Cell, Volume *127*

Supplemental Data

Evidence that Loading of Cohesin

Onto Chromosomes Involves

Opening of Its SMC Hinge

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Supplemental Experimental Procedures

Cloning of the Scc1-(TEV)3-Smc1 Fusion Protein

The *SCC1* coding sequence plus 300bp upstream sequence was cloned into the integrative vector YIplac128 using the XbaI site in the promoter of *SCC1*. The C-terminus of *SCC1* was fused by overlap extension PCR to the *SMC1* open reading frame including 400bp of its downstream region using linker peptide sequence containing three TEV sites: GGGGPRENLYFQGPRENLYFQGASENLYFQGGGGG. The plasmid (K4878) was linearized in the *LEU2* coding sequence and transformed into yeast.

Cloning of the Smc3-(TEV)3-Scc1 Fusion Protein

A SpeI restriction site was inserted after the Start codon of the *SCC1* coding sequence cloned as XbaI fragment into the vector YIplac211. The *SMC3* coding sequence was inserted as SpeI fragment after the Start codon of *SCC1*. Following peptide sequence was added between the last amino acid of *SMC3* and the first of *SCC1*: $G_4SG_3SG_4$TRAR ENLYFQGASENLYFQGELENLYFQGAS using a SpeI restriction site. The construct (K4879) was integrated into the yeast genome at the *URA3* locus. The Frb domain was fused to the C-terminus of the Smc3-Scc1 construct: ASGGGGGSGGGSGGGGAS-(I2021-mTOR(human)-K2113)-LE (K4882).

Cloning of the Smc1p14 and Smc3MP1 Constructs

The *SMC1* open reading frame with a C-terminal myc9 tag and endogeneous upstream and downstream sequences was cloned into the YIplac211 integrative vector. The hinge region was replaced by an AflII restriction site. Different versions of *P14* were cloned as AflII fragments into this vector. The final insertion sequences are given in the table below. The *SMC3* open reading frame with a HA3 tag at its C-terminus and genomic upstream and downstream sequences was inserted into YIplac128. The hinge region was replaced by the restriction site for XhoI. Different *MP1* cassettes were inserted as XhoI segments. Final sequences are given in the table below. The Smc1p14 and Smc3MP1 constructs used in experiments described in this paper are high-lighted by bold letters.

Smc3	linker	N- MP1 -C		linker	Smc3	Scc1 Binding
VNETMSRS(514)	**T**	**D(4)LKRF**	**LF(114)**		**L(685)ESLKNL**	YES
VNETMSRS(514)	T	D(4)LKRF	LFEEL(117)		L(685)ESLKNL	YES
VNETMSR(513)		L(5)KRF	LF(114)		L(685)ESLKNL	YES
VNET(510)		K(6)RF	LFEEL(117)		L(685)ESLKNL	No
VNET(510)		K(6)RF	LF(114)		L(685)ESLKNL	No

Smc1	linker	N- p14 -C		linker	Smc1	Scc1 Binding
SANQ(514)	**S**	**K(5)ALTQVL**	**QYLEE(117)**	**TTQS**	**K(679)EEY**	YES
SANQ(514)	S	K(5)ALTQVL	QYL(115)		K(679)EEY	No
SANQ(514)	S	K(5)ALTQVL	QYL(115)		E(680)EY	No
SANQ(514)	RE	A(6)LTQVL	QYL(115)		E(680)EY	YES
SANQ(514)	RE	A(6)LTQVL	QYL(115)		K(679)EEY	No
SANQ(514)		T(8)QVL	QYL(115)		E(680)EY	No
SAN(513)		L(7)TQVL	QYLEE(117)	TTQS	K(679)EEY	No

Smc1 and Smc3 constructs including upstream and downstream sequences were combined on a single YIplac211 vector:

K4885: Smc1 + Smc3-HA3
K4886: Smc1p14 + Smc3MP1-HA3
K4887: Smc1-myc9 + Smc3-HA3
K4888: Smc1p14-myc9 + Smc3MP1-HA3

Cloning of the Smc1FKBP12 and Smc3Frb Constructs

A NheI restriction site was inserted at three different positions into *SMC1* (after aa Q577, L597 and I647) and *SMC3* (after aa M583, S606 and K653) hinge domains of SMC genes cloned into yeast vectors as described in (Arumugam et al. 2003). NheI-XbaI fragments were used to insert following peptide sequence into NheI sites in *SMC1* and *SMC3*:

FKBP12: ARGSGSGGSS-(G2-FKBP12(human)-E108)-SSGSGSGSAS
Frb: ARGSGSGGSS-(I2021-mTOR(human)-K2113)-SSGSGSGSAS.

The Smc3 constructs (insertion at S606: K4884) were integrated into the genome at the *LEU2* locus. Smc1 constructs (insertion at L597: K4483) were integrated at the endogenous locus followed by loop out of the wild-type copy.

Cloning of the Scc1MP1-TEV3-p14 Construct

A HA6-cassette was inserted into a SpeI site directly after the Start codon of *SCC1*. A BssHII site was created in Scc1 at position 220 using site directed mutagenesis. Annealled oligos containing triple TEV sites were inserted into the BssHII site (K4880). A PCR product with MP1 and p14 linked by TEV sites were cloned into the BssHII site. The final sequence of the insert is as follows (K4881):

LAAA-(MP1mouse)-PRARENLYFQGASENLYFQGELENLYFQGARESSKH PTSLVPRGS-(p14mouse)-AAA

Cloning of hsSMC Overexpression Constructs

The hinge regions of in *SMC1* and *SMC3* constructs described in (Arumugam et al., 2003) were replaced by the corresponding region of Smc1p14 and Smc3MP1 constructs to result in plasmids K4889 (Smc1p14-TEV-protA), K4890 (Smc3MP1) and K4891 (Smc3MP1 K38I).

