

Follicular development of fetal gonads under the skin of adult mice

Jiyu Chen^{1,2,#}, Chang Liu^{1,2,#}, Yongqin Yu^{1,2}, Xiaoying Ye^{1,2}, Lin Liu^{1,2,3,*},
Zhengmao Zhu^{1,2,3,*}

¹Department of Genetics and Cell Biology, College of Life Science, Nankai University, Tianjin 300071, China

²State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300350, China

³Haihe Laboratory of Cell Ecosystem, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China

#These authors contributed equally to this work.

*Correspondence: liulin@nankai.edu.cn (L.L.), zhuzhengmao@nankai.edu.cn (Z.Z.)

Figure S1. Developmental defects of E12.5 gonads following subcutaneous transplantation.

(A) Morphology of SC grafts after transplantation for 16 days and 8 weeks. (B) Sixteen-day and 8-week subcutaneous grafts were embedded in paraffin, and 5- μ m-thick sections were prepared and stained with H&E. Scale bar: 100 μ m.

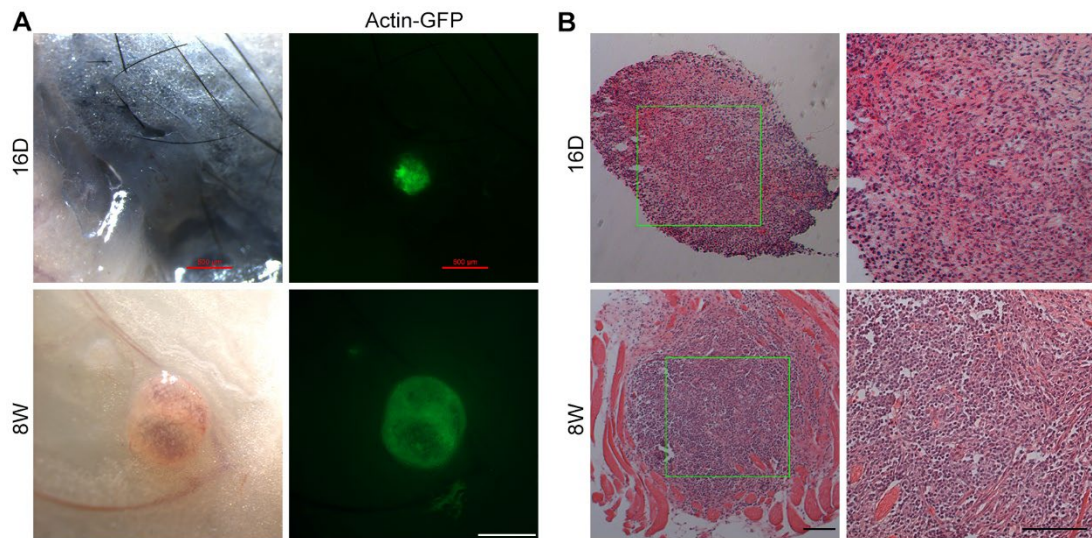


Figure S2. Decrease in germ cell number during development in subcutaneous grafts.

(A) Immunofluorescence staining of Ddx4 in gonads cultured *in vitro*, KC and SC grafts at 2, 4, 6 and 8 days. Scale bar: 20 μ m. (B) Number of Ddx4-positive cells in gonads cultured *in vitro*, KC and SC grafts at 2, 4, 6 and 8 days. Ddx4-positive cells were quantified by aggregating the counts from every fifth section throughout the entire tissue. $n \geq 3$. Mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Tukey's multiple comparisons test for (B).

