Supplemental Material for:

Meikin synergizes with shugoshin to protect cohesin Rec8 during meiosis I

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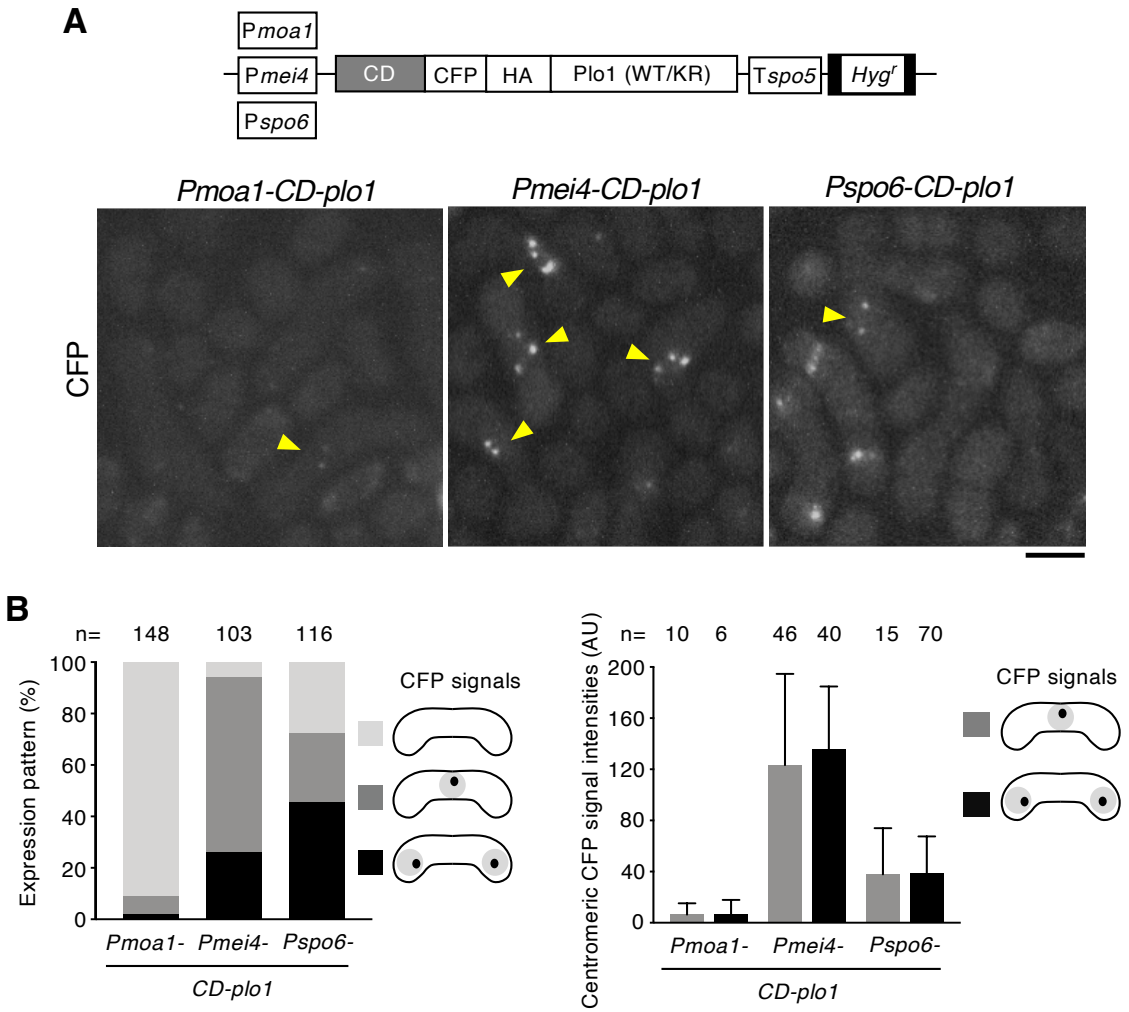
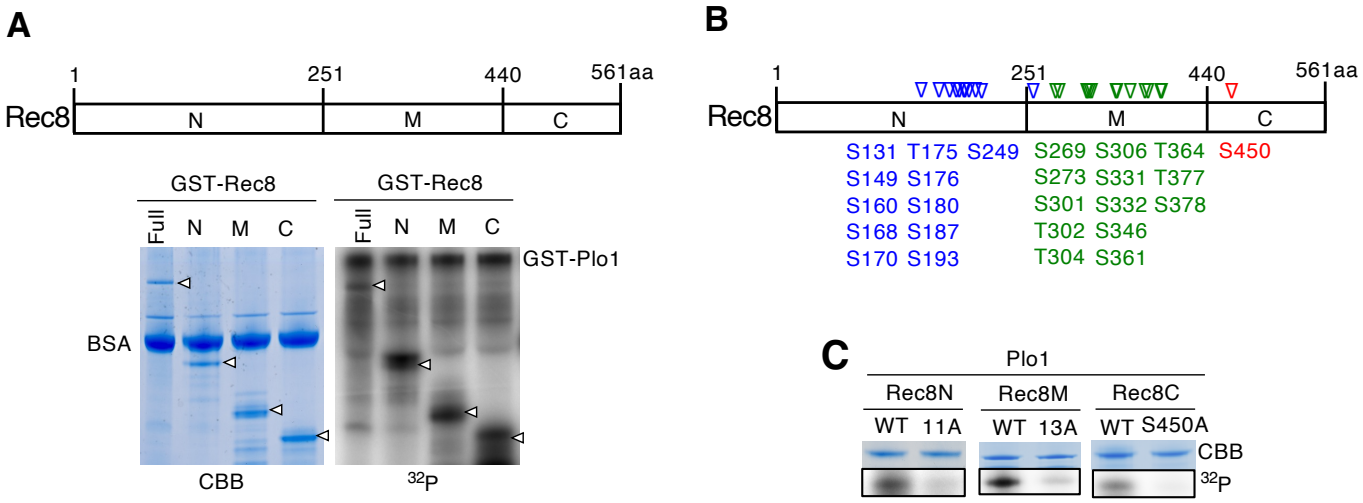
Contents **Page**

