

Supplementary Materials for

**ENTPD3 as a Novel Regulator of Endometrial Receptivity: Suppressing EMT
via the ATP-P2Y2 Axis in Patients with Recurrent Implantation Failure**

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The PDF file includes:

Figures. S1 to S12

Tables S1 and S6

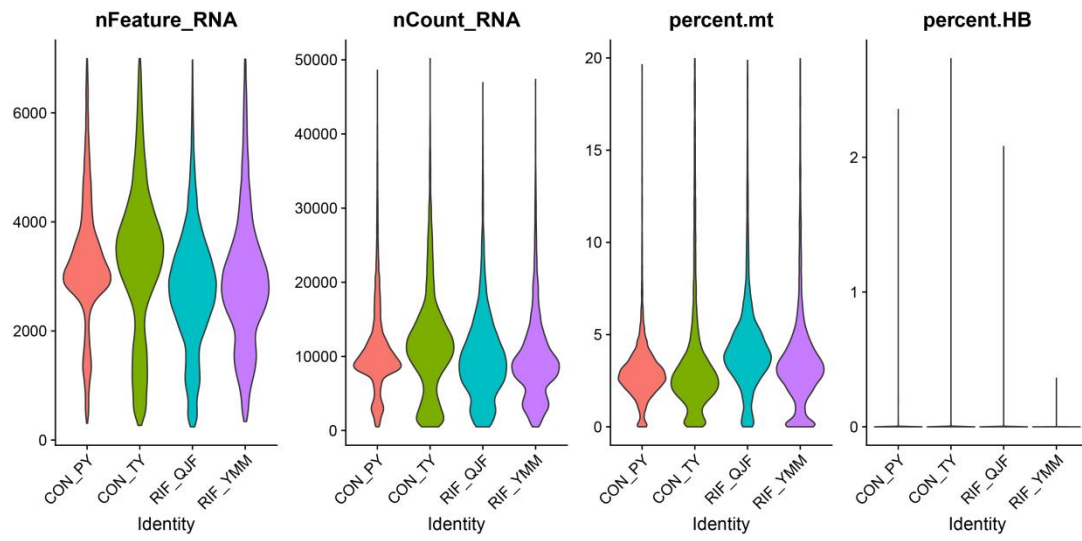


Fig. S1. Quality control metrics for scRNA-seq of endometrial tissues.

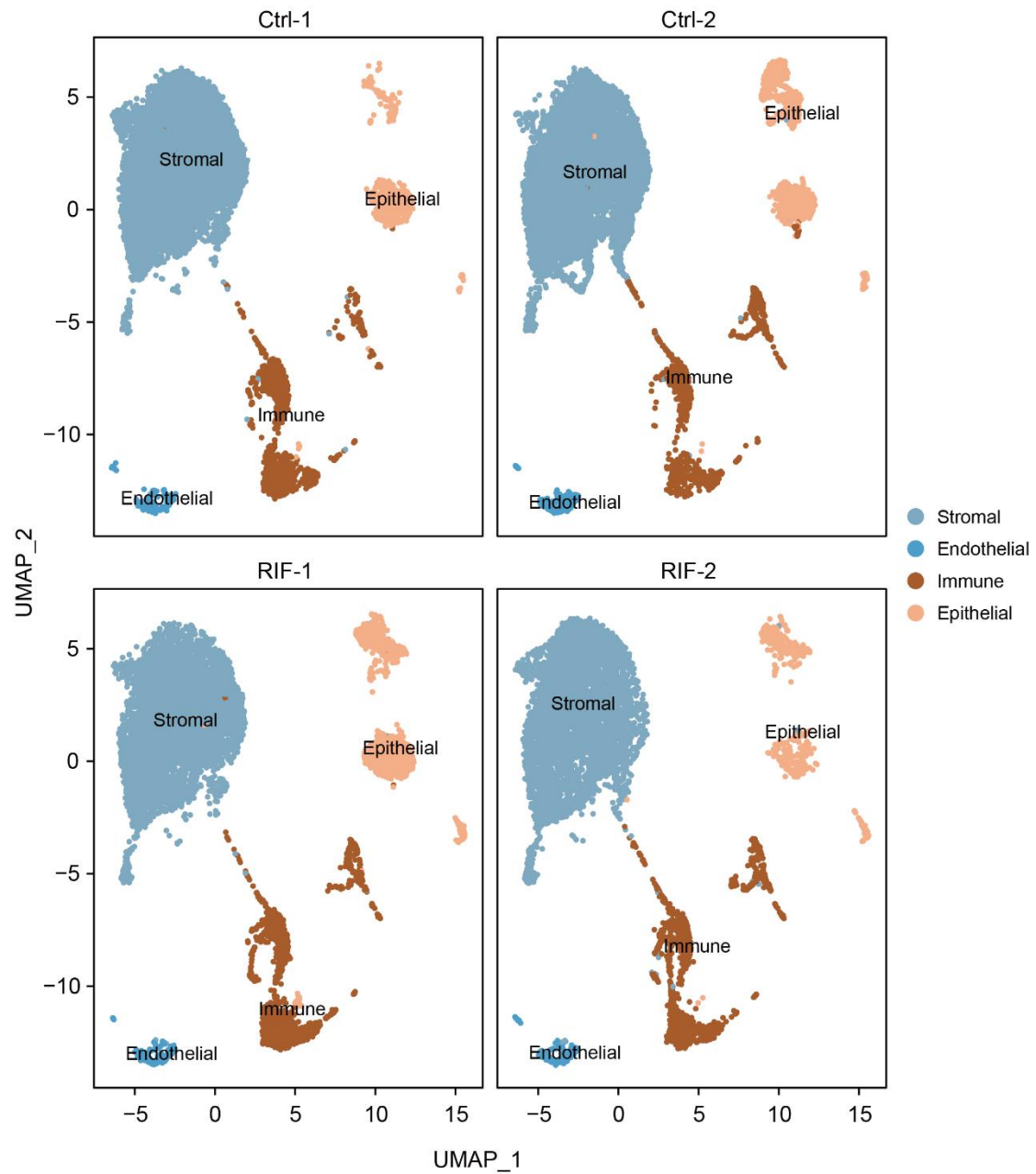


Fig. S2. UMAP showing the distribution of 4 major endometrial cells in two CTRL and two RIF patients.

