

Figure S1. Progression of germ cells in Wcd-RFP germarium.

Confocal Z-section projections of germaria from wild type (A) and bam-GFP; nos>wcd-RFP (B) stained for ORB (white). Note that ORB localization in the meiotic region and in stage 2 is similar in both genotypes. Scale bar; 20 μ m

Confocal Z-section projections of germaria from wild type (C) and bam-GFP; nos>wcd-RFP (D) stained for C(3)G (green) and DNA (DAPI, blue). Note that C(3)G is restricted to the selected oocyte (oocyte I) in meiotic region 3 and stage 2 in both genotypes (arrowheads, C and D), but the synaptonemal complex is not completely resolved in the second pro-oocyte (pro-oocyte II) in nos>wcd-RFP (open arrowheads, D). Scale bar; 20 μ m

(E) Graph plots C(3)G Fluorescence Intensity in the mitotic region and in the meiotic region 2 (WT, green, nos>wcd-RFP, magenta), meiotic region 3 and stage 2 (WT pro-oocyte I, green, WT pro-oocyte II, blue, nos>wcd-RFP pro-oocyte I, magenta and nos>wcd-RFP pro-oocyte II, grey). NS $p \geq 0.05$, * $p \leq 0.05$ (Mann-Whitney U-test). Number of samples analysed are below the bars.

(F) Graph plots the number of cysts in region 2 in wild type and bam-GFP, nos>wcd-RFP calculated using spectrin antibody labelling (not shown). NS $p \geq 0.05$ (Mann-Whitney U-test). (n) is the number of germarium analyzed for each genotype.

Figure S2. *mei-W68* mRNA signal is detected above background levels in the meiotic and mitotic regions.

(A, A') Confocal Z-section projection of a *WT* germarium labelled for *mei-W68* mRNA by HCR *in situ* hybridization using *mei-W68* HCR-initiator probes (A, green; A', white). The yellow dashed line delimits the boundary of mitotic and meiotic regions. α -Spectrin antibody labelling is in magenta. Scale bar: 10 μ m.

(B, B') Confocal Z-section projection of a *WT* germarium labelled by HCR *in situ* hybridization but excluding the HCR-initiator probes (compare with A, green; A', white). The yellow dashed line delimits the boundary of mitotic and meiotic regions. α -Spectrin antibody labelling is in magenta. Scale bar: 10 μ m.

(C) Graph plots the Fluorescence Intensity in the mitotic region of WT germarium labelled by HCR *in situ* hybridization without (white) and with *mei-W68* HCR-initiator probes (green). (n) is the number of germarium analyzed for each genotype.* $p \leq 0.05$ (Mann-Whitney U-test)

(D) Graph plots the Fluorescence Intensity in meiotic region of WT germarium labelled by HCR *in situ* hybridization without (white) and with mei-W68 HCR-initiator probes (green). (*n*) is the number of germarium analyzed for each genotype. **** $p \leq 0.0001$ (Mann-Whitney U-test).

Figure S3. *c(3)G*, *Nipped-B* and *mei-W68* mRNA expression in the meiotic region of *Wcd-RFP* germarium.

(A, B) Confocal Z-section projection of a *WT* (A) and *nos>wcd-RFP* (B) germarium labelled for *c(3)G* mRNA by HCR *in situ* hybridization (white). The yellow dashed line delimits the boundary of mitotic and meiotic regions. Scale bar: 10 μ m. (C) Graph plots *mei-W68* mRNA Fluorescence Intensity in the meiotic region of WT (green) and *nos>wcd-RFP* (magenta) germarium labelled by HCR *in situ* hybridization. (*n*) is the number of germarium analyzed for each genotype. NS $p \geq 0.05$ (Mann-Whitney U-test).

(D, E) Confocal Z-section projection of a *WT* (D) and *nos>wcd-RFP* (E) germarium labelled for *Nipped-B* mRNA by HCR *in situ* hybridization (white). The yellow dashed line delimits the boundary of mitotic and meiotic regions. Scale bar: 10 μ m. (F) Graph plots the *Nipped-B* mRNA Fluorescence Intensity in meiotic region of WT (green) and *nos>wcd-RFP* (magenta) germarium labelled by HCR *in situ* hybridization. (*n*) is the number of germarium analyzed for each genotype. NS $p \geq 0.05$ (Mann-Whitney U-test).

