

Figure S1

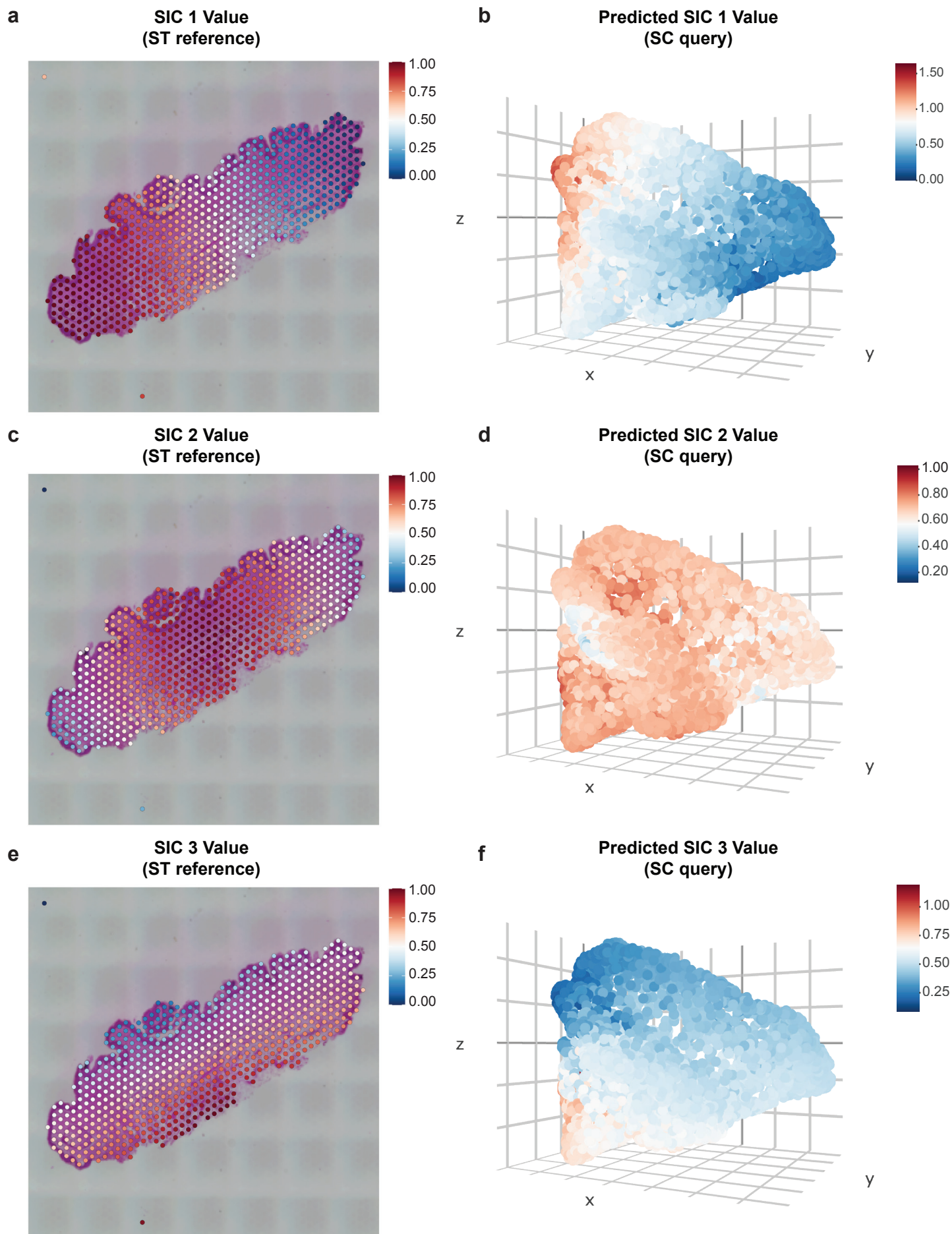


Figure S1. Spatial relevance of SICs. Top three SIC values in human skin ST data on the spatial image (left panels) and predicted top three SIC values in 3D visualization of scHolography reconstruction of human skin SC data (right panels). **a-b**, SIC 1 values in ST and predicted SIC 1 values in SC data. **c-d**, SIC 2 values in ST and predicted SIC 2 values in SC data. **e-f**, SIC 3 values in ST and predicted SIC 3 values in SC data.