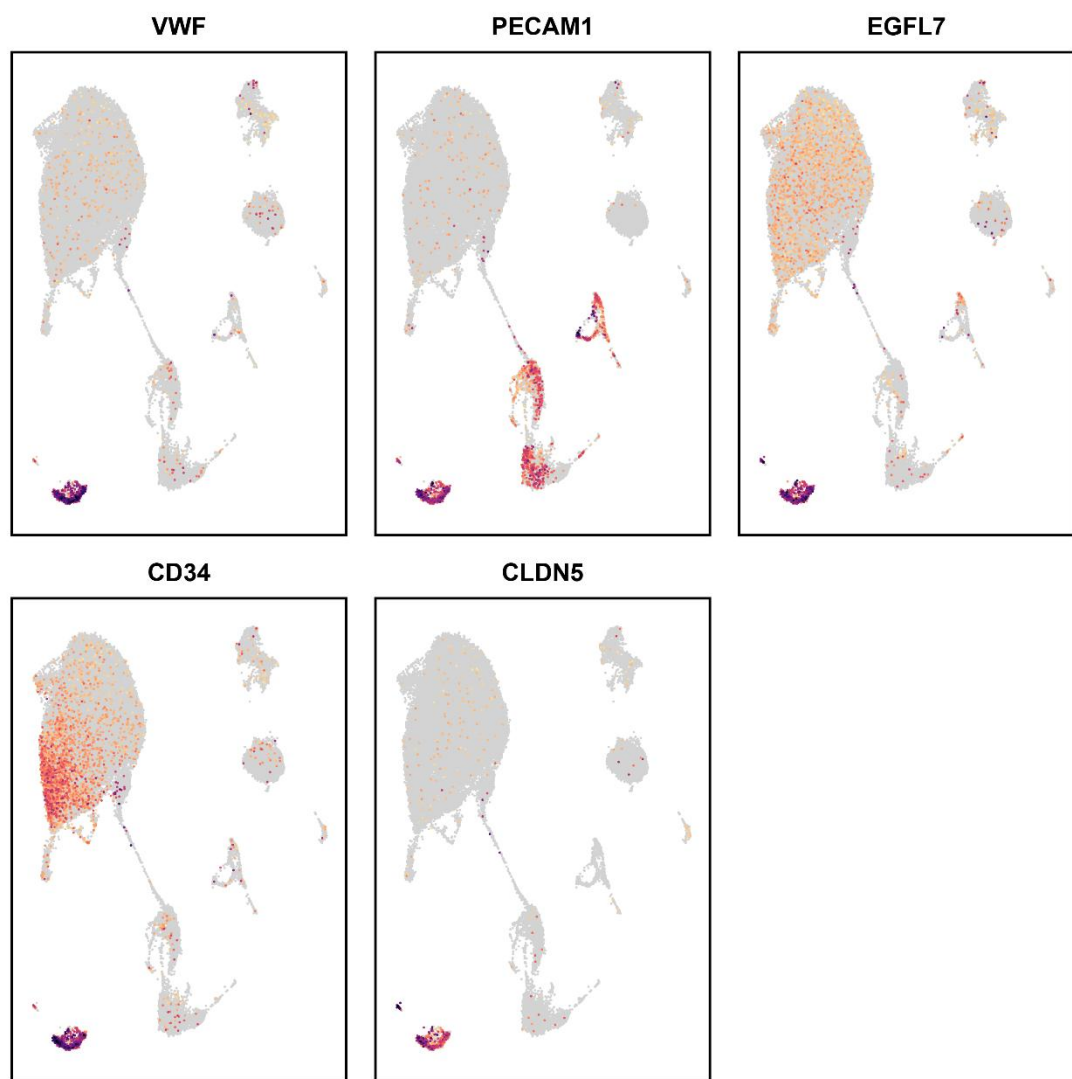


Fig. S3. UMAP showing the representative maker genes for endothelial cells.