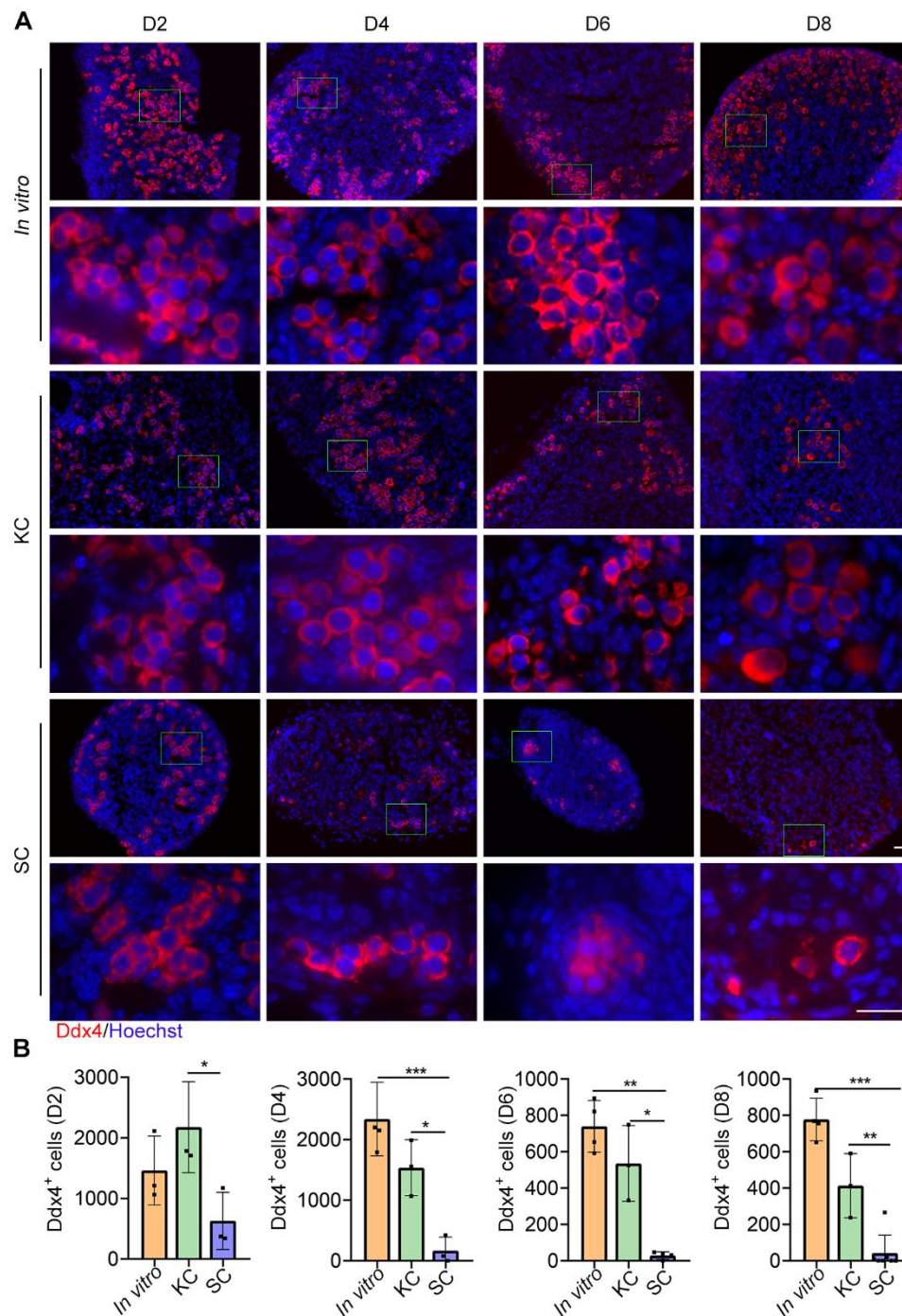


Figure S3. Follicle development of E12.5 gonads *in vitro*.

(A) Morphology of rOvaries reunited for one day and two days *in vitro* in various medium within a U-bottom 96-well plate. The culture media included GK15, GMEM plus 15% KSR. MF10 + Rocki + VC (MF10++), M199 plus 10% FBS, vitamin C and the Rock inhibitor ICI 182,780. Scale bar: 100 μ m. (B) Morphology of rOvaries cultured in transwell during development *in vitro*. Scale bar: 100 μ m. (C) Diameter changes of rOvaries during *in vitro* development. $n \geq 3$. (D) Twenty-day *in vitro* cultured rOvaries were embedded in paraffin, and sections were prepared and stained with H&E. Scale bar: 100 μ m. (E) Number of follicles in 20-day cultured rOvaries within the GK15 or MF10 + Rocki + VC culture system. (F) Percentage of follicles at various developmental stages in 20-day *in vitro* cultured rOvaries. $n \geq 3$. Mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Student's t test for (E), (F).

