Supplementary Figure legends:

Fig. S1. Zip3-focus formation in various mutants.

(A) Quantification of numbers of Zip3 foci was carried out in the *rad24 sml1* mutant as well as wild type and the *rad24* mutant. For each focus-positive chromosome spread, the numbers of Zip3 foci were counted and plotted as shown. The size of each circle represents the number of nuclei with each focus number (i.e., the sizes of circles are proportional to the numbers of nuclei with a given focus number). An average number of foci per nucleus are shown in red (top), and also as a red bar in the graph. Standard deviations of focus numbers are shown in parentheses. "N" at the top represents the number of nuclei analyzed for counting.

(B) Quantification of numbers of Zip3 foci was carried out in the *rad24 rad51* mutant as well as wild type, the *rad51* and the *rad24* mutant.

Fig. S2. Specificity of anti-Rad17 antiserum.

(A) Immunostaining analysis of chromosome spreads of the *MEC3-HA* cells. The spread was stained with anti-HA antibody (green). A representative image at 4 hr is shown. White bar; 4 μm.

(B) Immunostaining analysis of chromosome spreads of the *rad17* deletion mutant was performed using anti-Rad17 antiserum (green). As a control, the spread was costained with anti-Zip1 (red). White bar; 4 μm.

(C) Chromosome spreads from *MEC3-HA* diploids were stained with anti-HA (green), anti-Rad51 (red), and anti-Dmc1 (blue) antibodies. In addition to three-color and mono-color, different two-color combinations are shown. A representative image at 4 hr is shown. Scale bar (white): 4 μm.

(D) Colocalization frequencies of Rad51 (green) with Zip1-polycomplex (red) in the *spo11-Y135F* mutant. Immunostaining analysis of chromosome spreads for Zip1 and Rad51 were carried out in the *spo11-Y135F* mutant as described in Materials and Methods. A representative image at 4 hr is shown. White bar; 4 μm.

Fig. S3. Interaction of Zip3 with 9-1-1 clamp.

Pull-down assay of FATT(-Myc)-Zip3. *E. coli* lysates expressing either FATT-Zip3 or FATT-GFP were incubated with magnetic beads coated with anti-c-Myc antibodies. The beads (Input) were incubated with yeast meiotic cell lysates (at 4 hr) from wild type and *mec3* deletion. The beads were recovered, and eluates were analyzed by western blotting using anti-Mec3, anti-c-Myc, anti-Flag, or anti-HA antibodies. Anti-Flag can detect both Ddc1-Flag and FATT-Zip3-Flag. * indicates a non-specific band.

Fig. S4. Interhomolog bias in the *rad24* mutant.

(A) Schematic representation of the *HIS4-LEU2* recombination hotspot for the analysis of recombination intermediates: single-end invasion (SEI) and interhomolog (IH; red) and intersister (IS; green) double-Holliday junctions (dHJs). Sizes of relevant JM-containing fragments are indicated.

(B) Southern blotting of two-dimensional gel electrophoresis for recombination intermediates. A schematic representation for a typical gel is shown on the left. DNAs from the wild type (4 hr) and the *rad24* mutant (5 hr) were analyzed as described in the Materials and Methods. This is an independent analysis of Fig. 5.

(C) Kinetics of IH-dHJ, IS-dHJ, and the ratio of IH/IS dHJs are shown at the bottom. Wild type, open circles; the *rad24* mutant, closed circles.

Table S1. Strain list

Yeast strains used in this study.

