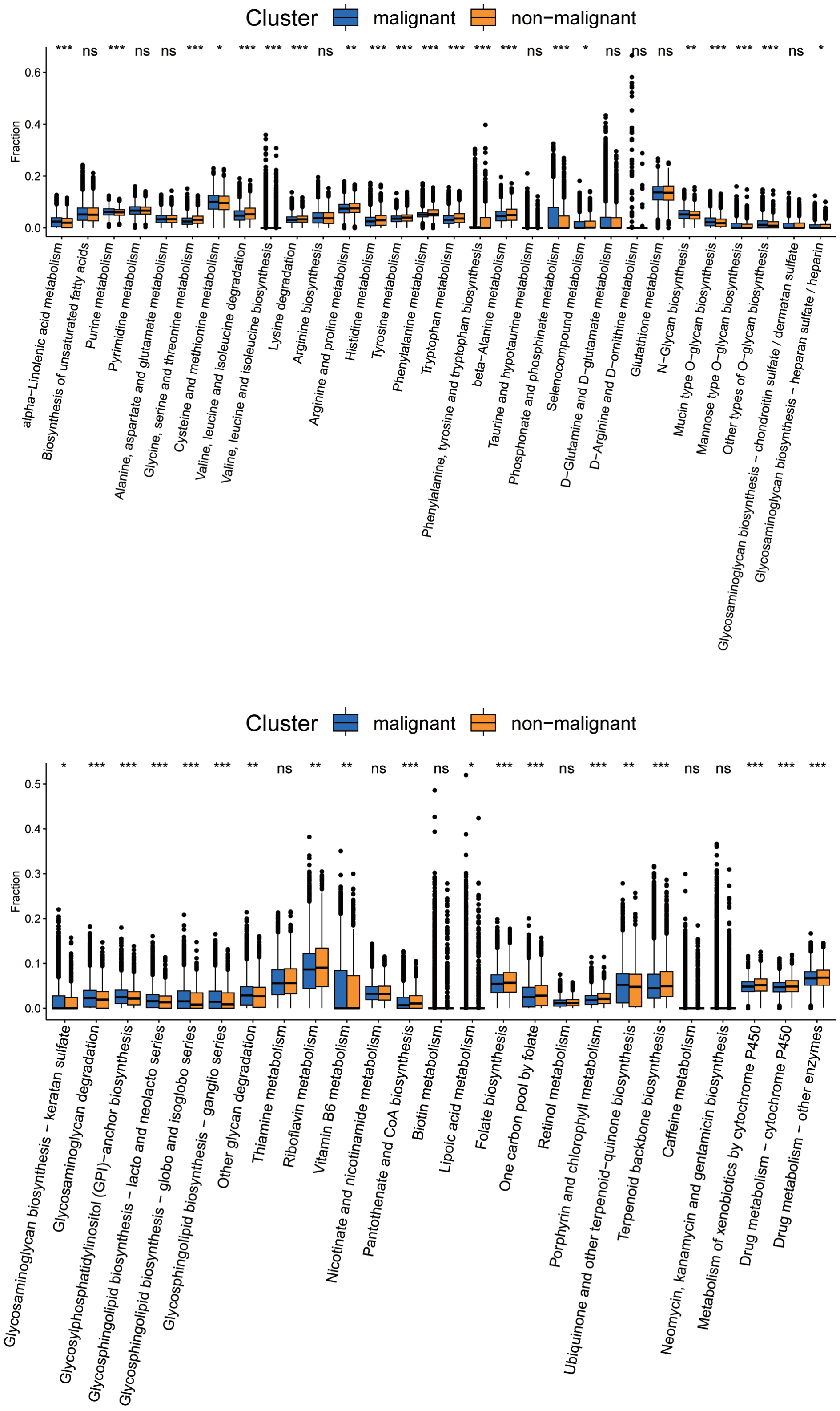

Figure S1 Analysis of metabolism between malignant and non-malignant epithelial cells. ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

