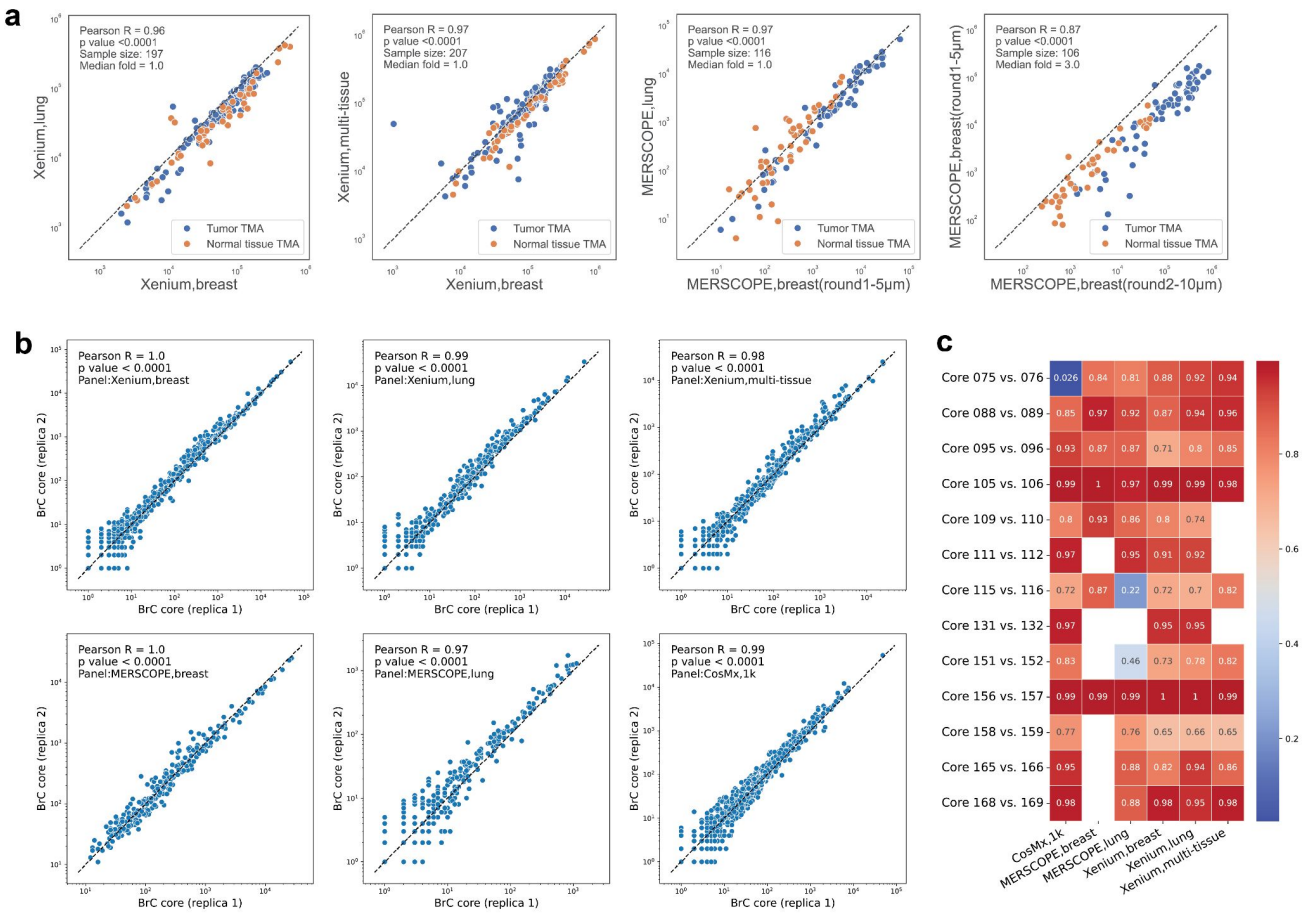
