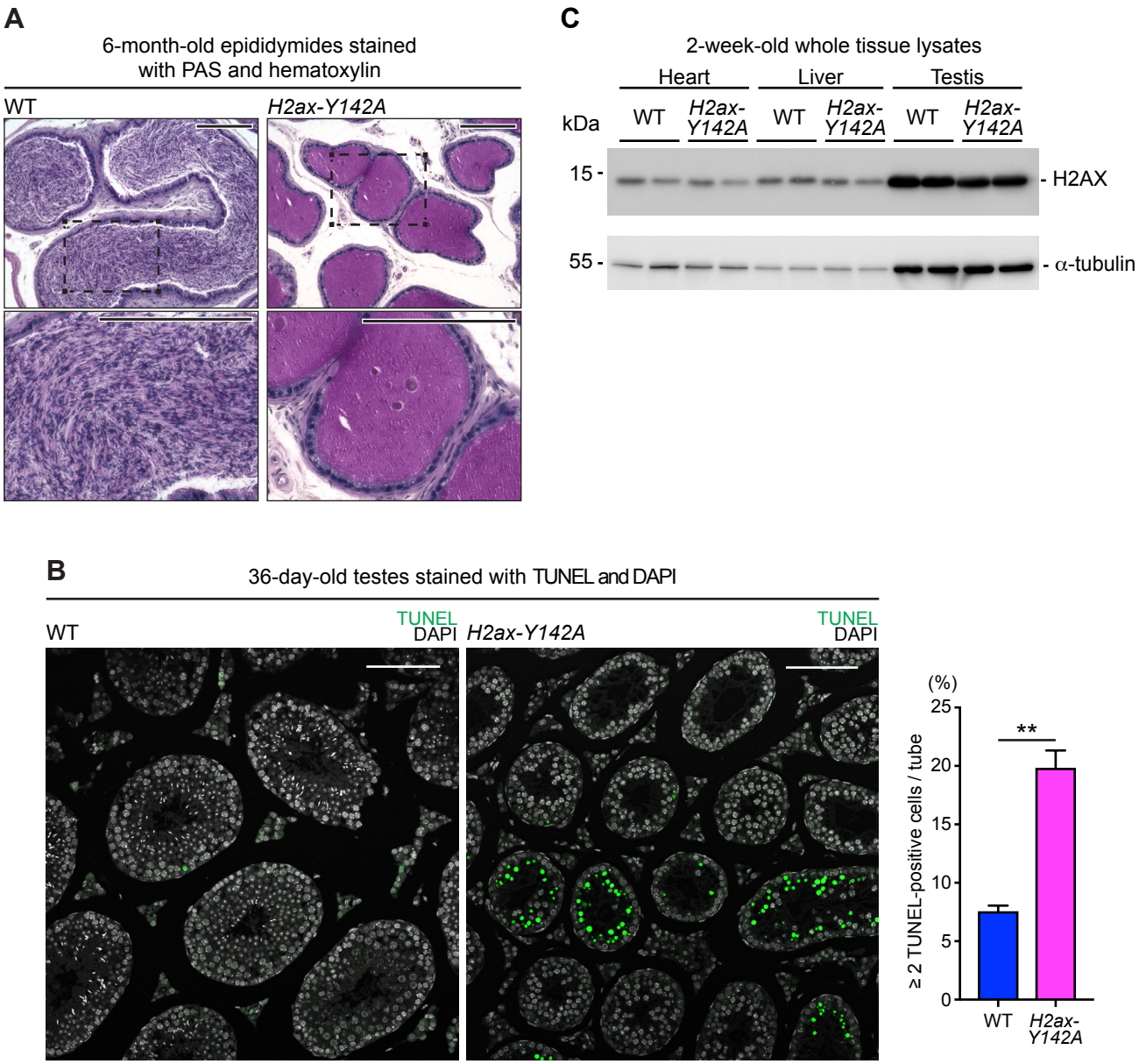

Figure S1. Characterization of *H2ax-Y142A* mice, Related to Figure 1.

(**A**) Epididymis sections from wild-type (WT) littermate control (left panels) and *H2ax-Y142A* (right panels) at 6 months of age subjected to periodic acid-Schiff (PAS) staining and hematoxylin counterstaining. Dashed squares are magnified in the bottom panels. Scale bars: 200 μm.

(**B**) Testis sections of WT littermate control (left panel) and *H2ax-Y142A* (middle panel) at 36 days of age subjected to TUNEL staining and DAPI counterstaining. Percentage populations of seminiferous tubules with ≥2 TUNEL-positive cells shown as mean ± s.e.m. for 3 independent littermate pairs (right panel). Total numbers of analyzed seminiferous tubules are indicated in the panel. ** $p < 0.01$, unpaired t test. Scale bars: 100 μm.

