

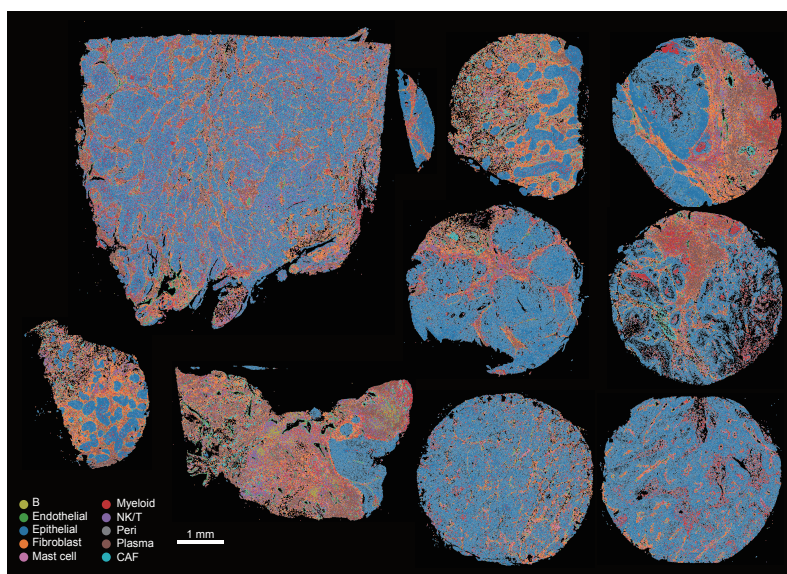
Single-Cell and Spatial Transcriptomic Profiling Reveals Distinct Tumor Microenvironment Dynamics in Cervical Adenocarcinoma and Squamous Cell Carcinoma

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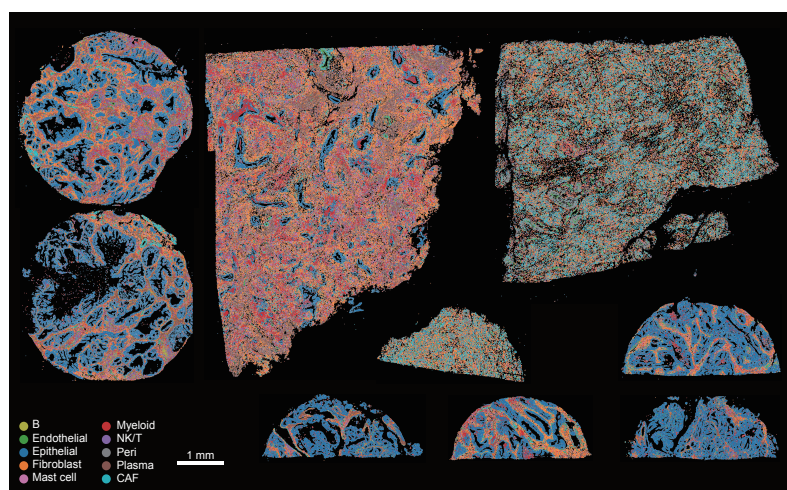
Supplementary information

Supplementary Figures 1-7 (available in this file)