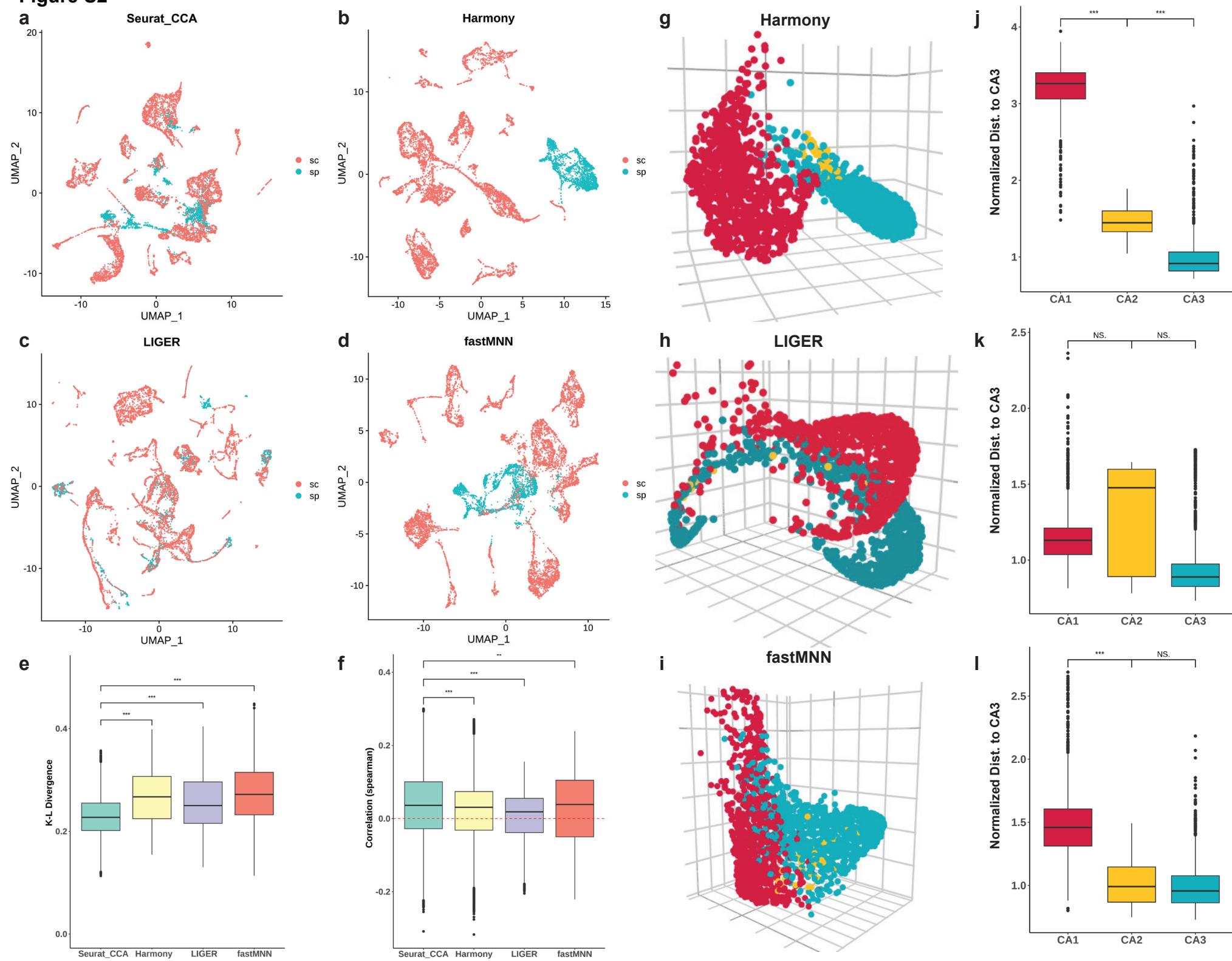
Figure S2

Figure S2. **a**, UMAP plot of Seurat CCA integration of scRNA-seq and ST data. **b**, UMAP plot of Harmony integration of scRNA-seq and ST data. **c**, UMAP plot of LIGER integration of scRNA-seq and ST data. **d**, UMAP plot of fastMNN integration of scRNA-seq and ST data. **e**, KL-divergence of scHolography predictions powered by different integration methods for simulated mouse hippocampus data as ground truth. Two-sided Wilcoxon tests are performed. **f**, Spearman correlation between scHolography cell-cell SMN distance powered by different integration methods and simulated mouse hippocampus cell-cell distance. Two-sided Wilcoxon tests are performed. **g-i**, Visualization of Harmony, LIGER, and fastMNN powered scHolography prediction results. **j-l**, Comparison of Harmony, LIGER, and fastMNN powered scHolography reconstruction results for CA1, CA2, and CA3 cell distance to CA3 cells. Cell distances were normalized for each method by the mean distance between CA3 cells and CA3 cells. Two-sided Wilcoxon tests are performed.

Figure S3

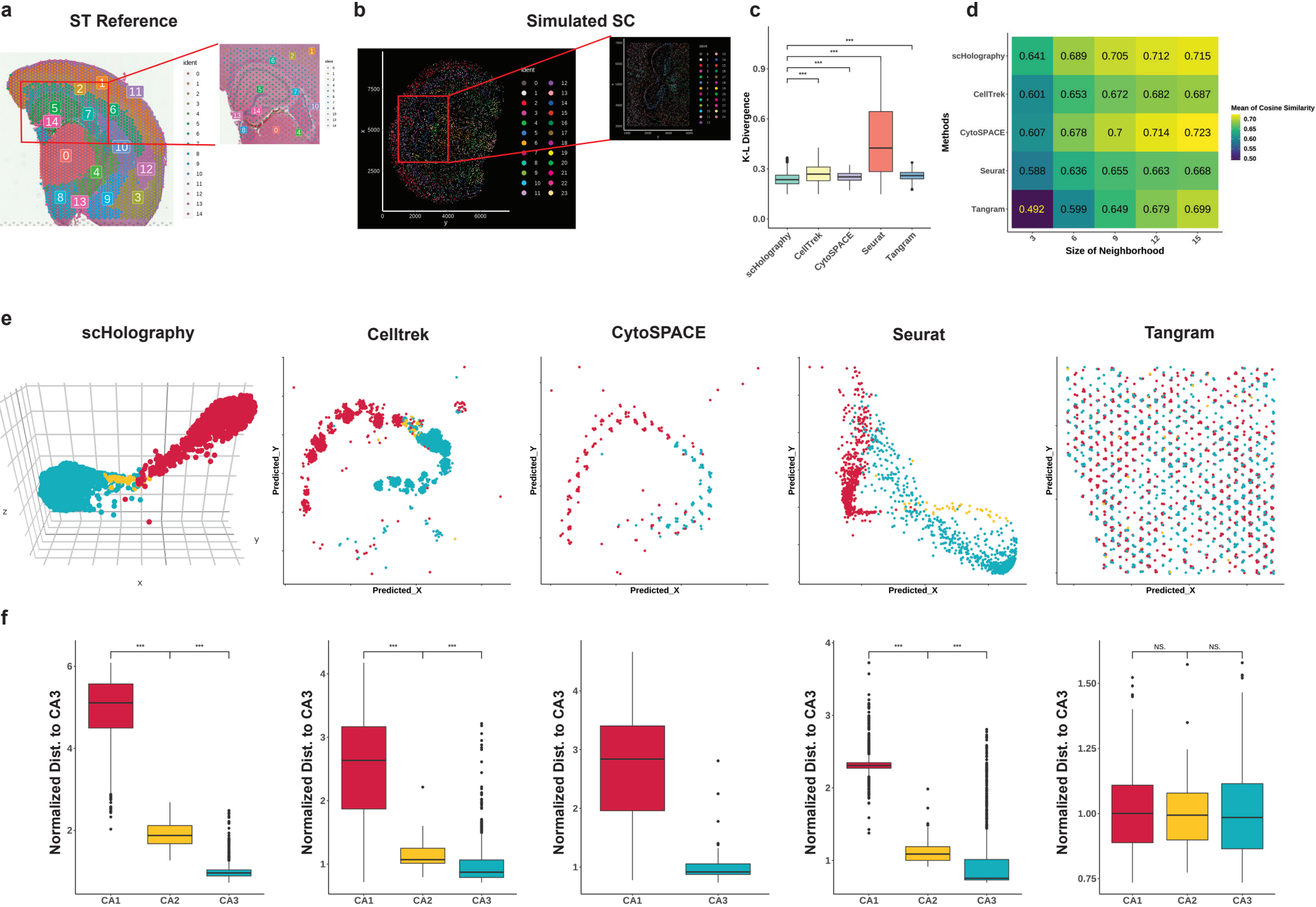


Figure S3. **a**, Illustration of 10X Visium mouse hippocampus ST reference region. **b**, Illustration of mouse hippocampus simulated SC query region. **c**, KL-divergence of spatial cell charting method predictions for simulated mouse hippocampus data as ground truth. Two-sided Wilcoxon tests are performed. **d**, Heatmap for the mean of cosine similarity between method-predicted spatial neighborhood accumulated expression and simulated mouse hippocampus spatial neighborhood accumulated expression. The size of the neighborhood varies from 3 to 15 cells. **e**, Visualization of scHolography, Celltrek, CytoSPACE, Seurat, and Tangram single-cell spatial charting results of a mouse hippocampus data. **f**, Comparison of scHolography, Celltrek, CytoSPACE, Seurat, and Tangram results for predicted CA1, CA2, and CA3 cell distance to CA3 cells. Cell distances were normalized for each method by the mean distance between CA3 cells and CA3 cells. Two-sided Wilcoxon tests are performed.