Cloning of the GAL1-10 TEV Plasmid
The GAL1-10 TEV protease expression cassette was transferred from an integrative vector (Uhlmann et al., 2000) into a yeast episomal plasmid carrying the *TRP1* gene (YEPlac112). The nine myc epitopes were removed (K4462).

Purification of WT and hsSMC Heterodimers
Proteins were expressed and purified as described in (Arumugam et al. 2006) with following modifications. Yeast cells were grown at 25ºC. TEV protease cleavage was done for 2hrs with 100µl TEV protease and repeated once. Fractions were pooled as indicated in Figure S2B.

Chromatin Immunoprecipitation
The ChIP protocol was modified from (Pidoux et al., 2004). 42ml of culture with optical density (at 600nm) of 0.5 was fixed at room temperature for 30min in 3% formaldehyde. Fixation was stopped by the addition of 100mM (final) glycine (5min). Cells were pelleted, washed in 20ml ice-cold PBS and incubated in 100mM PIPES-KOH pH9.3, 10mM DTT for 20min on ice. Cells were pelleted again and washed in HEMS buffer (100mM Hepes-KOH pH7.5, 1mM EDTA, 1mM $MgSO_4$, 1.2M sorbitol). Spheroplasts were prepared in 1ml HEMS buffer with 0.5mg/ml Zymolyase T-100 at 37ºC for 20min. Spheroplasts were spun down (7000g) and washed once in 1ml HEMS. The pellet was frozen and kept at -20ºC. Spheroplasts were thawed and resuspended in 2ml ice-cold lysis buffer (50mM Hepes-KOH pH7.55, 140mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% (w/v) sodium deoxycholate) and sonicated for 30sec and 20sec using a Bandolin Sonoplus UW2070 with a MS-72 tip with 38% power and 90% puls in a 5ml tube. Lysates were cleared by centrifugation and pre-cleared by incubation in 100µl proteinA Dyna beads for 90min. Beads were removed. 200µl extract was frozen at -20ºC as input sample. 2ug of antibody (MCA 1360, Serotec, for PK pull down; rabbit polyclonal, Gramsch, for myc pull down) was added to 1600µl extract and incubated for 90min. After addition of 100µl proteinA Dyna beads extracts were incubated overnight at 4ºC. Beads were washed in lysis buffer (1ml), lysis buffer (with 500mM NaCl), wash buffer (10mM Tris pH 8.0, 0.25M LiCl, 0.5% NP-40, 0.5% (w/v) sodium deoxycholate and 1mM EDTA) and TE. Samples were eluted and de-crosslinked by incubation in TES (50mM Tris 8.0, 10mM EDTA and 1% SDS) for 7hrs at 65ºC, followed by 2hrs proteinase K treatment. DNA was purified by phenol-chloroform extraction and ethanol precipitation. 3µl of input samples (1:200 dilution) and IP samples (1:20 dilution) were used in a 20µl real-time SYBR green PCR reaction (Applied Biosystems; following the instructions by the manufacturer). Following oligo pairs were used (at 0.3µM concentration) as primers:

CEN6:	ACGAACTTAAGGCCGCAGTA and CTTCGACAGGTTCCATAACG
Spb4:	GACGAAAGAACGGAAACTCG and TTGCCTTGGATAGCTTTGCT
Msh4:	TGACGTCGCTACACTTCCAG and TCCAGGATAGCCACTGCTTC
Met10-Smc2:	CGTAACGCAGGGTTTAGAGG and ATACATAGGCGCTGGTCGAA

Supplemental References

Arumugam, P., Gruber, S., Tanaka, K., Haering, C. H., Mechtler, K., and Nasmyth, K. (2003). ATP hydrolysis is required for cohesin's association with chromosomes. Curr Biol *13*, 1941-1953.

Pidoux, A., Mellone, B., and Allshire, R. (2004). Analysis of chromatin in fission yeast. Methods *33*, 252-259.

Uhlmann, F., Wernic, D., Poupart, M. A., Koonin, E., and Nasmyth, K. (2000). Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell *103*, 375-386.