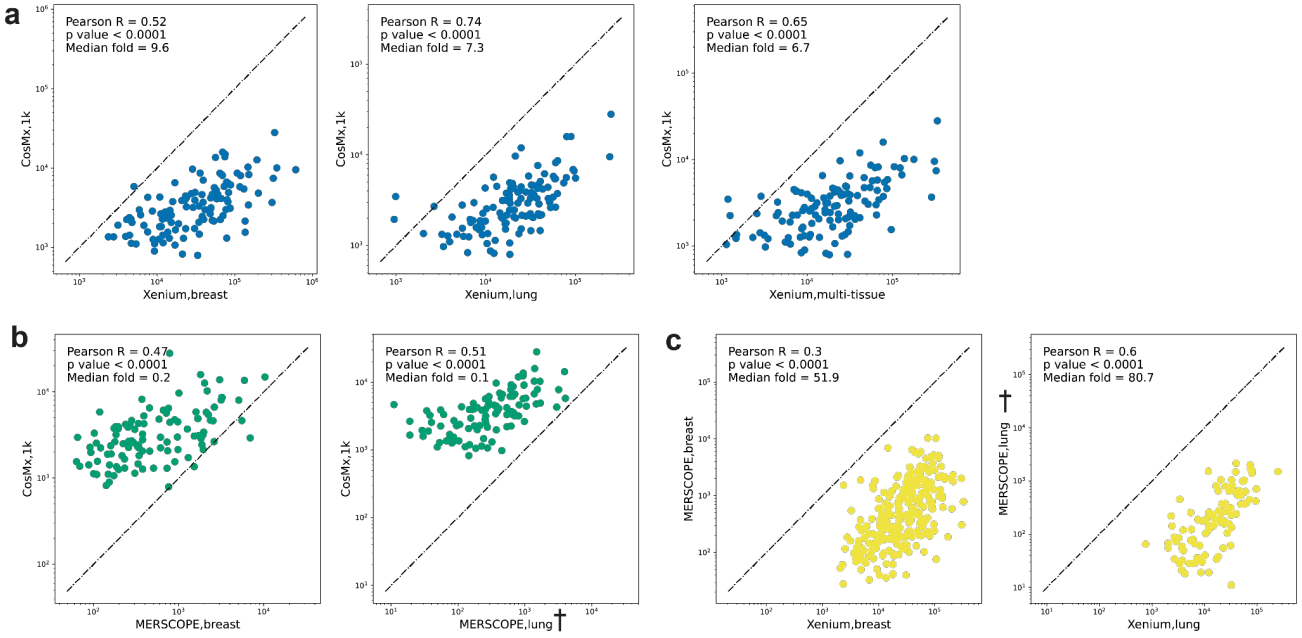