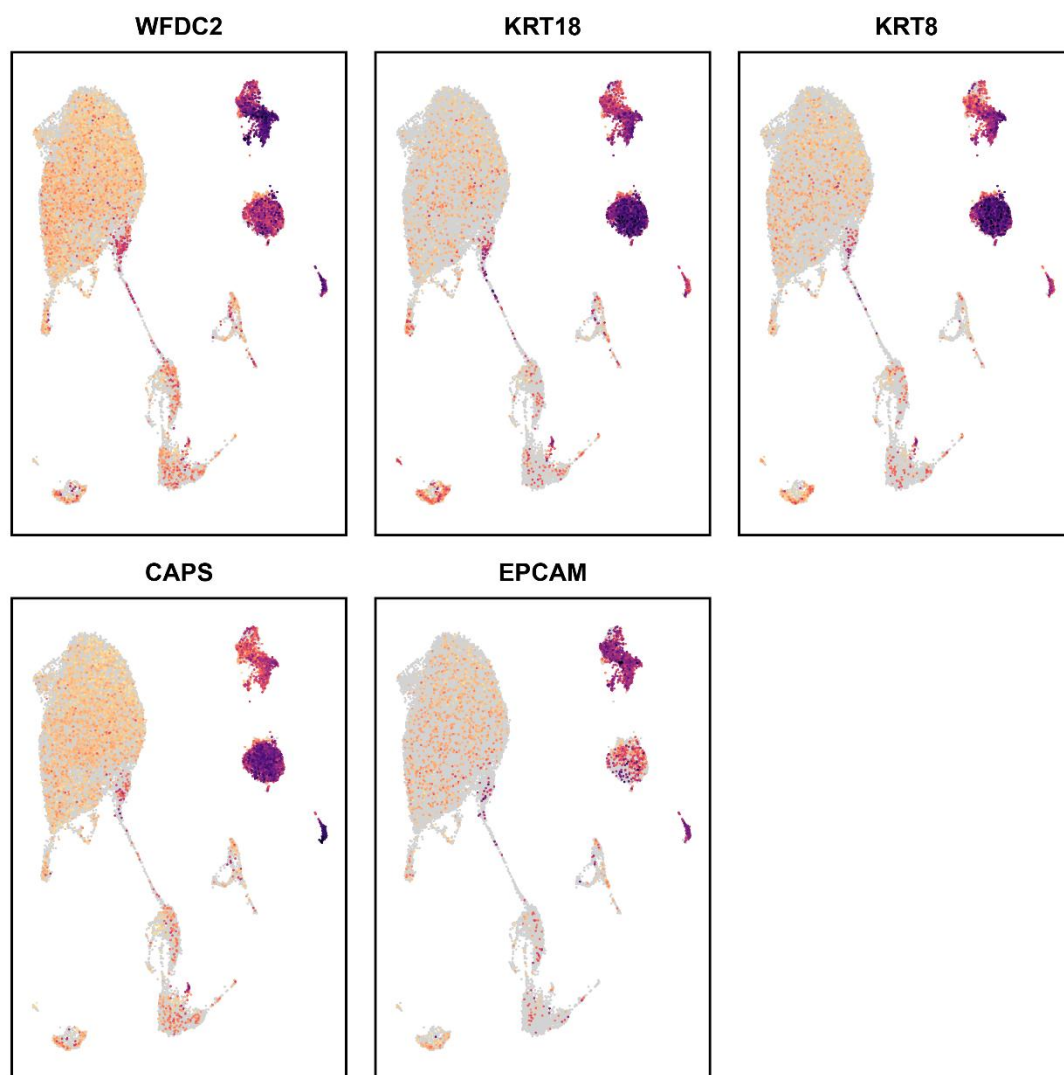


Fig. S4. UMAP showing the representative maker genes for epithelial cells.

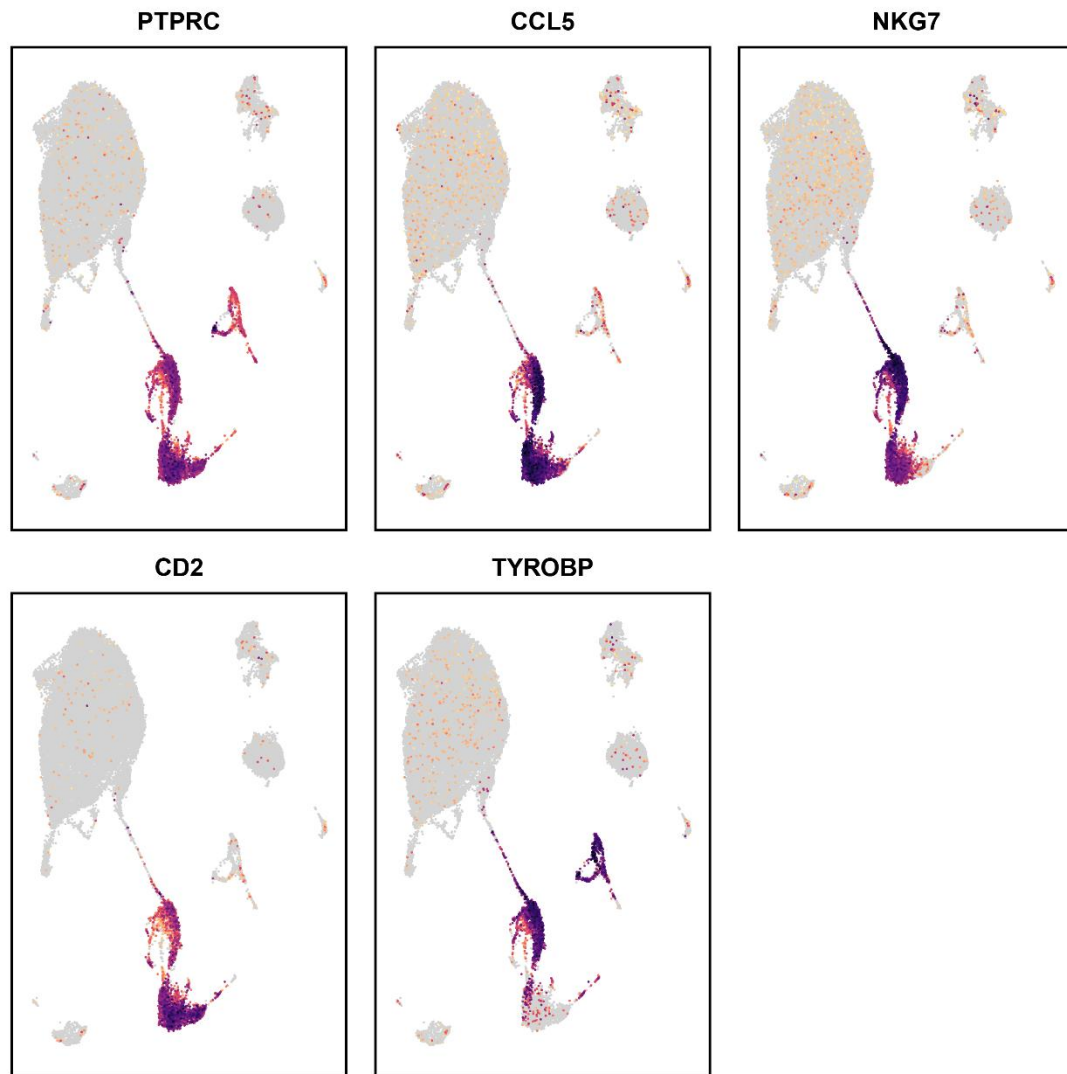


Fig. S5. UMAP showing the representative maker genes for immune cells.