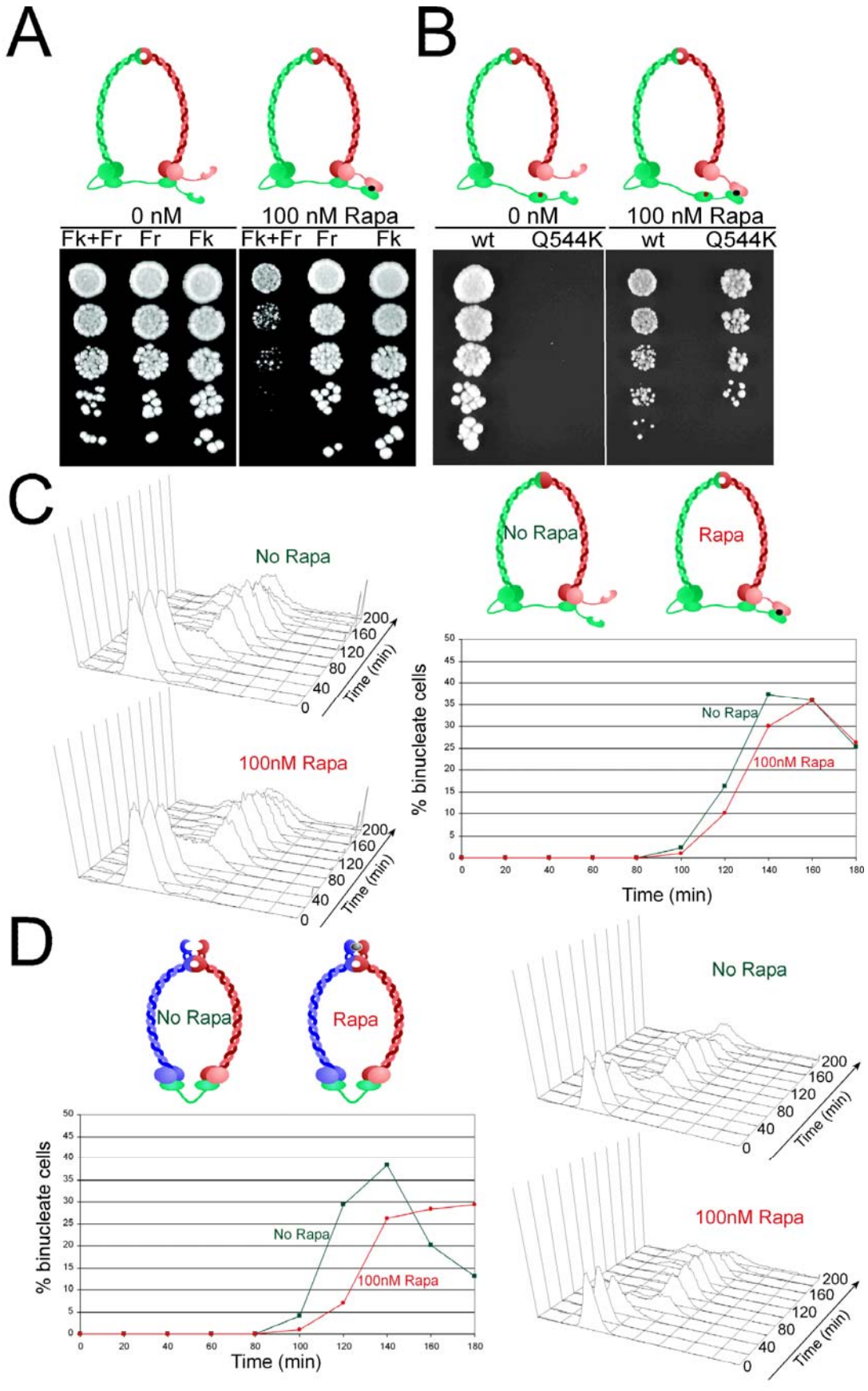
A
0 nM
100 nM Rapa
Fk+Fr
Fr
Fk
Fk+Fr
Fr
Fk
B
0 nM
100 nM Rapa
wt
Q544K
wt
Q544K
C
No Rapa
100nM Rapa
200
160
120
80
40
0
Time (min)
No Rapa
Rapa
% binucleate cells
No Rapa
100nM Rapa
Time (min)
D
No Rapa
Rapa
% binucleate cells
No Rapa
100nM Rapa
Time (min)
No Rapa
100nM Rapa
200
160
120
80
40
0
Time (min)

Figure S1. Linkage of Scc1 to Both SMC Heads Does Not Interfere with the Establishment of Sister-Chromatid Cohesion

(A) Linkage of Scc1 to both SMC heads does not abolish cohesin function. Strains K14172 (*MATα Δfpr1 TOR1-1 Δscc1 Δsmc3 P^{SCC1}SMC3-TEV3-SCC1-Frb SMC1-FKBP12*) (Fk+Fr), K14173 (*MATα Δfpr1 TOR1-1 Δscc1 Δsmc3 P^{SCC1}-SMC3-TEV3-SCC1-Frb*) (Fr) and 14174 (*MATα Δfpr1 TOR1-1 Δscc1 Δsmc3 P^{SCC1}-SMC3-TEV3-SCC1, SMC1-FKBP12*) (Fk) were plated onto YPD containing 0 or 100 nM rapamycin and grown for three days at 32°C.

(B) Linkage of Scc1 to both SMC heads suppresses a mutation in Scc1´s C-terminus. Strains K14356 (*MATα Δfpr1 TOR1-1 Δscc1 Δsmc3 P^{SCC1}-SMC3-TEV3-SCC1-Frb SMC1-FKBP12*) (wt) and K14358 (*MATα Δfpr1 TOR1-1 Δscc1 Δsmc3 P^{SCC1}-SMC3-TEV3-scc1(Q544K)-Frb, SMC1-FKBP12*) (Q544K) were pre-grown in the presence of 100nM Rapamycin and plated onto YPD containing 0 or 100 nM rapamycin. Yeast plates were incubated for four days at 32°C.

(C) Linkage of Scc1 to both SMC heads causes only a minor delay in nuclear division. Strain K30016 was synchronized using centrifugal elutriation and alpha factor arrest. Cells were released from the arrest in the presence or absence of 100nM rapamycin and samples were taken every 20min. Replication and nuclear division timing was monitored by flow cytometry and DAPI fluorescence microscopy, respectively.

(D) Linkage of Smc1 and Smc3 hinge domains causes a pronounced delay in nuclear division. As in (C) using strain K14668.

