

Supplemental information

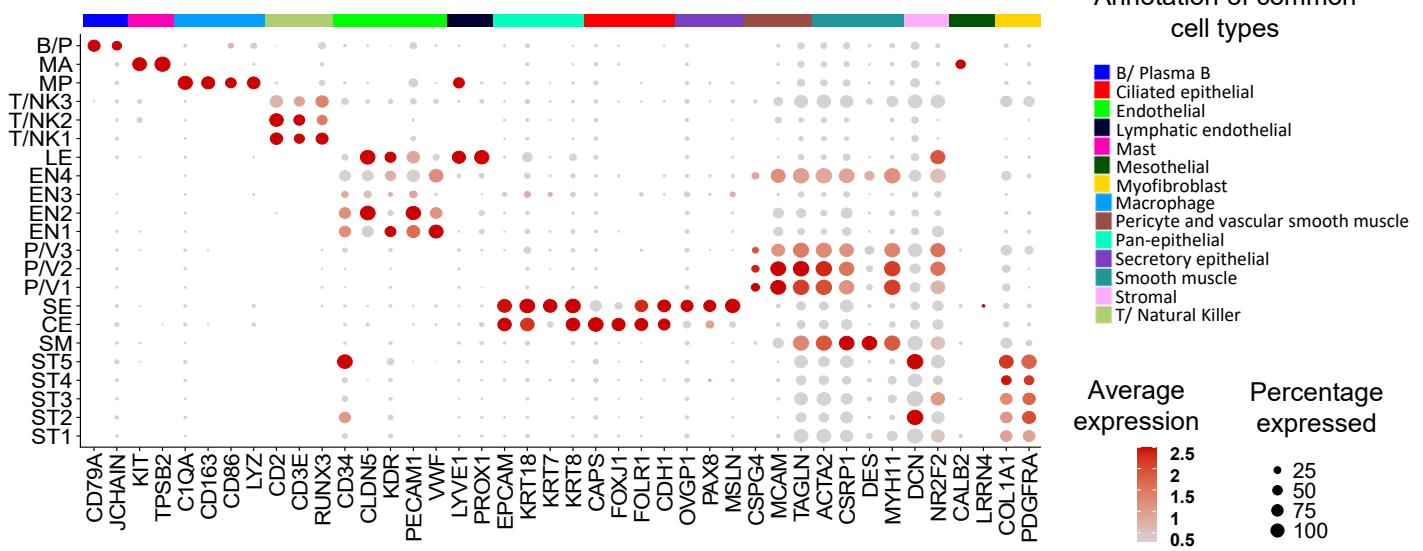
**A molecular atlas of the human
postmenopausal fallopian tube and ovary
from single-cell RNA and ATAC sequencing**

Ernst Lengyel, Yan Li, Melanie Weigert, Lisha Zhu, Heather Eckart, Melissa Javellana, Sarah Ackroyd, Jason Xiao, Susan Olalekan, Dianne Glass, Shilpa Iyer, Rahul Krishnan, Agnes Julia Bilecz, Ricardo Lastra, Mengjie Chen, and Anindita Basu

