1 **Supplemental Figures**

A

Sample info	PD0	ZENPD0	PD3	ZENPD3
Estimated Number of Cells	5000	5000	5000	5000
Valid Barcodes	97.4%	97.5%	97.5%	98.0%
Mean Reads per Cell	121,362	119,385	112,621	108,866
Median Genes per Cell	3,134	3,997	2,918	4,142
Total Genes Detected	22,280	22,799	21,795	22,677
Reads Mapped to Genome	92.4%	95.5%	93.4%	95.1%
Reads Mapped Confidently to Transcriptome	66.6%	64.9%	67.2%	63.9%
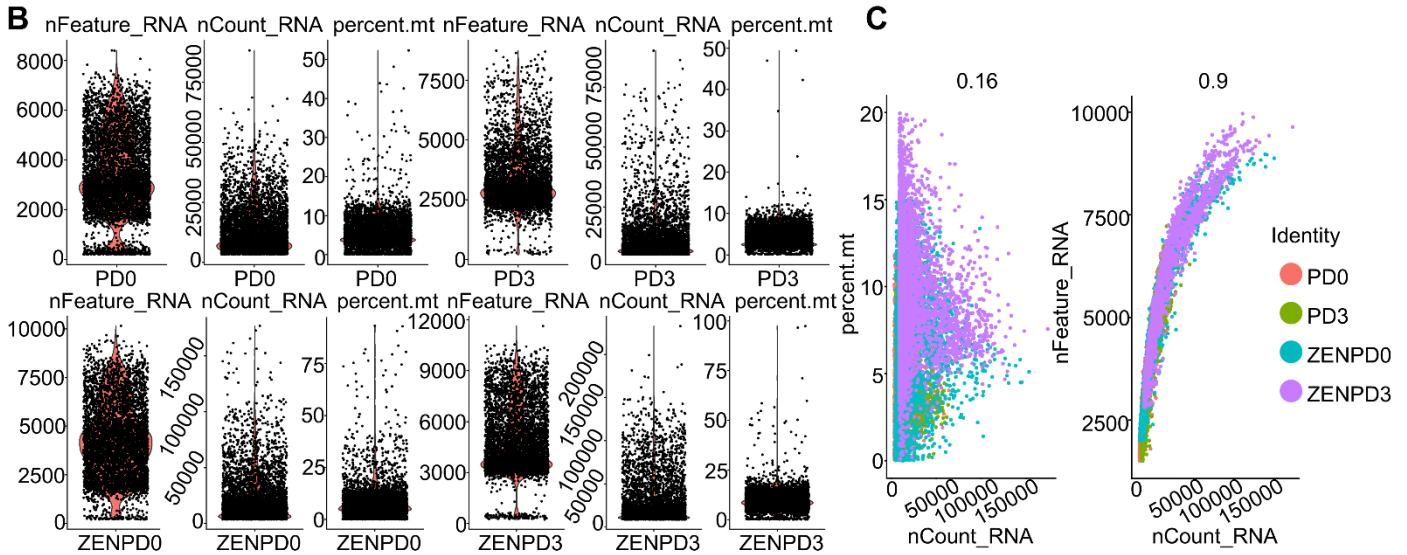
2