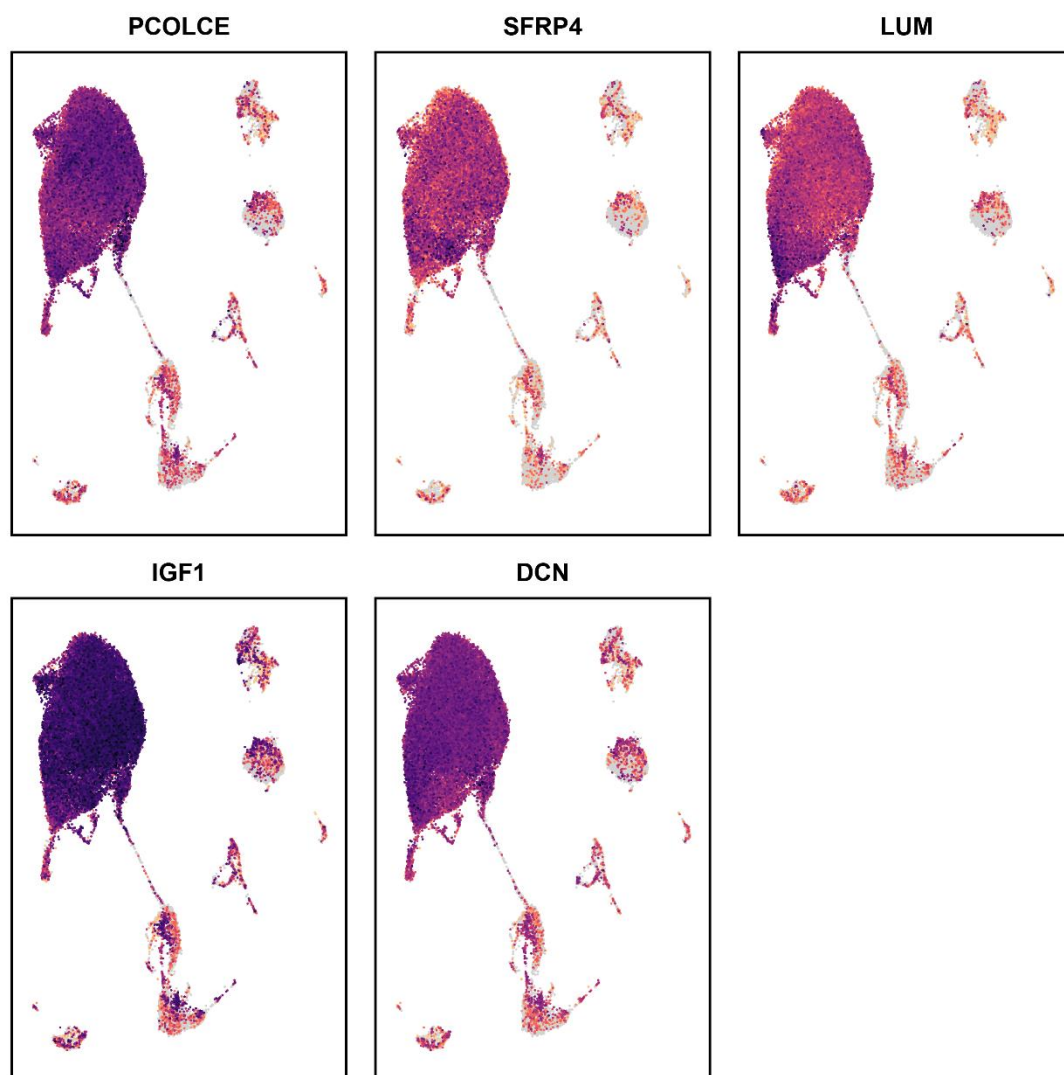


Fig. S6. UMAP showing the representative maker genes for stromal cells.

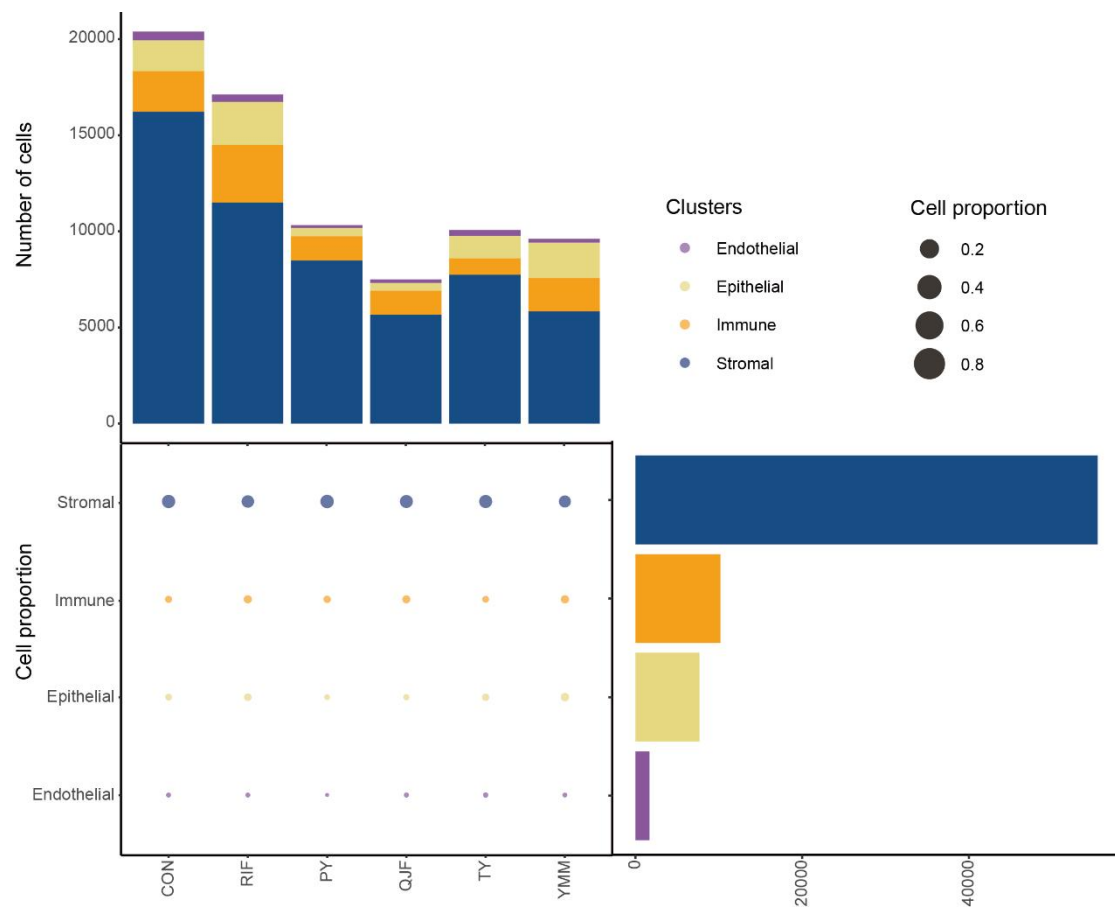


Fig. S7. Total cell number and cell proportion of four major endometrial cells in the endometrium of different samples.

