SI Appendix

# Comprehensive transcriptional atlas of human adenomyosis deciphered by the integration of single-cell RNA-sequencing and spatial transcriptomics

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**Figure S1**

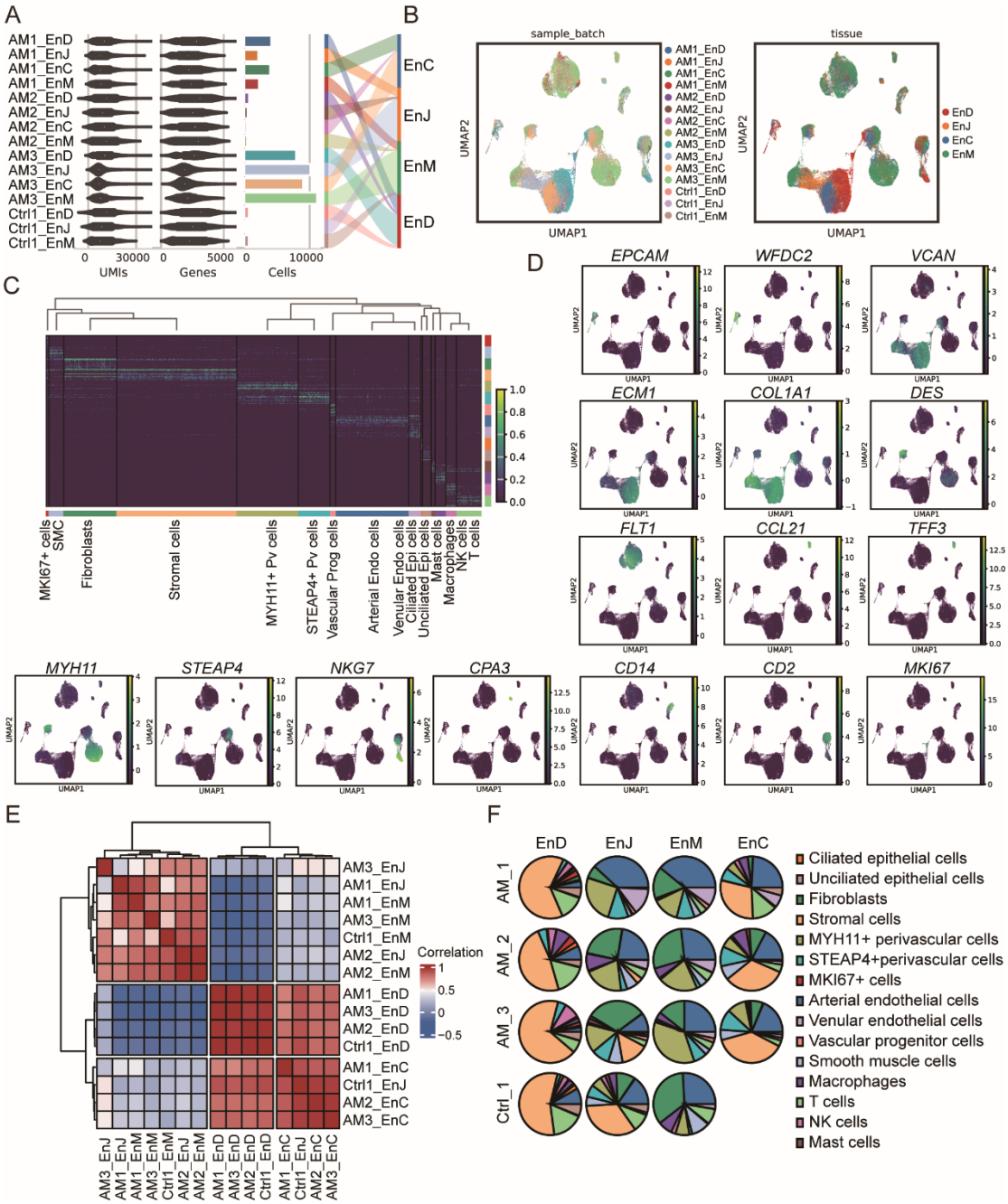



**Figure S1. Quality control and standardization of scRNA-seq, expressions of marker genes and distribution of each sample in various uterine regions.** (A) Diagram showing scRNA-seq metrics per sample (left) after quality control. These metrics indicate unique molecular identifier (UMIs) and total genes per cells across samples. The cord diagram (right) represents each sample in each tissue types. (B) Origin of droplet cells by sample (left) and tissue (right). (C) Heatmap revealing the scaled expression of differentially expressed genes for each cell type. (SMC: smooth muscle cells; Pv: perivascular; Prog: progenitor; Endo: endothelial; Epi: epithelial). (D) UMAP plot showing marker genes pattern in each cell type. (E) Correlation based on cluster frequencies, across all specimens profiled by scRNA-seq. (F) Pie chart represents major cell type proportions for each specimen.

