Developmental Cell, Volume *59*

Supplemental information

Characterization of the human fetal gonad and reproductive tract by single-cell transcriptomics

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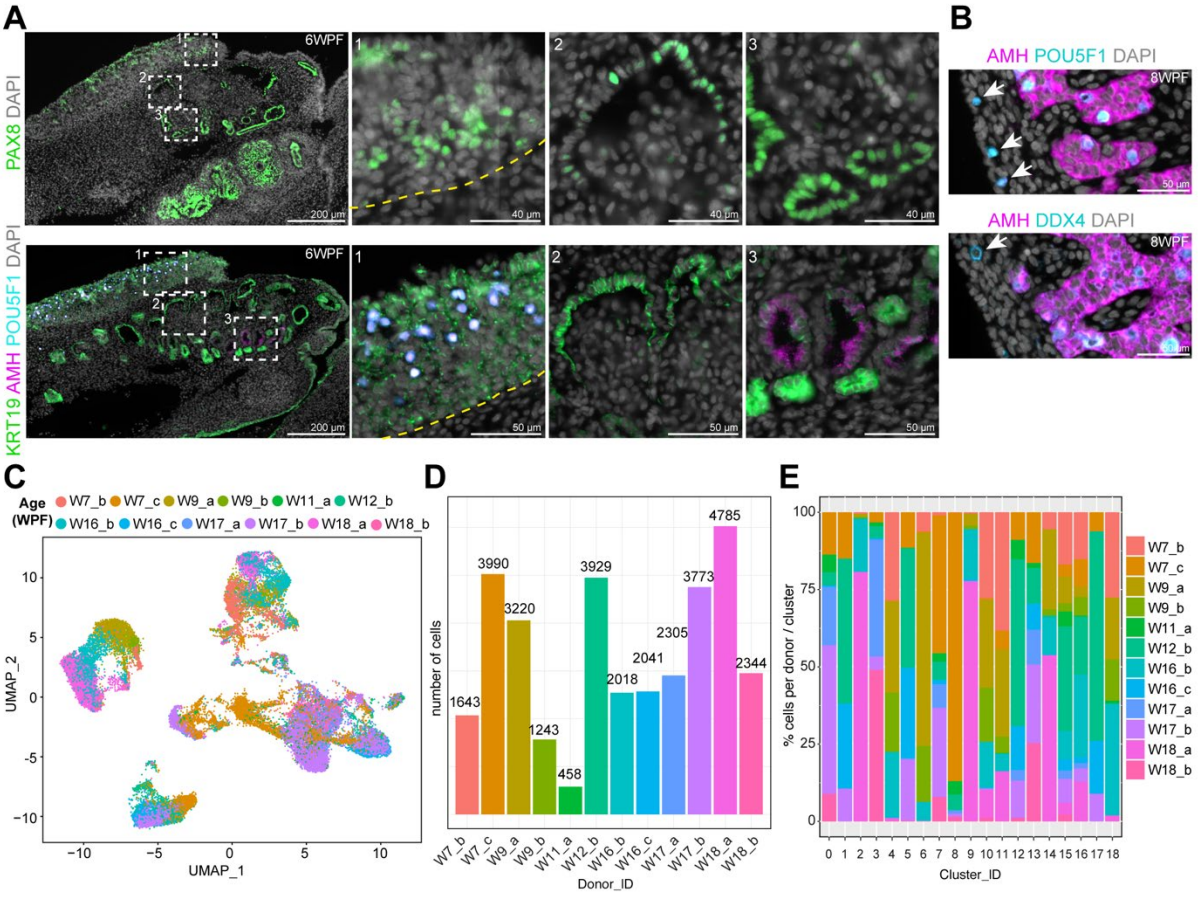