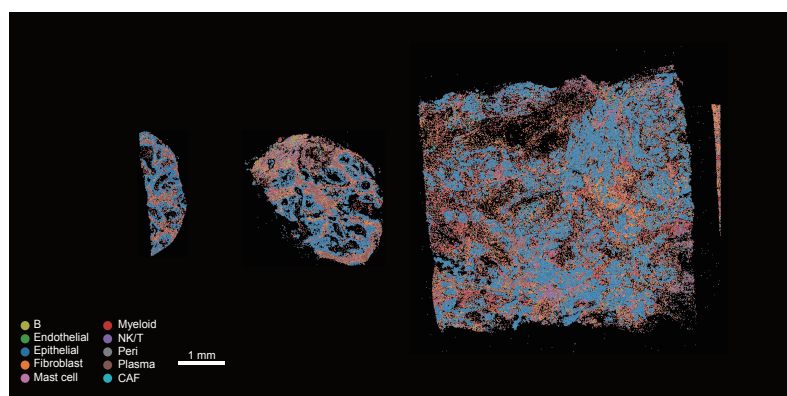
SCC



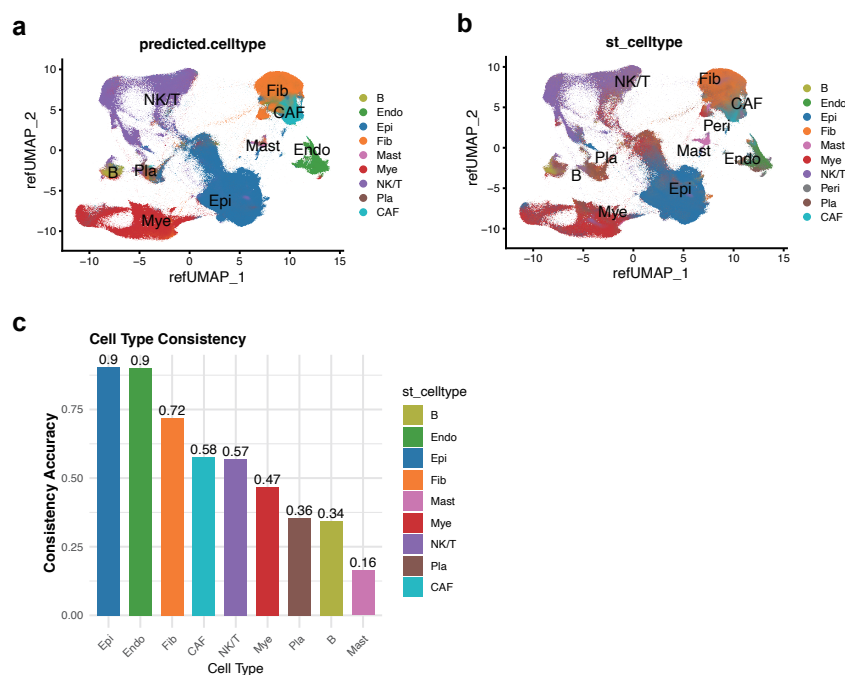
AC



ASC



Supplementary Fig. 1 Spatial distributions of different major cell types on different cervical cancer samples categorized at different histological subtypes. Dots with different colors indicate different cell types, scale bar is 1 mm.

