

Supplementary Material 8

Supplementary figures (Suppl. Figure S1–S14)

Laser microdissection-based multi-omics integration unveils the pathological atlas and tumor differentiation network of adamantinomatous craniopharyngioma

Bowen Wu¹ #, MM, Chunming Xu¹ #, MD, Chenxing Yang¹ #, MM, Shenhao Xie¹ #, MM, Suping Duan², BMed, Jiye Ye³, MD, Jinshi Zhang⁴, MD, Shaoyang Li⁵, MD, Furong Liang¹, BMed, Zhicheng Ye¹, BMed, Hai Luo¹, MM, Rilege Su¹, BMed, Laisheng Pan¹, MM, Zhizheng Liu¹, MM, Shizhou Xing¹, BMed, Tianming Dong¹, BMed, Yiguang Chen⁶, MD, Like Chen¹, MM, Suyue Zheng¹, MM, Jie Wu¹ *, MD, Tao Hong¹ *, MD

¹ Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, 330006, China

² Department of Neurology, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, 330006, China

³ Department of Neurosurgery, Hunan University of Medicine General Hospital, 418000, Huaihua, China

⁴ Department of Neurosurgery, Ganzhou Hospital-Nanfang Hospital, Southern Medical University (Ganzhou People's Hospital), Ganzhou, 341000, China

⁵ School of public health, Jiangxi medical college, Nanchang University, Nanchang, 330006, China

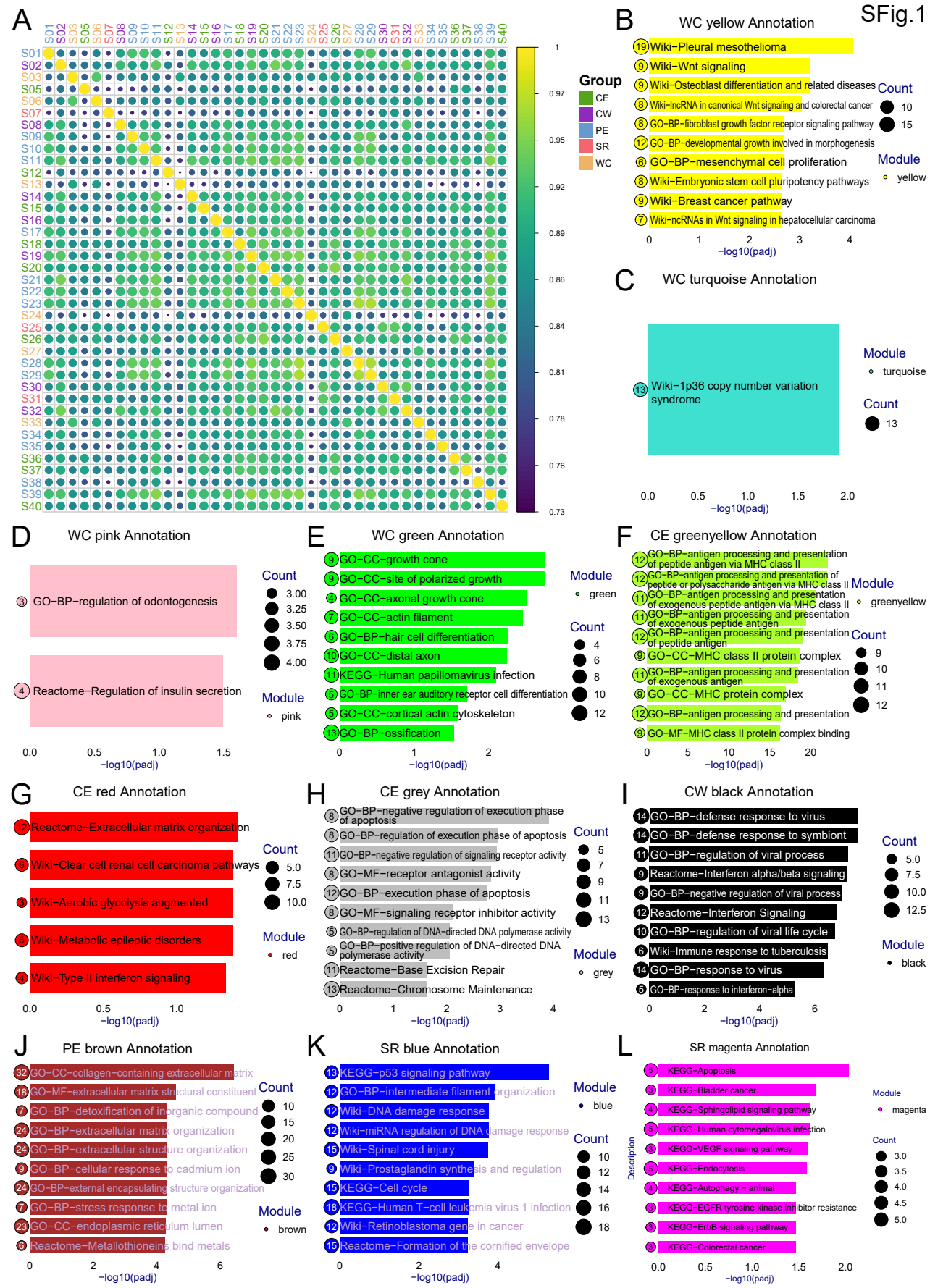
⁶ Department of Neurosurgery/Neuro-oncology, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, SunYat-sen University Cancer Center, 510060, Guangzhou, China

Bowen Wu, Chunming Xu, Chenxing Yang and Shenhao Xie contributed equally to this work.

* Corresponding author:

Jie Wu: Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 17 Yong Wai Zheng Street, Nanchang, 330006, China, E-mail: ndyfy10128@ncu.edu.cn

Tao Hong: Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 17 Yong Wai Zheng Street, Nanchang, 330006, China, E-mail: ndyfy00567@ncu.edu.cn



Supplementary Figure S1. Weighted gene co-expression network analysis based on laser microdissection combined with Smart-Seq2 data revealing the co-expression modules related to pathological structures and their functional enrichment.

A Heatmap showing the Spearman correlation clustering of the average gene expression profiles of the samples.

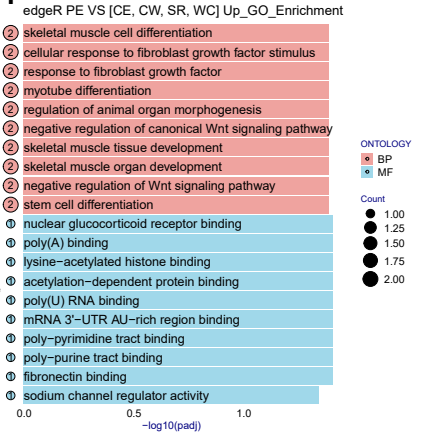
B–E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules in the whorl-like cell clusters (WC) (top 10 terms by adjusted p value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted p value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

F–H GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules in the constitutive epithelium (CE) (top 10 terms by adjusted p value).

I GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules in the cyst-wall epithelium (CW) (top 10 terms by adjusted p value).

J GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules in the palisading epithelium (PE) (top 10 terms by adjusted p value).