Figure S1

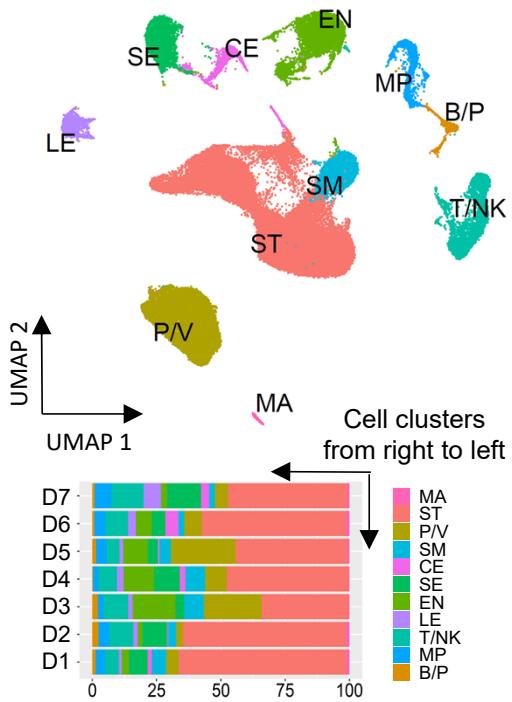


Fallopian tube

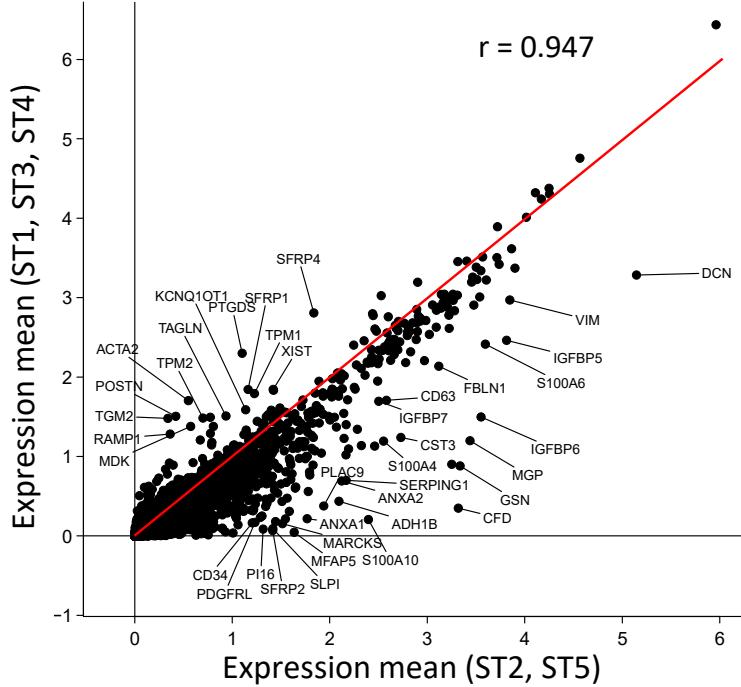


B

Fallopian tube

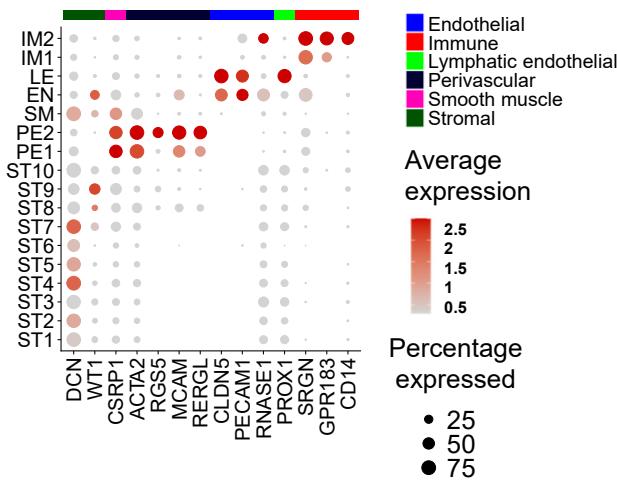


C Fallopian tube



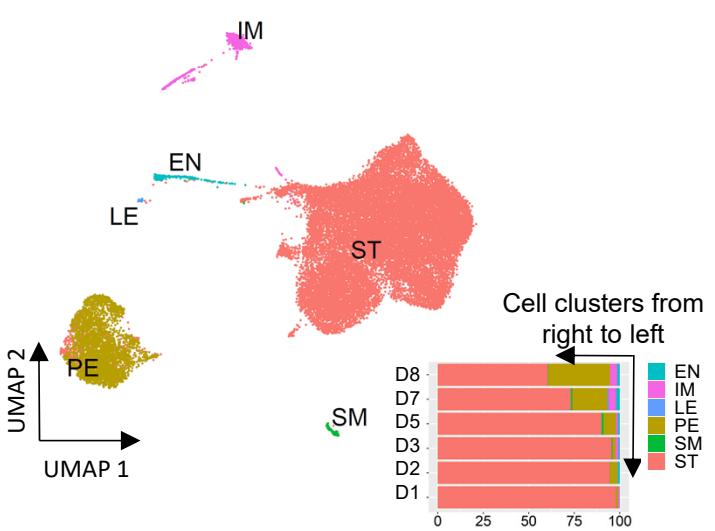
D

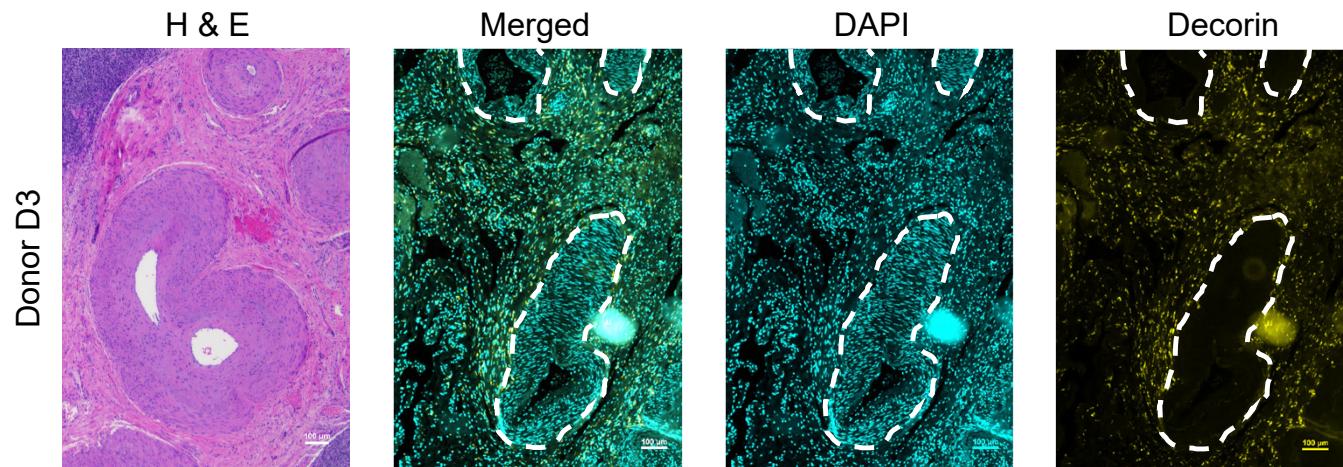
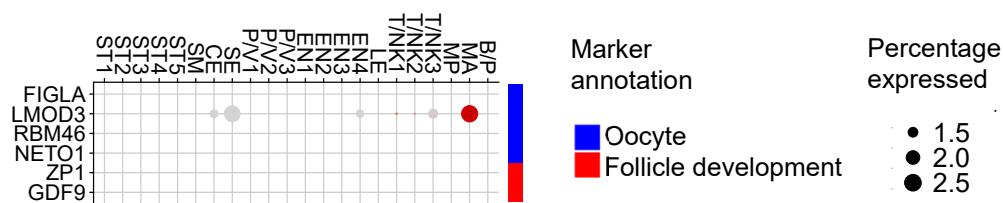
Ovary



E

Ovary



F**Ovary****G****Fallopian tube****H****Fallopian tube**

Cancer Cell, 2020

Cell Reports, 2021

Our study "HCA"

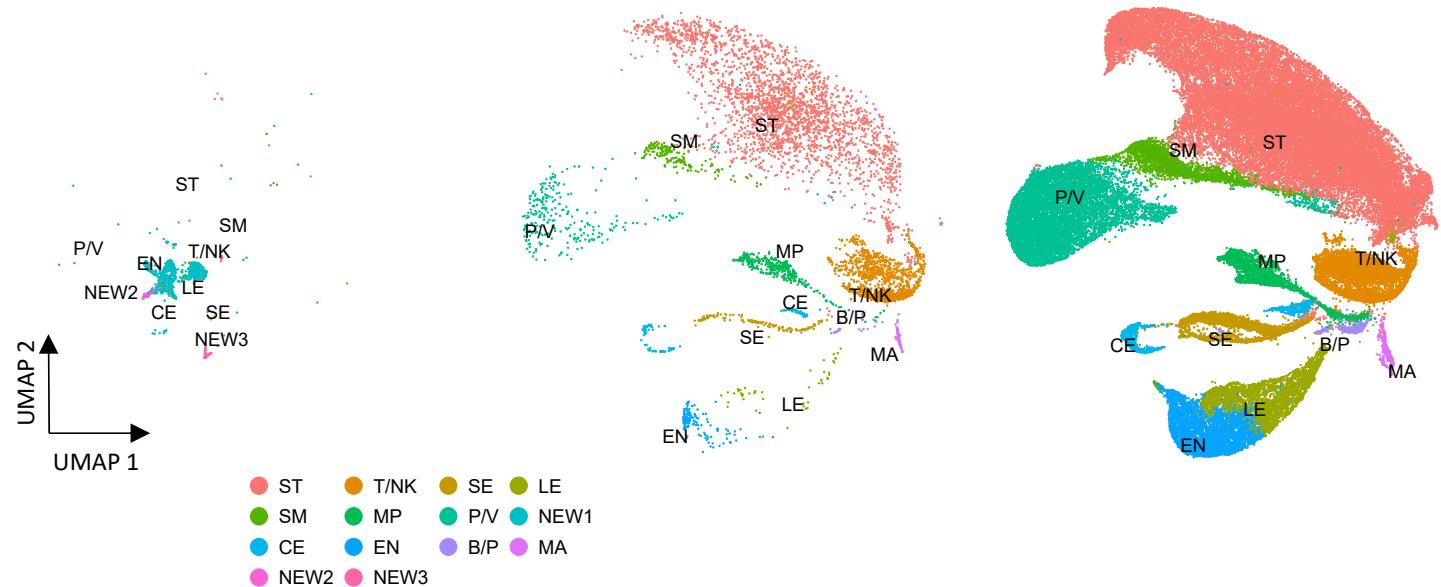


Figure S1 (related to Figure 1). Mapping major cell types of the normal human postmenopausal fallopian tube and ovary.

A) Dot plot showing normalized gene expression levels of known canonical marker genes for each cell type identified in the fallopian tube in Fig. 1B (n= 7).

B) Canonical cell types in the normal human postmenopausal fallopian tube. UMAP plot showing the 11 major cell clusters identified in the fallopian tube using scRNA-seq (n= 7). Data includes all three anatomic regions: fimbriae (n= 6), ampulla (n= 6), and isthmus (n=6). The lower panel shows the individual percentage of each cell type by the individual donor.

C) Scatter plot comparing gene expression levels of different stromal cell populations: ST2/5 versus ST1/3/4.

D) Dot plot showing normalized gene expression levels of known canonical marker genes for each cell type identified in the postmenopausal ovary in Fig. 1E.

E) Canonical cell types in the normal human postmenopausal ovary (n= 6). UMAP plot showing the six major cell clusters identified in the ovary using scRNA-seq. Relative abundance of the 6 major cell clusters found in the postmenopausal ovary using scRNA-seq. The graph shows the individual percentage of each cell type by the individual donor.

F) Hematoxylin and eosin (H&E) staining and RNA-fluorescent *in situ* hybridization (FISH) of decorin (DCN) in the postmenopausal ovary from donor D3. Every dot corresponds to one RNA transcript. Nuclei are stained with DAPI in cyan and DCN transcripts in yellow. Dashed white lines separate the stroma from blood vessels. Scale bar is 100 μ m.

G) Dot plot showing average gene expression of markers associated with oocyte and follicle development in the fallopian tube and ovary.

H) Fallopian tube, scRNA-seq: Comparison of our scRNA-seq data from the fallopian tube of 7 postmenopausal women (noted as 'HCA'), with normal epithelial cells from Hu, et al., Cancer Cell 2020, and one postmenopausal patient from Dinh, et al., Cell Reports, 2021 in the same UMAP space. Cells from the three datasets were integrated, clustered, and annotated based on our annotations. Three new clusters named 'NEW1/2/3' appear in 'Cancer Cell, 2020' data only; cell types identified in one postmenopausal patient from 'Cell Reports, 2021' showed good overlap with cell types identified by us.

Abbreviations: ST= stromal cells, T/NK= T cells and NK cells, SE= secretory epithelial cells, LE= lymphatic endothelial cells, SM= smooth muscle cells, MP= macrophages, P/V= pericytes and vascular smooth muscle cells, CE= ciliated epithelial cells, EN= endothelial cells, B/P= B cells and plasma B cells, MA= mast cells, NEW1-3= 3 unknown clusters detected in the Cancer Cell 2020 dataset only.