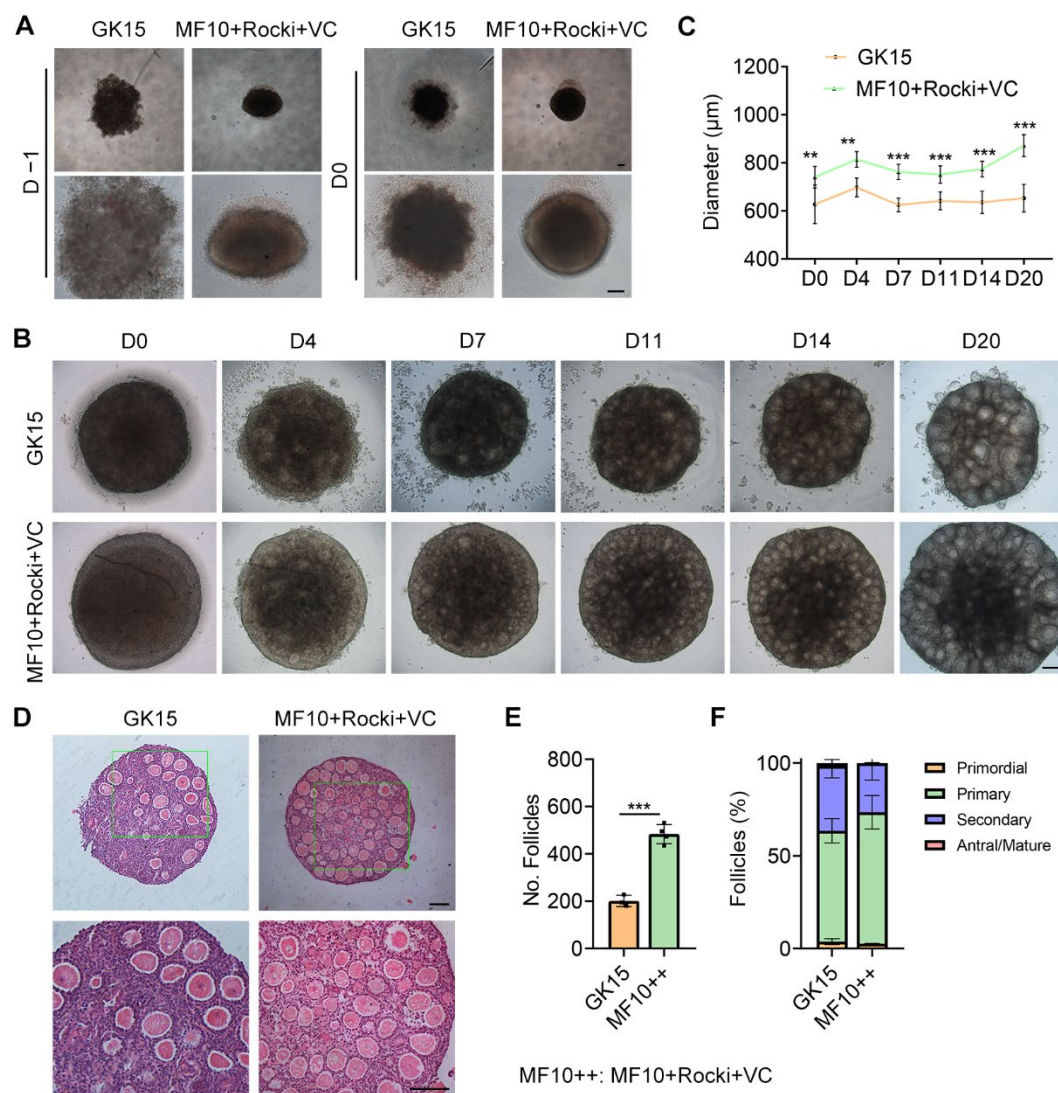


Figure S4. Folliculogenesis following subcutaneous transplantation of rOvary.

(A) Morphology of 10-day and 25-day grafts at the subcutaneous site after transplanting a 6-day *in vitro* cultured rOvary (D6–D10, D6–D25). Scale bar: 500 μ m. (B) Immunofluorescence staining of Ddx4 and Foxl2 in D6–D10 and D6–D25 subcutaneous transplants. Scale bar: 20 μ m. (C) H&E staining of D6–D10 and D6–D25 subcutaneous transplant sections. Scale bar: 100 μ m. (D) Number of follicles of D6–D10 and D6–D25 subcutaneous transplants. $n \geq 3$. Mean \pm SD. (E) Percentage of follicles at different developmental stages in D6–D10 and D6–D25 subcutaneous transplants. $n \geq 3$. Mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. Student's *t* test for (D), (E).