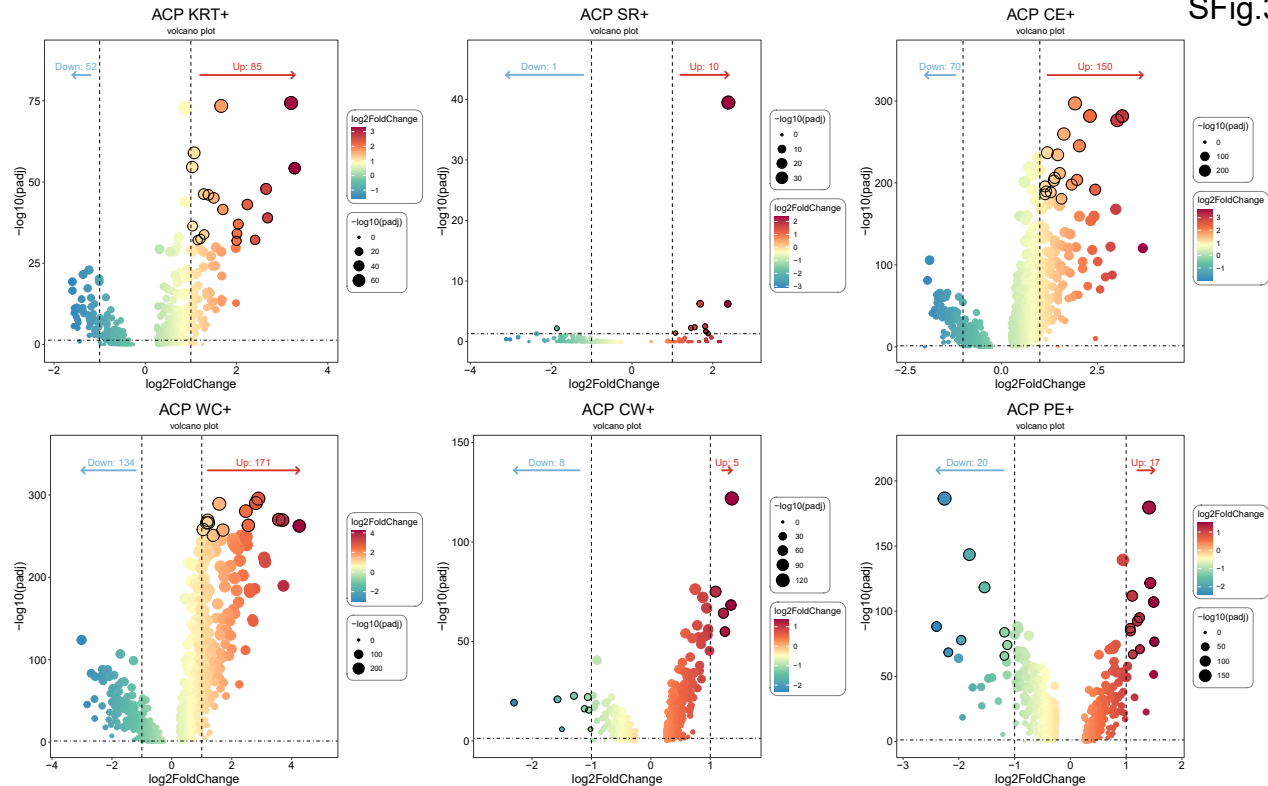
K, L GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules in the stellate reticulum (SR) (top 10 terms by adjusted p value).



Supplementary Figure S2. Multiple comparisons and functional enrichment analysis of differentially expressed genes based on Smart-Seq2 data.

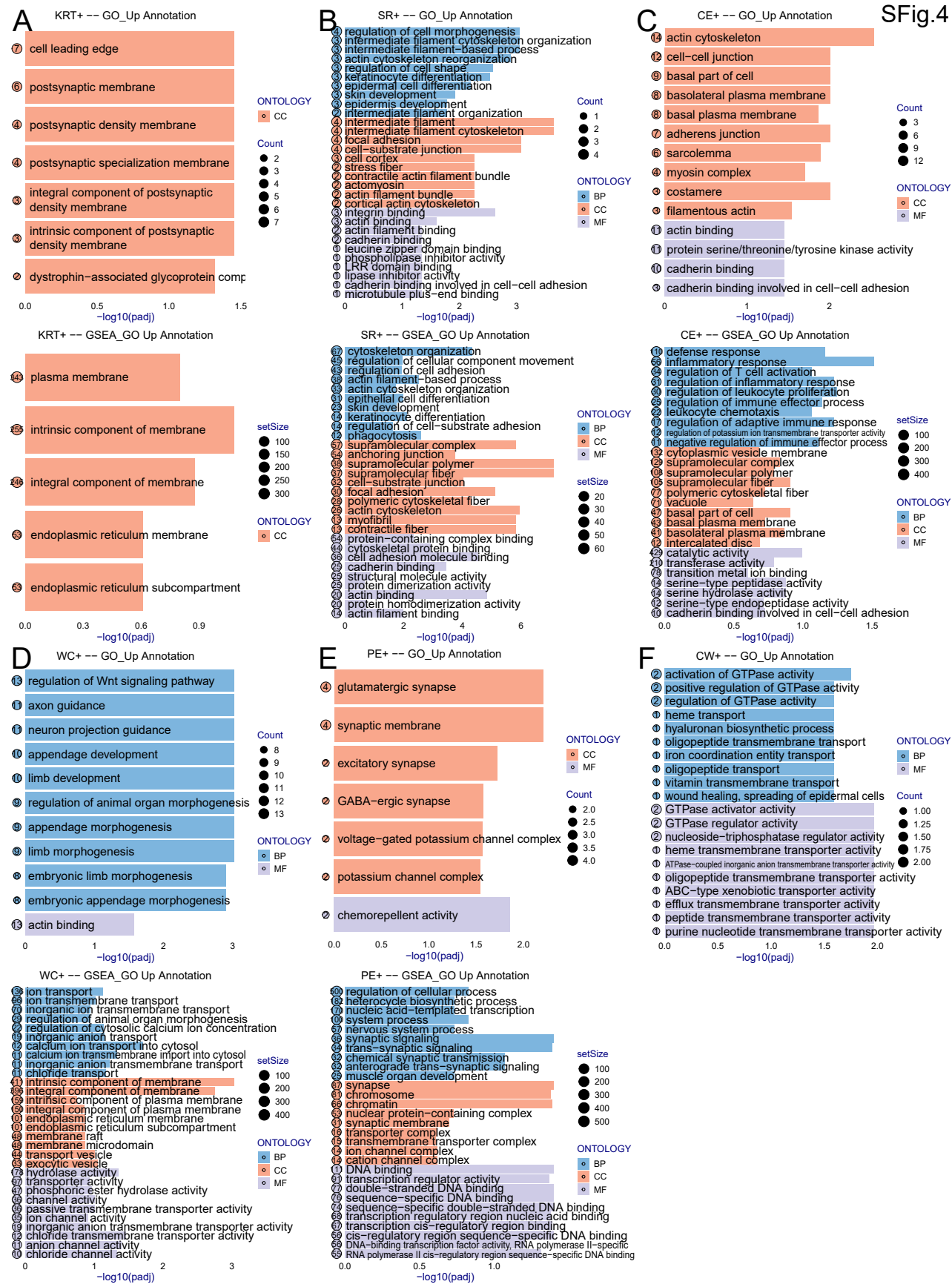
A Venn diagrams respectively showing the intersections of differentially expressed genes (DEGs) identified by edgeR between various pathological structures and others.