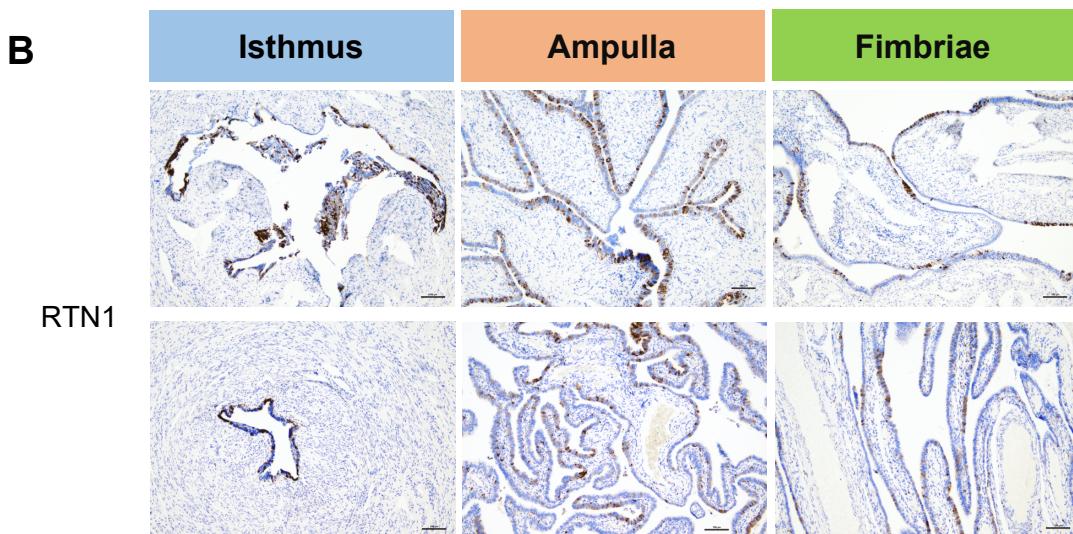
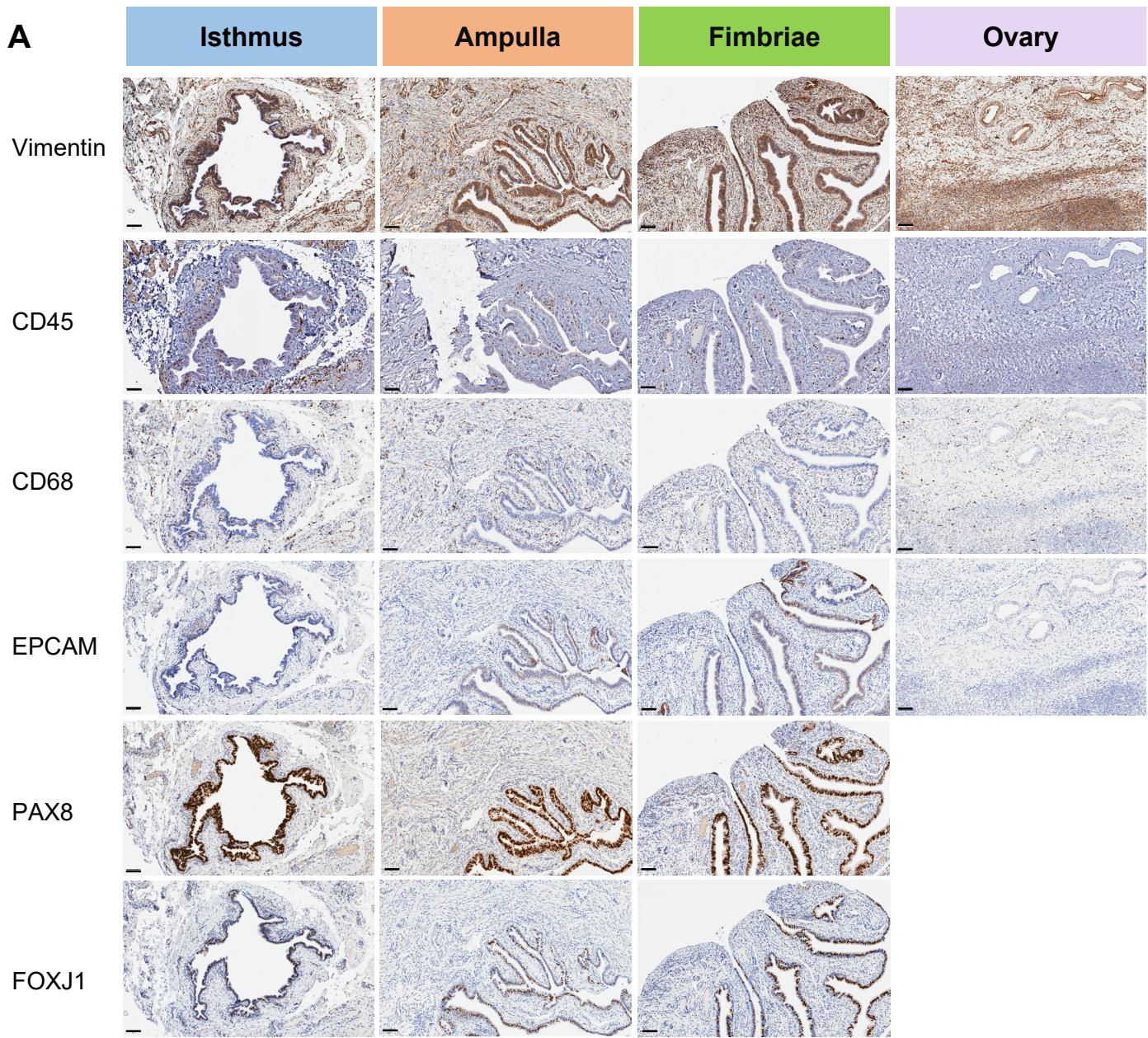


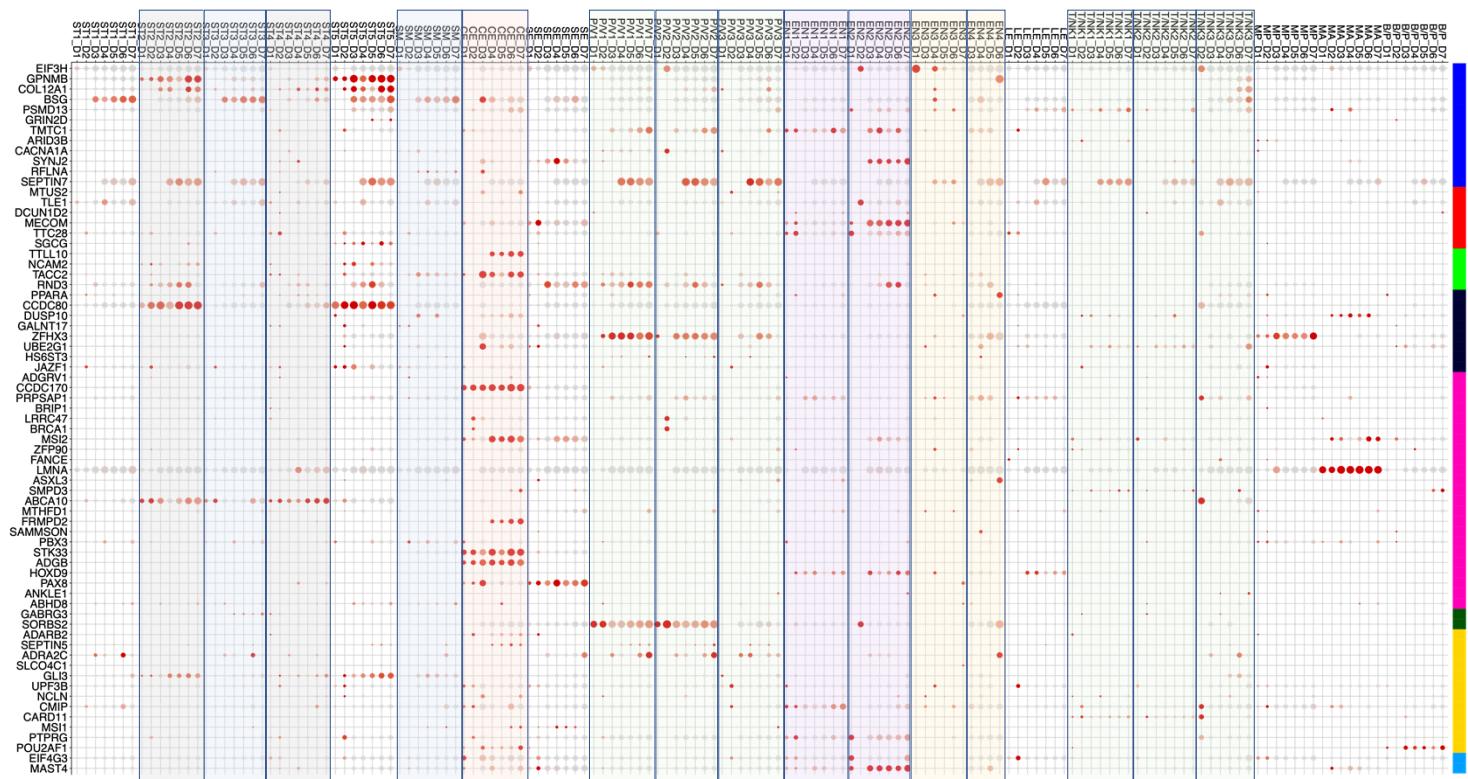
Figure S2 (related to Figure 2). Expression profiles of the isthmus, ampulla, and fimbrial regions of the postmenopausal fallopian tube.

A) Immunohistochemical staining of vimentin (mesenchymal cell marker), CD45 (hematopoietic cell marker), CD68 (monocyte and tissue macrophage marker) and EPCAM (epithelial cell marker) in the postmenopausal isthmus, ampulla, fimbriae, and ovary. PAX8 (secretory epithelial cell marker) and FOXJ1 (ciliated epithelial cell marker) expressions are shown in the isthmus, ampulla, and fimbriae of the fallopian tube (scale bar 100 µm).

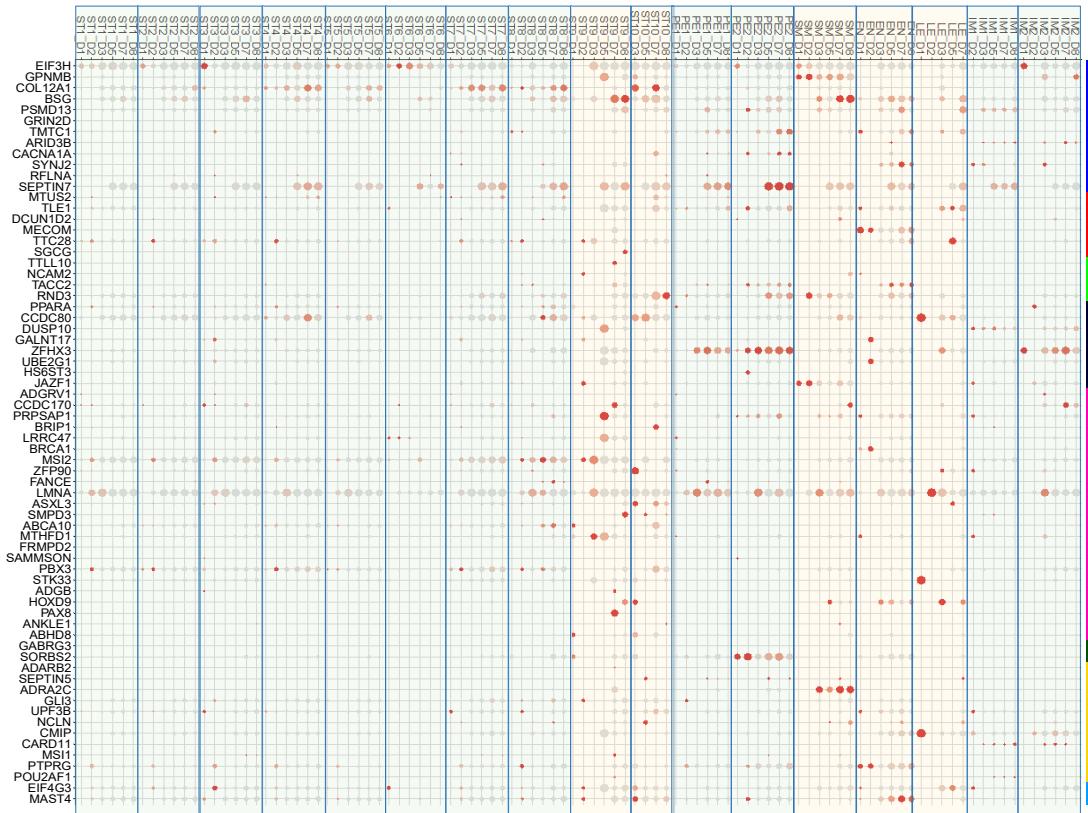
B) Immunohistochemical staining of RTN1 in the postmenopausal isthmus, ampulla, and fimbria (scale bar 100 µm).

