Figures and figure supplements

DMRT1 is a testis-determining gene in rabbits and is also essential for female fertility

Emilie Dujardin *et al*.

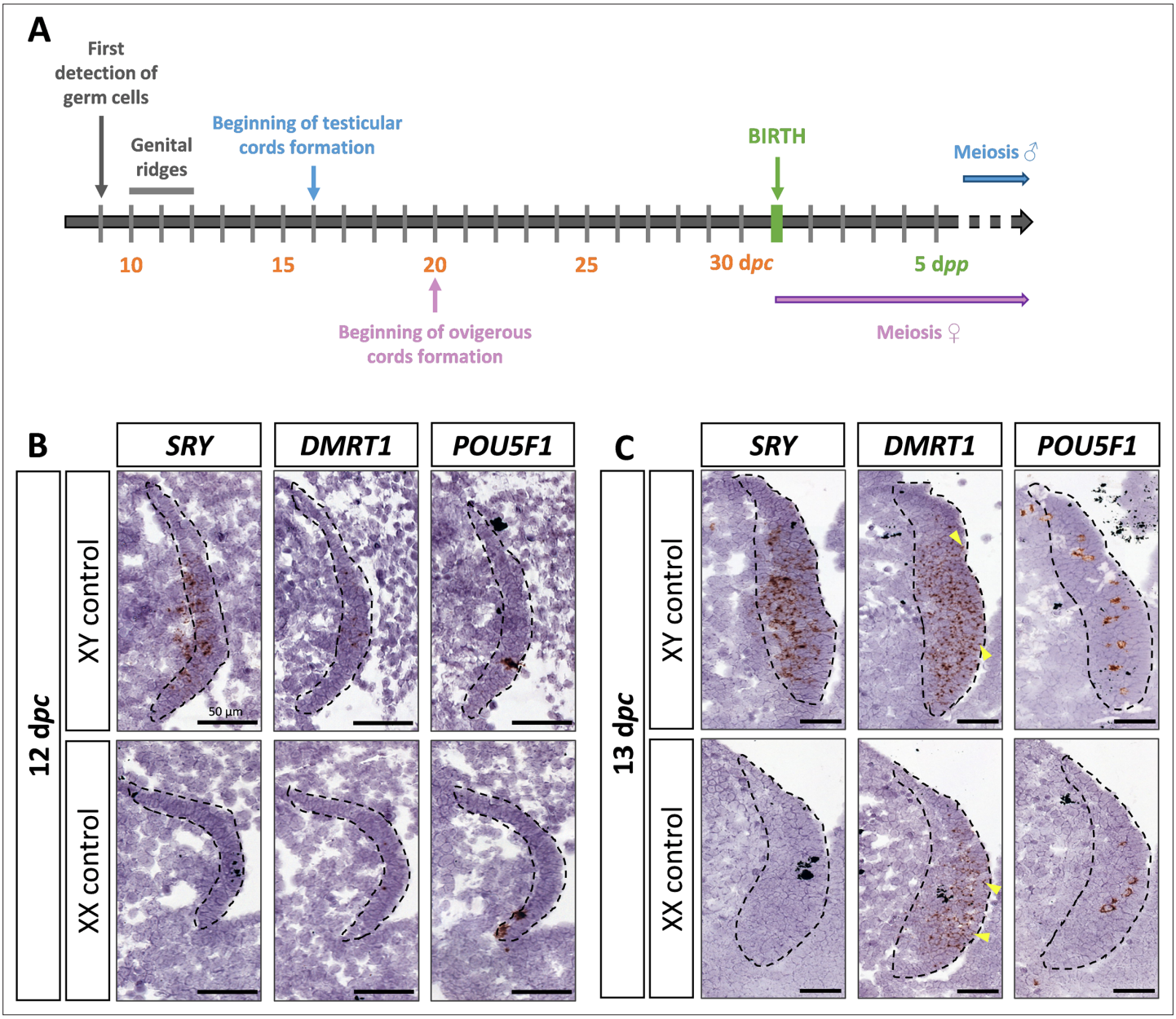

Figure 1. *SRY*, *DMRT1*, and *POU5F1* location during early gonadal development. (**A**) Key stages of gonadal development in rabbits with 31 days of gestation. Germ cells are first detected at 9 days *post-coïtum* (d*pc*), before the genital ridge formation, which occurs between 10 and 12 d*pc*. In XY gonads, testicular cords begin forming at 16 d*pc*, and germ cells enter meiosis a few months after birth. In XX gonads, the ovigerous cords appear at 20 d*pc*, and meiosis begins around birth. Location of *SRY*, *DMRT1*, and *POU5F1* by *in situ* hybridization (RNAscope technology) on XY and XX control gonads at (**B**) 12 d*pc* or (**C**) 13 d*pc*. Dotted line: developing genital crests. Yellow arrowheads: coelomic epithelial cells expressing *DMRT1*. Scale bar = 50 µm.