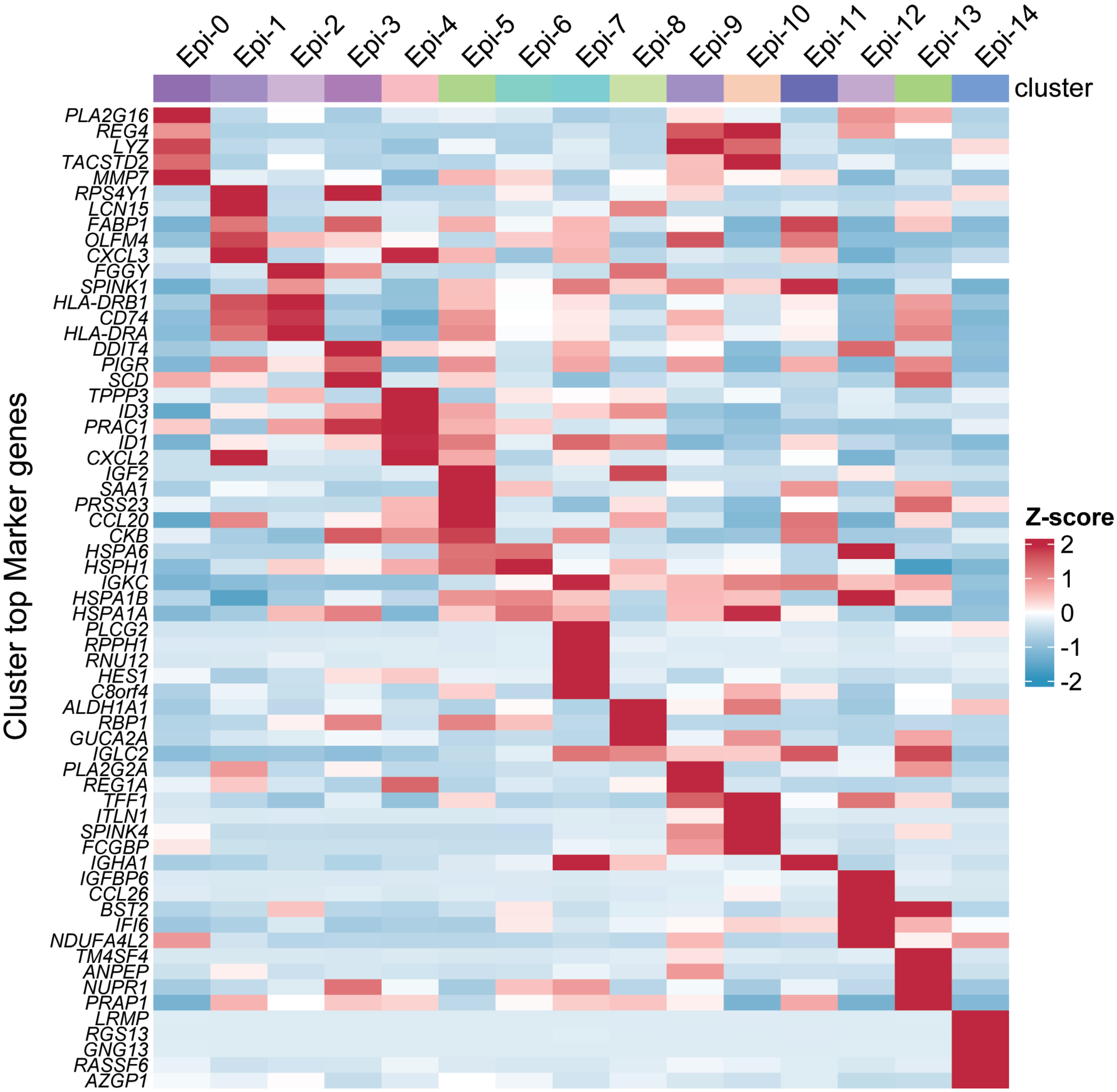

Figure S2 Heatmap of expression levels of the top 5 differentially expressed genes in 15 clusters in malignant epithelial cells.

