# Supporting Information

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### SI Materials and Methods

**Sporulation Conditions.** BR and S288C cells were inoculated into 5 mL YEPD medium overnight and inoculated at an $OD_{600}$ 0.2 in 50 mL YPA (S288C). When cultures reached a density of 4 to 5 × $10^7$ cells/mL, cells were washed in 2% KAC and resuspended in 50 mL 2% KAC 0.02% raffinose, and 0.01% Antifoam 204 (Sigma), pH 7.0. BR strains were inoculated directly from overnight cultures of YEPD (5 mL) to 50 mL 2% KAC + 0.01% Antifoam 204 (Sigma), pH 7.0. Cells from the BR and S288C strains were harvested at 20 to 24 h when > 50% of wild-type cells have progressed to pachytene. The *zip* mutants have similar kinetics to the wild-type strains until mid to late prophase I (1–3).

**Generation, Purification, and Validation of Hoffmann Laboratory α-Zip1 Antibody.** To generate an α-Zip1 antibody, the last 264 amino acids of Zip1 was fused to GST in plasmid pGEX-KG (pMB114/R1640) and expressed in bacteria, as described previously (1). Protein expression was induced by the addition of 0.1 mM IPTG to 2 L of BL21 cells at an $OD_{600}$ of 0.4 to 0.5. Cells were grown at 37°C, shaking at 200 rpm, and collected 3 h after induction. To extract Zip1-GST, cells were resuspended in 20 mL lysis buffer containing PBS, 150 mM NaCl, 1 mM EDTA, 2 mM DTT, 1% Triton-X-100, Roche complete EDTA-free protease inhibitor mixture, and 40 units/mL DNase. Next, 100 μg/mL lysozyme was added and cells were incubated for 30 min on ice before being lysed by sonication (two cycles of 30A for 1.5 min in 5-s intervals on ice; Ultrasonic Processor, Jencons Scientific Ltd.) After centrifugation (20 min in a Sorvall RC6, SS34 rotor, 10 K rpm, 4°C), Zip1-GST was present in the supernatant/soluble fraction. The fusion protein was bound to a 1-mL GSTTrap Fast Flow Column (GE Healthcare) equilibrated with 5 volumes of buffer A (5 mL, PBS, 150 mM NaCl, 1 mM EDTA, 2 mM DTT, 1% Triton X-100) at a flow rate of 1 mL/min. The protein was bound at a flow rate of 0.25 mL/min for 4 min, followed by washing in buffer A at a flow rate of 1 mL/min for 10 min. Zip1-GST was eluted in buffer B [PBS, 150 mM NaCl, 1 mM EDTA, 2 mM DTT, 10 mM reduced glutathione (Sigma), pH 8] at a flow rate of 1 mL/min for 10 min. After elution, the Zip1 antibody was immediately frozen at –80°C and aliquots stored at 4°C were used within 1 month. This step is important to detect the low levels of Zip1 after pachytene.

Generation of antibody to the Zip1-GST fusion protein was carried out at EUROGENTEC. The preimmune serum of 12 rabbits was screened; 8 of these recognized tubulin and proteins associated with the spindle. The Hoffmann laboratory antibody was generated in two rabbits, whose preimmune serum did not show any staining to tubulin or associated proteins in meiotic metaphase I. Furthermore, to validate that the α-Zip1 antibodies recognized Zip1 specifically, the antibody was preincubated with 5-μM Zip1-GST on ice for 30 min before adding the solution to the surface-spread nuclei. This treatment eliminated the signal in the channel detecting Zip1. The antibody was also tested against nuclear spreads prepared from *zip1*.

**Cytology and Antibodies.** Meiotic surface-spread nuclei were generated as described previously (2). For the BR, Y55, and S288C strain backgrounds, 2 to 8 mL were harvested at various time points and resuspended in 0.5 mL 2% KAC, 1 M sorbitol, 10 μL 10 mg/mL zymolyase 100 T (Seiguku Corporation), and 5 mL 1 M DTT in a round-bottomed 14-mL Falcon tube. This solution was incubated for 30 min at 30°C, or until > 50% of cells were spheroplasted. At this stage, 2 mL of a cold (4°C) solution containing 0.1 M Mes, 1 mM EDTA, 0.5 mM $MgCl_2$, and 1 M sorbitol was added to the solution and the spheroplasts were gently pelleted. The pellet was resuspended in 50 μL of 0.1 M Mes, 1 mM EDTA, and 0.5 mM $MgCl_2$, the tube gently flicked for ∼5 s, and 100 μL 4% formaldehyde (pH 8.5), prepared from paraformaldehyde, was added. The suspension was dotted onto two coverslips and 100% ethanol-treated slides were placed on top and then inverted. The slides were left to dry for 1 h at room temperature before removing the coverslip and rinsing the spreads three times with 1 mL 0.4% photoflo (KODAK). Following rinse, the slides were left for another hour to dry at room temperature.