Figure S1. Characteristics of the human fetal male dataset, Related to **Figure 1**, **Figure 2** and **Table S1**.
(A) Immunofluorescence for PAX8 (top) and KRT19, AMH and POU5F1 (bottom) in human fetal testis and developing reproductive tract at 6-6.5WPF (n=2). White dashed boxes in the left image indicate selected magnified regions on the right. Yellow dashed line indicates border between testis and mesonephros. **(B)** Immunofluorescence for AMH and POU5F1 (top) and for AMH and DDX4 (bottom) in human fetal testis at 8WPF (n=2). White arrows point to POU5F1+ cells (top) and DDX4+ cells (bottom) outside the seminiferous tubes. **(C)** Uniform manifold approximation and projection (UMAP) plot showing cell clusters obtained for the human fetal male dataset depicting the developmental age (WPF) and donor. **(D)** Bar plot showing the number of cells per donor. **(E)** Bar plot showing the percentage of cells per donor per cluster.

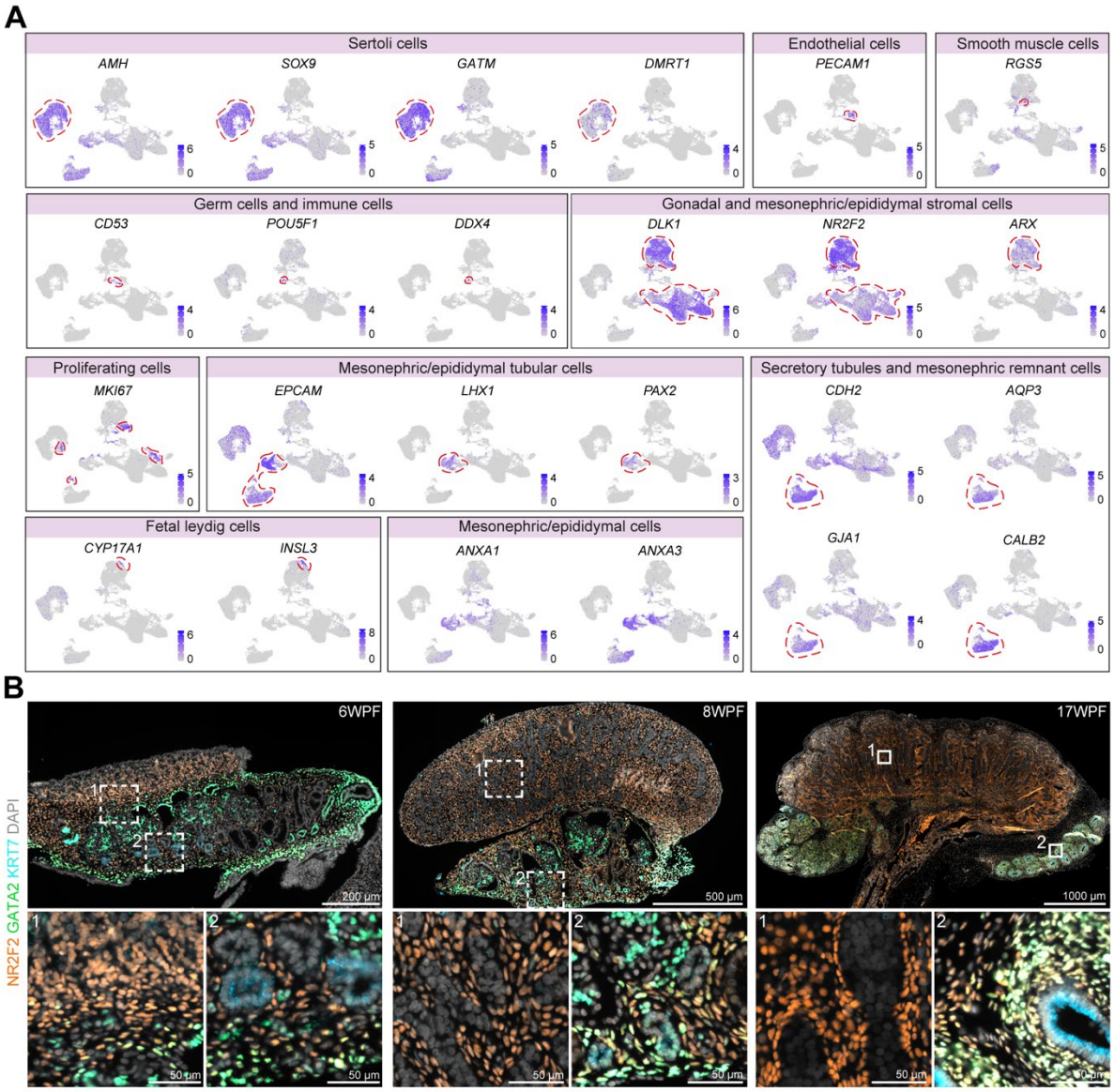

Figure S2. Identification of cell clusters in the human fetal male dataset, Related to **Figure 2** and **Table S1**.

(**A**) UMAP plot showing expression of selected markers for the main cell types in the fetal male dataset. Red dashed circles indicate cell populations with specific marker expression. (**B**) Immunofluorescence for NR2F2, GATA2 and KRT7 in human fetal testis and developing reproductive tract at 6-6.5WPF (n=2) (left), 8WPF (n=2) (middle) and 16-20WPF (n=7) (right). White dashed boxes indicate selected magnified regions on the bottom.