(**C**) Western blotting of whole tissue lysates from mouse heart, liver, and testis of WT and *H2ax-Y142A* at 2 weeks of age with anti-H2AX antibody. 20-μg protein samples were loaded in each lane. Two independent samples for WT and *H2ax-Y142A* are shown. Loading control: α-tubulin.

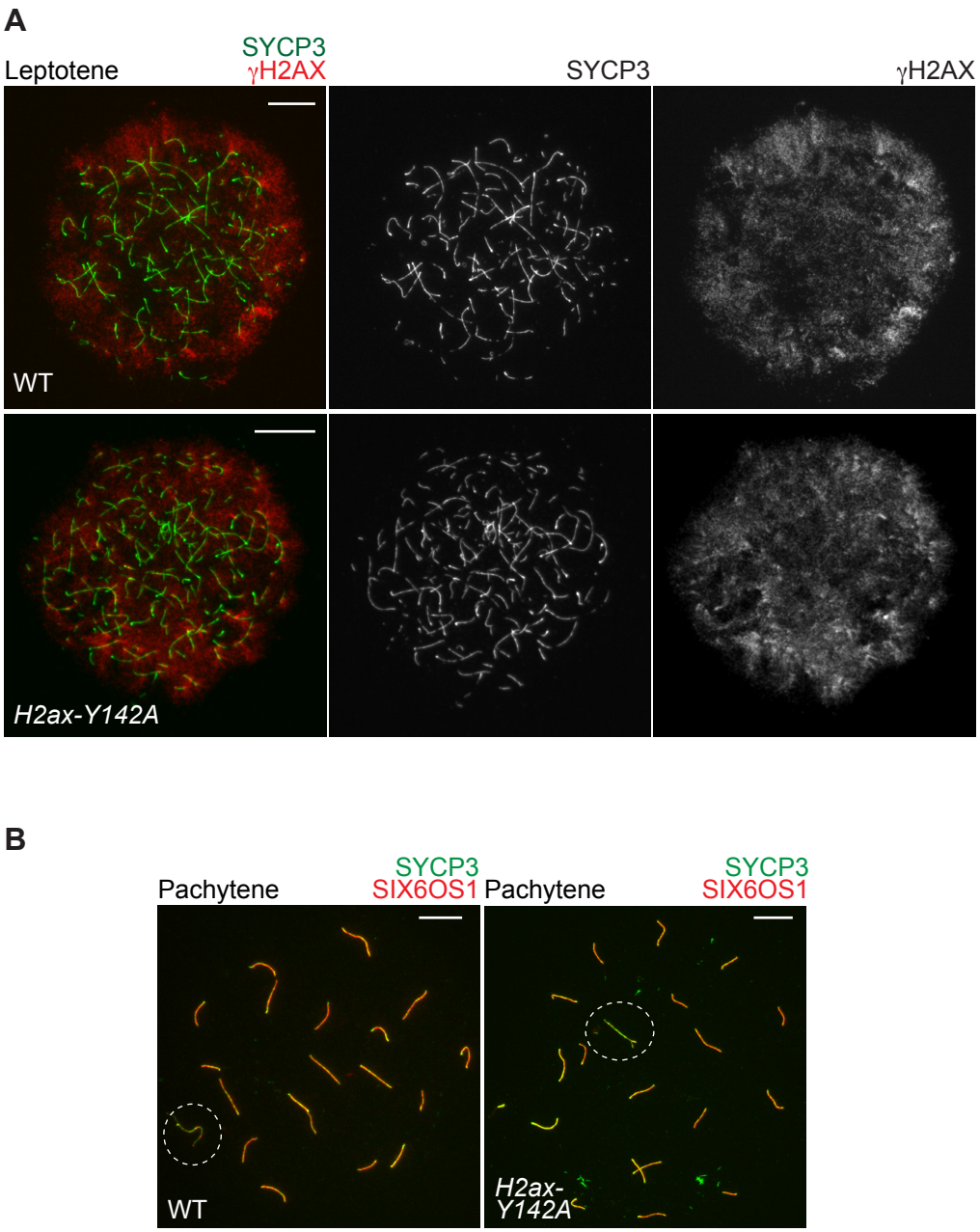

Figure S2. Normal formation of nuclear-wide γH2AX and autosomal synapsis in *H2ax-Y142A* mice, Related to Figure 2.

