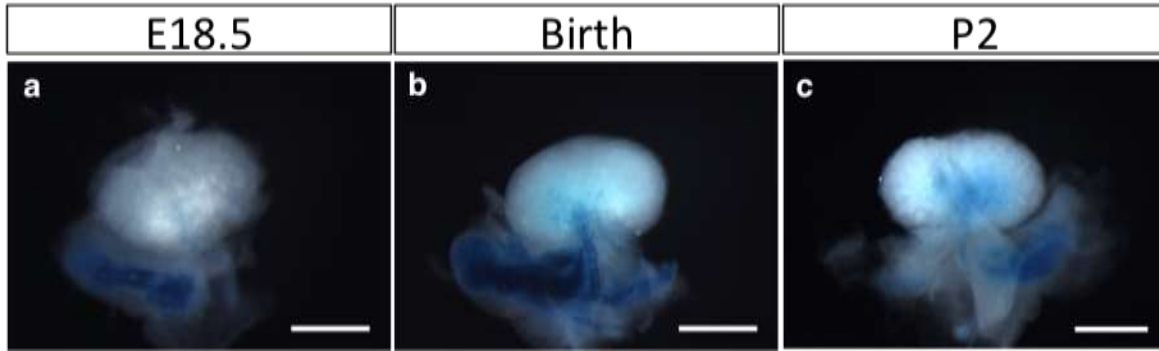
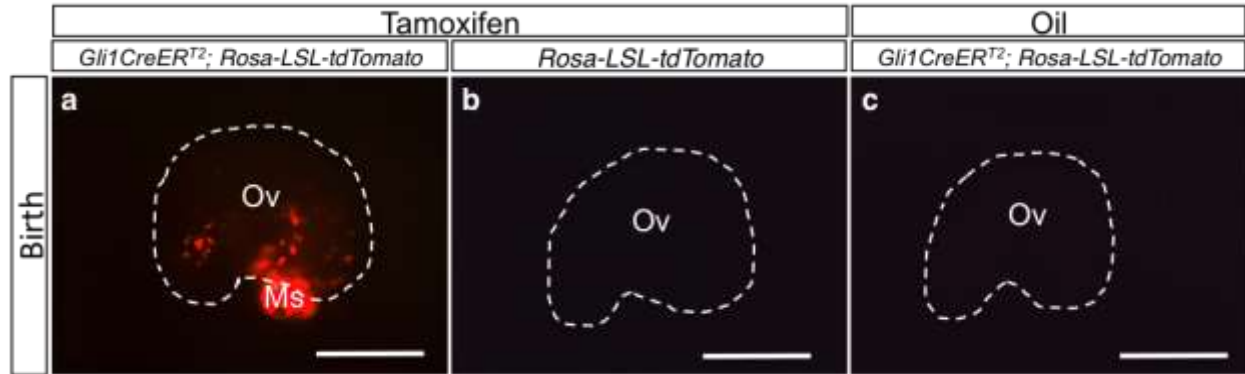


