

Figure S1. Depletion of P180 in germ cells induces a decrease of H3:RFP incorporation into chromatin and a concomitant downregulation of CAF-1 median subunit P105.

(A-B) Control (A) and P180-depleted (B) germaria carrying one copy of a *H3:RFP* transgene (RFP fluorescence in red and A'-B'), with DNA staining (blue). (C-D) Control (C) and P180-depleted (D) germaria (depletion induced for four days at the adult stage), with P105 (red and C'-D') and DNA (blue) stainings. A close-up view of the framed GSCs is shown in the upper right corner of each panel. Scale bars: 15μm.

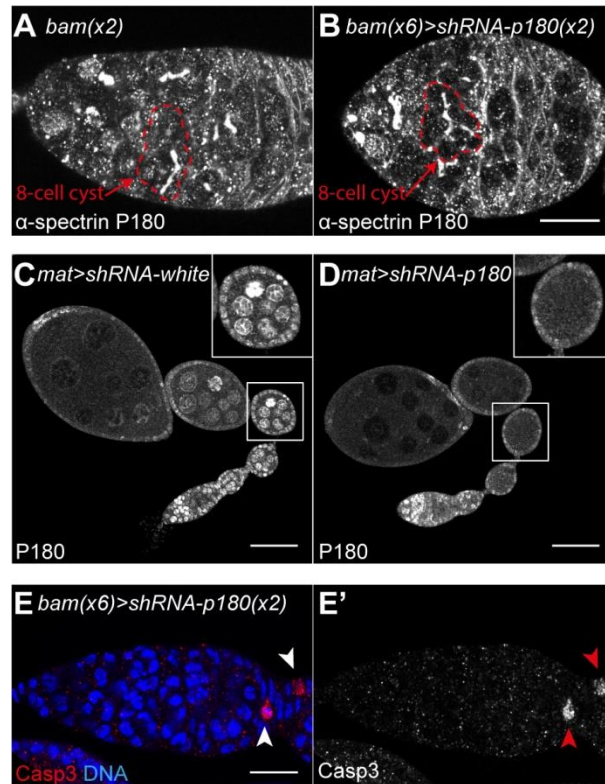


Figure S2. Depletion of P180 in more differentiated germ cells does not arrest oogenesis and does not induce apoptosis in germ cells.

(A-B) P180 together with α -spectrin stainings in a control germarium (A) and in a germarium expressing a shRNA against *p180* under the control of *bam-gal4* (B). The number in brackets indicates the number of copies of the corresponding transgene. Arrows point to 8-cell cysts either with P180 protein (A) or depleted of P180 protein (B). (C-D) P180 staining in a control ovariole (C) and in an ovariole expressing a shRNA against *p180* under the control of *mat-gal4* (D). (E) Cleaved caspase 3 (Casp3, red and E') and DNA (blue) stainings in a germarium expressing a shRNA against *p180* under the control of 6 copies of *bam-gal4*. Arrowheads point to two apoptotic somatic cells (Casp3 positive), validating the efficiency of the staining. Scale bars: 15 μ m (B and E) and 50 μ m (C-D).

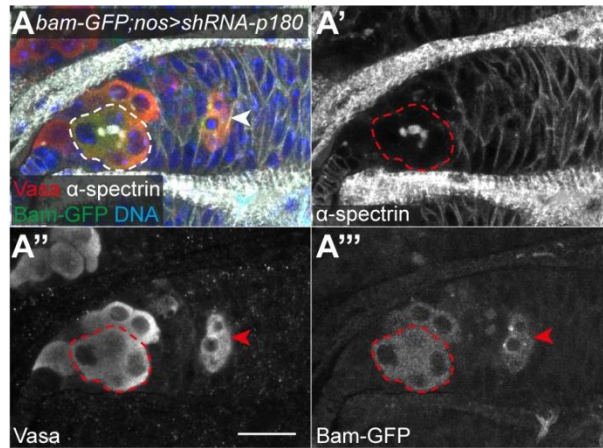


Figure S3. P180-depleted germ cells are able to initiate differentiation.