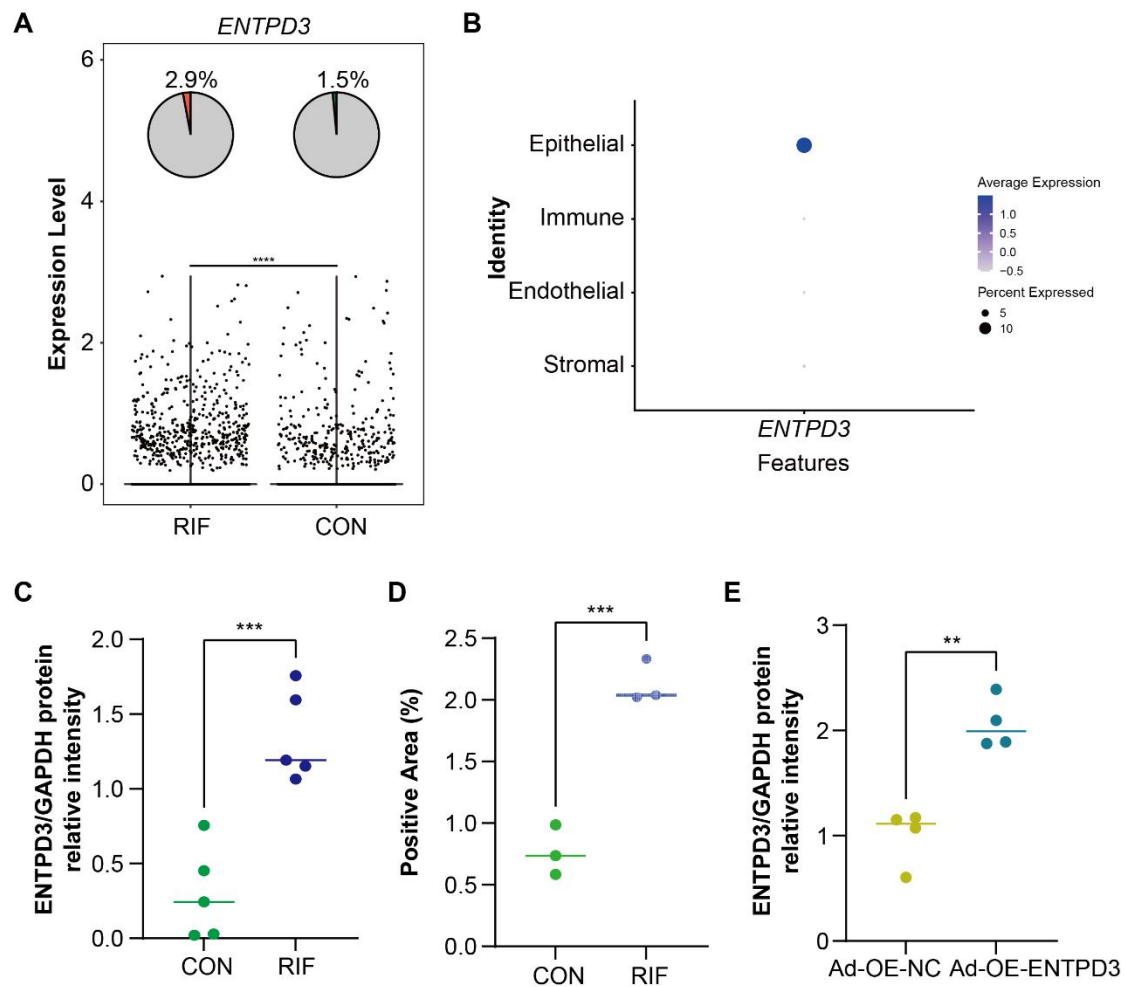


Fig. S8. (A) Comparative analysis of ENTPD3 expression levels in endometrial tissues between RIF patients and CTRL. (B) The expressive identity of *ENTPD3* among four major endometrial cells. (C) Relative protein expression of ENTPD3 normalized to GAPDH in endometrial tissues between RIF and CON patients (n=5). (D) Quantification of ENTPD3-positive immunostaining area (%) in endometrial tissues of RIF patients and CON patients (n=3). (E) Relative ENTPD3/GAPDH protein levels in mouse endometrial tissues transfected with OE-ENTPD3 versus the OE-NC control group (n=4). All results above are representative of three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

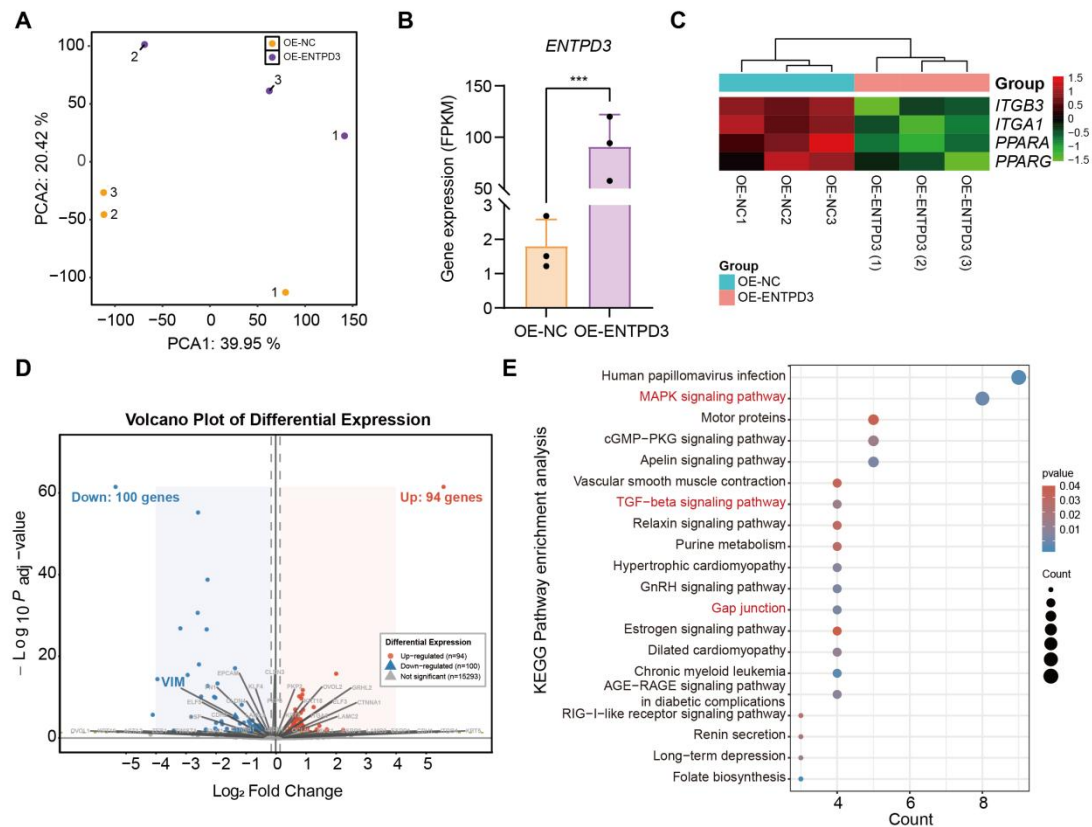


Fig. S9. Transcriptomic profile of Ishikawa cells between OE-NC and OE-ENTPD3 groups. (A) 2D scatter plot of the PCA analysis. Samples in the OE-NC (n = 3) and OE-ENTPD3 group (n = 3) are represented as blue and yellow dots, respectively. (B) FPKM expression of *ENTPD3* between the OE-NC and OE-ENTPD3 group. (C) Heatmap displaying the expression levels of *ITGB3*, *ITGA1*, *PPARA*, and *PPARG*. (D) Volcano plot of differentially expressed genes (DEGs), with upregulated and downregulated DEGs (FDR < 0.05) highlighted in light blue and light orange, respectively. (E) KEGG pathway enrichment analysis of the DEGs (FDR < 0.05) between the OE-NC and OE-ENTPD3 groups. *** $P < 0.001$.

