

Figure S1. Chromosome morphology in ndt804 and hopls cells during meiosis. (A) Meiotic cells arrested at pachytene by ndt804. Yeast cells were induced for 8 h to undergo synchronous meiosis, and nuclear spreads were prepared. Rec8-3HA (red) and Sgo 1-9Myc (green) were detected with anti-HA (12CA5) and anti-Myc (9E10) antibodies; DNA (blue) was stained with DAPI. (B) Hop 1 localization in wild-type (WT) and $P_{\text {CLB2 }}$ PDS5 cells at pachytene. Yeast nuclear spreads were prepared from synchronous meiotic cultures and subjected to immunofluorescence as in A. Rec8-3HA (red) and Hop 1 (green) were detected using anti-HA (12CA5) and anti-Hop1 antibodies; DNA (blue) was stained by DAPI. (C) Chromosome morphology in $P_{\text {CLB2 }}$ PDS5 hop $1 \Delta$ cells at pachytene. Rec8-3HA (red), Sgol-9Myc (green), and DNA (blue) were detected as described in A.


Figure S2. Immunoblot analysis of Rec8 in wild-type and $P_{\text {CLB2 }}$ PDS5 cells during meiosis. Yeast cultures were induced to enter meiosis synchronously. Protein extracts were prepared at the indicated time points. Rec8 was detected with an anti-HA antibody. $\beta$-Tubulin served as a loading control. Rec8 persists in $P_{\text {CLB2 }}$ PDS5 cells because they are blocked at prophase I. (A) Protein samples were prepared on two separate blots. (B) Selected protein samples from A were prepared on the same blot. Four time points are shown from each strain. WT, wild type.


Figure S3. FACS analysis of Shase progression during meiosis. Yeast cultures were induced to undergo synchronous meiosis. Aliquots were withdrawn at the indicated times, fixed with $70 \% \mathrm{EtOH}$, stained with propidium iodide, and sorted by a cell-sorting system (FACSAria; BD). WT, wild type.


Figure S4. Zip3 is required for SC formation and Rec8 is required for LE formation. Yeast cultures were induced to undergo synchronous meiosis. Nuclear spreads were prepared for immunofluorescence after 8 h of induction. (A) Zip 1 localization in zip3 3 and zip3 $3 \Delta P_{\text {CLB2 }} P D S 5$ cells. Zip 1 (green) was detected with anti-Zipl antibody and DNA (red) stained by DAPI. Arrows indicate the polycomplex. (B) Quantification of polycomplex formation in zip3D and zip3 $P_{\text {CIB2 }}$ PDS5 cells at pachytene. At least 25 cells were counted. Error bars indicate SD. (C) Red localization in rec84 and rec $8 \Delta P_{\text {CLB2 }} P D S 5$ cells. Red 1 (green) was detected with anti-Red1 antibody and DNA (red) stained by DAPI.


Figure S5. Localization of Zipl and Sgol in meiotic cells without Spollactivity. Yeast cultures were induced to undergo synchronous meiosis. Nuclear spreads were prepared for immunofluorescence after 6 h of induction. Note that Zipl staining only partially overlaps with that of Sgol.