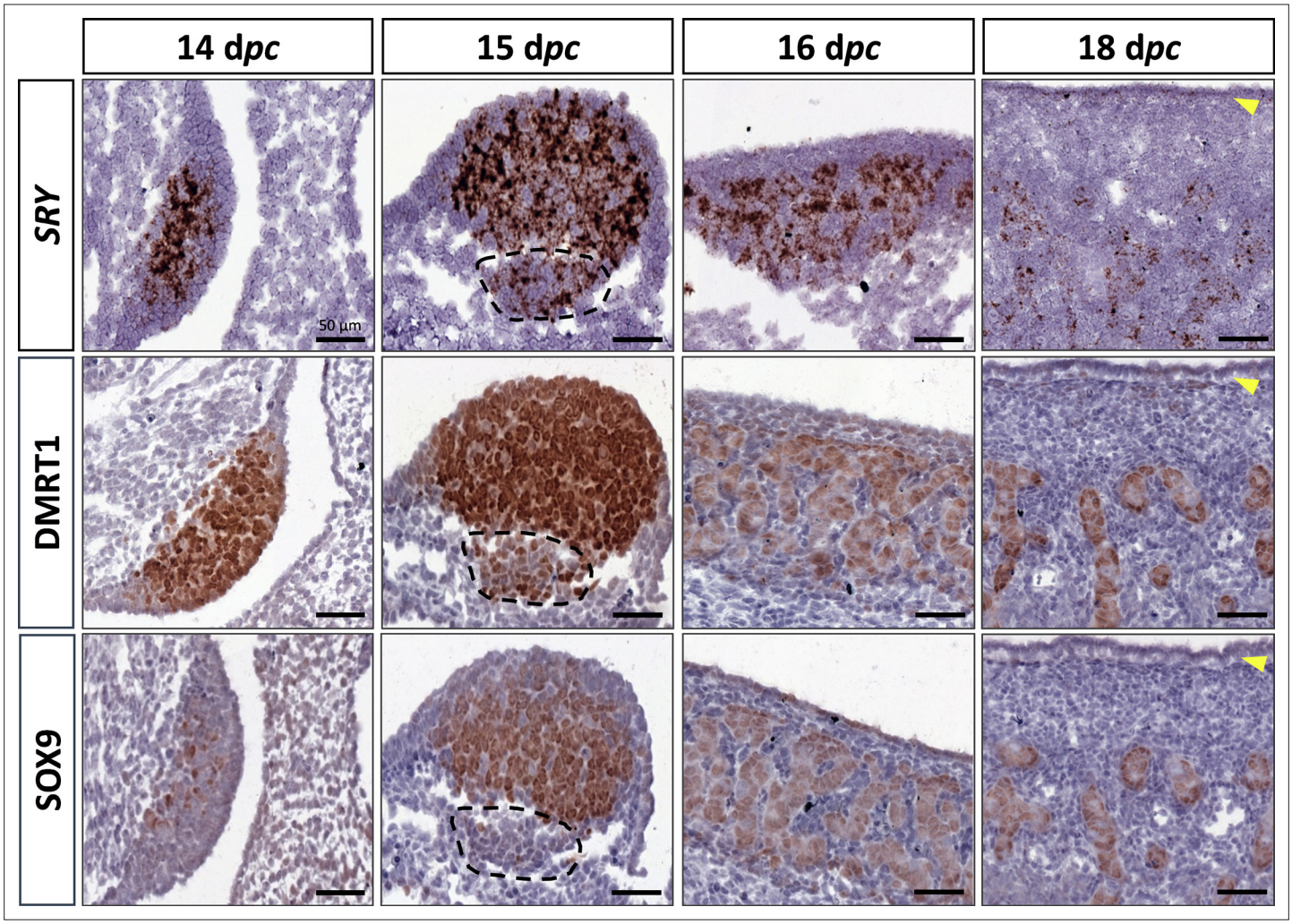

Figure 2. Somatic markers location during testis differentiation. Location of *SRY* by *in situ* hybridization (RNAscope technology), DMRT1, and SOX9 by immunohistochemistry on XY control testes from 14 to 18 d*pc*. The dotted line at 15 d*pc*: territory with cells expressing *SRY* and DMRT1 but not SOX9. Yellow arrowheads: tunica albuginea in formation. Scale bar = 50 µm.

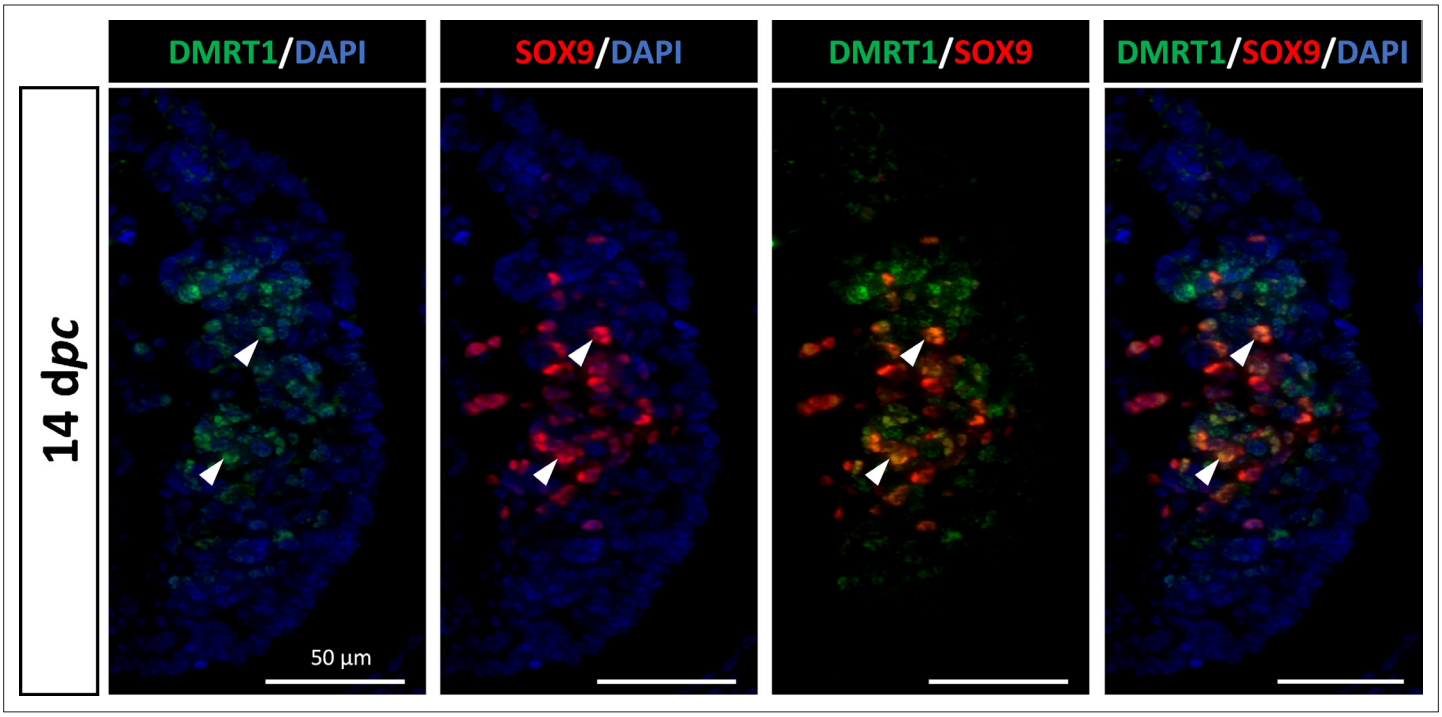

Figure 2—figure supplement 1. DMRT1 and SOX9 co-location on 14 d*pc* XY control gonad. DMRT1 (green) and SOX9 (red) immunodetection in XY control gonad at 14 d*pc*. Nuclei were stained in blue (DAPI). Arrowheads: cells co-expressing DMRT1 and SOX9. Scale bar = 50 µm.