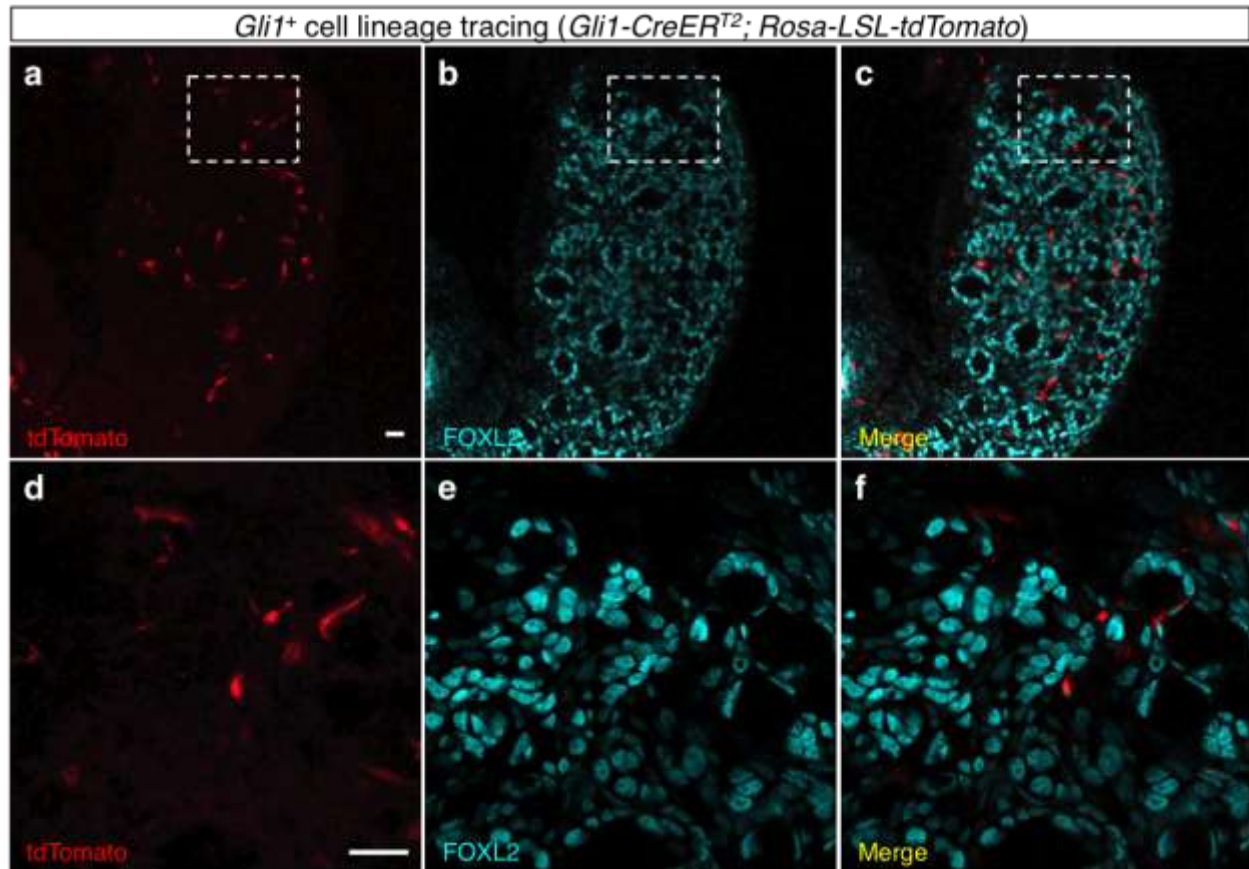
Supplementary Figure 1: Expression of *Gli1-lacZ* in E17.5 ovary and mesonephros. a, Transverse sections of E17.5 ovary and mesonephros from *Gli1-LacZ* reporter embryos (n=3) after LacZ staining (blue). The sections were counterstained with nuclear FastRed (red). **b,** Higher magnification of the outlined area (mesonephros) in (a). Ov, ovary; Ms, mesonephros. Scale bar: 100 μ m.



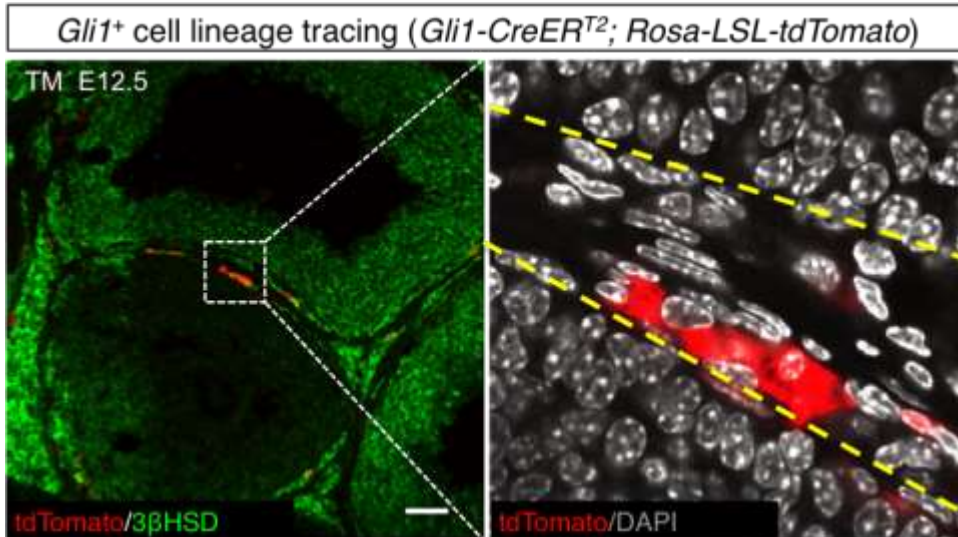
Supplementary Figure 2: Expression of *Gli1-lacZ* in perinatal ovaries. a-c, Expression of *Gli1-LacZ* in the developing ovaries (from E18.5 to P2) was detected by whole mount beta-galactosidase staining. N=3-5 for each specimen. Scale bar: 500 μ m.