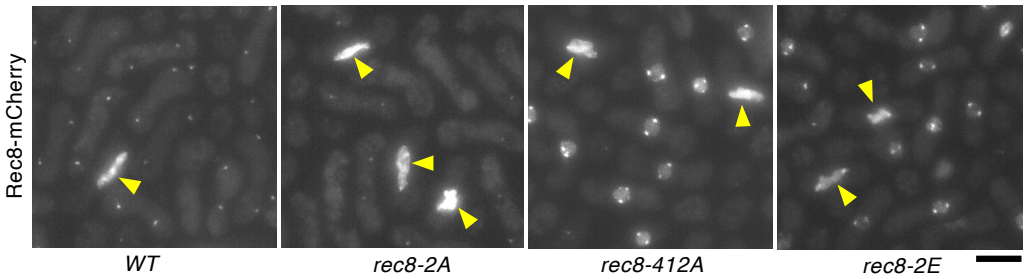


Supplemental Figure S1. Comparison of three meiotic promoters.
(A) To express the Plo1 fusion proteins in meiosis I, we examined three meiotic promoters, P*moa1*, P*mei4* and P*spo6*. The indicated *mes1-B44* cells were spotted on SPA plate and incubated at 26°C for 10 h (rather than 14 h). Arrowheads indicate the prophase I cells with CFP signals. Scale bars 5µm.
(B) CD-CFP fused Plo1 was monitored for their expression pattern (left). Centromeric CFP signal intensities were measured in prophase I and prophase II (right). n = cell or nuclear number used for assay. Error bars, SD.

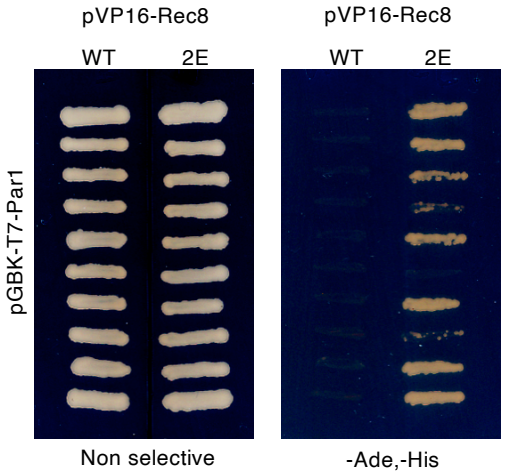
The *mei4*$^{+}$ promoter acted appropriately, showing the CFP signals mainly in prophase I, the period when Moa1 fulfills its function. In contrast, the *moa1*$^{+}$ promoter was weak, whereas the *spo6*$^{+}$ promoter was moderate but more active after prophase I.