Figure S4

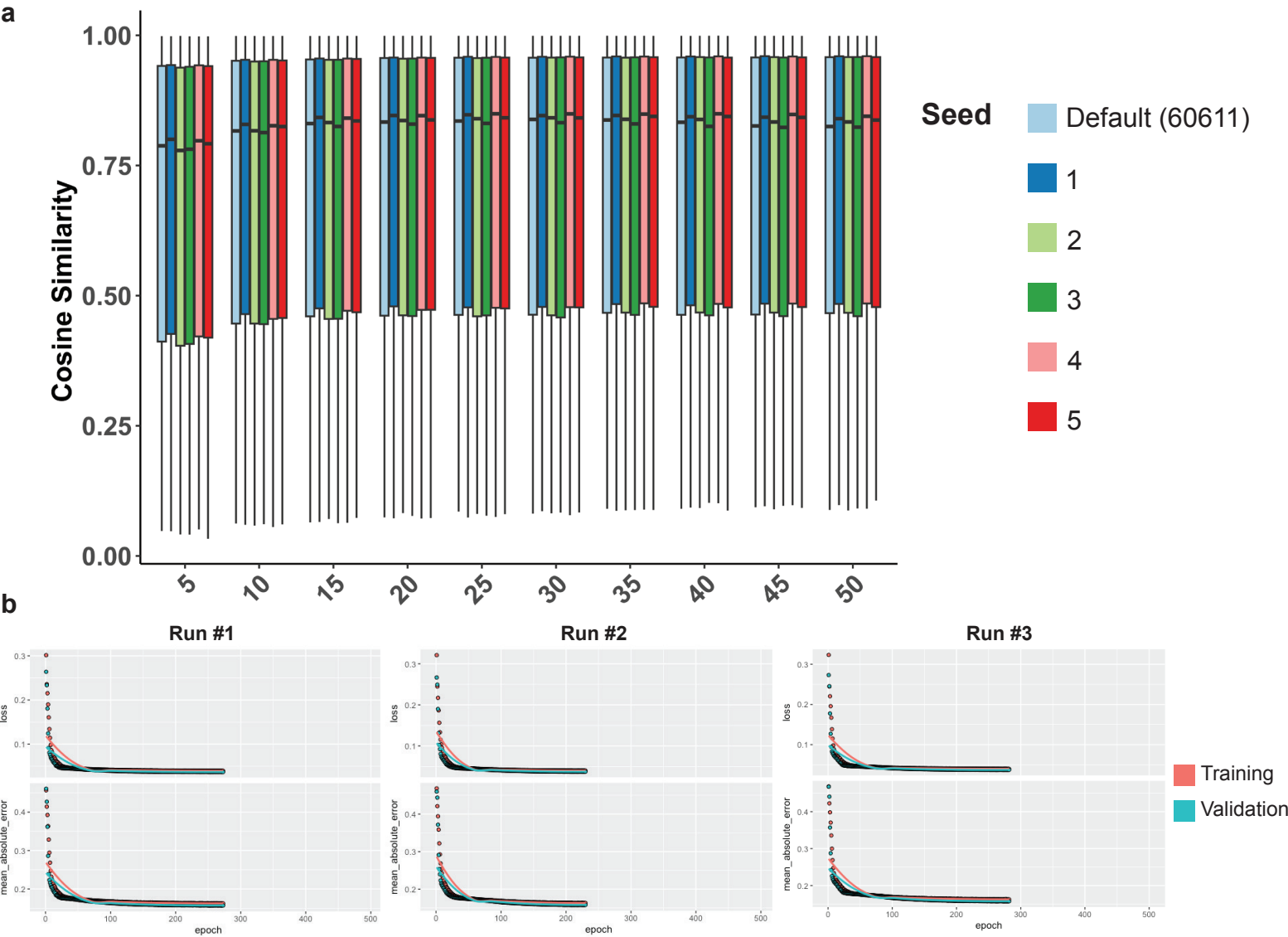
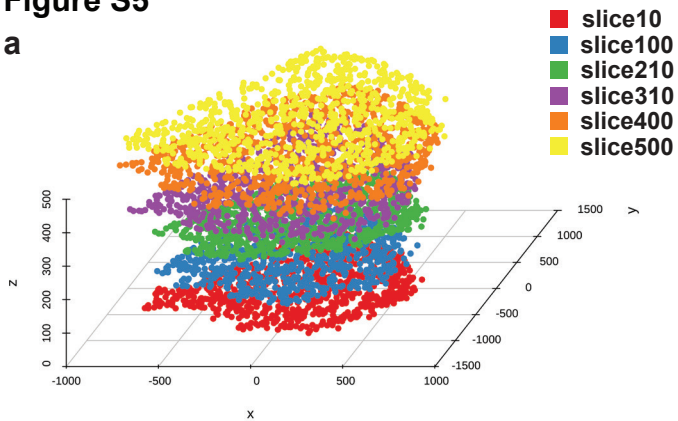


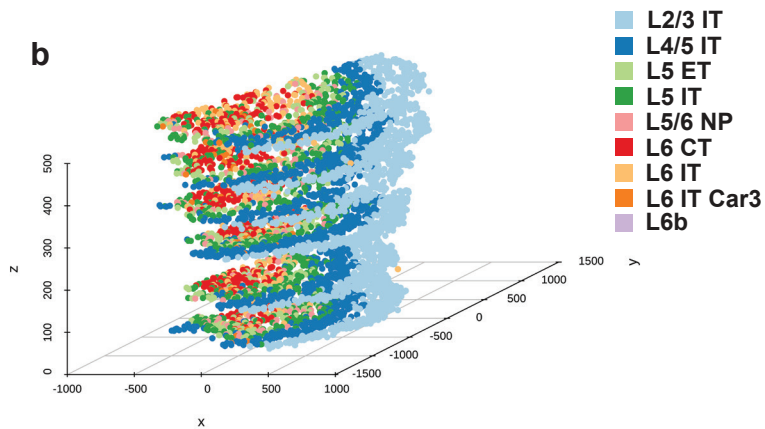
Figure S4. a, Boxplot for the mean of cosine similarity between scHolography-predicted spatial neighborhood accumulated expression under different initialization seeds and simulated mouse hippocampus spatial neighborhood accumulated expression. The size of the neighborhood varies from 5 to 50 cells. **b,** Training and validation loss and mean absolute error curves for the first three runs of 30 repeat runs of neural network training.