B–F Functional enrichment analysis of high-confidence DEGs upregulated in each pathological structure. The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted p value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S3. DEGs of the Scissor-mapped pathological structure feature subpopulations

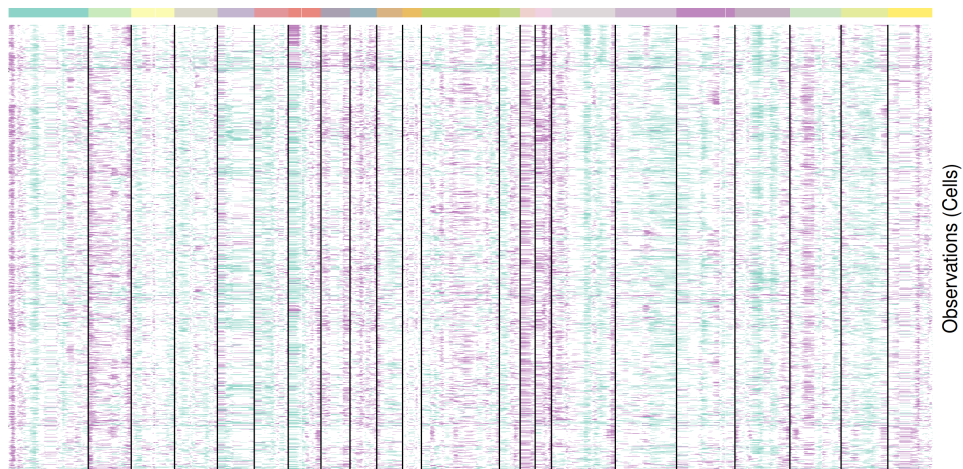
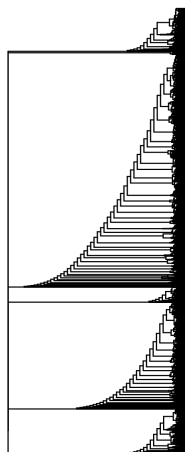
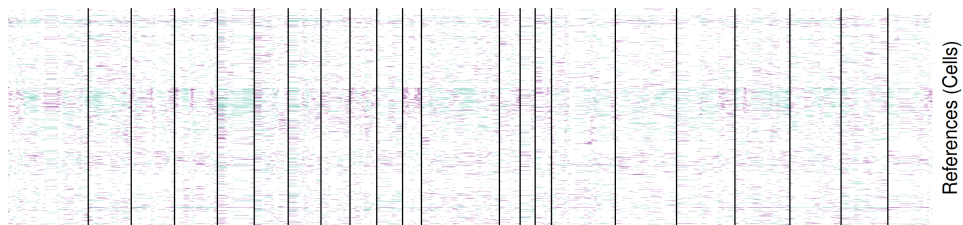
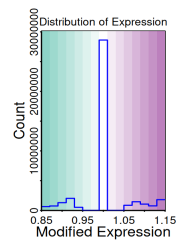
Volcano plots respectively showing the distribution of DEGs in each pathological structure feature subpopulation.



Supplementary Figure S4. Functional enrichment analysis of the Scissor-mapped pathological structure feature subpopulations.

One is GO functional enrichment analysis of DEGs upregulated in each pathological structure feature subpopulation. Another is the results of gene set enrichment analysis (GSEA) of GO terms. The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted p value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

inferCNV



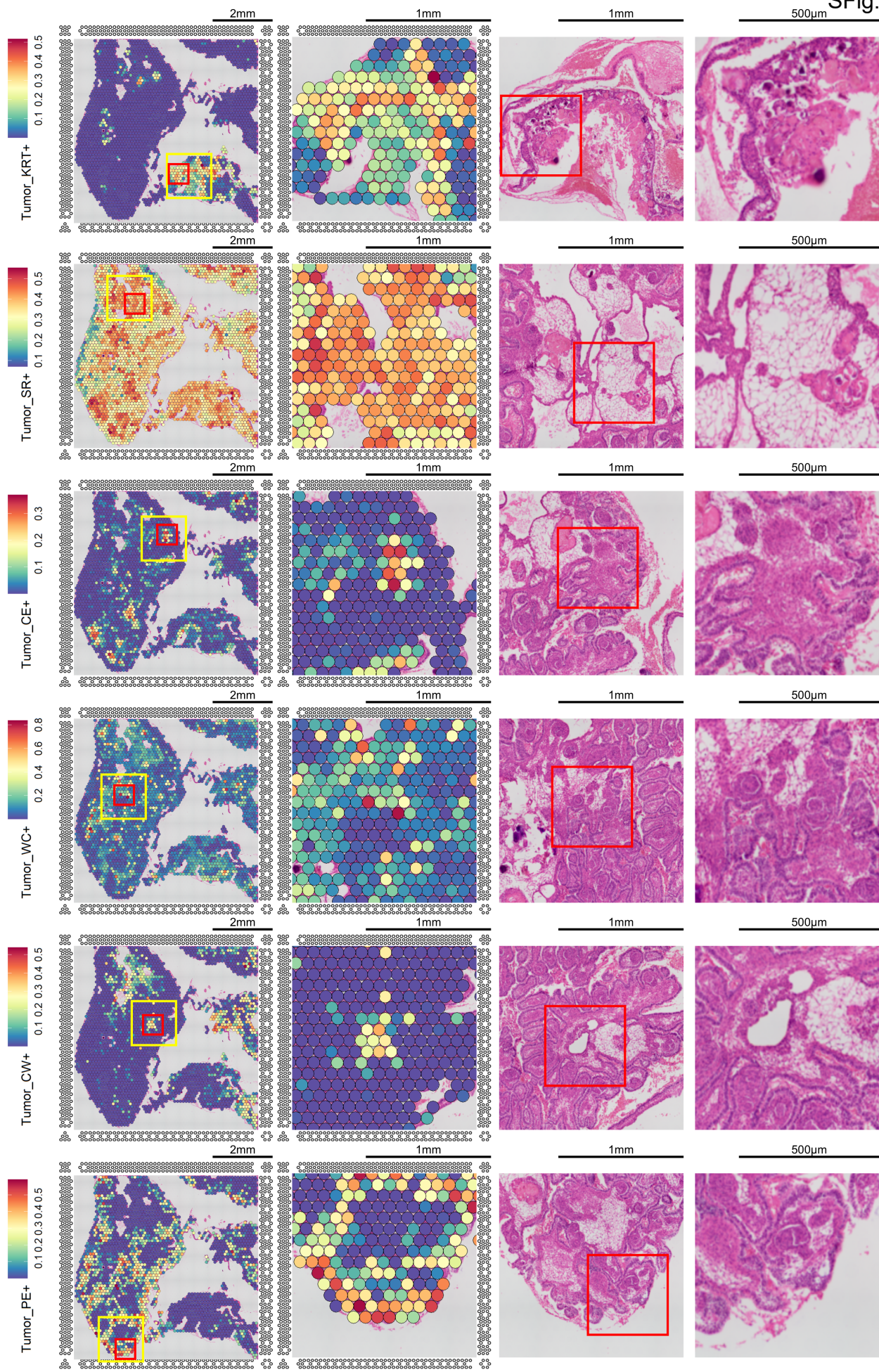
chr15

Genomic Region

ACP_Tu_CE+ ACP_Tu_CW+ ACP_Tu_KRT+ ACP_Tu_PE+ ACP_Tu_SR+ ACP_Tu_WC+

Supplementary Figure S5. Heatmap of copy number variations landscape of ACP cells.

Heatmap of copy number variations (CNVs) derived from the snRNA-seq data. Pituitary cells from the GSE178452 dataset¹ were used as references, with purple indicating chromosomal region amplification and cyan indicating deletion.