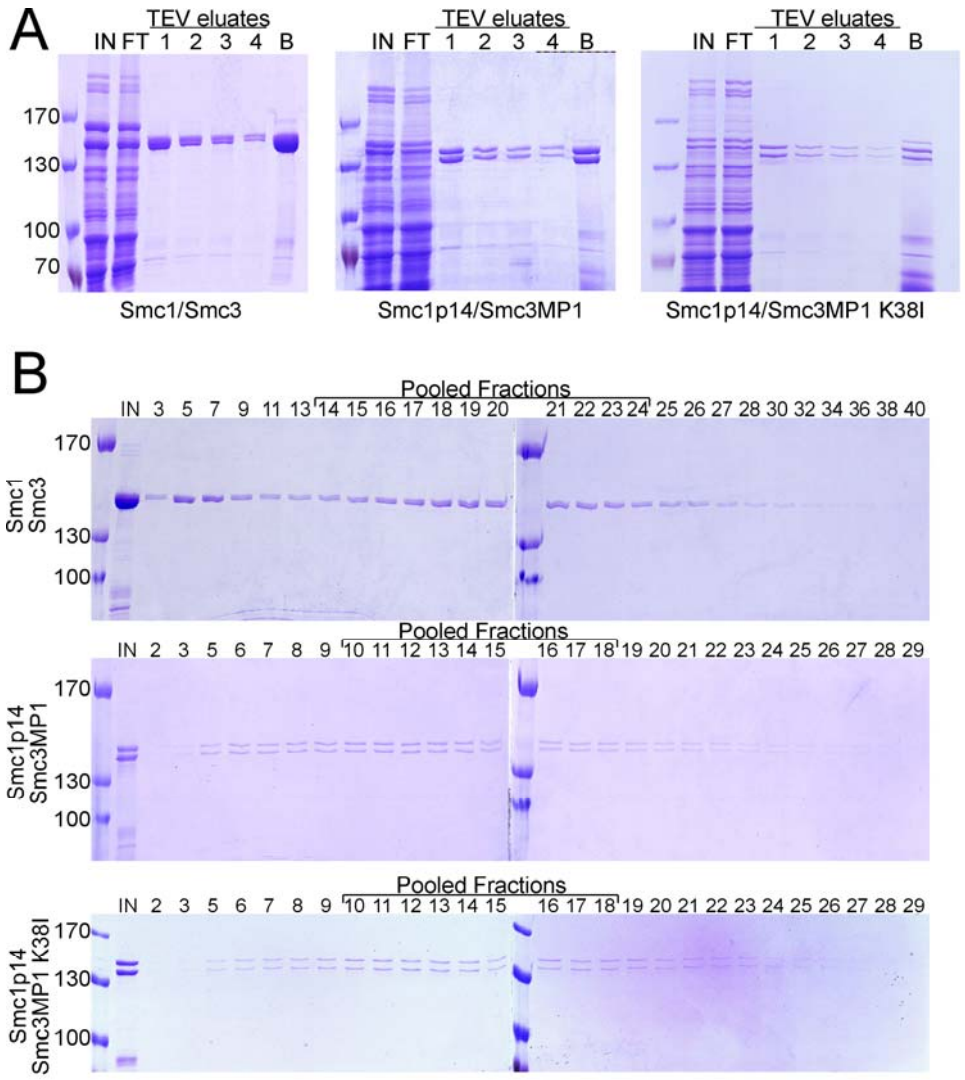

Figure S2. Purification of Smc1/Smc3 Heterodimers

(A) Purification by Protein A affinity and TEV protease cleavage. Crude extracts (IN) derived from strains K14331 (*Matα pM346-LEU2D::SMC1-TEV-protA pM346-URA3::SMC3*), K14330 (*Matα pM346-LEU2D::smc1p14-TEV-protA pM346-URA:: smc3MP1*) and K14476 (*Matα pM346-LEU2D::smc1p14-TEV-protA pM346-URA:: smc3(K38I)MP1*) were incubated with human IgG agarose beads (Sigma). Supernatant was discarded (FT), and beads were washed and incubated in 10ml buffer with TEV protease for 2hrs. Supernatant was collected (EL1) and beads were washed with 5ml buffer (EL2). Elution and washing was repeated once more (EL3, EL4). Beads were boiled in SDS buffer. Samples were analyzed by SDS-PAGE and Commassie staining. Amounts loaded into input and flow-through lanes equal one tenth of the other lanes.

(B) Gel filtration of purified SMC dimers. Samples were concentrated to 5 ml using a Vivaspin 20 (MWCO 100000) and loaded onto a 100ml Sephacryl S200 column (IN). 2ml fractions were collected and analyzed by SDS-PAGE followed by Commassie staining. Indicated fractions were pooled and concentrated in a Vivaspin 6 (MWCO 10000).