(A) α -spectrin (white and A'), vasa (red and A''), and DNA (blue) stainings in a gerarium expressing a shRNA against *p180* under the control of *nanos-gal4* together with the *bam-GFP* transgene (GFP fluorescence in green and A'''). The dotted lines demarcate a 4-cell cyst expressing Bam-GFP and Vasa. The arrowhead in A, A'' and A''' shows a group of dying (based on the presence of characteristic pyknotic nuclei visualized with DAPI staining) Vasa positive germ cells expressing Bam-GFP. The image is a maximum intensity projection of several confocal images. Scale bar: 15 μ m.

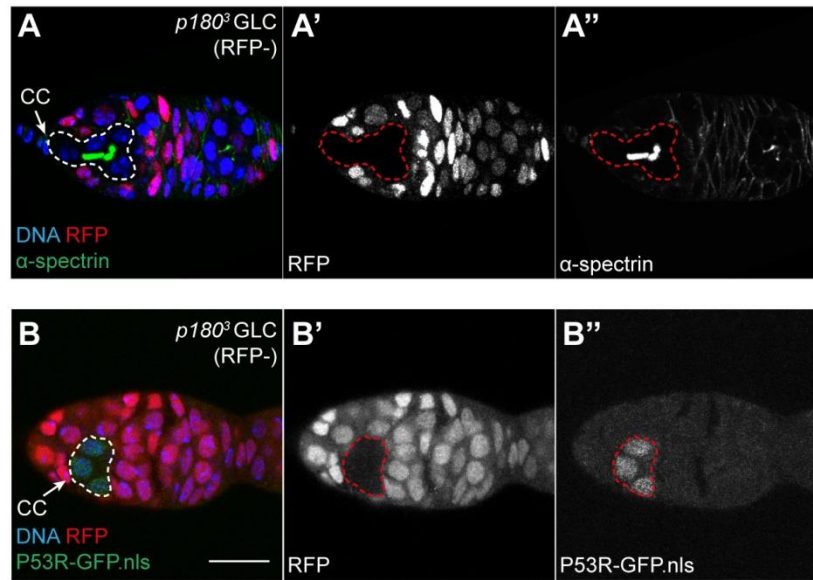


Figure S4. *p180*³ GLC exhibit branched fusomes originating from GSCs and show P53 activation.

(A-B) Germaria containing *p180*³ germline clones (GLC, dotted lines), identified by the absence of RFP fluorescence (A'-B') combined with a positive DNA staining (blue). The germarium in (A) was stained with an antibody against α-spectrin (green and A'') and the one in (B) contains a *p53R-GFP.nls* transgene (GFP fluorescence in green and B''). Cap cells (CC) are indicated. Scale bar: 15μm.