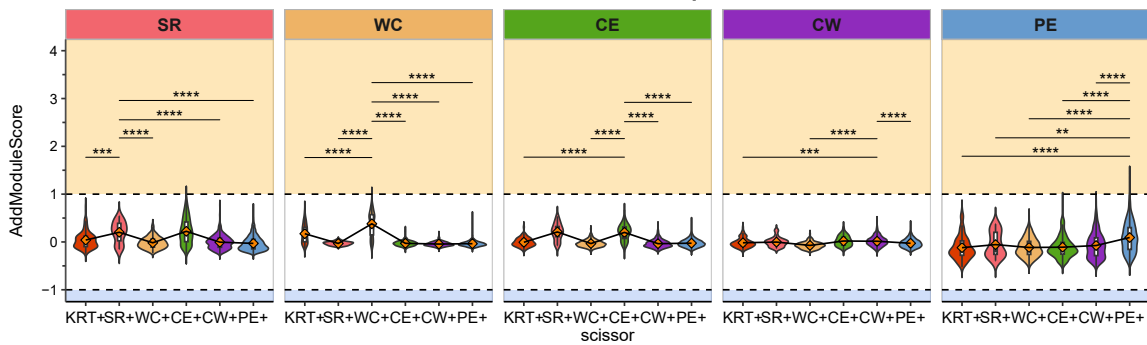


Supplementary Figure S6. Validation of the spatial localization of single-cell subpopulations based on the RCTD algorithm.

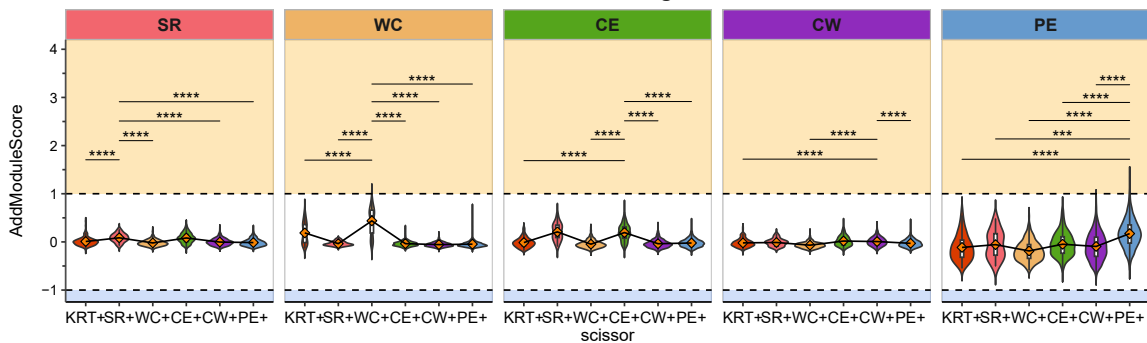
Scale bar: 2 mm, 1 mm, 1 mm, 500 μm (from left to right).

A

LMD DEGs DESeq2

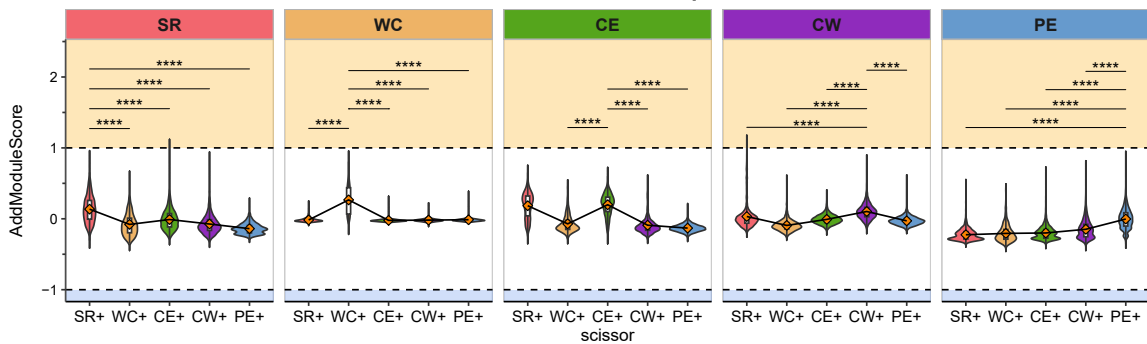


LMD DEGs edgeR

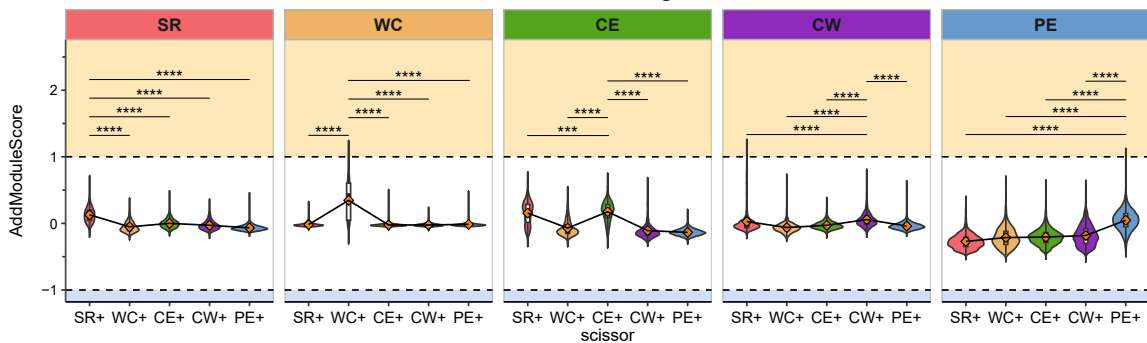


B

LMD DEGs DESeq2



LMD DEGs edgeR



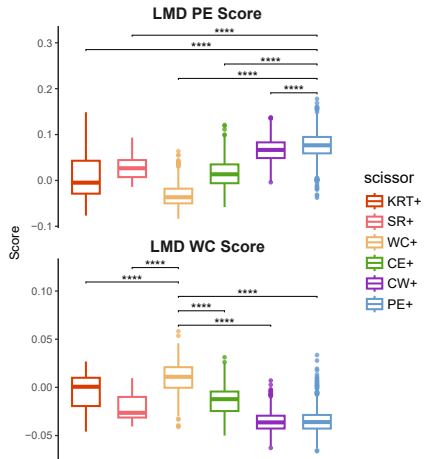
Supplementary Figure S7. Validation of single-cell clustering of high-confidence DEGs.

Violin plots showing the scores of high-confidence DEGs (identified by DESeq2 and edgeR) calculated based on the AddModuleScore method for the five pathological structure feature subpopulations. Panel A shows the results from our internal dataset, while Panel B presents validation using an external dataset (DOI: 10.5281/zenodo.7199080)². Statistical significance is assessed by Wilcoxon test: ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