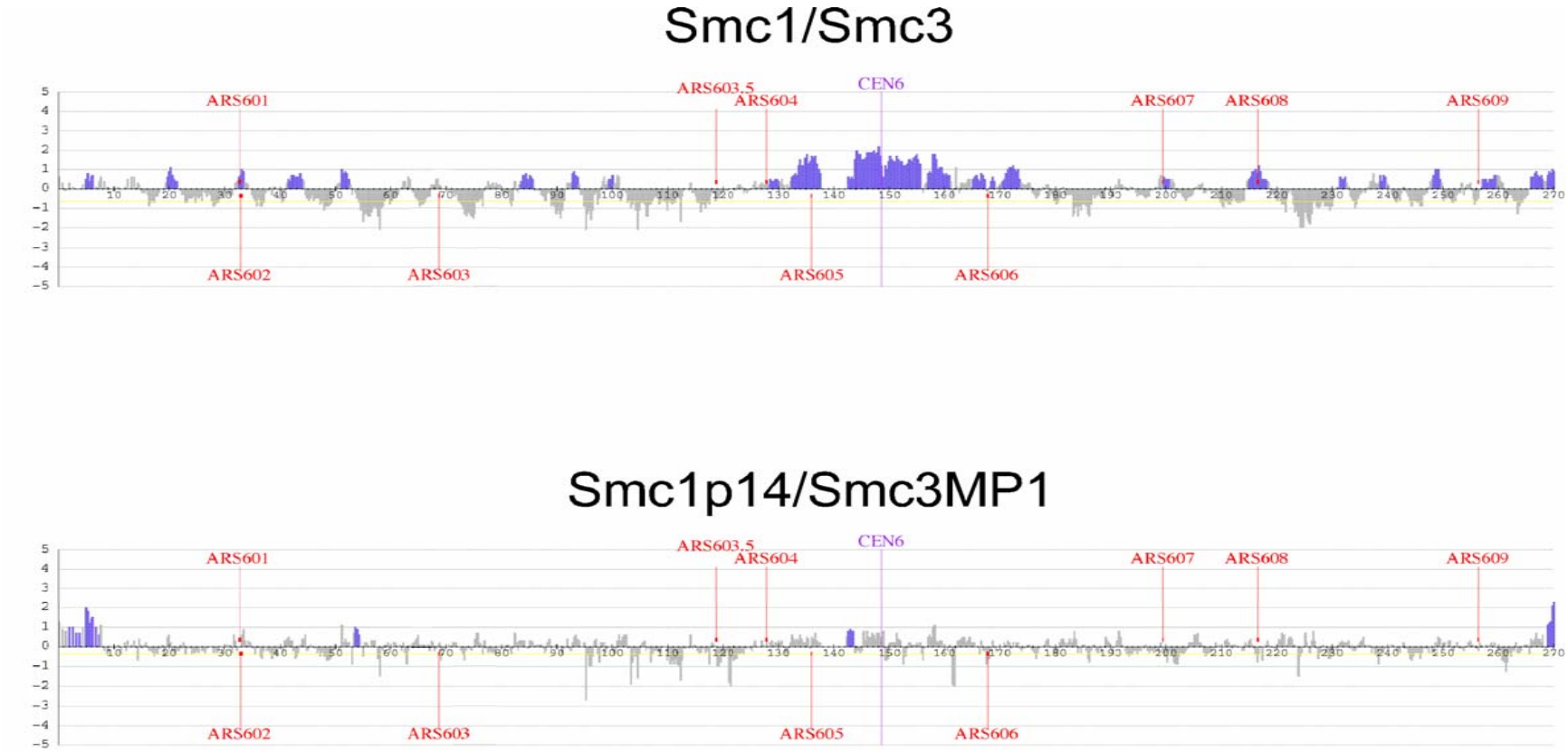

Figure S3. Association of Wild-Type and Hinge-Substituted SMCs with Chromosome VI

Chromosome-wide maps of the data shown in Figure 4E.

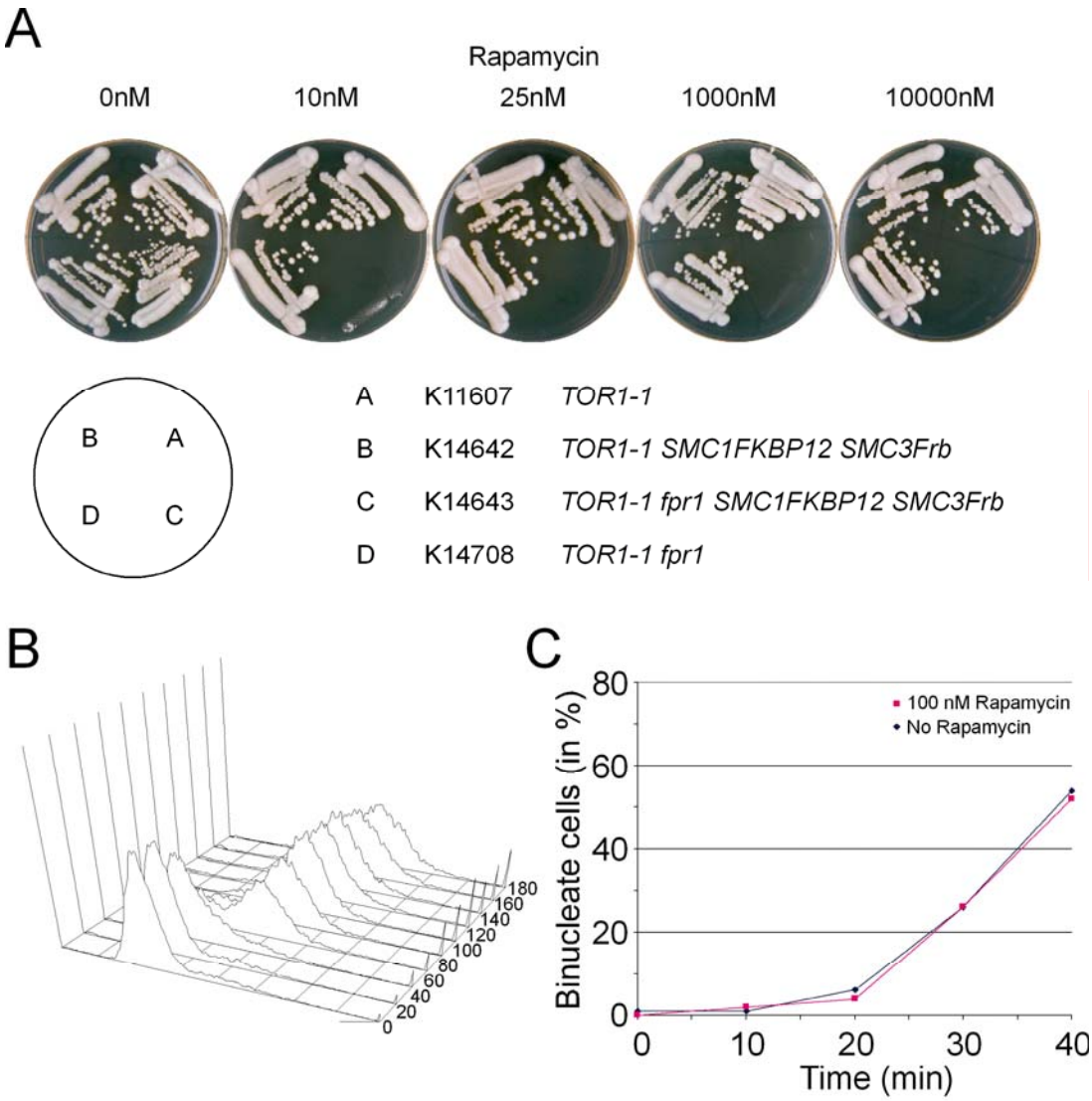

Figure S4.

(A) Sensitivity of Smc3Frb-Smc1FKBP12 cells with or without the Fpr1 gene to rapamycin. Cells were streaked onto plates containing YPD with different concentrations of rapamycin and incubated for 48hrs at 30ºC. Genotypes of strains are indicated.

(B) Monitoring of the DNA content of cells using flow cytometry in samples used in experiment shown in Fig. 6A.

