

Supplemental Figures

Fig S1. Detailed dataset for pre-processing and cell types identified in ovaries

(A) UMAP plot of single and doublet detection in four datasets. (B) The detailed information for sample pre-processing parameters during CellRanger and Seurat workflow. (C) Heatmap of the top five marker genes identified in each cell cluster in ovaries. (D) Violin plots of representative genes for germ cells, granulosa cells, stromal cells, erythrocytes, immune cells, and endothelial cells.

Fig S2. Histological section staining of germ cells and granulosa cells

(A) Histological section staining of STAT3 (green) for germ cells in ovaries with and without DEHP treatment at PD3. Cell nuclei were stained with PI (red). (B) Histological section staining of AMHR2 (green) for granulosa cells in ovaries with and without DEHP treatment at PD3. Cell nuclei were stained with Hoechst 33342 (blue). Scale bar = 50 μ m.

Fig S3. Single-cell analysis of germ cell population following DEHP exposure

(A) Cell-cycle-phase score of germ cell population using the gene list adopted from Tirosh et al. [76]. (B) Heatmap of the newly identified top five marker genes in germ cell clusters. (C) Feature plots of key genes with regard to meiosis, follicle formation, and oocyte growth in germ cell clusters. (D) DEHP down-regulated the expression of genes critical to follicle assembly. (E) Pathways and processes enriched by 14 key DEGs at the pre-stage of PF formation.

Fig S4. Stage-specific DEG enrichment in germ cells

(A) Enrichment of DEG lists with PD0-DEHP vs. PD0 and PD3-DEHP vs. PD3 at the pre-stage of PF formation in germ cells. (B) Enrichment of DEG lists with PD0-DEHP vs. PD0 and PD3-DEHP vs. PD3 at the early-stage of PF formation in germ cells. (C) Enrichment of DEG lists with PD0-DEHP vs. PD0 and PD3-DEHP vs. PD3 at the late-stage of PF formation in germ

cells.

Fig S5. Single-cell pseudotime in germ cell trajectories

(A) The representative genes mark different developmental States in germ cell trajectories. (B) Expression of representative genes in each gene set regarding fate transition in germ cells colored by cell States. (C) Enrichment of gene set 4 for protein-protein interaction by Metascape. (D) Heatmap of two gene sets between different fates of germ cells at branch point two. Several representative genes of each gene set are listed in the right panel. (E) Expression plots of meiotic (*Smc1b*, *Taf7l*) and oocyte-specific (*Ooep*, *Padi6*) genes along pseudotime.

Fig S6. Differential expression of two groups of pre-granulosa cells

(A) Feature plots of marker gene expression in BPGs based on UMAP. (B) Feature plots of marker gene expression in EPGs based on UMAP. (C) Venn diagram of DEGs at PD0 and PD3 in BPGs. (D) Venn diagram of DEGs for PD0 and PD3 in EPGs. (E) Enrichment analysis of 192 key genes in BPGs for protein-protein interaction (upper) and transcriptional factors of TRRUST (below) in Metascape. (F) Enrichment analysis of 248 key genes in EPGs for protein-protein interaction (upper) and transcriptional factors of TRRUST (below) in Metascape.

Fig S7. Enrichment analysis of key DEGs both in germ cells and pre-granulosa cells

(A) UMAP plot of germ cell and granulosa cell populations colored by sample groups. (B) UMAP feature plots of marker genes for germ cells (left) and granulosa cells (right). (C) Venn diagram of DEGs in germ cells for pre-, early-, and late-stages of follicle formation and State in Monocle. Key DEGs are marked with a dashed red line. (D) Venn diagram of DEGs of BPGs and EPGs. (E) Enrichment terms of 194 key genes affected by DEHP in germ cells. (F) Enrichment terms of 138 key genes affected by DEHP in pre-granulosa cells. (G) RNA expression levels of *Bmpr1a* and *Smad3* in all ovarian cells with and without DEHP exposure. (H)

Detection of BMPR1A and SMAD3 protein levels in PD3 mouse ovaries with and without DEHP exposure. GAPDH as a loading control for relative protein levels of BMPR1A and SMAD3. Data are shown as mean $\pm$ SD (N represents independent repeats). **P < 0.01.

