

## SUPPLEMENTAL INFORMATION

### SUPPLEMENTAL FIGURE LEGENDS

#### **Figure S1: Distribution of single-cell RNA-seq data by embryonic and fetal age, related to Figure 12.**

**A:** Deconvolution of Fig. 1B according to donor age.

**B:** Dotplot comparing percentage expression of selected markers in cell clusters identified in Fig. 1C. Normalized expression is plotted on a high-to-low scale (purple-grey), with dot size increasing with the percentage of cells within a cluster (0% – 75%) that express a given gene.

**C:** Deconvolution of Fig. 2A progenitor cluster according to donor age.

#### **Figure S2: Gonadal somatic cell lineage specification during sex determination, related to Figure 2.**

**A:** Immunofluorescence staining for VASA (yellow) and NR2F2 (magenta) in fetal ovaries and testes at W7-15. Nuclei counterstained with DAPI (grey). Cortex (c) and medulla (m) or tunica albuginea (ta) and epididymis (e) labels included to show section image orientation. Scale bars 50  $\mu$ m.

**B:** Immunofluorescence for selected sex-indifferent markers in fetal testes from W7-16 – LAMININ (yellow), NR2F2 (magenta), PDGFRA (cyan) and DAPI nuclear stain (grey). Tunica albuginea (ta) and epididymis (e) labels included to show section image orientation. Scale bars 50  $\mu$ m.

**C:** Immunofluorescence for selected sex-specific markers FOXL2 (cyan) or SOX9 (yellow) in fetal ovaries or testes respectively from W7-12, co-stained with NR2F2 (magenta). Scale bars 50  $\mu$ m.

**D:** Model of testis development between W7-16.

#### **Figure S3: Gene expression dynamics during ovarian granulosa lineage specification, related to Figures 3 and 4.**

**A:** Violin plots showing upregulated genes in bipotential (green), bipotential transitional (blue) or pre-granulosa cells (purple) compared to common progenitor cells (yellow). Cell clusters along the x-axis from left to right are in the same order as the row in the heatmap from Fig. 3B.

**B:** Expression of additional markers specific for common progenitors or bipotential cells cast on the UMAP plot from Fig. 4A. Normalized expression is plotted on a high-to-low scale (purple-grey).

**C:** Immunofluorescence staining for KRT19 (yellow) and FOXL2 (magenta) in fetal ovary samples at W7-15 with DAPI counterstain in gray. Cortex (c) and medulla (m) labels included to show section image orientation. Scale bars 20  $\mu$ m.

**D:** Immunofluorescence staining for KRT19 (yellow) and FOXL2 (magenta) in fetal ovary samples at W6-7 with DAPI counterstain in gray. Cortex (c) and medulla (m) labels included to show section image orientation. Scale bars 15  $\mu\text{m}$ .

**E:** Violin plots showing upregulated genes in PG1 (red), PG2 (green) or epithelial (blue) pre-granulosa cells.

**Figure S4: Gene expression dynamics as ovarian PGCs differentiate, related to Figure 5.**

**A:** UMAP showing the developmental progression of combined fetal ovary and testis germ cells over time. Testis PGCs go from PGCs to state f0 cells; ovary PGCs generally progress into RA-responsive meiotic germ cells (MGCs), MGCs and on to primordial oocytes, but a small arm branches off into state f0 alongside testis germ cells.

**B:** UMAP plot from Fig. S4A with cells colored by Seurat analysis-designated cell groups (labelled 1 – 6). Genes enriched in the broader clusters (outlined in dashed lines) are indicated.

**C:** Expression of selected markers cast on the UMAP plot from Figure S4A identifying PGCs, RA-responsive MGCs, MGCs, primordial oocytes and state f0 cells. Normalized expression is plotted on a high-to-low scale (purple-grey).

**D:** Bar graph showing the proportion of each germ cell stage in each fetal sample analyzed.

**E:** Violin plots showing expression of DEGs in either ovary or testis PGCs at the early stage (cluster 0 in Fig. S4A). Most DEGs are on the sex chromosomes.

**F, G:** Violin plots showing expression of genes downregulated (**E**) or upregulated (**F**) in PGCs during development. Cell clusters along the x-axis from left to right are PGC clusters (0-2 in Fig. 4A) with RA-responsive MGCs (cluster 3 in Fig. 4A) as a control.

**Figure S5: Characterizing germline cells and ovarian State f0, related to Figure 5.**

**A:** Distribution of germ cells from W10 and W13 ovaries on the pseudotime trajectory, showing a small proportion cluster in the state f0 group.

**B:** Dotplot comparing percentage expression of selected markers in PGCs, RA-responsive MGCs, MGCs, primordial oocytes and state f0 cells. Normalized expression is plotted on a high-to-low scale (purple-grey), with dot size increasing with the percentage of cells within a cluster (0% – 75%) that express a given gene.

**C:** Top panel: UMAP plot of reanalyzed data of combined germ cells from fetal ovaries (16 cells) and testes (346 cells) from a previously published dataset <sup>1</sup>. Left: Cells are colored by donor origins, either ovary or testis. Bottom panel: PIWIL4 and DDX4 expression cast on the UMAP plot in Figure S5D. Normalized expression is plotted on a high-to-low scale (purple-grey).

**D:** Immunofluorescence staining for state f0 marker PIWIL4 (magenta) and germ cell marker VASA (cyan) in fetal ovaries at W10-15. Nuclei are counterstained with DAPI (grey).