3 **Figure S1. Ovarian single cell sequencing information and quality control results**
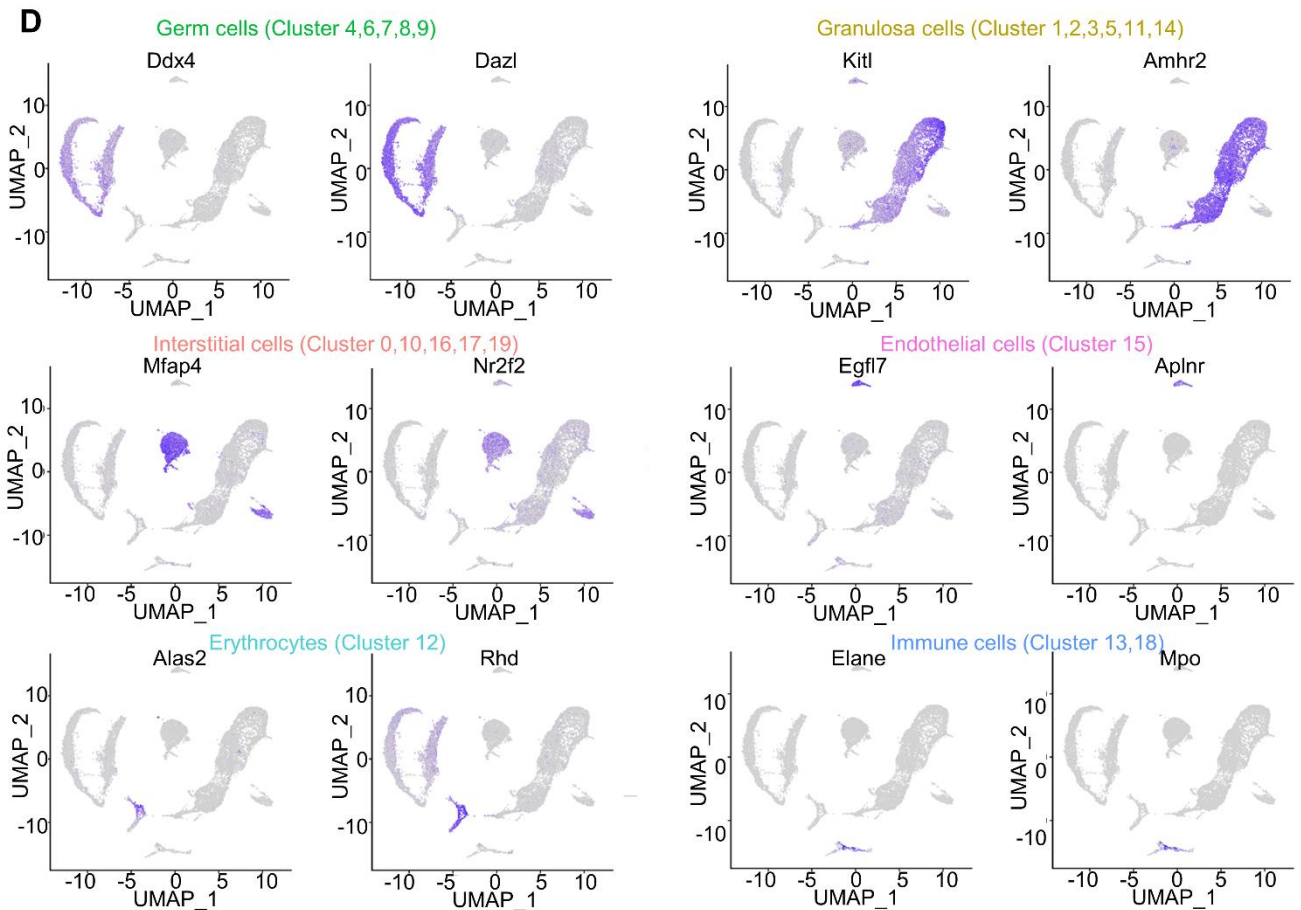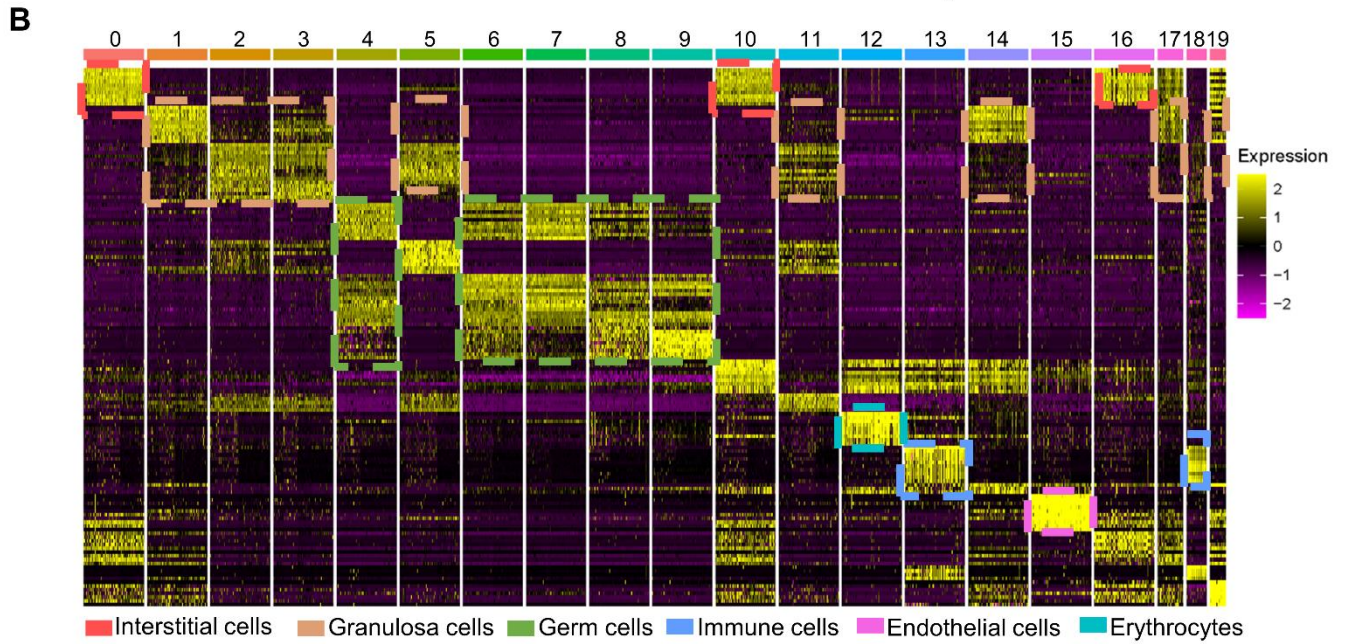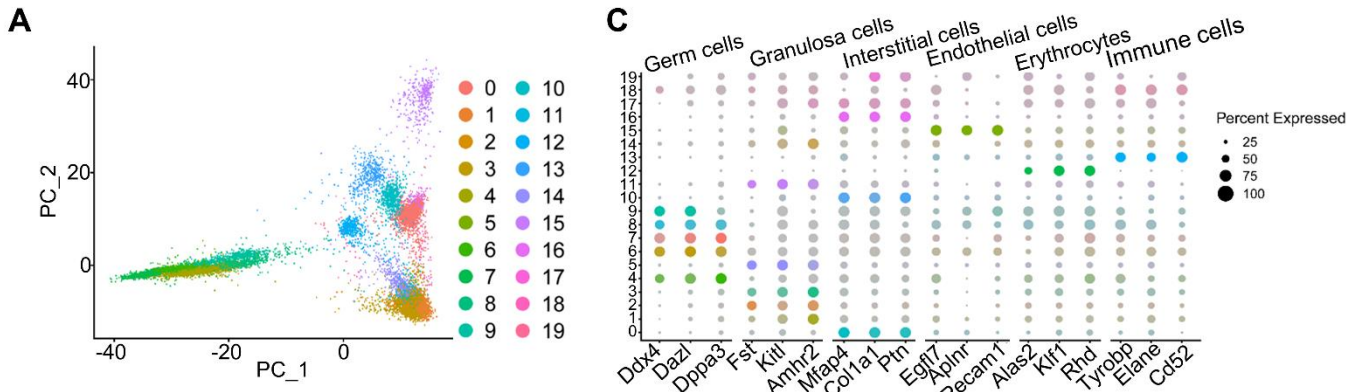
4 (A) Sequencing data statistics of four sets of single cell samples. (B) Violin plot demonstrating