Fig S8. Differential expression of immune cells and stromal cells

(A) UMAP plot of immune cell populations colored by sample groups (upper) and clusters (below). (B) Volcano plots of gene differential expression in immune cells for PD0-DEHP vs. PD0 and PD3-DEHP vs. PD3. (C) Top GO terms of DEGs in immune cells at PD0 and PD3. (D) UMAP plot of stromal cells colored by sample groups (upper) and clusters (below). (E) Volcano plots of gene differential expression in stromal cells for PD0-DEHP vs. PD0 and PD3-DEHP vs. PD3. (F) Top GO terms of DEGs in stromal cells at PD3.

Figure S1

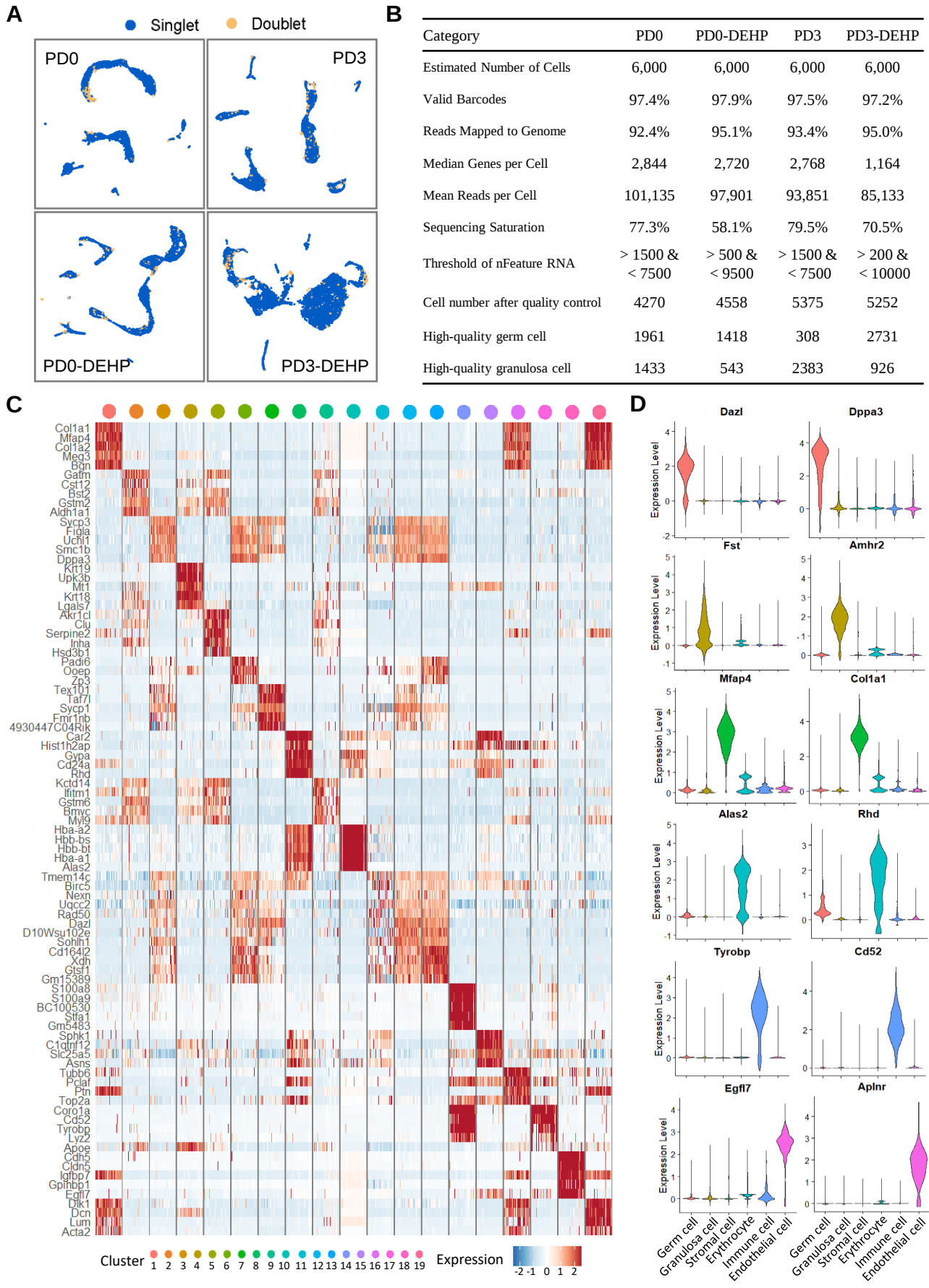


Figure S2

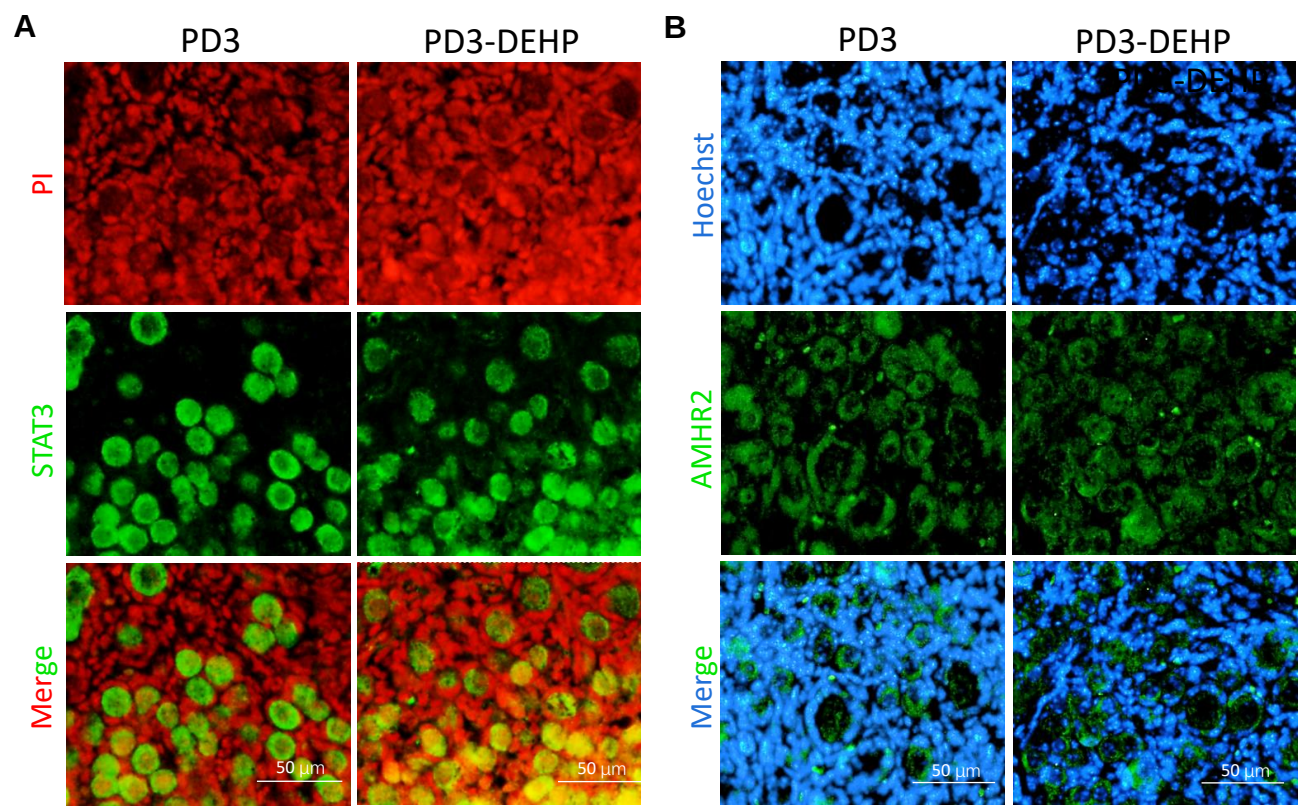


Figure S3

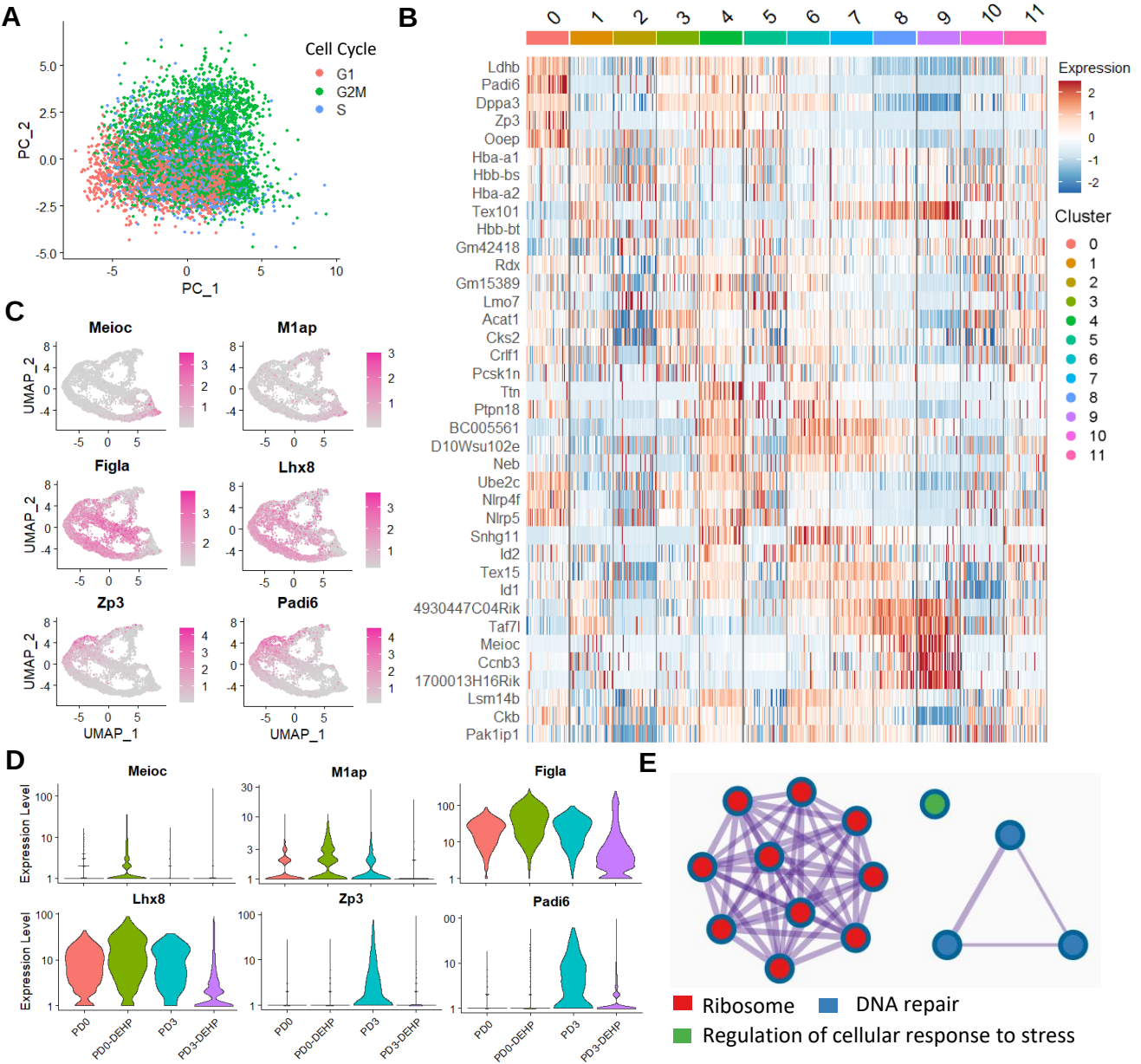
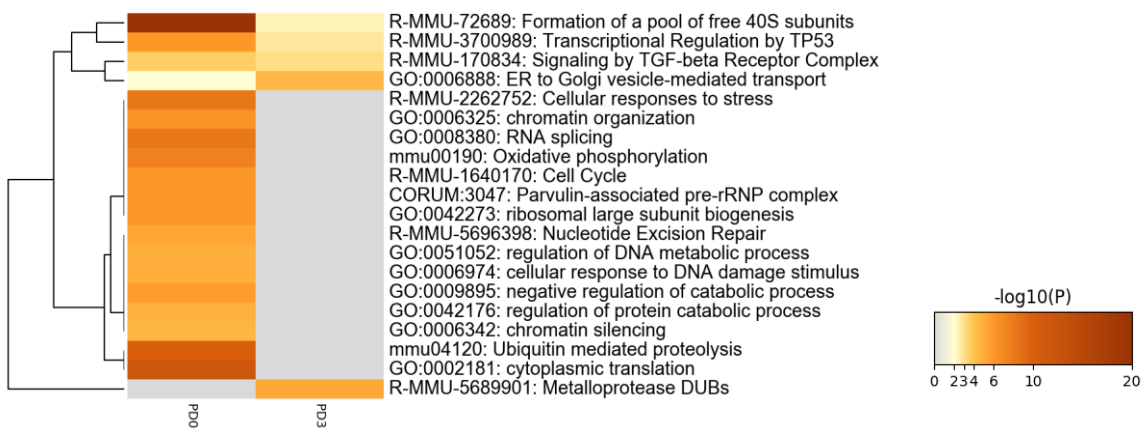