(C) Anaphase execution is not affected by linkage of Smc1's and Smc3's hinge domains. Strain K14690 grown in medium lacking methionine was arrested in G1 using alpha factor peptide. Cells were released from alpha factor into YEP medium supplemented with 2mM methionine to allow one round of replication followed by a metaphase arrest. 3hrs later methionine was washed away and formation of binucleates was monitored by DAPI staining of fixed cells.

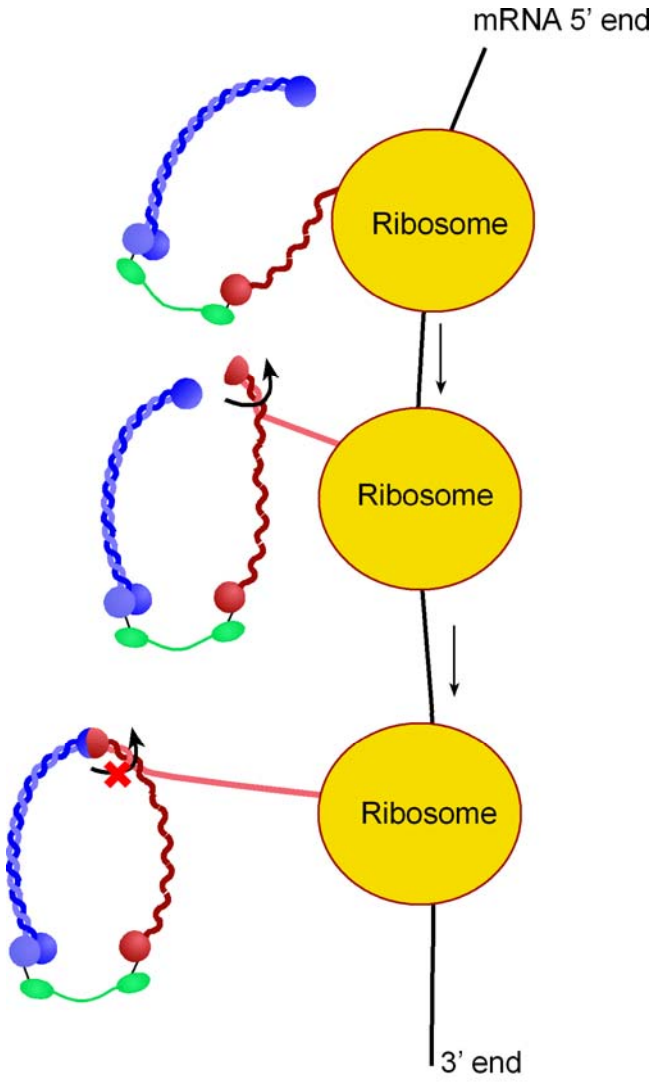

Figure S5. Proper Folding of a Smc3-Scc1-Smc1 Fusion Protein Might Be Hindered by Its Topology

Smc3-Scc1-Smc1 fusion proteins might form rings before the translation of the C-terminal coiled coil and head domain of Smc1 has finished. The nature of the ring would prevent the coiling of the C-terminal alpha helical domain around the N-terminal alpha helical domain of Smc1. This would produce miss-folded cohesin rings.

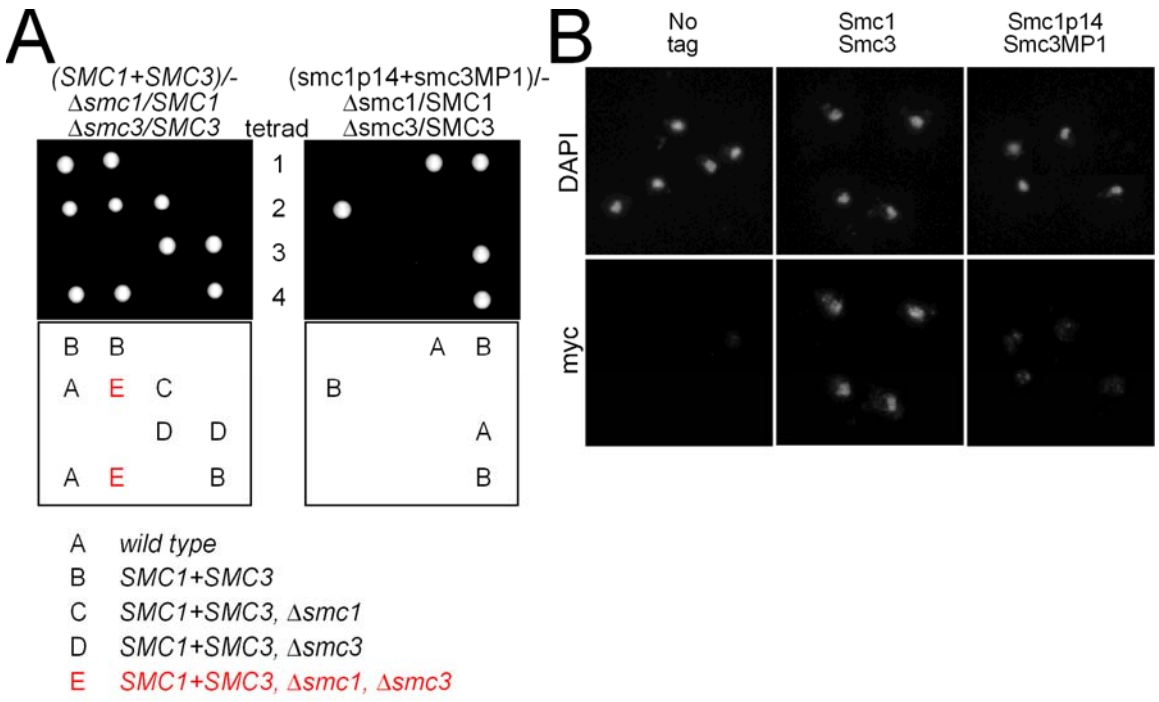

Figure S6. Hinge-Substituted SMC Heterodimers Are Dysfunctional and Do Not Associate with Chromosomes on Spreads

(A) Hinge-substituted SMC heterodimers cannot compensate for lack of *SMC1* and *SMC3* genes. Diploid yeast strains K13981 (MAT<u>a</u>/α Δ*smc1* Δ*smc3 (SMC1-myc9+SMC3-HA3)*) and K13974 (MAT<u>a</u>/α Δ*smc1* Δ*smc3 (smc1p14-myc9+smc3MP1-HA3)*) were sporulated. The four spores of each tetrad were dissected and grown for two days on YPD plates at 30°C. Colonies derived from four representative tetrads (out of 40 tetrads) are shown in the picture. The genotypes of growing spores were scored by genetic marker analysis and are indicated in the boxes.

