Stem Cell Reports, Volume *17*

Supplemental Information

FGFR3 is expressed by human primordial germ cells and is repressed after meiotic initiation to form primordial oocytes

Tsotne Chitiashvili, Fei-man Hsu, Iris Dror, Kathrin Plath, and Amander Clark

Supplemental information

FGFR3 is Expressed by Human Primordial Germ Cells and is Repressed after Meiotic Initiation to Form Primordial Oocytes

Tsotne Chitiashvili, Fei-man Hsu, Iris Dror, Kathrin Plath & Amander Clark

Figure S1. FGFR3 mRNA is expressed on germ cells in the prenatal human testis.

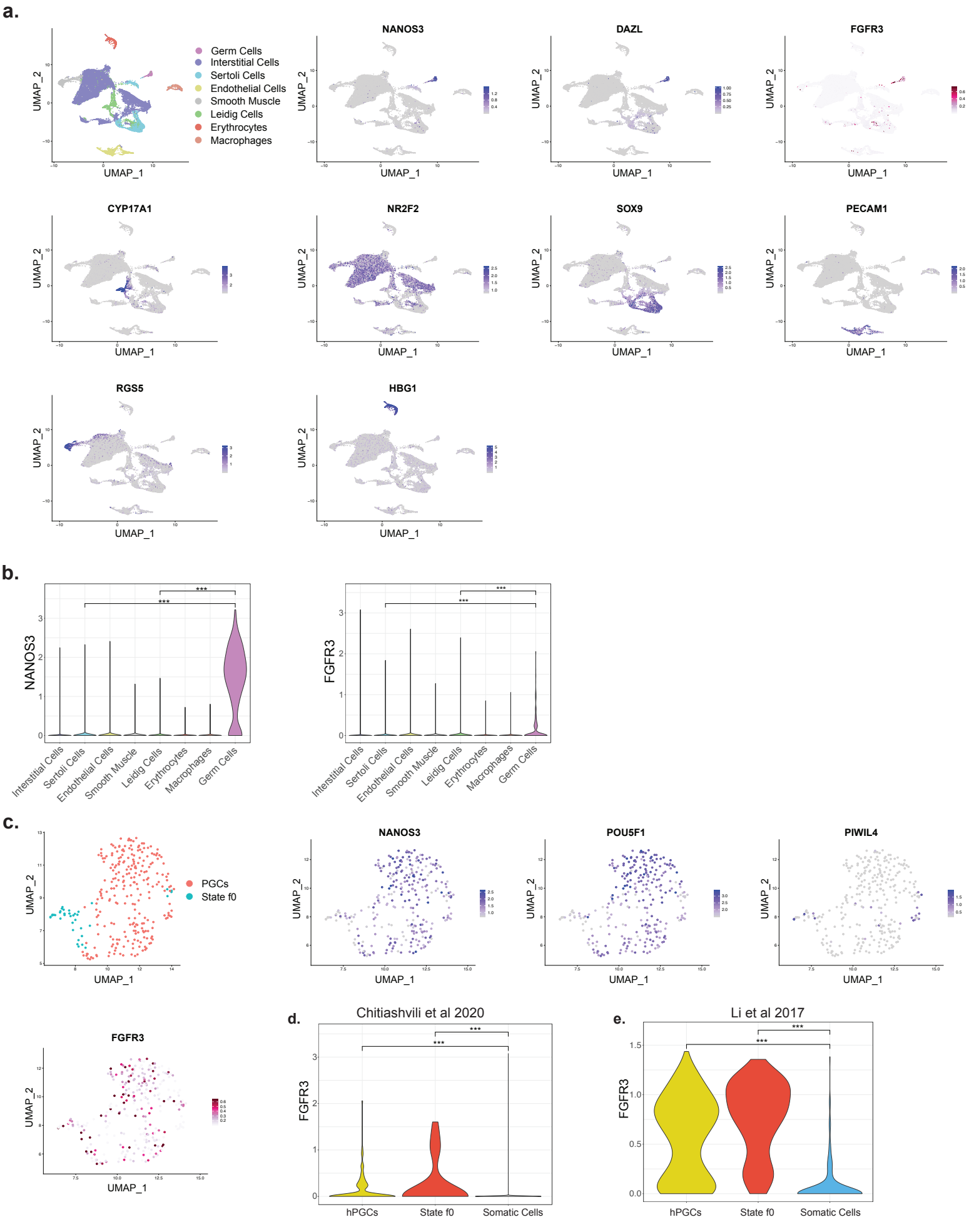
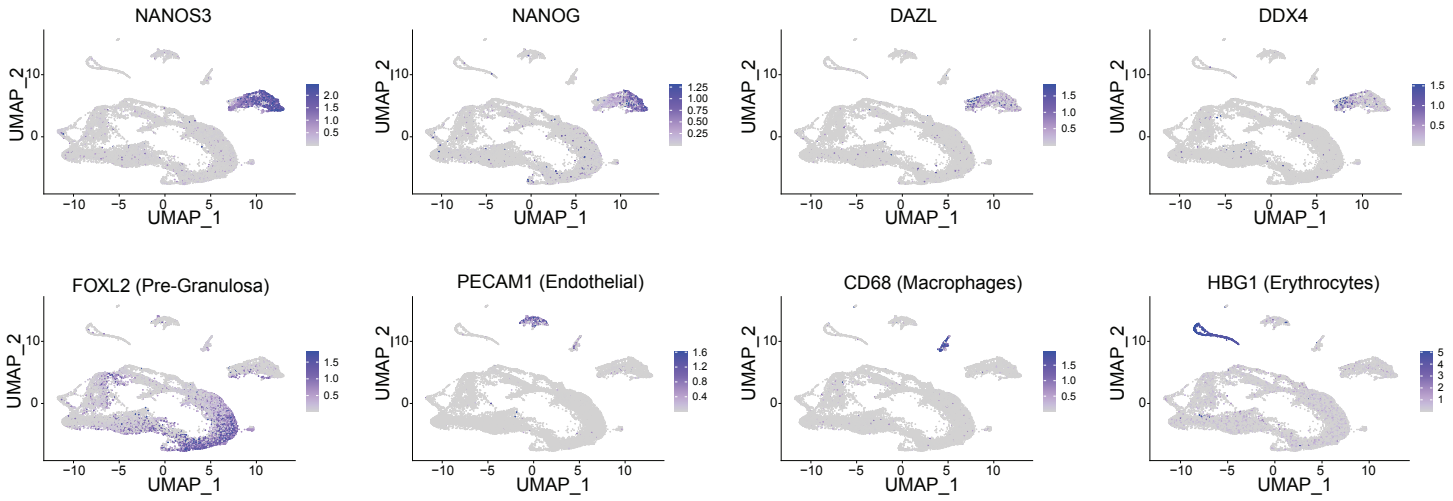