**Figure S2**

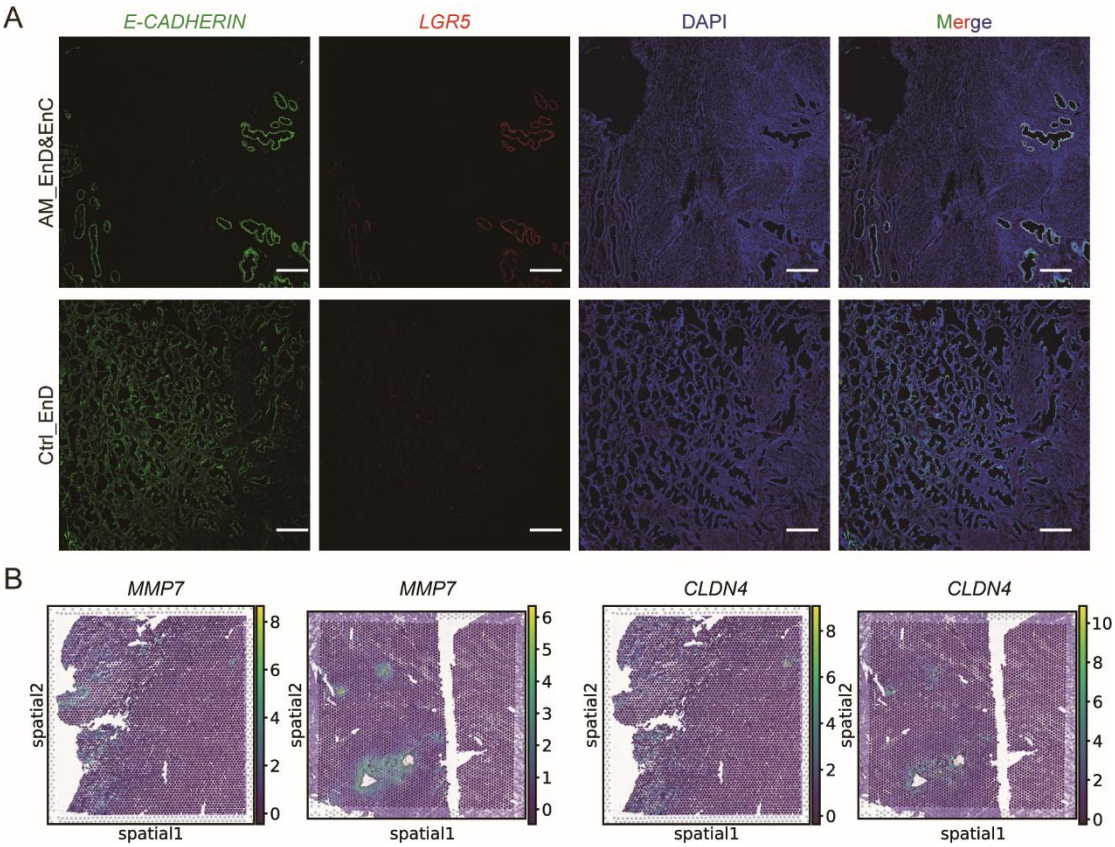



**Figure S2**. **Expression of characteristic genes of epithelial cells in adenomyosis lesions.** (A) Representative images of FISH for *LGR5* $^{+}$ cells in EnD and EnC of adenomyosis (upper panel), EnD of control (lower panel). Bar: 400 μm. (B) Visualization of *MMP7*$^{+}$ cells and *CLDN4*$^{+}$ cells in various regions of adenomyosis specimen by spatial transcriptomics.