(B) Hinge-substituted SMCs are not detected on chromosome spreads. Yeast strains K11990, K14022 and K14024 were arrested in mitosis using nocodazole. Chromosome spreads were prepared and stained with DAPI and antibodies against the myc-epitopes on Smc1 proteins.

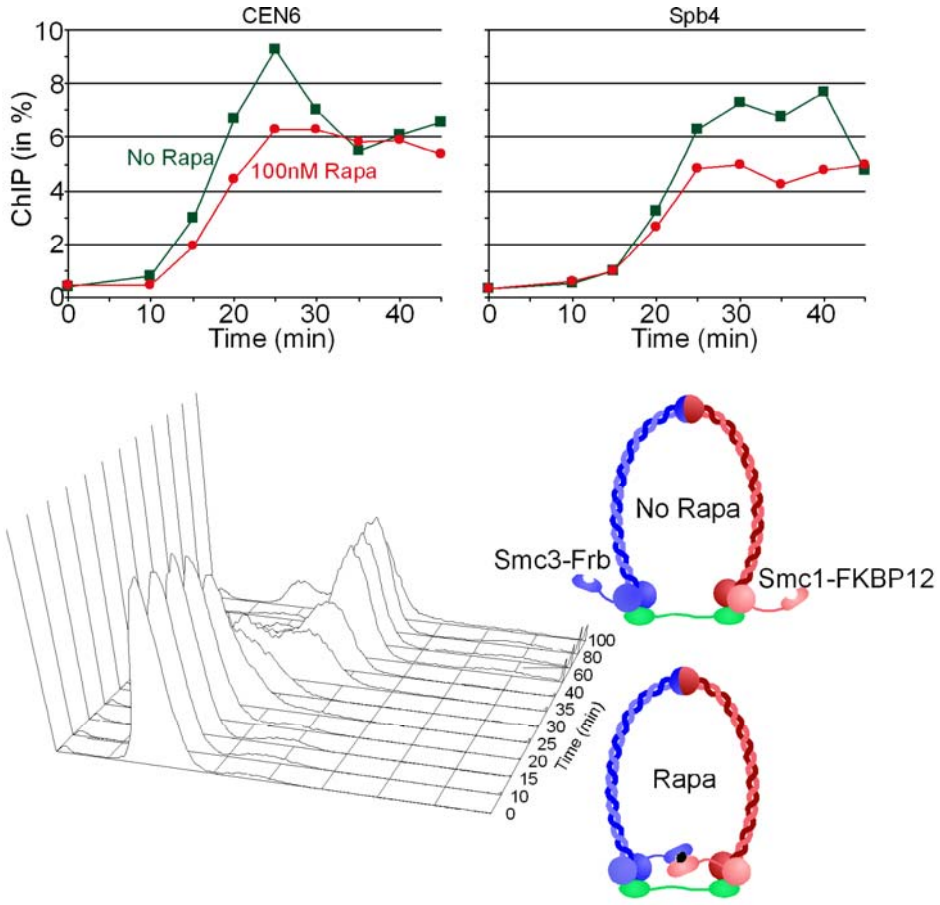

Figure S7. Smc1/Smc3 Head Crosslinking Does Not Block Loading of Cohesin at Centromeric and Pericentric Sites

Cells of strain 14709 (*MATa* Δ*fpr1 TOR1-1 SMC3-Frb SMC1-FKBP12 SCC1-Pk9*) were grown in YPD medium and arrested in G1 phase using α-factor peptide. Cells were released from the G1 arrest and incubated in YPD medium in the absence or presence of 100nM Rapamycin. Samples were taken every five minutes and cellular DNA content was monitored by flow cytometry (see bottom left panel). Sample aliquots were fixed in formaldehyde and subjected to chromatin immunoprecipitation using α-Pk-tag antibodies.

Cells of strain 14709 are sensitive to rapamycin and exhibit a severe cohesion defect in the presence of the drug (data not shown), but there is little effect of rapamycin on the loading of cohesin onto DNA at positions at which cohesin loads first.

