

Single-cell transcriptomics analysis reveals dynamic changes and prognostic signature in tumor microenvironment of PDAC

-Supplemental Materials-

Yongsheng Li ^{a 1}, Zhilong Ding ^{c 1}, Shasha Zhang ^{d 1}, Tingxin Cheng ^a,
Yihuai Hu ^a, Shiyong Ren ^{a *}, Fei Zhong ^{b *}, Shiyan Wang ^{a *}

^a School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huai'an, China;

^b Department of Laboratory Medicine, The Affiliated Huaian Hospital of Xuzhou Medical University and Huai'an Second People's Hospital, Huai'an, Jiangsu, China;

^c Department of Hepatobiliary Surgery, The Affiliated Huaian Hospital of Xuzhou Medical University and Huai'an Second People's Hospital, Huai'an, Jiangsu, China.

^d Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Engineering Research Center of Techniques and Instruments for Diagnosis and Treatment of Congenital Heart Disease, Institute of Developmental and Regenerative Medicine, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

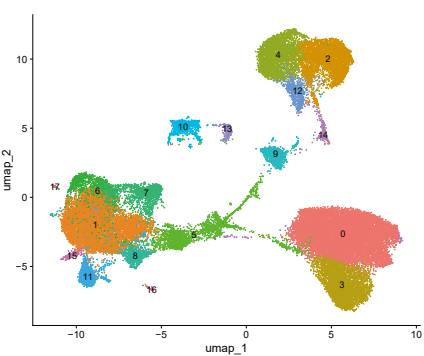
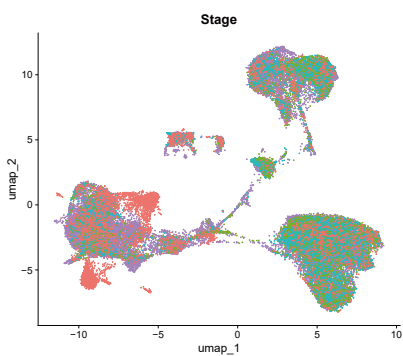
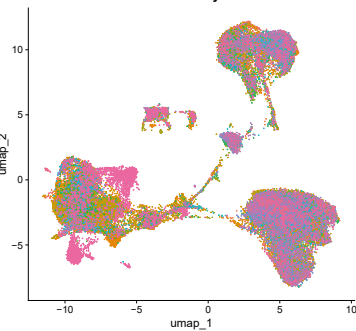
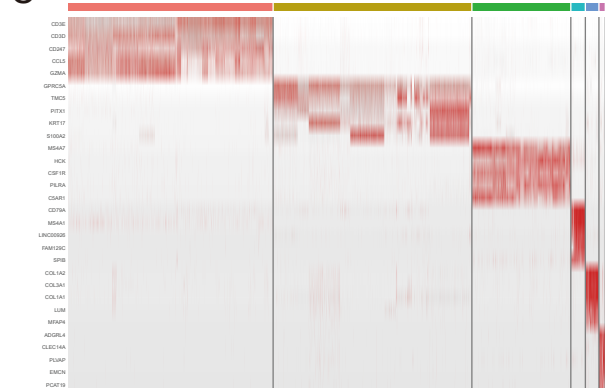
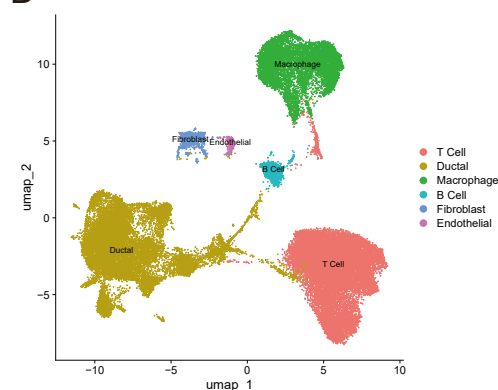
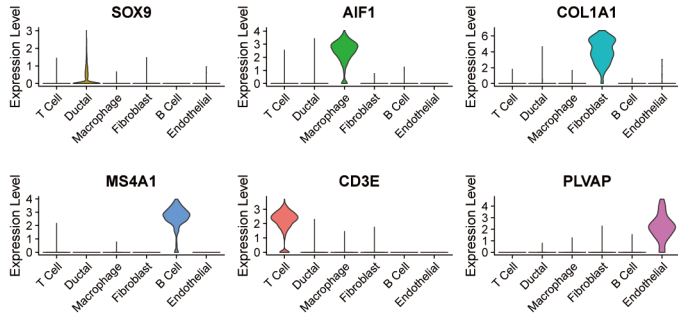
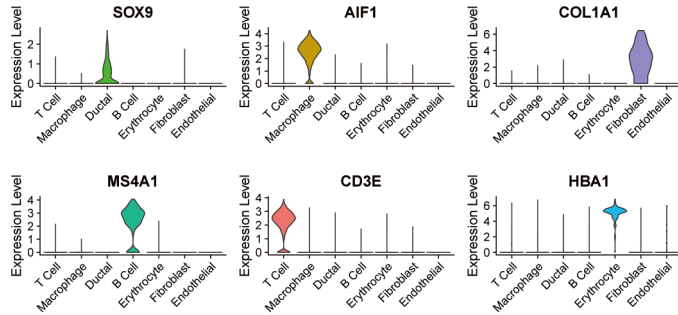
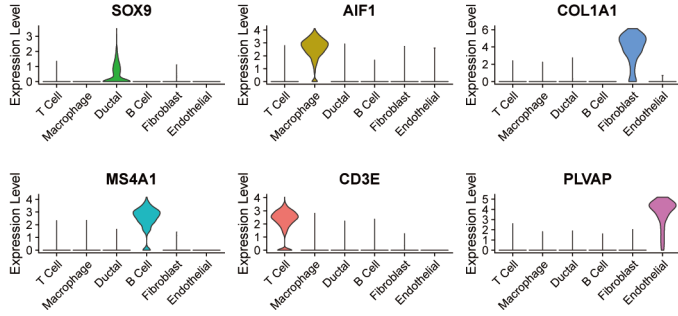
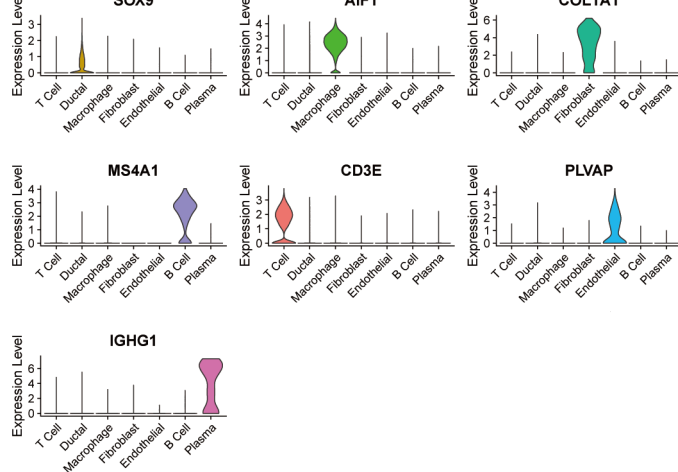
A**B****Harmony****C****D****E****F****G****H**