Figure S3

A Fallopian tube



Ovary



Average expression

Percentage expressed

GWAS phenotype

- 25
- 50
- 75
- 100

- High-grade serous carcinoma
- Borderline
- Low-grade serous carcinoma
- Mucinous carcinoma
- High-grade serous carcinoma/clear cell carcinoma
- Clear cell carcinoma
- Endometrioid adenocarcinoma

Figure S3

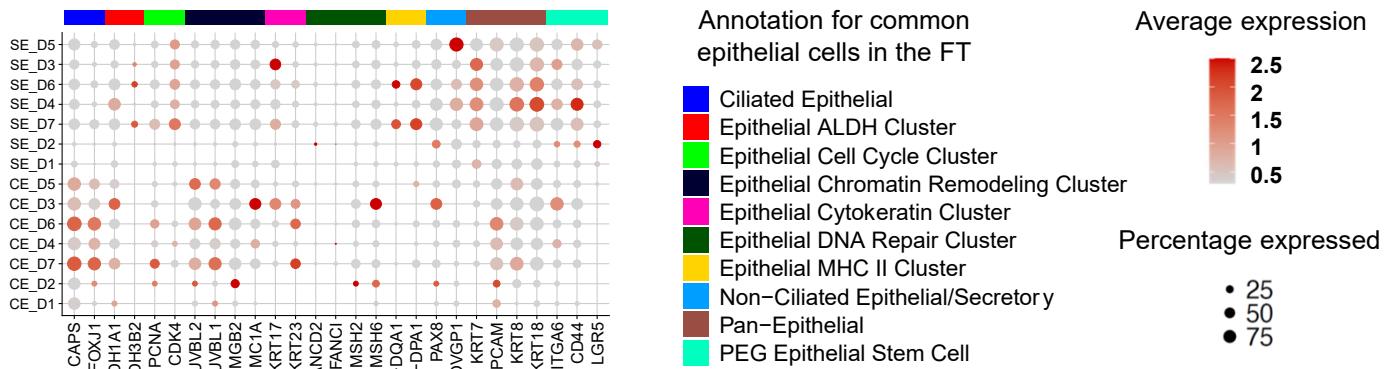
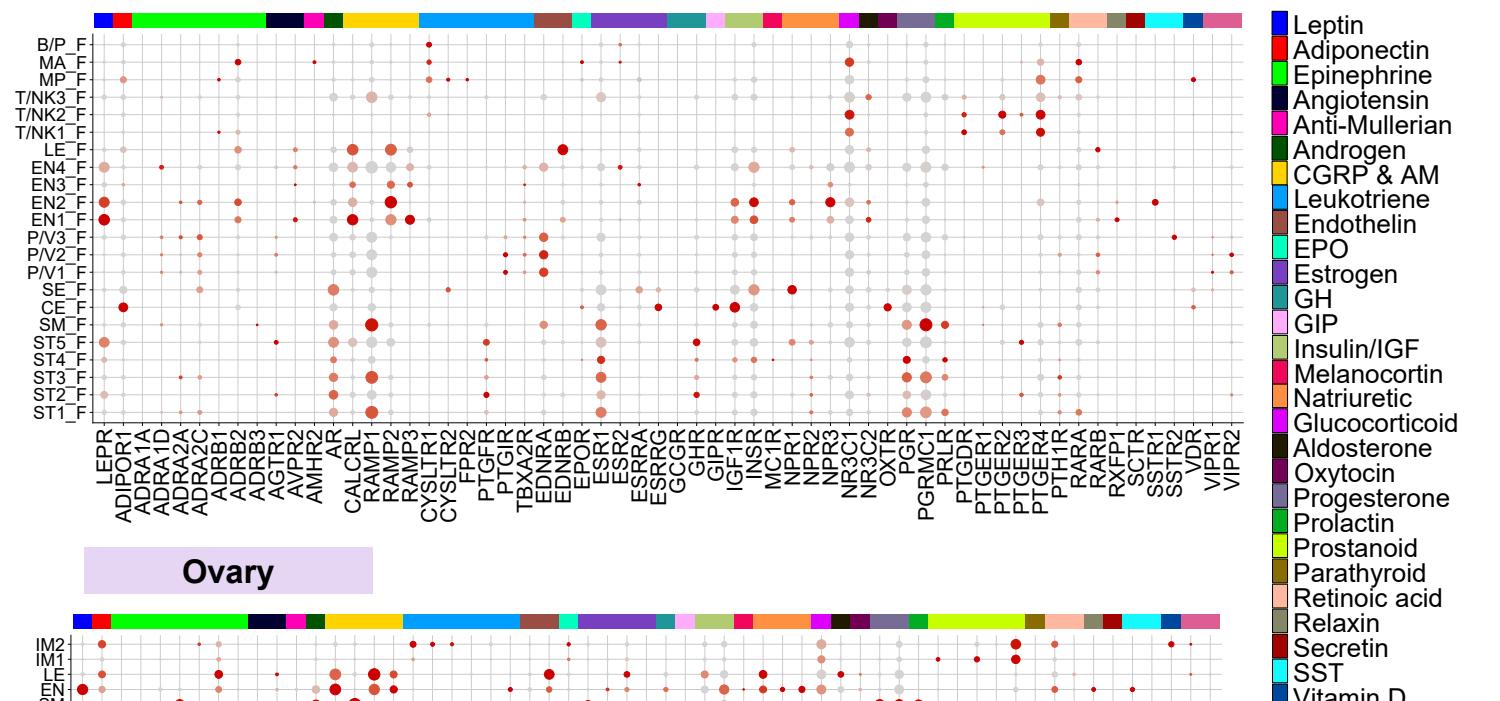
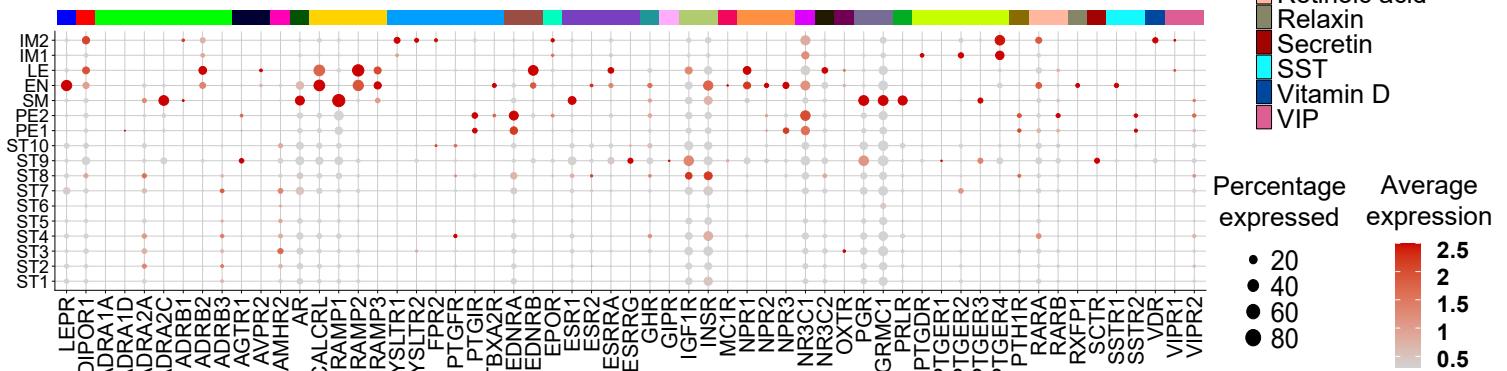
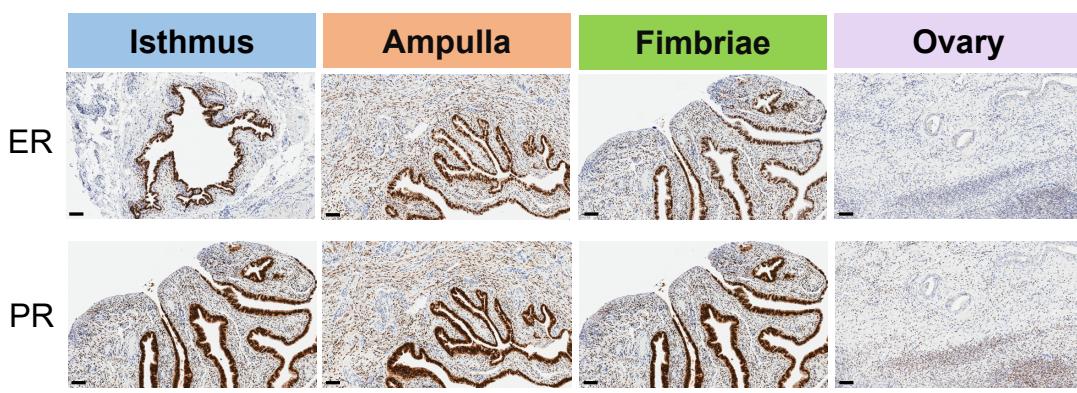
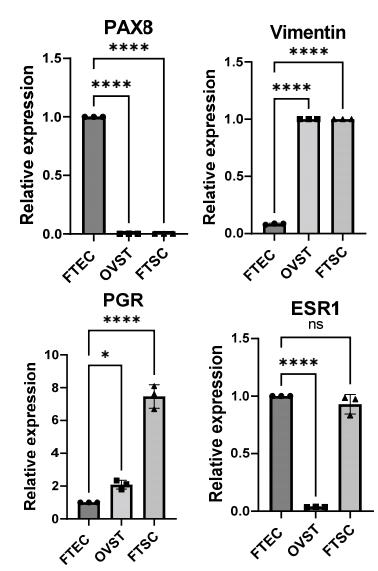
B Fallopian tube**C Fallopian tube****Ovary****D****E**

Figure S3 (related to Figure 3). Expression of GWAS and other marker genes in different regions of the postmenopausal fallopian tube and the ovary obtained from scRNA-seq.