Fig.S1

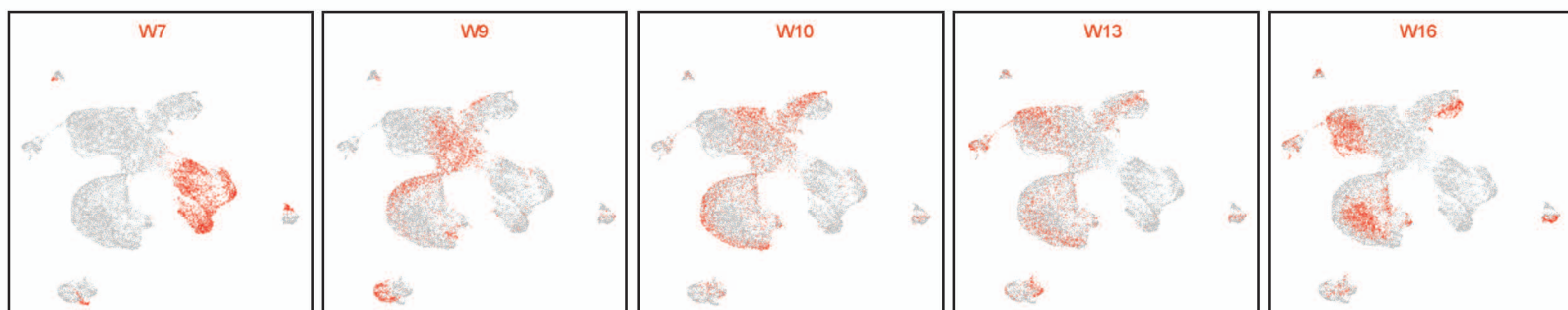
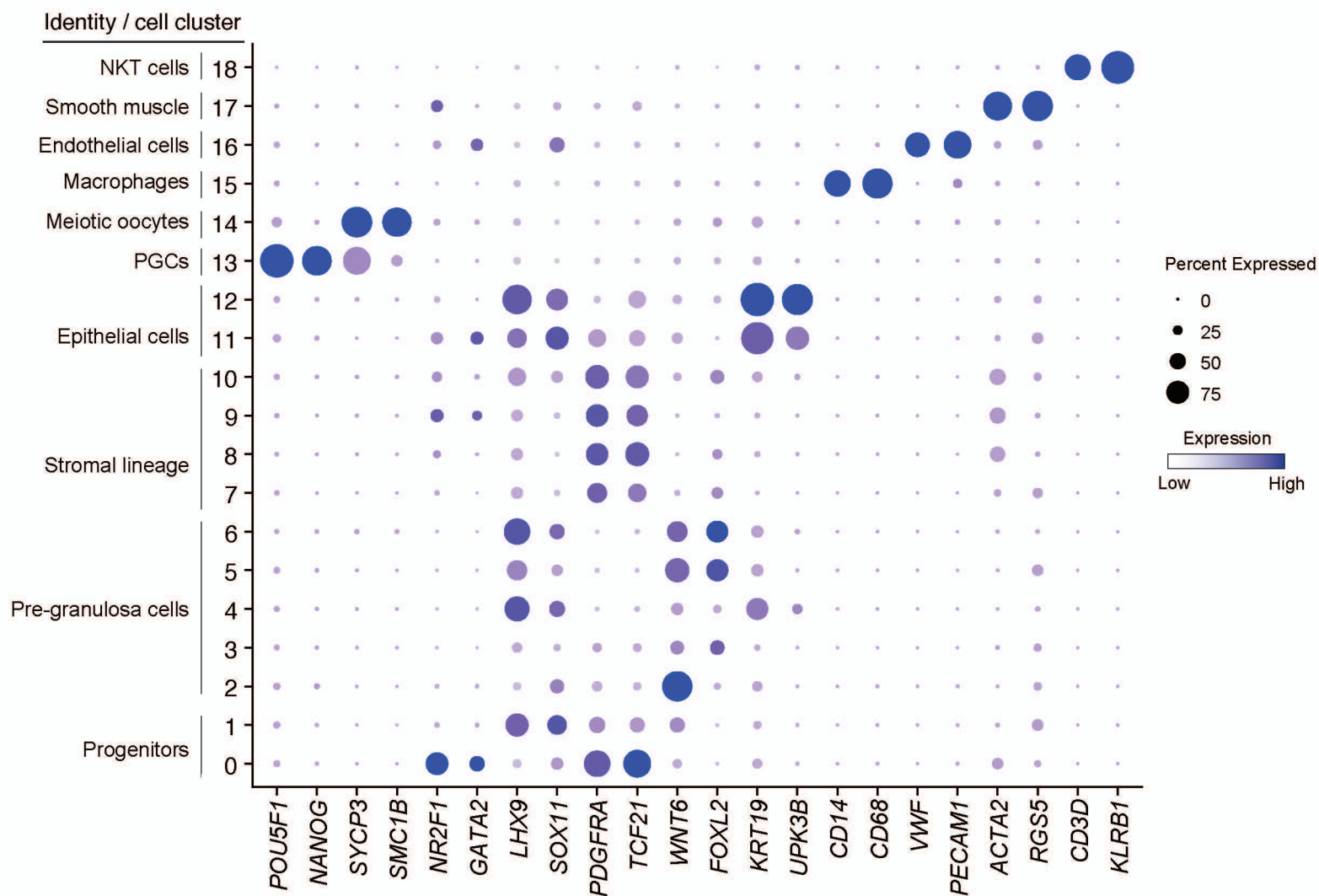
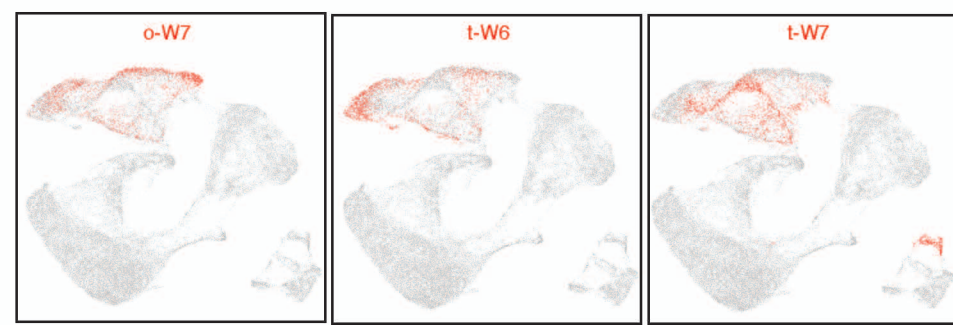