5 the number of genes (nFeature_RNA), unique molecular identifier (nCount_RNA), and

6 percentage of mitochondrial genes (percent.mt) in the different data sets. (C) The scatter plot
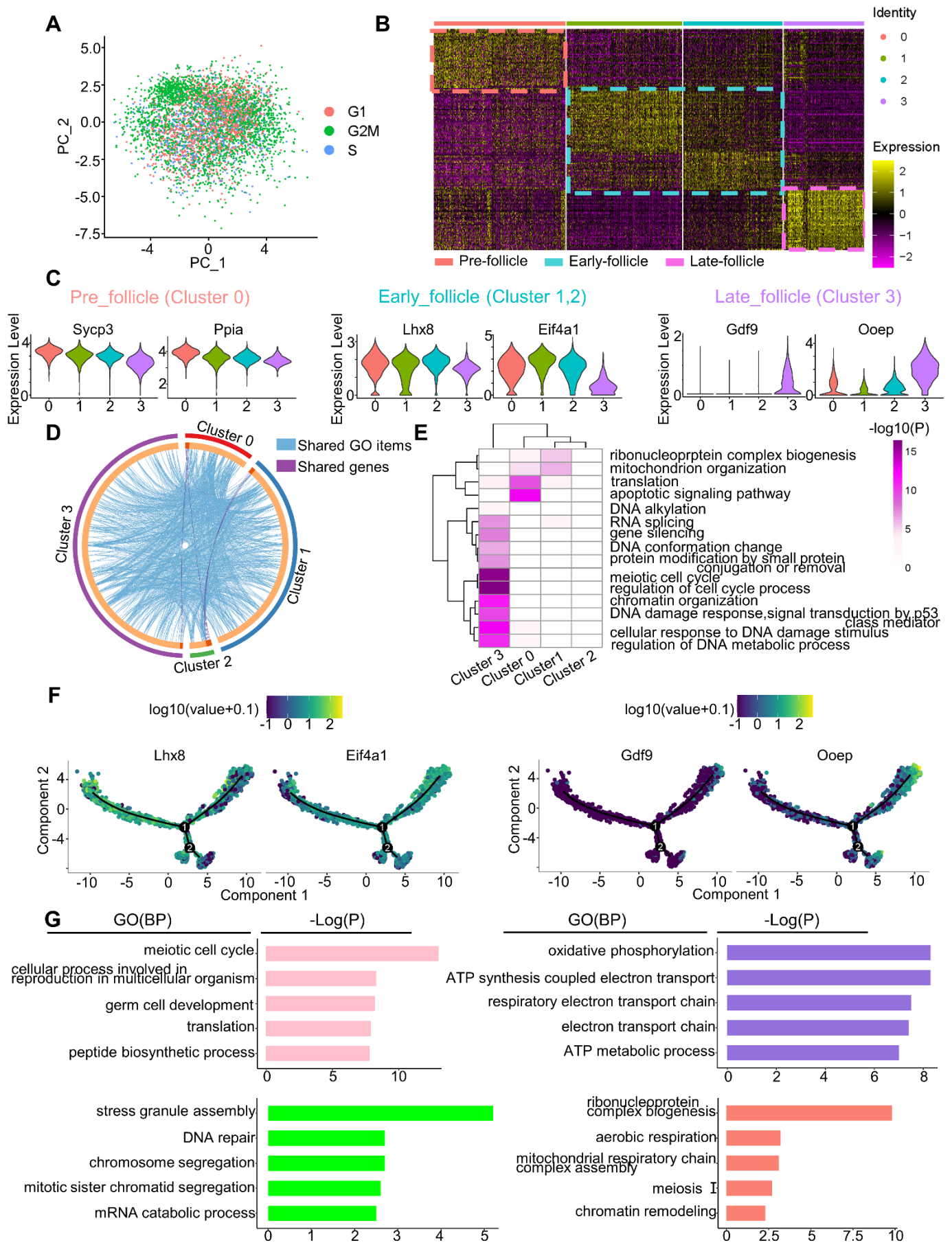
7 shows the correlation between nCount_RNA, nFeature_RNA, and percent.mt.

8

A

PC_2

40

20

0

-40 -20 0

PC_1

0 10
1 11
2 12
3 13
4 14
5 15
6 16
7 17
8 18
9 19

C

Germ cells Granulosa cells Interstitial cells Endothelial cells Erythrocytes Immune cells

19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

Ddx4 Dazl Dppa3 Fst Kitl Amhr2 Mfap4 Col1a1 Ptn Egfl7 Aplnr Pecam1 Alas2 Klf1 Rhd Tyrobp Elane Cd52

Percent Expressed
• 25
• 50
● 75
● 100

B

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Expression
2
1
0
-1
-2

Interstitial cells Granulosa cells Germ cells Immune cells Endothelial cells Erythrocytes

D

Germ cells (Cluster 4,6,7,8,9)

Ddx4

Dazl

UMAP_2

10

0

-10

-10 -5 0 5 10
UMAP_1

Granulosa cells (Cluster 1,2,3,5,11,14)

Kitl

Amhr2

10

0

-10

-10 -5 0 5 10
UMAP_1

Interstitial cells (Cluster 0,10,16,17,19)

Mfap4

Nr2f2

10

0

-10

-10 -5 0 5 10
UMAP_1

Endothelial cells (Cluster 15)

Egfl7

Aplnr

10

0

-10

-10 -5 0 5 10
UMAP_1

Erythrocytes (Cluster 12)

Alas2

Rhd

10

0

-10

-10 -5 0 5 10
UMAP_1

Immune cells (Cluster 13,18)

