Supporting Information

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SI Materials and Methods

Strain Construction. Sister chromatid cohesion (SCC) genes, SCC2 and SCC4, were FLAG epitope tagged as described (1). A carboxyl-terminal fusion of *SCC2* to an auxin-inducible degron (AID) cassette (2) was constructed by using standard approaches and pMK43 (provided by the National Bioresources Program of the MEXT, Japan). *CDC45* was epitope tagged by transformation with a plasmid containing a carboxyl-terminal region of *CDC45* fused to three HA epitopes (pRS306-*CDC45*-3XHA). Carboxyl-terminal V5 tagging of *MCD1* was constructed by using standard approaches and pFA6a-6xGLY-V5-hphMX4 (Addgene). Deletions spanning Scc2 amino acids 143–155 or 2–155 were constructed as described (3) by using multiple, ≤700 base-pair (bp) overlapping DNA fragments that deleted *SCC2* nucleotides 436–474 and AfeI/BstEII-digested pJW4, in the case of the Δ143–155 mutant, or deleted *SCC2* nucleotides 4–474 and BamHI-BstEII-digested pJW4, in the case of the Δ-2–155 mutant. pJW4 contains *SCC2* in YCplac22. The reassembled vectors containing *scc2Δ143–155*, pJW10, or *scc2Δ2-155*, pJW16, were introduced by transformation into CPY18, whose sole copy of *SCC2* resides on a plasmid expressing *URA3*. Cells were then plated on 8 mM 5-fluoro-orotic acid to screen for the ability of cells to lose wild-type *SCC2*. To construct a temperature-sensitive *SCC2* degron, a 217-bp fragment of the 5′ portion of *SCC2* was amplified by PCR with the addition of 5′ XbaI and 3′ NotI restriction sites, respectively, and then cloned into a yeast integrating vector, pRS306tet (EUROSCARF), to create a doxycycline-regulated dihydrofolate reductase (DHFR) fusion to the 5′ region of *SCC2*, creating plasmid, pNL6. Following linearization, the fusion construct was integrated at the endogenous *SCC2* locus by yeast transformation and selection for uracil prototrophs. The addition of 10 μg/mL doxycycline and shift to growth at the restrictive temperature (37 °C) for 1 h halts td-*SCC2* expression and facilitates rapid turnover of Scc2, respectively (4).

Yeast Cell Culture and Cell Cycle Staging. Unless otherwise indicated, cells were released from αF by washing twice in medium containing 0.1 mg/mL protease from *Streptomyces griseus* (Sigma-Aldrich) and returned to growth in protease-containing medium. Proteasome function was inhibited in Δ*erg6* mutants following treatment with 50 mM MG132 in DMSO for 3 h (5). Indole-3-acetic acid was added to culture medium at a final concentration of 2.5 mM in 1% DMSO to induce Scc2-AID turnover.

Chromatin Fractionation. After incubation in 100 mM Pipes/KOH at pH 9.4, 4×10^9 cells were spheroplasted with 500 μg/mL Zymolyase-100T in 0.4 M sorbitol/50 mM KPO_4 in the presence of the following protease inhibitors: 1 mM benzamidine, 0.5 mM sodium metabisulfite, 2.7 μg/mL pepstatin A, 4 mg/mL leupeptin, 1 mM phenylmethanesulfonyl fluoride and phosphatase inhibitors (2 mM sodium orthovanadate, 10 mM NaF, and 10 mM β-glycerophosphate). Spheroplasts were resuspended in an equal cell pellet volume and lysed with 0.25% Triton X-100 to create a whole cell extract (WCE). Lysates were then centrifuged at 12,000 × *g* for 10 min on 30% (wt/vol) sucrose gradients. As an added control, pellets were digested with 5 U DNase I, releasing chromatin-bound proteins into a soluble fraction, termed SN2. DNase I digestions were in 10 mM Tris·HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$ at pH 7.6 at 37 °C for 10 min. WCEs, soluble proteins from the supernatant (SN1), and the resuspended chromatin bound proteins (pellet) were then analyzed via immunoblot.