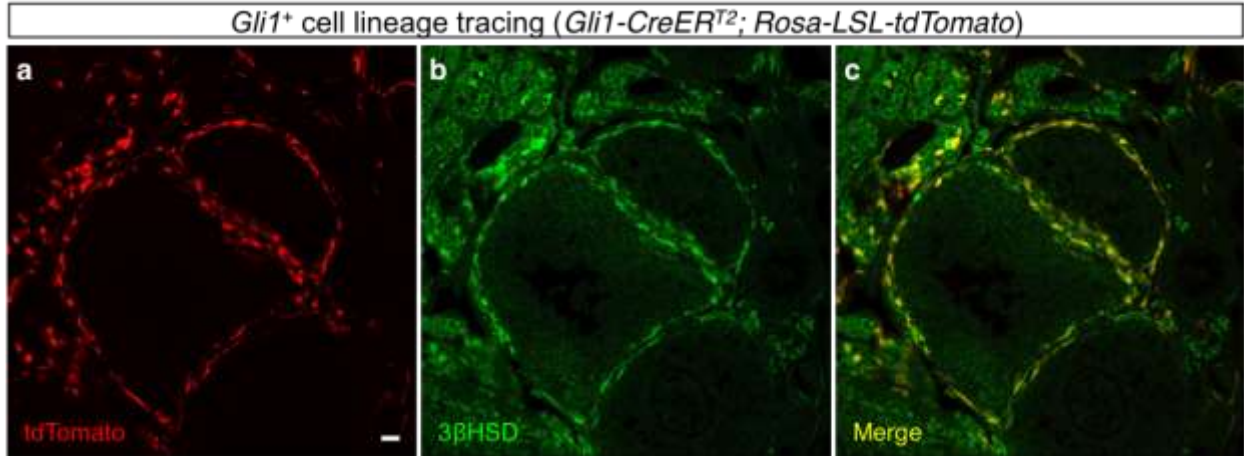
Supplementary Figure 3: Cre-mediated tdTomato expression is not observed in the absence of Cre or tamoxifen. a-c, Tamoxifen or oil was administered at E12.5 and the ovaries were analyzed for tdTomato (red) at birth. tdTomato expression is only detected in *Gli1-CreER^{T2}; Rosa-LSL-tdTomato* ovaries that were treated with tamoxifen (a), but not in the Cre-negative control (b), nor the oil-treated control (c). N=3-4 for each specimen. Scale bar: 500 μ m.