Figure S2. Cell type annotation of prenatal ovaries

a.



b.

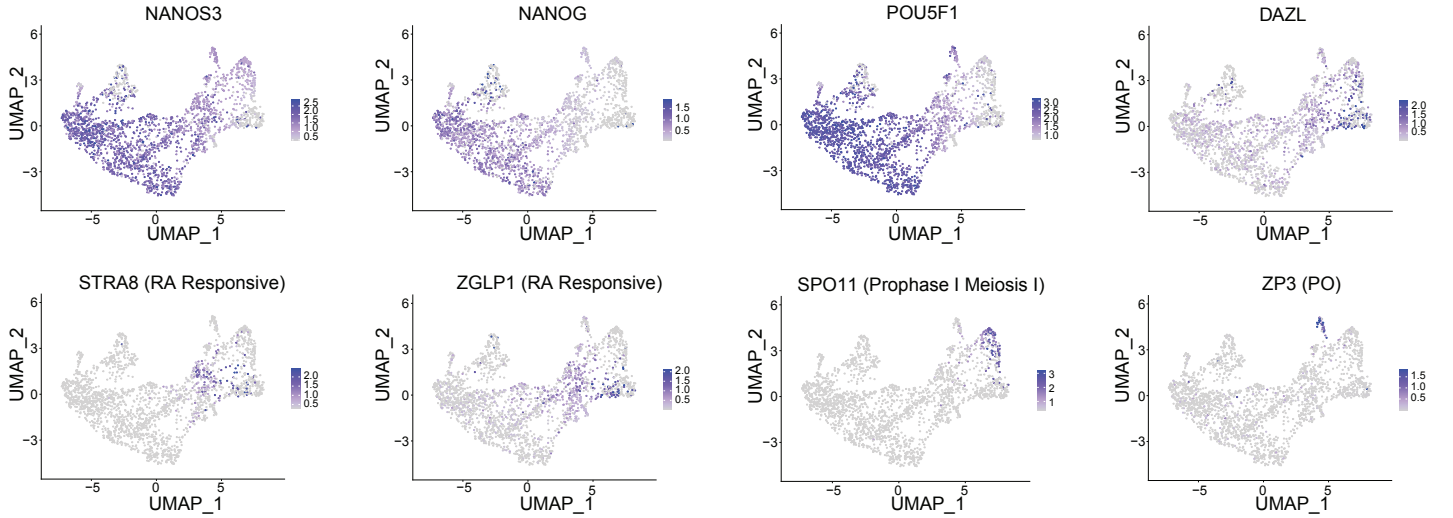


Figure S3. FGFR3 mRNA is predominantly expressed by ovarian germ cells

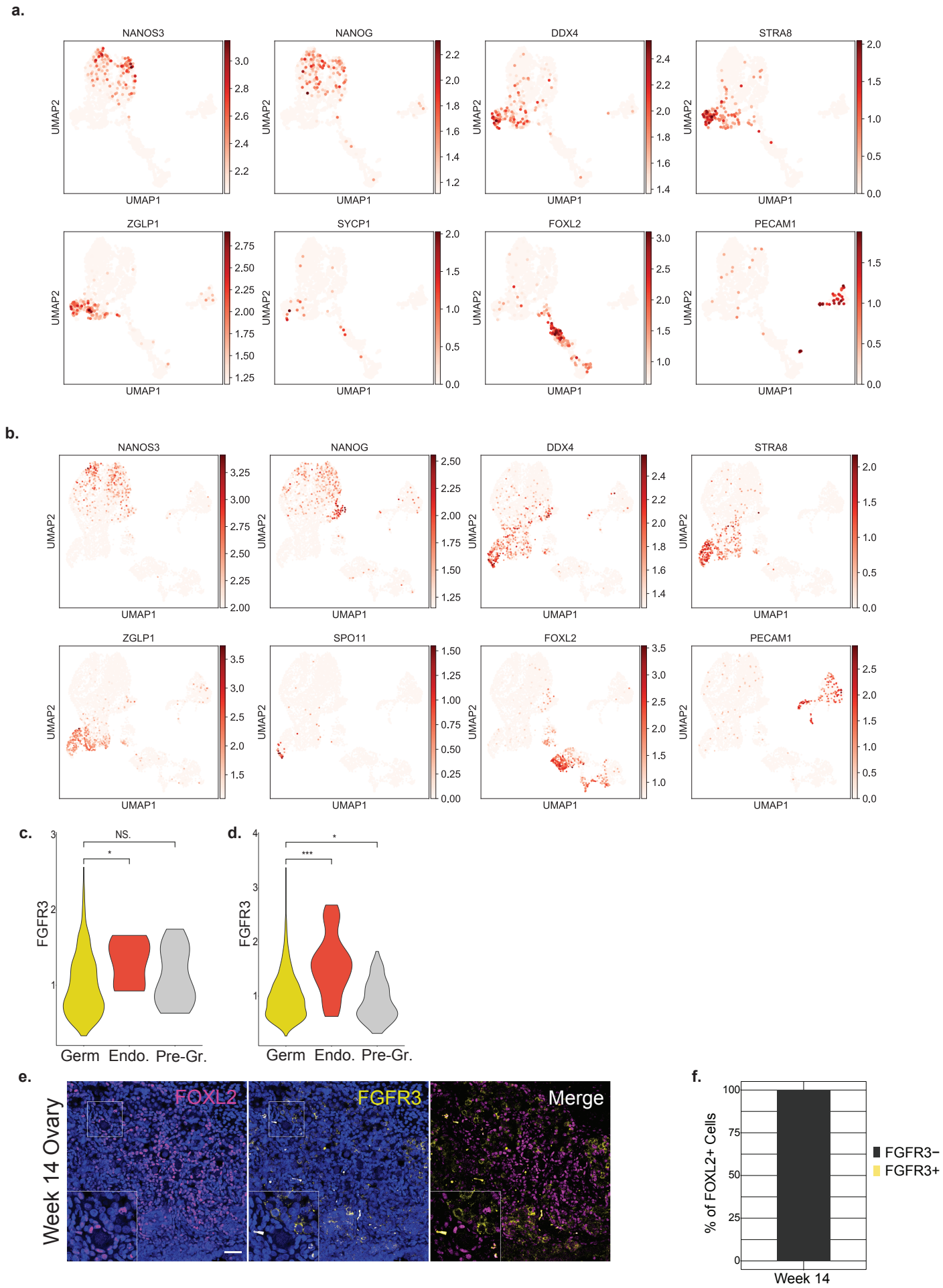
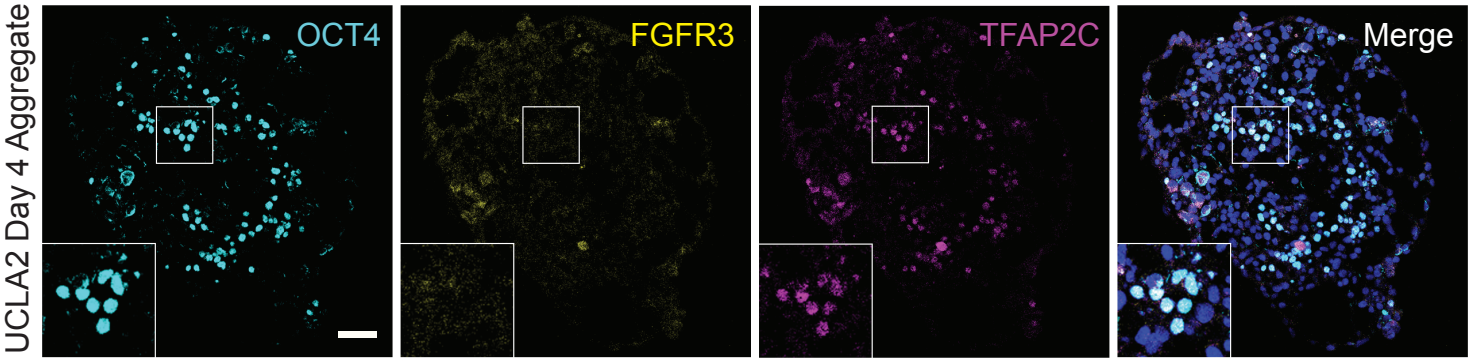


Figure S4. FGFR3 protein is not detectable in PGCLCs *in vitro*



Supplemental figure legends

Supplemental Figure 1. FGFR3 mRNA is expressed on germ cells in the prenatal human testis.

a. Annotation of testis cell types based on diagnostic markers for each cell type. NANOS3 and DAZL for germ cells. NR2F2 marks interstitial cells, CYP17A1 marks leidig cells, PECAM1 endothelial cells, HBG1 erythrocytes, RGS5 smooth muscle cells, SOX9 sertoli cells. **b.** Expression of PGC specific marker NANOS3 and FGFR3 in testicular somatic cells and PGCs. **c.** Annotation of testicular germ cells based on their stage specific marker expression. NANOS3 and POU5F1 marking PGCs and PIWIL4 marking stage f0 prospermatogonia. FGFR3 expression in PGCs and state f0 bottom panel. **d-e.** Expression of FGFR3 in male PGCs state f0 prospermatogonia and somatic cells, data from Chitiashvili et al [17] and Li et al dataset [18]. ***P<0.001, Statistical significance was assessed by Wilcoxon test.