Indirect immunocytology was carried out by incubating surface-spread nuclei with primary antibodies [guinea pig anti-GFP (1:100) (4), goat anti-GFP (Abcam ab5450-25), rabbit anti-Zip1 (1:100) (1), rabbit anti-Zip1 (1:100) generated by EUROGENTEC for the Hoffmann laboratory, mouse anti-Zip1 (1), YOL1/34 rat anti-tubulin antibody from Novus Biologicals (NB100-1639; 1:400), and mouse 9E10 anti-MYC antibody (Covance, MMS-150P, 1:100)], resuspended in 50 μL 1 part FBS: 2 parts PBS-4% BSA for 4 h at room temperature followed by 16-h incubation at 4°C in a humidity chamber. Primary antibodies were tested on negative controls where possible.

Secondary antibodies were obtained from Jackson ImmunoResearch and included FITC-conjugated donkey anti-rat IgG (712-095-153), Texas Red-conjugated donkey anti-rat IgG (712-075-153), Cy5-conjugated donkey anti-rat IgG (712-175-153), Texas Red-conjugated donkey anti-mouse IgG (715-075-151), Cy5-conjugated donkey anti-mouse IgG (715-175-151), Texas Red-conjugated donkey anti-GFP (706-075-148), Texas Red-conjugated donkey anti-guinea pig IgG (706-075-148), Cy5-conjugated donkey anti-guinea pig IgG, FITC-conjugated anti-rabbit (711-095-152), Texas Red-conjugated donkey anti-rabbit (711-075-152), and Cy5-conjugated donkey anti-rabbit (711-175-152). Secondary antibodies were used at 1:200 dilution in 50 μL of PBS-4% BSA and incubated for 2 h at room temperature in a humidity chamber in the dark. Spreads were mounted with anti-fade (1 mg/mL p-phenylenediamin in 0.1 × PBS and 90% glycerol) and DAPI (1 μg/mL mount).

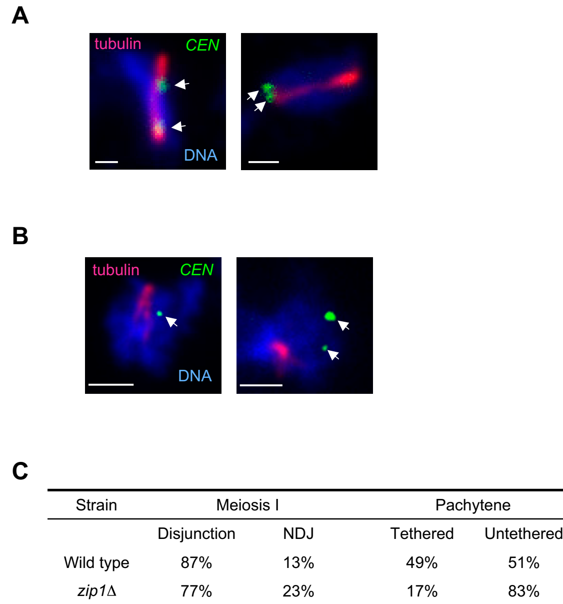
Immunofluorescence on fixed cells was carried out by adding one-tenth volume of 40% formaldehyde (Sigma) to 5 ×$10^8$ to $10^9$ cells and incubating at room temperature for 1 h. The fixation was followed by three washes in 2-mL SKP (1.2 M sorbitol, 0.05 M $KPO_4$, pH 7.0). Cells were transferred to an Eppendorf tube and the cell walls removed by adding 100 μl SKP, 1 μl DTT (1 M), and 2 μl zymolyase (100T, 10 mg/mL) and incubating cells at 37 °C for 15 to 60 min, depending upon the extent of spheroplasting. Next, 100 μl PBS (150 mM NaCl, 50 mM $NaPO_4$, pH 7.4) was added and cells were gently pelleted and resuspended in 67 μl PBS + 0.1% Nonidet P-40 by gently flicking the Eppendorf tube. This suspension was left for 30 min at room temperature followed by the addition of 33 μl FBS containing the primary antibodies. After incubation for 4 h at room temperature and overnight at 4°C, the cells were washed gently three times in PBS by resuspending (without pipetting) followed by gentle centrifugation. Next, 100 μl PBS-4%BSA containing secondary antibodies were added and incubated for 2 h at room temperature. After another three washes in PBS, cells were resuspended in a 50 μl mount containing DAPI diluted 1:2 with PBS (see above), applied to a coverslip, and a glass slide added followed by inversion. Antibody concentrations were used as for surface-spread nuclei.