Figure S1. Miki Shinohara

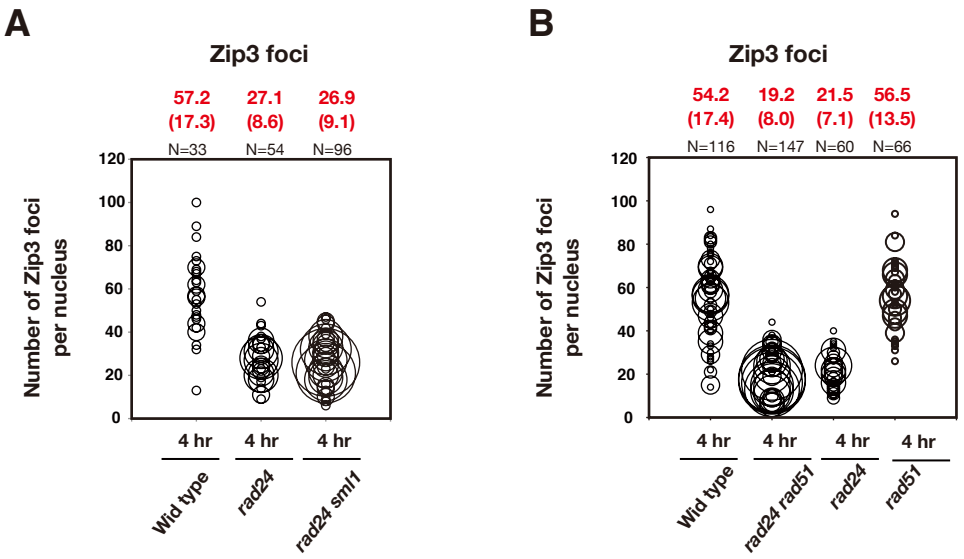

Figure S2. Miki Shinohara

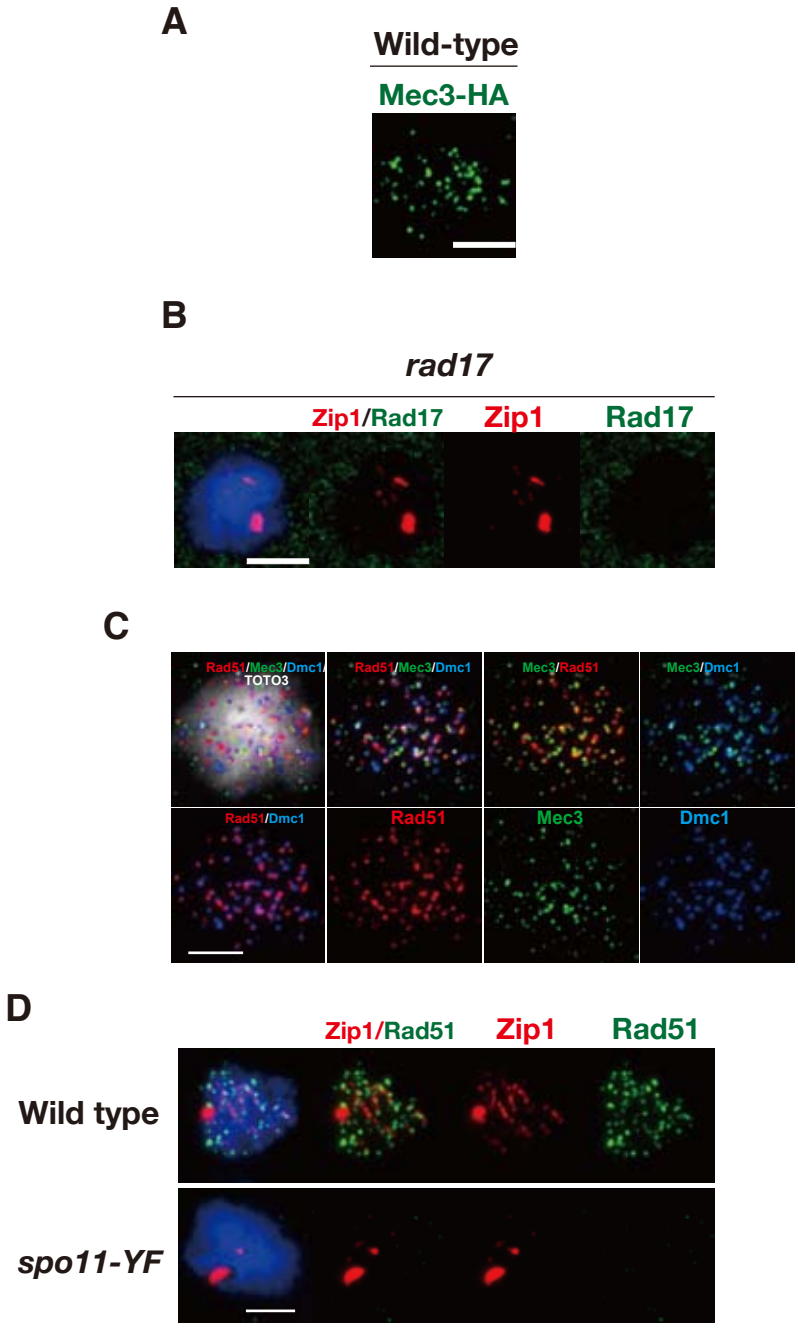

Figure S3. Miki Shinohara

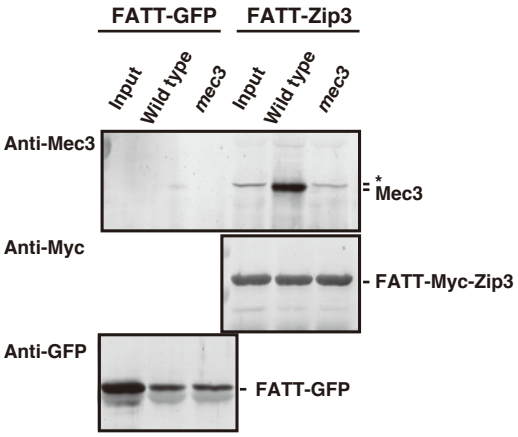

Figure S4. Miki Shinohara

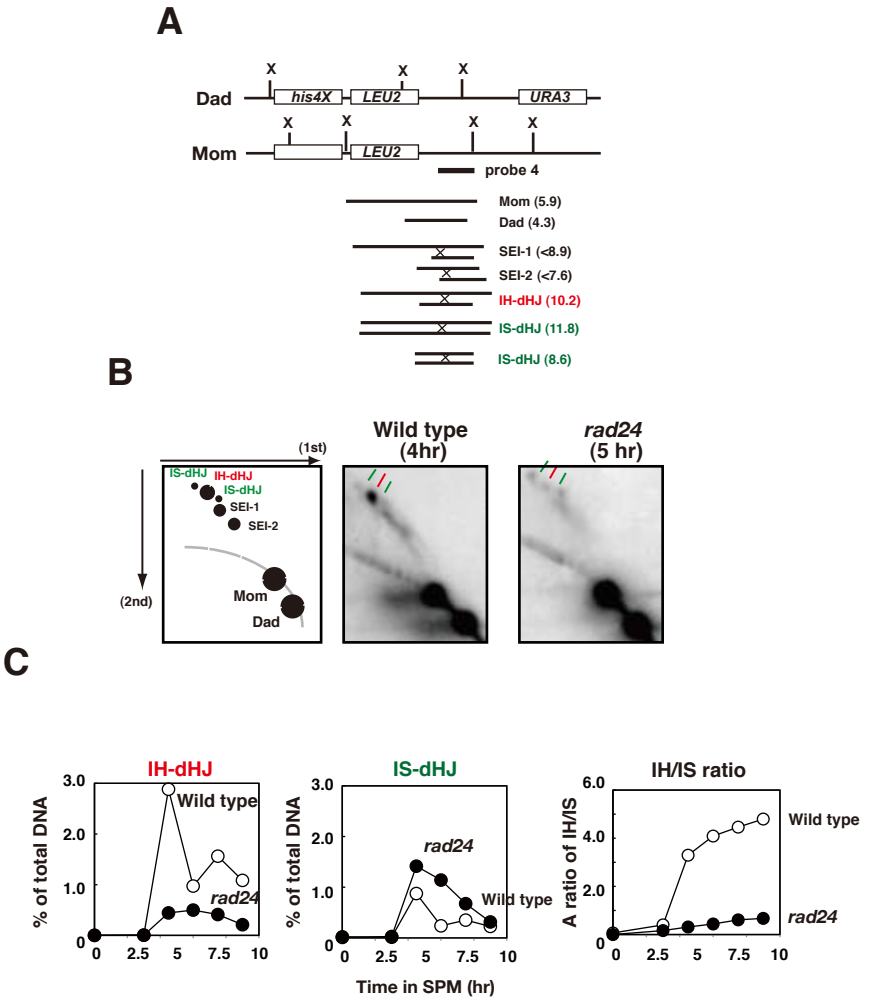

Table S1. Strain list

NKY1551	*MAT**a*** /α, *ho::LYS2/", lys2/", ura3/", leu2::hisG/", his4X-LEU2(BamHI)-URA3/his4B-LEU2(MluI), arg4-nsp/arg4-bgl*
	NKY1551 with *rad24::LEU2*
MSY717	NKY1551 with *zip1::LEU2*
MSY2820	NKY1551 with *mec3::LEU2*
MSY3967	NKY1551 with *ddc1::LEU2*
MSY3969	NKY1551 with *rad17::hisG*
MSY587	NKY1551 with *mec1::LEU2, sml1::KanMX6*
MSY3687	NKY1551 with *sml1::KanMX6*
MSY3699	NKY1551 with *spo11-Y135F::KanMX4*
MSY1737	NKY1551 with *rad50-K18I::URA3*