A

AddModuleScore

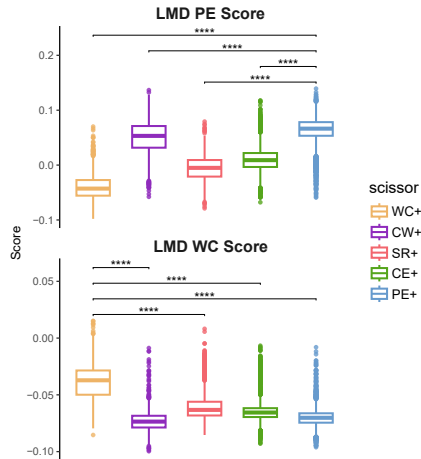
Gene sets source: PMID 29541918



B

AddModuleScore

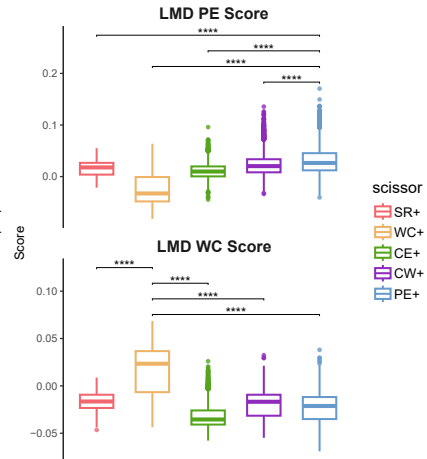
Gene sets source: PMID 29541918



C

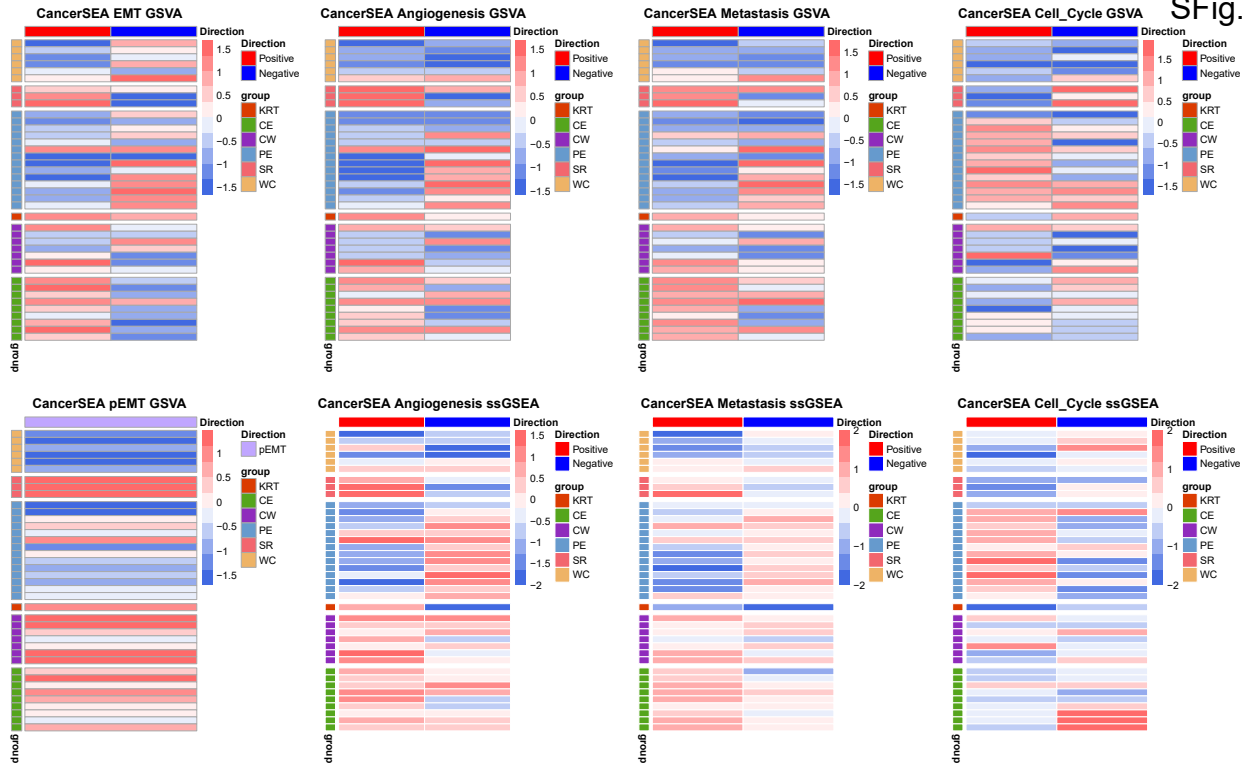
AddModuleScore

Gene sets source: PMID 29541918

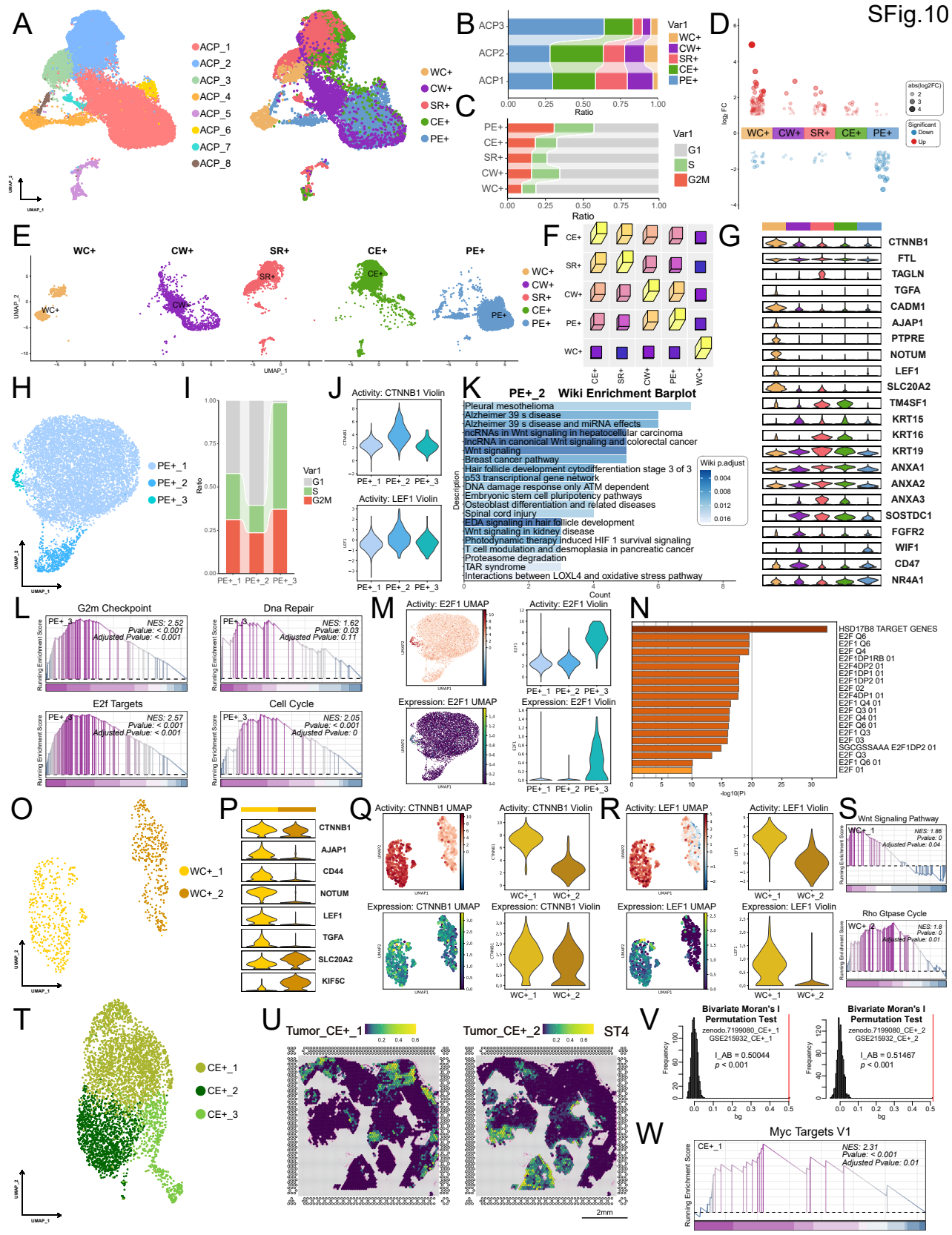


Supplementary Figure S8. Verification of single-cell clustering of DEGs in WC and PE areas obtained through previous external laser microdissection study.

Box plots showing the scores of DEGs in WC and PE areas obtained through previous external laser microdissection study (PMID 29541918)³ calculated based on the AddModuleScore method for all pathological structure feature subpopulations. Panel A shows the results from our internal dataset, while Panel B and C present validation using external datasets (one is from DOI: 10.5281/zenodo.7199080, and another is obtained from Chen Y)^{2,4}. Statistical significance is assessed by Wilcoxon test: **** $p < 0.0001$.



Supplementary Figure S9. ssGSEA and GSVA scores for gene sets in Smart-Seq2 data.