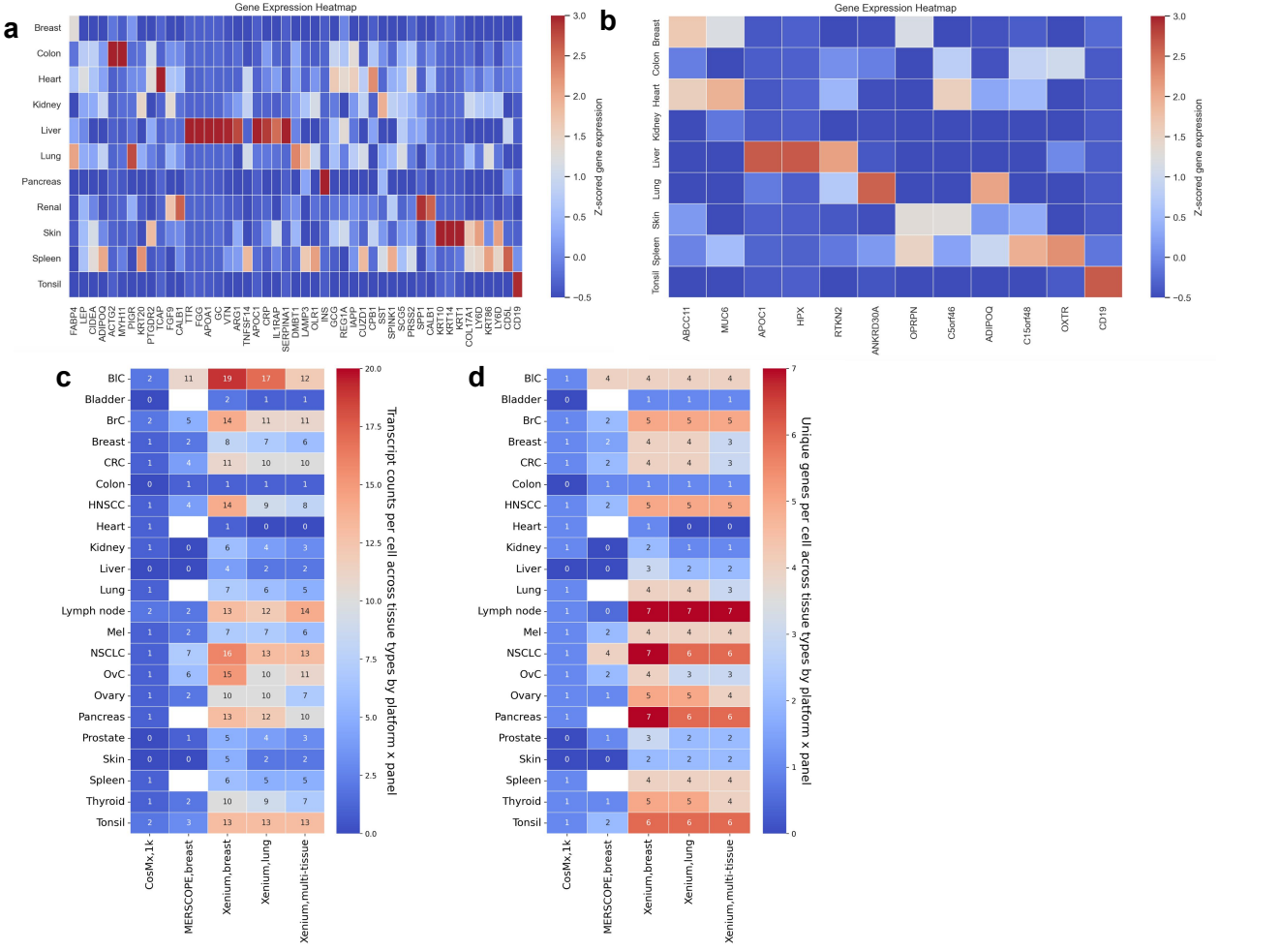
Supplementary Figures