A) Cell type specific GWAS gene expression in the fallopian tube (n= 7) and ovary (n= 6). Fallopian tube (top): Cell subtypes with matching colors (grey, blue, green, and purple) have that same set of GWAS genes expressed *among* and *across* subtypes, except for yellow where some patients express different sets of GWAS genes. Ovary (bottom): Cell subtypes highlighted in green show expression for the same set of GWAS genes among patients, and in yellow where some patients express different sets of GWAS genes.

B) Normalized expression of genes associated with ciliated (CE) and secretory (SE) epithelial cells of the fallopian tube in each donor (scRNA-seq). The genes are grouped by epithelial cell subtypes or functions. Note the expression of markers for intercalated or PEG cells in SE cells.

C) Dot plots of hormone receptor genes and their putative hormone ligands (color bars on top) in the postmenopausal fallopian tube fimbriae (Top) and ovary (Bottom). Colors indicate the specific hormones.

D) Immunohistochemical staining of estrogen (ER) and progesterone (PR) hormone receptors in the postmenopausal isthmus, ampulla, fimbriae, and ovary (scale bar 100 μ m).

E) qRT-PCR of the indicated RNA expressed in primary human fallopian tube epithelial cells (FTEC), fallopian tube stromal cells (FTSC) and ovarian stromal cells (OVST). Bar graphs show relative mean RNA expression \pm SD of one biological sample performed in triplicate of PAX8, Vimentin, progesterone receptor (PGR) and estrogen receptor 1 (ESR1) for each cell type. * p<0.05, **** p<0.0001

Abbreviations: ST= stromal cells, T/NK= T and natural killer cells, SE= secretory epithelial cells, LE= lymphatic endothelial cells, SM= smooth muscle cells, MP= macrophages, P/V= pericytes and vascular smooth muscle cells, CE= ciliated epithelial cells, EN= endothelial cells, B/P= B cells and plasma B cells, MA= mast cells, CGRP & AM= calcitonin gene-related peptide & adrenomedullin, EPO= erythropoietin, GH= growth hormone, GIP= glucose-dependent insulinotropic polypeptide, IGF= insulin-like growth factor, SST= somatostatin, VIP= vasoactive intestinal polypeptide.

Figure S4

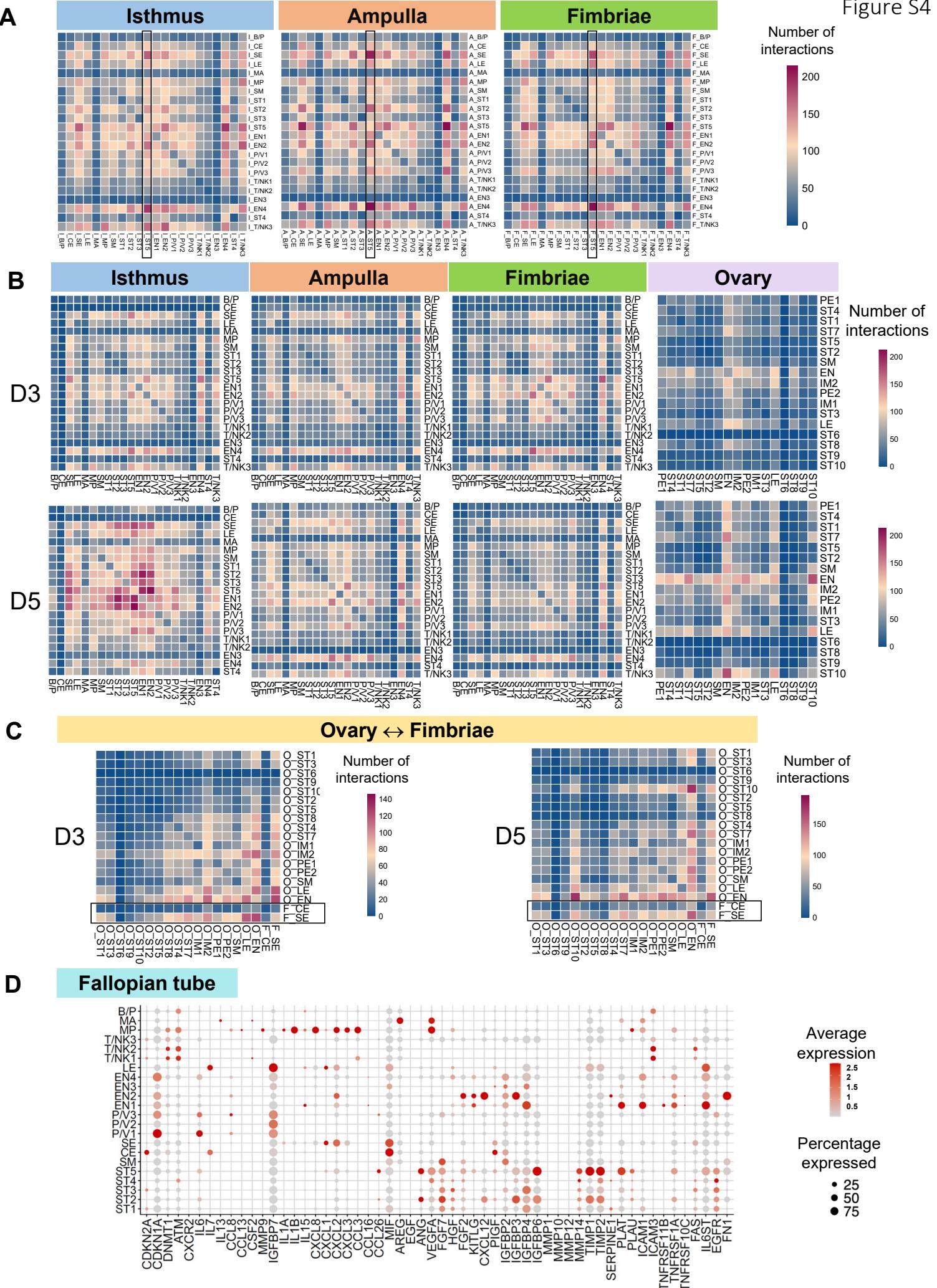


Figure S4 (related to Figure 4). Ligand-receptor interactions in the fallopian tube and ovary in select patients.

A) Fallopian tube. Heatmaps showing the frequency of interactions among the different cell types in the isthmus, ampulla, and fimbria of the fallopian tube as detected by CellPhoneDB for all scRNA-seq donors (n= 7). ST5 from the fallopian tube interacts strongly with CE, T/NK, SE, and EN cells in the fallopian tube.

B) Heatmaps showing the frequency of interactions among different cell types in the isthmus, ampulla, and fimbriae in the fallopian tube and ovary imputed by CellPhoneDB from scRNA-seq (n= 2).

C) Ovary ⇔ Fimbriae interactions. Heatmaps showing the frequency of interactions detected by CellPhoneDB among different cell types in the ovary (O) with ciliated (CE) and secretory epithelial cells (CE) in the fallopian tube using scRNA-seq (n= 2).

D) Aging and senescence-related gene expression in the fallopian tube.

Abbreviations: ST= stromal cells, T/NK= T and natural killer cells, SE= secretory epithelial cells, LE= lymphatic endothelial cells, SM= smooth muscle cells, MP= macrophages, P/V= pericytes and vascular smooth muscle cells, CE= ciliated epithelial cells, EN= endothelial cells, B/P= B cells and plasma B cells, MA= mast cells.