Elane

Mpo

10

0

-10

-10 -5 0 5 10
UMAP_1

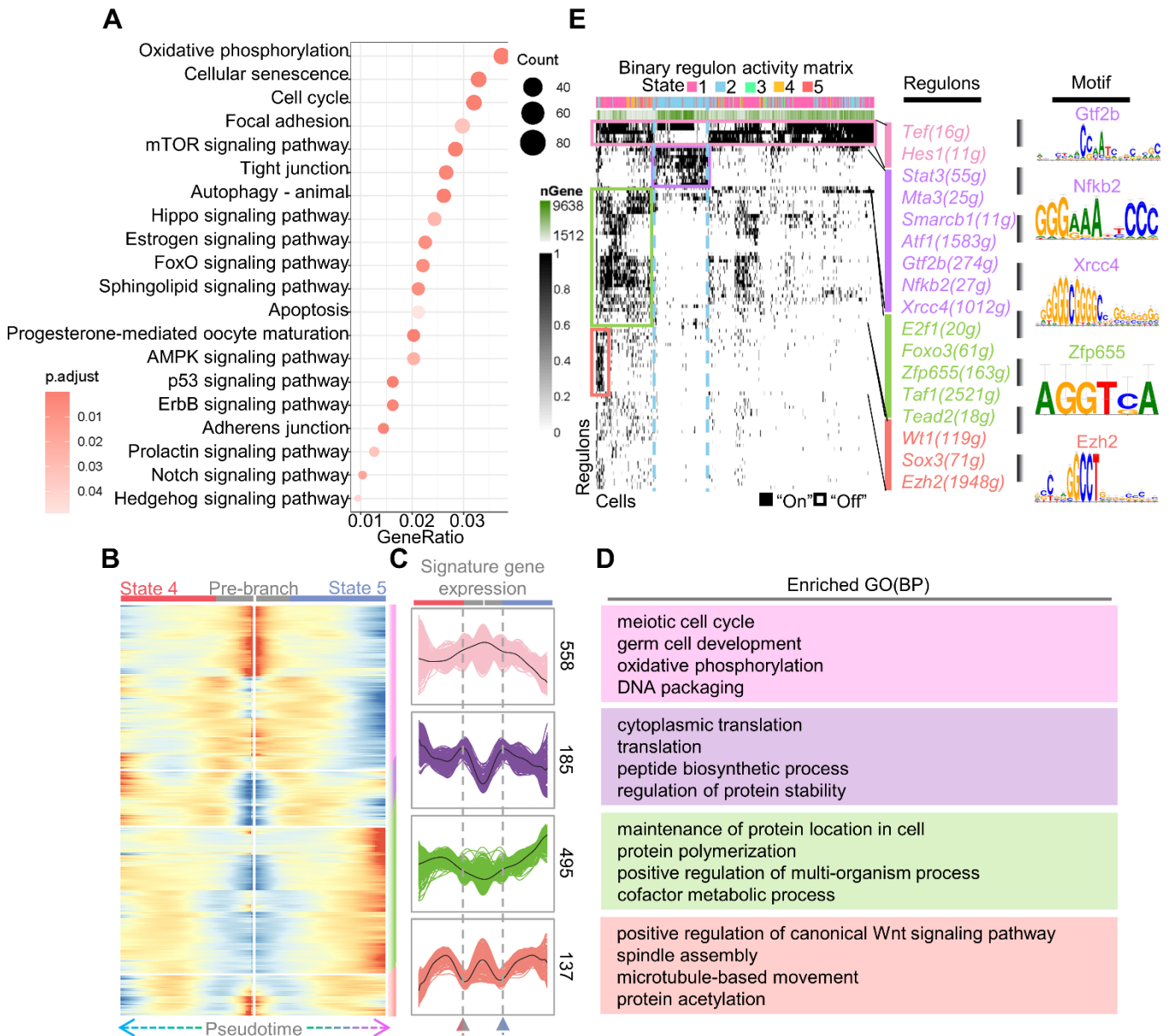10   **Figure S2. Expression of marker genes in each cell cluster**

11   (A) PCA chart shows the position of all cells. (B) The heatmap shows the top 10 DEGs of each

12   cell cluster. (C) The bubble chart shows the expression of genes specifically expressed in

13   different cell clusters. The size of the dot represents the percentage of cells expressing the

14   specified gene in each cluster, and the color intensity of the dot represents the average

15   expression level of the specified gene. (D) UMAP map of the expression level of cell marker

16   genes in each type. Germ cells: *Ddx4* and *Dazl*; Granulosa cells: *Kitl* and *Amhr2*; Interstitial

17   cells: *Mfap4* and *Nr2f2*; Endothelial cells: *Egfl7* and *Aplnr*; Erythrocytes: *Alas2* and *Rhd*;

18   Immune cells: *Elane* and *Mpo*.

19

A

B

C

Pre_follicle (Cluster 0)

Early_follicle (Cluster 1,2)

Late_follicle (Cluster 3)

D

E

F

log10(value+0.1)

log10(value+0.1)

G

GO(BP)	-Log(P)
meiotic cell cycle	
cellular process involved in reproduction in multicellular organism	
germ cell development	
translation	
peptide biosynthetic process	

GO(BP)	-Log(P)
oxidative phosphorylation	
ATP synthesis coupled electron transport	
respiratory electron transport chain	
electron transport chain	
ATP metabolic process	

21 **Figure S3. Dissecting germ cell subclusters**

22 (A) PCA diagram shows that after removing the effect of cell cycle from the germ cells, the

23 cells were no longer separated into stages according to G1, G2/M, and S phases. (B) The

24 heatmap shows the top 20 DEGs of each cell cluster. The germ cell clusters are divided into

25 Pre-, Early-, and Late-follicle. (C) Violin chart shows the average expression level of the three

26 stage marker genes in different cell clusters (Pre-follicle: *Sycp3* and *Ppia*; Early_follicle: *Lhx8*

27 and *Eif4a1*; Late_follicle: *Gdf9* and *Ooep*). The plot uses the color of the cell cluster in the

28 UMAP diagram. (D) Circos diagram shows the DEGs and GO terms shared between different

29 germ cell clusters. The purple line represents Shared DEGs, and the blue line represents Shared

30 GO terms. (E) Heatmap shows the GO term enrichment results of four germ cell clusters. (F)

31 Gene expression levels and positions of *Lhx8*, *Eif4a1*, *Gdf9,* and *Ooep* on single-cell

32 pseudotime trajectories. (G) The histogram shows the GO terms enrichment results of four

33 gene sets (top 50).