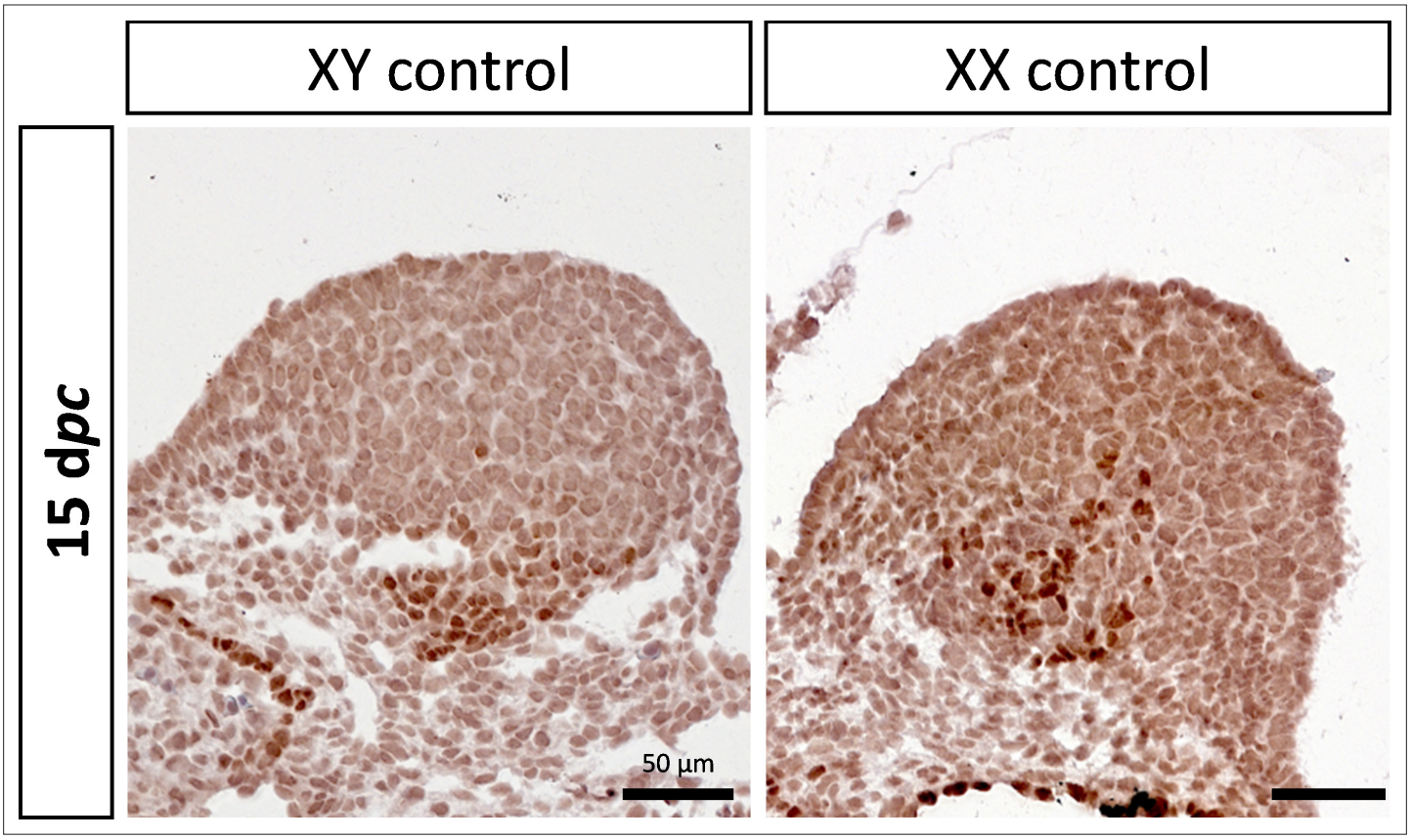

Figure 2—figure supplement 2. Identification of PAX8-positive cells in 15 d*pc* control gonads. Immunostaining of PAX8 in XY and XX control gonads at 15 d*pc*. Scale bar = 50 µm.