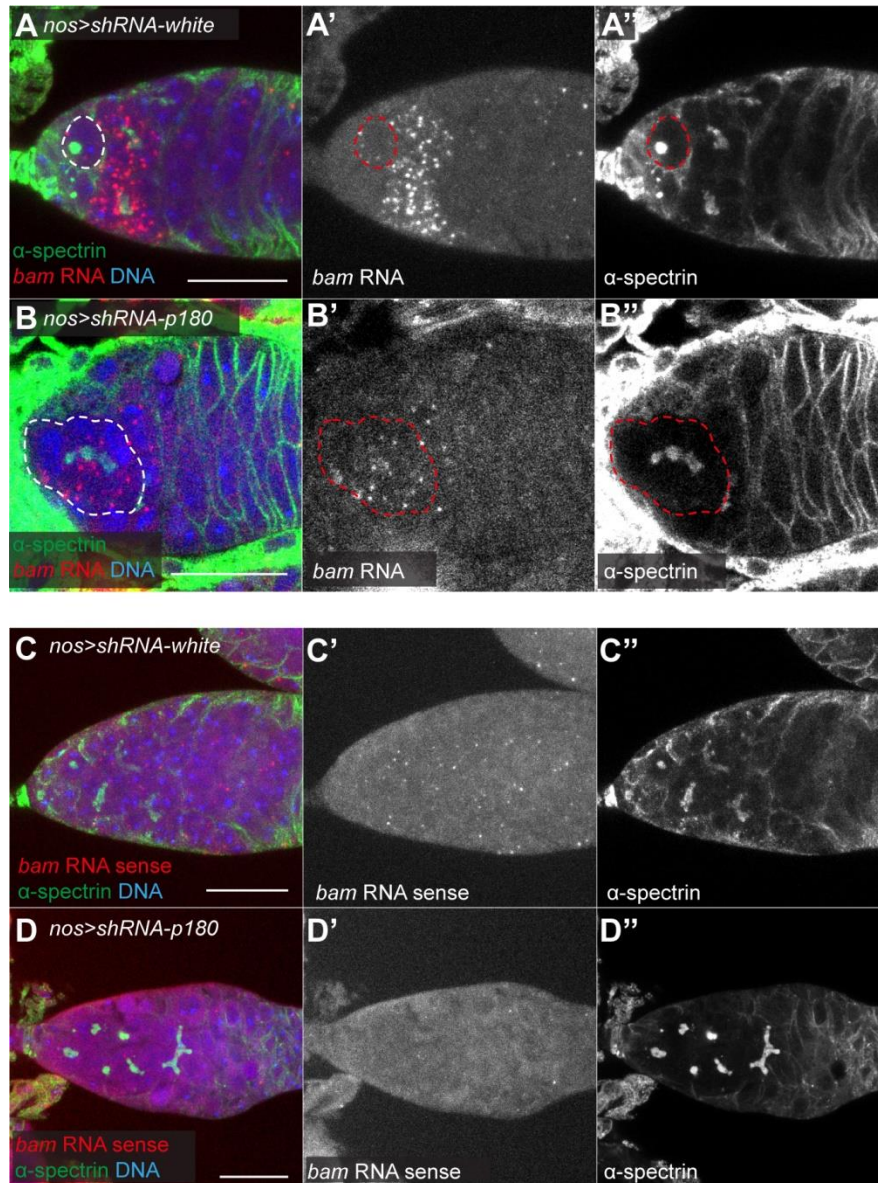


Figure S5: *bam* RNA accumulates in P180-depleted stem-cysts.

(A-B) Control (A) and P180-depleted (B) germaria, with α -spectrin (green and A''-B''), *bam* RNA (detected by RNA-FISH with an antisense probe, red and A'-B') and DNA (blue) stainings. Dotted lines delineate either a single GSC (A) or a stem-cyst (B). (C-D) Control (C) and P180-depleted (D) germaria, with α -spectrin (green and C''-D''), *bam* RNA sense probe (red and C'-D') and DNA (blue) stainings. Note that the *bam* RNA sense probe does not provide a specific signal, showing that the signal observed in A and B with the antisense probe is specific for *bam* RNA. Scale bars: 15 μ m.

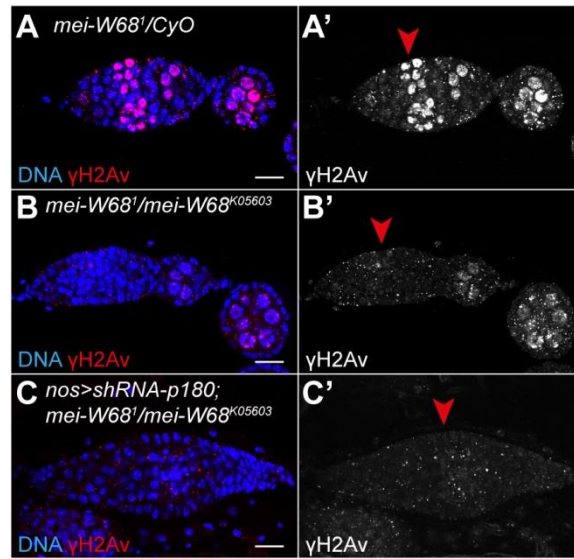


Figure S6. Suppression of programmed meiotic DSBs does not rescue the oogenesis arrest induced by depletion of P180 in germ cells.