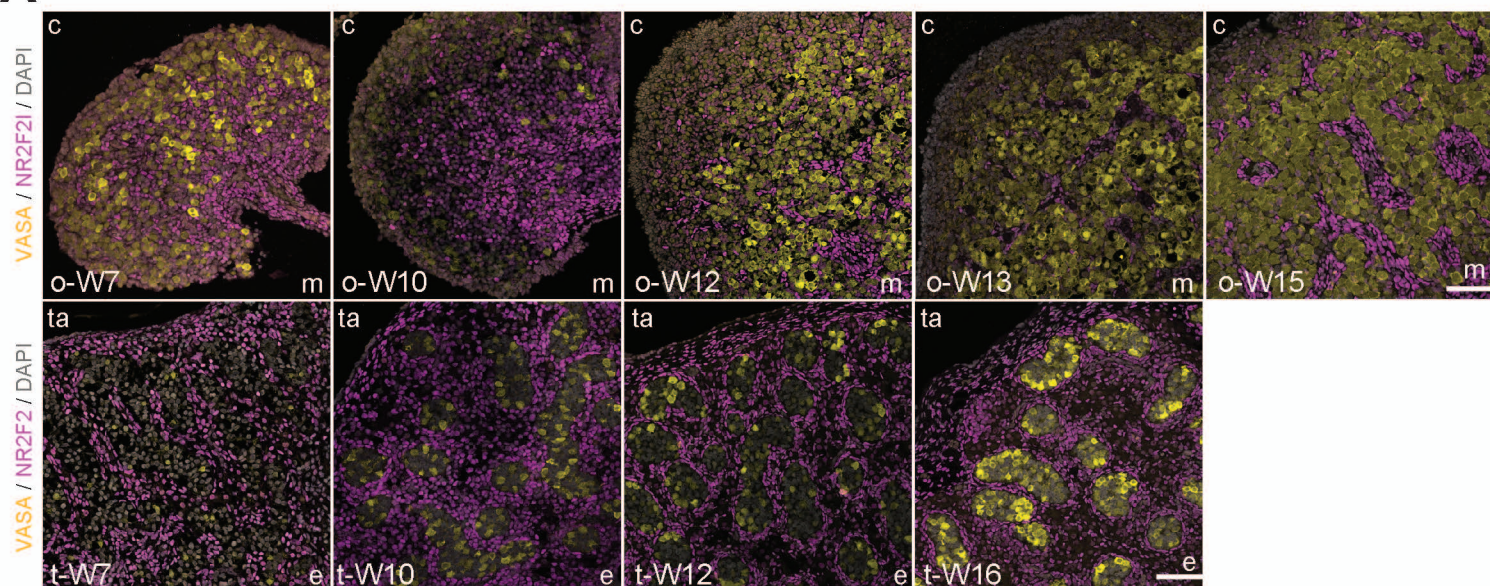
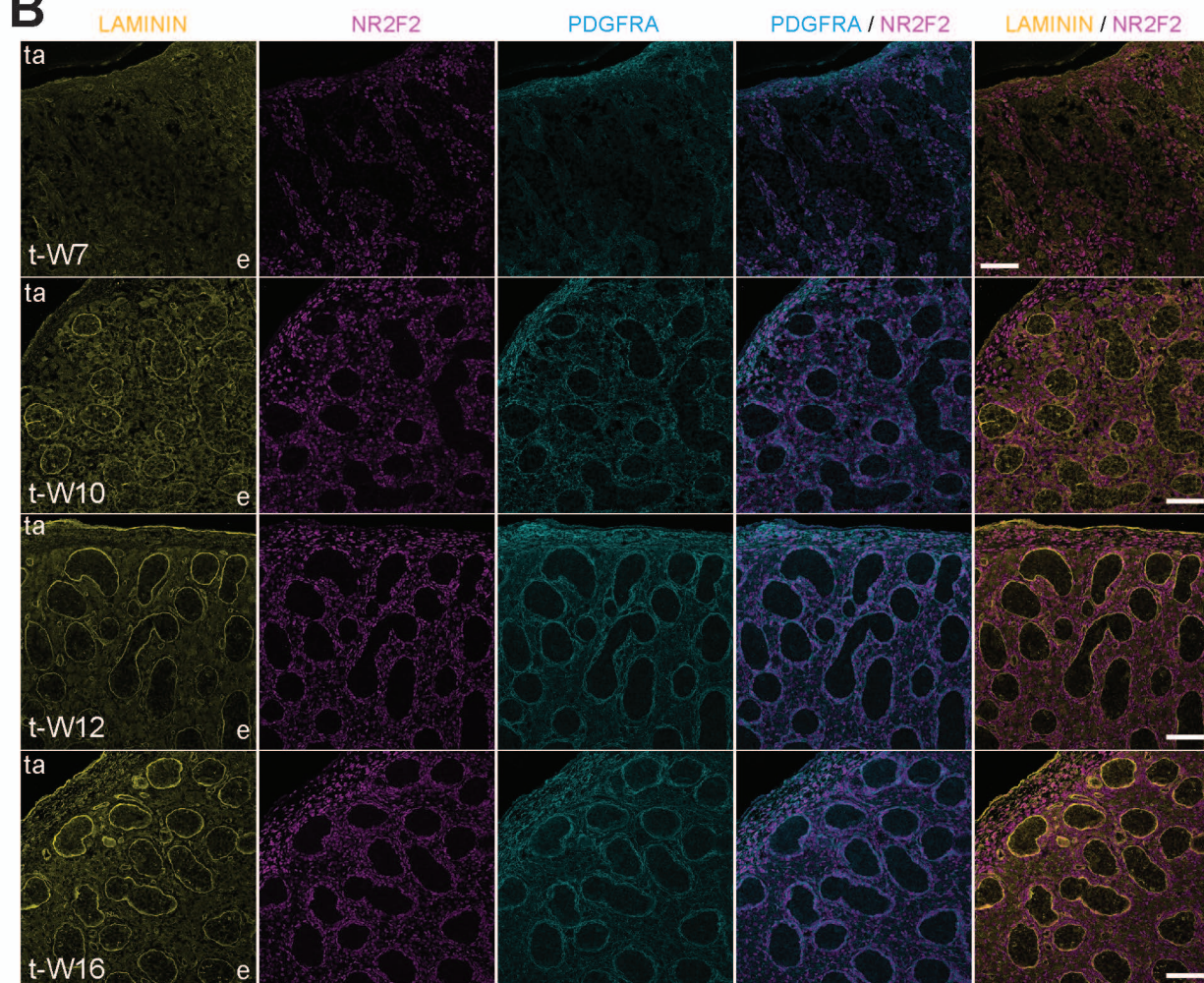
**A****B****C**