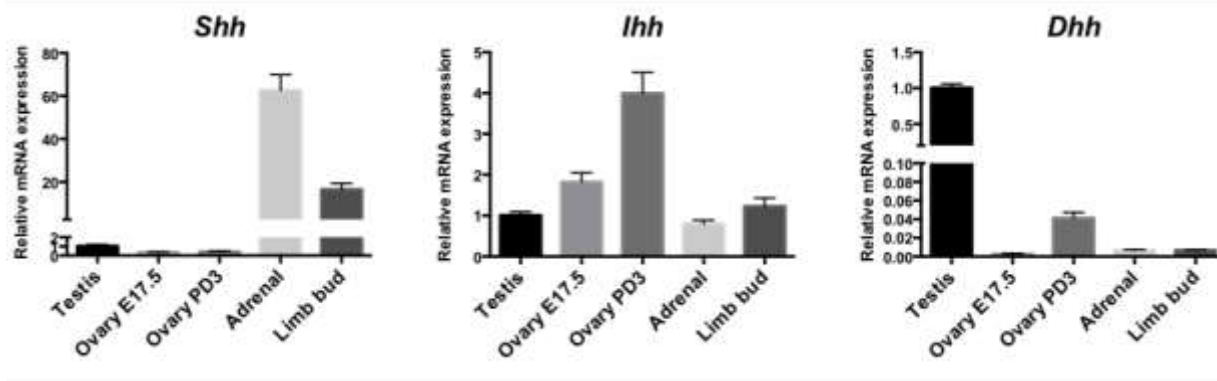
Supplementary Figure 4: Lineage-tracing experiments for the mesonephros-derived *Gli1*-positive cells in the perinatal ovary. **a-c**, The lineage-tracing of mesonephros-derived *Gli1*-positive cells in the *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* embryos was induced by tamoxifen administration from E12.5-E14.5, and the ovaries were analyzed at P5 for tdTomato (red) and granulosa cell marker FOXL2 (cyan). E, embryonic day; P, postnatal day. **d-f**, Higher magnification of the outlined areas. Scale bar: 25 μ m. (c & f) are merged images of (a & b) and (d & e), respectively. N=3 for the *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* specimens.



Supplementary Figure 5: The mesonephros-derived *Gli1*-positive cells are located immediately adjacent to the basal membrane in adult ovary. Lineage-tracing of the *Gli1*-positive cells in the *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* embryos were induced by tamoxifen (TM) administration at E12.5. The ovaries were examined at 2 months of age for tdTomato, 3βHSD, and DAPI. Yellow dotted line indicates the basal membrane that separates the theca cell layer from granulosa cells. N=6 for the *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* specimens. Scale bar. 25 μm.

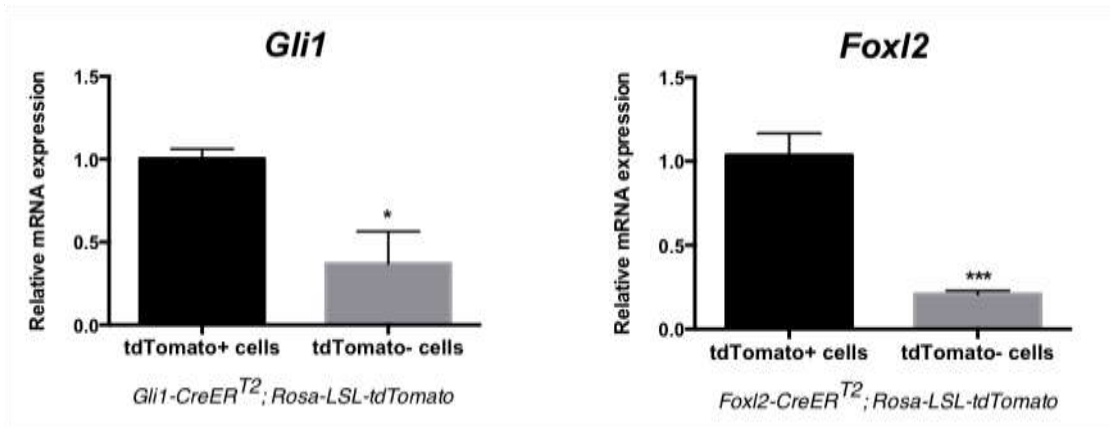


Supplementary Figure 6: Mesonephros-derived *Gli1*-positive cells are steroidogenically active in the adult ovary. a-c, The lineage-tracing of the mesonephros-derived *Gli1*-positive cells in *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* embryos was induced by tamoxifen administration from E12.5-14.5 and the ovaries were analyzed at 2 months of age for tdTomato (red) and steroidogenic cell marker 3βHSD (green). N=4 for the *Gli1-CreER^{T2}*; *Rosa-LSL-tdTomato* specimens. Scale bar: 25 μm.