Figure S5

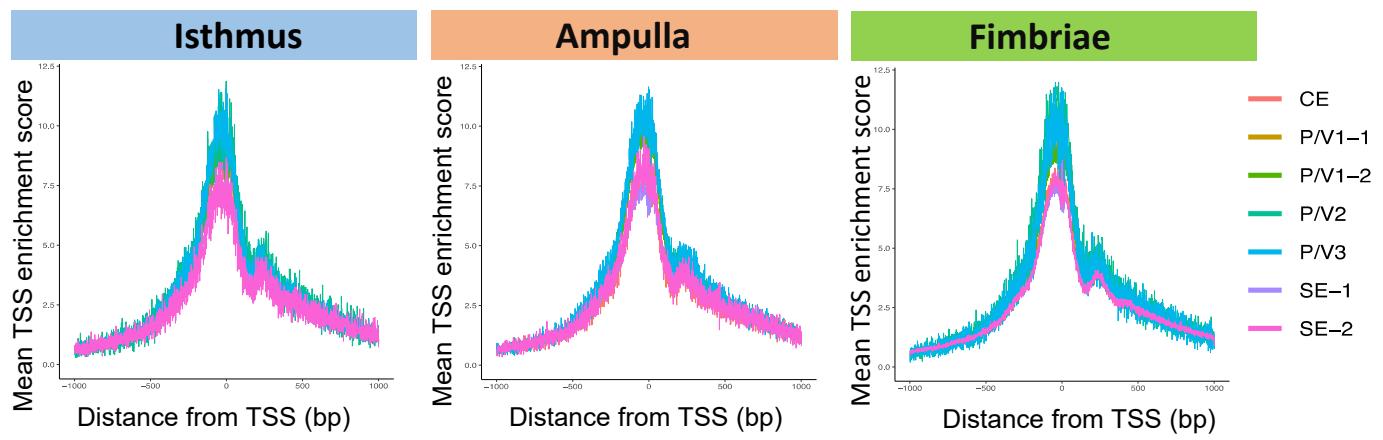
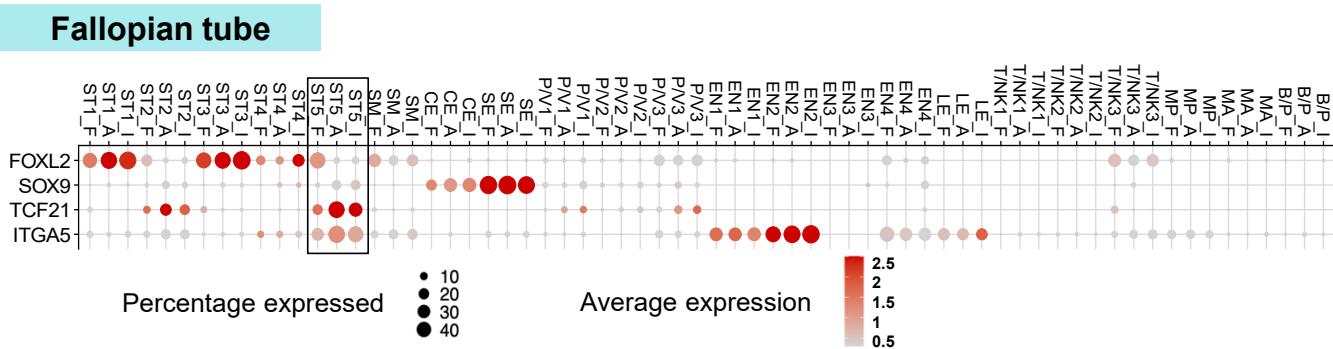
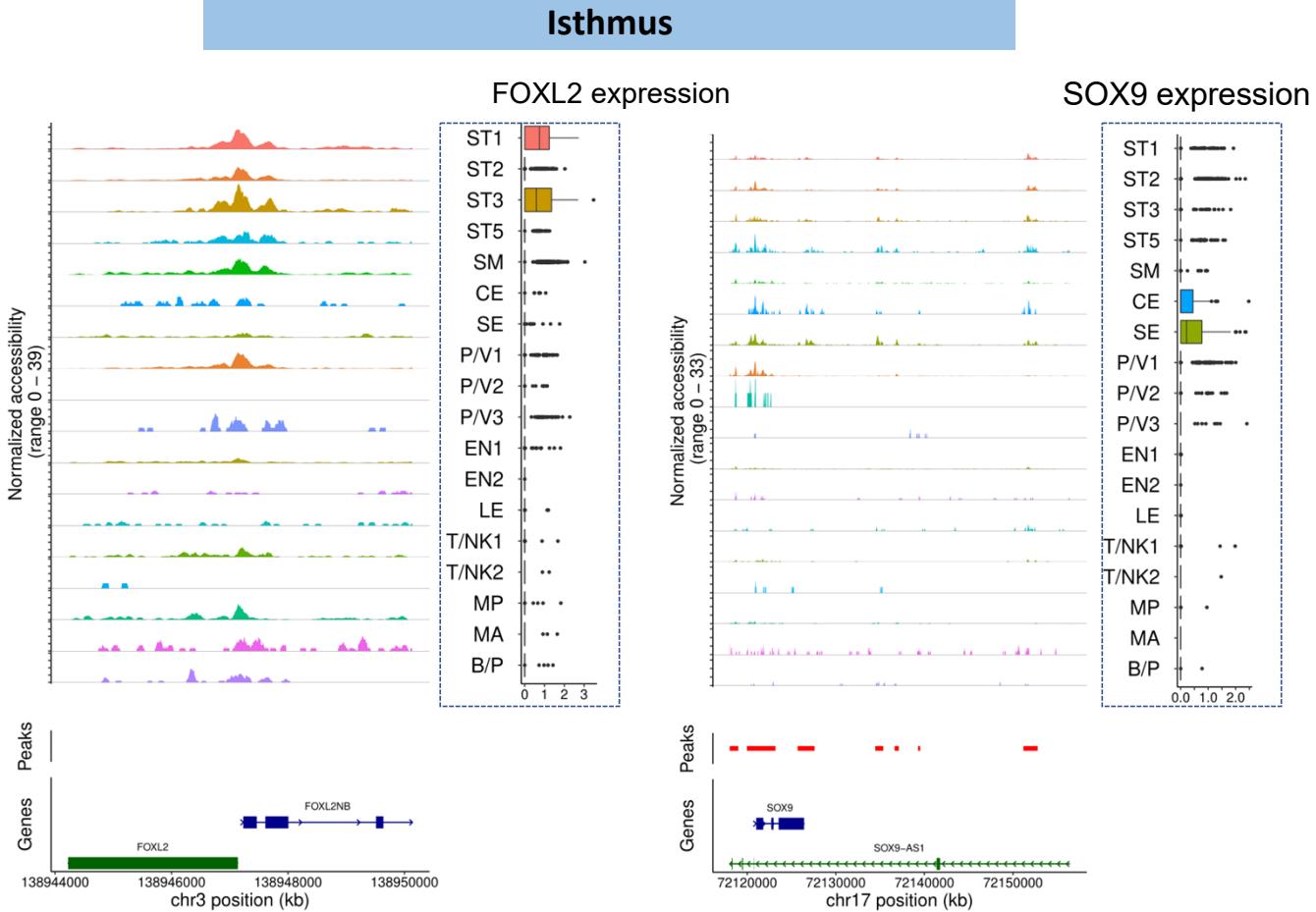
A**B****C**