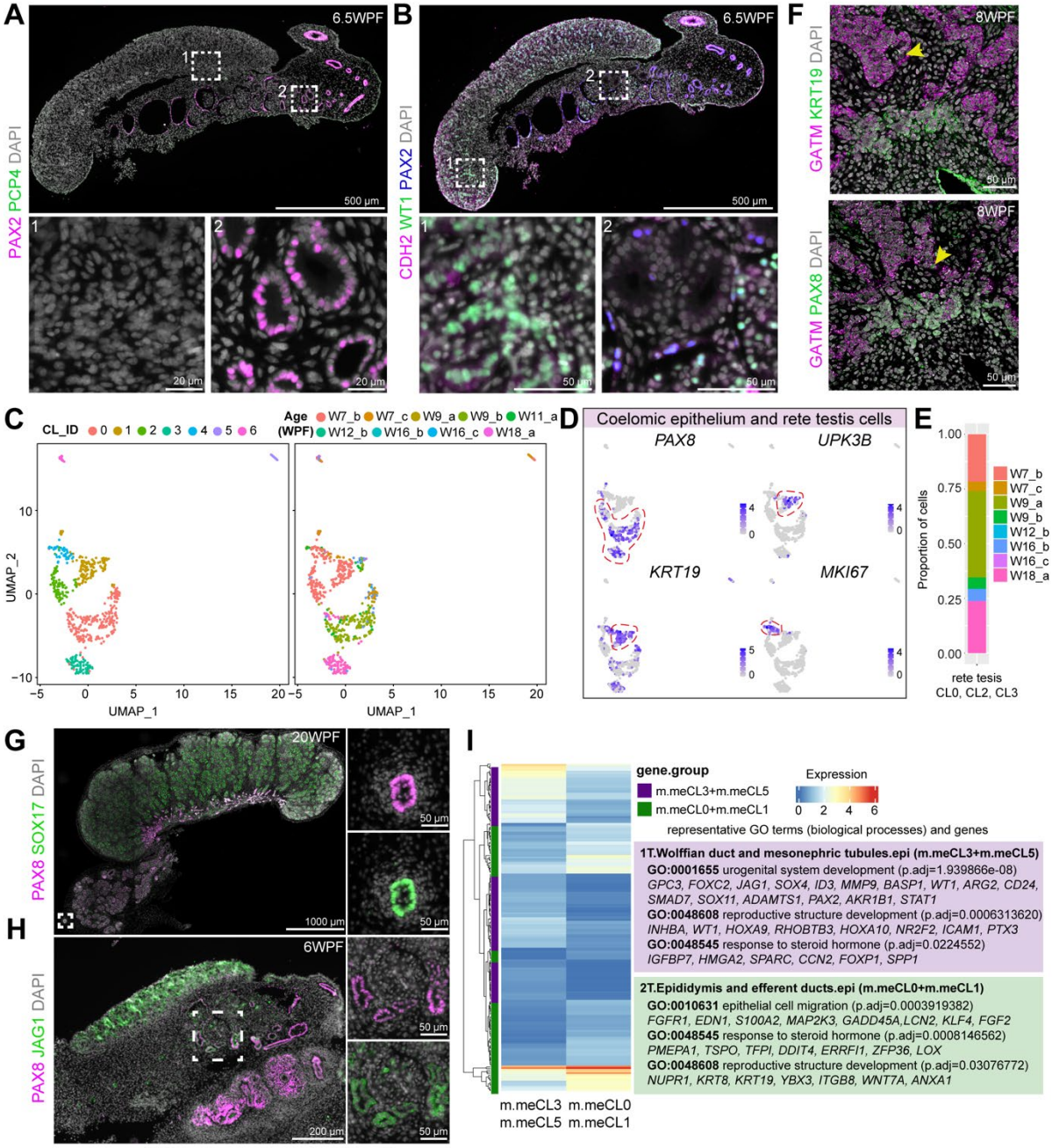

Figure S3. Characteristics of male rete and mesonephric epithelial cells, Related to **Figure 3**, **Figure 4** and **Table S3**.

(A-B) Immunofluorescence for PAX2 and PCP4 **(A)** and CDH2, WT1 and PAX2 **(B)** in male gonad and mesonephros at 6-6.5WPF (n=2). White dashed boxes indicate selected magnified regions at the bottom. **(C-D)** UMAP plots showing sub-clustering of mCL11, depicting cluster numbers (**C**, left), developmental age/donor (**C**, right), and expression the selected markers **(D)**. **(E)** Bar plot showing the proportion per donor of male rete testis cells. **(F)** Immunofluorescence for GATM and KRT19 (top) and GATM and PAX8 (bottom) in testis at 8WPF (n=2). Yellow arrows indicate GATM+KRT19+ or GATM+PAX8+ cells. **(G)** Immunofluorescence for PAX8 and SOX17 in testis and mesonephros at 16-20 WPF (n=7). White dashed boxes indicate selected magnified regions (right). **(H)** Immunofluorescence for PAX8 and JAG1 in male gonad and mesonephros at 6-6.5WPF (n=2). White dashed boxes indicate selected magnified regions (right). **(I)** Heatmap showing differentially expressed genes between cells from m.meCL3+m.meCL5 and cells from m.meCL0+m.meCL1, and associated representative GO terms.