In Vitro Cleavage Assay. To immuno-isolate Scc2, protein A magnetic beads (Invitrogen), prebound to FLAG antibody at a ratio of 2–5 μg of FLAG serum per 10 μL of a 50% bead suspension by overnight incubation at 4 °C, were then incubated with WCE at 4 °C for 2 h and washed thoroughly six times in 50 mM Hepes, 100 mM KCl, 2.5 mM $MgCl_2$, 10% (vol/vol) glycerol, 0.1% Triton X-100, 0.1% Tween 20, and 300 mM NaCl that also contained protease and phosphatase inhibitors with 10 min between washes. Beads were then incubated with a second WCE lacking FLAG-tagged proteins for 2 h at 4 °C and subsequently washed as described above. Proteins were eluted from beads in 1% SDS by boiling for 5 min, and eluates were analyzed via immunoblot. Where indicated, WCEs (~40 μg/mL total protein) were treated with 200 U of lambda phosphatase (New England Biolabs) in a 100-μL reaction incubated at 30 °C for 0.5–1 h.

Immunoblot Quantitation. SDS polyacrylamide gels (6%, vol/vol) were used to distinguish full-length and cleaved Scc2 species. Proteins were transferred to polyvinylidene difluoride membrane at 100 V for 1 h. Primary and secondary antibody solutions contained 1% BSA, 1% dry nonfat milk, and 0.1% Tween 20 in PBS. Mouse monoclonal FLAG (Sigma) and HA (Roche) antibodies, rabbit Scc2 polyclonal serum (this study), rabbit G6PDH (Sigma), and mouse polyclonal Myc serum (Santa Cruz) were used at 5,000-, 5,000-, 500-, 100,000-, and 500-fold dilutions, respectively. Goat anti-mouse and anti-rabbit horseradish peroxidase-conjugated secondary antibodies (BioRad) were used at 1:2,500 and 1:10,000, respectively. Quantitation was performed by using the BioRad ChemiDoc System and ImageLab software.

Antibody Production. An approximate 480-bp fragment encoding amino acids 40–200 of Scc2 was amplified by PCR as a BamHI-SalI fragment and cloned into BamHI, SalI-digested pGEX-6P-1 GST bacterial expression vector (GE Healthcare). Following a 3-h induction with 0.8 mM IPTG, GST-Scc2 was purified from Rosetta (Novagen) cell extracts by using glutathione-sepharose beads (Amersham Biosciences). GST-Scc2 was eluted from the beads by the addition of glutathione (6.15 mg/mL), whereas an Scc2 polypeptide lacking GST was obtained by treatment of GST-Scc2–bound beads with PreScission protease (GE Healthcare). Rabbits were first inoculated with the intact GST-Scc2 and with Scc2 alone in subsequent boosts, performed by Covance Research Products.

Mass Spectrometry. Bead-eluted Scc2-FLAG samples were loaded onto a 1.5-mm-thick NuPAGE Bis-Tris 4–12% gradient gel (Invitrogen). The BenchMark Protein Ladder (Invitrogen) was used as a protein molecular mass marker. The electrophoretic run was performed by using Mes SDS running buffer, in an X-Cell II mini gel system (Invitrogen) at 200 V, 120 mA, and 25 W per gel for 30 min. The gel was stained by using SimplyBlue SafeStain (Invitrogen) stain and destained with water according to the manufacturer's protocol. After excision, gel pieces were destained in 200 μL of 25 mM ammonium bicarbonate in 50% vol/vol acetonitrile (ACN) for 15 min and washed twice with 200 μL of 50% (vol/vol) ACN. Disulfide bonds in proteins were reduced by incubation in 10 mM DTT at 60 °C for 30 min, and cysteine residues were alkylated with 20 mM iodoacetamide in the dark at room temperature for 45 min. Gel pieces were subsequently washed with 100 μL of distilled water followed by the addition of 100 μL of ACN and dried by SpeedVac (Savant ThermoFisher). One hundred nanograms of trypsin was then added