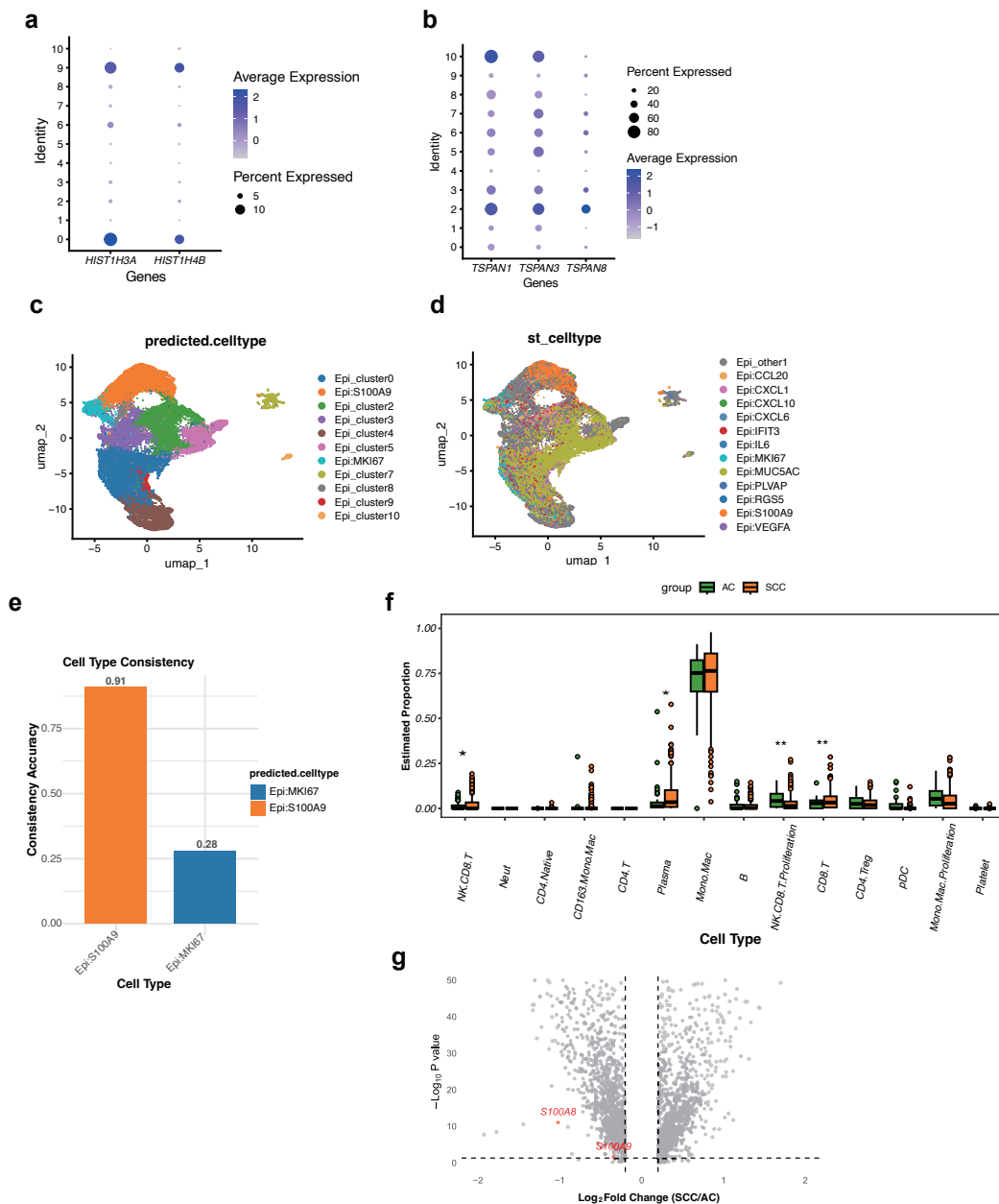


Supplementary Fig. 2 Integration of the scRNA-seq data and Xenium spatial transcriptomic data.

a Projection of the Xenium single cells into the scRNA-seq data UMAP, and labeling single cells with the predicted cell identities from the scRNA-seq data annotations.

b Projection of the Xenium single cells into the scRNA-seq data UMAP, and labeling single cells with the original ST annotations.

c The histogram showing consistency of the major cell type annotations by comparing the predicted annotations and the original ST annotations, the y-axis represents the percentage of consistently annotated cells in each major cell type in Xenium data.



Supplementary Fig. 3 Subclustering the epithelial/tumor cells using scRNA-seq data and integration with Xenium data.

a The dotplot showing expression profiles of two marker genes found in cluster 0, *HIST1H3A* and *HIST1H4B*, in different subclusters of all epithelial cells in the scRNA-seq data.

b The dotplot showing expression profiles of three marker genes found in cluster 2, *TSPAN1/3/8*, in different subclusters of all epithelial cells in the scRNA-seq data.

c Projection of the Xenium single cells into the corresponding scRNA-seq data UMAP for the epithelial/tumor cells, and labeling single cells with the predicted cell identities from the scRNA-seq data subclustering and annotations.