**Figure S3**

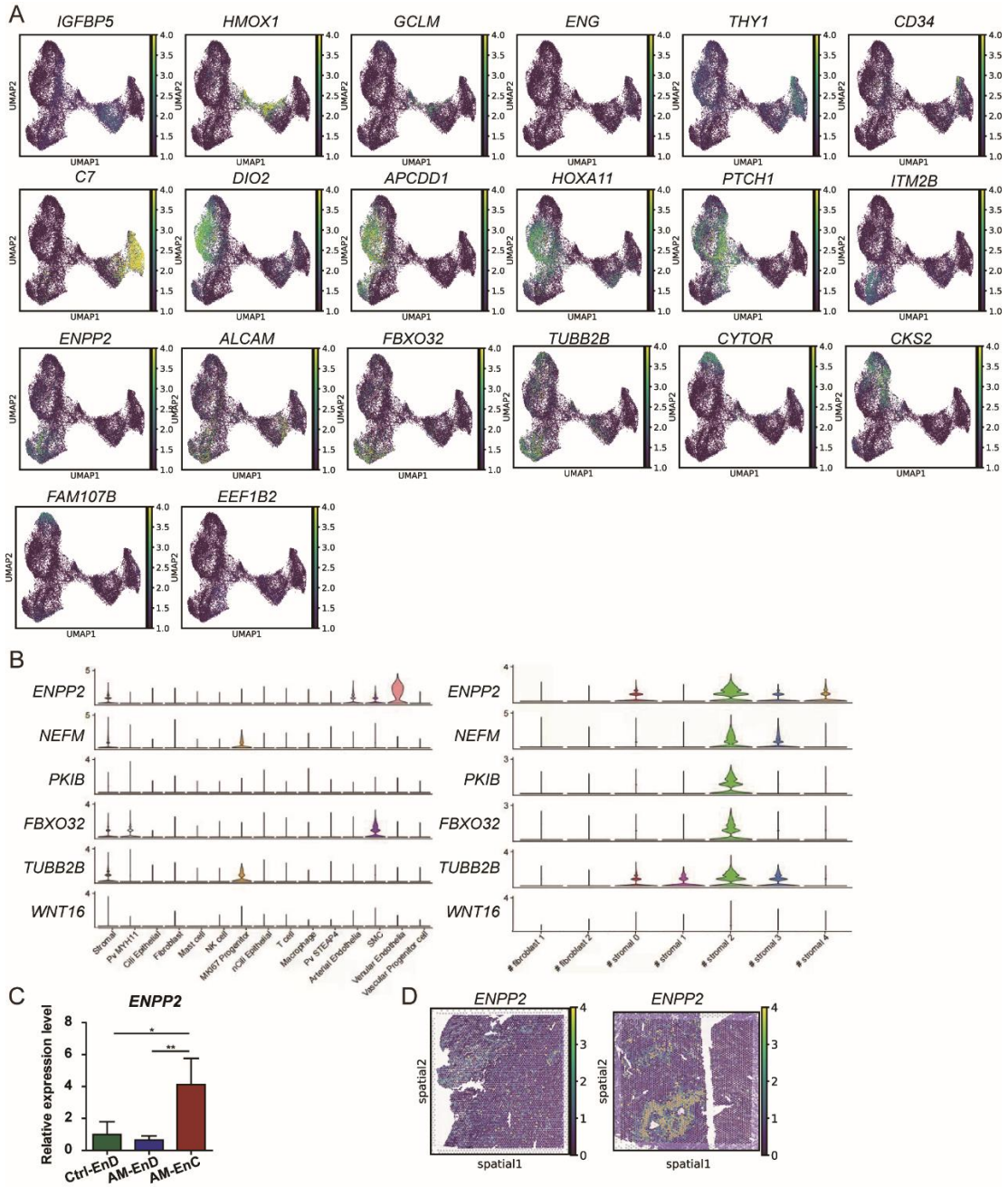
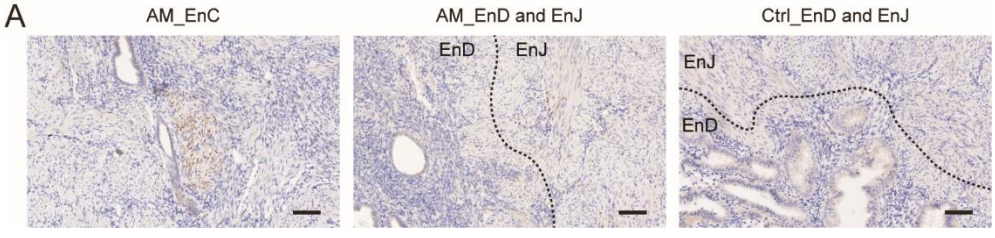



**Figure S3**. **Characteristic gene expression pattern in stromal subpopulations.** (A)Marker genes pattern in each stromal subclusters by UMAP plot. (B)The expression of marker genes of stromal 2 in all cell types and stromal subpopulations by Violin plot.