Supplementary Figure S10. Validation of key molecular markers and pathological structure heterogeneity using external dataset 1. Dataset source (DOI: 10.5281/zenodo.7199080)²

A Left: UMAP dimensionality reduction visualization of epithelial tumor cells, including a total of 25250 cells, showing 8 tumor clusters in different colors. Right: Distribution of 5 pathological structure feature-positive cells in UMAP space based on the Scissor algorithm mapping.

B Stacked bar chart showing the proportion of cells belonging to different pathological structure features in each sample.

C Stacked bar chart showing the proportion of cells in each cell cycle stage in the 5 pathological structure feature subpopulations.

D Multiple volcano plots showing the distribution of differentially expressed genes in the 5 pathological structure feature subpopulations.

E UMAP dimensionality reduction visualization of cell clusters, split by the results of the Scissor algorithm mapping.

F Heatmap showing the Spearman correlation clustering of the average gene expression profiles of the 5 subpopulations.

G Multiple violin plots showing the expression levels of representative marker genes in each cell subpopulation.

H Palisading epithelium-positive (PE⁺) cells are divided into three subpopulations in the UMAP plot with different colors.

I Stacked bar chart showing the proportion of cells in each cell cycle stage in the three PE⁺ subpopulations.

J Violin plots of CTNNB1 and LEF1 activity in each PE⁺ pathological structure subpopulation.

K WikiPathways enrichment analysis of DEGs upregulated in the PE⁺_2 subpopulation. The fill color represents the adjusted *p* value, and the length of the column represents the number of genes involved in the pathway.

L The results of gene set enrichment analysis (GSEA) conducted on PE⁺_3 subpopulation. NES is the normalized enrichment score, and NES > 1 indicates that the pathway is positively enriched.

M Left: UMAP visualization of the distribution of E2F1 transcription factor activity and expression derived from decoupleR in all PE⁺ cells. Right: Violin plots of E2F1 activity and expression in each PE⁺ subpopulation.

N Bar chart showing the enrichment of transcription factors among the upregulated differentially expressed genes in the PE⁺_3 subpopulation generated by Metascape⁵.

O Whorl-like cell clusters-positive (WC⁺) cells are divided into two subpopulations in the UMAP plot with different colors.

P Multiple violin plots showing the expression levels of representative marker genes in each WC⁺ cell subpopulation.

Q Left: UMAP visualization of the distribution of CTNNB1 transcription factor activity and expression derived from decoupleR in all WC⁺ cells. Right: Violin plots of CTNNB1 activity and expression in each WC⁺ subpopulation.

R Left: UMAP visualization of the distribution of LEF1 transcription factor activity and expression derived from decoupleR in all WC⁺ cells. Right: Violin plots of LEF1 activity and expression in each WC⁺ subpopulation.

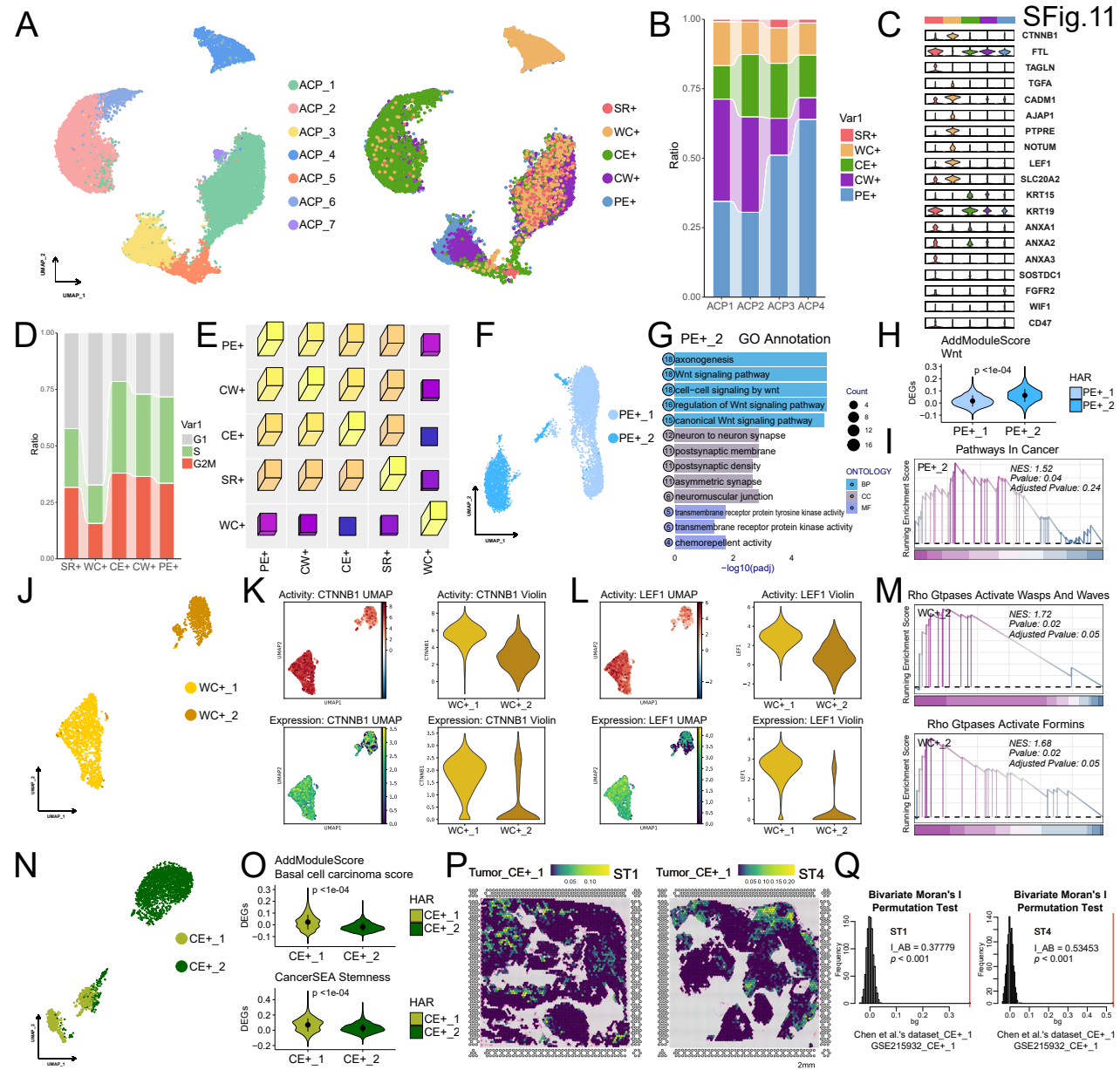
S The result of GSEA conducted on WC⁺_1 and WC⁺_2 subpopulations. NES is the normalized enrichment score, and NES > 1 indicates that the pathway is positively enriched.

T Constitutive epithelium-positive (CE⁺) cells are divided into three subpopulations in the UMAP plot with different colors.

U Feature scores of CE⁺_1 and CE⁺_2 subpopulations in each spatial spot of ST4 calculated based on the RCTD deconvolution algorithm. Scale bar: 2 mm.

V Histogram showing the permutation distribution of the bivariate Moran's I for spatial colocalization analysis of CE⁺_1 and CE⁺_2 subpopulations from an external dataset (10.5281/zenodo.7199080) and our internal dataset, respectively, in each spatial spot of the ST4 sample.

W The result of GSEA conducted on CE⁺_1 subpopulation. NES is the normalized enrichment score, and NES > 1 indicates that the pathway is positively enriched.



Supplementary Figure S11. Validation of key molecular markers and pathological structure heterogeneity using external dataset 2. Dataset source (Chen Y)⁴

A Left: UMAP dimensionality reduction visualization of epithelial tumor cells, including a total of 26428 cell nuclei, showing 8 tumor clusters in different colors. Right: Distribution of 5 pathological structure feature-positive cells in UMAP space based on the Scissor algorithm mapping.