(**A**) Chromosome spreads of wild-type (WT) littermate control and *H2ax-Y142A* leptotene spermatocytes immunostained with antibodies raised against SYCP3 and γH2AX. Scale bars: 10 μm.

(**B**) Chromosome spreads of WT littermate control and *H2ax-Y142A* pachytene spermatocytes immunostained with antibodies raised against SYCP3 and SIX6OS1. Dashed circles indicate the sex chromosomes. Scale bars: 10 μm.

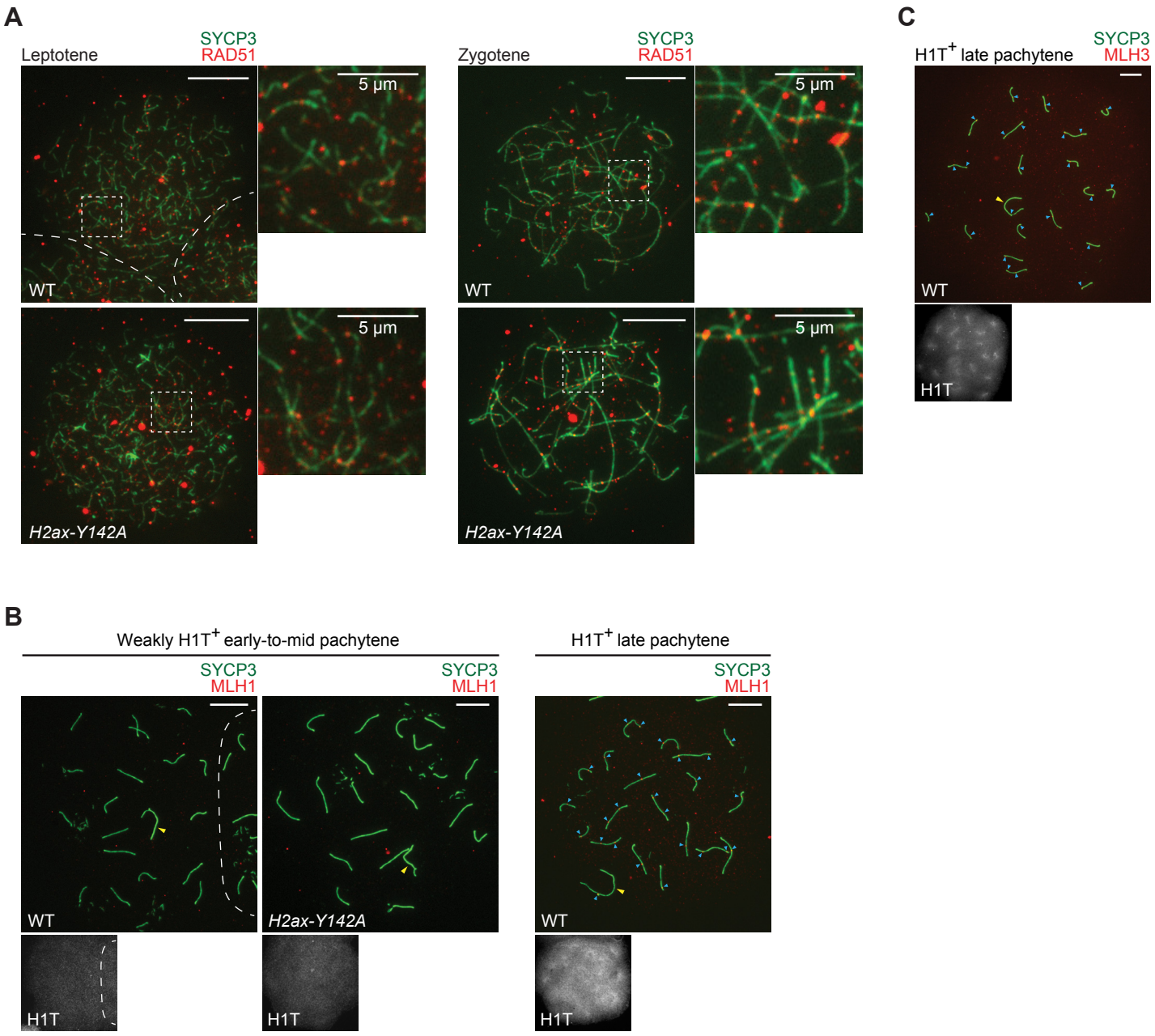

Figure S3. Normal DSB-formation in *H2ax-Y142A* mice and timely accumulation of MLH1 and MLH3 at the late pachytene stage, Related to Figure 4.