Fig.S2

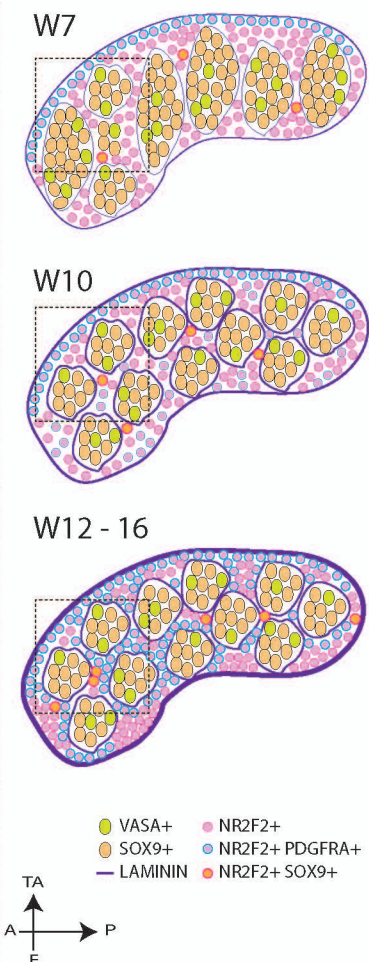
A



B



D



C

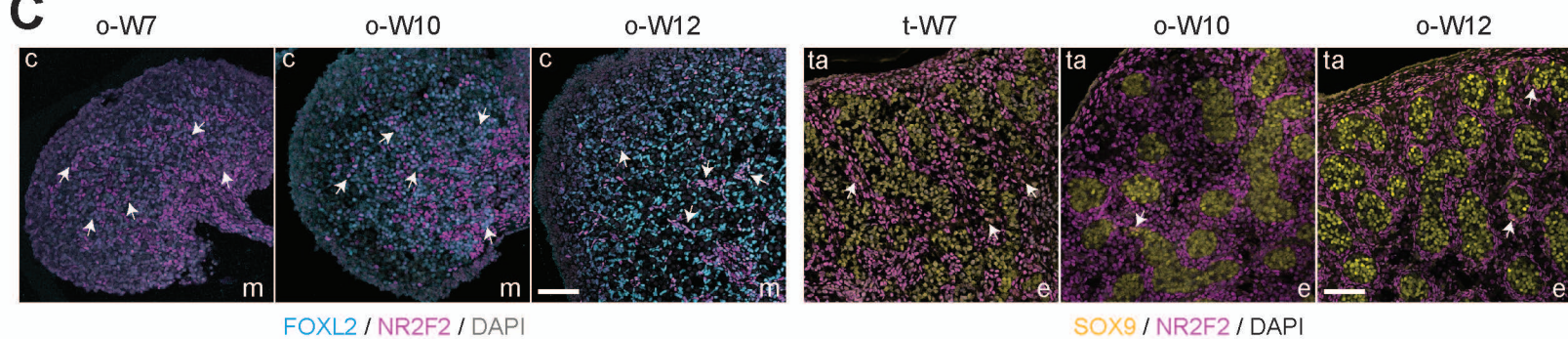