34

A

Oxidative phosphorylation
Cellular senescence
Cell cycle
Focal adhesion
mTOR signaling pathway
Tight junction
Autophagy - animal
Hippo signaling pathway
Estrogen signaling pathway
FoxO signaling pathway
Sphingolipid signaling pathway
Apoptosis
Progesterone-mediated oocyte maturation
AMPK signaling pathway
p53 signaling pathway
ErbB signaling pathway
Adherens junction
Prolactin signaling pathway
Notch signaling pathway
Hedgehog signaling pathway

Count
40
60
80

p.adjust
0.01
0.02
0.03
0.04

GeneRatio
0.01 0.02 0.03

E

Binary regulon activity matrix
State 1 2 3 4 5

Regulons
Tef(16g)
Hes1(11g)
Stat3(55g)
Mta3(25g)
Smarcb1(11g)
Atf1(1583g)
Gtf2b(274g)
Nfkb2(27g)
Xrcc4(1012g)
E2f1(20g)
Foxo3(61g)
Zfp655(163g)
Taf1(2521g)
Tead2(18g)
Wt1(119g)
Sox3(71g)
Ezh2(1948g)

Motif
Gtf2b
Nfkb2
Xrcc4
Zfp655
Ezh2

nGene
9638
1512
1

Regulons
Cells   "On" "Off"

B

State 4   Pre-branch   State 5

Pseudotime

C

Signature gene expression

558
185
495
137

D

Enriched GO(BP)

meiotic cell cycle
germ cell development
oxidative phosphorylation
DNA packaging

cytoplasmic translation
translation
peptide biosynthetic process
regulation of protein stability

maintenance of protein location in cell
protein polymerization
positive regulation of multi-organism process
cofactor metabolic process

positive regulation of canonical Wnt signaling pathway
spindle assembly
microtubule-based movement
protein acetylation

35

36 **Figure S4. Single-cell pseudotime trajectories of germ cells**

37 (A) KEGG enrichment results of DEGs (top 20). (B) The heatmap shows the changes in gene

38 expression of all germ cells in the two cell fate branches at point 2. (C) The graph shows the

39 expression of the genome in the pseudotime trajectory. (D) GO term enrichment results of four

40 gene sets (top 50). (E) The SCENIC binary regulon activity matrix shows the regulon

41 enrichment of a specific cell state. Each column represents a single cell, and each row

42   represents a rule. "On" represents an effective regulon, and "Off" represents an ineffective

43   regulon.

44

45
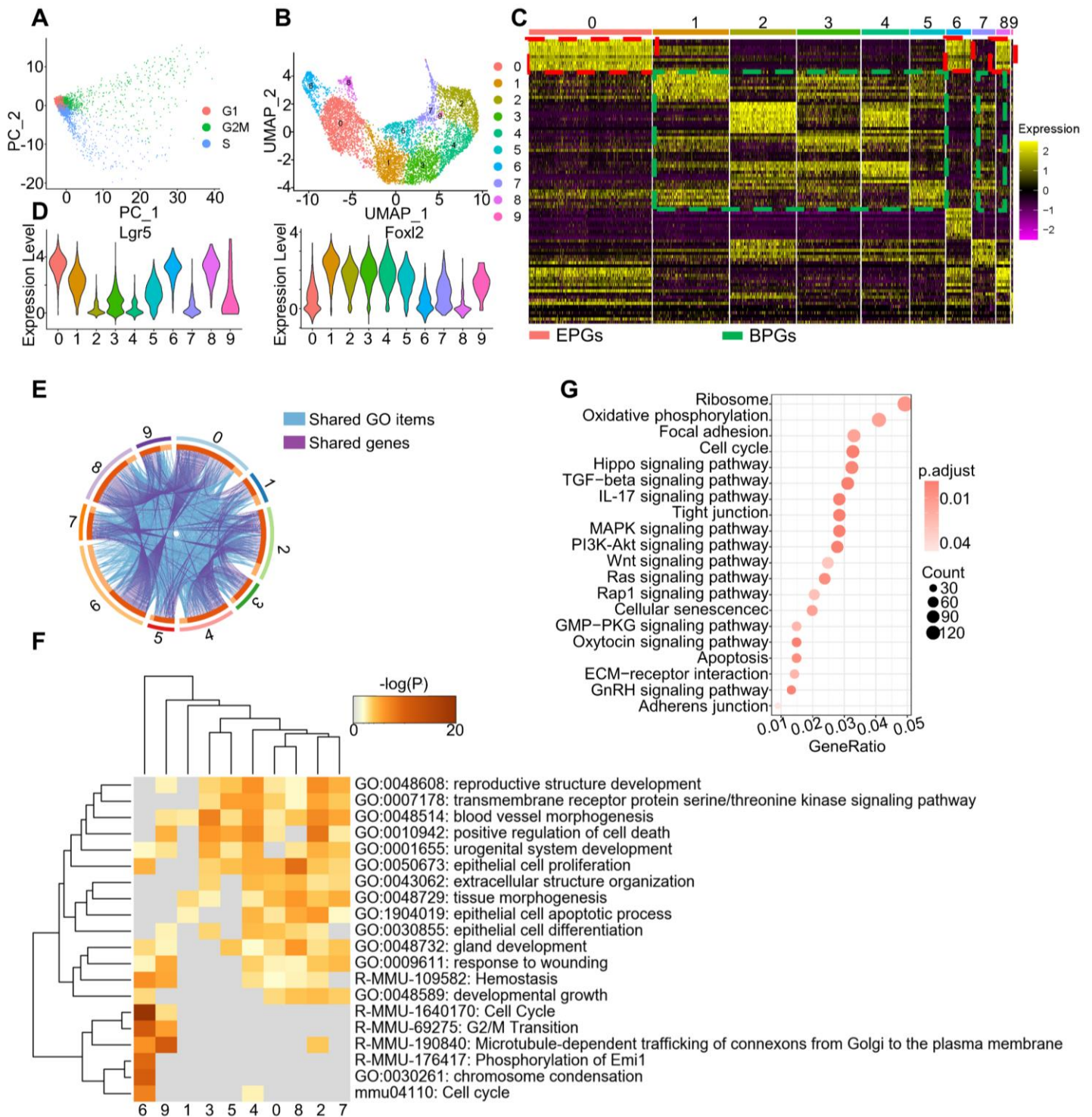
Figure S5. Dissection of granulosa cell subclusters