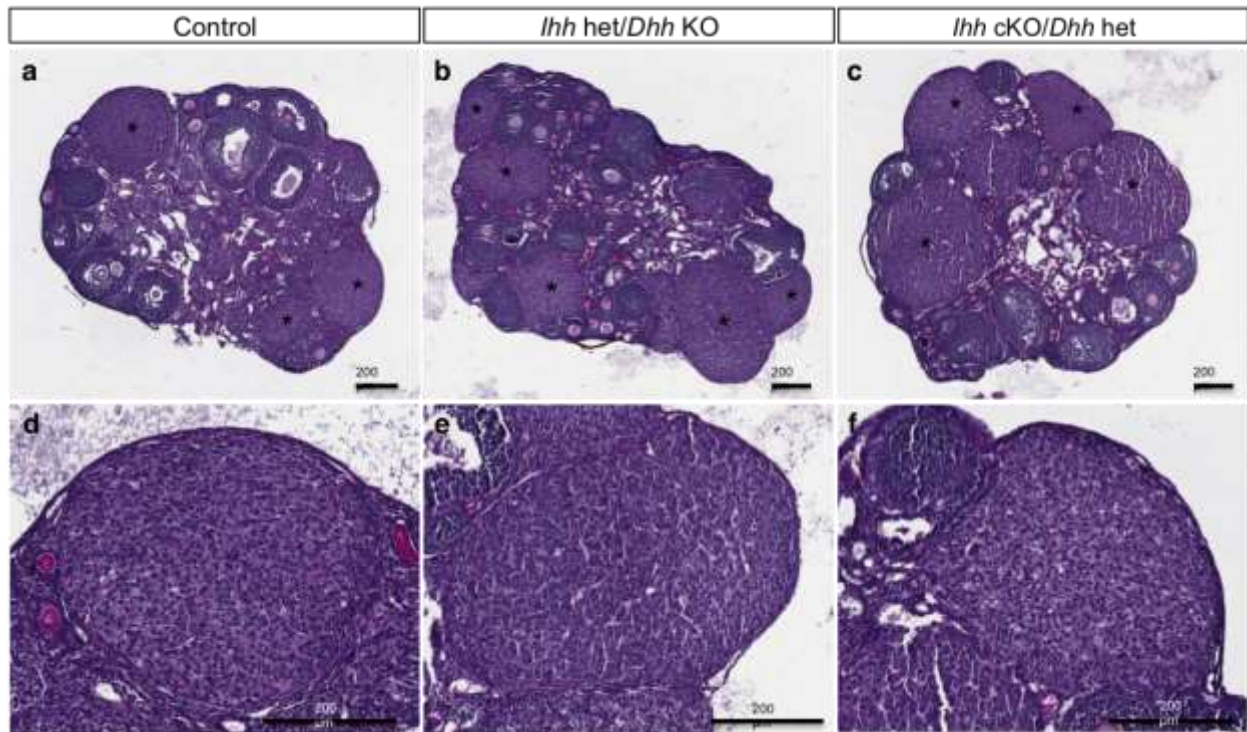


Supplementary Figure 7: qPCR analysis of *Shh*, *Ihh*, and *Dhh* in the perinatal ovaries.