Supplementary Figure 1: Reproducibility across different panels and cores from same patient.
(a) Scatter plots of cumulative gene expression levels (on a logarithmic scale) of shared genes between two panels within each platforms, captured from matched tissue cores. Column 1: Xenium breast vs. Xenium lung; Column 2: Xenium breast vs. Xenium multi-tissue; Column 3: MERSCOPE breast round 1(5 μm) vs. MERSCOPE breast round 2(10 μm). Each data point corresponds to a TMA core. **(b)** Scatter plots of gene expression levels (on a logarithmic scale) of every shared gene between two cores of the same tissue type from the same patient. In this example, cores are from breast cancer tissue. Each data point corresponds to a gene. **(c)** Heatmap of correlation coefficient expressed as Pearson's r values, indicating good core-to-core or sample-to-sample reproducibility. Core pairs are selected from same tissue/tumor type from the same patients.

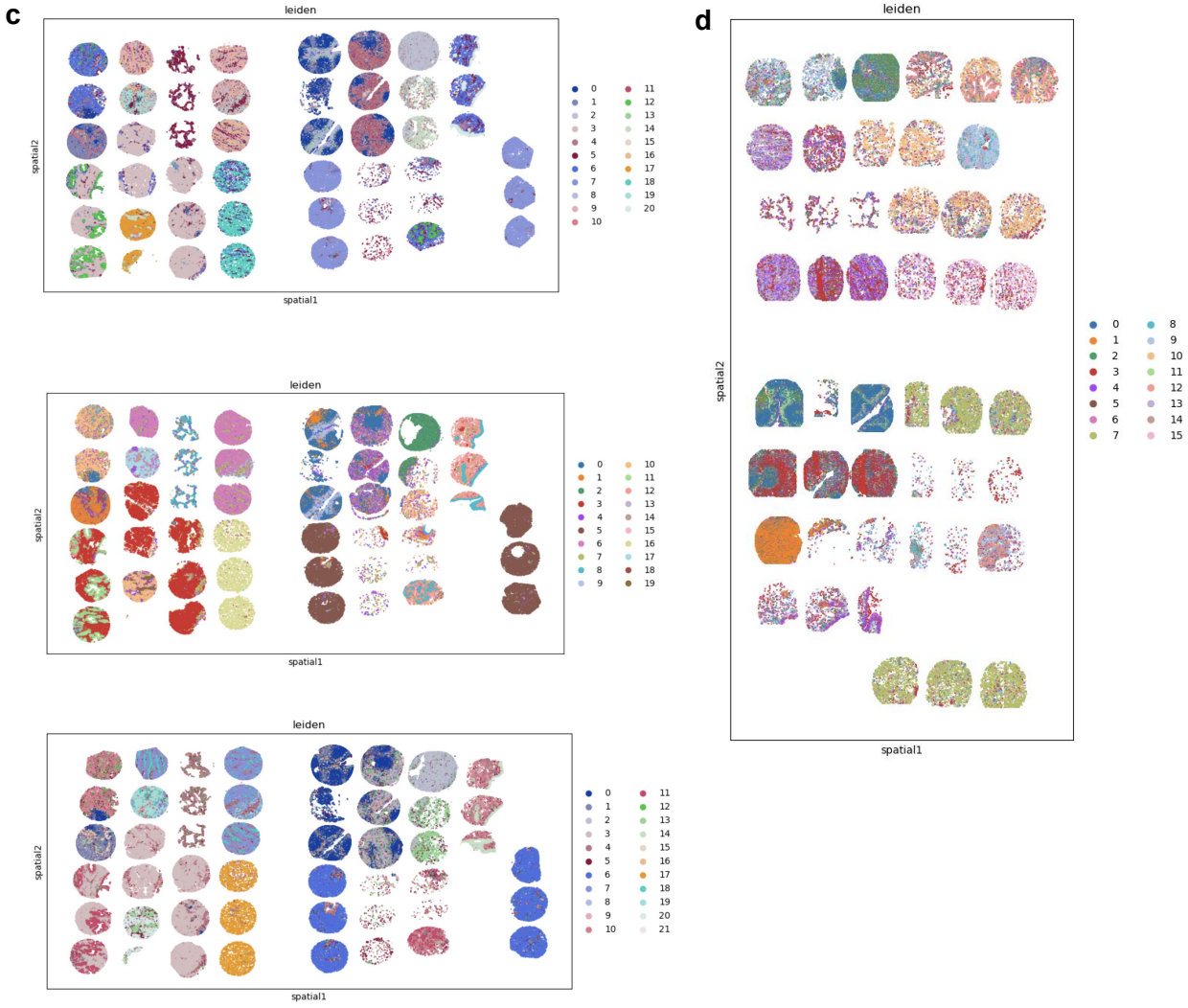


Supplementary Figure 2: Gene by gene plots of iST results by panel and by tissue microarray.
(a) Scatter plots of summed gene expression levels (on a logarithmic scale) of every shared gene between Xenium (breast/lung) and CosMx (1k) data, captured from matched normal tissue TMA cores. Each data point corresponds to a gene. **(b)** Same as (a) but between MERSCOPE (breast/lung) and CosMx(1k). **(c)** Same as (a) but between Xenium(breast/lung) and MERSCOPE(breast/lung). **(d)** Same as (a) but between Xenium(multi-tissue) and CosMx(1k). † denotes the MERSCOPE lung panel acquired with a 5 µm imaging thickness.