https://dx.doi.org/10.21037/tcr-2025-586

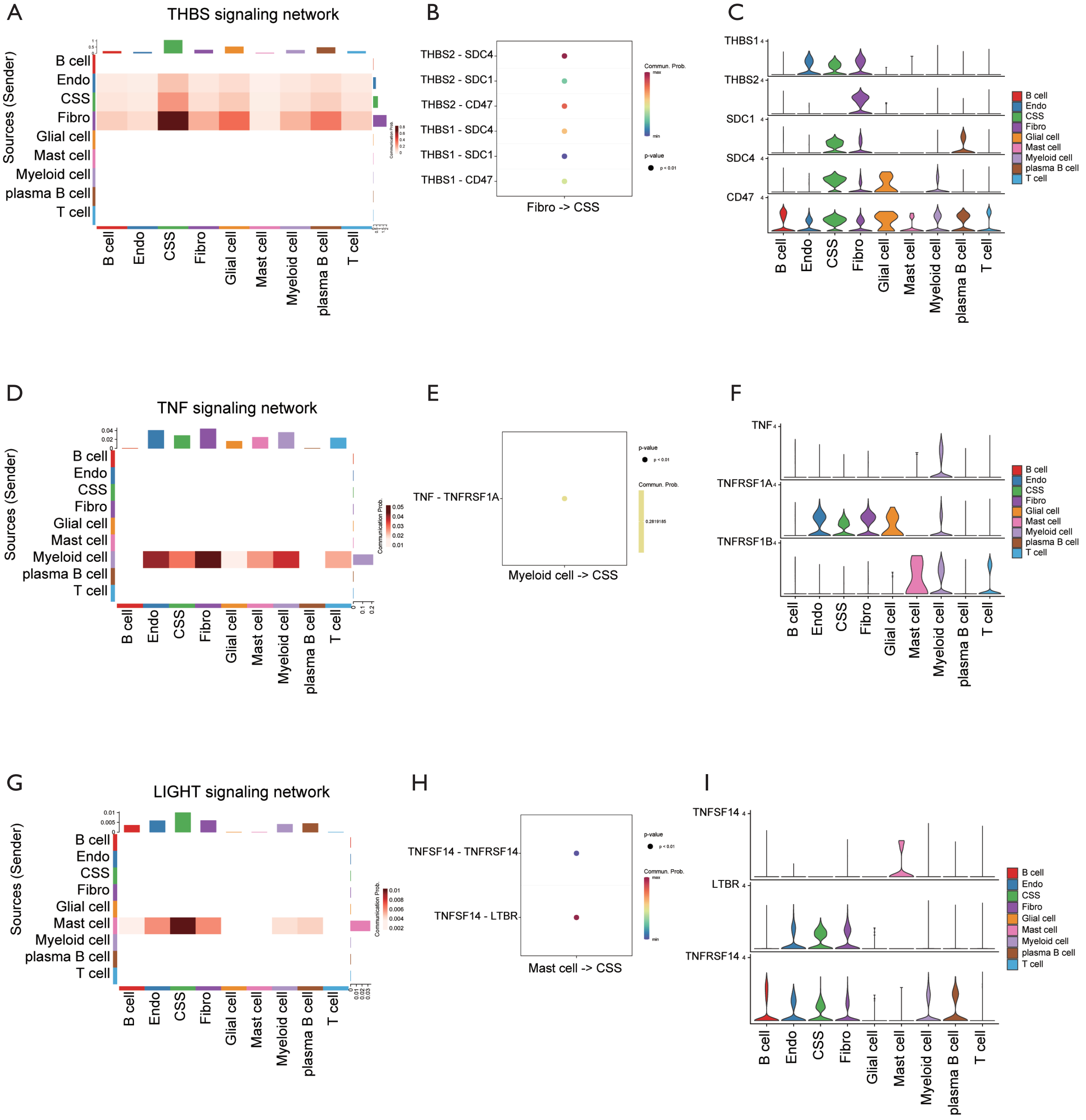

Figure S3 Cell-cell communication analysis of signaling pathways. (A) Contribution heatmap of cell communication in THBS signaling pathways. (B) Dot plots of the significant differentially expressed ligand-receptor pairs in THBS signaling pathways. (C) Violin plot visualizing ligand-receptor pairs THBS signaling pathways in all cell clusters. (D) Contribution heatmap of cell communication in TNF signaling pathways. (E) Dot plots of the significant differentially expressed ligand-receptor pairs in TNF signaling pathways. (F) Violin plot visualizing ligand-receptor pairs of TNF signaling pathways in all cell clusters. (G) Contribution heatmap of cell communication in LIGHT signaling pathways. (H) Dot plots of the significant differentially expressed ligand-receptor pairs in LIGHT signaling pathways. (I) Violin plot visualizing ligand-receptor pairs of LIGHT signaling pathways in all cell clusters.