(A) PCA chart shows that granulosa cells are not separated in stages according to G1, G2/M,

and S phases. (B) Subpopulation of granulosa cell clusters. (C) Heatmap shows the top 20

49   DEGs of each cell cluster. Granulosa cell clusters are divided into EPGs and BPGs. (D) Violin

50   chart shows the average expression levels of the marker genes at the two stages in different cell

51   clusters (EPGs: *Lgr5*; BPGs: *Foxl2*). This plot uses the color of the cell cluster in the UMAP

52   diagram. (E) Circos diagram shows the DEGs and GO terms shared between different

53   granulosa cell clusters. The purple line represents Shared DEGs, and the blue line represents

54   Shared GO terms. (F) Heatmap shows the enrichment results of the granulosa cell clusters. (G)

55   KEGG enrichment results of DEGs (top 20).

56

57
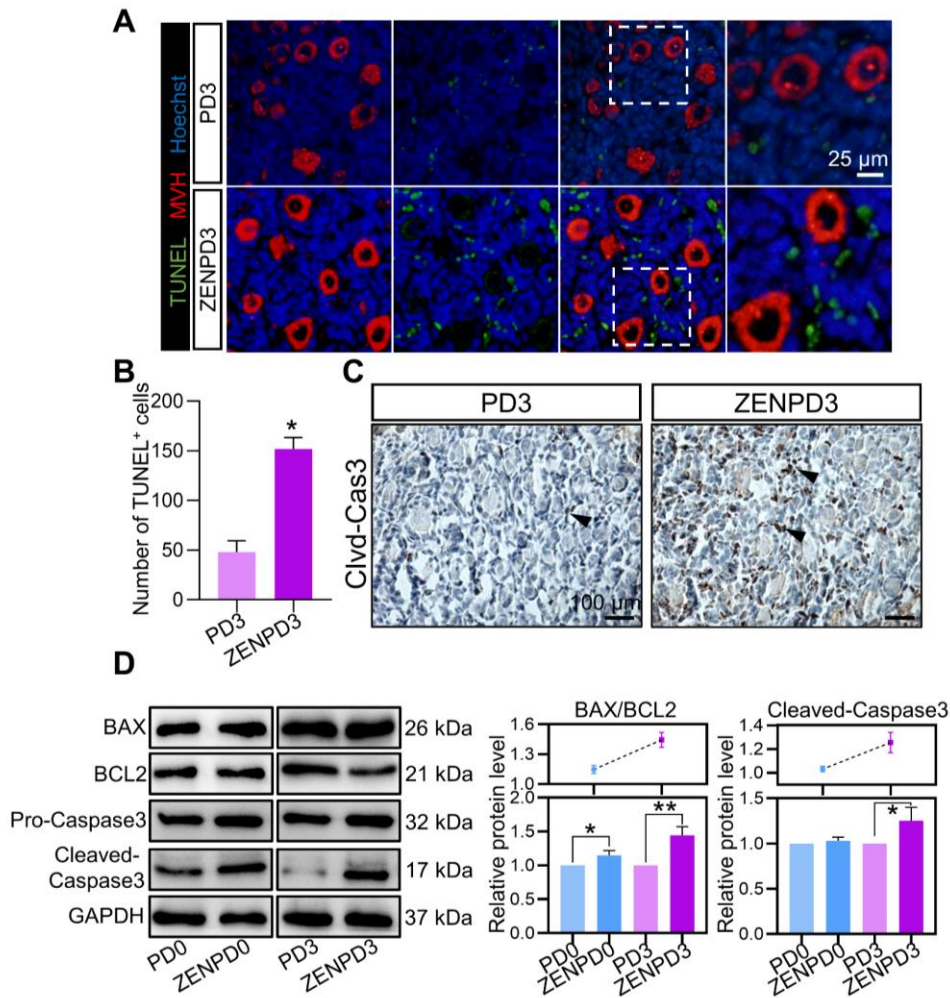
Figure S6. ZEN exposure promoted apoptosis of ovarian granulosa cells

(A) TUNEL staining of PD3 ovary. MVH labeled oocytes (red); TUNEL labeled apoptotic cells