(C) mRNA expression levels of *ENPP2* for stromal 2 markers in Ctrl_EnD, AM_EnD and AM_EnC examined by qRT-PCR (n = 4 per group). Data are presented as the mean ± SEM, **p* < 0.05, ** *p* < 0.01. (D) Visualization of *ENPP2*$^{+}$ cells in various regions of adenomyosis specimen by spatial transcriptomics.

**FigureS4**



**FigureS4.The expression of WFDC1 in adenomyosis specimen by immunohistochemistry (IHC) staining.** Scale bar: 400 μm.

**Figure S5**

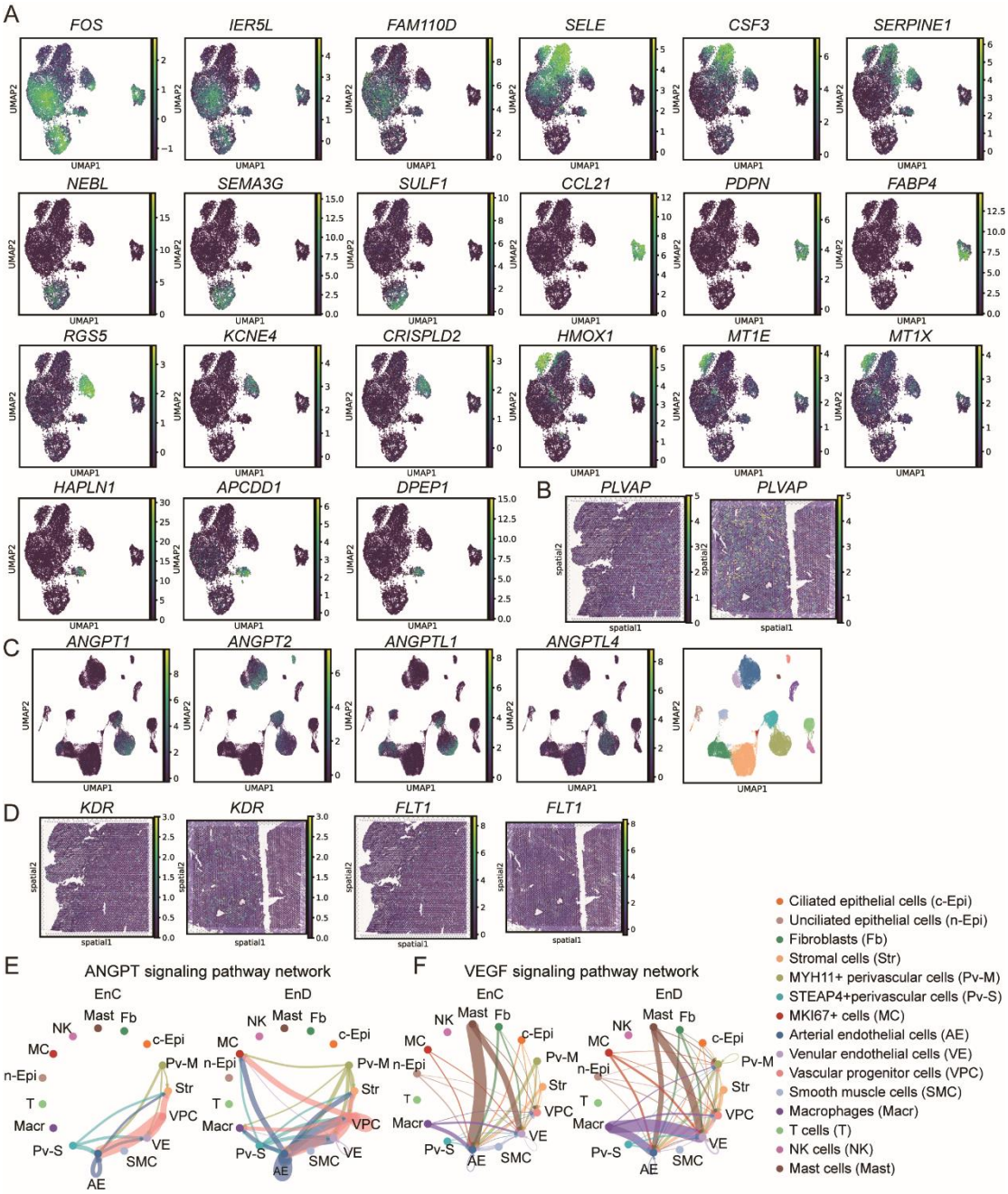



**Figure S5. Characteristic gene expression patterns of endothelial subpopulations in adenomyosis.** (A) Marker genes expression pattern in each endothelial subclusters