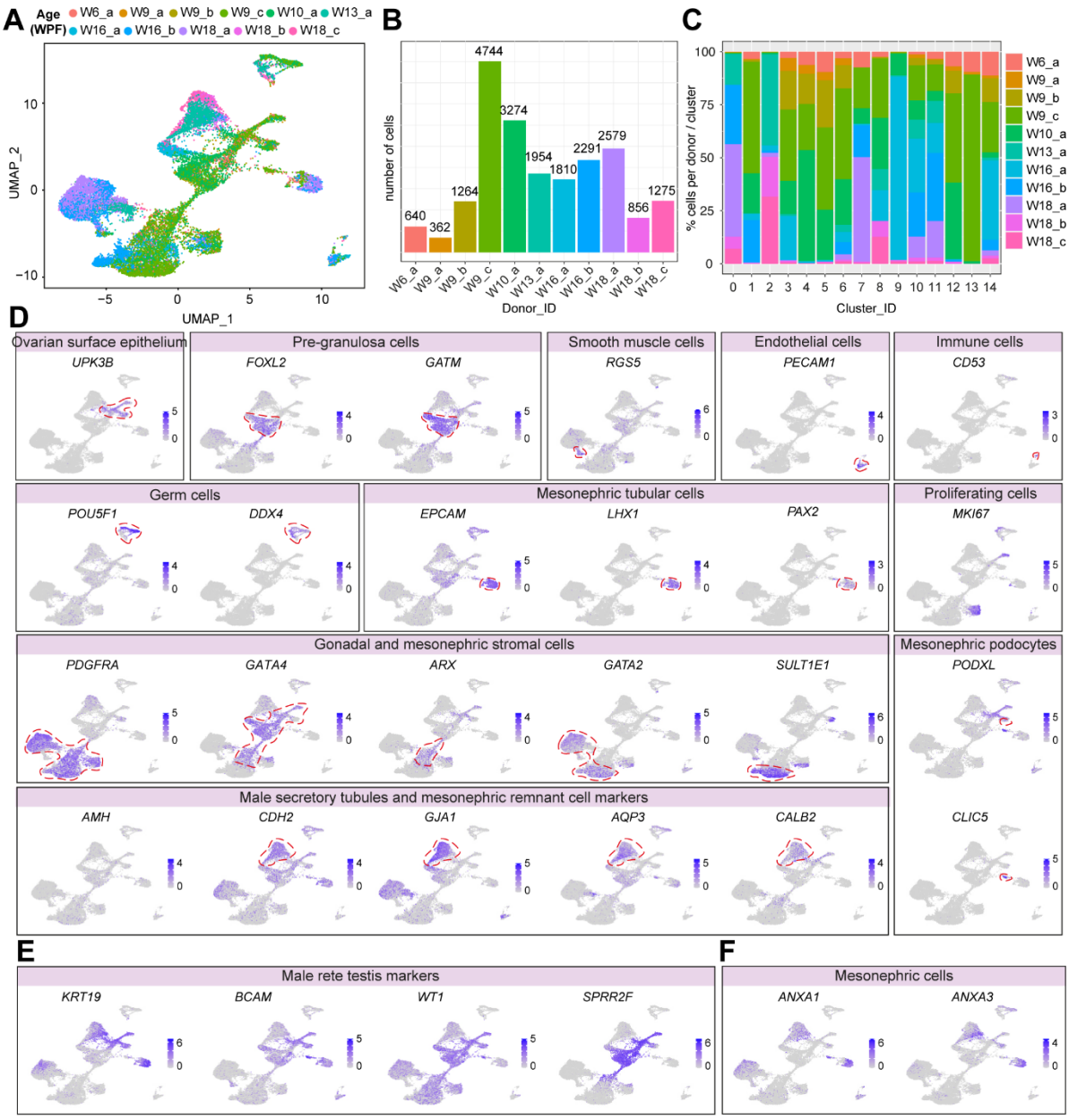

Figure S4. Characteristics of the human fetal female dataset, Related to **Figure 5** and **Table S4**. **(A)** UMAP plot showing female dataset colored by developmental age/donors. **(B)** Bar plot showing the number of cells per donor. **(C)** Bar plot showing the percentage of cells per donor per cluster. (**D**) UMAP plot showing expression of selected markers for the main cell types in the fetal female dataset. Red dashed circles indicate cell populations with specific marker expression. **(E-F)** UMAP plot showing expression of rete testis **(E)** and mesonephric cell markers in the female dataset **(F)**.