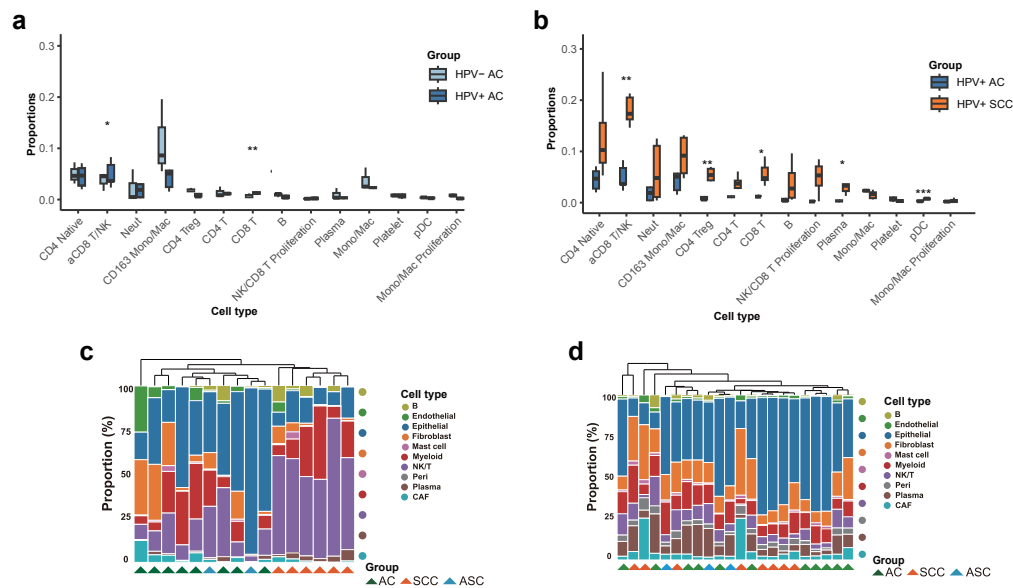
d Projection of the Xenium single cells into the scRNA-seq data UMAP for the

epithelial/tumor cells, and labeling single cells with the original ST annotations.

e The histogram showing two subclusters in epithelial cells with consistency accuracy more than 0.25 by comparing the predicted annotations and the original ST annotations, the y-axis represents the percentage of consistently annotated cells in each subcluster in Xenium data.

f Boxplot indicating comparison of different immune cell types between AC and SCC cervical cancer with deconvolution analysis with the TCGA dataset; Student's t-test was used to perform statistical analysis, and “*” means statistical p value < 0.05, “***” means statistical p value < 0.01.

g The volcano plot showing differentially expressed genes within all myeloid cells in AC or SCC samples based on the scRNA-seq data. X axis indicates Log₂ Fold change (SCC/AC), y axis indicates the -Log₁₀ (p values); two MDSC marker genes *S100A8*/*S100A9* were highlighted in red.