B Stacked bar chart showing the proportion of cell nuclei belonging to different pathological structure features in each sample.

C Multiple violin plots showing the expression levels of representative marker genes in each cell subpopulation.

D Stacked bar chart showing the proportion of cell nuclei in each cell cycle stage in the 5 pathological structure feature subpopulations.

E Heatmap showing the Spearman correlation clustering of the average gene expression profiles of the 5 subpopulations.

F PE⁺ cells are divided into two subpopulations in the UMAP plot with different colors.

G GO functional enrichment analysis of DEGs upregulated in PE⁺_2 subpopulation. The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

H Violin plot showing the scores of WNT signaling pathway gene sets calculated based on the AddModuleScore method for the two PE⁺ subpopulations. Statistical significance is assessed by Wilcoxon test.

I The result of GSEA conducted on PE⁺_2 subpopulation. NES is the normalized enrichment score, and NES > 1 indicates that the pathway is positively enriched.

J WC⁺ cells are divided into two subpopulations in the UMAP plot with different colors.

K Left: UMAP visualization of the distribution of CTNNB1 transcription factor activity and expression derived from decoupleR in all WC⁺ cells. Right: Violin plots of CTNNB1 activity and expression in each WC⁺ subpopulation.

L Left: UMAP visualization of the distribution of LEF1 transcription factor activity and expression derived from decoupleR in all WC⁺ cells. Right: Violin plots of LEF1 activity and expression in each WC⁺ subpopulation.

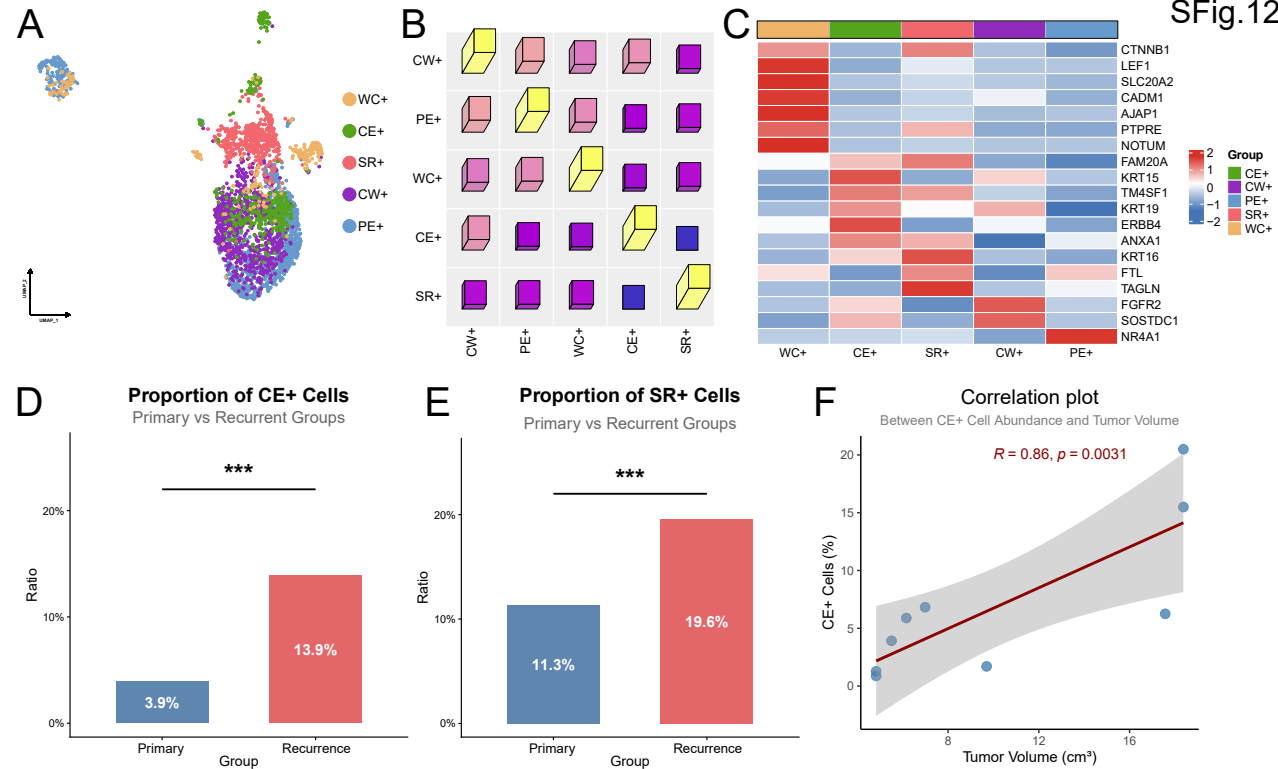
M The results of GSEA conducted on WC⁺_2 subpopulation.

N CE⁺ cells are divided into two subpopulations in the UMAP plot with different colors.

O Violin plots showing the scores of basal cell carcinoma score and stemness gene sets calculated based on the AddModuleScore method for the two CE⁺ subpopulations. Statistical significance is assessed by Wilcoxon test.

P Feature scores of CE⁺_1 subpopulation in each spatial spot of ST1 and ST4 calculated based on the RCTD deconvolution algorithm. Scale bar: 2 mm.

Q Histogram showing the permutation distribution of the bivariate Moran's I for spatial colocalization analysis of CE⁺_1 (result from Chen et al.'s dataset) and CE⁺_1 (result from our internal dataset GSE215932) subpopulations in each spatial spot of the ST1 and ST4 samples.



Supplementary Figure S12. Validation of key molecular markers and pathological structure heterogeneity using external dataset 3. Dataset source (GSE292083)⁶

A Distribution of 5 pathological structure feature-positive cells in UMAP space based on the Scissor algorithm mapping.