by UMAP plot. (B) Visualization of *PLVAP*$^{+}$ cells in spatial transcriptomics. (C) The expression of *ANGPT1*, *ANGPT2*, *ANGPTL1* and *ANGPTL4* in various cell types by UMAP plot. (D) Visualization of *KDR*$^{+}$ cells and *FLT1*$^{+}$ cells in spatial transcriptomics.

(E) Ligand-receptor pairs network of ANGPT signaling pathways in EnC and EnD. (F) Ligand-receptor pairs network of VEGF signaling pathways in EnC and EnD.

**Figure S6**

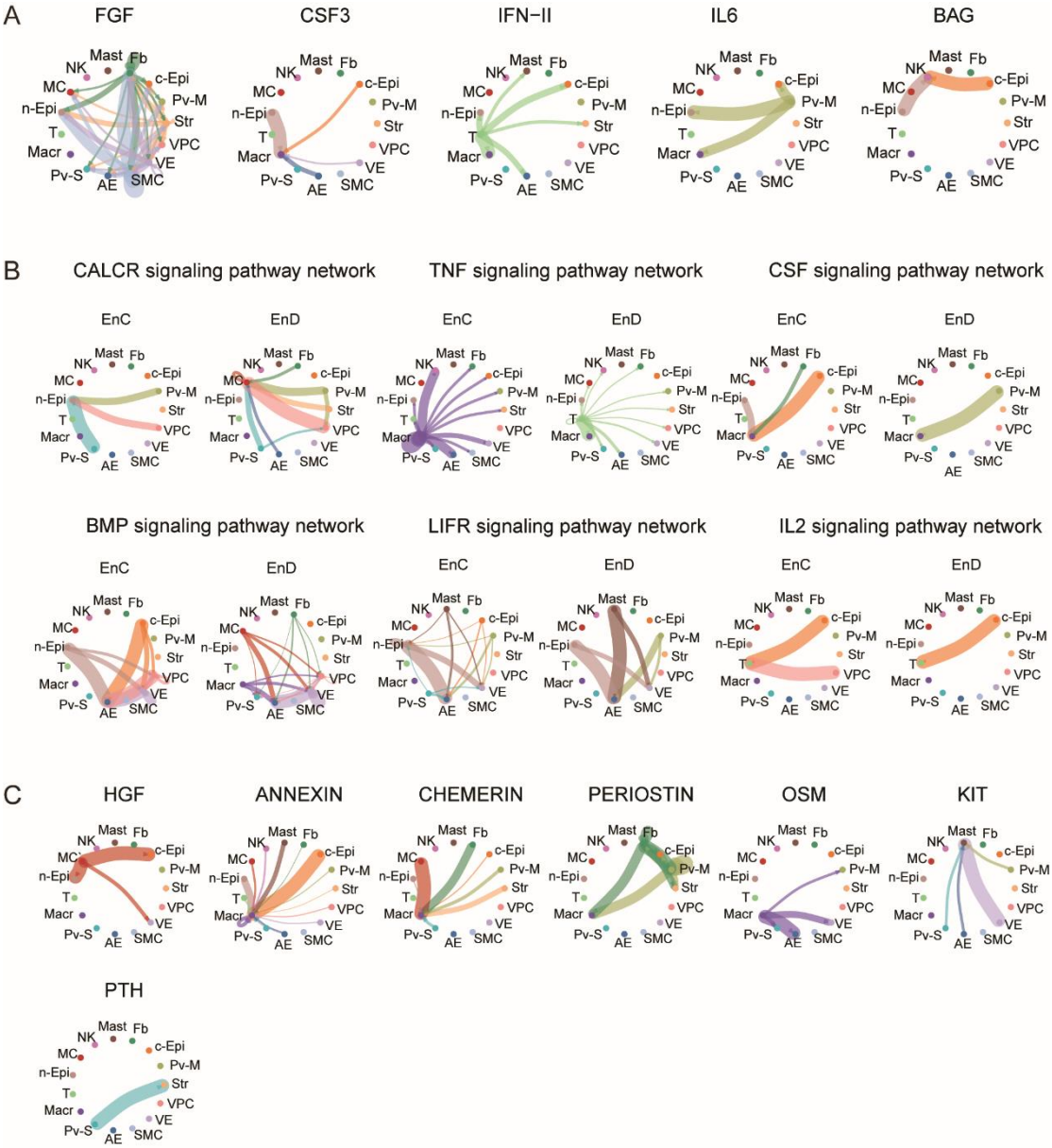



**Figure S6. Correlated signaling networks among different cell types in EnC and EnD of adenomyosis by CellPhoneDB.** (A) Network of intercellular interactions of specific signaling pathways for FGF, CSF3, IFN-II, IL6 and BAG in EnC. (B) Network of intercellular interactions of specific signaling pathways for CALCR, TNF, CSF, BMP, LIFR and IL2 in EnD and EnC. (C) Network of intercellular interactions of specific signaling pathways for HGF, ANNEXIN, CHEMERIN, PERIOSTIN, OSM, KIT and PTH in EnD.

**Table S1. Clinical characteristics of adenomyosis patient samples used in this study. Related to Figure 1.**

| Disease | Location | Sample No. | Age | Pregnancy |
|---|---|---|---|---|
| Ademomyosis | Eutopic endometrium (EnD) | Sample A1-0 | 49 | 1-0-1-1 |
| | Junctional zone (EnJ) | Sample A1-1 | | |
| | Adnomyophathy lesion (EnC) | Sample A1-2 | | |
| | Myometrium (EnM) | Sample A1-3 | | |
| Ademomyosis | Eutopic endometrium (EnD) | Sample A2-0 | 49 | 1-0-2-1 |
| | Junctional zone (EnJ) | Sample A2-1 | | |
| | Adnomyophathy lesion (EnC) | Sample A2-2 | | |