Figure S1. Single-cell transcriptomic analysis in PDAC tissues.

(A) The UMAP plot showing the distribution of cell populations, color-coded by cluster. UMAP uniform manifold approximation and projection.

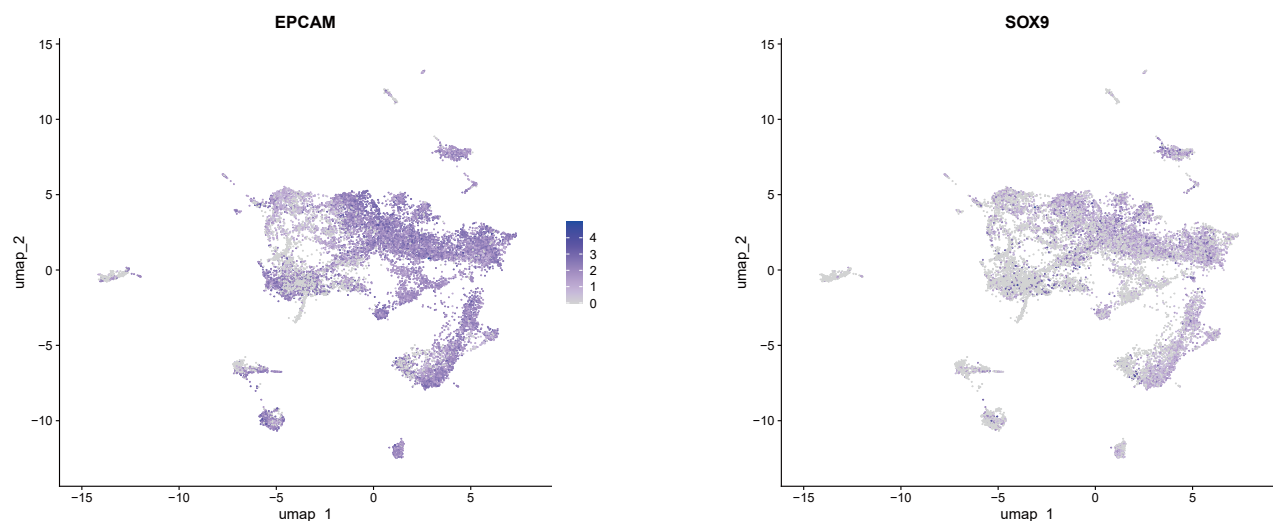
(B) The UMAP plots showing the distribution of cell populations, according to cancer stage (left) and patient sample (right).

(C) Heatmap presenting the expression levels of top 5 cell type-specific genes among cell populations.

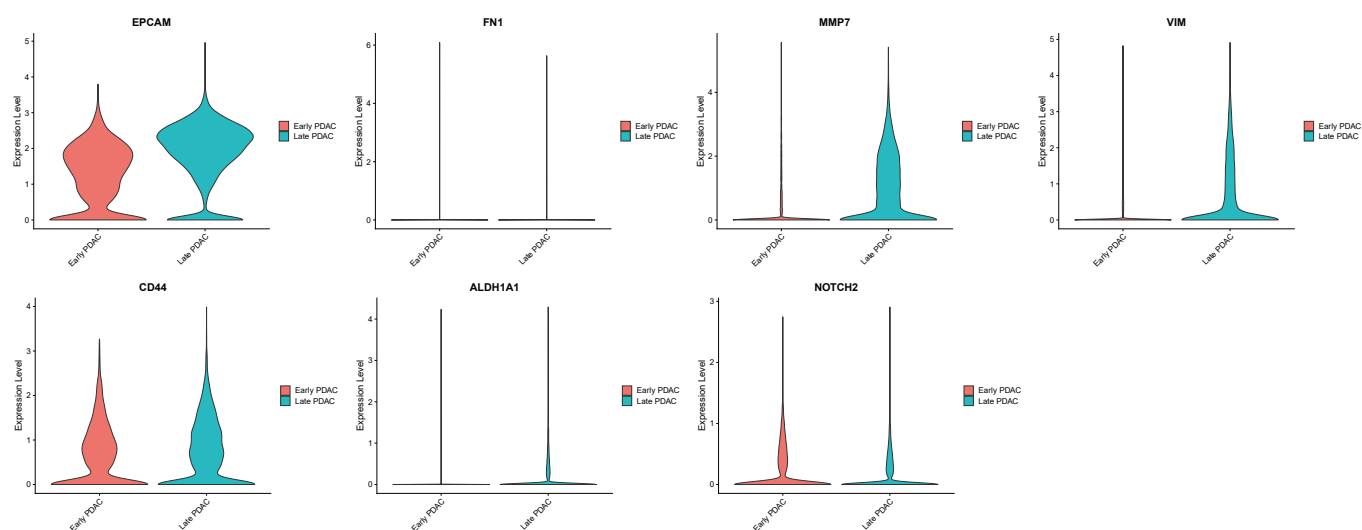
(D) The UMAP plot showing annotation of 6 cell types.

(E-H) The violin plots demonstrating the identity of each cluster through analyzing the expression of known cell type specific markers. Specifically, E for PDAC I, F for PDAC II, G for PDAC III, and H for PDAC IV.