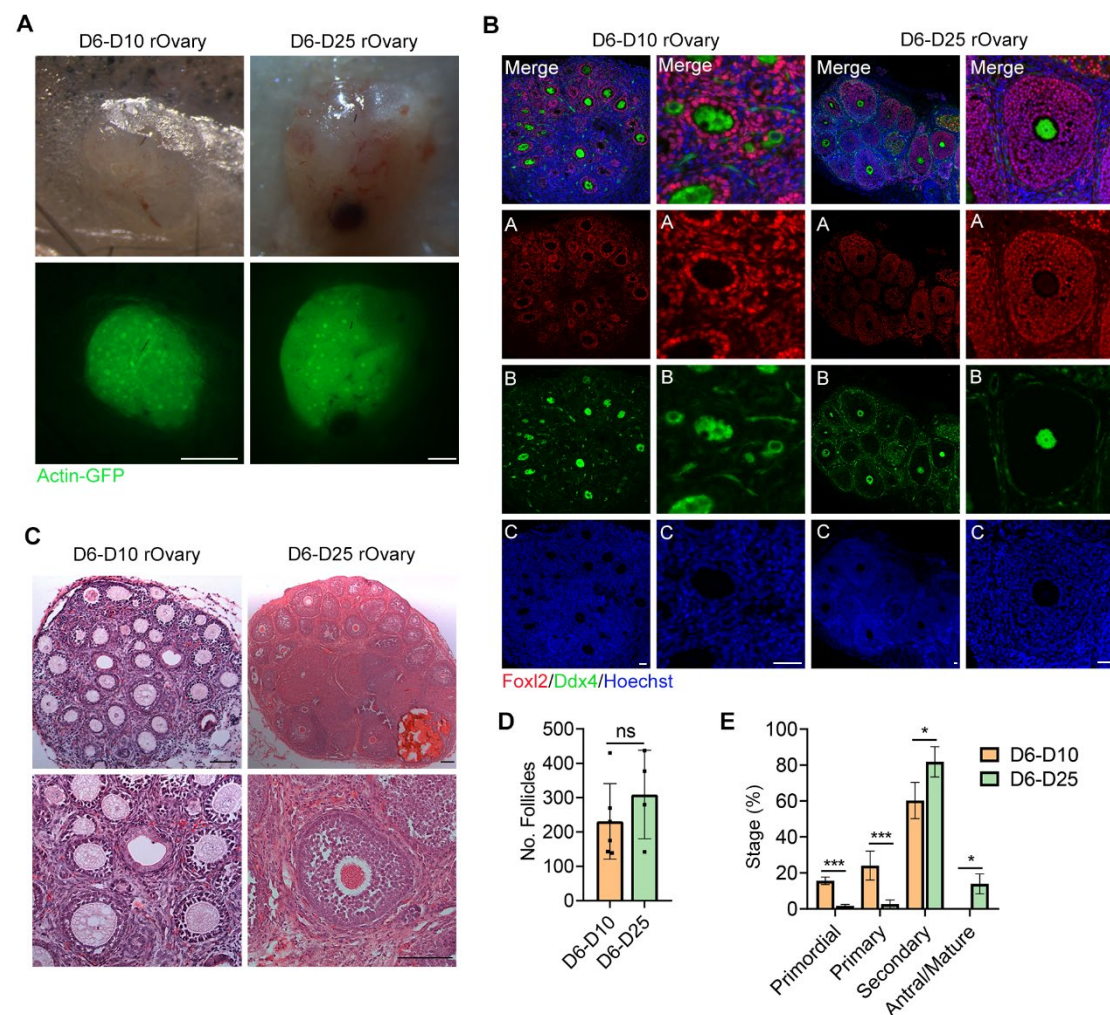


Figure S5. Completion of meiosis in 8-day *in vitro* cultured gonads.

(A) Immunofluorescence staining of Ddx4 and Foxl2 in 6-day and 8-day cultured gonads. Scale bar: 20 μ m. (B) Immunofluorescence staining of SYCP3 in 6-day and 8-day cultured gonads. Scale bar: 20 μ m.