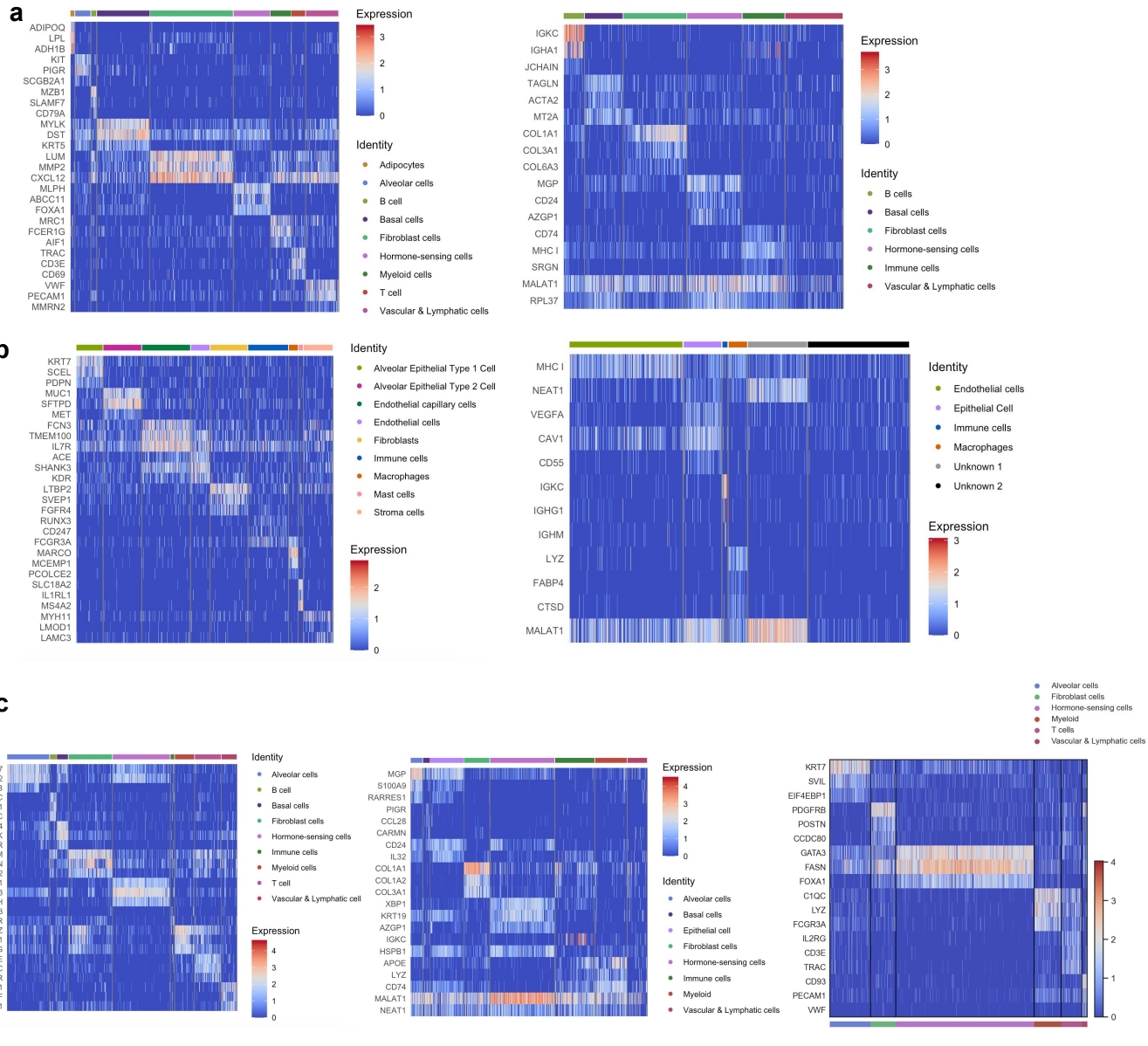
Supplementary Figure 3: Tissue marker analyses and cell level measurements.
(a) Heatmap of Z-scored gene expression showing CosMx's ability to specifically identify known lineage markers. We focused on the normal tissue TMA profiled with multi-tissue panel and selected genes with canonical expression patterns for this analysis. **(b)** Same as (a) but for MERSCOPE (breast panel). **(c)** Heatmap of transcripts per cell after filtration. Only shared genes (40) are considered here for each panel. **(d)** Same as (c) but showing unique transcripts from the same gene set.