Supplemental Figure 2. Cell type annotation of prenatal ovaries.

a. Expression of diagnostic markers for cell type annotation of ovarian cells. NANOS3, NANOG, DDX4 and DAZL marking germ cells, FOXL2 pre-granulosa cells, PECAM1 endothelial cells, CD68 macrophages and HBG1 erythrocytes. **b.** Expression of germ cell stage specific markers in female germ cells. NANOS3, NANOG, POU5F1 and DAZL expressed in PGCs. STRA8 and ZGLP1 in retinoic acid (RA) responsive meiotic germ cells. SPO11 in prophase I of meiosis I and ZP3 marker of primordial oocytes.

Supplemental Figure 3. FGFR3 mRNA is predominantly expressed by ovarian germ cells.

a. Expression of diagnostic markers for cell type annotation in week 8 and week 13 (**b**) ovarian samples. NANOS3 and NANOG marking PGCs, DDX4, STRA8, ZGLP1 and SYCP1 meiotic germ cells, while FOXL2 and PECAM1, pre-granulosa and endothelial cells accordingly. **c-d.** Expression levels of FGFR3 in week 8 and 13 Germ cells (Germ), Endothelial (Endo) and Pre-granulosa cells (Pre-Gr) respectively with detectable levels of FGFR3. Cells from each category that had no detectable FGFR3 values were excluded from the analysis. **e.** Immunostaining of Week 14 ovary for pre-granulosa marker FOXL2 (magenta) and FGFR3 (yellow) n=2 experiments. **f.** Quantification of FGFR3- and FGFR3+ cell proportion that are FOXL2+ (70 cells counted from week 14 ovary from 2 independent experiments). ***P<0.001, Statistical significance was assessed by Wilcoxon test.

Supplemental Figure 4. FGFR3 protein is not detectable in PGCLCs *in vitro*.

UCLA2 hESCs differentiated into PGCLCs for 4 days. At day 4 aggregates were immunostained for OCT4 (cyan), FGFR3 (yellow) and TFAP2C (magenta), n=2 differentiation experiments. Lower panel week 14 ovary staining with VASA (cyan), FGFR3 (yellow) and TFAP2C (magenta) used as a positive control for FGFR3 staining.

Supplemental methods

Immunofluorescence

Slides of paraffin-embedded sections were deparaffinized by successive treatment with xylene and 100%, 95%, 70% and 50% ethanol. Antigen retrieval was performed by incubation with 10 mM Tris pH 9.0, 1 mM EDTA, 0.05% Tween-20 at 95 °C for 40 min. The slides were cooled to room temperature and washed with 1× PBS and 1× TBS (PBS + 0.2% Tween-20). Afterwards, the samples were permeabilized with 0.5% Triton X-100 in 1× PBS, then washed with 1× TBS and blocked with 5% normal donkey serum in 1× TBS. Primary antibody incubation was conducted with 5% normal donkey serum overnight at 4C. Samples were again washed with 3× TBS-Tween-20 and incubated with fluorescent secondary antibodies at 1:100 for 1 hour, then washed and counterstained with DAPI for 5 min and mounted using Vectashield. A list of the primary antibodies used for immunofluorescence in this study is provided in Supplementary Table 1 under the antibody list tab. The secondary antibodies used in this study were all obtained from Life technologies and were used at 1:400 dilution. Images were taken using LSM 880 Confocal Instrument (Zeiss) or Zeiss Axio Imager M1. For image processing and analysis, Fiji (ImageJ) was used. For nuclear size quantification, images were converted into 8-bit images and then analyzed using profile plot tool. Intensity values were exported as a CSV file and then R Studio and the ggplot2 package was used for plotting.