to each sample and allowed to rehydrate the gel plugs at 4 °C for 45 min and incubated at 37 °C overnight. The tryptic mixtures were acidified with formic acid (FA) to a final concentration of 1%. Peptides were extracted two times from the gel plugs by using 1% FA in 50% ACN. The collected extractions were pooled with the initial digestion supernatant, and the volume was reduced by using SpeedVac. Samples were measured on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) coupled to an Eksigent nanoLC-2D system through a nano-electrospray LC−MS interface. Eight microliters of sample was injected into a 10-μL loop by using the autosampler. To desalt the sample, material was flushed out of the loop and loaded onto a trapping column (ZORBAX 300SB-C18, dimensions 5 × 0.3 mm × 5 μm) and washed with 5% (vol/vol) ACN and 0.1% FA at a flow rate of 10 μL/min for 5 min. At this time, the trapping column was put online with the nano-pump at a flow rate of 350 nL/min. The mobile phase included water with 0.1% FA (solvent A) and 99.9% ACN with 0.1% FA (solvent B). A 90-min gradient from 6% ACN to 40% (vol/vol) ACN was used to separate the peptides. Peptides were separated on a house-made 100 μm inner diameter × 150 mm fused silica capillary packed with Jupiter C_{18} resin (Phenomex). Data acquisition was performed by using the instrument supplied Xcalibur (version 2.0.6) software. The mass spectrometer was operated in the positive ion mode; the peptide ion masses were measured in the Orbitrap mass analyzer, whereas the peptide fragmentation was performed by using either higher energy collisional dissociation (HCD) or electron transfer dissociation (ETD) in the linear ion trap analyzer by using default settings. Ten most intense ions were selected for fragmentation in each scan cycle; fragmented masses were excluded from further sequencing for 90 s.

MS/MS spectra were extracted from raw data files and converted into Mascot generic format (MGF) files by using a PAVA script (University of California, San Francisco). These mgf files were then independently searched against the *Saccharomyces cerevisiae* subset of the SwissProt database by using an in-house Mascot server (Version 2.2.06, Matrix Science). Mass tolerances were ±15 ppm for MS peaks, and ±0.6 Da for MS/MS fragment ions. For all HCD spectra, fragment ion tolerances were set to 0.05 Da. Trypsin specificity was used, allowing for one missed cleavage. Met oxidation, protein N-terminal acetylation, and peptide N-terminal pyroglutamic acid formation were allowed for variable modifications while carbamidomethyl of Cys was set as a fixed modification.

1. Kogut I, Wang J, Guacci V, Mistry RK, Megee PC (2009) The Scc2/Scc4 cohesin loader determines the distribution of cohesin on budding yeast chromosomes. *Genes Dev* 23(19):2345–2357.
2. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M (2009) An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat Methods* 6(12):917–922.
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4. Dohmen RJ, Wu P, Varshavsky A (1994) Heat-inducible degron: A method for constructing temperature-sensitive mutants. *Science* 263(5151):1273–1276.
5. Hwang GW, Furuchi T, Naganuma A (2002) A ubiquitin-proteasome system is responsible for the protection of yeast and human cells against methylmercury. *FASEB J* 16(7):709–711.