Figure S5

a



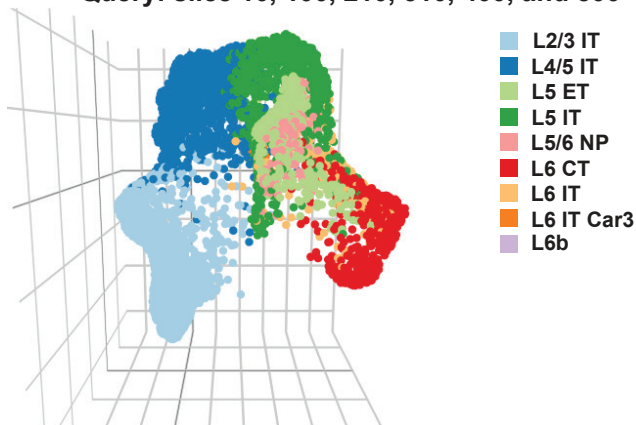
b



c

Reference: slice 10

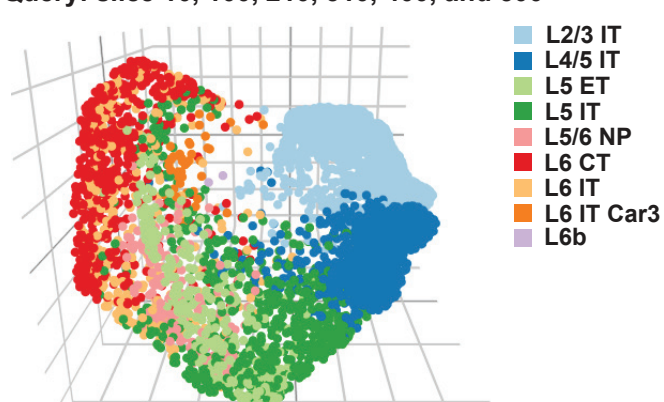
Query: slice 10, 100, 210, 310, 400, and 500



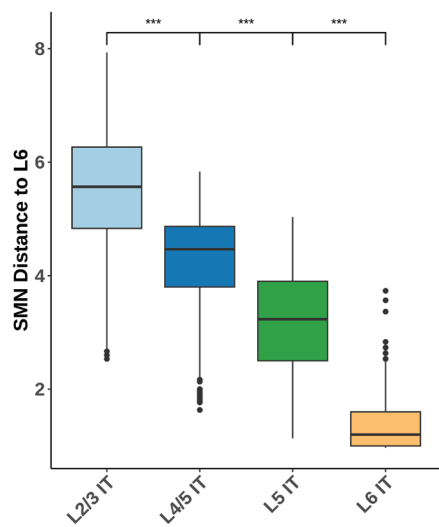
d

Reference: slice 500

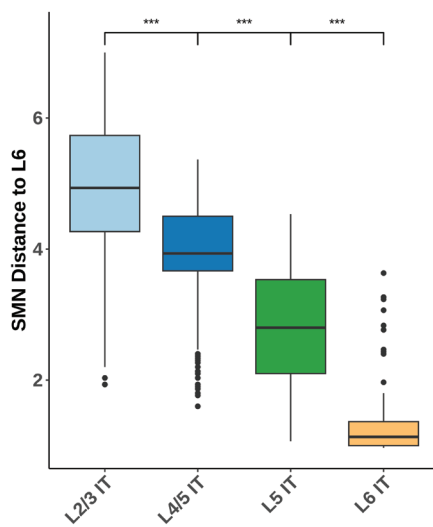
Query: slice 10, 100, 210, 310, 400, and 500



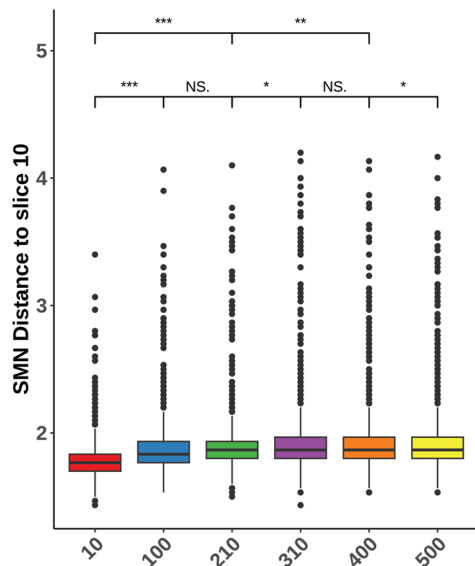
e



f



g



h

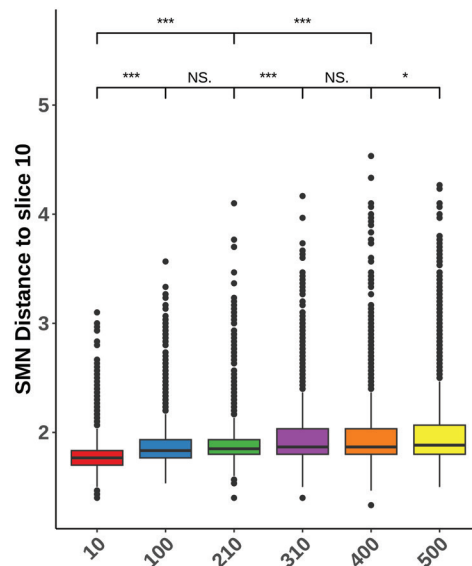


Figure S5. **a**, Stacked-2D plot of Merfish mouse cortex sample 1 (slice 10, 100, 210, 310, 400, and 500) colored by the slice. **b**, Stacked-2D plot of Merfish mouse cortex sample 1 (slice 10, 100, 210, 310, 400, and 500) colored by cell types. **c-d**, 3D visualization of Merfish sample1 scHolography reconstruction result. **c**, use slice 10 as the reference; **d**, use slice 500 as the reference. **e-f**, SMN distances from L2/3 IT, L4/5 IT, L5 IT, L6 IT to L6 IT in Merfish sample1 scHolography prediction. Two-sided Wilcoxon tests are performed. **e**, use slice 10 as the reference; **f**, use slice 500 as the reference. **g-h**, SMN distances from slice 10, 100, 210, 310, 400, and 500 to slice 10 in Merfish sample 1 scHolography prediction. Two-sided Wilcoxon tests are performed. **g**, use slice 10 as the reference; **h**, use slice 500 as the reference.