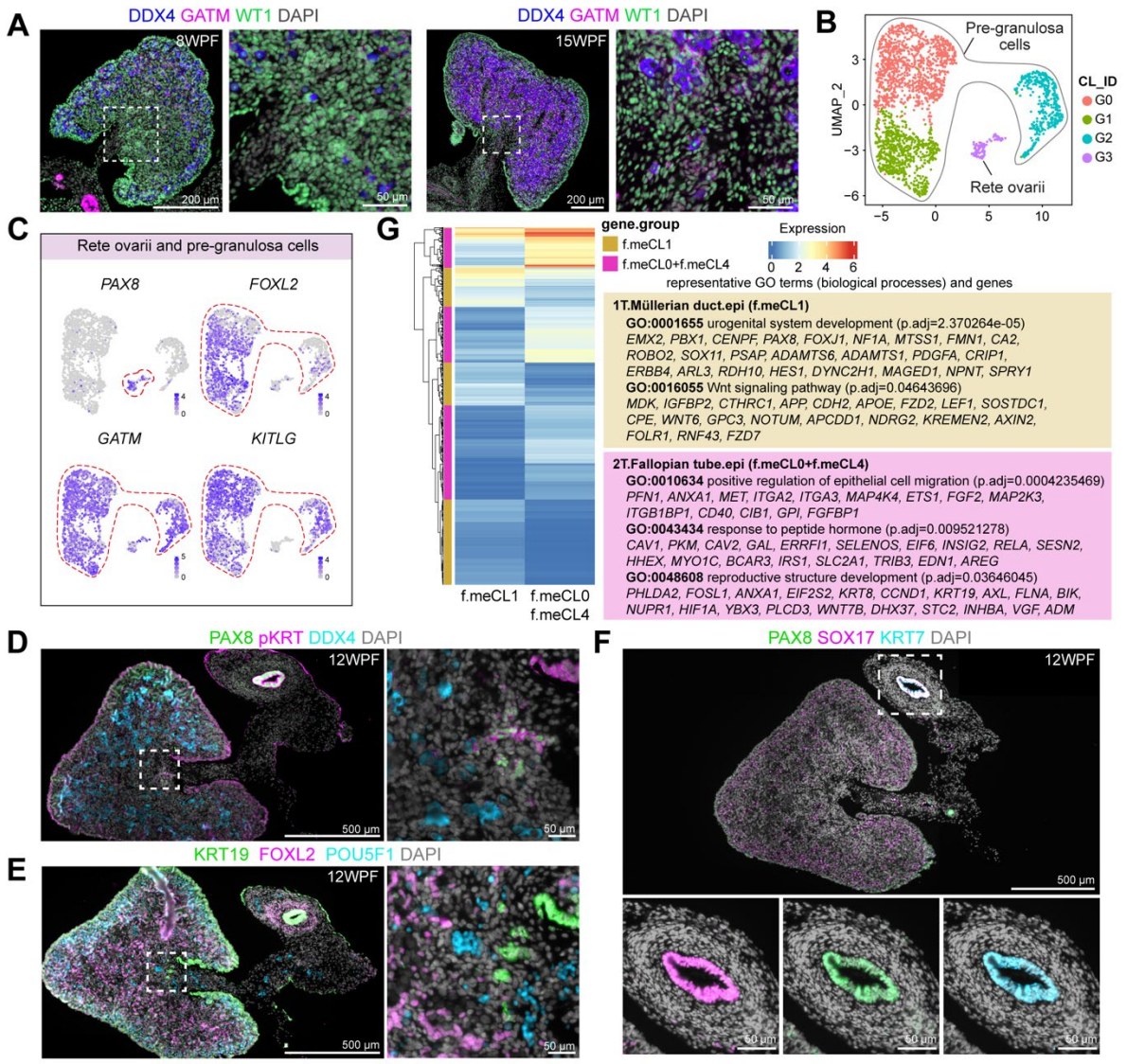

Figure S5. Characteristics of female rete and mesonephric epithelial cells, Related to **Figure 6** and **Table S5**.