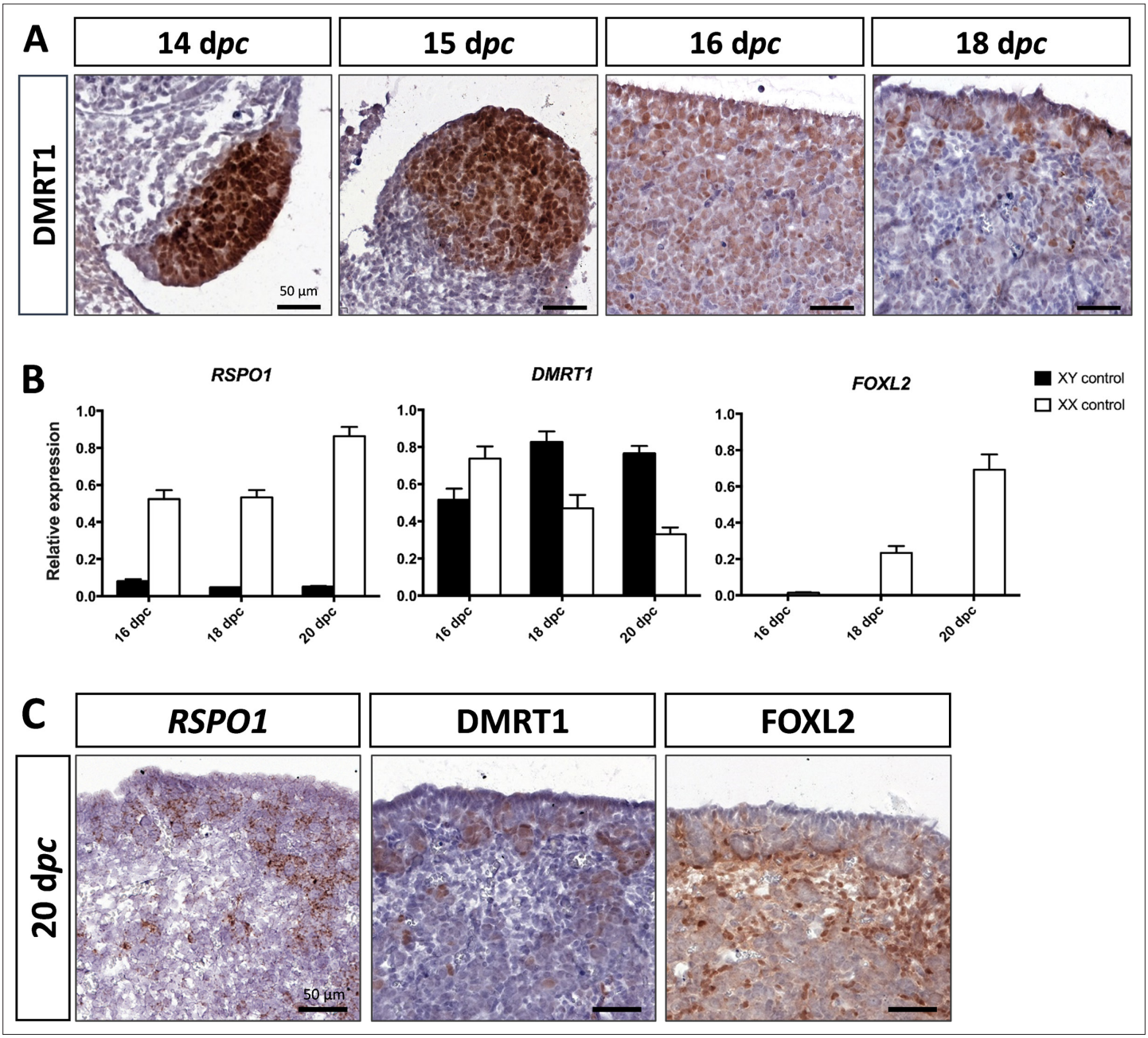

Figure 3. Somatic markers location and expression during ovarian differentiation. (**A**) Immunostaining of DMRT1 on XX control ovaries from 14 to 18 d*pc*. (**B**) Quantitative RT-PCR (RT-qPCR) analyses of *RSPO1*, *DMRT1*, and *FOXL2* expression from 16 to 20 d*pc* in control gonads of both sexes. The error bars correspond to the standard error of the mean (n=3-5) (**C**) *RSPO1 in situ* hybridization (RNAscope technology), immunostaining of DMRT1 and FOXL2 on 20 d*pc* control ovaries. Scale bar = 50 µm.

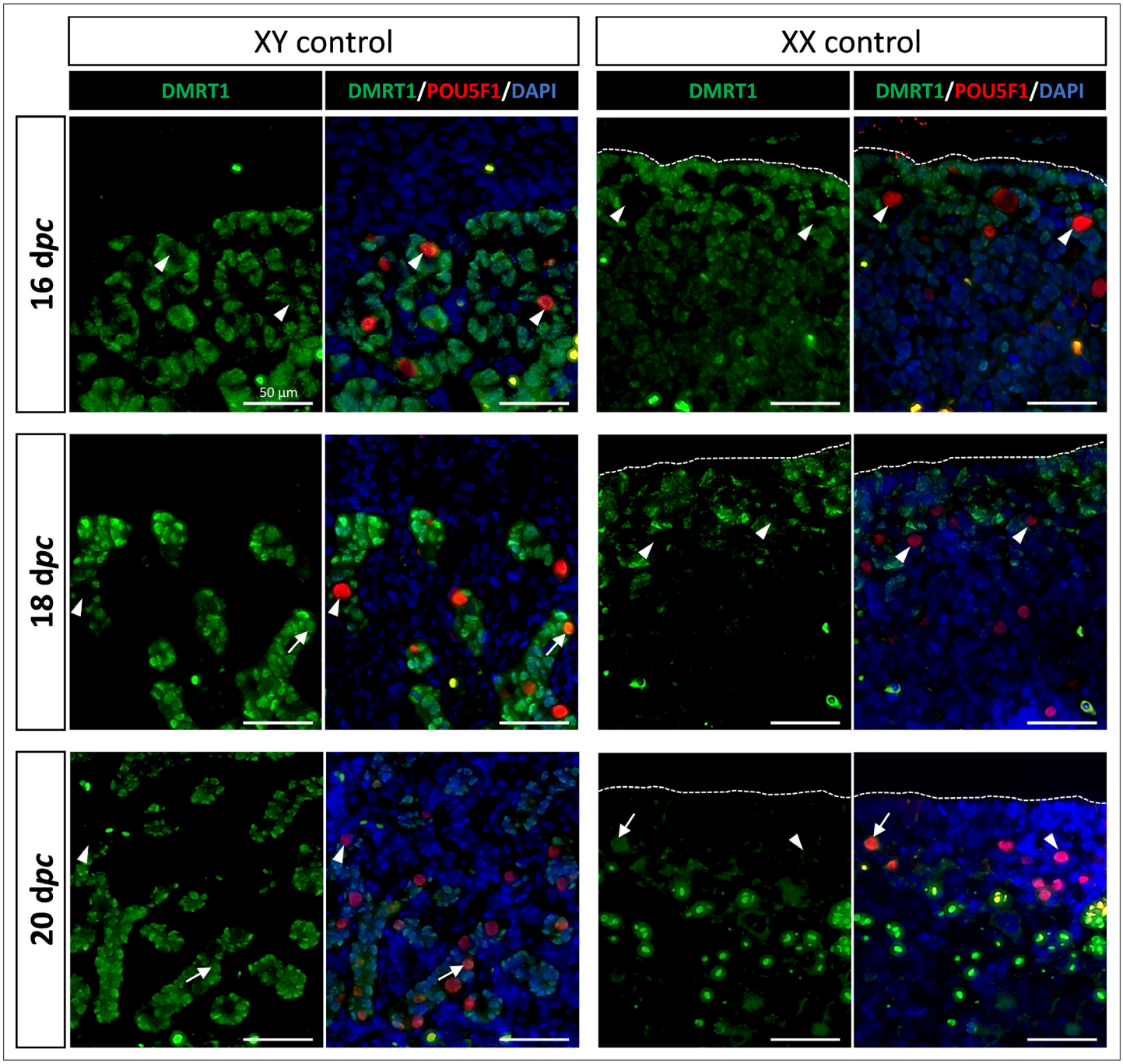

Figure 3—figure supplement 1. DMRT1 and POU5F1 co-detection in control gonads. DMRT1 (green) and POU5F1 (red) immunodetection in XY and XX control gonads from 16 to 20 d*pc*. Nuclei were stained in blue (DAPI). Arrowheads: cells expressing POU5F1 only. Arrows: cells co-expressing POU5F1 and DMRT1. Dotted line: delimitation of the ovarian surface epithelium. Dots with intense green labeling: auto-fluorescence of red blood cells. Scale bar = 50 µm.