FISH was carried out as described previously (2). Probes to the centromeres of chromosome *I* (*ADE1*, ORF) and *III* (*CEN3*, 114530–115044 bps on chromosome *III*) were generated as described previously (2). DAPI-stained "worm-like" spreads

were selected as pachytene to assess pairing (5). A combination of FISH with immunofluorescence was carried out by adding primary antibody against tubulin after hybridization and the stringency washes of the FISH probes. Secondary conjugated antibodies were added after three washes in PBS (see above).

Images were captured using a Deltavision IX70 system (Applied Precision) using the *softWoRx* software, and an Olympus Plan Apo 100× 1.4 numerical aperture objective lens or an Olympus Plan Apo 60× 1.4 numerical aperture lens. Emission and excitation filters for DAPI (DAPI-5060B, FF01-387/11–25 and FF409-Em02-25), FITC (FITC-3540B, FF506-Ex04-25 and FF506-Em02-25), Texas Red (TR-4040B, FF593-Ex03-25 and FF593-Em02-25), and Cy5 (Cy5-4040A, FF660-Ex03-25 and FF660-Em02-25) were obtained from Semrock. Images were captured by a CoolSnap HQ CCD camera (Roper Scientific) and deconvolved using the Constrained Iterative Deconvolution algorithm associated with *softWoRx*.


1. Sym M, Engebrecht JA, Roeder GS (1993) ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* 72:365–378.
2. Chua PR, Roeder GS (1998) Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. *Cell* 93:349–359.
3. Agarwal S, Roeder GS (2000) Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. *Cell* 102:245–255.
4. Fung JC, Rockmill B, Odell M, Roeder GS (2004) Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. *Cell* 116:795–802.
5. Stewart MN, Dawson DS (2004) Potential roles for centromere pairing in meiotic chromosome segregation. *Cell Cycle* 3:1232–1234.


| Strain | Meiosis I | | Pachytene | |
|---|---|---|---|---|
| | Disjunction | NDJ | Tethered | Untethered |
| Wild type | 87% | 13% | 49% | 51% |
| *zip1*Δ | 77% | 23% | 17% | 83% |

**Fig. S1.** Nondisjunction and centromere pairing of a doubly monosomic chromosome *I* and *III* "pair." (*A*) Representative images of disjunction (*Left*) and nondisjunction (*Right*). *CEN1* and *CEN3* (*green*; indicated by arrows) were detected by FISH with probes against *ADE1* (*CEN1*) and *CEN3*. Nuclear spreads were costained for tubulin (*red*) and DAPI (*blue*). Disjunction (*Left*) and nondisjunction (*Right*) were measured by analyzing cells that were at anaphase I (spindles longer than 4 μm) or later stages. (*B*) Examples of *zip1* cells in pachytene (9 h in sporulation medium; "bush"-like spindles in red) with tethered (*Left*) *CEN1* and *CEN3* (*green*, indicated by arrows) or untethered *CEN1* and *CEN3* (*Right*). (Scale bars, 2 μm.) (*C*) Quantification of nondisjunction (NDJ) and centromere tethering in wild-type (Y29) and *zip1* (Y119) strains. One hundred meiotic nuclear spreads were assessed per strain.