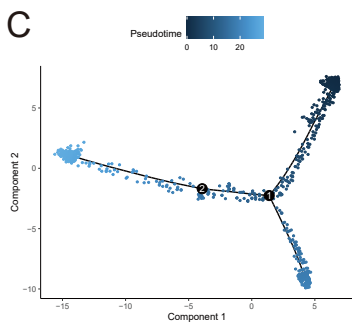
A



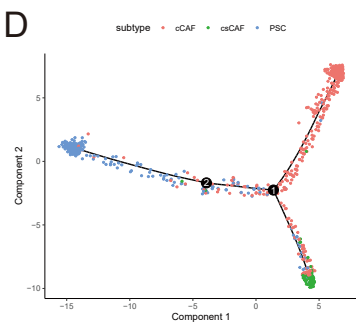
B



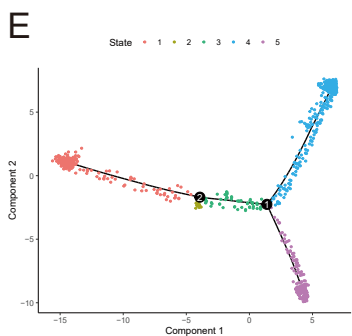
C



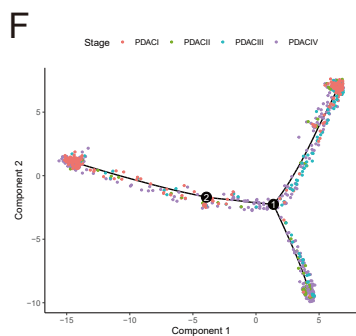
D



E



F



G

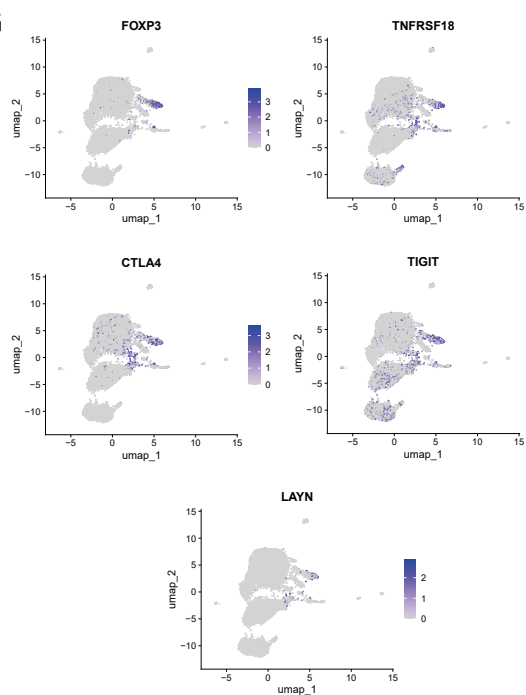


Figure S2. The distribution of specific genes and Pseudotime analysis.

(A) The feature plots showing the expression of SOX9 and EPCAM.

(B) The violin plots demonstrating that late PDAC had significantly higher expression levels of mesenchymal and CSC markers, and EPCAM (ductal cell marker) confirming their ductal cell identity.

(C-F) Pseudotime analysis exploring the development trajectories of CAFs.

(G) The UMAP plot showing the expression levels of marker genes for Treg/exhausted T cell subpopulations.