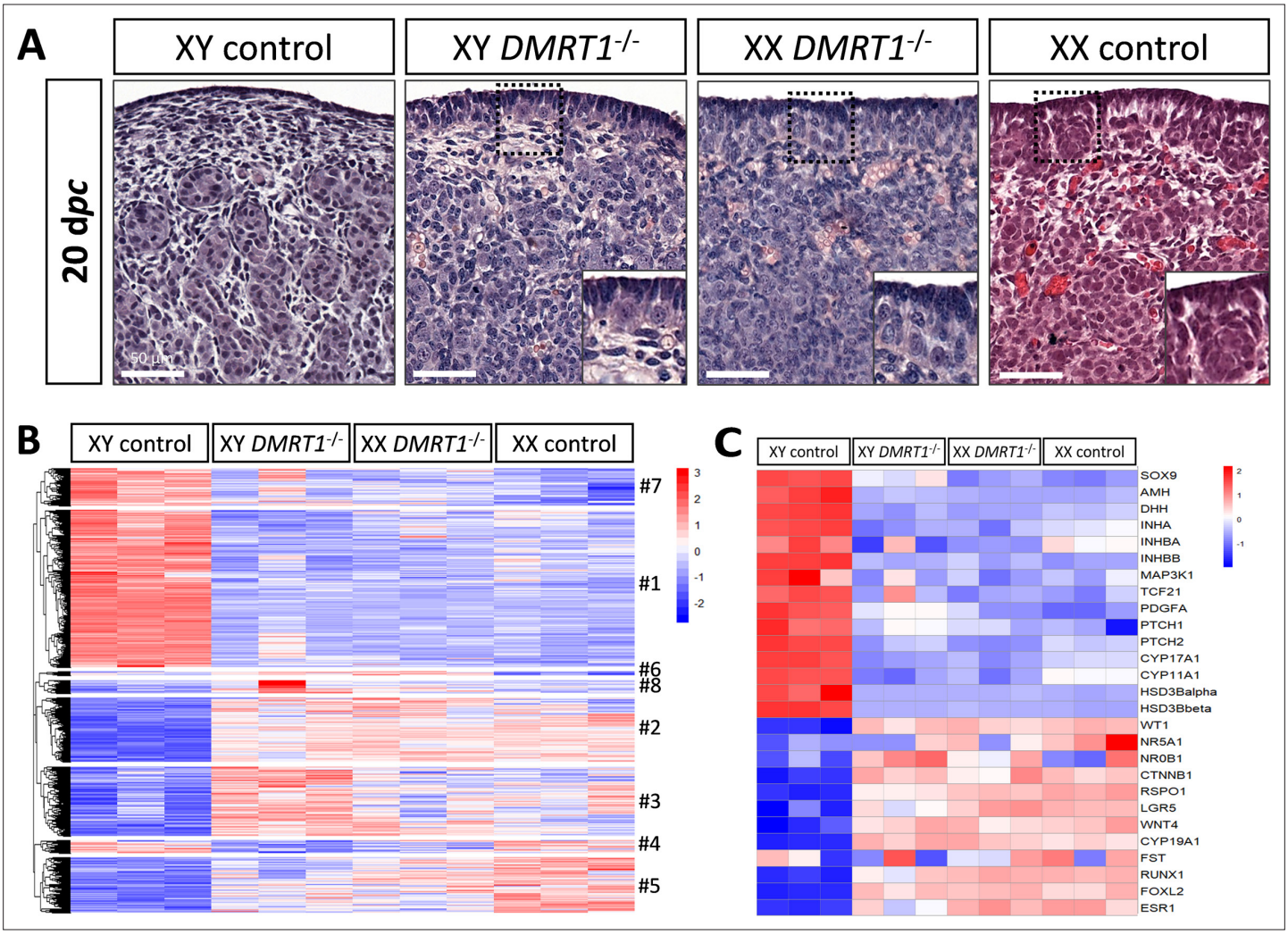

Figure 4. Ovarian-like morphology and transcriptomic signature of XY *DMRT1*$^{-/-}$ gonads at 20 d*pc*. (**A**) Hematoxylin and eosin staining of gonads sections from control and *DMRT1*$^{-/-}$ 20 d*pc* rabbits. The enlarged area shows the characteristic ovarian surface epithelium found on XY *DMRT1*$^{-/-}$ gonads. Scale bar = 50 µm. Heatmap representation of (**B**) 3460 deregulated genes (adjusted p-value <0.05 and |log2FC| > 1) or (**C**) 27 selected genes between XY control, XY *DMRT1*$^{-/-}$, XX *DMRT1*$^{-/-}$, and XX control at 20 d*pc*.