Figure S6

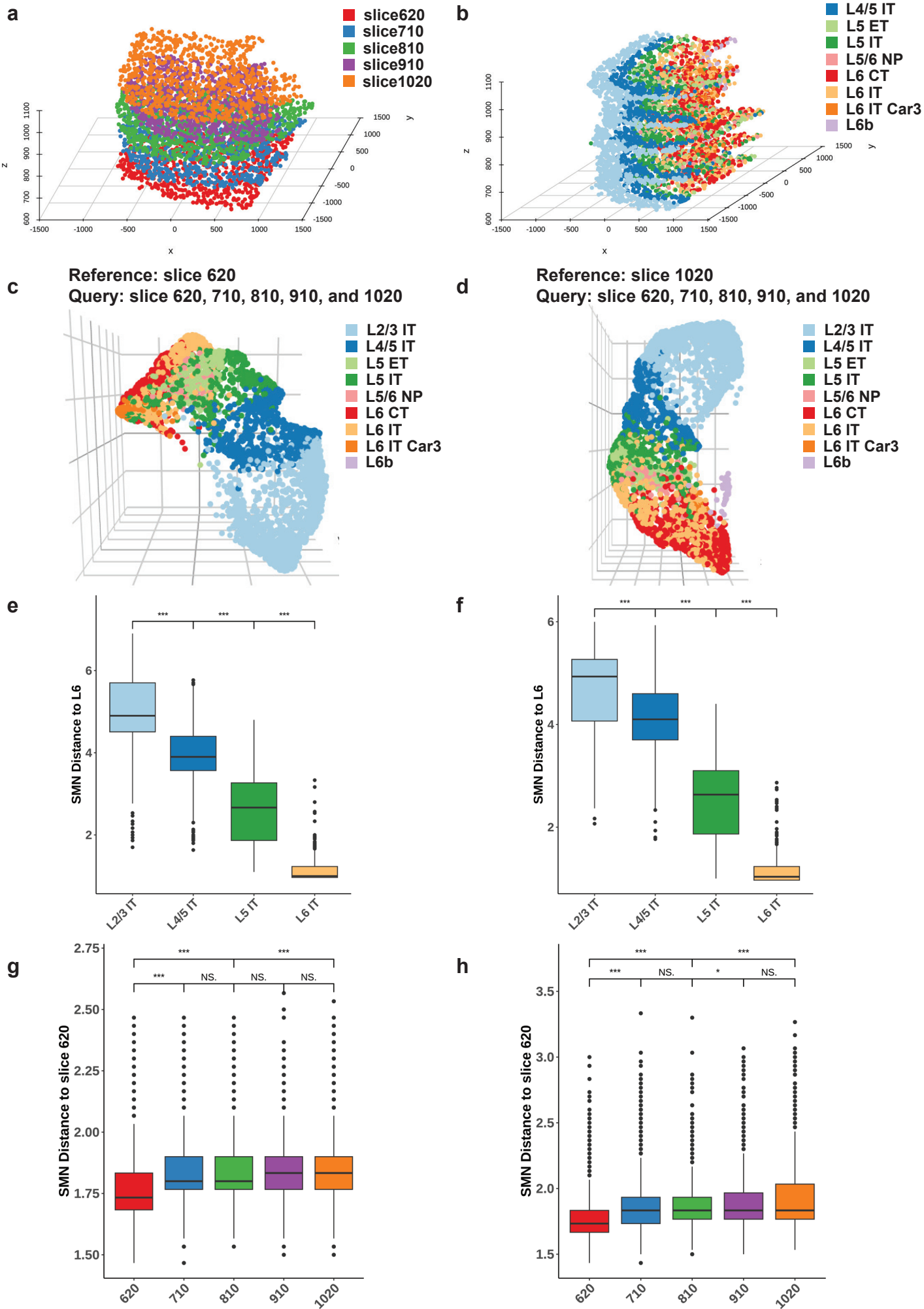


Figure S6. **a**, Stacked-2D plot of Merfish mouse cortex sample 2 (620, 710, 810, 910, and 1020) colored by the slice. **b**, Stacked-2D plot of Merfish mouse cortex sample 1 (620, 710, 810, 910, and 1020) colored by cell types. **c-d**, 3D visualization of Merfish sample2 scHolography reconstruction result. **c**, use slice 620 as the reference; **d**, use slice 1020 as the reference. **e-f**, SMN distances from L2/3 IT, L4/5 IT, L5 IT, L6 IT to L6 IT in Merfish sample2 scHolography prediction. Two-sided Wilcoxon tests are performed. **e**, use slice 620 as the reference; **f**, use slice 1020 as the reference. **g-h**, SMN distances from slice 620, 710, 810, 910, and 1020 to slice 620 in Merfish sample 2 scHolography prediction. Two-sided Wilcoxon tests are performed. **g**, use slice 620 as the reference; **h**, use slice 1020 as the reference.

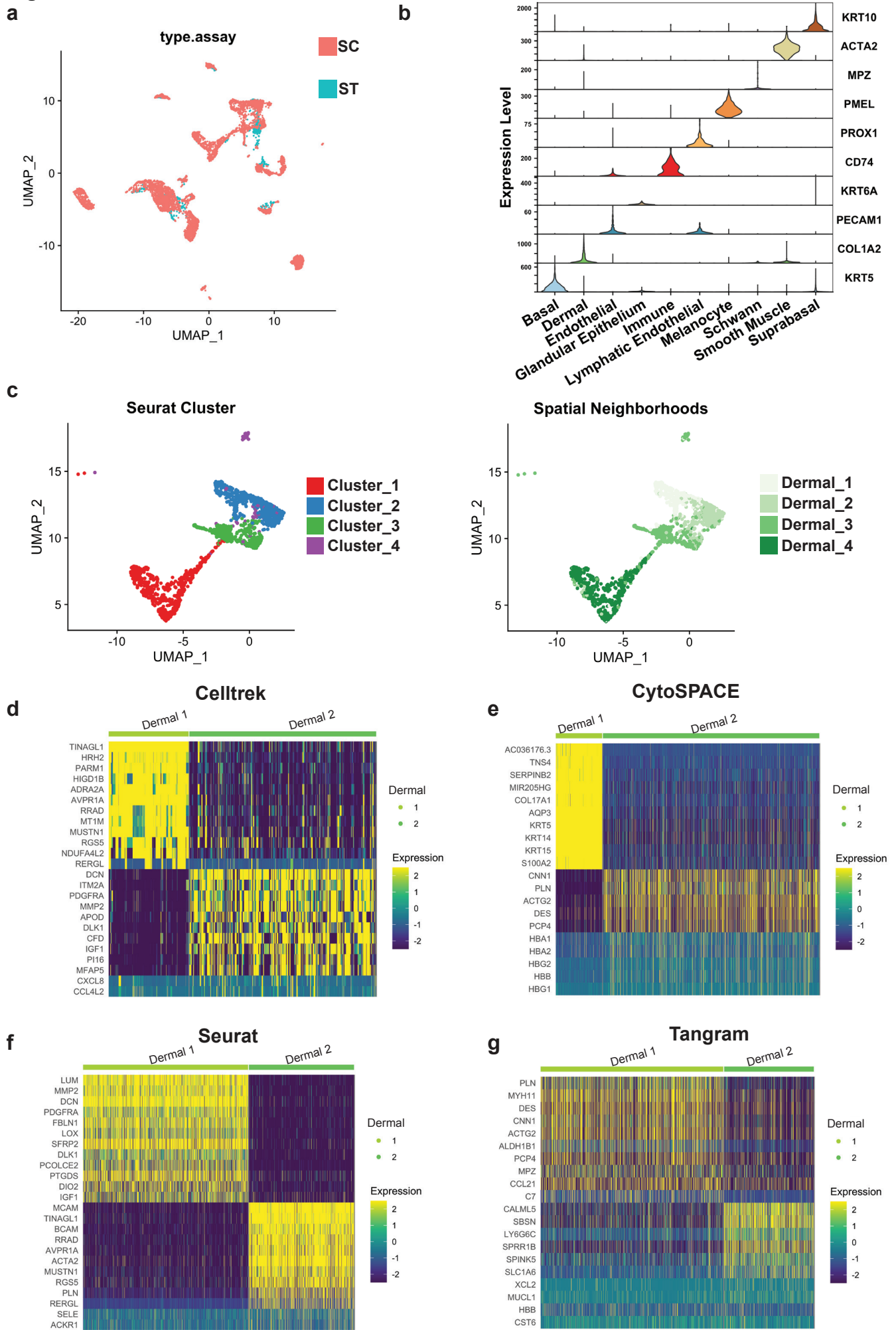
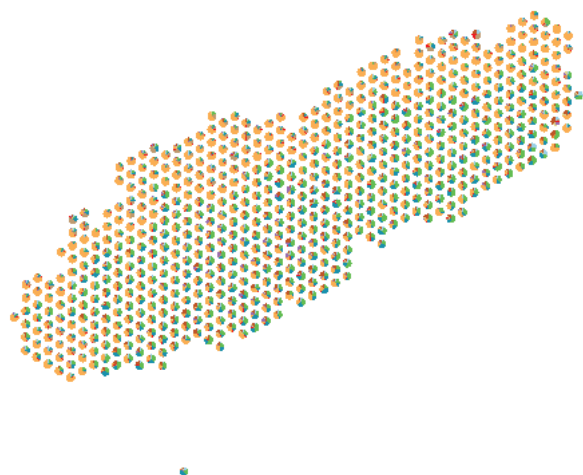
Figure S7