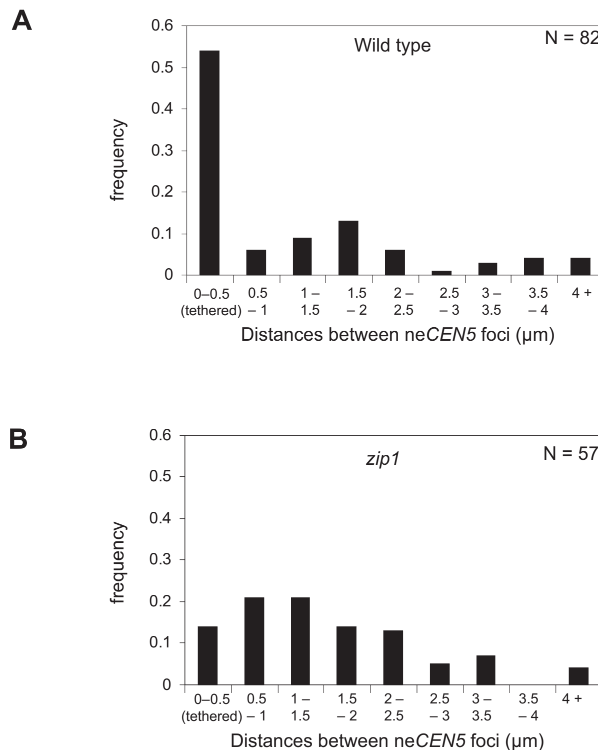



**Fig. S2.** The distribution of distances between the two ne*CEN5*s in wild-type and *zip1* strains. Meiotic spreads were prepared from wild type (Y712) (*A*) and *zip1* (Y787) (*B*) and the distances between the two ne*CEN5*s were measured and categorized as shown on the *x* axis. The frequency of each category is shown on the *y* axis. The number of meiotic spreads analyzed for each strain (N) is shown in the upper right-hand corner.

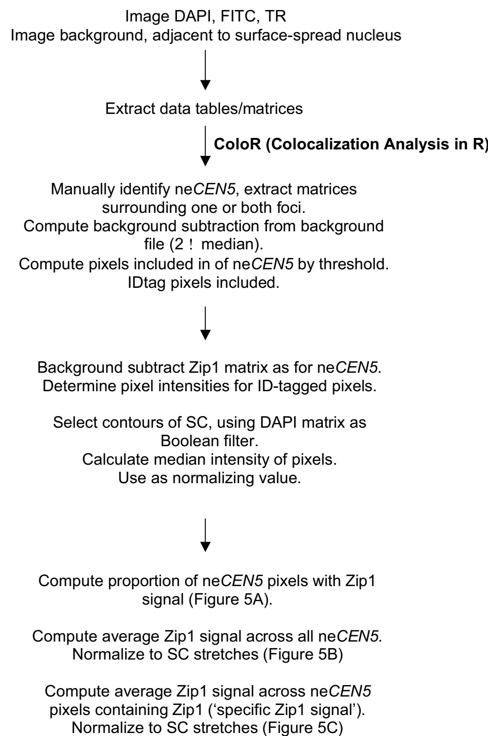



**Fig. S3.** Flow diagram of the ne*CEN5* and Zip1 colocalization analysis. Briefly, ne*CEN5* pixels were selected and the proportion of these containing Zip1 signal was quantified. Furthermore, the intensity of Zip1 was determined and normalized to the intensity of Zip1 along homologously synapsed chromosome pairs.

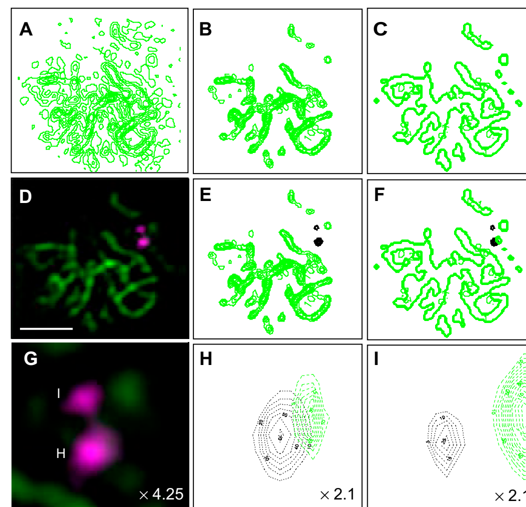



**Fig. S4.** A representative example of an image analysis. Contour of Zip1 signal (raw) (*A*), after subtraction of background (*B*), and the total area included after background subtraction (Boolean, *C*). (*D*) Corresponding image from the Deltavision IX70, with Zip1 in green and ne*CEN5* in magenta. (Scale bar, 2 μm.) (*E*) Contour image of ne*CEN5* and Zip1, and colocalization of ne*CEN5* with Zip1 positive pixels (Boolean, *F*). (*G*) Enlargement of the area containing the two ne*CEN5* foci (4.25-fold) compared to (*D*) followed by contour plots of the individual ne*CEN5* (*black*) and Zip1 (*green*) signals (*H* and *I*). Fold enlargement compared to (*G*) is shown in the right hand corner. Image: Y712_29.