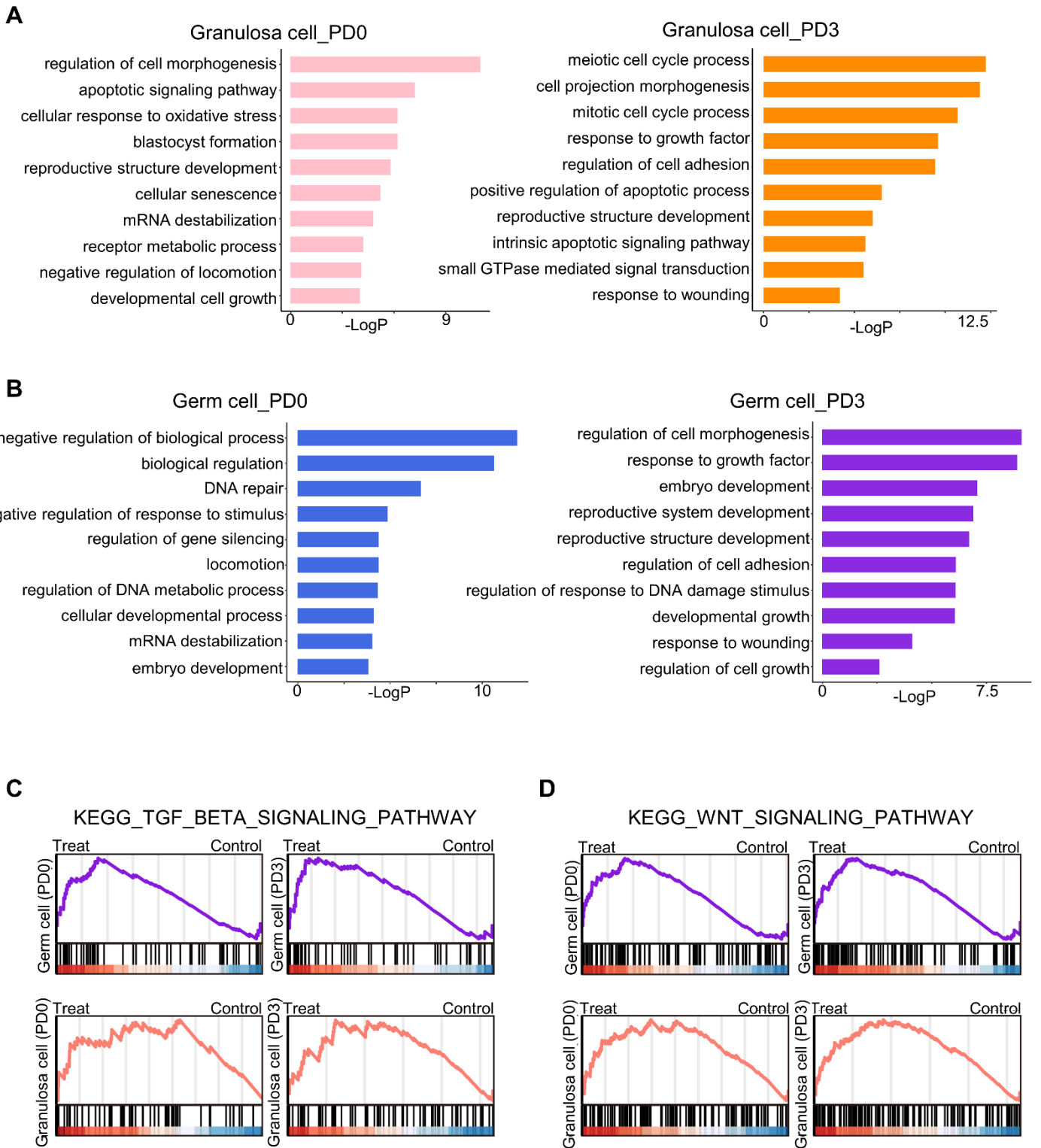(green); nuclei were stained with Hoechst (blue). (B) Quantitative analysis of apoptotic cells.

(C) Cleaved-Caspase3 staining of PD3 ovary in the control group and the treatment group. (D)

Western blot analysis results of BAX, BCL2, Pro-Caspase3, Cleaved-Caspase3, and GAPDH.

The percentage of each group is presented as the mean ± standard deviation. All experiments

were repeated at least three times (*$P < 0.05$; **$P < 0.01$).

65

A

Granulosa cell_PD0

- regulation of cell morphogenesis
- apoptotic signaling pathway
- cellular response to oxidative stress
- blastocyst formation
- reproductive structure development
- cellular senescence
- mRNA destabilization
- receptor metabolic process
- negative regulation of locomotion
- developmental cell growth

0   -LogP   9

Granulosa cell_PD3

- meiotic cell cycle process
- cell projection morphogenesis
- mitotic cell cycle process
- response to growth factor
- regulation of cell adhesion
- positive regulation of apoptotic process
- reproductive structure development
- intrinsic apoptotic signaling pathway
- small GTPase mediated signal transduction
- response to wounding

0   -LogP   12.5

B

Germ cell_PD0

- negative regulation of biological process
- biological regulation
- DNA repair
- negative regulation of response to stimulus
- regulation of gene silencing
- locomotion
- regulation of DNA metabolic process
- cellular developmental process
- mRNA destabilization
- embryo development

0   -LogP   10

Germ cell_PD3

- regulation of cell morphogenesis
- response to growth factor
- embryo development
- reproductive system development
- reproductive structure development
- regulation of cell adhesion
- regulation of response to DNA damage stimulus
- developmental growth
- response to wounding
- regulation of cell growth