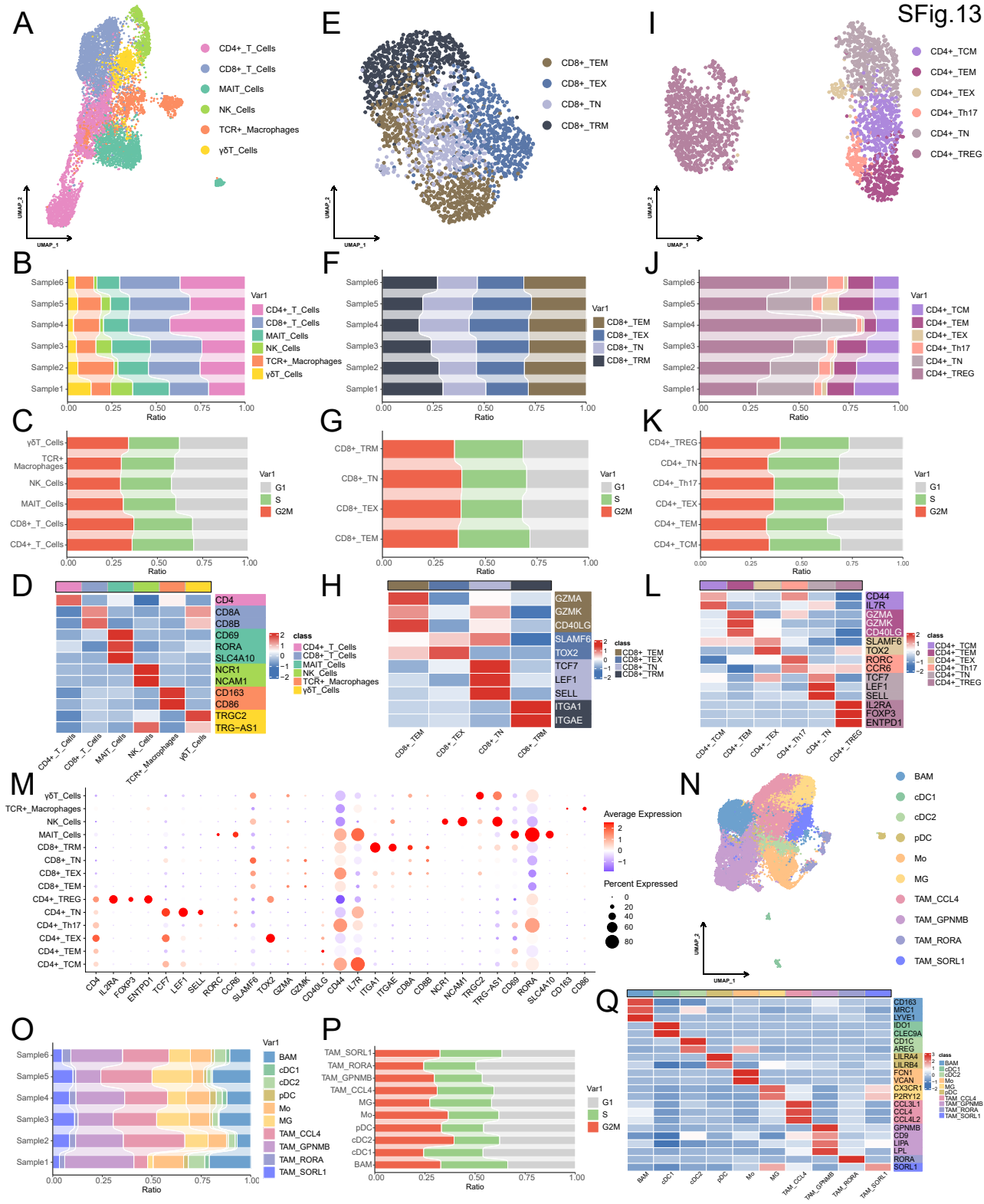
B Heatmap showing the Spearman correlation clustering of the average gene expression profiles of the 5 subpopulations.

C Heatmap showing the average expression levels of representative marker genes in each cell subpopulation.

D Column charts showing the relationships between the proportions of CE⁺ cells and tumor recurrence. Statistical significance is assessed by Fisher's test: *** $p < 0.001$.

E Column charts showing the relationships between the proportions of SR⁺ cells and tumor recurrence. Statistical significance is assessed by Fisher's test: *** $p < 0.001$.

F Scatter plot showing the correlation between the proportion of CE⁺ cells and tumor volume.



Supplementary Figure S13. Characteristics of all immune cells in ACP.

A, E, L UMAP dimensionality reduction visualization of lymphoid populations: (A) all lymphoid immune cells, (E) CD8⁺ T cells, (L) CD4⁺ T cells, showing various clusters in different colors.

B, F, J Stacked bar charts showing the proportion of cell nuclei belonging to different lymphoid populations in each sample: (B) all lymphoid immune cells, (F) CD8⁺ T cells, (J) CD4⁺ T cells.

C, G, K Stacked bar charts showing the proportion of cell nuclei in each cell cycle stage in lymphoid populations: (C) all lymphoid immune cells, (G) CD8⁺ T cells, (K) CD4⁺ T cells.

D, H, L Heatmaps showing the average expression levels of the marker genes for different lymphoid populations: (D) all lymphoid immune cells, (H) CD8⁺ T cells, (L) CD4⁺ T cells.

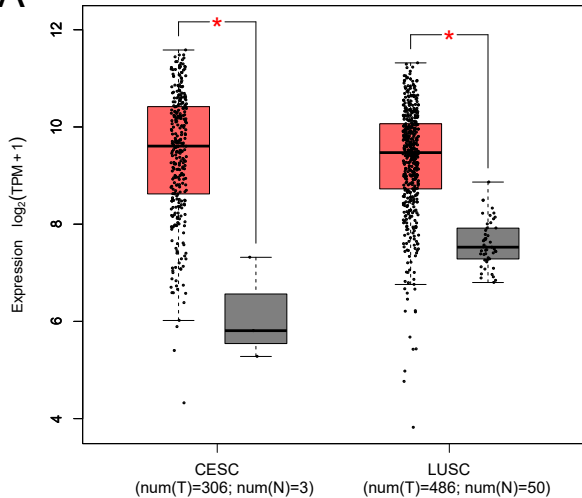
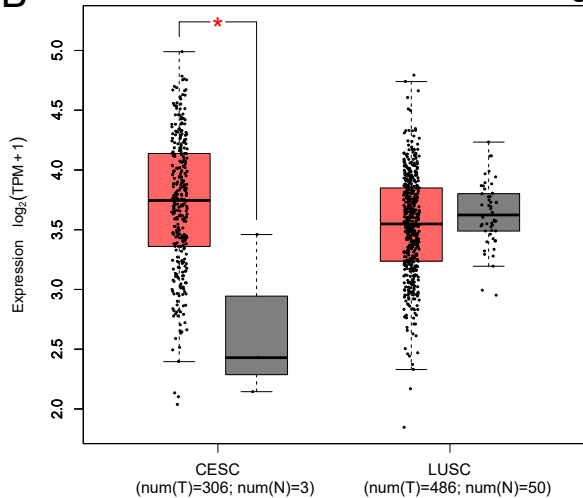
M Bubble plot showing the expression levels of the signature marker genes for all types of lymphoid populations.

N UMAP dimensionality reduction visualization of all types of myeloid immune cells, showing 10 clusters in different colors.

O Stacked bar chart showing the proportion of cell nuclei belonging to different myeloid populations.

P Stacked bar chart showing the proportion of cell nuclei in each cell cycle stage in myeloid populations.

Q Heatmap showing the average expression levels of the marker genes for different myeloid populations.

A**B**

Supplementary Figure S14. Analysis of the expression of CE⁺ cell signature genes in two squamous cell carcinomas using GEPIA2⁷.

A Box plot comparing the expression of four core CE⁺ signature genes (KRT15, KRT19, ANXA1, ANXA2) between tumor and normal samples (CESC: cervical squamous cell carcinoma; LUSC: lung squamous cell carcinoma; *: $p < 0.05$).

B Box plot comparing the expression of top 50 DEGs of CE⁺ subpopulation between tumor and normal samples.

References:

1. Zhang Z, Zamojski M, Smith GR et al. Single nucleus transcriptome and chromatin accessibility of postmortem human pituitaries reveal diverse stem cell regulatory mechanisms. *Cell Rep.* 2022;38(10):110467
2. Jiang Y, Yang J, Liang R et al. Single-cell RNA sequencing highlights intratumor heterogeneity and intercellular network featured in adamantinomatous craniopharyngioma. *Sci Adv.* 2023;9(15):eadc8933-eadc8933
3. Apps JR, Carreno G, Gonzalez-Meljem JM et al. Tumour compartment transcriptomics demonstrates the activation of inflammatory and odontogenic programmes in human adamantinomatous craniopharyngioma and identifies the MAPK/ERK pathway as a novel therapeutic target. *Acta Neuropathol.* 2018;135(5):757-777
4. Chen Y, Liu X, Ainiwan Y et al. Axl as a potential therapeutic target for adamantinomatous craniopharyngiomas: Based on single nucleus RNA-seq and spatial transcriptome profiling. *Cancer Lett.* 2024;592:216905
5. Zhou Y, Zhou B, Pache L et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun.* 2019;10(1):1523-1523
6. Matsuda T, Kono T, Taki Y et al. Deciphering craniopharyngioma subtypes: Single-cell analysis of tumor microenvironment and immune networks. *iScience.* 2024;27(11):111068
7. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.* 2019;47(W1):W556-W560