| | Myometrium (EnM) | Sample A2-3 | | |
| Ademomyosis | Eutopic endometrium (EnD) | Sample A3-0 | 48 | 1-0-2-1 |
| | Junctional zone (EnJ) | Sample A3-1 | | |
| | Adnomyophathy lesion (EnC) | Sample A3-2 | | |
| | Myometrium (EnM) | Sample A3-3 | | |
| uterine fibroids | Eutopic endometrium (EnD) | Sample C1-0 | 44 | 1-0-1-1 |
| | Junctional zone (EnJ) | Sample C1-1 | | |
| | Myometrium (EnM) | Sample C1-2 | | |

**Table S2. stromal subpopulation in samples. Related to Figure 3.**

| | Fibroblast 1 | Fibroblast 2 | Stromal 0 | Stromal 1 | Stromal 2 | Stromal 3 | Stromal 4 |
|---|---|---|---|---|---|---|---|
| EnD | 36 | 0 | 5419 | 5 | 24 | 2723 | 347 |
| EnJ | 2919 | 48 | 468 | 606 | 74 | 114 | 751 |
| EnC | 406 | 249 | 139 | 45 | 2910 | 18 | 1490 |
| EnM | 104 | 2219 | 0 | 0 | 0 | 0 | 0 |

**Table S3. Primer sequneces used for qRT-PCR in this study. Related to Figure 2, 3, 4.**

| Gene | Primer sequence |
|---|---|
| LGR5 rt F | CCTTGGCCCTGAACAAAATA |
| LGR5 rt R | ATTTCTTTCCCAGGGAGTGG |
| PKIB rt F | CCTCAAACTGGAGGCTCTCTCC |
| PKIB rt R | AGCACTCTTGATAGATTATGAGCC |
| ENPP2 rt F | TCAGAGGACGAATCAAAATGGG |
| ENPP2 rt R | CAGGTATGTCTTGAGTGTCAGG |
| WNT16 rt F | AAGTGAAGGCTGGCACTGG |
| WNT16 rt R | GGCAGTCTACTGACATCAACTTGG |
| APCDD1 rt F | GGAGTCACAGTGCCATCACATG |
| APCDD1 rt R | GGACCTTGTGATGAACTCTGGG |
| VWC2 rt F | GGAGTTCGTGGTGTCTCCATG |
| VWC2 rt R | CAAAGCAGTTTGGACCATTTTTGC |
| SFRP5 rt F | CACAAGTTCCCCCTGGACAA |
| SFRP5 rt R | TGTGCTCCATCTCACACTGG |
| GAPDH rt F | CTGCACCACCAACTGCTT |
| GAPDH rt R | TTCTGGGTGGCAGTGATG |