https://dx.doi.org/10.21037/tcr-2025-586

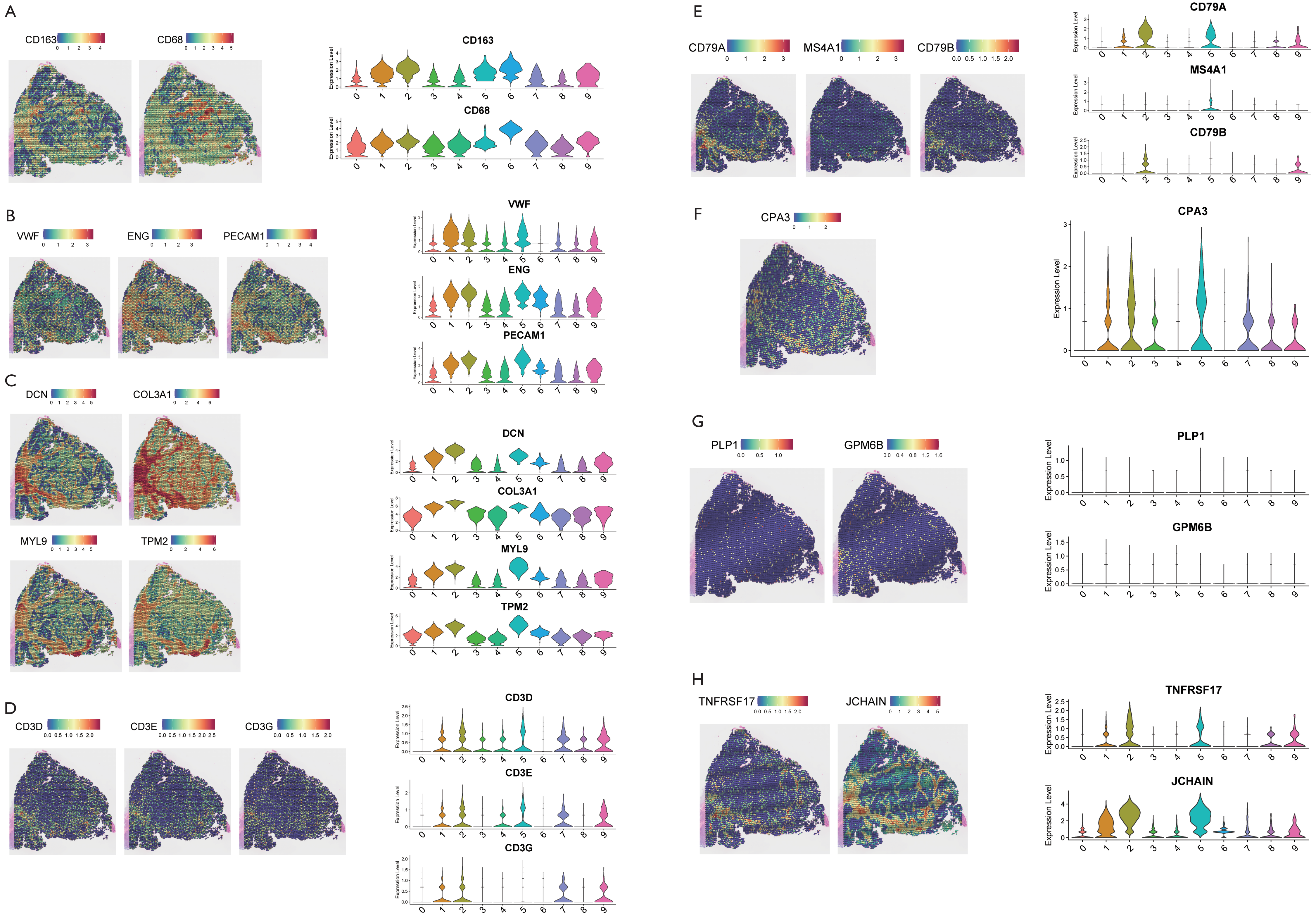

Figure S4 Cell type-specific marker validation. (A) Expression of markers for macrophages identified in slide 1. (B) Expression of markers for endothelial cells identified in slide 1. (C) Expression of markers for fibroblasts identified in slide 1. (D) Expression of markers for T cells identified in slide 1. (E) Expression of markers for B cells identified in slide 1. (F) Expression of markers for mast cells identified in slide 1. (G) Expression of markers for glial cells identified in slide 1. (H) Expression of markers for plasma cells identified in slide 1.