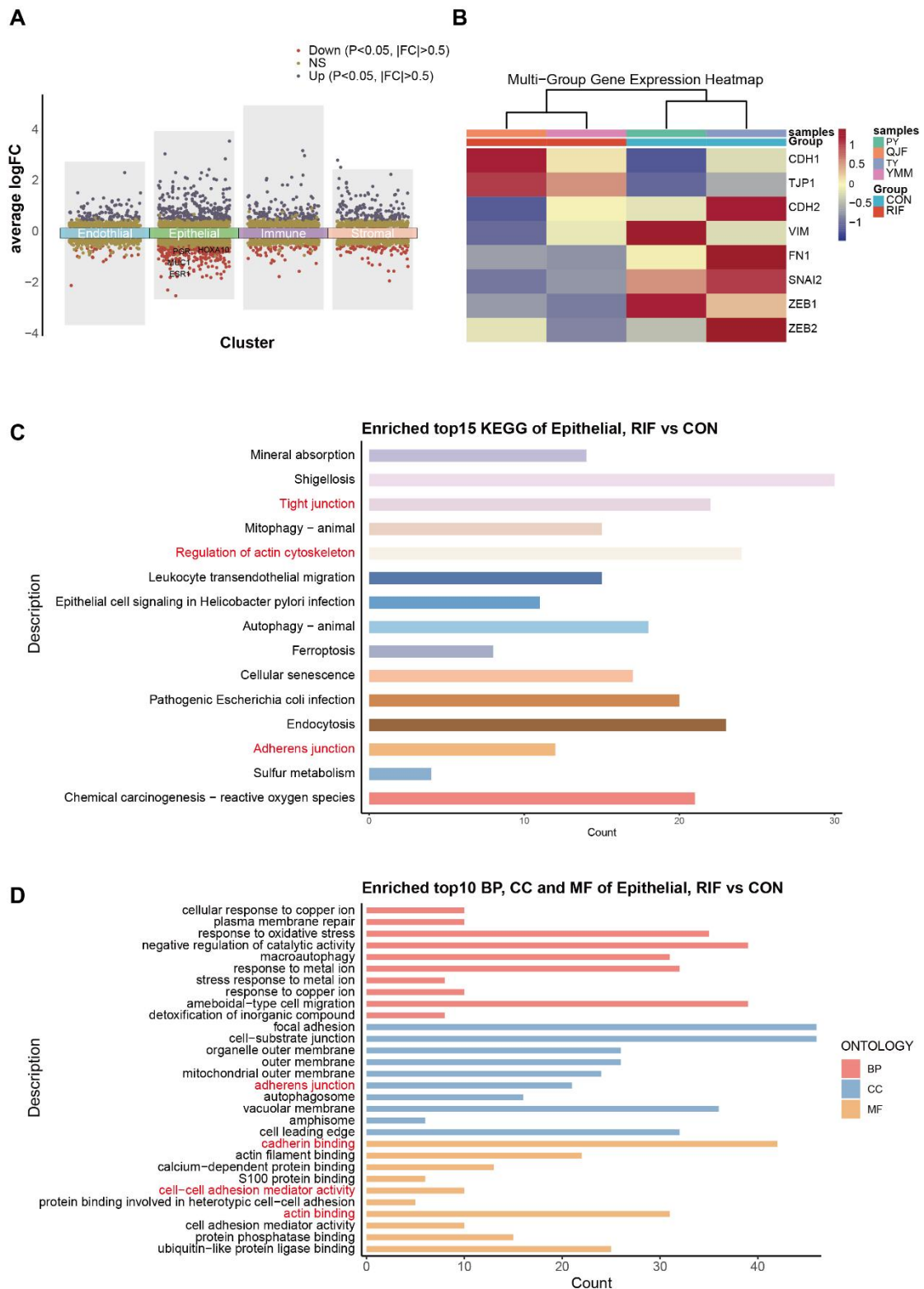


Fig. S10. Single-Cell Transcriptomic Analysis Reveals DGE and Functional Enrichment in Endometrial Cells between CTRL and RIF Patients (A) Volcano plot of differentially expressed genes (DEGs) in four major endometrial cell types, showing upregulated and downregulated DEGs (adjust $P < 0.01$) in CTRL and RIF patients. (B) Heatmap displaying the expression levels of epithelial-related makers (CDH1, TJP1) and mesenchymal-related markers (CDH2, VIM, FN1, SNAI2, ZEB1, and ZEB2) between CTRL and RIF patients. (C) Top 15 enriched

KEGG pathways in epithelial cells. **(D)** Top 15 enriched Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF) in epithelial cells.