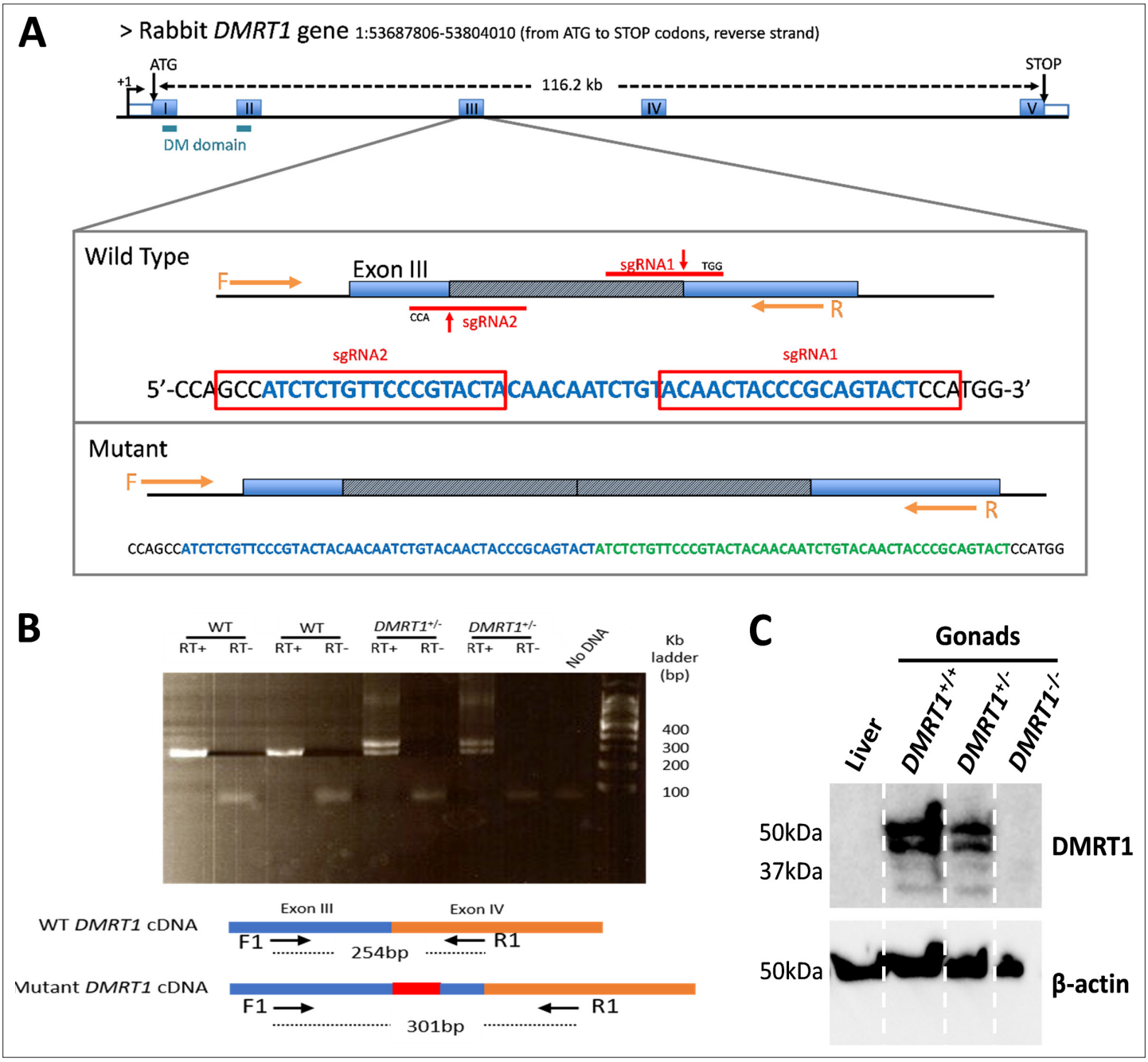

Figure 4—figure supplement 1. *DMRT1* mutation using CRISPR/Cas9 in rabbits. (**A**) Position of the two guides (sgRNA1 and sgRNA2) on the rabbit *DMRT1* transcript. Blue boxes represent the translated exons. The DM domain expands from exon I to exon II. The vertical red arrow indicates the Cas9-induced cleavage. The red box points to the sequence of the sgRNAs. The hatched box and bold blue letters point to the sequence between the theoretical and expected cleavage points. The cleaved fragment was inserted tandemly in the mutant allele at the cleavage site. Thus, by sequencing, a repeat was found (the repeats are written in blue and green bold letters). Consequently, the wild-type and the mutant allele were characterized through PCR using the F/R set of primers and gel electrophoresis. +1: putative transcription start site; ATG: site of initiation of translation; STOP: stop codon. (**B**) Total RNA were reverse transcribed (RT+), and the amplified products were analyzed through gel electrophoresis. A unique amplicon of 254 bp was observed in PCR products from wild-type rabbits and two (254 and 301 bp) in PCR products from heterozygous rabbits. RT−: reverse transcription control (no reverse transcriptase). (**C**) Western blot with nuclear proteins extracted from the liver of control rabbits and from 7 to 8 gonads of 1–3 d*pp* *DMRT1*$^{+/+}$, *DMRT1*$^{+/-}$, and *DMRT1*$^{-/-}$ rabbits.

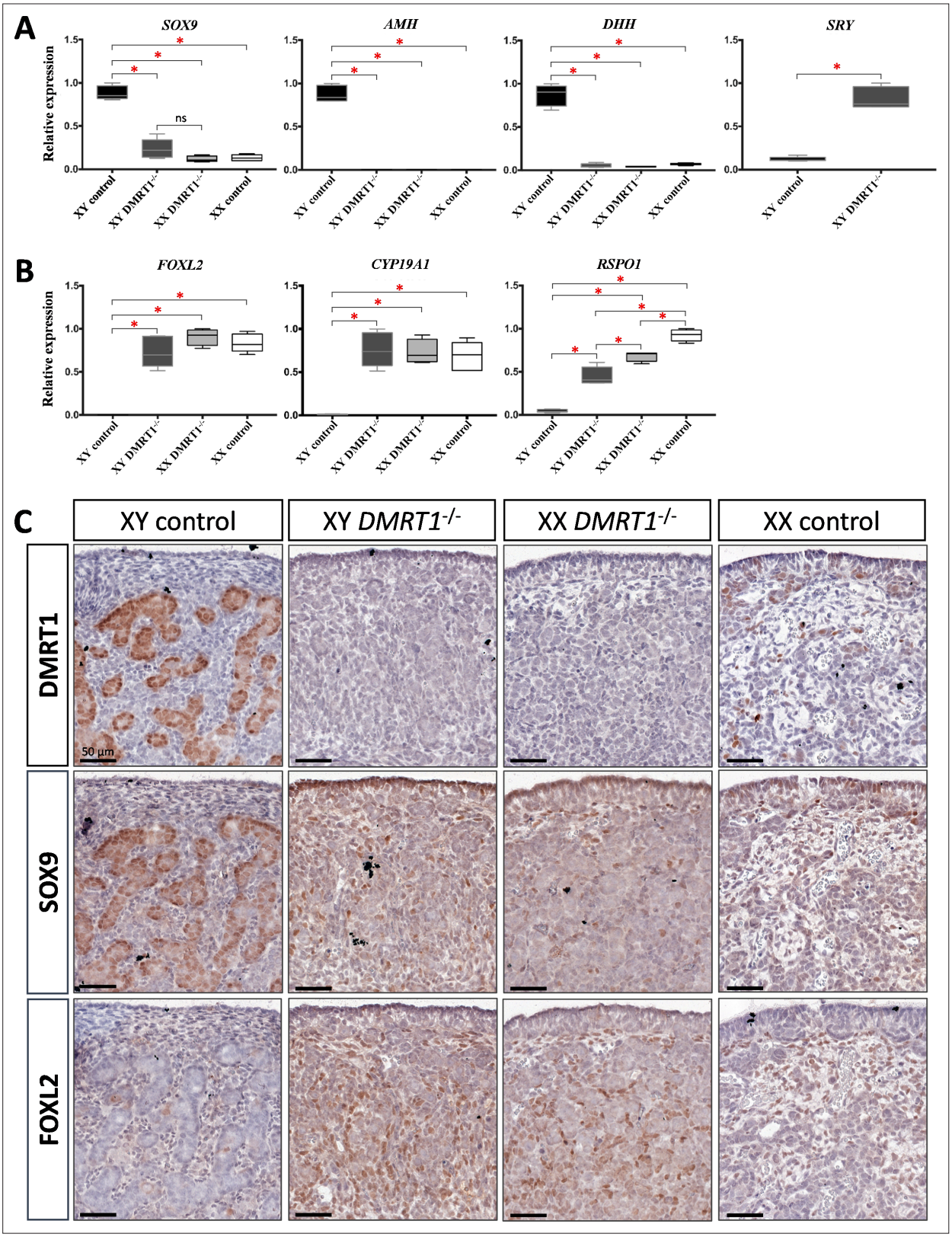

Figure 5. Somatic markers expression and location on control and *DMRT1*$^{-/-}$ gonads at 20 d*pc*. Quantitative RT-PCR (RT-qPCR) analyses of (**A**) testicular-related differentiation genes (*SOX9*, *AMH*, *DHH*, and *SRY*) or (**B**) ovarian-related differentiation genes (*FOXL2*, *CYP19A1*, and *RSPO1*) in XY control, XY *DMRT1*$^{-/-}$, XX *DMRT1*$^{-/-}$, and XX control gonads (n = 4–5) at 20 d*pc*. Statistical analyses were performed using the non-parametric Kruskal–Wallis test, followed by a pairwise permutation test: *p-value <0.05; ns: non-significant. (**C**) Immunostaining of DMRT1, SOX9, and FOXL2 on XY control, XY *DMRT1*$^{-/-}$, XX *DMRT1*$^{-/-}$, and XX control gonad sections at 20 d*pc*. Scale bar = 50 µm.