0   -LogP   7.5

C KEGG_TGF_BETA_SIGNALING_PATHWAY

D KEGG_WNT_SIGNALING_PATHWAY
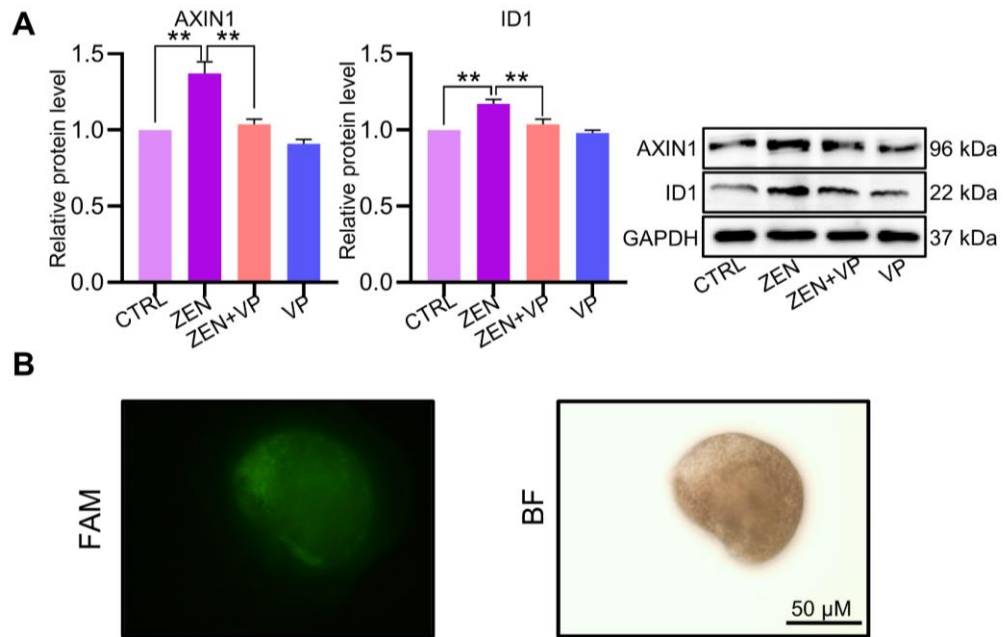
66

67 **Figure S7. DEGs enrichment analysis**

68 (A) The bar graph shows the results of the GO term enrichment of the DEGs of PD0 and PD3

69 ovarian granulosa cells. (B) The histogram shows the results of the GO term enrichment of

70 PD0 and PD3 ovarian germ cell DEGs. (C) GSEA of the TGF-beta signaling pathway. (D)

71 GSEA of the Wnt signaling pathway.

72

73

Figure S8. *In vitro* culture of ovaries

(A) Representative Western blot and analysis results of AXIN1, ID1, and GAPDH. (B)

Representative image of mouse ovary cultured with the *Yap1* siRNA group. The percentage of

each group is presented as the mean ± standard deviation. All experiments were repeated at

least three times (*$P < 0.05$; **$P < 0.01$).

79

Supplemental Tables

Table S1. Antibodies used in this paper

Primary antibodies	Vendor	Dilution	Source
AXIN1 (WB)	Affinity (DF9264)	1:1000	Rabbit
BMP2 (WB)	Affinity (AF5163)	1:1000	Rabbit
BMPR1B (WB)	Affinity (DF6748)	1:500	Rabbit
BAX (WB)	CST (2772S)	1:1000	Rabbit
BCL2 (WB)	Beyotime (AB112)	1:1000	Rabbit
Caspase3 (IHC/WB)	Abcam (ab13847)	1:200/1:1000	Rabbit
FZD2 (WB)	Affinity (AF5282)	1:5000	Rabbit
GAPDH(WB)	Affinity (T0004)	1:1000	Mouse
ID1 (WB)	Affinity (DF2932)	1:1000	Rabbit
LHX8 (IHC/WB)	Sigma (SAB2101342)	1:200/1:1000	Rabbit
MVH (WB)	Abcam (ab13840)	1:1000	Rabbit
SMAD4 (WB)	Affinity (AF5247)	1:1000	Rabbit
P-SMAD4 (Thr276) (WB)	Affinity (AF8316)	1:500	Rabbit
TEAD1 (WB)	ABclonal (A6768)	1:1000	Rabbit
TEAD2 (WB)	ABclonal (A17407)	1:1000	Rabbit
WNT4 (WB)	Affinity (DF9040)	1:500	Rabbit
YAP1 (IHC/WB)	Affinity (AF6328)	1:100/1:500	Rabbit
P-YAP (S127) (WB)	Affinity (AP0489)	1:500	Rabbit
γ-H2AX (IF/WB)	Bioworld (P16104)	1:200/1:1000	Mouse
Secondary antibodies			
CY3-conjugated goat anti-rabbit (IF)	Beyotime (A0516)	1:200	Goat
FITC-conjugated goat anti-rabbit (IF)	Beyotime (A0562)	1:200	Goat
FITC-conjugated goat anti-mouse (IF)	Beyotime (A0568)	1:150	Goat
HRP-conjugated goat anti-Mouse IgG (WB)	Beyotime (A0216)	1:1000	Goat
HRP-conjugated goat anti-rabbit IgG (IHC/WB)	Beyotime (A0258)	1:200/1:1000	Goat

84 **Table S2. log2 (Foldchange) of key genes**

Gene symbol	Germ cell_PD0	Germ cell_PD3	Granulosa cell_PD0	Granulosa cell_PD3
Wnt4	1.154	1.229	2.499	2.787
Fzd2			1.565	1.190
Bmp2			0.798	0.733
Bmpr1b	1.270	1.187	0.638	0.585
Smad4	1.602	1.902	1.629	1.981
Yap1	0.639	0.609	0.543	0.599
Tead1	1.175	1.531	1.113	1.598
Tead2		0.370	-0.905	-1.230
Axin1	1.272	1.436	1.433	1.859
Id1	0.580	0.329	0.959	0.818

85

86

87 **Table S3. Software and Algorithms**

ImageJ	v 1.48
AlphaView SA	3.4.0
Image-Pro Plus	5.1
R-3.6.3	https://www.r-project.org
CellRanger	https://www.10xgenomics.com/
Seurat V3.1	https://satijalab.org/seurat/v3.1/immune_alignment.html
Metascape	http://metascape.org
Cytoscape	https://cytoscape.org/
Monocle 2	http://cole-trapnell-lab.github.io/monocle-release/docs/
SCENIC	https://github.com/aertslab/SCENIC
CellPhoneDB	https://www.cellphonedb.org/

88

89

90 **Table S4. Primers Used for Quantitative RT-PCR**

Genes	Forward primer sequence	Reverse primer sequence
Gapdh	GTCGGAGTGAACGGATTTGGC	CACCCCATTTGATGTTGGCG
Yap1	ACCCTCGTTTTGCCATGAAC	TGTGCTGGGATTGATATTCCGTA

91