Fig.S3

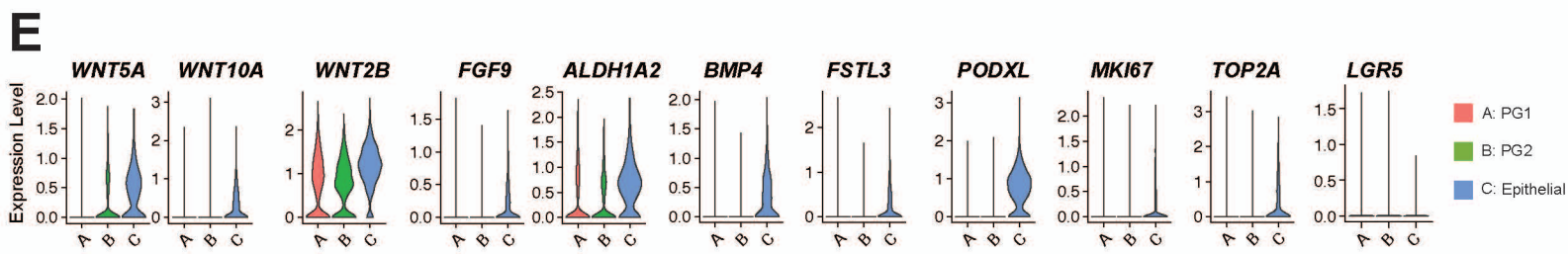
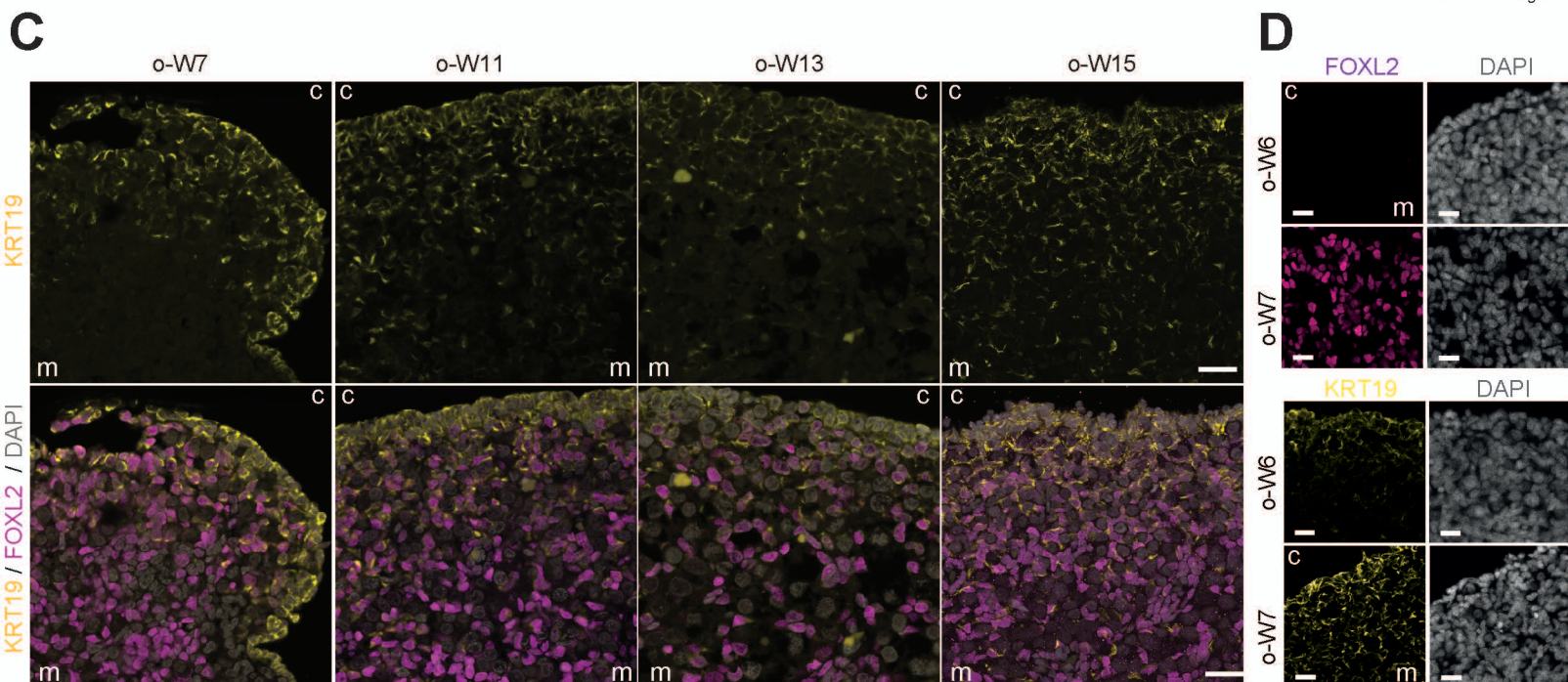