PGCLC differentiation

PGCLCs were induced from primed UCLA1 and UCLA2 hESCs as described previously (Chen, Liu, et al. 2017) starting with human pluripotent stem cells grown on MEFs. In brief, hESCs and hiPSCs

were dissociated into single cells with 0.05% trypsin-EDTA (GIBCO, 25300-054) and plated onto human-plasma-derived fibronectin-coated (Invitrogen, 33016-015) 12-well plates at a density of 200,000 cells per well in 2 ml per well of iMeLC medium (15% KSR (GIBCO, 10828-028), 1× penicillin–streptomycin–glutamine (GIBCO, 10378-016), 0.1 mM 2-mercaptoethanol (GIBCO, 21985-023), 1 mM sodium pyruvate (GIBCO, 11360-070), 1× NEAA (GIBCO, 11140-050), 3 mM CHIR99021 (Stemgent, 04-0004), 10 mM of ROCKi (Y27632, Stemgent, 04-0012-10), 50 ng ml^{-1} activin A (Peprotech, AF-120-14E), and 50 ng ml^{-1} primocin in Glasgow's MEM (GMEM) (GIBCO, 11710-035)). After 24 h, using 0.05% trypsin, iMeLCs were dissociated into single cells and plated into ultra-low cell attachment U-bottom 96-well plates (Corning, 7007) at a density of 3,000 cells per well in 200 ml per well of PGCLC medium, which is composed of 15% KSR (GIBCO, 10828-028), 1× NEAA (GIBCO, 11140-050), 0.1 mM 2-mercaptoethanol (GIBCO, 21985-023), 1 mM sodium pyruvate (GIBCO, 11360-070), 10 ng per ml^{-1} human LIF (Millipore, LIF1005), 1× penicillin–streptomycin-–glutamine (GIBCO, 10378-016), 200 ng ml^{-1} human BMP4 (R&D systems, 314-BP), 50 ng ml^{-1} human EGF (R&D systems, 236-EG), 10 mM of ROCKi (Y27632, Stemgent, 04-0012-10) and 50 ng ml^{-1} primocin in GMEM (GIBCO, 11710-035). Day 4 aggregates were collected and embedded in a paraffin block that was afterwards used for sectioning as described before (Chen, Liu, et al. 2017).

Tissue processing for scRNA-seq

Fetal tissues were processed 24–48 h after termination. On arrival, tissues were washed with PBS and dissociated using mix of collagenase IV 10 mg ml^{-1} (Life Technologies, 17104-019), dispase II 250 µg ml^{-1} (Life Technologies, 17105041), DNase I 1:1,000 (Sigma-Aldrich, 4716728001), 10%

fetal bovine serum (Life Technologies, 10099141) in 1× PBS. Tissues were dissociated for 15 min at 37 °C. In every 5 min, the tissues were pipetted against the bottom of Eppendorf tube using p1000 pipette. Afterwards, cells were centrifuged for 5 min at 500*g*, resuspended in 1× PBS with 0.04% BSA, strained through a 40 µm strainer to get rid of clumps and counted using an automated cell counter (Thermo Fisher Scientific, Countess II). Afterwards cells were used for FACS sorting.

FACS sorting

For FACS sorting tissues were dissociated as described above. The dissociated cells were stained with conjugated antibodies, washed with FACS buffer (1% BSA in PBS) and resuspended in FACS buffer with 7-AAD (BD PharMingen, 559925) as viability dye. The conjugated antibodies used in this study FGFR3 conjugated with PE (R&D systems FAB766P), 1:60 dilution. Prior to sorting cells suspension was filtered through 40micron strainer. Sorting was performed for further experiments using BD FACSAria FACS machine. FACS data were analyzed using FlowJo v.10. Dead cells were excluded from this analysis (by selecting 7AAD negative cells), and negative gates were set based on unstained cells from the same samples. For scRNA-seq experiments 7-AAD negative and FGFR3 positive cells were collected in PSB+0.04% BSA. Cells from week 8 and week 13 tissues were mixed at 1:3 ratio to generate a single 10X library.

scRNA-seq library preparation

scRNA-seq libraries were generated using the 10x Genomics Chromium instrument and Chromium Single Cell 3’ Reagent Kit v3. Library was designed to target 10,000 cells and library

was generated according to the manufacturer's instructions and library fragment size distribution was determined using a Tapestation instrument. Library was sequenced using an Illumina Novaseq 6000 platform, at an average depth of 300–350 million reads per sample.