Supplemental Figure S2. ***In vitro*** **Phosphorylation of Rec8 by Plo1.**
(A) Recombinant GST-fused Rec8 fragments (Full length, N-terminal, Middle, and C-terminal domains) were incubated with recombinant GST-Plo1 in the presence of [γ-^{32}P] ATP. The incorporation of radioactive phosphate groups was visualized by autoradiography (^{32}P) and compared with protein levels (CBB, Coomassie Brilliant Blue). The arrow heads indicate GST-Rec8 fusion proteins.
(B) Schematic depiction of the N-terminal, Middle, and C-terminal domains of Rec8. Serine or threonine in the indicated sites was substituted to alanine.
(C) Recombinant GST-fused Rec8 fragments (wild-type and mutants with alanine substitutions) were incubated with recombinant GST-Plo1 in the presence of [γ-^{32}P] ATP.



Supplemental Figure S3. Distributions of Rec8-mCherry in *WT, rec8-2A, rec8-S412A* and *rec8-2E* cells are intact in prophase I.
Logarithmically growing *mes1-B44* cells were collected and suspended in 20 mg/ml leucine, spotted on sporulation-inducing medium (SPA) and incubated at 26°C for 14 h. Most cells were arrested at prophase II, whereas a small number of cells were in prophase I. In all strains, Rec8-mCherry was observed at full levels in the nucleus during prophase I, although the pattern differed after meiosis I. Arrowheads indicate prophase I nuclei. Scale bar 5µm.



Supplemental Figure S4. Yeast two-hybrid assay suggests that the mutant Rec8-2E gained increased affinity with Par1.
The construct of Par1 was amplified by PCR, cloned into pGBKT7 vector and used as bait. The constructs of Rec8 (246-561aa) and the 2E mutant version were amplified by PCR, cloned into pVP16 vector and used as prey. These plasmids were transformed into the L40 strain of *Saccharomyces cerevisiae* (*MATa leu2 trp1 his3 ade2 URA3:lexA:lacZ LYS2:lexA:HIS3 gal80*). Plates lacking histidine and adenine were used as selective media.

Supplemental Methods

In vitro kinase reactions

To generate recombinant GST-fusion proteins, the open reading frames of *hhp1*$^{+}$, *plo1*$^{+}$, *rec8*$^{+}$, *rec8N* (residues 1-268 aa), *rec8M* (251-440 aa), *rec8C* (450-561 aa) were amplified by PCR and cloned into the pGEX4T-2 vector (GE Healthcare). The plasmids were expressed in *Escherichia coli* strain BL21C+ and the recombinant proteins were purified with glutathione-Sepharose beads (GE Healthcare). GST-HA-Plo1 and substrates were incubated with kinase buffer (50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 0.5% Triton X-100 and 5 mM dithiothreitol) at 30 °C for 30 min in the presence of [γ-^{32}P] ATP (Miyazaki et al. 2017). The incorporation of the radioactive phosphate group was visualized by means of autoradiography, and protein loading was analyzed by staining with Coomassie brilliant blue (CBB) or silver stain.

In vitro dephosphorylation reactions

To generate phosphorylated Rec8 substrates, GST-tagged Rec8C1-WT and -2E (residues 391–561 aa) were expressed in *Escherichia coli* strain BL21C+ and purified with glutathione-Sepharose beads, then labelled with [γ-^{32}P] ATP with recombinant GST-CK1 (Hhp1) kinase. The immunoprecipitated PP2A complexes from yeast cell extracts were collected after washing with immunoprecipitation buffer without phosphatase inhibitors (25 mM MOPS pH 7.2, 15 mM $MgCl_2$, 15 mM EGTA, 10% Triton X-100, 1 mM dithiothreitol (DTT)). The equivalent of 10 µl of immunoprecipitated beads was preincubated for 10 min with or without okadaic acid (Wako) at room temperature (20 °C), followed by the addition of ^{32}P-labelled Rec8-C1 substrates in a dephosphorylation buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.01% Brij35, 2 mM $MnCl_2$, 2 mM DTT) to a total reaction volume of 20 µl, then incubated at 30 °C with gentle agitation.