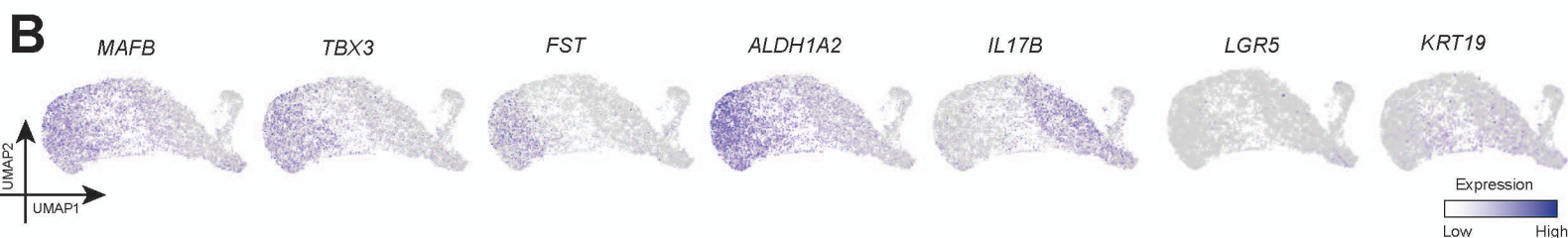
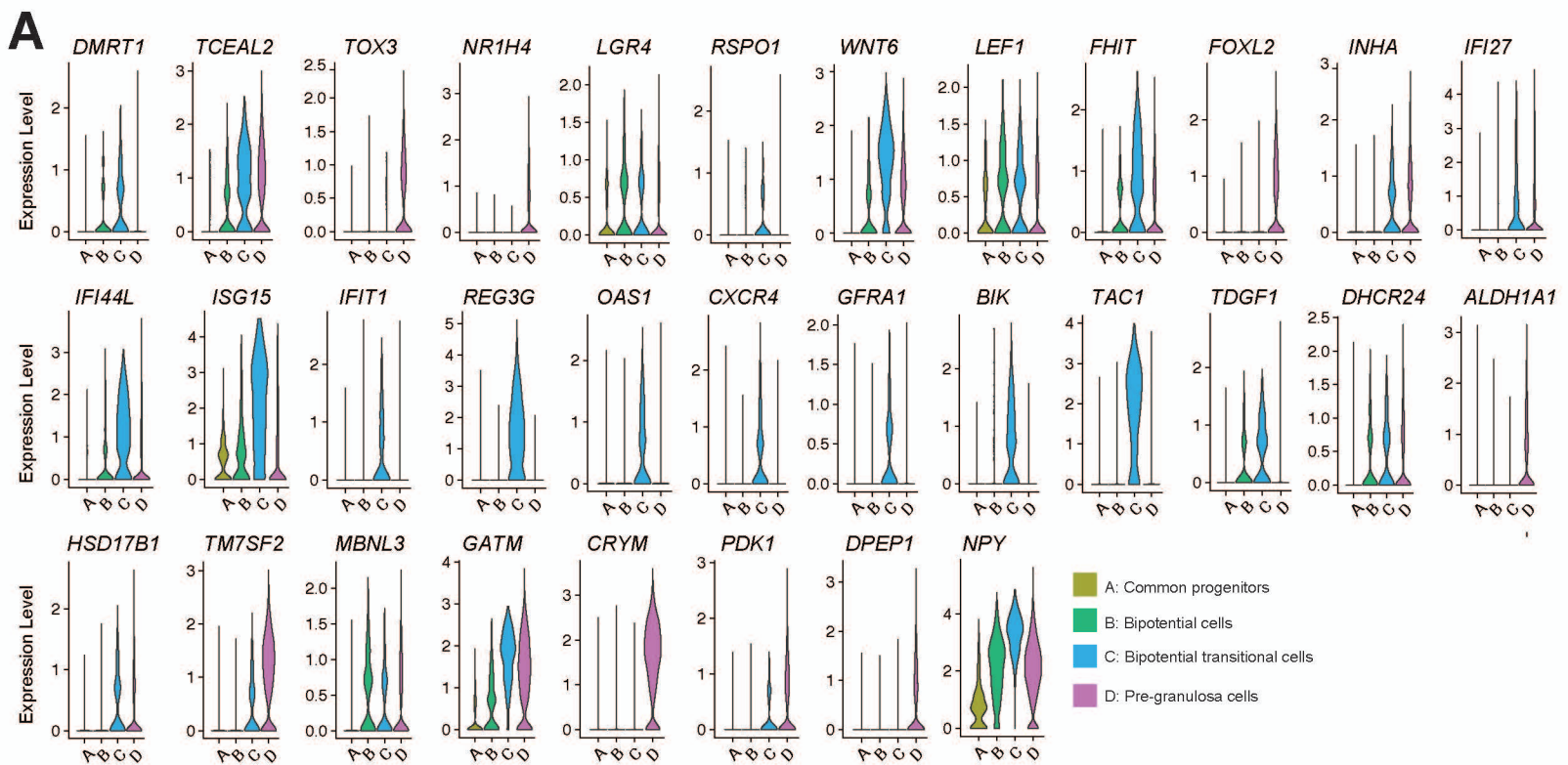
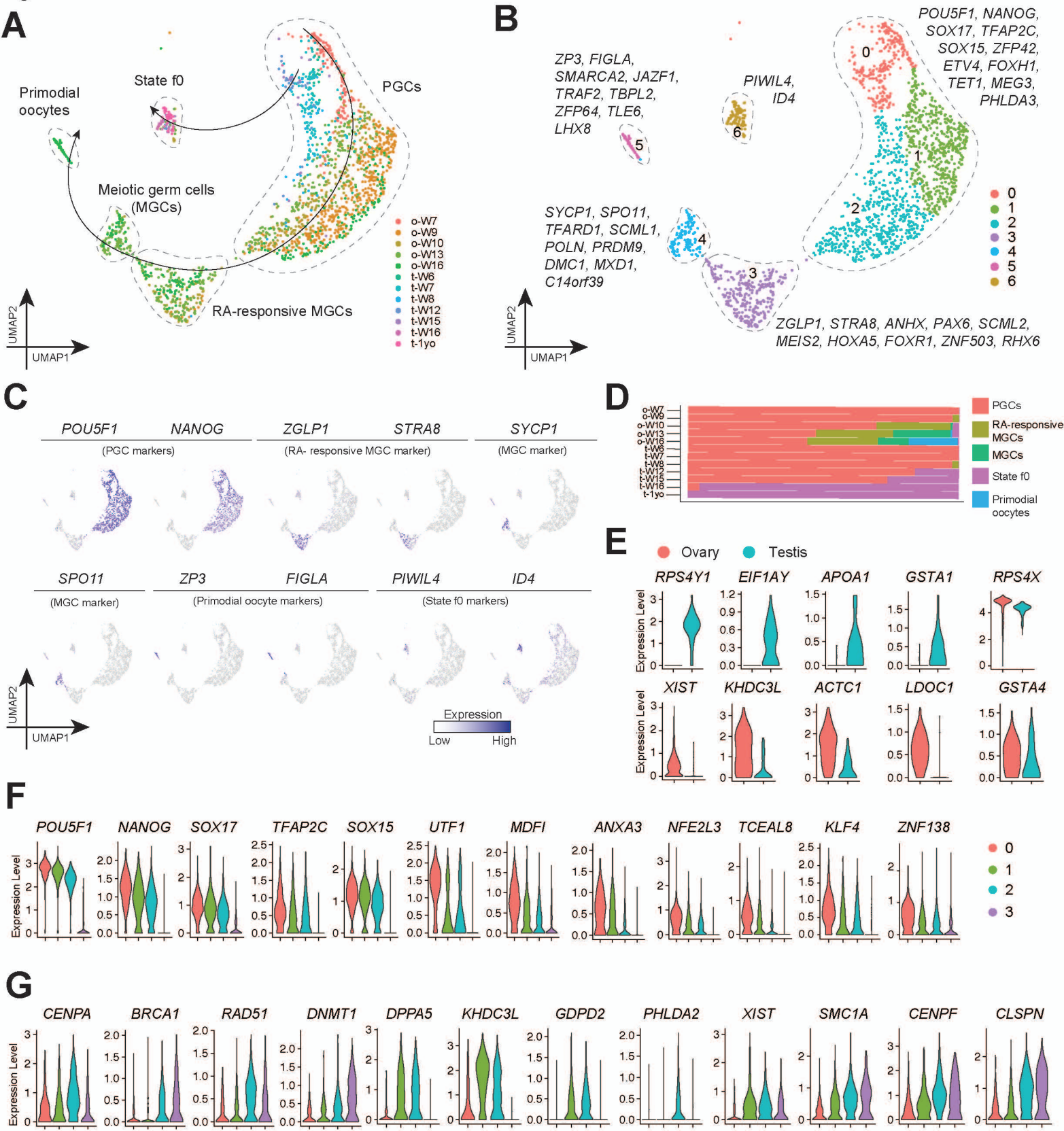