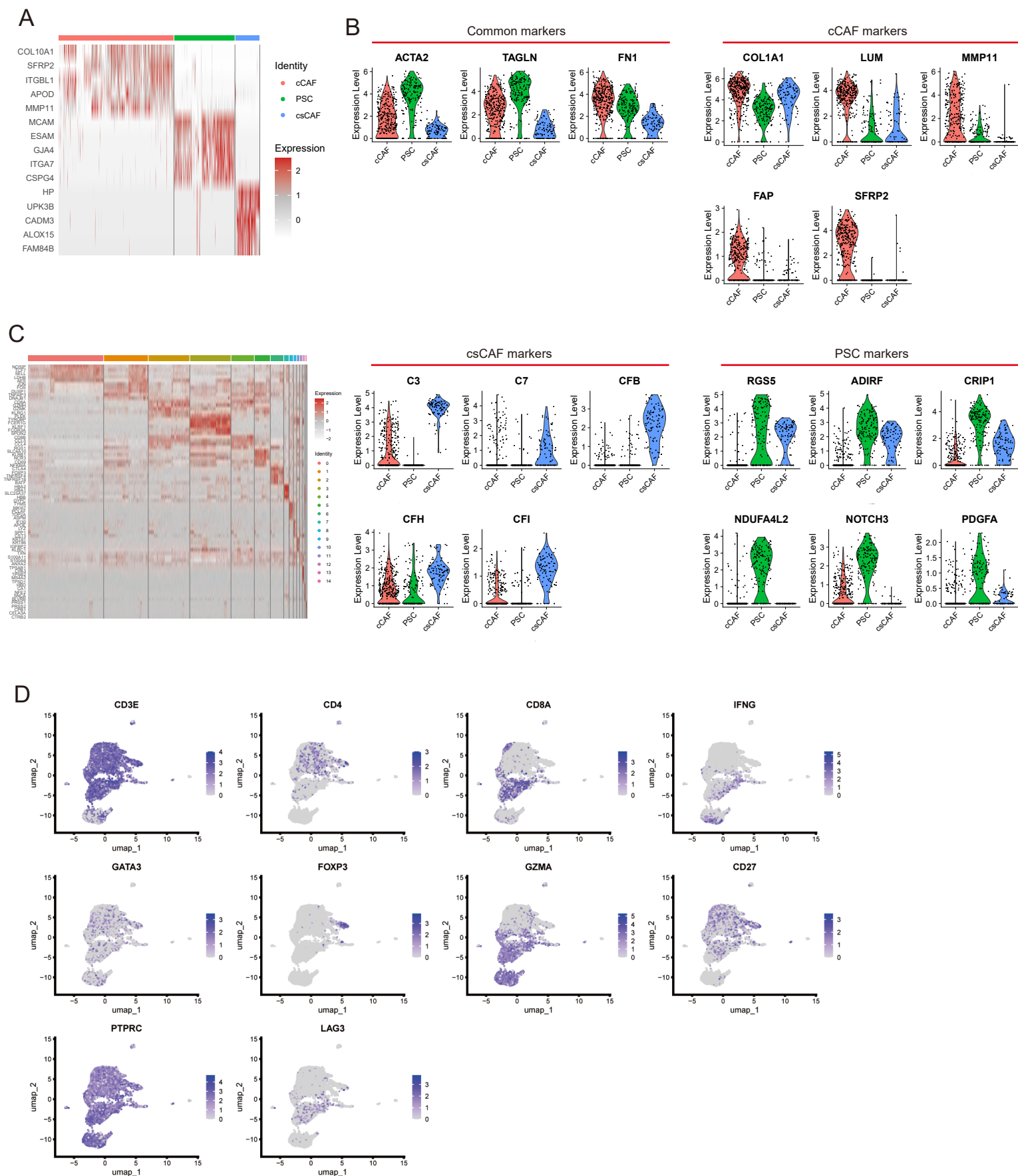


Figure S3. Relevant analysis of fibroblast and T cell subpopulations.

(A) Heatmap presenting the expression levels of top 5 cell type-specific genes among CAF subpopulations.

(B) The violin plots demonstrating the identity of each CAF subcluster, including pan-CAF common markers (top left), cCAFs markers (top right), csCAFs markers (bottom left), PSCs markers (bottom right).

(C) Heatmap comparing the expression levels of top 5 cell type-specific genes among T cell subclusters, each column and row represented a single cell and gene respectively.

(D) The UMAP plots showing the expression level of specific T cell subsets marker gene.