Antibody production

To generate a phospho-specific antibody for S450 of Rec8 (anti-Rec8-pS450), rabbits were immunized with a S450-phosphorylated peptide corresponding to amino acids 445–455 (PALESpSQFHET) of Rec8. The antiserum was passed through a column conjugated to the non-phosphorylated peptide and then subjected to affinity-purification using the phosphorylated peptide. Antibodies that recognizes the Rec8 protein (anti-Rec8) were obtained in the same immunization but from different rabbit. The antiserum was passed through a column conjugated to the phosphorylated peptide and then subjected to affinity-purification using the non-phosphorylated peptide.

Protein preparation and immunoblot analysisCells (2 x10^8) were suspended in HB buffer (25 mM MOPS pH 7.2, 60 mM ß-glycerophosphate, 15 mM p-nitrophenylphosphate, 15 mM $MgC1_2$, 15 mM EGTA , 1 mM DTT, 0.1 mM sodium vanadate, 1% Triton X-100, 1 mM PMSF, 20 µg/ml leupeptin, 40 µg/ ml aprotinin) and boiled for 5 min. Ice cold 1.5 ml of glass beads (500 µm in diameter) were added and the cells were broken by using Freezer mill (SPEX). A total of 10 µg of protein from each sample was

subjected to electrophoresis in SDS-polyacrylamide gels and immunoblotted. Rec8-pS450 were detected with anti-Rec8-pS450 polyclonal antibodies at 1:500 dilution, Rec8 with anti-Rec8 polyclonal antibodies at 1:1,000 dilution and Rec8-FLAG with anti-FLAG M2 antibody (Sigma) at 1:2,000 dilution. Tubulin was detected with anti-tubulin antibody TAT-1 at 1:2,000 dilution. For second antibodies, we used horseradish peroxidase (HRP)-conjugated donkey anti-rabbit (NA934, GE Healthcare) or goat anti-mouse (STAR120P, AbD SeroTEC) IgG at 1:5,000 dilution.

Supplemental table S1. List of *S. pombe* strains

Fig 1B	YW28	*h- Δmoa1::kan imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP mes1-B44*
	YW29	*h+ Δmoa1::kan mes1-B44*
Fig 1C	YW27	*h- leu1 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP mes1-B44*
	YW28	*h- Δmoa1::kan imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP mes1-B44*
	YW29	*h+ Δmoa1::kan mes1-B44*
	YW34	*h+ Δmoa1::kan mes1-B44 Z::Pmei4-2CD-CFP-plo1<<natr*
	YW35	*h+ Δmoa1::kan mes1-B44 Z::Pmei4-2CD-CFP-plo1KR<<natr*
	YW36	*h+ Δmoa1::kan mes1-B44 Z::Pmei4-cnp3C-CFP-plo1<<natr*
	YW37	*h+ Δmoa1::kan mes1-B44 Z::Pmei4-cnp3C-CFP-plo1KR<<natr*
Fig 2A	YJ120	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8<<hygr*
	YJ125	*h- leu1 mes1-B44 ade6-M216 rec8<<hygr*
	YJ138	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-N-11A<<hygr*
	YJ139	*h- leu1 mes1-B44 ade6-M216 rec8-N-11A<<hygr*
	YJ140	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-M-13A<<hygr*
	YJ141	*h- leu1 mes1-B44 ade6-M216 rec8-M-13A<<hygr*
	YJ123	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S450A<<hygr*
	YJ128	*h- leu1 mes1-B44 ade6-M216, rec8-S450A<<hygr*
Fig 2B	YJ100	*h+ pat1-114 kanr<<Prad21-slp1+ par1-FLAG<<NATr rec8-13myc<<hygr*
	YJ101	*h+ pat1-114 kanr<<Prad21-slp1+ par1-FLAG<<NATr rec8-S450A-13myc<<hygr*
Fig 2C	YW119	*h- pat1-114 rec8-FLAG<<natr*
	YW120	*h- pat1-114 moa1::hygr rec8-FLAG<<natr*
	YJ168	*h- pat1-114 rec8-S450A-FLAG<<natr*
Fig 2D	YJ120	*h+ leu1, mes1-B44, imr1<<lacO<<ura4+, his7+<<Pdis1-lacI-GFP, rec8<<hygr*
	YJ125	*h- leu1, mes1-B44, ade6-M216, rec8<<hygr*
	YJ133	*h+ leu1, mes1-B44, imr1<<lacO<<ura4+, his7+<<Pdis1-lacI-GFP, Δrec8::hygr*
	YJ134	*h- leu1, mes1-B44, ade6-M216, Δrec8::hygr*
	YJ121	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP Δsgo1::hygr*
	YJ126	*h- leu1 mes1-B44 ade6-M216 Δsgo1::hygr*
	YJ122	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S449A<<hygr*
	YJ127	*h- leu1 mes1-B44 ade6-M216 rec8-S449A<<hygr*
	YJ123	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S450A<<hygr*
	YJ128	*h- leu1 mes1-B44 ade6-M216 rec8-S450A<<hygr*
	YJ124	*h+ leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S449AS450A<<hygr*
	YJ129	*h- leu1 mes1-B44, ade6-M216 rec8-S449AS450A<<hygr*
Fig 2E	YJ221	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP*
	YJ169	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S449E<<hygr*
	YJ173	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S450E<<hygr*
	YJ98	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP rec8-S449ES450E<<hygr*