Supplementary Fig: 4 Global clustering analyses
(a) Global Clustering results of tumor TMA from Xenium breast panel (top), Xenium lung panel (middle), and Xenium panhuman panel (bottom). **(b)** Global Clustering results of tumor TMA from MERFISH breast panel (top), MERSCOPE lung panel (middle), and CosMx multitissue panel (bottom).



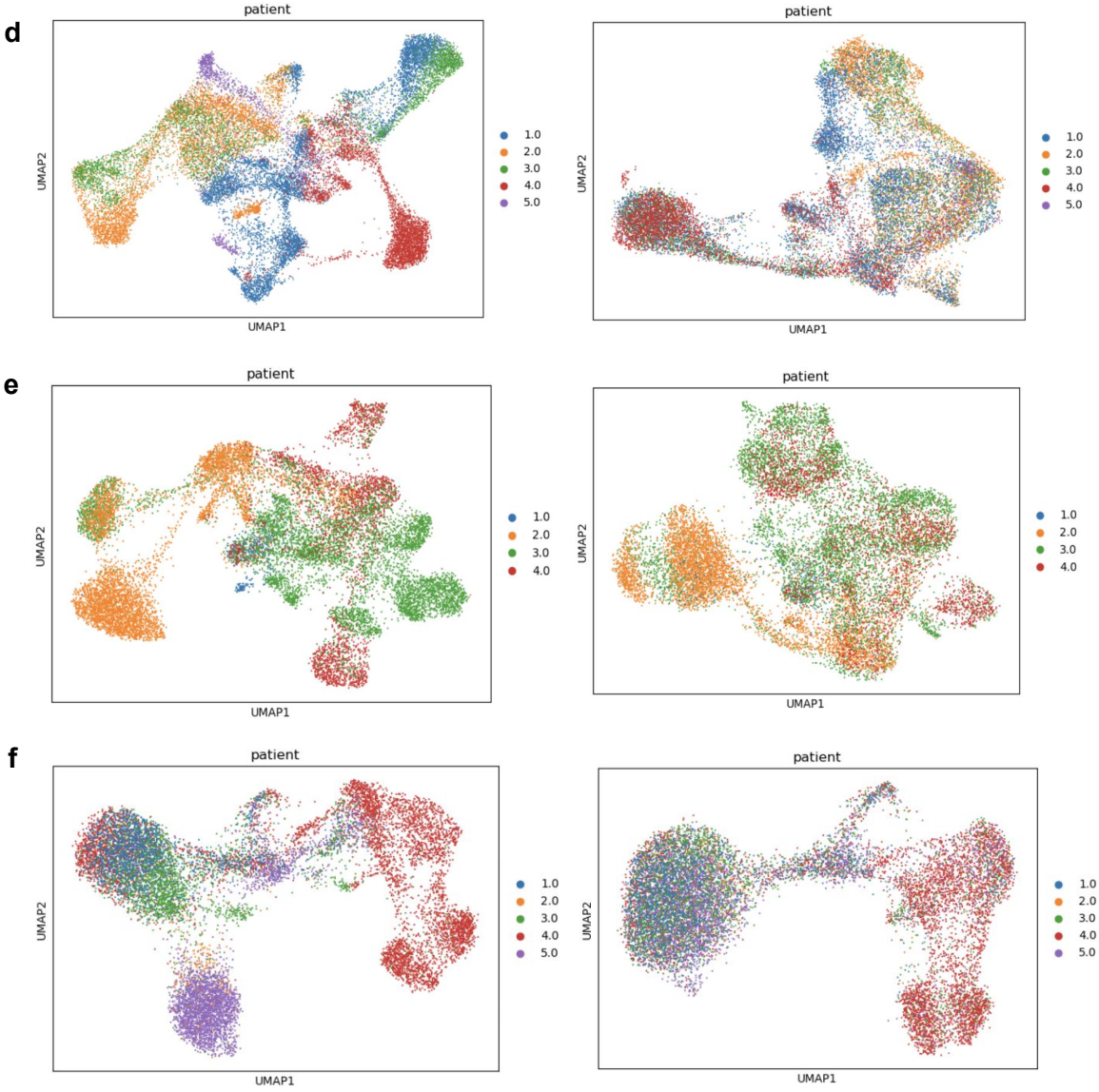
Supplementary Fig: 4 Global clustering analyses

(c) Global Clustering results of normal TMA from Xenium breast panel (top), Xenium lung panel (middle), and Xenium panhuman panel (bottom). **(d)** Global Clustering results of normal TMA from CosMx multitissue panel (bottom).