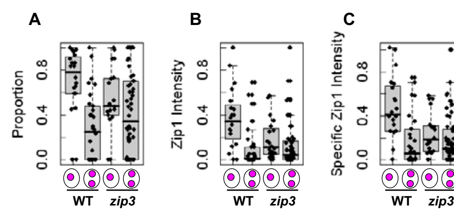



**Fig. S5.** Analysis of Zip1 colocalization to ne*CEN5* using ColoR. (*A*) The proportion of ne*CEN5* with Zip1 colocalized was determined for wild type and *zip3* according to whether a single (tethered) or two (untethered) ne*CEN5* foci were detected. The behavior of ne*CEN5* is illustrated in magenta. The proportion of each centromere containing Zip1 is shown as a black dot. These are overlaid on a Tukey-Kramer box-and-whisker plot. The gray-shaded boxes demark the interquantile range (25th to 75th percentile) and the whiskers illustrate 1.5 × the interquantile range or the maximum or minimum value, whichever is lower. (*B*) The average signal of Zip1 *per* centromere was calculated as the total Zip1 signal divided by the total area of each ne*CEN5*. This was normalized against Zip1 signal from stretches of SCs. (*C*) The specific intensity of the Zip1 signal was calculated as the total Zip1 signal divided by the number of Zip1-positive pixels from each ne*CEN5*. This value was normalized as in (*B*). Importantly, (*B*) and (*C*) yield similar results, suggesting that the Zip1 signal associated with the ne*CEN5* is generally decreased in *zip3* mutants and when the two ne*CEN5*s are untethered in the wild type.

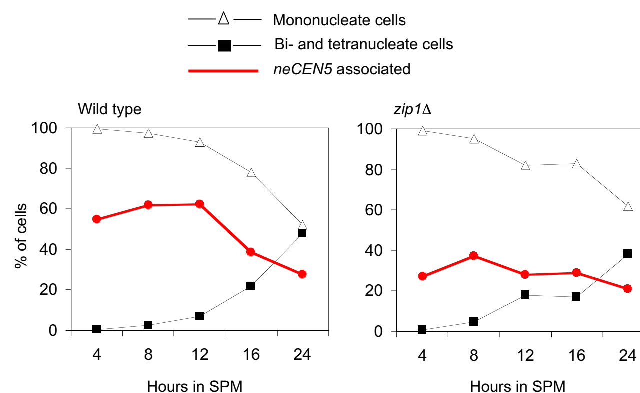



**Fig. S6.** Time-course analysis of ne*CEN5* tethering in wild-type and *zip1* strains. Cells from wild-type and *zip1* strains were taken at the indicated time points and assessed for ne*CEN5* tethering (single GFP focus). Meiotic nuclear divisions were monitored simultaneously in wild type (Y712) and *zip1* (Y787) by fixing cells with 70% ethanol and staining with DAPI to determine the proportions of mononucleate versus bi- or tetranucleate cells. Greater than 100 nuclei were scored for each time point.

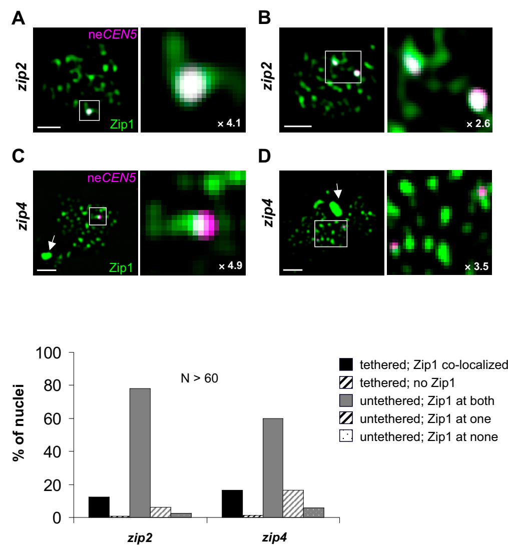



**Fig. S7.** Localization of Zip1 to ne*CEN5*s at midprophase in *zip2* and *zip4* mutants. (*A*–*D*) Meiotic nuclear spreads were stained for ne*CEN5* (*magenta*) and Zip1 (*green*) at midprophase stages. The boxed areas shown in the left panel are magnified in the right panel. Magnification is given in the lower right-hand corner. Arrows indicate polycomplexes. (*A* and *B*) Examples of *zip2* (Y813) (see Table S1) nuclei with tethered ne*CEN5*s and Zip1 colocalized (*A*) or untethered ne*CEN5*s with Zip1 also colocalized (*B*). (*C* and *D*) Examples of *zip4* (Y1133) (see Table S1) nuclei with tethered (*C*) or untethered (*D*) ne*CEN5s* and associated Zip1. (*E*) Percent of nuclei with Zip1 colocalized, according to whether ne*CEN5*s were tethered or untethered.