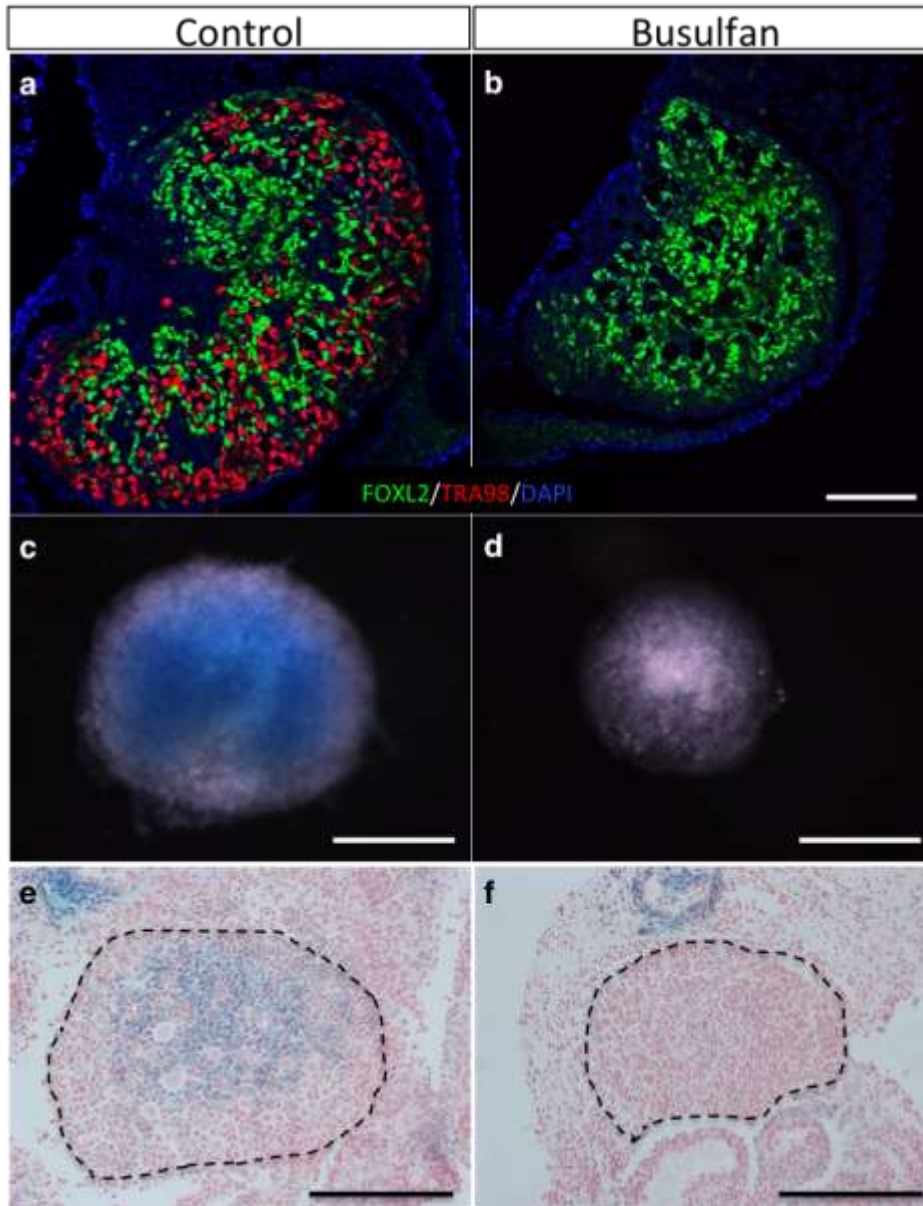
Testis (P3) (n=4), adrenal (P3) (n=4) and limb bud (E17.5) (n=4) were included as negative (testis for *Shh*) and positive control (adrenal for *Shh*; limb bud for *Shh* and *Ihh*; testis for *Dhh*). n=5 for the E17.5 ovaries and n=5 for the P3 ovaries. Results were normalized to *18S*, and the expression levels in the testis were set as 1.