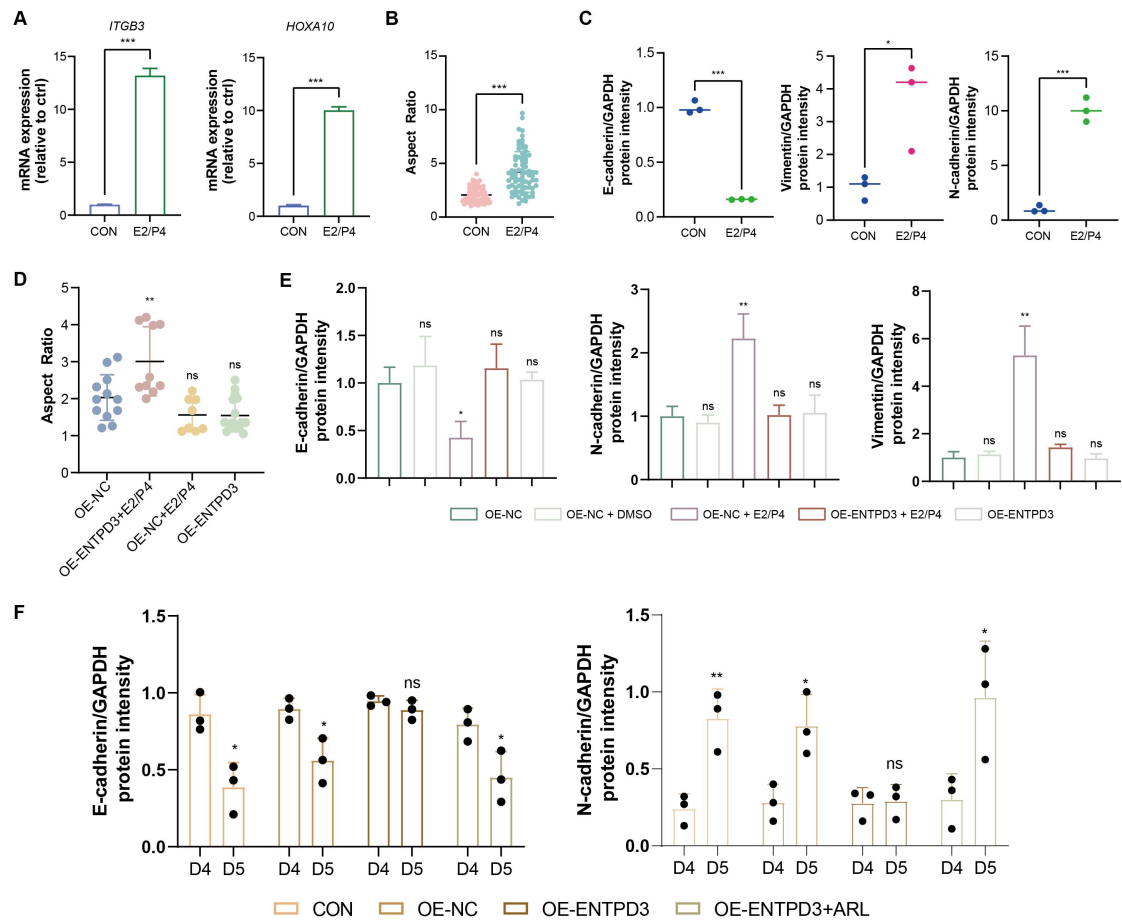


Fig. S11. (A) The mRNA expression of *ITGB3* and *HOXA10* in Ishikawa cells with or without E2/P4 induced (n=3). (B) Quantitative analysis of the cell aspect ratio based on phase-contrast images in Ishikawa cells treated with or without E2/P4 for 24 hours. (C) Relative protein expression of E-cadherin, N-cadherin and vimentin normalized to GAPDH in Ishikawa cells with or without E2/P4 induced (n=3). (D) Quantitative analysis of the cell aspect ratio based on phase-contrast images of Ishikawa cells under different conditions: OE-NC, OE-ENTPD3, and their combination with E2/P4. (E) Relative protein expression of E-cadherin, N-cadherin and vimentin normalized to GAPDH in Ishikawa cells under the conditions of OE-NC, OE-ENTPD3, and their combination with E2/P4 for 24 hours (n=3). (F) Relative protein expression of E-cadherin and N-cadherin normalized to GAPDH in mouse uterine tissues collected at D4 09:00 h and D5 09:00 h from the following groups: Control, OE-NC, OE-ENTPD3, and OE-ENTPD3 + ARL (n=3). Data are presented as mean $\pm$ SD. All results above are representative of three independent experiments. Ns. no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

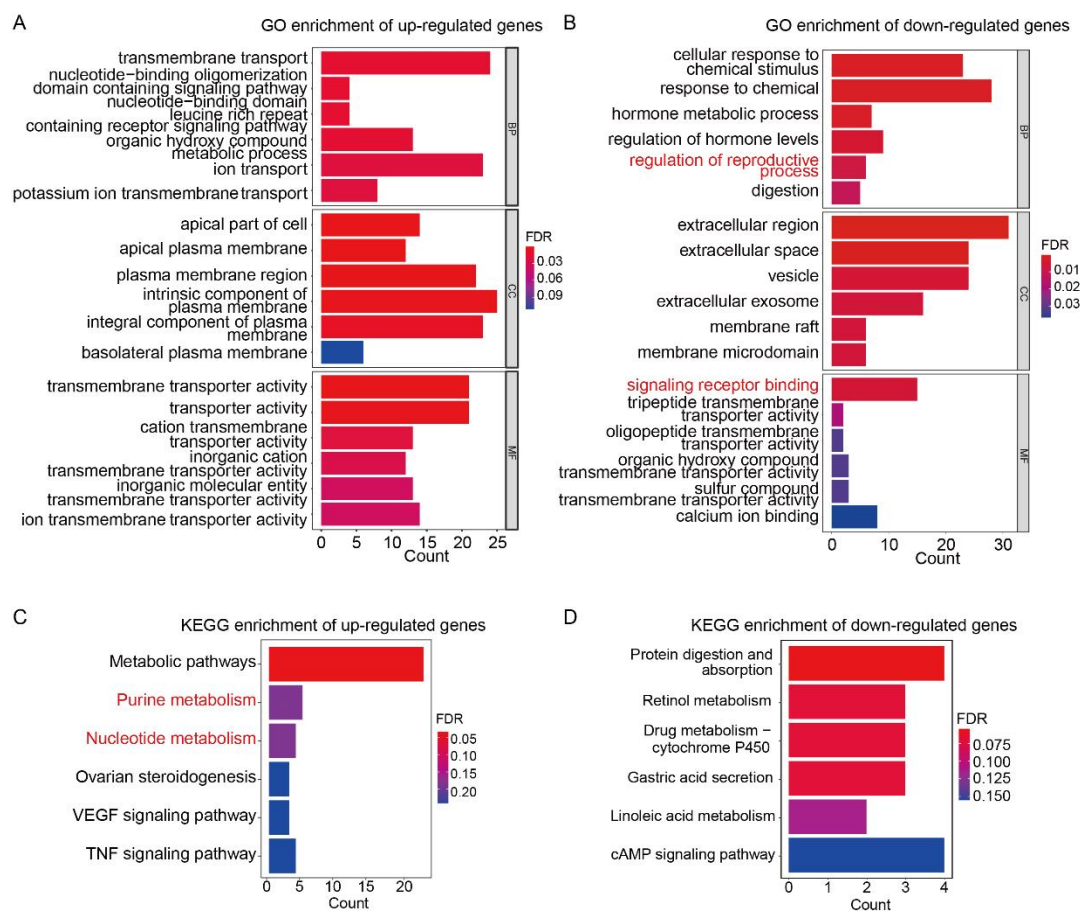


Fig. S12. Functional enrichment analysis of differentially expressed genes in RIF patients. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on DEGs identified from integrated transcriptomic profiling of publicly available endometrial datasets. Top six significantly enriched terms are shown. **(A)** GO enrichment of up-regulated genes. **(B)** GO enrichment of down-regulated genes. **(C)** KEGG enrichment of up-regulated genes. **(E)** KEGG enrichment of down-regulated genes.