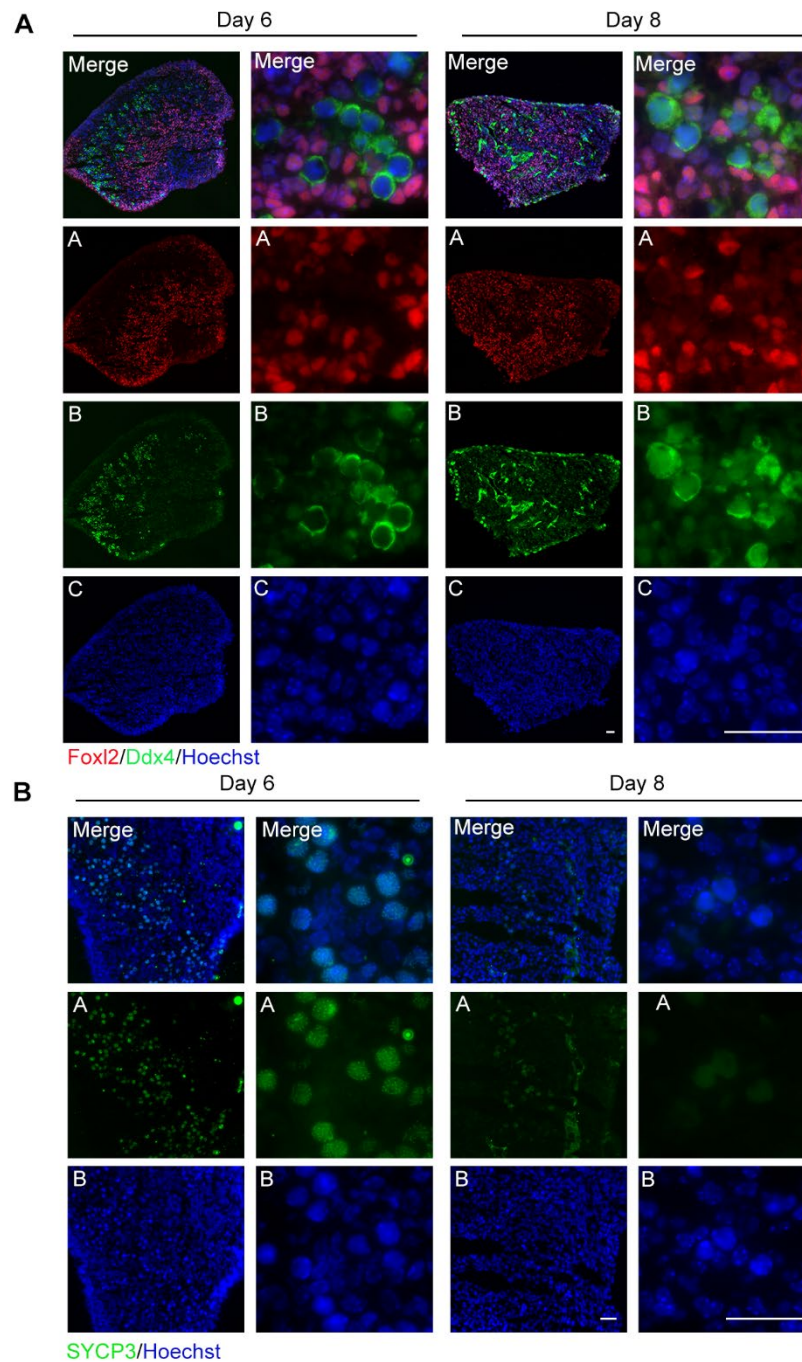


Figure S6. Follicles derived from the SC-transplanted fetal gonad fail to produce live offspring.

(A) GV oocytes isolated from control ovary, KC grafts, and SC grafts, along with embryo development following IVM and IVF. Scale bar: 100 μ m. (B) Summary of GV oocytes development to the blastocyst stages. (C) Germline competency assessment of Control-ovary-derived, KC-grafts-derived, and SC-grafts-derived mice by injection 2-cell embryos into surrogate mothers. Pseudo-pregnant ICR mice served as surrogate mothers. (D) Summary of Control-ovary-derived, KC-grafts-derived, and SC-grafts-derived pups following the injection of the 2-cell embryos into surrogate mothers.