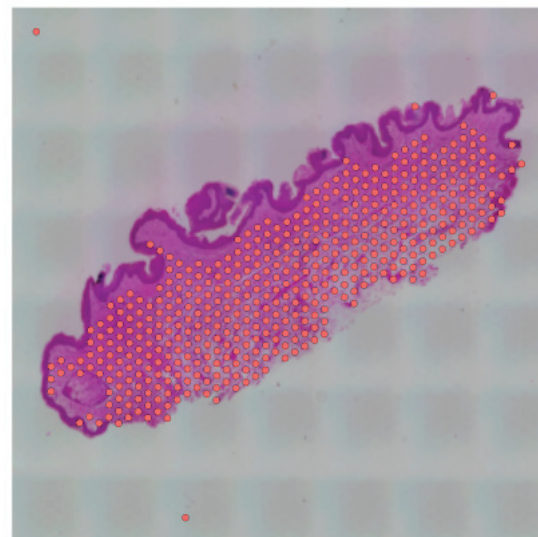
Figure S7. a, Human foreskin SC and ST data integration. **b,** Identification of major cell types in human foreskin samples by cell lineage markers. *KRT10*, suprabasal epithelial cells; *ACTA2*, smooth muscle cells.; *MPZ*, Schwann cells; *PMEL*, melanocytes; *PROX1*, lymphatic endothelial cells; *CD74*, immune cells; *KRT6A*, glandular epithelial cells; *COL1A2*, dermal cells; *PECAM1*, endothelial cells; *KRT5*, basal epithelial cells. **c,** Comparison between transcriptome-based Seurat dermal cell clusters (left) and scHolography identified dermal spatial neighborhoods (right). **d-g,** Heatmaps of top 10 differentially expressed genes for the dermal spatial neighborhood accumulated SMN expression profile. The spatial neighborhoods were identified based on Celltrek, CytoSPACE, Seurat, and Tangram prediction.