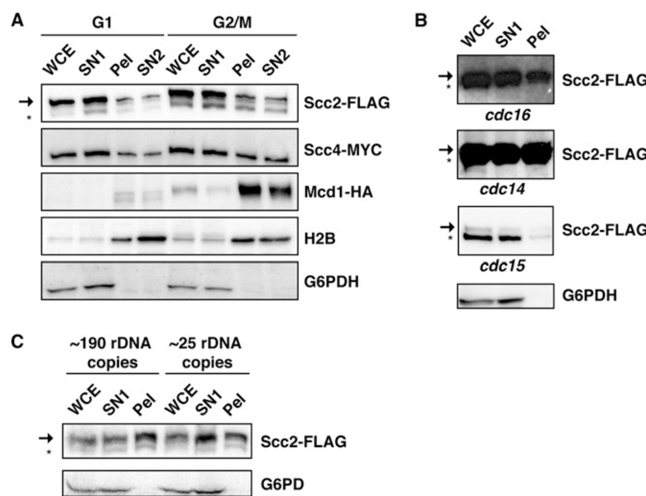

Fig. S1. Scc2 chromatin association is cell cycle regulated. (*A*) Scc2-FLAG Scc4-MYC Mcd1-HA (1891-32C) cells were staged in G_1 or M by using αF or a conditional *cdc16* mutant (37 °C, 3 h), respectively, and then subjected to chromatin fractionation. Levels of the indicated proteins were determined by immunoblotting WCE or fractions of nonchromatin bound supernatants (SN1), chromatin-bound pellets (Pel), or the supernatant generated by DNase I digestion of pellets (SN2). Chromatin-bound Mcd1 and H2B and nonchromatin-bound glucose-6-phosphate dehydrogenase (G6PDH) serve as fractionation controls. Release of proteins into SN2 by DNase I treatment of pellets indicates the chromatin dependence of pellet fraction enrichment. The arrow and asterisk indicate slower and faster migrating Scc2 species, respectively. (*B*) Conditional *cdc16*, *cdc14*, or *cdc15* mutant cells (PMY679, PVY1, and 1886-24A, respectively) containing Scc2-FLAG were αF-arrested, released into fresh media at 37 °C for 3 h, and then fractionated and analyzed via immunoblot by using FLAG and G6PDH antibodies. (*C*) Immunoblots of chromatin fractionations of asynchronously growing cells having either ∼190 or ∼25 copies of rDNA (MTY084 and MTY086, respectively) are shown by using FLAG and G6PDH antibodies.

Fig. S2. Post S-phased inactivation of *scc2-4* cells. *scc2-4* (1875-39B) cells were first synchronized in G_1 with αF and then released from the arrest under different growth conditions, indicated in the text. DNA histograms generated by propidium iodide staining from three independent experiments of cycling cells (asynchronous, red), αF-arrested cells (blue), and cells at the time of shift to the restrictive temperature (green) are indicated. Unreplicated (1C) and replicated (2C) DNA contents are indicated.

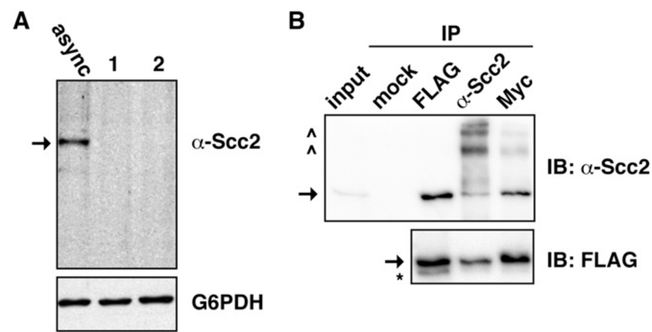

Fig. S3. A rabbit polyclonal serum is specific for Scc2. (*A*) WCE of asynchronously growing td-*SCC2* control cells (2650) and two biological replicates (labeled 1 and 2) of these cells treated with 10 μg/mL doxycycline to inhibit td-*SCC2* expression and shifted to growth at 37 °C for 1 h to induce Scc2 turnover were subjected to immunoblot by using a rabbit polyclonal serum raised against amino acids 40–200 of *S. cerevisiae* Scc2, or anti-G6PDH antibody as a loading control. The arrow indicates full-length Scc2. (*B*) An extract of Scc2-FLAG Scc4-13Myc cells [1891-32C, input (2%)] was subjected to reciprocal coimmunoprecipitation with the indicated antibodies [none (mock), FLAG, rabbit polyclonal Scc2 serum, or Myc], followed by immunoblot with either the rabbit polyclonal Scc2 serum (*Upper*) or anti-FLAG antibody (*Lower*). The arrow, asterisk, and caret indicate full-length, cleaved, and phosphorylated forms of Scc2, respectively.

