Supplemental material

JCB

Bohr et al., http://www.jcb.org/cgi/content/full/jcb.201409035/DC1

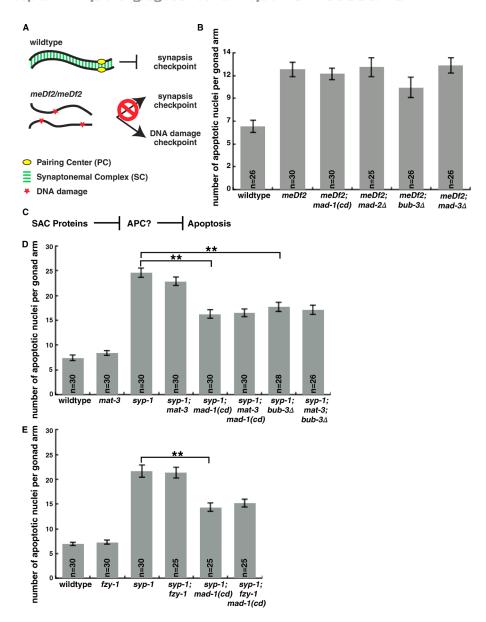


Figure S1. SAC components MAD-1, MAD-2, and BUB-3 are not required for the DNA damage checkpoint, and loss of APC components does not affect the synapsis checkpoint. (A) DNA damage checkpoint response in meDf2 homozygotes. (B) Mutations in mad-1, mad-2, or bub-3 do not affect apoptosis in meDf2 homozygotes. (C) Schematic of the possible role of the APC in the synapsis checkpoint. (D) Mutation of mat-3 does not affect germline apoptosis in syp-1, syp-1;mad-1(cd), or $syp-1;bub-3\Delta$ mutants. (E) Mutation of fzy-1 does not affect germline apoptosis in syp-1 or syp-1;mad-1(cd) mutants. Error bars represent \pm SEM. **, P < 0.0001.

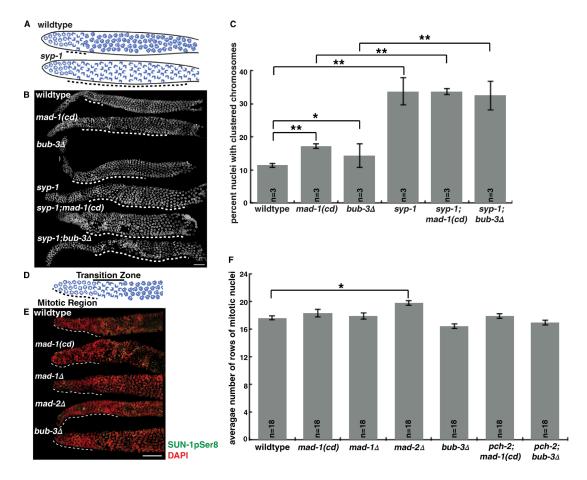


Figure S2. Loss of MAD-1 or BUB-3 does not affect meiotic entry or meiotic progression. (A) Cartoon depicts wild-type and syp-1 germlines. (B) Images of wild-type, mad-1(cd), $bub-3\Delta$, syp-1, syp-1; mad-1(cd), and syp-1; $bub-3\Delta$ germlines stained with DAPI. Regions of clustered chromosomes are indicated by dashed lines. (C) Mutation of mad-1 and bub-3 results in slightly more nuclei with clustered chromosomes than wild-type germlines but does not reduce the percentage of nuclei with clustered chromosomes in syp-1 mutants. (D) Cartoon depicts mitotic and early meiotic region (transition zone) of the germline. (E) Images of the mitotic and early meiotic region of germlines in wild-type, mad-1(cd), $mad-1\Delta$, $mad-2\Delta$, and $bub-3\Delta$ mutants stained with DAPI and an antibody against SUN-1 pSer8. The length of the mitotic region is indicated by dashed lines. (F) $mad-2\Delta$ delays meiotic entry, whereas mad-1(cd), $mad-1\Delta$, $bub-3\Delta$, pch-2; mad-1 (cd), and pch-2; $bub-3\Delta$ double mutants do not affect meiotic entry. Bars, 30 μ m. Error bars represent \pm SEM. *, μ 0.001; **, μ 0.0001.