(A) Immunofluorescence for GATM, WT1 and DDX4 in 8WPF and 15WPF (8-20WPF; n=5) fetal ovaries. White dashed boxes indicate the magnified region at the right. **(B)** UMAP plot showing the sub-clustering of fCL4. **(C)** UMAP plot showing expression of rete and pre-granulosa cell markers in sub-clusters of fCL4. **(D-F)** Immunofluorescence for pan (p)KRT, PAX8 and DDX4 **(D),** POU5F1, KRT19 and FOXL2 **(E),** and PAX8, SOX17 and KRT7 **(F)** in ovaries and mesonephros at 12WPF (n=1). White dashed boxes indicate the magnified region in the right **(D,E)** or at the bottom **(F)**. **(G)** Heatmap showing differentially expressed genes between cells from f.meCL1 and cells from f.meCL0+f.meCL4, and associated representative GO terms.

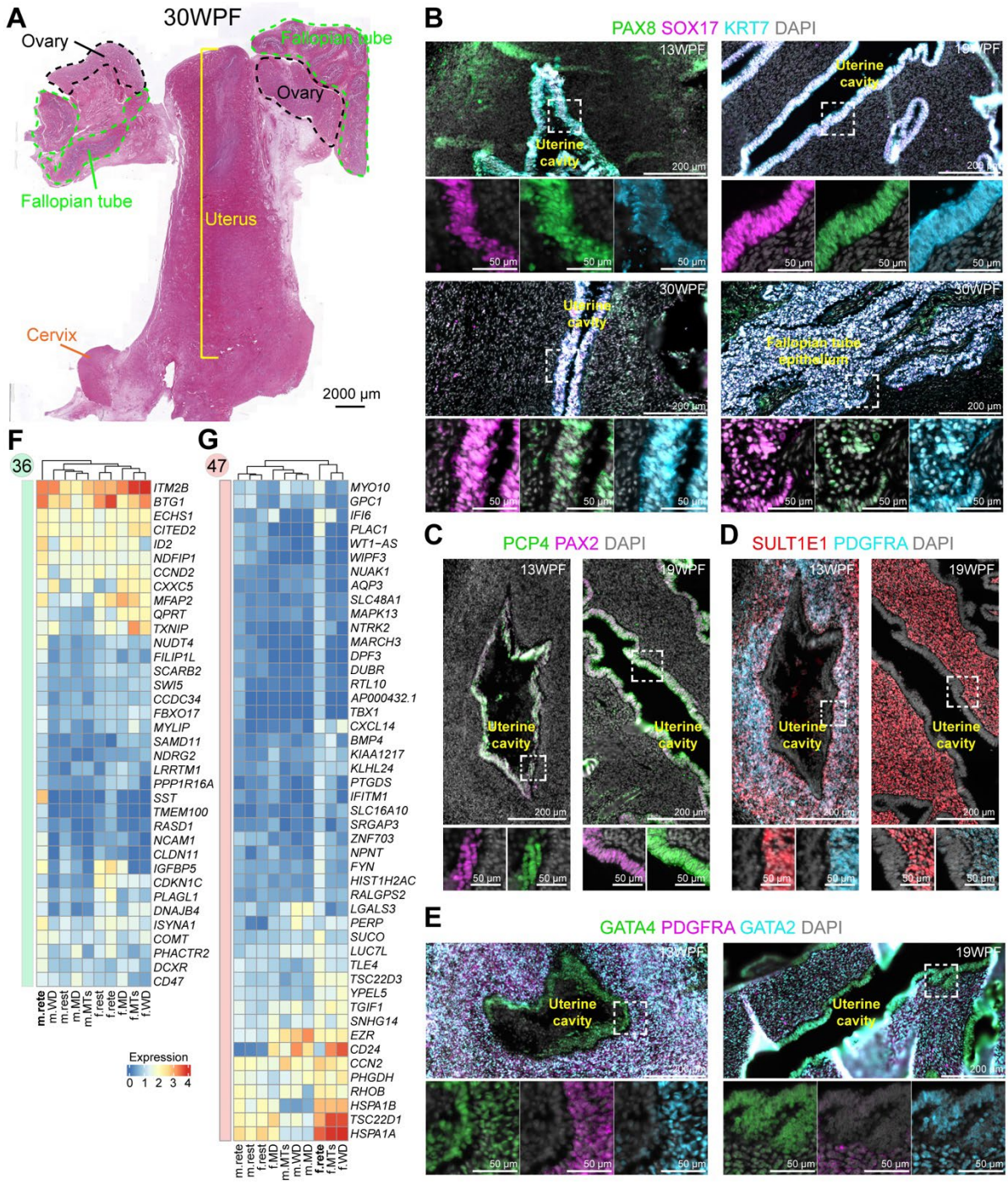

Figure S6. Characteristics of female reproductive tract and unique rete markers, Related to **Figure 7**, **Table S6** and **Table S7**.

(A) Hematoxylin and eosin staining of 3T female gonad and reproductive tract (30WPF; n=1). **(B)** Immunofluorescence for PAX8, SOX17 and KRT7 in uterus at 13WPF (n=1) (top left), 19WPF (n=1) (top right), 30WPF (n=1) (bottom left), and in fallopian tube at 30WPF (n=1) (bottom right). White dashed boxes indicate selected magnified regions at the bottom. **(C-E)** Immunofluorescence for PAX2 and PCP4 **(C)**, PDGFRA and SULT1E1 **(D)** and GATA4, PDGFRA and GATA2 **(E)** in uterus at 13WPF (n=1) and 19WPF (n=1). White dashed boxes indicate selected magnified regions at the bottom. **(F)** Heatmap showing mean expression of 36 DEGs specifically upregulated in 1T rete testis in 1T male and female cell types of interest. **(G)** Heatmap showing mean expression of 47 DEGs specifically upregulated in 1T rete ovarii in 1T male and female cell types of interest.