Figure S4

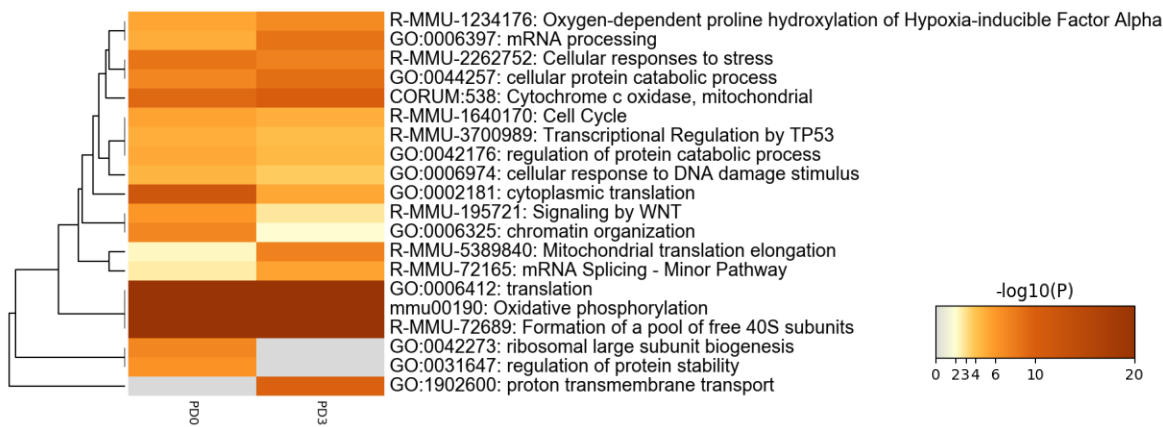
A

Enrichment analysis of DEG lists at pre-stage



B