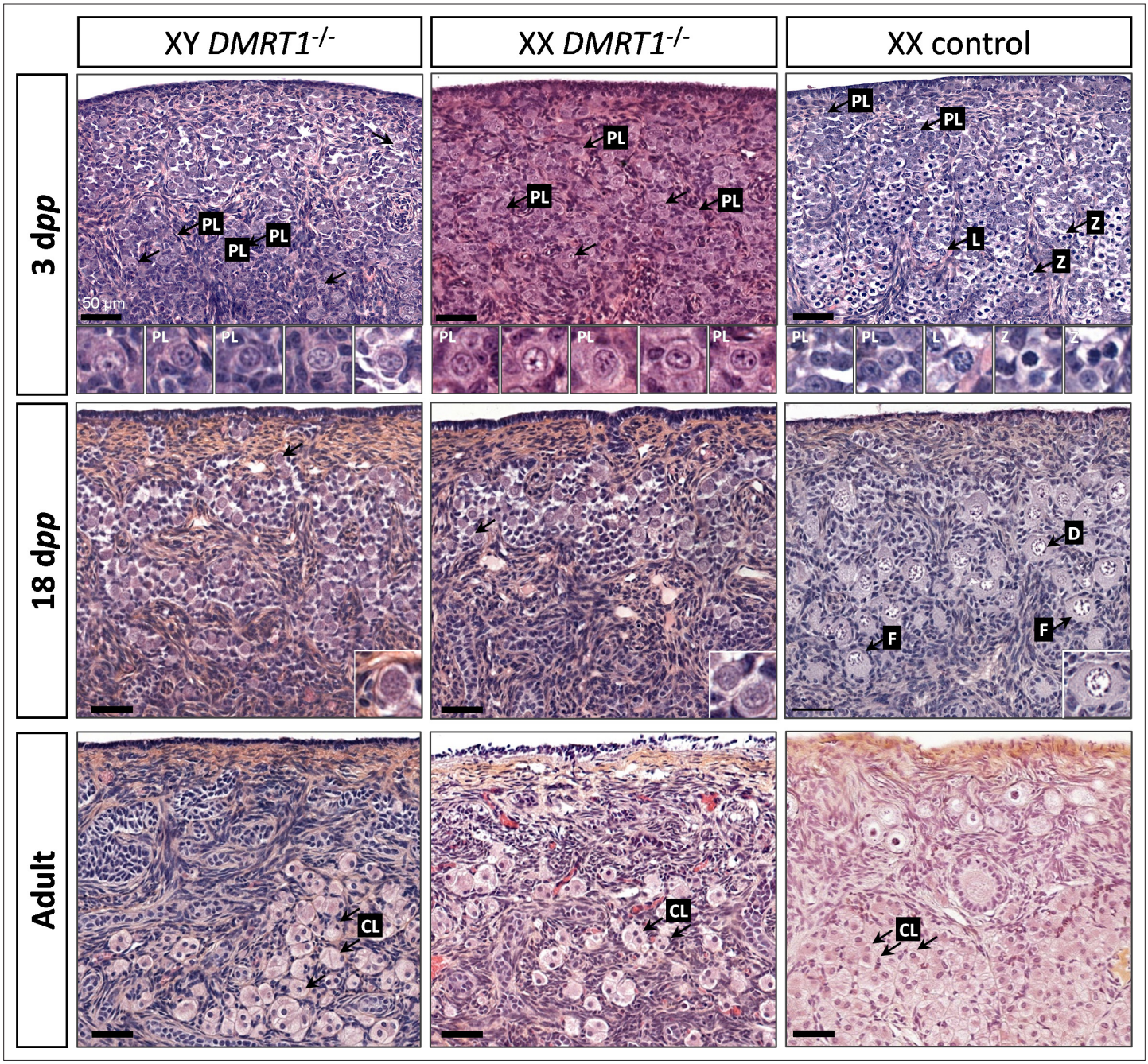

Figure 6. Evolution of gonadal morphogenesis in XY and XX *DMRT1*$^{-/-}$ rabbits. Hematoxylin and eosin staining of gonad sections from XY and XX *DMRT1*$^{-/-}$ gonads and XX control ovaries at 3 days *post-partum* (d*pp*), 18 d*pp*, and in adulthood (4–9 months). The enlargements for the first two panels correspond to the nuclei pointed by an arrow. PL: preleptotene stage; L: leptotene stage; Z: zygotene stage; D: diplotene stage; F: ovarian follicle; CL: luteal cells. Scale bar = 50 µm.