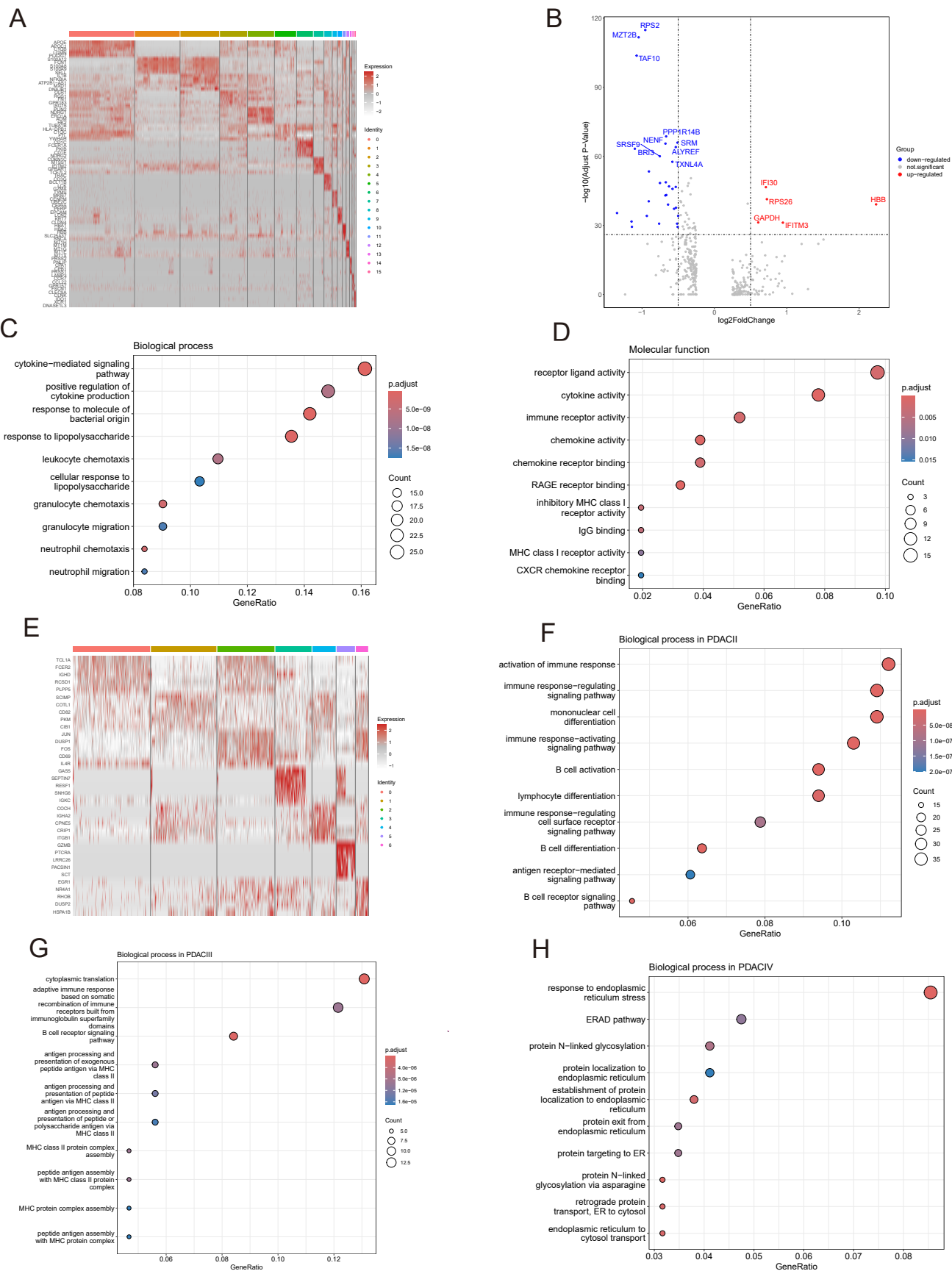


Figure S4. Relevant analysis of macrophage subsets and B cell subsets.

(A) Heatmap of macrophage subclusters and top 5 specific marker genes.

(B) The DEGs between late PDAC (PDAC II, III and IV) and early PDAC (PDAC I) in macrophage subcluster analysis shown by volcano plot, red and blue dots represented the genes upregulated and downregulated respectively (PDAC II, III and IV vs PDAC I).

(C-D) GO analysis revealing the biological processes and molecular functions enriched by upregulated genes in macrophages in late PDAC.

(E) Heatmap of B cell subclusters and related marker genes.

(F-H) GO analysis revealing the biological processes enriched by upregulated genes in B cells across PDAC stages II, III, and IV.