(**A-C**) Chromosome spreads of wild-type (WT) littermate control and *H2ax-Y142A* pachytene spermatocytes immunostained with antibodies raised against the following proteins: SYCP3 (**A-C**), RAD51 (**A**), MLH1 (**B**), MLH3 (**C**), and H1T (**B, C**). Dashed squares are magnified in the panels to the right. Yellow arrowheads indicate sex chromosomes (**B, C**) and blue arrowheads indicate MLH1 (**B**) or MLH3 (**C**) foci, which were recruited to all chromosomes, each chromosome bearing a minimum of 1 focus or maximum of 2 foci. Scale bars: 10 μm unless otherwise described in the panels.

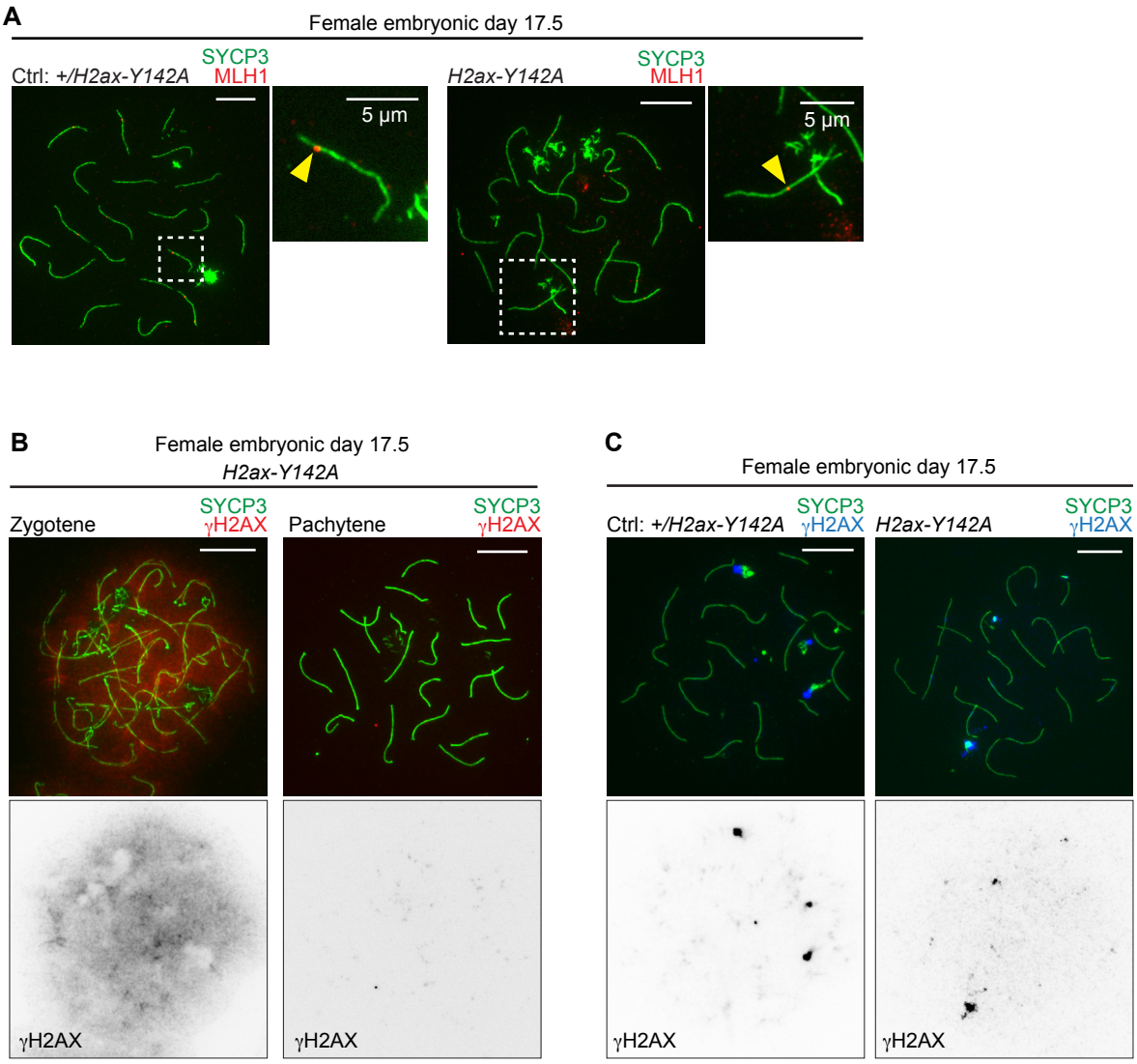

Figure S4. Meiotic prophase I in *H2ax-Y142A* oocytes is normal, Related to Figure 4.
(**A–C**) Chromosome spreads of heterozygote (Ctrl) littermate control and *H2ax-Y142A* oocytes at embryonic day 17.5 (i.e., in either the zygotene or pachytene stage) immunostained with antibodies raised against SYCP3 (**A, B, C**), MLH1 (**A**), and γH2AX (**B, C**). Yellow arrowheads indicate MLH1 foci (**A**). Dashed squares are magnified in the panels to the right (**A**). Scale bars: 10 µm.