Table S1. Yeast strains used in this study

| Strain names | Genotype |
| :---: | :---: |
| NH144 | MATa, leu2dhisG, his4-x, ura3, lys2, hoدLYS2 MATa, leu2-k, arg4-Nsp, ura3, lys2, hodLYS2 |
| HY1276 | MATa, his4, leu2, P ClB2 PDS5::KANMX4, REC8-3HA::URA3,SGO1-9myc MATa, arg4, leu2, $P_{\text {СІв2 } 2}$ PDS5::KAN, REC8-3HA::URA3, SGO1-9myc |
| HY1276C | MATa, arg4, leu2, his4, REC8-3HA::URA3,SGO1-9MYC MATa, his4, leu2, REC8-3HA::URA3,SGO1-9MYC |
| HY1277 | MATa, his4-x, ura3, leu2, P CLB2 PDS5::KANMX4 MAT , arg4-Nsp, ura3, leu2, P ${ }_{\text {CLB2 }}$ PDS5::KANMX4 |
| HY1285 | MATa arg4, leu2, his4, REC8-3HA::URA3,SGO1-9MYC, P ClB2 PDS5::KAN, ndt80ДKAN MATa leu2, his4, REC8-3HA:: $_{\text {P }}$ URA3,SGO1-9MYC, P ClB2 $2^{\text {PDS } 5:: K A N, ~ n d+80 \triangle K A N ~}$ |
| HY1297 | MATa arg4, leu2, P ${ }_{\text {ClB2 } 2}$ PDS5::KAN, REC8-3HA::URA3,SGO1-9myc, spoll-Y135F::HB MATa leu2, $P_{\text {ClB2 } 2}$ PDS5::KAN, REC8-3HA::URA3,SGO1-9myc, spol1-Y135F::HB |
| HY1297C | MATa arg4, leu2, his4, REC8-3HA::URA3,SGO1-9myc, spol1-Y135F::HB MATa leu2, his4, REC8-3HA::URA3,spoll-Y135F::HB |
| HY1298 |  |
| HY1298C | MATa arg4-Nsp his4-x leu2 trp 1 ura3 MATa arg4-Bgl his4-B leu2 trp 1 ura3 |
| HY1299 | MATa his4, leu2, REC8-3HA::URA3,SGO1-9myc, ndt80DKAN MATa arg4, his4, leu2, REC8-3HA::URA3,SGO1-9myc, ndt80دKAN |
| HY1325 | MATa ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, P ${ }_{\text {ClB2 } 2}$ PDS5::KAN, nd+804HB, REC8-3HA::URA3/ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, P ClB2 $^{\text {PDS5::KAN, ndt80 }}$ HB, REC8-3HA::URA3 |
| HY1325C | MATa arg4, ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, ndt80AHB, REC8-3HA::URA3 MAT $\alpha$ ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, ndt80دHB, REC8-3HA::URA3 |
| HY1332 | MATa his4, ura3, leu2, P ${ }_{\text {ClB2 }}$ PDS5::KANMX4, REC8-3HA::URA3,SGO1-9myc, zip $1 \Delta$ HB MAT $\alpha$ arg4, ura3, leu2, $P_{\text {СІв } 2 P D S 5:: K A N, ~ R E C 8-3 H A:: U R A 3, ~ S G O 1-9 m y c, ~ z i p ~}^{1 \Delta H B}$ |
| HY1333 | MATa leu2, his4, REC8-3HA::URA3,SGO1-9myc, zip 1 4 HB MATa leu2, his4, REC8-3HA::URA3, SGO1-9myc, zip 1 1 HB |
| HY1391 | MATa ura3, leu2, CEN4::224lacO::CLONAT,TEL4::224lacO::CLONAT, his3::HIS3::Iacl-GFP::KAN, $P_{\text {CIB2 } 2}$ PDS5::HB MATa his3, leu2, $P_{\text {ClB2 }}$ PDS5::KANMX4, REC8-3HA::URA3, SGO1-9myc |
| HY1392 | MATa arg4, leu2, his4, REC8-3HA::URA3,SGO1-9MYC MATa ura3, leu2, CEN4::224lacO::CLONAT, TEL4::224lacO::CLONAT, his3::HIS3::lacl-GFP::KAN |
| HY1419 |  |
| HY1419C | MATa his4, ura3, leu2, rec84HB MAT arg4, ura3, leu2, rec8 ${ }^{\text {HB }}$ |
| HY1534 | MAT $\alpha$ leu2, ura3, arg4, REC8-3HA::URA3, sir2دCLONAT |
| HY1535 | MAT $\alpha$ leu2, ura3, arg4, REC8-3HA::URA3, $\mathrm{P}_{\text {CIB2 }}$ PDS5::KANMX4, sir2::CLONAT |
| HY1541 | ura3, leu2, his $4, P_{\text {cup } 1} M C D 1:: K A N, P_{\text {ClB2 } 2} P D S 5:: K A N M X 4$, rec $8 \Delta H B$ MAT $\alpha$ ura3, leu2, $P_{\text {Cup } 1 M C D ~} 1:: K A N$, $P_{\text {Cl132 } 2}$ PDS5::KANMX4, rec8 8 HB |
| HY1542 |  |
| HY1608 |  |
| HY1609 | MATa arg4, leu2, zip 1 H HB MATa arg4, ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, zip 1 4 HB |
| HY1611 | MATa arg4, ura3::tetOx224::URA3, leu2::tetR-GFP::LEU2, zip1AHB, P ${ }_{\text {CIB2 }}$ PDS5::KANMX4 MATa leu2, his4, Rec8-3HA::URA3, zip 14HB, $P_{\text {ClB2 }}$ PDS5::KANMX4 |
| HY1636 | MATa ura3, leu2, P Cup MCD $1:: K A N, P_{\text {Clв2 }}$ PDS5::KAN, rec8 8 HB, URA3 ::tetO::URA3, LEU2::tetR-GFP MAT $\alpha$ ura3, leu2, his $4, P_{\text {Cup } 1}$ MCD $1:: K A N, P_{\text {Cl\|22 }}$ PDS5::KAN, rec $8 \Delta H B$ |
| HY1637 | MATa ura3, leu2, arg4, $P_{\text {Cup } 1 \text { MCD } 1:: K A N, ~ r e c 8 \Delta H B, ~ U R A 3:: t e t O:: U R A 3, ~ L E U 2:: t e t R-G F P ~ M A T \alpha ~ u r a 3, ~ l e u 2, ~ h i s 4, ~}^{\text {, }}$ $P_{\text {Cup } 1 M C D 1:: K A N, ~ r e c 8 \Delta H B ~}^{\text {I }}$ |