Fig. S4





## SUPPLEMENTAL TABLES

**Supplemental Table 1: Red blood cell marker genes excluded from cell cluster analysis, related to Figures 1 and 2.**

<i>HBE1</i>	<i>EPB42</i>	<i>CAT</i>	<i>MYT1</i>	<i>NPL</i>
<i>HBZ</i>	<i>NUDT4</i>	<i>RGCC</i>	<i>KRT1</i>	<i>TMEM56</i>
<i>HBG2</i>	<i>SLC39A8</i>	<i>LXN</i>	<i>TUBA4A</i>	<i>SLC38A5</i>
<i>HBA1</i>	<i>UCP2</i>	<i>SMIM1</i>	<i>PIM1</i>	<i>TUBB6</i>
<i>HBA2</i>	<i>KLF1</i>	<i>UBAC1</i>	<i>SOD3</i>	<i>HIST1H1C</i>
<i>HBG1</i>	<i>SLC2A1</i>	<i>CREG1</i>	<i>YWHAH</i>	<i>TFR2</i>
<i>HBB</i>	<i>FAM210B</i>	<i>GATA1</i>	<i>TRIM58</i>	<i>SLC29A1</i>
<i>HBM</i>	<i>SELENBP1</i>	<i>GLRX5</i>	<i>FAM46C</i>	<i>RP11-386G11.10</i>
<i>AHSP</i>	<i>DCAF12</i>	<i>EIF2AK1</i>	<i>GDF15</i>	<i>GPR146</i>
<i>SLC25A37</i>	<i>ADIPOR1</i>	<i>CHPT1</i>	<i>KEL</i>	<i>TANGO2</i>
<i>ALAS2</i>	<i>RGS10</i>	<i>GSTO1</i>	<i>SIAH2</i>	<i>FAXDC2</i>
<i>MT1G</i>	<i>TMCC2</i>	<i>ANKRD9</i>	<i>ANK1</i>	<i>GABARAPL2</i>
<i>BLVRB</i>	<i>S100A6</i>	<i>BNIP3L</i>	<i>HSF1</i>	<i>MAP2K3</i>
<i>MT1H</i>	<i>STRADB</i>	<i>R3HDM4</i>	<i>HK1</i>	<i>GRAP2</i>
<i>GYPA</i>	<i>MT1M</i>	<i>ICAM4</i>	<i>PPP1R14C</i>	<i>LMO2</i>
<i>GYPC</i>	<i>OAZ1</i>	<i>RP11-20B24.2</i>	<i>TAL1</i>	<i>PKLR</i>
<i>GYPB</i>	<i>LYL1</i>	<i>GMPR</i>	<i>PITX1</i>	<i>ALAD</i>
<i>MT2A</i>	<i>FTH1</i>	<i>PHOSPHO1</i>	<i>NARF</i>	<i>RAP1GAP</i>
<i>BPGM</i>	<i>LINC00152</i>	<i>TALDO1</i>	<i>FAM104A</i>	<i>CORO1C</i>
<i>MT1E</i>	<i>DMTN</i>	<i>RHCE</i>	<i>TUBB1</i>	<i>MAP1LC3B</i>
<i>SNCA</i>	<i>CCNDBP1</i>	<i>FKBP8</i>	<i>FBXO7</i>	<i>ACKR1</i>
<i>MT1X</i>	<i>RHAG</i>	<i>UROD</i>	<i>ESPN</i>	<i>TF</i>
<i>HEMGN</i>	<i>NFE2</i>	<i>RFESD</i>	<i>ST8SIA1</i>	<i>CDKN2D</i>
<i>HMBS</i>	<i>AC104389.1</i>	<i>RP11-470P21.2</i>	<i>MARCH2</i>	<i>NR1H2</i>
<i>SLC4A1</i>	<i>S100A4</i>	<i>HAGH</i>	<i>GADD45A</i>	<i>HPS1</i>
<i>HBQ1</i>	<i>CTSE</i>	<i>MKRN1</i>	<i>YPEL4</i>	<i>FAM83A</i>
<i>MYL4</i>	<i>TMOD1</i>	<i>ARL4A</i>	<i>PLEK2</i>	<i>SNX22</i>
<i>SLC25A39</i>	<i>PCGF5</i>	<i>MTURN</i>	<i>YBX3</i>	<i>XXbac-BPG252P9.9</i>
<i>FECH</i>	<i>BCL2L1</i>	<i>TESC</i>	<i>RILP</i>	<i>USP15</i>
<i>MT1F</i>	<i>RP11-797H7.5</i>	<i>CR1L</i>	<i>TSPAN32</i>	<i>OPTN</i>
<i>FTL</i>	<i>SMIM5</i>	<i>CD36</i>	<i>RUNDC3A</i>	
<i>MPP1</i>	<i>GYPE</i>	<i>RAD23A</i>	<i>MIR4435-2HG</i>	
<i>NCOA4</i>	<i>C17orf99</i>	<i>RNF10</i>	<i>EPOR</i>	
<i>PRDX2</i>	<i>CPOX</i>	<i>GFI1B</i>	<i>CTA-363E6.6</i>	