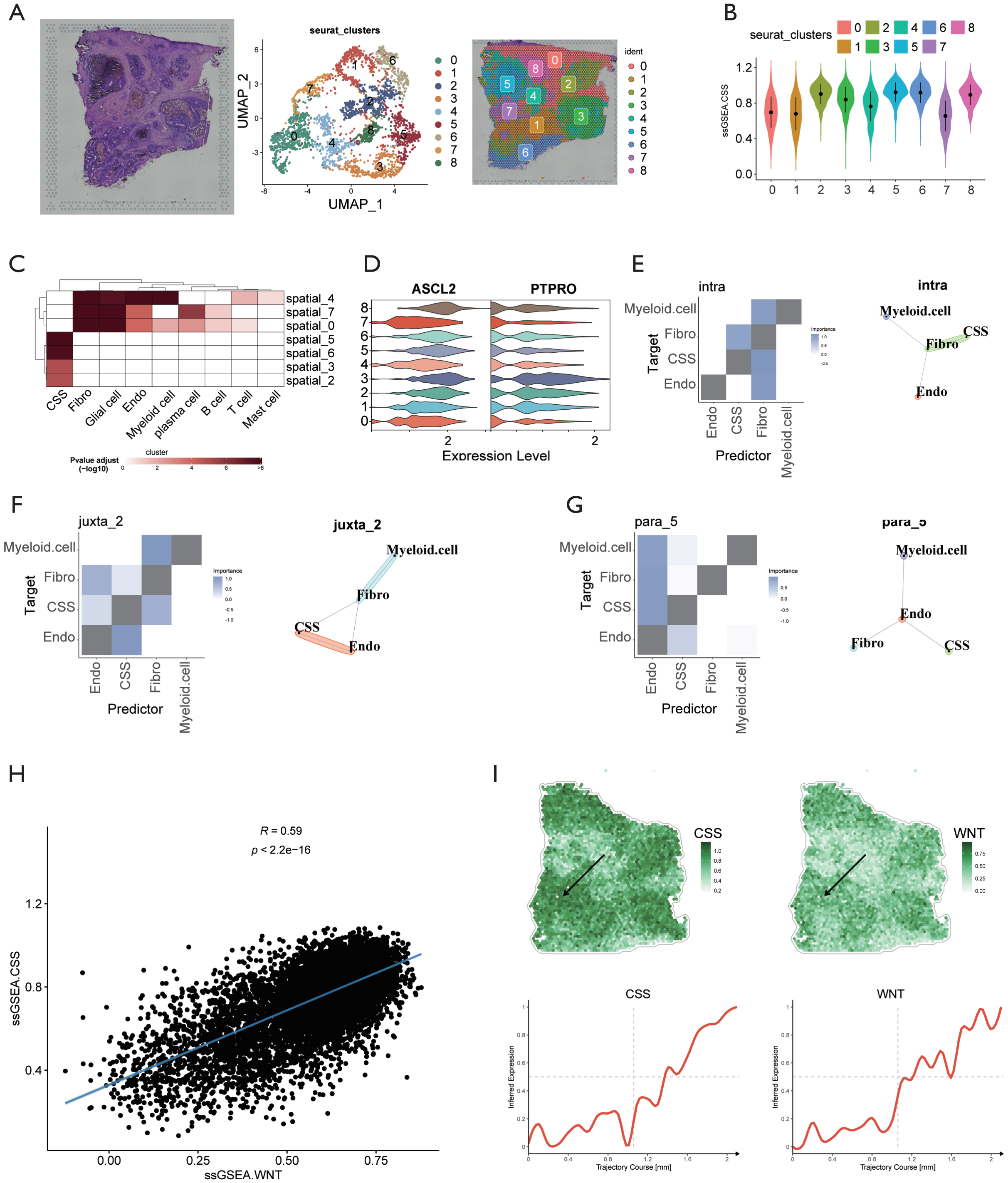

Figure S5 Spatial co-localization of fibroblasts and their regulatory impact on Co-CSS was verified in slide 2. (A) Hematoxylin and eosin staining of tissue sections and UMAP plot showing the spot clusters identified using unbiased clustering of the ST spots. (B) Violin plots and bubble plots of CSS scores for each cluster. (C) The MIA map of scRNA-seq-identified cell types (Co-CSS and other cell types) and ST-defined regions in slide 2. (D) Violin plot visualizing the marker genes of Co-CSS. (E) Mean importance of the abundance of major cell types within a spot. (F) Mean importance of the abundance of major cell types within a 3-spot radius. (G) Mean importance of the abundance of major cell types within a 5-spot radius. (H) Correlation between CSS score and WNT signaling pathway score. (I) Spatial feature plots display the ssGSEA scores for CSS and WNT signaling pathway in slide and corresponding line graphs show the ssGSEA scores along the trajectory, providing a detailed view of CSS and WNT signaling pathway activity changes. The spatial transcriptomics data were obtained from 10x Genomics public repository (https://www.10xgenomics.com/datasets/human-colorectal-cancer-11-mm-capture-area-ffpe-2-standard and https://www.10xgenomics.com/datasets/human-colorectal-cancer-whole-transcriptome-analysis-1-standard-1-2-0) and processed following their standard FFPE protocol (CG000518, CG000520, CG000495).