MSY1758	NKY1551 with *rad51::hisG-URA3-hisG*
MSY2746	NKY1551 with *rad52::hisG-URA3-hisG*
MSY2777	NKY1551 with *trp1::hisG/"*
MSY845/846	*MAT**a*** /α, *ho::LYS2/", lys2/", ura3/", leu2::hisG/", trp1::hisG/"*
MSY831/833	MSY831/833 with *ndt80::LEU2*
MSY5137	MSY831/833 with *ndt80::LEU2, rad24::LEU2*
MSY5123	MSY831/833 with*, rad24::HygMX6, sml1::KanMX6*
MSY5357/5358	MSY845/MSY846 with *msh4::TRP1*
MSY2987	MSY845/MSY846 with *msh5::TRP1*
MSY3935	MSY845/MSY846 with *spo22/zip4::TRP1*
MSY3162	MSY831/833 with *MEC3-3HA::KanMX4*
MSY2925	MSY831/833 with *MEC3-3HA::KanMX4, rad17::hisG*
MSY2989	MSY831/833 with *DDC1-3FLAG::KanMX4*
MSY3805	NKY1551 with *DDC1-3FLAG::KanMX4/"*
GTY82 KHY235	NKY1551 with *DDC1-3FLAG::KanMX4/", RAD17-3HA::KanMX4/"*