with anti-myc antibody (1:10000, Gramsch Laboratories) or anti-Pgk1 antibody (1:10000, Invitrogen) in PBS-T containing 5% milk. After 3 extensive washing, the membrane was incubated with a corresponding horseradish peroxidase-conjugated secondary antibody. The membranes were developed with ECL reagents (GE Healthcare) and the chemiluminescence signal was detected by the ChemiDoc XRS+ system (Bio-Rad). To detect the Smc1myc9 or Pgk1 protein, the membrane was exposed to supercooled high-resolution CCD camera (Bio-Rad) for 5 minutes or 20 seconds. The image was analyzed using Quantity One 1-D Analysis Software (Bio-Rad). Protein bands were digitized, and the net band intensities for each band were recorded and expressed as pixels.

Live Cell Imaging

Live cell imaging was performed as described in Rowland *et al*. [5] using diploid yeast cells homozygous for Smc1-GFP integrated at the *ura3* locus. For imaging Smc3, diploid cells heterozygous for endogenous Smc3-GFP were used. Preferentially, cells with small buds, reflecting G2/M phase were analysed. Photobleaching was performed by using 100% laser intensity for 200 ms. Post-bleaching images were acquired at every 15 sec for 8 minutes.

Image quantification was carried out with ImageJ 1.3v software. The relative fluorescence intensity (RFI) for bleached and unbleached signals was calculated as using the following equation [S1]:

$$RFI = \frac{(I_{Rt}-I_{Bt})x(I_{N0}-I_{B0})}{(I_{Nt}-I_{Bt})x(I_{R0}-I_{B0})}$$

where, I_{Rt} is the mean intensity of the region of interest (either bleached or unbleached portion of the barrel structure) at time t, I_{Bt} is the mean intensity of the image background at time t outside the nucleus, and I_{Nt} is the mean intensity of the nuclear GFP signal around the barrel structure at time t. I_{N0}, I_{B0} and I_{R0} are the signal intensities at t=0. To calculate the half time of recovery, curve fitting was performed by using single exponential curve using Prism 5 (GraphPad).

Other Techniques

Chromosome spreads and minichromosome cohesion assays were performed as described previously [3, S2].

Yeast Strains

All yeast strains used in this study were derived from W303. The list of yeast strains is provided in Table S1.

Supplemental References

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