https://dx.doi.org/10.21037/tcr-2025-586

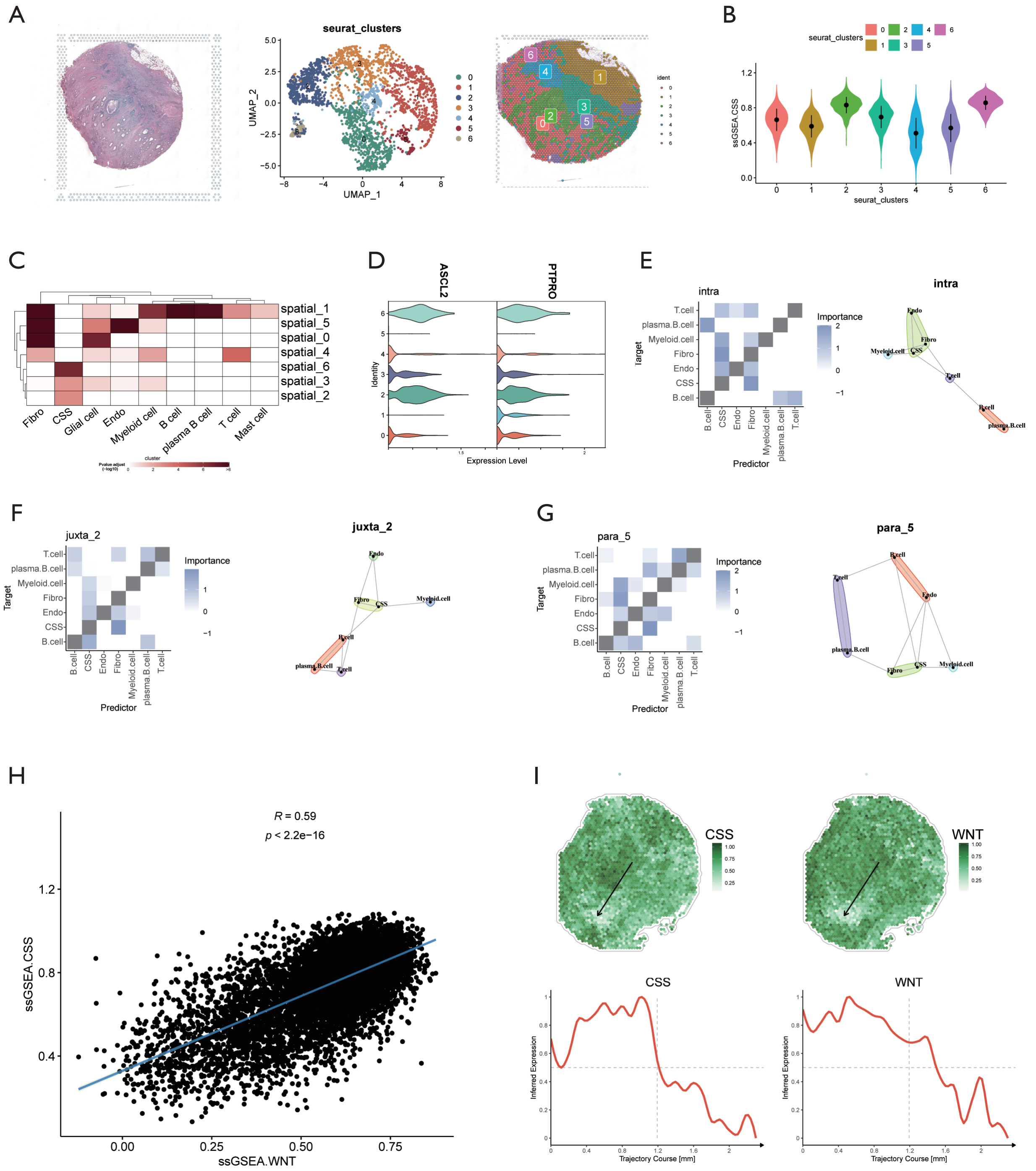

Figure S6 Spatial co-localization of fibroblasts and their regulatory impact on Co-CSS was verified in slide 3. (A) Hematoxylin and eosin staining of tissue sections and UMAP plot showing the spot clusters identified using unbiased clustering of the ST spots. (B) Violin plots and bubble plots of CSS scores for each cluster. (C) The MIA map of scRNA-seq-identified cell types (Co-CSS and other cell types) and ST-defined regions in slide 3. (D) Violin plot visualizing the marker genes of Co-CSS. (E) Mean importance of the abundance of major cell types within a spot. (F) Mean importance of the abundance of major cell types within a 3-spot radius. (G) Mean importance of the abundance of major cell types within a 5-spot radius. (H) Correlation between CSS score and WNT pathway score. (I) Spatial feature plots display the ssGSEA scores for CSS and WNT pathway in slide and corresponding line graphs show the ssGSEA scores along the trajectory, providing a detailed view of CSS and WNT pathway activity changes. The spatial transcriptomics data were obtained from 10x Genomics public repository (https://www.10xgenomics.com/datasets/human-colorectal-cancer-11-mm-capture-area-ffpe-2-standard and https://www.10xgenomics.com/datasets/human-colorectal-cancer-whole-transcriptome-analysis-1-standard-1-2-0) and processed following their standard FFPE protocol (CG000518, CG000520, CG000495).

https://dx.doi.org/10.21037/tcr-2025-586