Enrichment analysis of DEG lists at early-stage



C

Enrichment analysis of DEG lists at late-stage

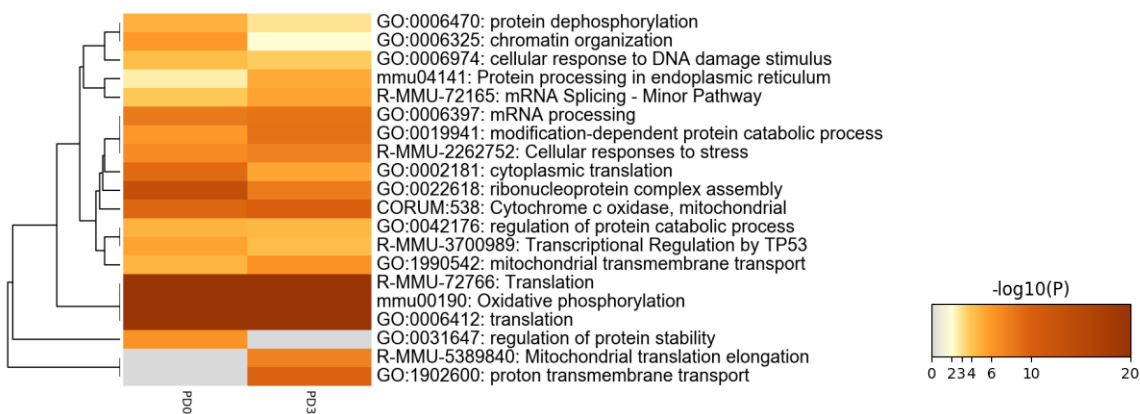


Figure S5

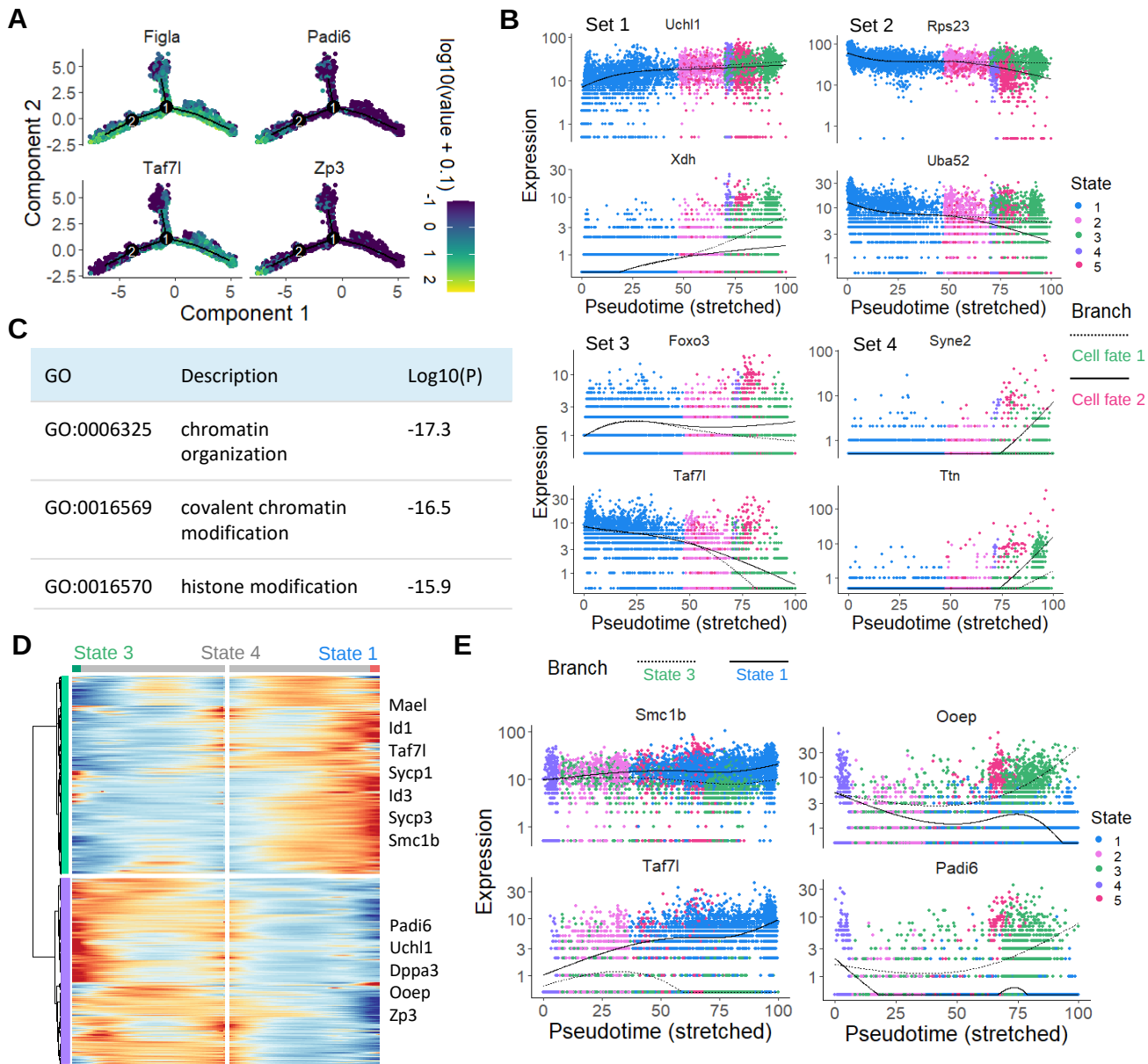


Figure S6