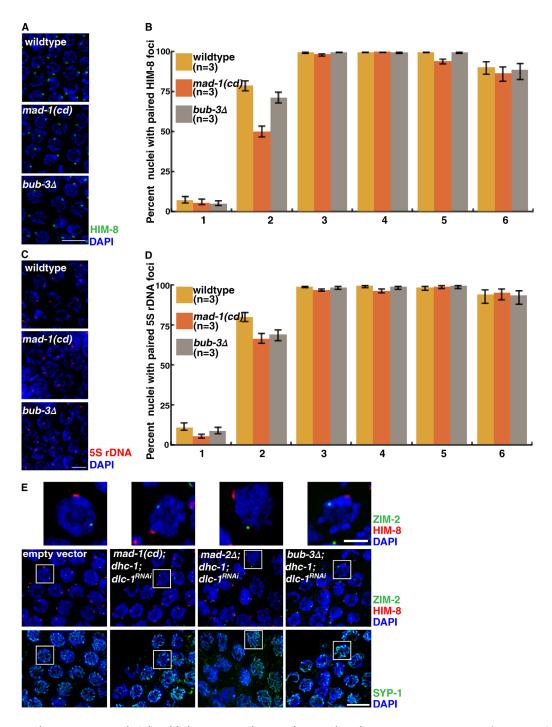


Figure S3. **Meiotic chromosomes in mad-1(cd) and bub-3Δ mutants do not undergo nonhomologous synapsis.** (A) Images of meiotic nuclei in wild-type worms and mad-1(cd) and bub-3Δ mutants stained to visualize DNA (blue) and HIM-8. (B) The X chromosome PC pairs similarly in wild-type worms and in mad-1(cd) and bub-3Δ mutants. (C) Images of meiotic nuclei in wild-type worms and mad-1(cd) and bub-3Δ mutant worms stained to visualize DNA (blue) and the 5S rDNA locus. (D) The 5S rDNA locus pairs similarly in wild-type worms and in mad-1(cd) and bub-3Δ mutants. (E) Synapsis is homologous in mad-1(cd);dhc-1;dlc-1^{RNAi}, mad-2Δ;dhc-1;dlc-1^{RNAi}, and bub-3Δ;dhc-1;dlc-1^{RNAi} mutants. Images of meiotic nuclei in wild-type, mad-1(cd);dhc-1;dlc-1^{RNAi} mutants germlines stained to visualize DNA (blue), the autosomal PC protein ZIM-2, and the X chromosome PC protein HIM-8. Also depicted are the same nuclei stained with SYP-1 to verify the presence of synapsed chromosomes. White boxes outline individual fully synapsed nuclei that have paired HIM-8 and ZIM-2 signals. These nuclei are depicted as enlarged images in the top panels. Bars: (A, C, and E [bottom]) 5 μm; (E, top) 2 μm. Error bars represent 95% confidence intervals.

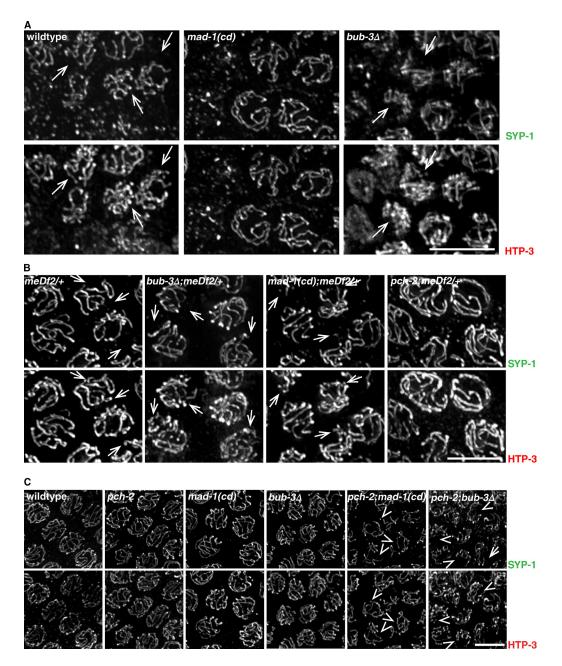


Figure S4. **Grayscale images of Fig. 2 (C and F) and Fig. 4 B.** (A) Images of nuclei during synapsis initiation in wild-type worms and mad-1(cd) and $bub-3\Delta$ mutants stained to visualize SYP-1 (top; green in Fig. 2 C) and HTP-3 (bottom; red in Fig. 2 C). (B) Images of meiotic nuclei in meDf2/+, mad-1(c-d); meDf2/+, $bub-3\Delta$; meDf2/+, and pch-2; meDf2/+ mutants stained to visualize SYP-1 (top; green in Fig. 2 F) and HTP-3 (bottom; red in Fig. 2 F). (C) Images of nuclei in wild-type worms and pch-2, mad-1(cd), $bub-3\Delta$, pch-2; mad-1(cd), and pch-2; $bub-3\Delta$ mutants stained to visualize SYP-1 (top; green in Fig. 4 B) and HTP-3 (bottom; red in Fig. 4 B). Arrows/arrowheads indicate unsynapsed chromosomes. Bars, 5 μ m.