

Notch signaling maintains Leydig progenitor cells in the mouse testis

DEV024786 Supplementary Material

Files in this Data Supplement:

- [Supplemental Figure S1](#) -**Fig. S1. Notch2 and Notch3 are expressed at very low levels in XX gonads. (A-F)** Whole-mount in situ hybridization for *Notch2* (A,B) and *Notch3* (D,E) on XX gonads at 11.5 and 12.5 dpc (10×). (C,F) 12.5 dpc XX *Notch2^{LacZ}* and *Notch3^{LacZ}* gonads were stained for β-gal and sectioned (20×). (**G,H**) Notch2 and Notch3 are both expressed in the interstitium of XY gonads by 13.5 dpc. 13.5 dpc XY *Notch2^{LacZ}* (G) and *Notch3^{LacZ}* (H) gonads were stained for β-gal and then sectioned (100×). g, gonad; m, mesonephros; TC, testis cord; I, interstitium. Scale bars: 20 μm.
- [Supplemental Figure S2](#) -**Fig. S2. In *Rosa^{Notch}; Sf1-cre* gonads, Notch signaling is constitutively active in somatic cell precursors at 11.5 dpc, and in differentiated Sertoli and Leydig cells. (A-E)** Immunofluorescent staining of PECAM1 (red, germ cells and vasculature) and NICD-GFP (green, active Notch) in gonads from *Rosa^{Notch}; Sf1-cre* mice, and in *Rosa^{Notch}* controls at 10.5 dpc (B). At 10.5 dpc, no GFP was detected above background in experimental versus controls without Sf1-Cre (A,B). At 11.5 dpc, GFP was enriched in nuclei of pre-Sertoli cells and other somatic cell (C). (D,E) After testis cords formed (broken lines), GFP expression was detected in Sertoli cells and some interstitial cells (Leydig progenitors). (**F,G**) Lower magnification views of Fig. 4C,D. Compared with wild type (F), *Rosa^{Notch}; Sf1-cre* gonads (G) showed loss of SF1 (green)-positive cells in the interstitium at 13.5 dpc. PECAM1 (red) labels vasculature and germ cells; SF1 labels both Sertoli cells around germ cells, and Leydig cells in the interstitium. Scale bars: 50 μm.
- [Supplemental Figure S3](#) -**Fig. S3. The early progenitor cell marker LHX9 labels some interstitial cells at all stages. (A)** 11.5 and (**B**) 13.5 dpc XY gonads stained by immunofluorescence for LHX9 (red, nucleus), PECAM1 (green, germ cell and vasculature) and DNA (blue, Syto13). LHX9-positive cells are detected in the coelomic epithelium and inside the gonad at both stages (arrows). Scale bars: 50 μm. g, gonad; m, mesonephros; TC, testis cord (broken lines); CE, coelomic epithelium.
- [Supplemental Figure S4](#) -**Fig. S4. Germ cell loss in *Rosa^{Notch}; Sf1-cre* gonad at postnatal day 1. (A,B)** An increase in caspase 3-positive cells (red) inside testis cords (broken lines) suggests germ cells undergo apoptosis

in the *Rosa^{Notch}; Sf1-cre* gonad (B), compared with wild type (A). (Syto13 stains DNA, blue.)