Table S1. List of Yeast Strains Used in This Study

699	*MATa*	*ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL psi+*
11607	*MATa*	*TOR1-1*
11680	*MATa*	*leu2::P^{SCC1}-SCC1-TEV3-SMC1-myc18::LEU2 Δscc1::HIS3 Δsmc1::KAN*
11969	*MATa*	*YCplac33-$P^{GAL1-10}$CSCC1(268-566)-HA3*
11970	*MATa*	*Δscc1::HIS3 Δsmc1::KAN leu2::P^{SCC1}-SCC1-TEV3-SMC1-myc18::LEU2 YCplac33-$P^{GAL1-10}$CSCC1(268-566)-HA3*
11990	*MATa*	*SCC1-Pk6::KAN*
12314	*MATa/α*	*Δscc1::URA3/SCC1 Δsmc1::KAN/SMC1*
12385	*MATa*	*Δscc1::URA3 Δsmc1::KAN* leu2::*P^{SCC1}-SCC1-TEV3-SMC1::LEU2*
12542	*MATa/α*	*Δscc1::KAN/SCC1 Δsmc3::HIS3/SMC3*
12822	*MATα*	*LEU2::P^{MET3}CDC20 Δscc1::KAN ura3::HA6-SCC1(TEV220)::URA3 YEplac112*
12823	*MATα*	*LEU2::P^{MET3}CDC20 Δscc1::KAN ura3::HA6-SCC1(TEV220)::URA3 YEplac112-$P^{GAL1-10}$TEV*
12824	*MATa*	*LEU2::P^{MET3}CDC20 Δscc1::KAN ura3::HA6-SCC1MP1-(TEV220)-p14::URA3 YEplac112*
12825	*MATa*	*LEU2::P^{MET3}CDC20 Δscc1::KAN ura3::HA6-SCC1MP1-(TEV220)-p14::URA3 YEplac112-$P^{GAL1-10}$TEV*
12988	*MATα*	*Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1::URA3*
13581	*MATa*	*SMC1::(SMC1 SMC3-HA3)::URA3*
13585	*MATa*	*SMC1::(smc1p14 smc3MP1-HA3)::URA3*
13630	*MATa/α*	*Δscc1::URA3/SCC1 Δsmc1::KAN/SMC1 leu2::P^{SCC1}-SCC1-TEV3-SMC1::LEU2/leu2*
13638	*MATa/α*	*Δscc1::KAN/SCC1 Δsmc3::HIS3/SMC3 ura3::P^{SCC1}-SMC3-TEV3-SCC1::URA3/ura3*
13653	*MATα*	*Δscc1::KAN ura3::HA6-SCC1(TEV220)::URA3 YEplac112-$P^{GAL1-10}$TEV*
13654	*MATa*	*Δscc1::KAN ura3::HA6-SCC1MP1-(TEV220)-p14::URA3 YEplac112-$P^{GAL1-10}$TEV*
13974	*MATa/α*	*Δsmc1::KAN/SMC1 Δsmc3::NAT/SMC3 ura3::(smc1p14-myc9 smc3MP1-HA3)::URA3/ura3*
13981	*MATa/α*	*Δsmc1::KAN/SMC1 Δsmc3::NAT/SMC3 ura3::(SMC1-myc9 SMC3-HA3)::URA3/ura3*
14022	*MATa*	*SCC1-Pk6::KAN ura3::(SMC1-myc9 SMC3-HA3)::URA3*
14024	*MATa*	*SCC1-Pk6::KAN ura3::(smc1p14-myc9 smc3MP1-HA3)::URA3*
14081	*MATa/α*	*TRP1::$P^{GAL1-10}$SCC1/SCC1 ura3::myc9-Scc1TEV268-myc9::URA3/ura3::(SMC1-myc9 SMC3-HA3)::URA3*
14082	*MATa/α*	*TRP1::$P^{GAL1-10}$SCC1/SCC1 ura3::myc9-Scc1TEV268-myc9::URA3/ura3::(smc1p14-myc9 smc3MP1-HA3)::URA3*
14084	*MATa/α*	*TRP1::$P^{GAL1-10}$SCC1/SCC1 ura3::myc9-Scc1TEV268-myc9::URA3/ura3*
14172	*MATα*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1-Frb::URA3 SMC1-FKBP12::KAN*
14173	*MATα*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1-Frb::URA3*
14174	*MATα*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1::URA3 SMC1-FKBP12::KAN*
14195	*MATa*	*Δfpr1::NAT TOR1-1 SCC1-Frb::HIS3 SMC1-FKBP12::KAN*
14196	*MATa*	*Δfpr1::NAT TOR1-1 scc1(S525N)-Frb::HIS3 SMC1-FKBP12::KAN*
14197	*MATa*	*Δfpr1::NAT TOR1-1 scc1(Q544K)-Frb::HIS3 SMC1-FKBP12::KAN*
14330	*MATα*	*pM346-LEU2D::smc1p14-TEV-protA pM346-URA3::smc3MP1*

14331	*MATα*	*pM346-LEU2D::SMC1-TEV-protA pM346-URA3::SMC3*
14356	*MATα*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HPH ura3::P^{SCC1}-SMC3-TEV3-SCC1-Frb::URA3 SMC1-FKBP12::KAN*
14358	*MATα*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HPH ura3::P^{SCC1}-SMC3-TEV3-scc1(Q544K)-Frb::URA3 SMC1-FKBP12::KAN*
14476	*MATα*	*pM346-LEU2D::smc1p14-TEV-protA pM346-URA3::smc3(K38I)MP1*
14642	*MATa*	*TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2 SMC1FKBP12*
14643	*MATα*	*Δfpr1::NAT TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2 SMC1FKBP12*
14644	*MATa*	*Δfpr1::NAT TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2*
14645	*MATα*	*Δfpr1::NAT TOR1-1 SMC1FKBP12*
14668	*MATa*	*Δfpr1::NAT TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2 SMC1FKBP12*
14690	*MATa*	*Δfpr1::NAT TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2 SMC1FKBP12 TRP1::P^{MET3}-CDC20 ura3::tetOs::URA3 his3::TETR-GFP::HIS3*
14697	*MATa*	*Δfpr1::NAT TOR1-1 Δsmc3::HPH leu2::SMC3Frb::LEU2 SMC1FKBP12 SCC1-Pk9::KAN*
14708	*MATα*	*Δfpr1::NAT TOR1-1*
14709	*MATa*	*Δfpr1::NAT TOR1-1 SMC3-Frb::HIS3 SMC1-FKBP12::KAN SCC1-Pk9::KAN*
14829	*MATa*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1-Frb::URA3 SMC1-FKBP12::KAN SCC3-myc18::TRP1*
30016	*MATa*	*Δfpr1::NAT TOR1-1 Δscc1::KAN Δsmc3::HIS3 ura3::P^{SCC1}-SMC3-TEV3-SCC1-Frb::URA3 SMC1-FKBP12::KAN*

Strains are derivatives of W303. All markers not stated are as in strain 699. A dash connecting two gene names (as in SMC1-FKBP12) indicates a gene fusion. Joined gene names (as in SMC1FKBP12) indicates the insertion of the latter into the former.