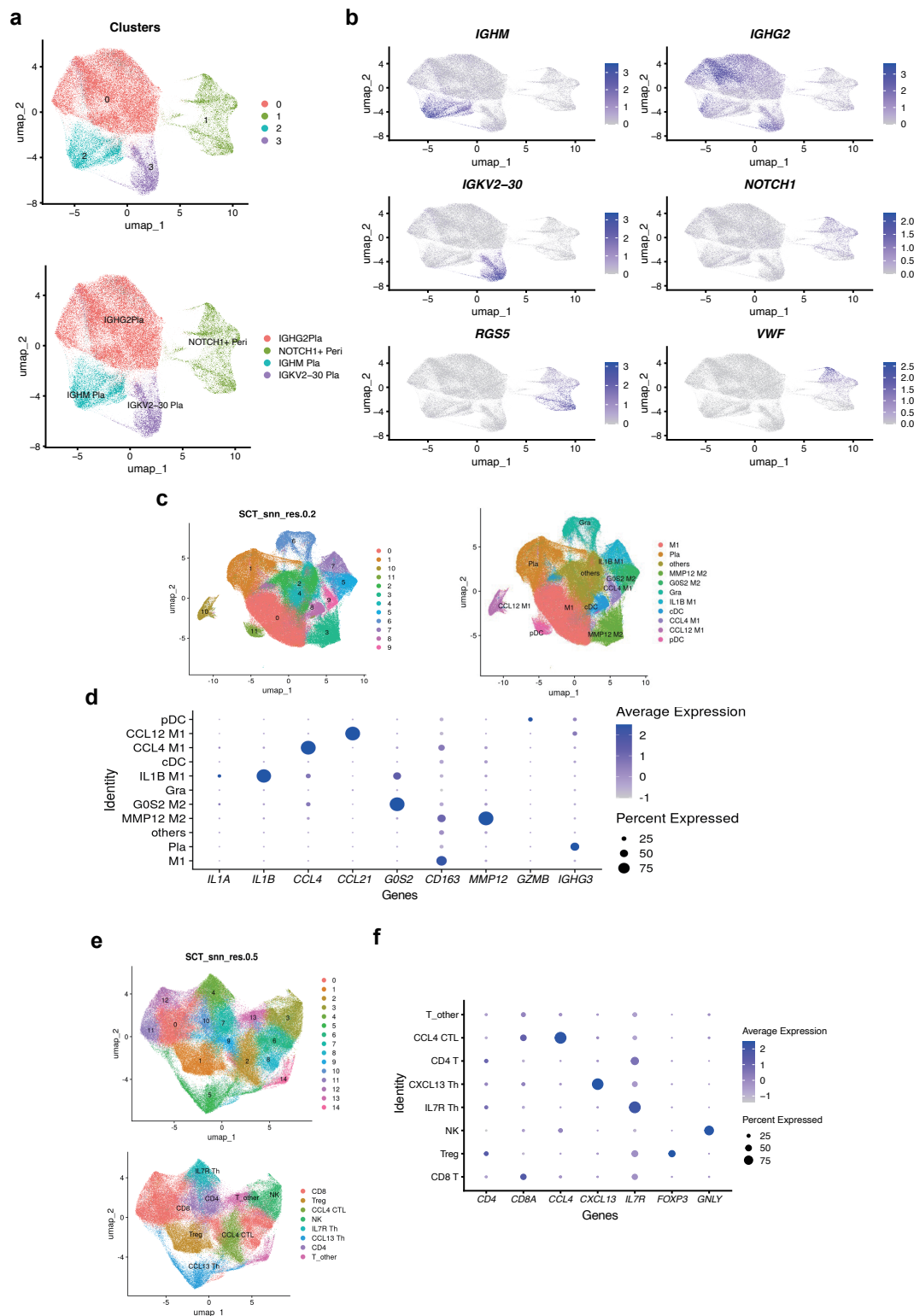
Supplementary Fig. 4 Sub-analyses stratifying HPV status or combining all three cancer subtypes together.

a Comparative distribution of immune cells between HPV+ AC and HPV- AC in scRNA-seq datasets; Student's t-test was used to perform statistical analysis, and “*” means statistical p value < 0.05, “**” means statistical p value < 0.01.

b Comparative distribution of immune cells between HPV+ AC and HPV+ SCC in scRNA-seq datasets; Student's t-test was used to perform statistical analysis, and “*” means statistical p value < 0.05, “**” means statistical p value < 0.01, “***” means statistical p value < 0.001.

c Sample-specific distribution patterns of cellular subpopulations identified through scRNA-seq data. Colors in the histograms indicate different major cell types; triangles with different colors at the bottom indicate AC, ASC or SCC cancer subtypes.

d Sample-specific distribution patterns of cellular subpopulations identified through Xenium ST data. Colors in the histograms indicate different major cell types; triangles with different colors at the bottom indicate AC, ASC or SCC cancer subtypes.