(G, H) Confocal Z-section projection of a *WT* (G) and *nos>wcd-RFP* (H) germarium labelled for *mei-W68* mRNA by HCR *in situ* hybridization (white). The yellow dashed line delimits the boundary of mitotic and meiotic regions. Scale bar: 10 μ m. (I) Graph plots the *mei-W68* mRNA Fluorescence Intensity in the meiotic region of WT (green) and *nos>wcd-RFP* (magenta) germarium labelled by HCR *in situ* hybridization. (*n*) is the number of germarium analyzed for each genotype. NS $p \geq 0.05$ (Mann-Whitney U-test).

Figure S4 Mei-W68 reagents

(A) Schematic representation of *Drosophila melanogaster mei-W68* locus showing neighbouring genes CG7744 and *par-1* (top in grey). Enlargement of *mei-W68* and *par-1* RNA showing introns (black acute lines for *mei-W68* and dashed lines for *par-1*), untranslated (magenta box) and translated regions (green box). Triangle represents *mei-W68^l* insertion site of approximately 5kb (McKim and Hayashi-Hagihara). Positions of set of primers A, B and C used for RT-PCR are indicated.

(B) RT-PCR gene expression levels of *mei-W68^{1/DfBSC782}* using primers A, B relative to *WT*.
 (C) Representation of Mei-W68 catalytic (magenta box) and TOPRIM domains (blue box).
 Mei-W68^{CD} is a substitution in the catalytic domain of the two conserved Tyrosine (Y80Y81)
 into Phenylalanine (*F80*F81). Mei-W68^{HA} is tagged at the C-terminus with HA (green box)
 connected by a linker to His (yellow box)

Figure S5. Absence of DSBs in Mei-W68^{HA} flies

Confocal Z-section projections of wild type (A, A'), *mei-W68^{HA}/+* (B, B') and *mei-W68^{HA}/mei-W68^{HA}* (C, C') germaria stained for DSBs (γ-H2Av, magenta), fusome (α-Spectrin, green) and DNA (DAPI, blue). The yellow dashed line delimits the boundary of mitotic and meiotic regions. Note that DSBs are absent in *mei-W68^{HA}/mei-W68^{HA}*. Scale bar; 10 μm

Figure S6. Mei-W68 and Mei-P22 mutant flies do not produce DSBs

Confocal Z-section projections of wild type (A, A'), *mei-W68^{1/DfBSC782}* (B, B'), *mei-W68^{CD/CD}* (C, C') and *mei-P22^{P22/P22}* (D, D') germaria stained for DSBs (γ-H2Av, magenta), fusome (α-Spectrin, green) and DNA (DAPI, blue). The yellow dashed line delimits the boundary of mitotic and meiotic regions. Note that DSBs are absent in all the mutants. Scale bar; 10 μm

Figure S7. RPA::GFP foci transiently overlap with γ-H2Av.

(A-A'') Confocal Z-section projection of *RpA-70 EGFP* pro-oocyte stained for DSBs (γ-H2Av, A, A'', magenta) and DNA (DAPI, A'', blue). GFP is in green (A', A''). Scale bar= 10 μm.

(B) Line profile plots the normalized intensity for γ-H2Av (magenta) and RPA::GFP (green) from A'' (yellow dashed line). The RPA::GFP peak partially overlaps with the γ-H2Av peak on the right side, but not with the γ-H2Av on the left side.

(C-C'') Confocal Z-section projection of *RpA-70 EGFP; spn-D²* pro-oocyte stained for DSBs (γ-H2Av, C, C'', magenta) and DNA (DAPI, C'', blue). GFP is in green (C'', C''). Scale bar= 10 μm.

(D) Line profile plots the normalized intensity for γ-H2Av (magenta) and RPA::GFP (green) from C'' (yellow dashed line). The two RPA::GFP peaks overlap with the two γ-H2Av peaks.

(E) Percentages of γ-H2Av overlapping with RPA::GFP (magenta) and RPA::GFP overlapping with γ-H2Av (green) in *RpA-70 EGFP* and *RpA-70 EGFP; spn-D²* pro-oocytes. The number of analyzed nuclei is indicated under each genotype.

99 **Movie S1:** Live-imaging of RPA::GFP in wild type germarium. Maximum intensity
100 projection. (1 frame each 3 mn).
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102 **Movie S2:** Live-imaging of RPA::GFP in *spn-D²* mutant germarium. Maximum intensity
103 projection. (1 frame each 3 mn).
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