Figure S8

a

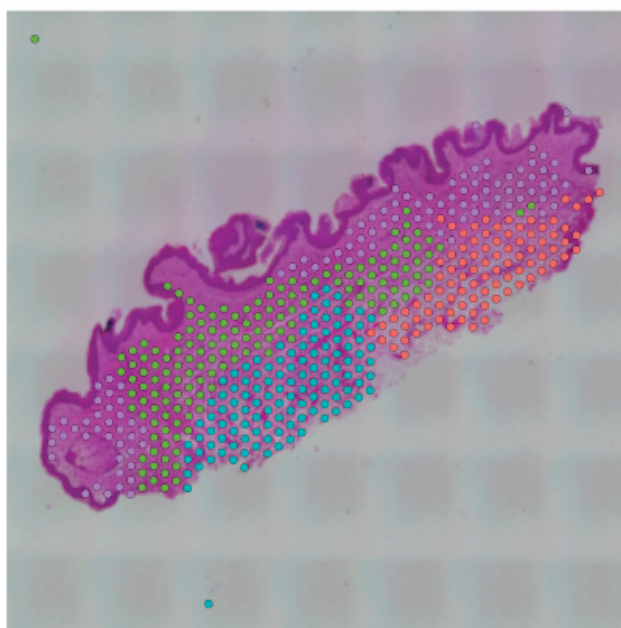


b



■ Dermal Cell Containing Spot

c



■ Dermal Niche 1
■ Dermal Niche 2
■ Dermal Niche 3
■ Dermal Niche 4

d

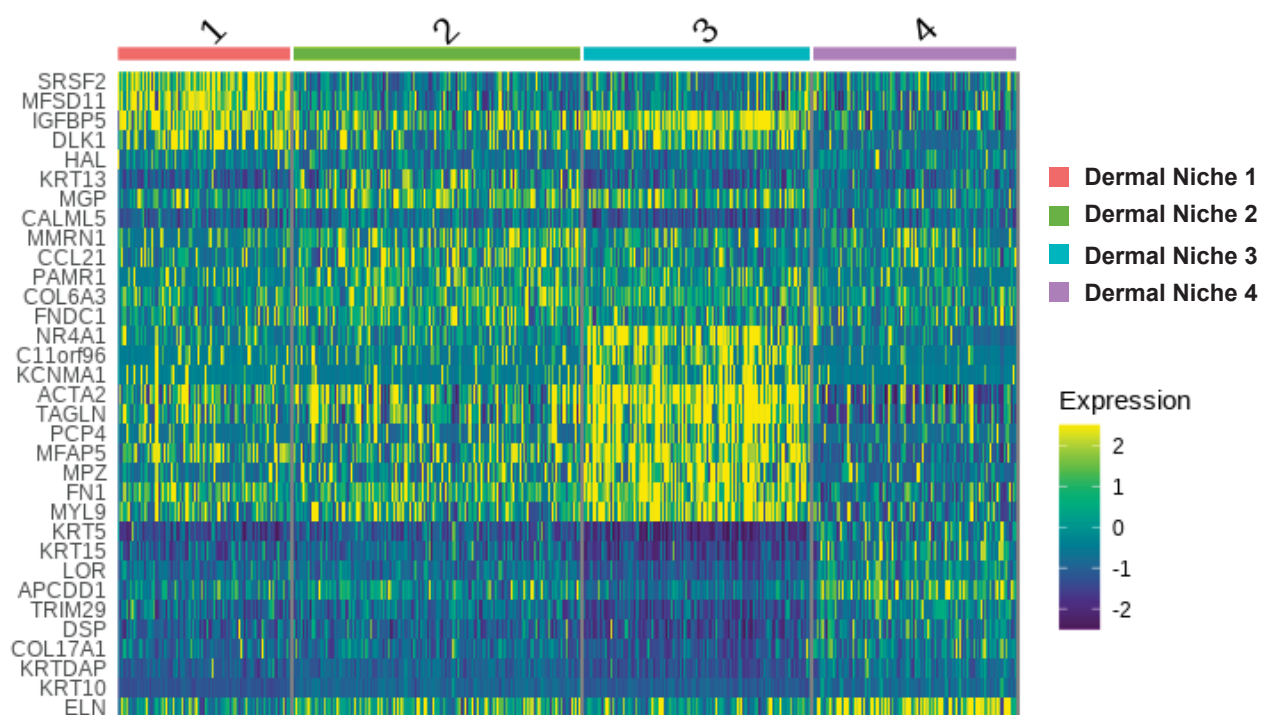
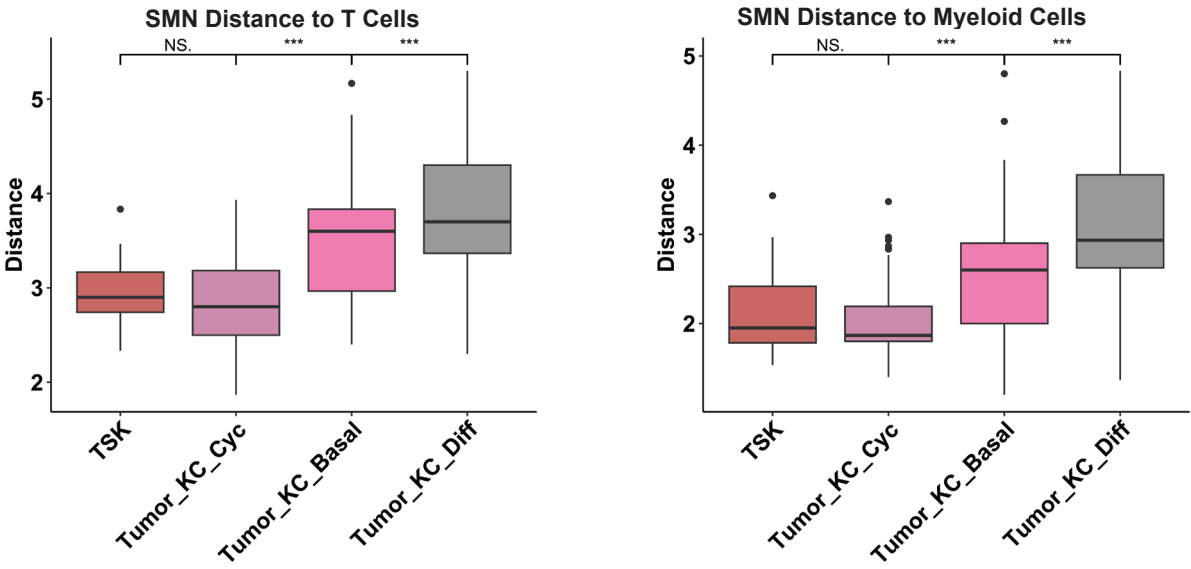


Figure S8. **a**, SPOTlight deconvolution results for human skin 10X Visium data. **b**, Defined dermal cell-containing spatial spots. A spot is defined as a dermal cell-containing spatial spot when its dermal cell composition is inferred to be greater than 0.15 by SPOTlight. **c**, Spatial niche analysis for dermal cell-containing spatial spots. **d**, Heatmap of top DEG for each spatial niche.