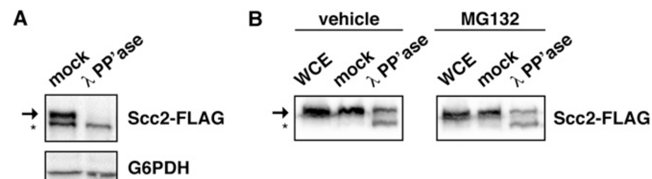

Fig. S4. Scc2 cleavage is independent of vacuolar proteases and the proteasome. (*A*) Mock treatment or λ phosphatase treatment of WCE prepared from asynchronously growing *pep4-3 prb1-1122 prc1-407* Scc2-FLAG cells (MTY046) was immunoblotted with FLAG and G6PDH antibodies. The arrow and asterisk indicate full-length and cleaved Scc2 species, respectively. (*B*) Scc2-FLAG is cleaved upon phosphatase treatment in WCE from asynchronous Δ*erg6* cells (MTY096) treated with DMSO or proteasome inhibitor MG132.

Fig. S5. Amino-terminal Scc2 residues 2–155 are essential for viability. (*A*) Parental strain CPY18, containing a chromosomal *SCC2* deletion rescued with pPCM102, a *URA3*/*CEN4*/ARS1 vector carrying wild-type *SCC2,* was transformed with YCplac22 (*TRP1*/*CEN4*/ARS1) alone (JWY154), or the same vector containing wild-type *SCC2* (JWY140), *scc2Δ143-155* (JWY139), or *scc2Δ2-155* (JWY211). The growth of these strains is shown on synthetic dextrose complete (SDC) medium lacking tryptophan and on SDC medium containing 5′-fluroorotic acid (5′-FOA) to demonstrate the ability of cells carrying the various plasmids to lose the *SCC2*/*URA3* plasmid. (*B*) Coimmunoprecipitation of Scc2-FLAG and Scc2Δ143-155-FLAG with Scc4-HA in cells containing untagged wild-type Scc2 (JWY143 and JWY148) was tested. Beads alone IP (mock) fails to interact with Scc2, as expected. IP samples represent 10% of the material present in the immunoprecipitations. The arrow and asterisk indicate full-length and cleaved Scc2 species, respectively. (*C*) FLAG and G6PDH immunoblots of chromatin fractionations of asynchronously growing *SCC2* and *scc2Δ143-155* cells (JWY140 and JWY139, respectively) are shown. Note that these strains retain untagged *SCC2* on *URA3* plasmids. (*D*) Scc2 remains sensitive to cleavage upon phosphatase treatment in *SCC2* or *scc2Δ143-155* cells (JWY140 and JWY139, respectively).

Fig. S6. Scc2 cleavage is constitutive following dephosphorylation. (*A*) Scc2-FLAG cells (1891-32C) were staged in G_1 with αF. Samples taken at the G_1 arrest (0 min), and every 15 min upon release were analyzed via immunoblot with α–Scc2 serum. The abundance of slower migrating, phosphorylated Scc2 species (^) and full-length Scc2 (arrow) throughout the cell cycle is shown. (*B*) After immuno-isolation from asynchronous cells (1891-32C), Scc2-FLAG–bound beads were first phosphatase-treated or mock-treated. The beads were then incubated with extracts from untagged cells (1891-36D) staged at specific times throughout the cell cycle, followed by extensive washing and FLAG immunoblot analysis to determine Scc2 species distributions. The arrow and asterisk indicate full-length and cleaved Scc2, respectively.