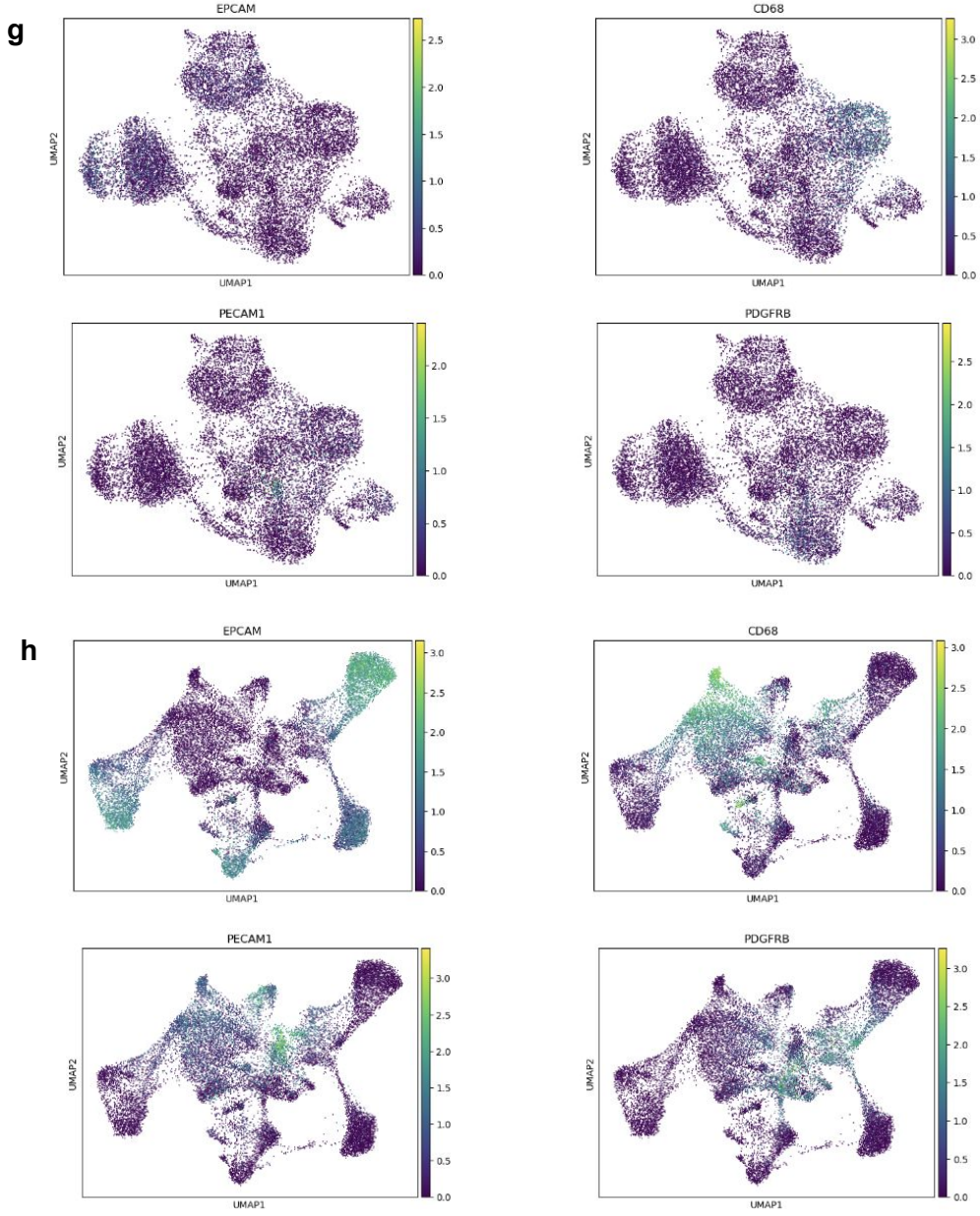
Supplementary Fig: 5. Cell type recovery and UMAPs