(A-C) Control (A), *mei-W68^l/mei-W68^{K05603}* mutant (B) and P180-depleted *mei-W68^l/mei-W68^{K05603}* mutant (C) germaria with DNA (blue) and γ H2Av (red, A'-C') stainings. Arrowheads point to the meiotic region of germaria where meiotic DSBs normally accumulate. Scale bars: 15 μ m.

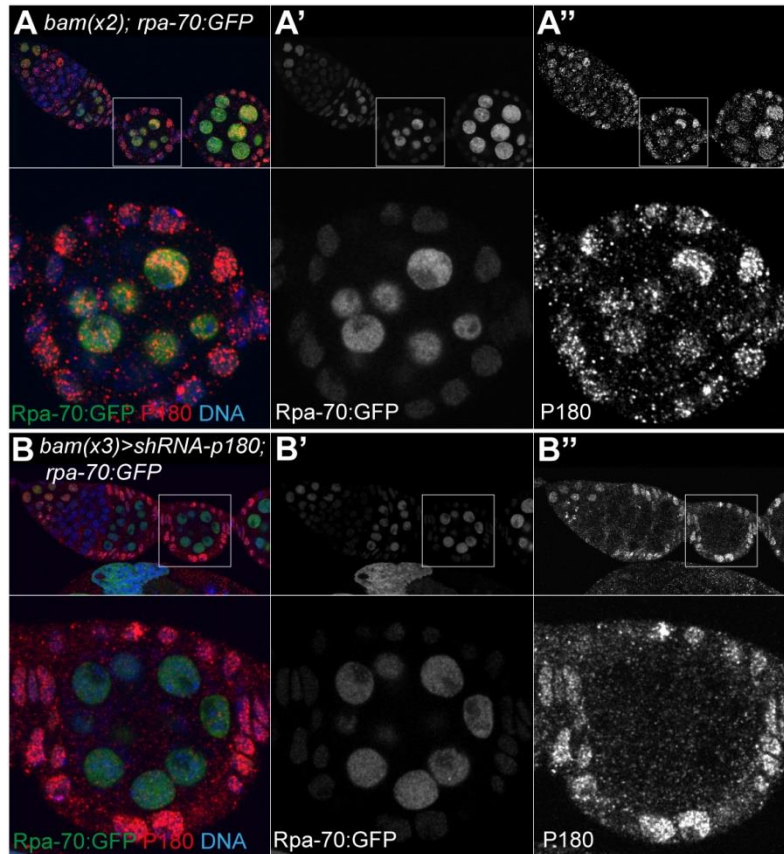


Figure S7. Depletion of P180 at later stages of germ cell development does not induce an accumulation of Rpa-70:GFP foci.

(A-B) Egg chambers from control flies (A) and flies expressing a shRNA targeting p180 under the control of 3 copies of *bam-gal4* (B) together with the *rpa-70:GFP* transgene (GFP fluorescence in green and A'-B'), with P180 (red and A''-B'') and DNA (blue) stainings. Close-up views of the framed egg chambers are shown below each panel.