Table S1. Clinical characteristics of the women involved in this research

Variables	Control patients (n = 10)	RIF patients (n = 10)	p value
Age (y)	29.30±4.19	33.20±3.29	0.703
BMI (kg/m ²)	21.83±1.48	21.42±1.57	0.735
FSH (mIU/ml)	8.11±0.89	8.03±0.86	0.948
LH (mIU/ml)	5.55±0.77	6.03±0.87	0.694
Estradiol (pg/ml)	46.08±5.14	43.61±3.37	0.191

Measurement data were expressed as mean ± SD, and normally distributed data were analyzed by unpaired t test.

Table S2. Inclusion and exclusion criteria

	Control patients	RIF patients
Inclusion criteria		
1. Age: 20~39		
2. The inability to achieve a clinical pregnancy despite the transfer of at least four high-quality embryos over a minimum of three embryo transfer cycles.		
Exclusion criteria		
1. Parental chromosome abnormalities		
2. Reproductive abnormalities (severe hydrosalpinx, moderate to severe intrauterine adhesions, uterine malformations, severe endometriosis or adenomyosis)		
3. Endocrinologic dysfunction (such as diabetes, insulin resistance, polycystic ovary syndrome, and hypothyroidism)		
4. Immunologic dysfunction (thrombosis, and autoimmune diseases)		
5. Unwilling to sign informed consent		

Table S3. Primers for qRT-PCR

Gene Symbol	Forward Primer Sequence (5' to 3')	Reverse Primer Sequence (5' to 3')
<i>ENTPD3</i>	ACAGAGGAGACTTGGCCCCA	ATGGGCAGACGGATCAGAGG
<i>P2Y2</i>	GCTACAGGTGCCGCTTCAAC	AGACACAGCCAGGTGGAACAT
<i>CDH1</i>	ATTCTGATTCTGCTGCTCTTG	AGTAGTCATAGTCCTGGTCTT
<i>CDH2</i>	GATGTTGAGGTACAGAATCGT	GGTCGGTATGGATGGCGA
<i>VIM</i>	AGAGAGAGGAAGCCGAAAAC	TGGATTTCCTCTTCGTGGAGTT
<i>ITGB3</i>	TTCAATGCCACCTGCCTCAA	TTGGCCTCAATGCTGAAGCTC
<i>LIF</i>	CCCTGGTCCCTACTCAACAA	CTGGACCCTGACACCCTAAA
<i>HOXA10</i>	TCACGGCAAAGAGTGGTC	AGTTTCATCCTGCGGTTCTG
<i>GAPDH</i>	GGTATCGTGGAAGGACTCATGAC	ATGCCAGTGAGCTTCCCGTTCAG
<i>Entpd3</i>	ACCTGTCCCGTGCTTAAATG	AGACAGAGTGAAGCCCCTGA
<i>Hand2</i>	TCGGTTATCTAGTGCTGTC	ATACTTACAATGTTTACACCTTCA
<i>Muc1</i>	AGCCCCTATGAGGAGGTTTCG	AAGTGGTCACCACAGCTGGG
<i>Gapdh</i>	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTGATGGGCTTCCCG
<i>Hoxa10</i>	GGCAGTTCCAAAGGCGAAAA	CAAAAAAAGCCAGAACAAAC
<i>Ihh</i>	CATCTTCAAGGACGAGGAGAACA	CATGACAGAGATGGCCAGTGA

Table S4. Quality control metrics for scRNA-Seq of endometrial tissues

Group	Number of cells	Number of genes detected	Total UMIs	Mitochondrial %
Control1	12391	31431	122618577	3.20
Control2	11452	32341	138993494	5.57
RIF1	7763	26672	76598735	4.98
RIF2	10062	28524	101058816	4.16

Abbreviations: UMIs, Unique Molecular Identifiers; RIF, Recurrent Implantation Failure.

Table S5. Sequence alignment of human ENTPD3 wild-type and S224A mutant

ENTPD3 ectonucleoside triphosphate diphosphohydrolase 3 [*Homo sapiens* (human)]

Gene ID: 956

Transcript variants: Canonical transcript: NM_001248.4; Alternative transcript: NM_001291960.2;

WT 1590bp

atgttcactgtgctgaccgccaacatgtgagcaagcaggcctcaaggccctctaccgaactccaacatcattgccttgggtggtcttg
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S224A

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WT Amino Acids

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QKVKGQVPSHLHGSTPIHLGATAGMRLRLQNETAANEVLESIQSYFKSQPFDFRGAQ
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QLPLLLPKFDEVYARSYCFSSANYIYHLFVNGYKFTEETWPQIHFEKEVGNSSIAWSLG
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DHAVDSD*

S224A Amino Acids

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QLPLLLPKFDEVYARSYCFSSANYIYHLFVNGYKFTEETWPQIHFEKEVGNSSIAWSLG

YMLSLTNQIPAESPLIRLPIEPPVFVGTLAFFTAALLCLAFLAYLCSATTRKRHSEHAF
DHAVDSD*

Table S6. Molecular Docking Scores of ATP-P2Y2 Interaction

ATP-P2Y2 -7.6

mode	affinity (kcal/mol)	dist from best mode	
		rmsd l. b.	rmsd u. b.
1	-7.6	0.000	0.000
2	-7.3	1.279	2.006
3	-7.2	2.497	3.312
4	-6.8	2.562	4.973
5	-6.7	4.361	5.963
6	-6.4	2.385	5.290
7	-6.2	1.601	2.405
8	-6.1	1.297	2.024
9	-6.1	1.843	3.139
10	-6.0	3.211	4.849
11	-6.0	6.116	8.362
12	-6.0	6.102	8.330
13	-5.9	3.022	3.979
14	-5.9	2.390	4.302
15	-5.8	3.055	5.886
16	-5.8	3.530	5.264
17	-5.7	4.220	6.372
18	-5.5	3.922	6.451
19	-5.5	2.507	3.959
20	-5.4	3.720	6.237

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