Table S1. *Saccharomyces cerevisiae* strains

Strain	Relevant genotype	Source
A364a*		
1891–32C	*MATa SCC2*-6His-3FLAG::kanMX *MCD1*-6HA *SCC4*-6His-13Myc::clonNAT *his3 cdc16-1 ura3 bar1 gal1*	This study
PMY679	*MATa SCC2*-6His-3FLAG::kanMX *cdc16-1 his3Δ200 MCD1-6HA ura3 bar1 gal1*	This study
1891–36D	*MATa SCC4*-6His-13Myc::clonNAT *his3 leu2 MCD1*-6HA *ura3 bar1 gal1*	This study
PVY1	*MATa* SCC2-6His-3FLAG::kanMX *cdc14-1 ura3 his3Δ200 leu2 trp1 bar1*	This study
1886–24A	*MATa SCC2*-6His-3FLAG::kanMX *cdc15-2 MCD1*-6HA his3Δ1 *leu2 trp1 bar1 cyh2*	This study
PMY715	*MATa SCC2*-6His-3FLAG::kanMX *SMC3*-6MYC::*his5*+ *CDC45*-3HA*::URA3 ura3 leu2 bar1 trp1*	This study
MTY096	*MATa SCC2*-6His-3FLAG::clonNAT Δ*erg6*::kanMX *ura3 leu2 bar1 his3Δ200*	This study
W303*		
2650	*MATa ura3-1 URA3*::tetdeg-*scc2 his3-11,15 leu2-3,112 LEU2*::pCM245 TetR-*SSN6 HIS3*::pRS403*HIS3*::pRS403-188 CMV tTA *ade2-1 trp1-1*	This study
MTY084	*MATa SCC2*-6His-3FLAG::kanMX *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100* Δ*fob1*::*HIS3 (*∼*190 rDNA* copies*)*	This study
MTY086	*MATa SCC2*-6His-3FLAG:: kanMX *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100* Δ*fob1*::*HIS3* (∼25*rDNA* copies)	This study
JWY139	*MATa* Δ*scc2*::clonNAT *ura3 trp1-1 leu2-3,112 his3 can1-100* GAL psi+ *SMC3*-6MYC::*his5*+ pJW10 and pPCM102 (*SCC2*/*URA3*/*CEN4*/ARS)	This study
JWY140	*MATa* Δ*scc2*::clonNAT *ura3 trp1-1 leu2-3,112 his3 can1-100* GAL psi+ *SMC3*-6MYC::his5+ pJW4 and pPCM102 (*SCC2*/*URA3*/*CEN4*/ARS)	This study
JWY154	*MATa* Δ*scc2*::clonNAT *ura3 trp1-1 leu2-3,112 his3 can1-100* GAL psi+ *SMC3*-6MYC::his5+ YCplac22 and pPCM102 (*SCC2*/*URA3*/*CEN4*/ARS)	This study
JWY211	*MATa* Δ*scc2*::clonNAT *ura3 trp1-1 leu2-3,112 his3 can1-100* GAL psi+ *SMC3*-6MYC::his5+ pJW16 and pPCM102 (*SCC2*/*URA3*/*CEN4*/ARS)	This study
JWY143	*MATa SCC2*-6His-AID::KAN *ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 SCC4*-6His-3HA::hph Δ*bar1::LEU2* pJW4	This study
JWY148	*MATa SCC2*-6His-AID::KAN *ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 SCC4*-6His-3HA::hph Δ*bar1::LEU2* pJW10	This study
1875–39B	*MATa scc2-4 leu2 ura3 his3 SCC3*-18MYC::*TRP1 ade2-1 can1-100*	This study
JWY214	*MATa ura3-1*::*ADH1*-AtTIR1-9MYC *URA3 SCC2*-6HIS-AID::clonNAT *trp1-1 can1-100 SCC4*-6HIS-3FLAG::KAN *MCD1*-6G-V5::hph pJW4	This study
JWY215	*MATa ura3-1*::*ADH1*-AtTIR1-9MYC *URA3 SCC2*-6HIS-AID::clonNAT *trp1-1 can1-100 SCC4*-6HIS-3FLAG::KAN *MCD1*-6G-V5::hph pJW16	This study
S288C*		
MTY046	*MATa SCC2*-6His-3FLAG:: kanMX *pep4-3 prb1-1122 prc1-407 leu2 trp1 ura3-52*	This study

*Strain background.