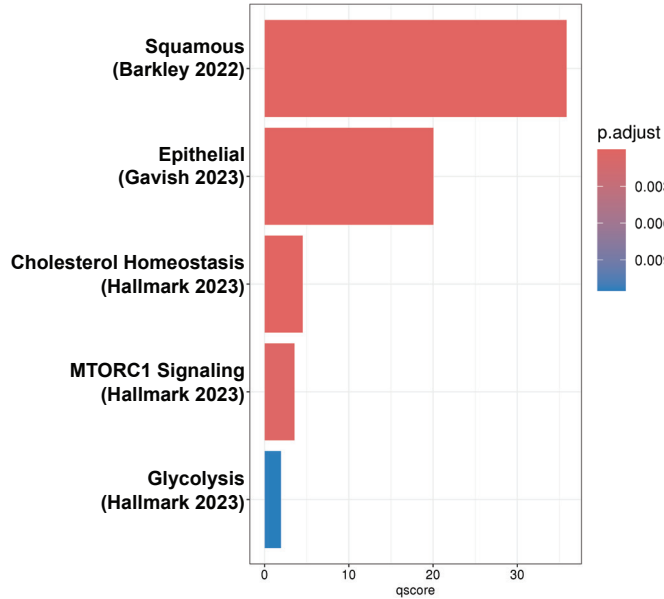
Figure S9

a



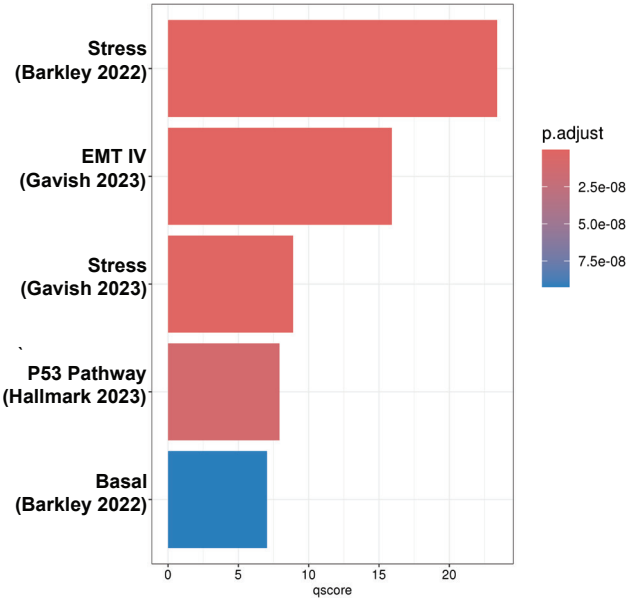
b

Enrichment Analysis of DEGs for Tumor KC1 Spatial Neighborhood



c

Enrichment Analysis of DEGs for Tumor KC2 Spatial Neighborhood



d

Enrichment Analysis of DEGs for Tumor KC3 Spatial Neighborhood

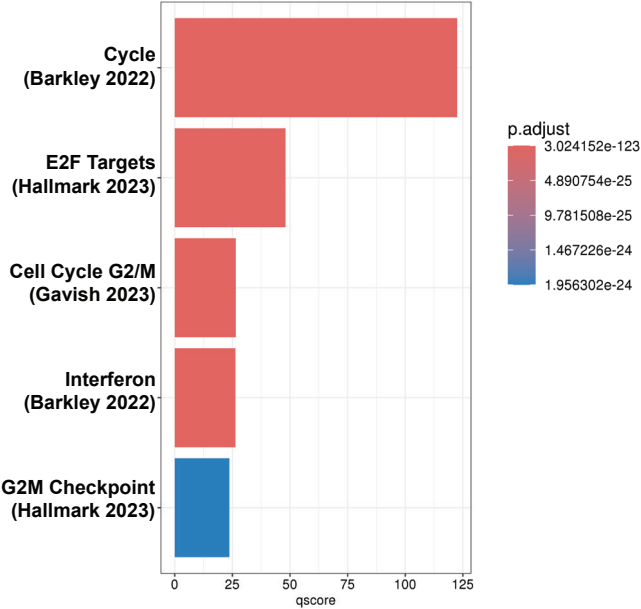


Figure S9. **a**, Violin plot of SMN distance between different Tumor KC cell types and T cells (left). Violin plot of SMN distance between different Tumor KC cell types and myeloid cells (right). **b-d**, Enrichment analysis of DEGs (Mann Whitney Wilcoxon test, $p_{\text{adjusted}} < 0.05$) for Tumor KC1 (**b**), Tumor KC2 (**c**), and Tumor KC3 (**d**) accumulated spatial neighborhood expression profiles. Hypergeometric tests were performed for enrichment analysis, and the top 5 term hits were displayed.

Figure S10. **a**, Composition of spatial neighborhood assignment for different Tumor KC cell types. **b-c**, Violin plot of SMN distance between different Tumor KC spatial neighborhoods and T cells (**b**). Violin plot of SMN distance between different Tumor KC spatial neighborhoods and myeloid cells (**c**). **d**, SMN distances between myeloid cells and T cells vs. SMN distances between myeloid cells and tumor keratinocyte cells, break down for each myeloid cell type. **e**, Violin plot of SMN distance between different myeloid cell types and T cells (left). Violin plot of SMN distance between different myeloid cell types and tumor keratinocyte cells (right). **f**, Overall signaling strengths inferred by CellChat for different cell types in cSCC. Based on scHolography, each cell type is split into (1) distal and (2) proximal groups. Cells in the distal group are not SMNs of any tumor keratinocyte cells, while cells in the proximal group are SMNs of at least one tumor keratinocyte cell.

Figure S11

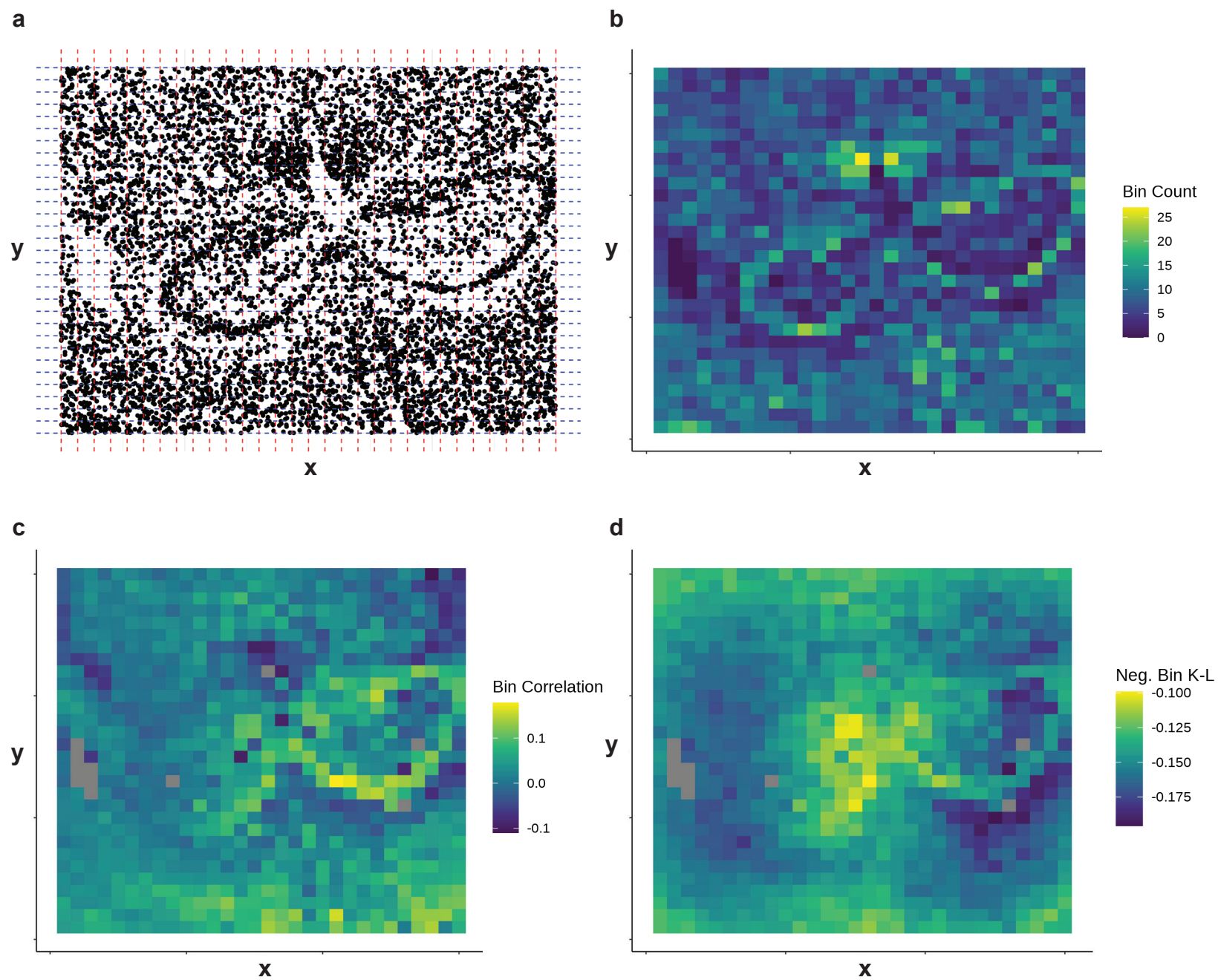


Figure S11. **a**, Binning of Vizgen data. The image region was binned with 30 by 30 bins on both axes. **b**, The number of cell centroid in each bin. Left open right closed intervals were applied. **c**, The mean Spearman correlation of between SMN graph distance and FR embedding distance between each cell pairs within each bin. **d**, The mean K-L Divergence of scHolography predictions for simulated mouse hippocampus data as ground truth within each bin.