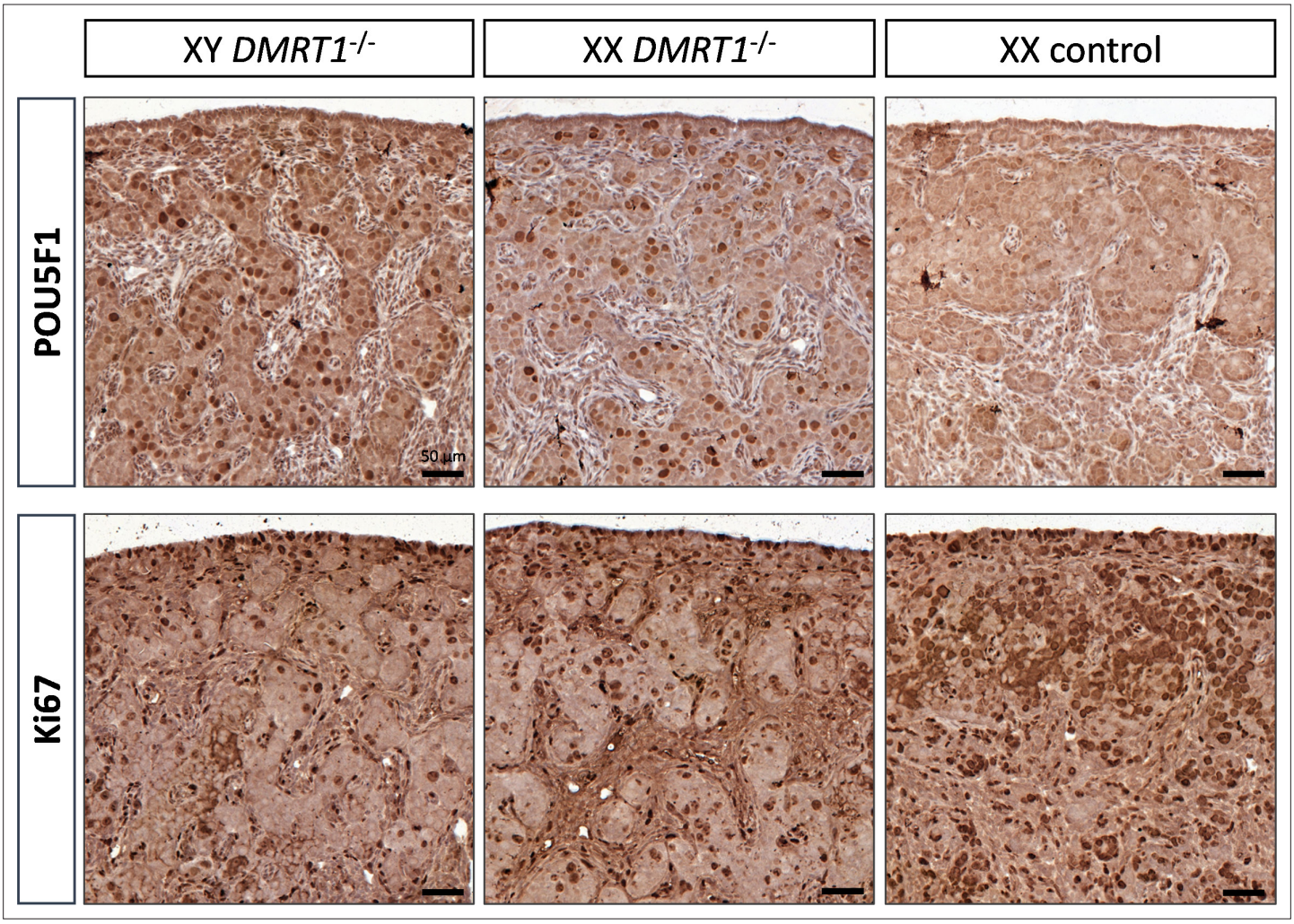

Figure 6—figure supplement 1. POU5F1 and Ki67 location on control and *DMRT1*$^{-/-}$ gonads at 3 d*pp*. Immunostaining of POU5F1 (pluripotency marker) and Ki67 (a marker of the exit of the G0 phase of the cell cycle) on gonad sections from XY *DMRT1*$^{-/-}$, XX *DMRT1*$^{-/-}$, and XX control at 3 d*pp*. Scale bar = 50 µm.

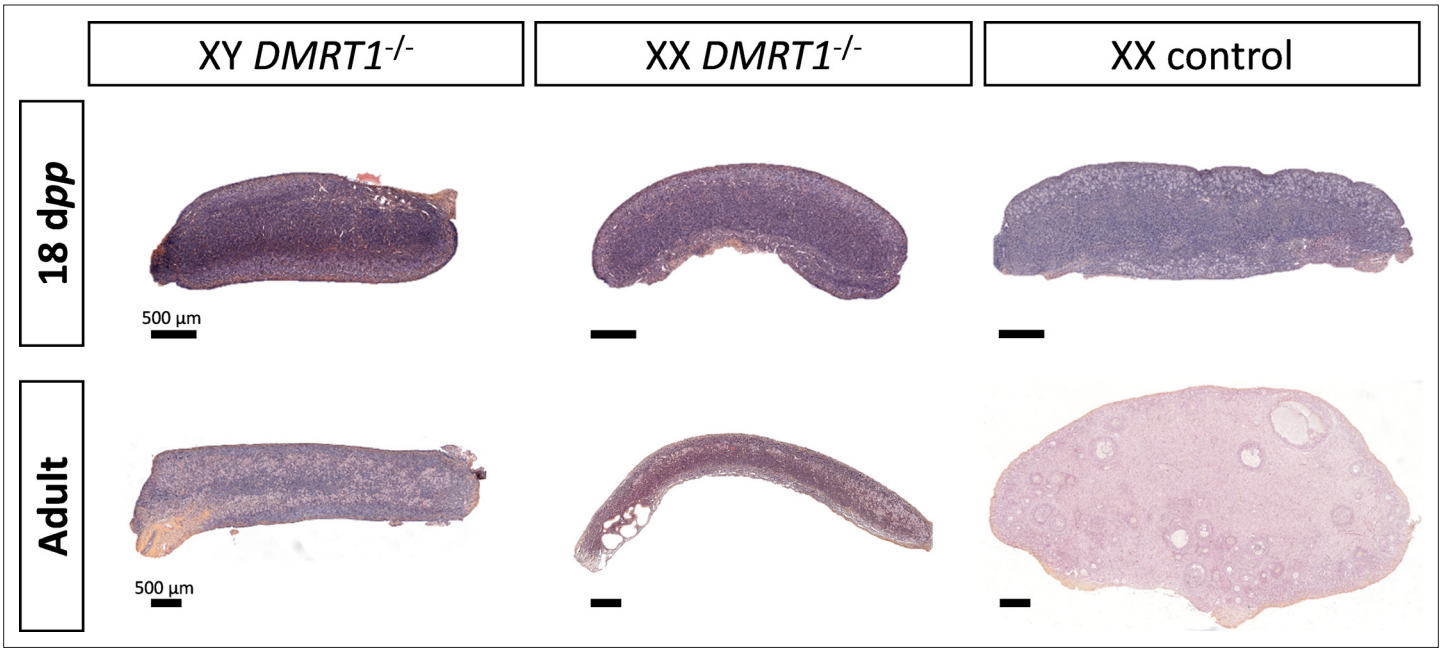

Figure 6—figure supplement 2. Evolution of gonadal size in XY and XX *DMRT1*$^{-/-}$ rabbits. Hematoxylin and eosin staining of gonad sections from XY and XX *DMRT1*$^{-/-}$ gonads and XX control ovaries at 18 d*pp*, and in adulthood (4–9 months). Scale bar = 500 µm.