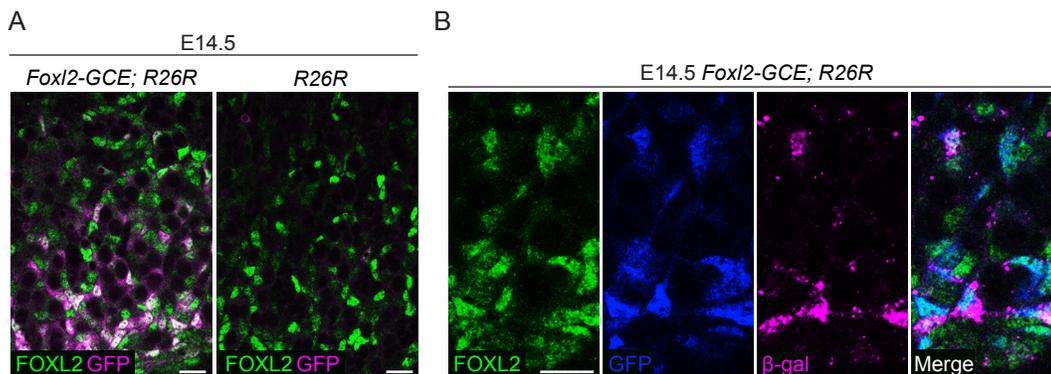


Supplemental Figure S1

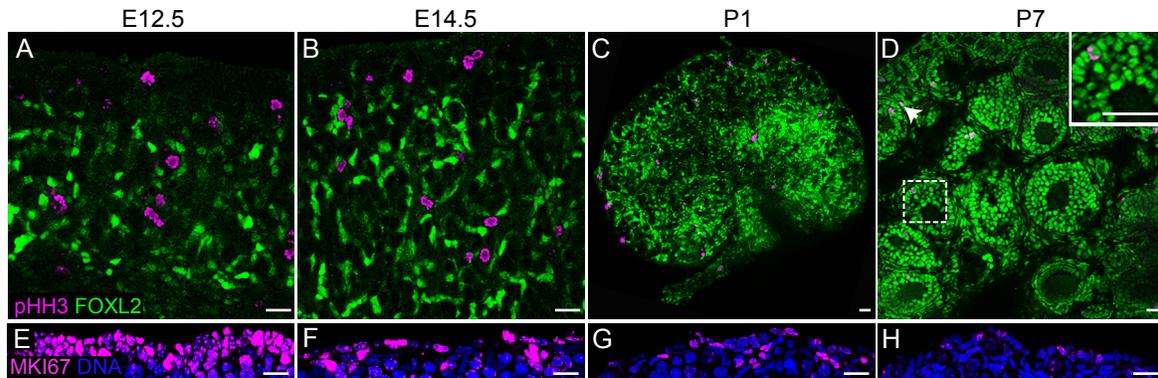
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Supplemental Figure S1. Specificity and activation of the *Foxl2-GCE* transgene in the fetal ovary. A, Ovaries from E14.5 transgenic *Foxl2GCE; R26R* and control *R26R* embryos show that the GFP-CreERT2 fusion protein is specifically expressed in a subpopulation of FOXL2-expressing cells. B, Ovary from a transgenic *Foxl2-GCE; R26R* embryo injected with tamoxifen at E12.5 and dissected at E14.5. β -galactosidase expression was specifically detected in a subset of FOXL2/GFP double-positive cells. Confocal images of whole-mount immunostained gonads were taken at 40 \times . Scale bars represent 20 μ m.

Supplemental Figure S2

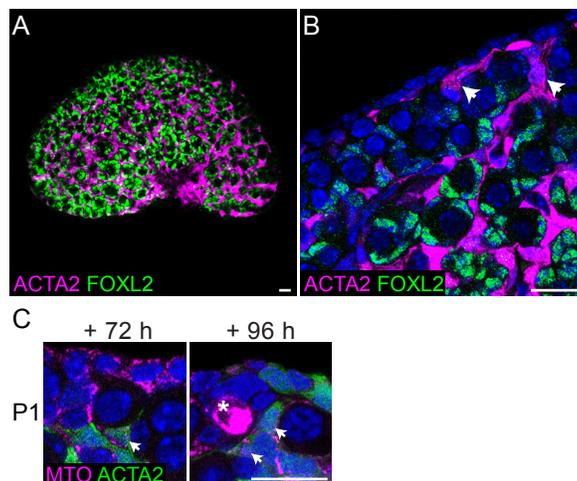
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Supplemental Figure S2. FOXL2-positive cells are not mitotically dividing, but the surface epithelium is actively cycling during early ovary development. Cells expressing FOXL2 (green) were negative for phospho-histone H3 (pHH3, magenta) staining at E12.5 (A) and E14.5 (B). At birth (C), pHH3 staining was generally excluded from strongly FOXL2-positive cells in the newly formed follicles. By P7 (D), a subset of follicles had progressed into primary and secondary stages and contained pHH3-positive cells (inset). Dividing cells were also observed in the stroma (arrowhead). E-H, The surface epithelium contained MKI67-positive cells (magenta) at all stages examined. Nuclei (blue) were stained with syto13. A, B, E, and F are images of whole-mount immunostained ovaries taken at 40 \times . C, D, G, and H are images of cryosectioned gonads taken at 20 \times (C-D) or 40 \times (G-H). Scale bars represent 20 μ m.

Supplemental Figure S3

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Supplemental Figure S3. Interstitial cells also emerge from the surface epithelium after birth. A, Ovary from a P2 *Acta2-EYFP* mouse showing strong reporter activity in the interstitial cells between newly formed follicles. Magenta, EYFP; green, FOXL2. B, Some of the FOXL2-negative proliferative cells under the surface epithelium expressed the *Acta2-EYFP* reporter (magenta, arrowheads) at P1. C, Ovaries from P1 *Acta2-EYFP* mice were labeled with MitoTracker (magenta) and cultured for 72 or 96 h. A subset of ingressing cells expressed the *Acta2-EYFP* reporter (green; arrowheads). The asterisk marks an oocyte that had incorporated MitoTracker. Nuclei (blue) were stained with syto13. Confocal images were taken at a magnification of 10× (A) or 40× (B, C). Scale bars represent 20 μm.