Figure S5

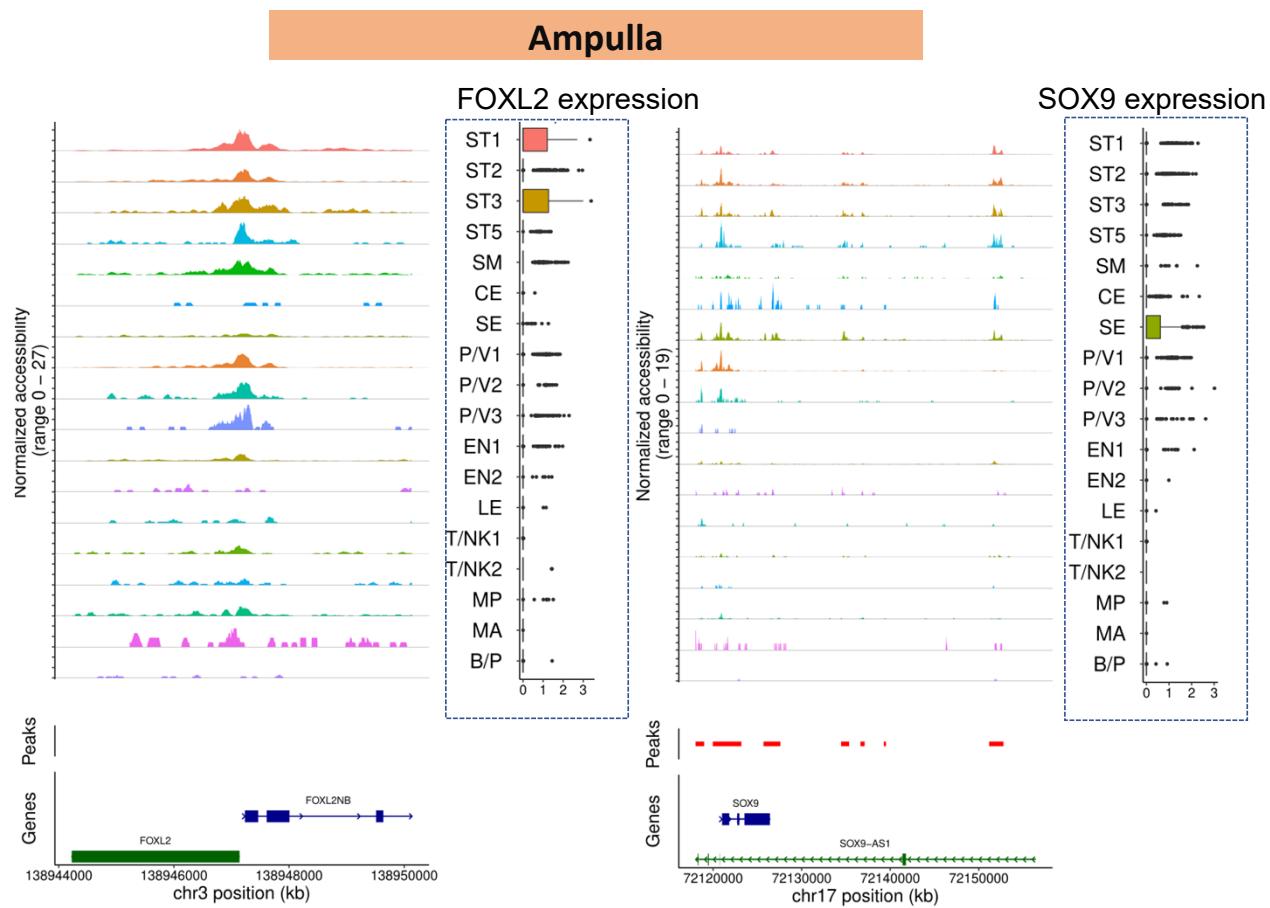
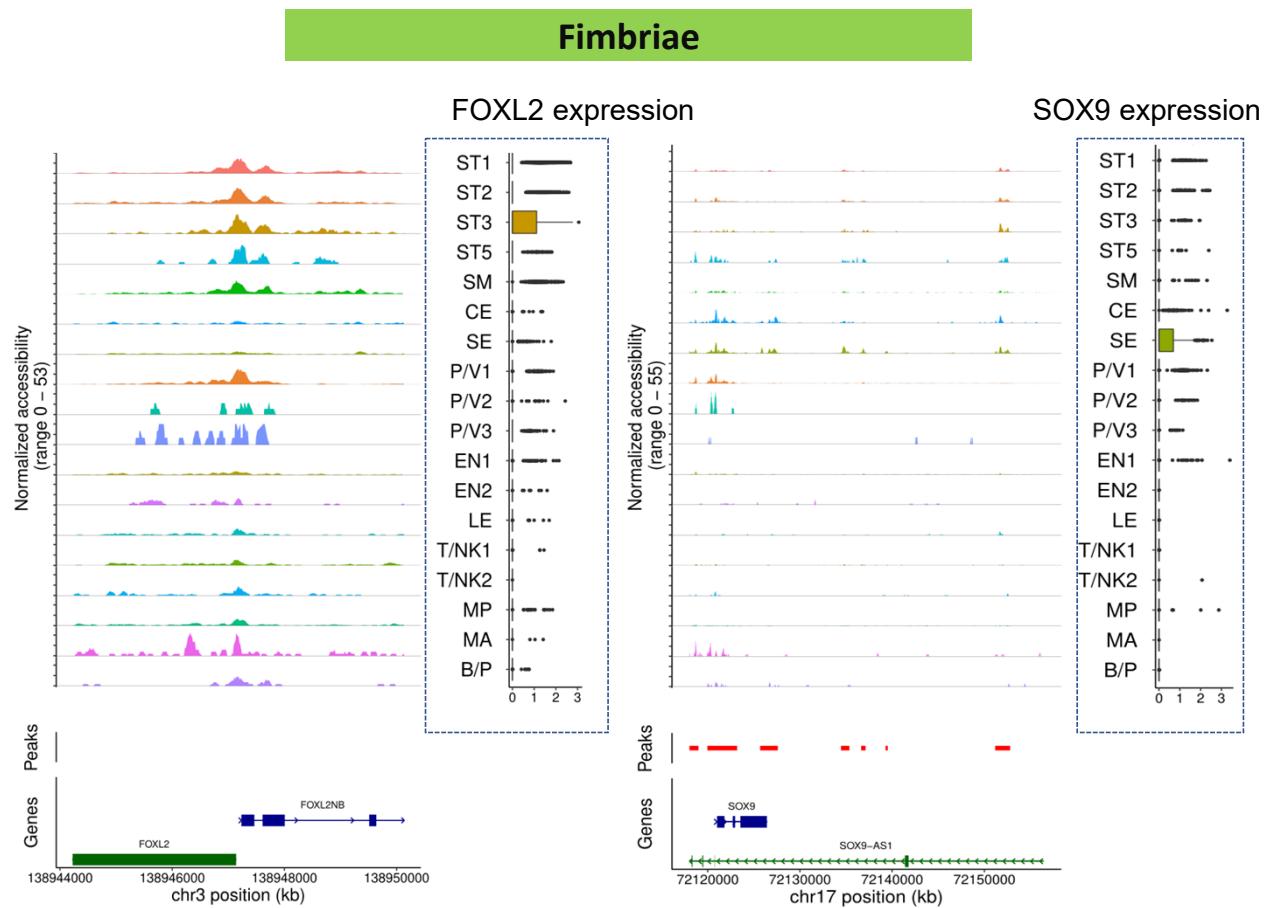
D**E**

Figure S5

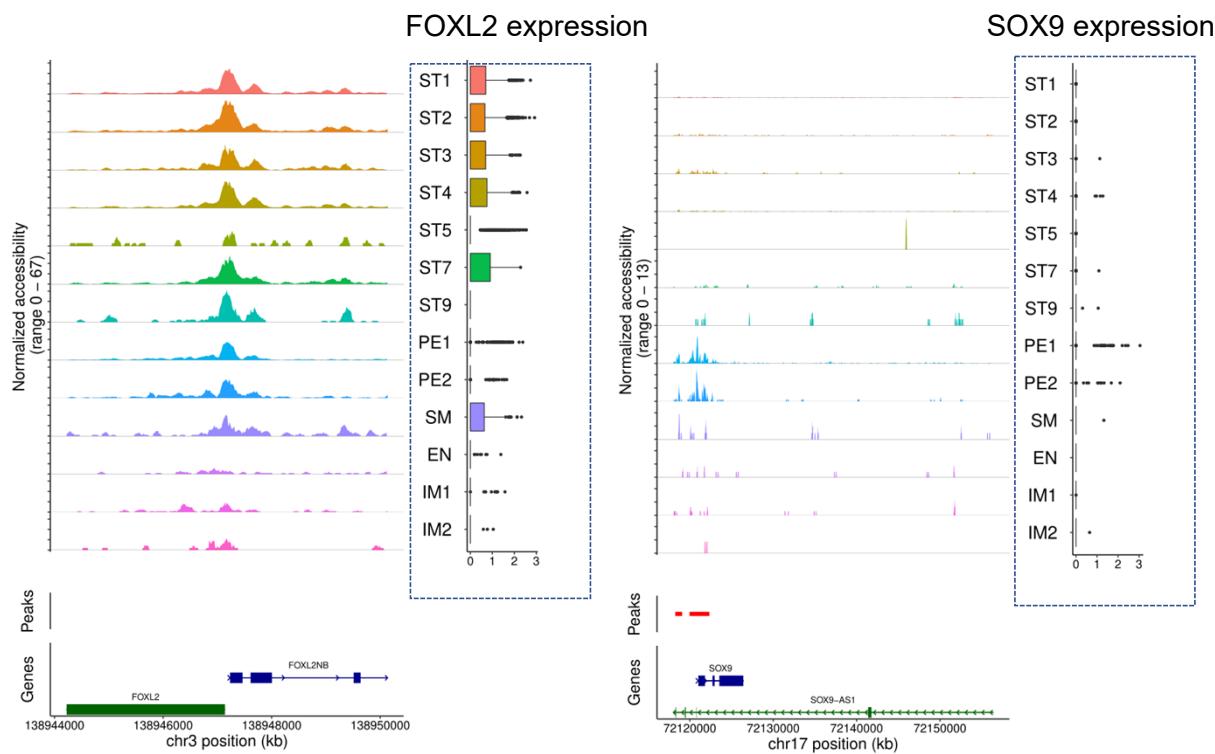
F**Ovary**

Figure S5 (related to Figure 5). Analysis of select transcription factors in the postmenopausal fallopian tube and ovary using scRNA-seq and scATAC-seq.

A) ATAC seq. Transcription Start Site (TSS) enrichment scores calculated across all genes for select cell types, 1000 bp upstream and downstream from their respective TSS in the isthmus (n= 4), ampulla (n= 3), and fimbria (n= 4).

B) scRNA-seq Dot plot of select epithelial-mesenchymal transition (EMT) markers in the isthmus, ampulla and fimbriae of the fallopian tube based on scRNA-seq.

C) Track plots from scATAC-seq showing the accessibility profiles at FOXL2 and SOX9 loci across all cell sub-types (left) in the isthmus, ampulla, fimbriae, and ovary. Matching scRNA-seq expressions of FOXL2 and SOX9 are shown in the boxplots (right).

Abbreviations: ST= stromal cells, T/NK= T and natural killer cells, SE= secretory epithelial cells, LE= lymphatic endothelial cells, SM= smooth muscle cells, MP= macrophages, P/V= pericytes and vascular smooth muscle cells, CE= ciliated epithelial cells, EN= endothelial cells, B/P= B cells and plasma B cells, MA= mast cells.