**Table S1. Strains used in this study**

| Strain name | Genotype |
|---|---|
| Y713/TB105 (S288C) | *MAT*a *his3 leu2,112 lys2Bgl*II *CEN5-lacO-LEU2* $P_{CYC1}$*LacI-GFP-HIS3 ilv1 (Saccharomyces carlsbergensis* chromosome *V*) (1) |
| Y714/TB109 (S288C) | *MATα his3 leu2,112 lys2Bgl*II *CEN5-lacO-LEU2* $P_{CYC1}$*LacI-GFP-HIS3 ilv1 (Saccharomyces cerevisiae* chromosome *V*) (1) |
| Y712 (S288C) | Y713 × Y714 |
| Y784 (S288C) | Y712 but *mad3Δ::NATMX4/ mad3Δ::NATMX4* |
| Y787 (S288C) | Y712 but *zip1Δ::KANMX4/zip1Δ::KANMX4* |
| Y790 (S288C) | Y712 but *msh4Δ::KANMX4/msh4Δ::KANMX4* |
| Y813 (S288C) | Y712 but *zip2Δ:: HPHMX4/ zip2Δ:: HPHMX4* |
| Y1010 (S288C) | Y712 but *zip3Δ::KANMX4/ zip3Δ::KANMX4* |
| Y1133 (S288C) | Y712 but *zip4Δ::KANMX4/ zip4Δ::KANMX4* |
| Y1155 (S288C) | Y712 but *mad3Δ::NATMX4 zip1Δ::KANMX4/ mad3Δ::NATMX4 zip1Δ::KANMX4* |
| Y2172 (S288C) | Y712 but *mer3Δ::HPHMX4/ mer3Δ:: HPHMX4* |
| Y29 (SK1) | VG61-17B × VGGALCEN (monosomic for chromosomes *I* and *III*) (2) |
| Y119 (SK1) | Y29 but *zip1Δ::KANMX4/zip1Δ::KANMX4* |
| Y636 (BR) | *MAT***a***/MATα LacO::CEN3/LacO::CEN3 LacI-GFP::URA3/LacI-GFP::URA3 ura3/ura3 CTF19-13MYC:KANMX4/CTF19-13MYC: KANMX4* (3) |

1. Cheslock PS, Kemp BJ, Boumil RM, Dawson DS (2005) The roles of *MAD1, MAD2* and *MAD3* in meiotic progression and the segregation of nonexchange chromosomes. *Nat Genet* 37: 756–760.
2. Guacci V, Kaback DB (1991) Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. *Genetics* 127:475–488.
3. Tsubouchi T, Roeder GS (2005) A synaptonemal complex protein promotes homology-independent centromere coupling. *Science* 308:870–873.

**Table S2. Meiosis I nondisjunction and centromere tethering frequencies of the nonexchange chromosome *V* pair in *zmm* mutants**

| Strain | % MI nondisjunction (SEM)[a] | % *neCEN5* tethering (pachytene)[b] |
|---|---|---|
| Wild type | 10.7 (+/− 0.9) | 55.5 (+/− 2.5) |
| *zip1Δ* | 26.5 (+/− 0.6) | 13.5 (+/− 2.5) |
| *zip2Δ* | 26.3 (+/− 1.0) | 13 (+/− 0.0) |
| *zip3Δ* | 23.8 (+/− 0.4) | 22 (+/− 1.0) |
| *zip4Δ* | 29.5 (+/− 1.8) | 18.5 (+/− 1.5) |
| *mer3Δ* | 11.2 (+/− 0.1) | 55 (+/− 2.0) |
| *msh4Δ* | 13.3 (+/− 1.8) | 50 (+/− 3.0) |

[a]Greater than 100 tetrads were assessed for nondisjunction for at least three independent experiments.
[b]Greater than 60 pachytene nuclei for tethering frequencies.