A

Testis and male reproductive tract

Cell type	Markers validated by IF
Germ cells	POU5F1+ DDX4+
Sertoli cells	AMH+ GATM+
Male gonadal stromal cells	PDGFRA+ GATA4+
Male rete cells	PAX8+ PCP4+ PAX2+ WT1+ KRT19+
Male mesonephric and epididymis stromal cells	PDGFRA+ SULT1E1+ GATA2+ NR2F2+
Male mesonephric tubule epithelial cells/glomeruli	PAX8+ CDH2+ JAG1+ PODXL-
Male Wolffian duct epithelial cells	PAX8+ GATA3+
Male Müllerian duct epithelial cells	PAX8+ SOX17+
Epididymis and efferent duct epithelial cells	PAX8+ KRT7+ PAX2+ KRT19+
Mesonephric podocytes	PODXL+

B

Ovary and female reproductive tract

Cell type	Markers validated by IF
Germ cells	POU5F1+ DDX4+
Pre-granulosa cells	FOXL2+ GATM+
Female gonadal stromal cells	PDGFRA+ NR2F2+ GATA4+
Female rete cells	PAX8+ PCP4+ KRT19+ WT1+
Female mesonephric and fallopian tube stromal cells	PDGFRA+ SULT1E1+ GATA2+
Female mesonephric tubule epithelial cells/glomeruli	CDH2+ PCP4+
Female Wolffian duct epithelial cells	GATA3+
Female 1T Müllerian duct epithelial cells	PAX8+ SOX17+ PCP4+ KRT19+
Female 2T Müllerian duct epithelial cells and uterine epithelial lining	PAX8+ SOX17+ PCP4+ KRT7+ PAX2+ GATA2+ GATA4+
Uterine mesenchyme	PDGFRA+ SULT1E1+ GATA2+

Figure S7. Summary of markers validated by immunofluorescence in gonads and reproductive tract, Related to **STAR METHODS**.
(A) Markers validated by immunofluorescence (IF) in testis and male reproductive tract, separated by cell type. **(B)** Markers validated by IF in ovaries and female reproductive tract, separated by cell type.