**Supplemental Table 2: Differential gene expression analysis of common progenitor cells, related to Figure 2.**

Parameters: log2fc=1; p_adjust<0.05		
DEGs upregulated in ovarian common progenitors	DEGs upregulated in testicular common progenitors	
None	<i>RPS4Y1</i>	
Parameters: log2fc=0.3; p_adjust<0.05		
DEGs upregulated in ovarian common progenitors	DEGs upregulated in testicular common progenitors	
<i>XIST</i>	<i>RPS4Y1</i>	<i>CTGF</i>
<i>RPS10</i>	<i>RPS26</i>	<i>MIF</i>
<i>LAPTM4B</i>	<i>JUN</i>	<i>GNAS</i>
	<i>ID3</i>	<i>TPM2</i>
	<i>ACTA2</i>	<i>PART1</i>
	<i>FOS</i>	<i>NDUFA4L2</i>
	<i>TAGLN</i>	<i>MYL9</i>
	<i>TPM1</i>	<i>HIST1H4C</i>
	<i>MALAT1</i>	<i>DNAJC15</i>
	<i>CNN2</i>	

**Supplemental Table 3: Differential gene expression analysis of bipotential cells, related to Figure 2.**

Parameters: log2fc=1; p_adjust<0.05			
DEGs upregulated in ovarian bipotential cells		DEGs upregulated in testicular bipotential cells	
None		<i>RPS4Y1</i>	
Parameters: log2fc=0.3; p_adjust<0.05			
DEGs upregulated in ovarian bipotential cells		DEGs upregulated in testicular bipotential cells	
<i>SULT1E1</i>	<i>CD24</i>	<i>RPS4Y1</i>	<i>TXNDC17</i>
<i>XIST</i>	<i>FBLN5</i>	<i>RPS26</i>	<i>MEG3</i>
<i>IGFBP3</i>	<i>PRSS23</i>	<i>RNASE1</i>	<i>RPS27</i>
<i>IFI6</i>	<i>TOP2A</i>	<i>BEX1</i>	<i>EMX2</i>
<i>PTN</i>	<i>NR2F1</i>	<i>DLK1</i>	<i>RPS28</i>
<i>CPE</i>	<i>CDH2</i>	<i>JUNB</i>	<i>ATP5I</i>
<i>SHISA3</i>	<i>POSTN</i>	<i>DCN</i>	<i>NR0B1</i>
<i>SFRP2</i>	<i>CLDN11</i>	<i>MIF</i>	<i>PKM</i>
<i>PLAT</i>	<i>TYMS</i>	<i>FOS</i>	<i>LHX9</i>
<i>RPS10</i>	<i>CSRP2</i>	<i>IER2</i>	<i>ELK1</i>
<i>AKAP12</i>	<i>H2AFX</i>	<i>LDHA</i>	<i>MT-ND3</i>
<i>DHRS3</i>	<i>TAGLN</i>	<i>RPS29</i>	<i>MT-CO3</i>
<i>ANXA2</i>	<i>TUBB2B</i>	<i>ENO1</i>	<i>GATA4</i>
<i>CDK1</i>	<i>TUBB4B</i>	<i>JUN</i>	<i>LRRN3</i>
<i>ISG15</i>	<i>TGFBI</i>	<i>GSTA1</i>	<i>IL17B</i>
<i>ALCAM</i>	<i>PCSK1N</i>	<i>NSG1</i>	
<i>MXRA8</i>	<i>PCOLCE</i>	<i>SOX4</i>	

## REFERENCES

1. Li, L., Dong, J., Yan, L., Yong, J., Liu, X., Hu, Y., Fan, X., Wu, X., Guo, H., Wang, X., et al. (2017). Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells and Gonadal Niche Interactions. *Cell Stem Cell* 20, 858-873.e854. [10.1016/j.stem.2017.03.007](https://doi.org/10.1016/j.stem.2017.03.007).