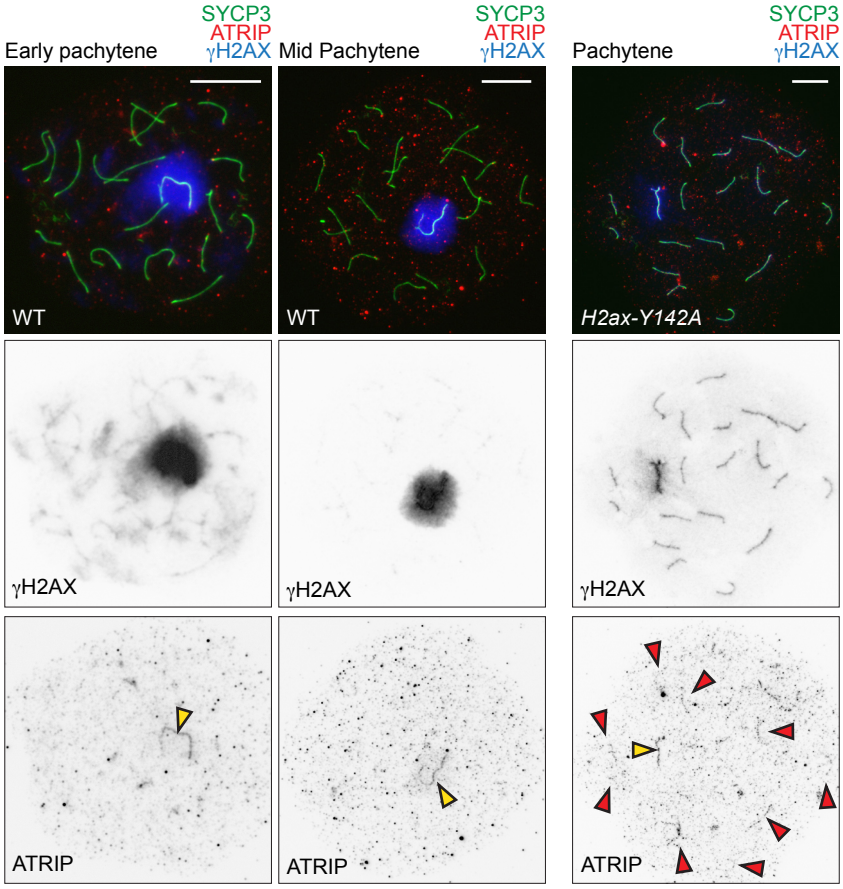

Figure S5. ATRIP persists on the autosomes of *H2ax-Y142A* pachytene spermatocytes, Related to Figure 5. Chromosome spreads of wild-type (WT) littermate control and *H2ax-Y142A* pachytene spermatocytes immunostained with antibodies raised against SYCP3, γH2AX, and ATRIP. Yellow arrowheads indicate the sex chromosomes. Red arrowheads indicate ATRIP foci that persist on *H2ax-Y142A* autosomes. Scale bars: 10 μm.

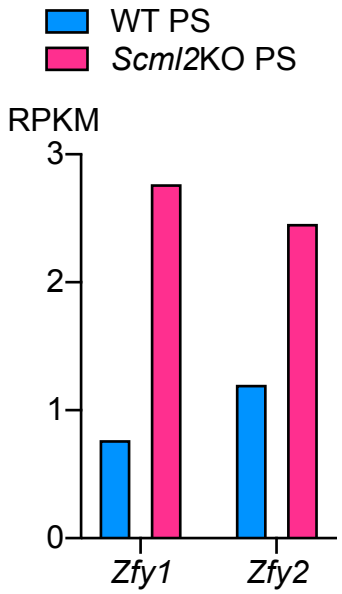

Figure S6. Derepression of *Zfy1* and *Zfy2* genes in *Scml2*KO pachytene spermatocytes, Related to Figure 6.
RNA-seq RPKM values for the genes *Zfy1* and *Zfy2* in wild-type (WT) and *Scml2*KO pachytene spermatocytes.