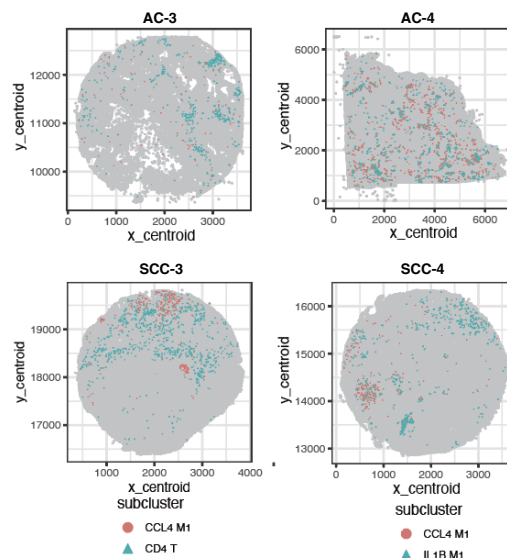


Supplementary Fig. 5 Subclustering and annotating subtypes of plasma cells, myeloid cells and lymphocytes using Xenium data.

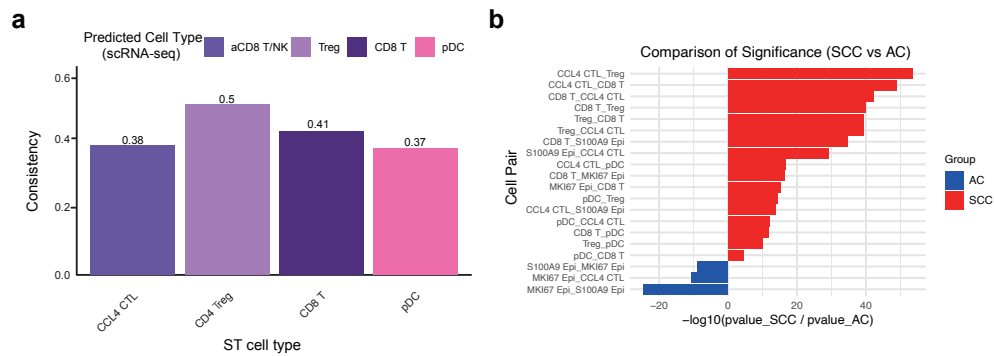
a Two-dimensional UMAP showing subclusters (upper) and annotations (lower) of plasma cells in the Xenium data based on the relative marker genes expression.

b The featureplot showing expression profiles of specific marker genes for different subtypes; expression of *NOTCH1*, *RGS5* and *VWF* is shown.

- c** Two-dimensional UMAP showing subclusters (left) and annotations (right) of myeloid cells in the Xenium data based on the relative marker genes expression.
- d** The dotplot showing expression profiles of specific marker genes for different subtypes; “Pla” indicates plasma cells due to the high expression of *IGHG3* in that cluster.
- e** Two-dimensional UMAP showing subclusters (upper) and annotations (lower) of lymphocytes in the Xenium data based on the relative marker genes expression; subclusters without any markers passing the threshold are combined together and annotated accordingly.
- f** The dotplot showing expression profiles of specific marker genes for different subtypes; expression of *CD4*, *CD8A*, *CCL4*, *CXCL13*, *IL7R*, *FOXP3* and *GNLY* is shown.



Supplementary Fig. 6 Representative images illustrating the spatial distributions of two cell-type pairs (CCL4+ M1 & CD4+ T cells, CCL4+ M1 & IL1B+ M1) in AC and SCC samples.



Supplementary Fig. 7 Validation of cell-cell interactions among six important cell subtypes.

a The histogram showing consistency of the four specific immune cell type annotations by comparing the predicted annotations and the original ST annotations, the y-axis indicates the percentage of consistently annotated cells per cell type in Xenium data.

b The histogram revealing stronger interactions among 6 specific cell types in SCC samples, consistent with the Cellchat results based on scRNA-seq data; X-axis means $-\log_{10}(p_{\text{SCC}} / p_{\text{AC}})$, where p_{SCC} and p_{AC} are permutation test *p*-values for specific cell pairs in SCC and AC groups separately.