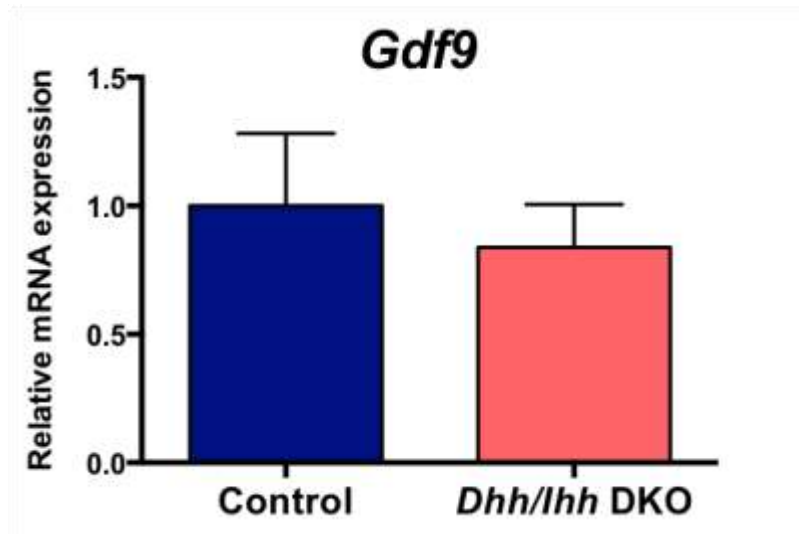
Supplementary Figure 8: qPCR validation of *Gli1*⁺ theca progenitor cells and *Foxl2*⁺ granulosa cells. Tamoxifen was administered to pups from P1-2 through lactating dams to induce tdTomato expression in theca progenitor cells (*Gli1-CreER^{T2}; Rosa-LSL-tdTomato* mice) (n=3) and granulosa cells (*Foxl2-CreER^{T2}; Rosa-LSL-tdTomato* mice) (n=5). Cells isolated from a pair of ovaries were pooled as n of 1. The ovaries from P5 pups were subjected to fluorescence-activated cell sorting. tdTomato⁺ cells and tdTomato⁻ cells were obtained for qPCR analysis for the expression of *Gli1* and *Foxl2*. Results were normalized to *18S*, and the expression levels in tdTomato⁺ cells were set as 1. *P < 0.05; ***P < 0.001; Two-tailed Student's *t*-test. Values in all graphs are presented as means±s.e.m.



Supplementary Figure 9: Ovarian folliculogenesis appears normal in the absence of *Dhh* or *Ihh* in the adult ovary. **a-c**, PAS/ Hematoxylin staining of ovarian sections from control (*Sfl-Cre; Ihh*^{+/+}; *Dhh*^{+/+}) (n=7), *Ihh* het/*Dhh* KO (*Sfl-Cre; Ihh*^{f/+}; *Dhh*^{-/-}) (n=3) and *Ihh* KO/*Dhh* het (*Sfl-Cre; Ihh*^{f/-}; *Dhh*^{+/+}) ovaries (n=3) at 2 months of age. Asterisks indicate the presence of corpora lutea. **d-f**, Higher magnification images of corpus luteum. Scale bar: 200 μm.



Supplementary Figure 10: Effects of *in utero* busulfan treatment on oocytes and the expression of *Gli1* in the ovary. a-b, E18.5 control (n=9) and busulfan-treated ovaries (n=15) were analyzed by immunofluorescence detection for granulosa cell marker FOXL2 (green), germ cell marker TRA98 (red), and nuclear counterstain DAPI (blue). Scale bar: 100 μ m. **c-f**, *Gli1-LacZ* expression (blue) in the control and busulfan-treated ovaries after 3 days of culture (c & d are whole mount) and (e & f are sections counterstained with FastRed). Scale bar: 500 μ m.



Supplementary Figure 11: qPCR analysis of *Gdf9* expression in control and *Dhh/Ihh* DKO (*Sf1-Cre; Ihh^{f/-}; Dhh^{-/-}*) ovaries. n=4 for control ovaries and n=3 for *Dhh/Ihh* DKO ovaries. Results were normalized to *18S*, and the expression levels in the control (*Sf1-Cre; Ihh^{f/+}; Dhh^{+/-}*) ovaries were set as 1. Two-tailed Student's *t*-test was applied and P=0.67. Values are presented as means±s.e.m.

Genes of interest	Fold change (Ovary derived <i>Gli1</i> ⁺ cells vs. mesonephros-derived <i>Gli1</i> ⁺ cells)
<i>Pgr</i>	4.113
<i>Wt1</i>	2.374
<i>Esr1</i>	2.881
<i>Bmp2</i>	2.029
<i>Cxcl2</i>	3.800
<i>Cxcl3</i>	4.890
<i>Cxcl12</i>	2.487
<i>Foxo3</i>	1.869
<i>HSD17b3</i>	-1.549

Supplementary Table 1: Fold changes of genes of interest from microarray analysis of ovary-derived *Gli1*-positive cells versus mesonephros-derived *Gli1*-positive cells from 2 months of age mice. n=3 for independent pools of ovary-derived *Gli1*-positive cells and mesonephros-derived *Gli1*-positive cells.