Fig 2F	YJ106	*h90 leu1, mes1-B44, imr1<<lacO<<ura4+, his7+<<Pdis1-lacI-GFP, pHBCA13-CFP-atb2+, rec8-mCherry-Tspo5<<NAT, ⊿ rec12::hygr*
	YJ107	*h90 leu1, mes1-B44, imr1<<lacO<<ura4+, his7+<<Pdis1-lacI-GFP, pHBCA13-CFP-atb2+, rec8-S449E,S450E-mCherry-Tspo5<<NATr, ⊿ rec12::hygr*
Fig 3A, 3B	YJ149	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-mCherry-Tspo5<<natr*
	YJ152	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-mCherry-Tspo5<<natr sgo1::kanr*
	YW15	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S449A S450A-mCherry-Tspo5<<natr*
Fig 3B, 3C	YJ151	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S449E S450E-mCherry-Tspo5<<natr*
	YJ154	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S449E,S450E-mCherry-Tspo5<<natr sgo1::kanr*
Fig 3D	YW160	*h- ura4? Padh1-rec8-3HA<<ura4+ lys1::hygr<<Pnmt81-par1+-CFP-CD*
	YW161	*h- ura4? Padh1-rec8-S449AS450A-3HA<<ura4+ lys1::hygr<<Pnmt81-par1+-CFP-CD*
	YW162	*h- ura4? Padh1-rec8-S449ES450E-3HA<<ura4+ lys1::hygr<<Pnmt81-par1+-CFP-CD*
	Y380	*h+ ura4?, Padh1-rec8-3HA<<ura4+, Pnmt81-sgo1-FLAG-GFP<<kanr*
	Y381	*h+ ura4?, Padh1-rec8-S449AS450A-3HA<<ura4+, Pnmt81-sgo1-FLAG-GFP<<kanr*
	Y382	*h+ ura4?, Padh1-rec8-S449ES450E-3HA<<ura4+, Pnmt81-sgo1-FLAG-GFP<<kanr*
Fig 4A	YJ149	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-mCherry-Tspo5<<natr*
	YJ150	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S412A-mCherry-Tspo5<<natr*
	YJ153	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+, his7+<<Pdis1-lacI-GFP, pHBCA13-CFP-atb2+, rec8-S412A-mCherry-Tspo5<<natr, sgo1::kanr*
Fig 4D	PB345	*h+ pat1-114 kanr<<Prad21-slp1+ par1-FLAG<<natr*
Sup Fig S1	YW46	*h+ Δmoa1::kan mes1-B44 Z::Pmoa1-2CD-CFP-plo1<<natr*
	YW34	*h+ Δmoa1::kan mes1-B44 Z::Pmei4-2CD-CFP-plo1<<natr*
	YW30	*h+ Δmoa1::kan mes1-B44 Z::Pspo6-2CD-CFP-plo1<<natr*
Sup Fig S3	YJ149	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-mCherry-Tspo5<<natr*
	YW15	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S449A S450A-mCherry-Tspo5<<natr*
	YJ150	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S412A-mCherry-Tspo5<<natr*
	YJ151	*h90 leu1 mes1-B44 imr1<<lacO<<ura4+ his7+<<Pdis1-lacI-GFP pHBCA13-CFP-atb2+ rec8-S449E,S450E-mCherry-Tspo5<<natr*