Figure S6

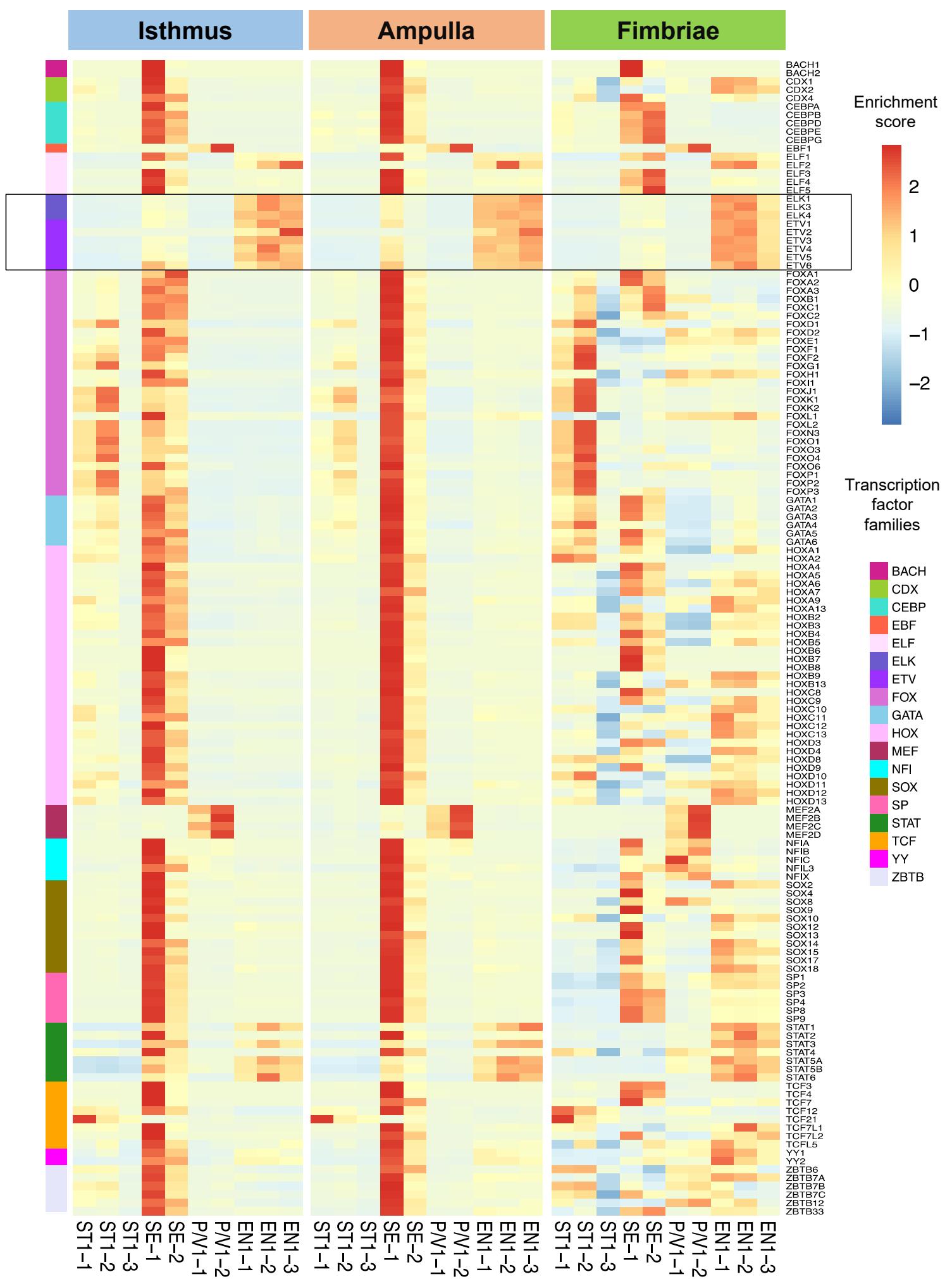


Figure S6

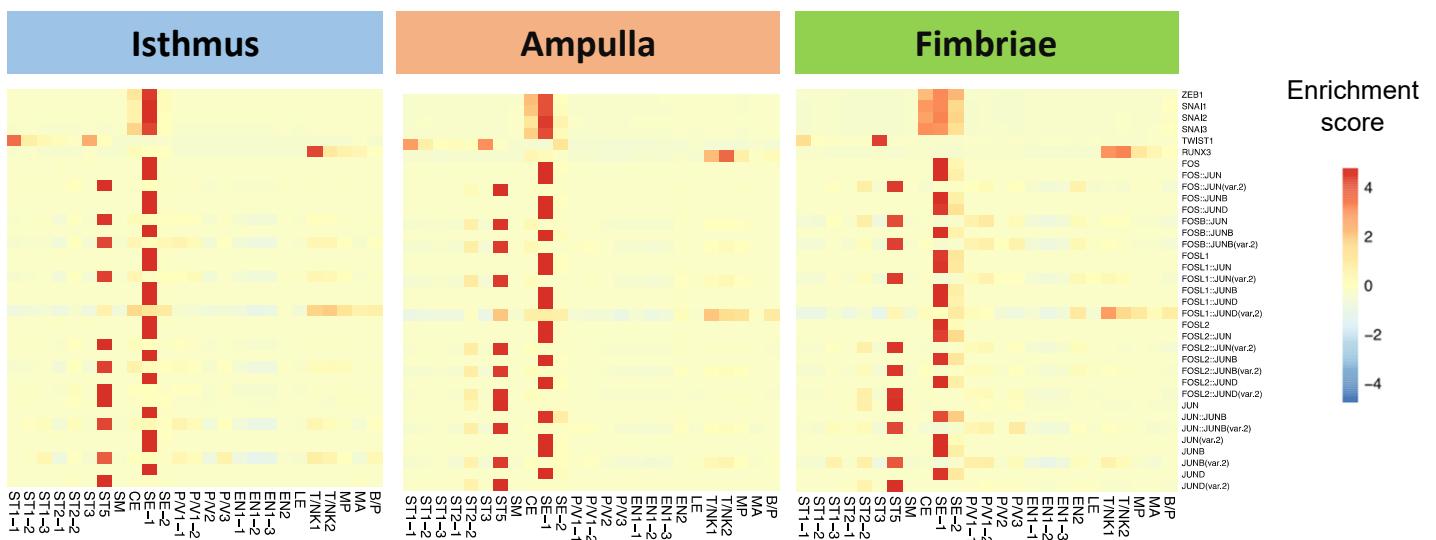
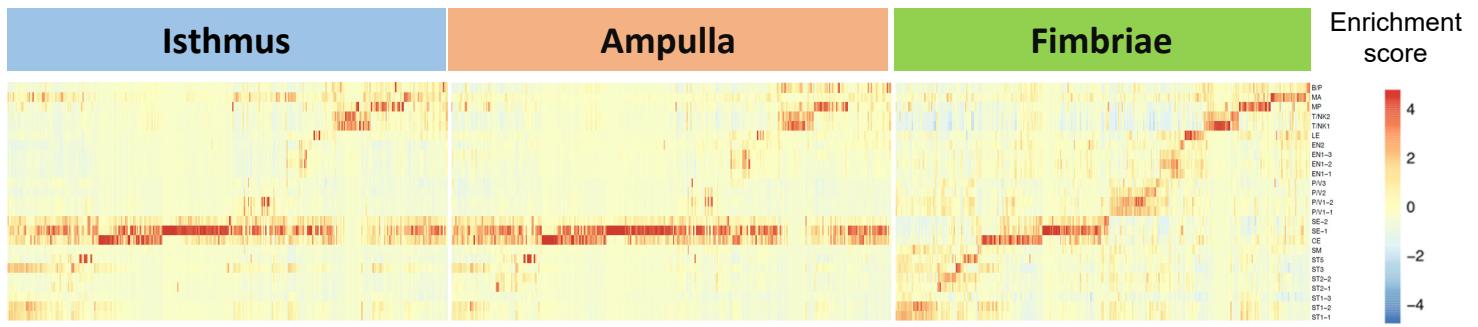
B**C**

Figure S6 (related to Figure 6). Transcription factor analysis of scATAC-seq data in the different anatomical regions of the fallopian tube.

A) Heat maps depicting select transcription factor (TF) accessibility in the isthmus (n= 4), ampulla (n= 3), and fimbria (n= 4) by cell type using scATAC-seq alone. TF families ELK and ETV are highlighted.

B) TF enrichment of JUN/FOS family and other epithelial-mesenchymal transition (EMT) TFs including ZEB1, SNA12, SNA13, TWIST1 and RUNX3 in the in the isthmus, ampulla and fimbria (using the JASPAR transcription factor database). For a few TFs, the splicing variant (var2) is expressed in one cell type but not in the other.

C) Motif enrichment analysis of 330 expressed transcription factors across cell types in the isthmus, ampulla and fimbria of the fallopian tube.

Abbreviations: ST= stromal cells, T/NK= T and natural killer cells, SE= secretory epithelial cells, LE= lymphatic endothelial cells, SM= smooth muscle cells, MP= macrophages, P/V= pericytes and vascular smooth muscle cells, CE= ciliated epithelial cells, EN= endothelial cells, B/P= B cells and plasma B cells, MA= mast cells.

Table S1 (related to Figure 1): Patient cohort characteristics.

Patient ID	Age (years)	Race	Hispanic	Gravida Para	MP age (years)	BMI	RNA-seq	ATAC-seq	Surgery indication
D1	70	White	No	G3P2	51	27.58	^a A, F, I, O	-	Vaginal prolapse
D2	72	White	No	G2P2	52	28.47	^a A, I, O	-	Vaginal prolapse
D3	62	White	No	G3P3	56	31.48	A, F, I, O	A, F, I, O	Vaginal prolapse
D4	55	White	No	G4P4	53	21.45	F, I	F, I	Vaginal prolapse
D5	65	Asian	Unknown	G3P2	50	22.73	A, F, I, O	A, F, I, O	Vaginal prolapse
D6	61	White	Yes	G4P3	51	26.74	A, F, I	A, F, I	Vaginal prolapse
D7	62	White	No	G4P2	52	31.31	A, F, O	-	Vaginal prolapse
D8	58	White	No	G6P6	50	21.57	O	O	Vaginal prolapse

All patients were non-smokers.

Abbreviations: donor (D), ampulla (A), isthmus (I), fimbria (F), ovary (O), Menopausal age (MP), Gravida (G) = number of pregnancies, Para (P) = number of births of viable offspring. ^aDrop-seq data