(a) Heatmap showing the top gene markers for cell types annotated in breast samples of normal TMA from Xenium breast (left) and Cosmx multitissue (right). **(b)** Heatmap showing the top gene markers for cell types annotated in lung samples of normal TMA from Xenium lung (left) and Cosmx multitissue (right). **(c)** Heatmap showing the top gene markers for cell types annotated in breast cancer samples of tumor TMA from Xenium breast (left), Cosmx multitissue (middle), NERSCOPE breast (right).

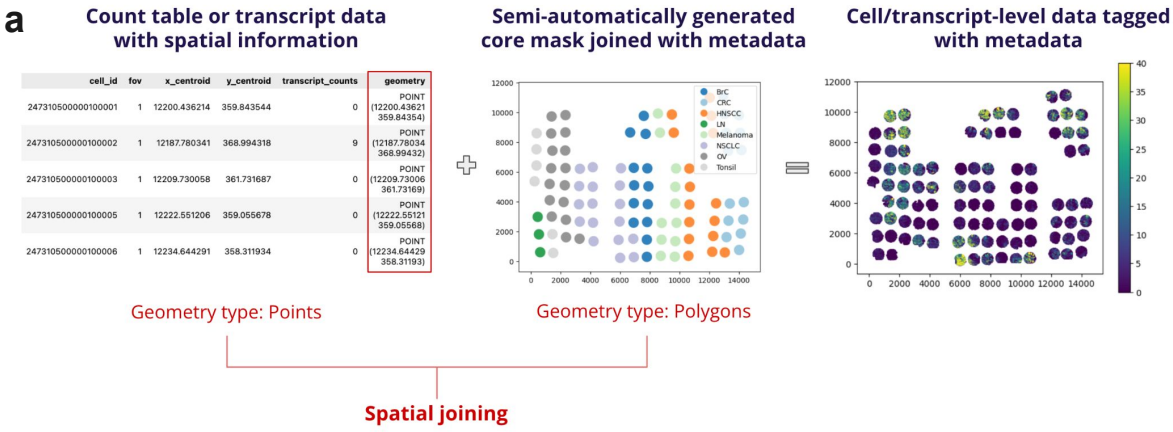


Supplementary Fig: 5. Cell type recovery and UMAPs

(d) UMAP of breast cancer samples of tumor TMA from Xenium breast panel pre (left) and post (right) batch effect removal. **(e)** UMAP of breast cancer samples of tumor TMA from Cosmx multitissue panel pre (left) and post (right) batch effect removal. **(f)** UMAP of breast cancer samples of tumor TMA from MERFISH breast panel pre (left) and post (right) batch effect removal



Supplementary Fig: 5. Cell type recovery and UMAPs
(g) UMAP plot of well-known gene markers for BrC, in breast cancer samples of tumor TMA from CosMx multitissue panel. **(h)** UMAP plot of well-known gene markers for BrC, in breast cancer samples of tumor TMA from Xenium multitissue panel.



Supplementary Figure 6: Workflow for tagging imaging spatial transcriptomics data

(a) To facilitate standardized data formatting and subsequent analytical processes, we built this data ingestion pipeline with the following objectives: 1) to grab cell-level and transcript-level data from diverse platforms and normalize the data structure; 2) to tag each cell and transcript with essential metadata including tissue type, tumor status, PD-L1 status, among others; and 3) to transform the data into various formats tailored to the requirements of particularized analyses. Specifically, to tag the data, core centers in the TMA were pinpointed using DAPI images (Xenium) or cell metadata that contains global coordinates (MERSCOPE and CosMx) using QGIS(version:3.16.10-Hannover). Cells or transcripts within a specified radius were then labeled with core metadata via spatial joining (implemented by GeoPandas, version:0.13.0). In instances where the cores are in close proximity or when a uniform radius cannot be applied effectively, we manually generated the core boundary masks.