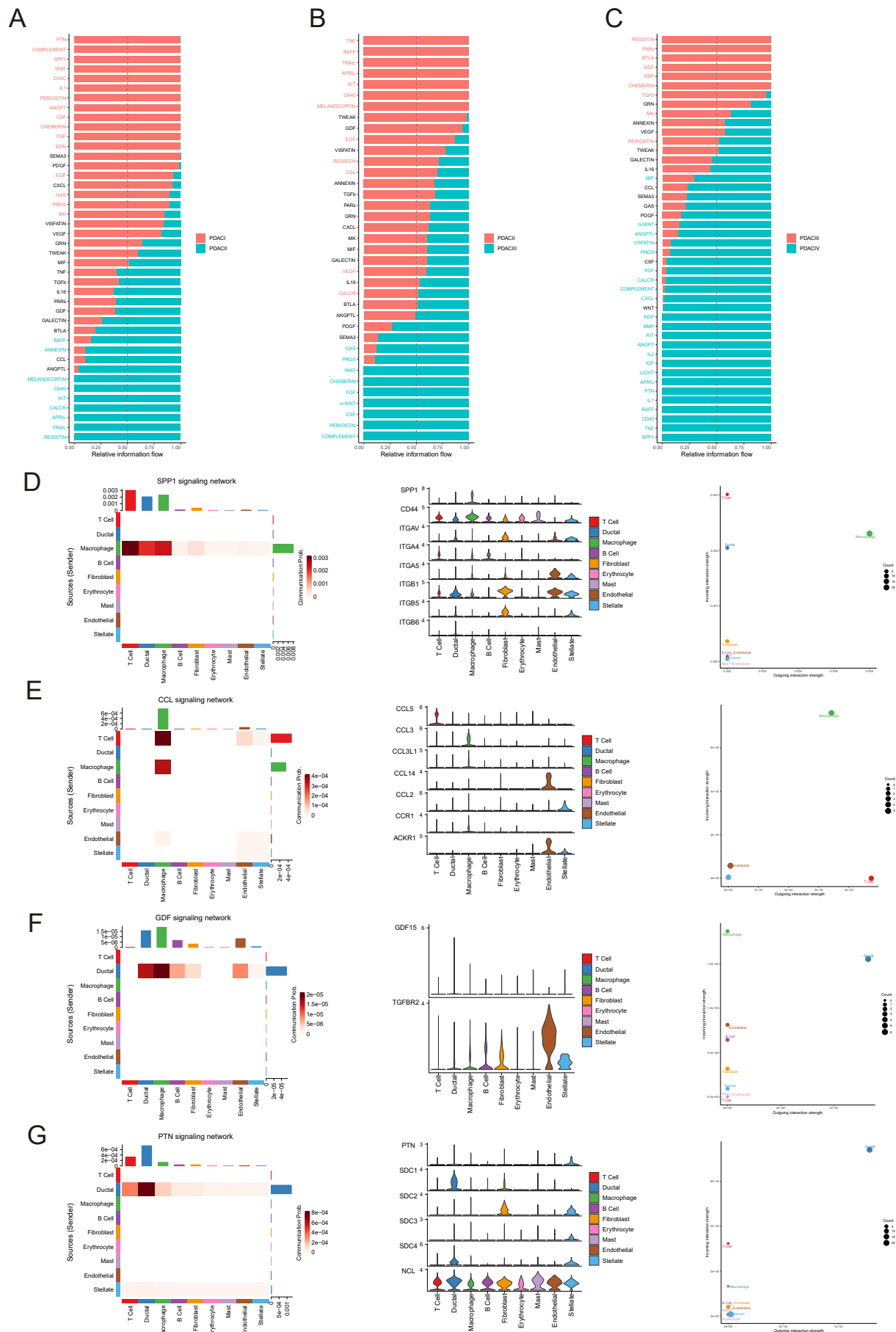


Figure S5. Analysis of intercellular interactions across different stages of PDAC.

(A) Comparison of cellular communication between PDAC I and PDAC II.

(B) Comparison of cellular communication between PDAC II and PDAC III.

(C) Comparison of cellular communication between PDAC III and PDAC IV.

(D-G) The left panel displays a network diagram illustrating the interaction network of the signaling pathway, the middle panel features a violin plot showcasing the expression of signaling pathway-related genes, and the right panel illustrates the incoming and outgoing strength of the signaling pathway.