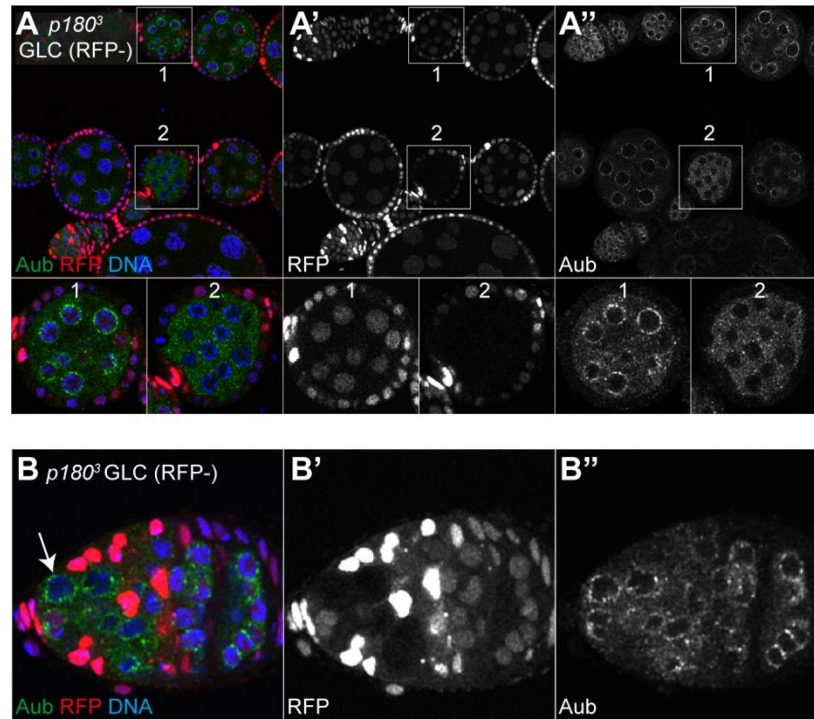


Figure S8. P180 is required for the “nuage” formation in female germline egg chambers.

(A-B) *p180*³ mutant germline clones (GLC) identified by the absence of RFP fluorescence (A' and B'), with Aubergine (Aub, green and A''-B'') and DNA (blue) stainings. Close-up views in A show control (1) and *p180*³ mutant (2) egg chambers in which the “nuage” is altered. The white arrow in B shows a *p180*³ mutant GSC in the germarium, in which the “nuage” is intact.

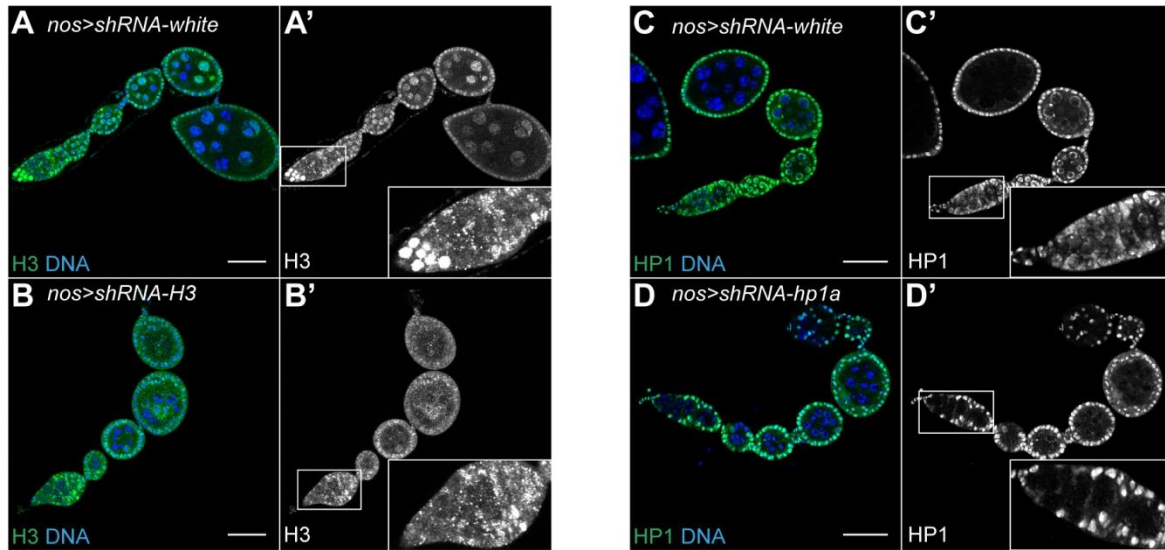


Figure S9. Histone H3 and HP1a are efficiently depleted in germ cells upon expression of cognate targeting shRNAs under the control of *nanos-gal4*.

(A-B) Control (A) and H3-depleted (B) ovarioles with DNA (blue) and histone H3 (green, A'-B') stainings. Scale bars: 50μm. (C-D) Control (C) and HP1a-depleted (D) ovarioles with DNA (blue) and HP1 (green, C'-D') stainings. Scale bars: 50μm.