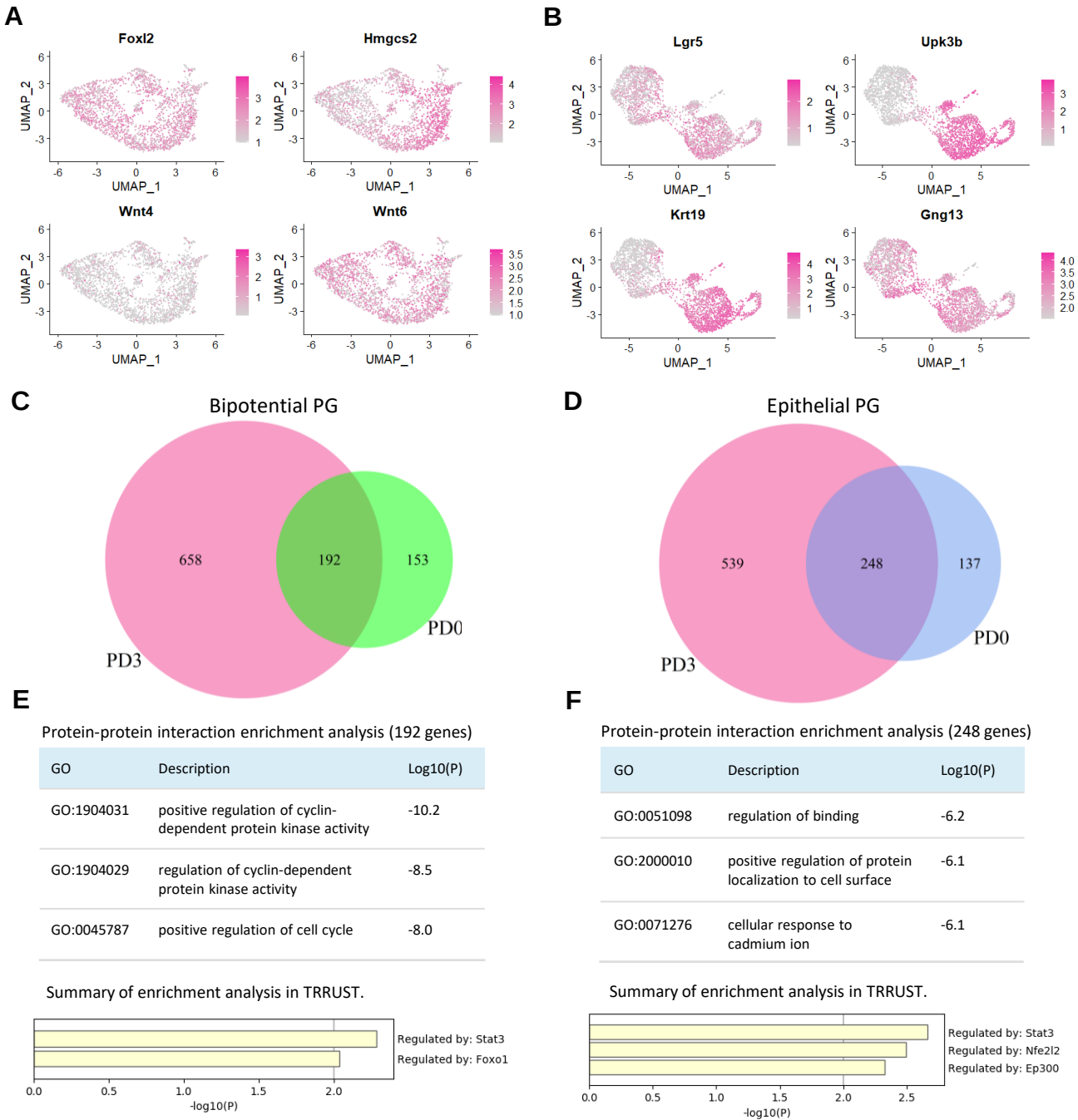


Figure S7

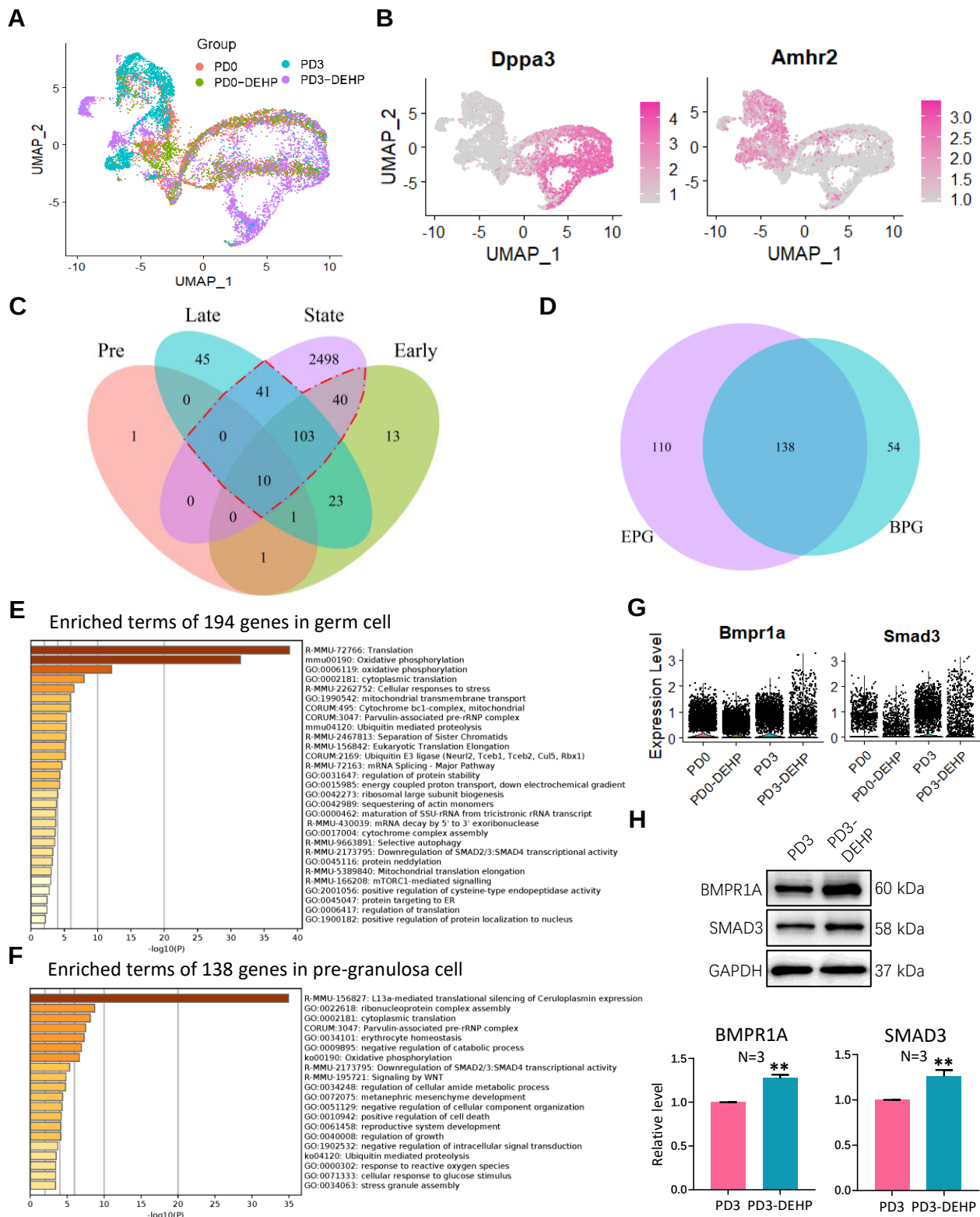


Figure S8

