

Fig. S1. Cell annotation and gene expression of three Stereo-seq-FFPE sections. (A) Spatial maps show the spatial distribution of cell types is similar among three sections generated by the Stereo-seq-FFPE protocol. (B) The heatmaps show scaled expression of classic cell markers is similar among three sections generated by the Stereo-seq-FFPE protocol.

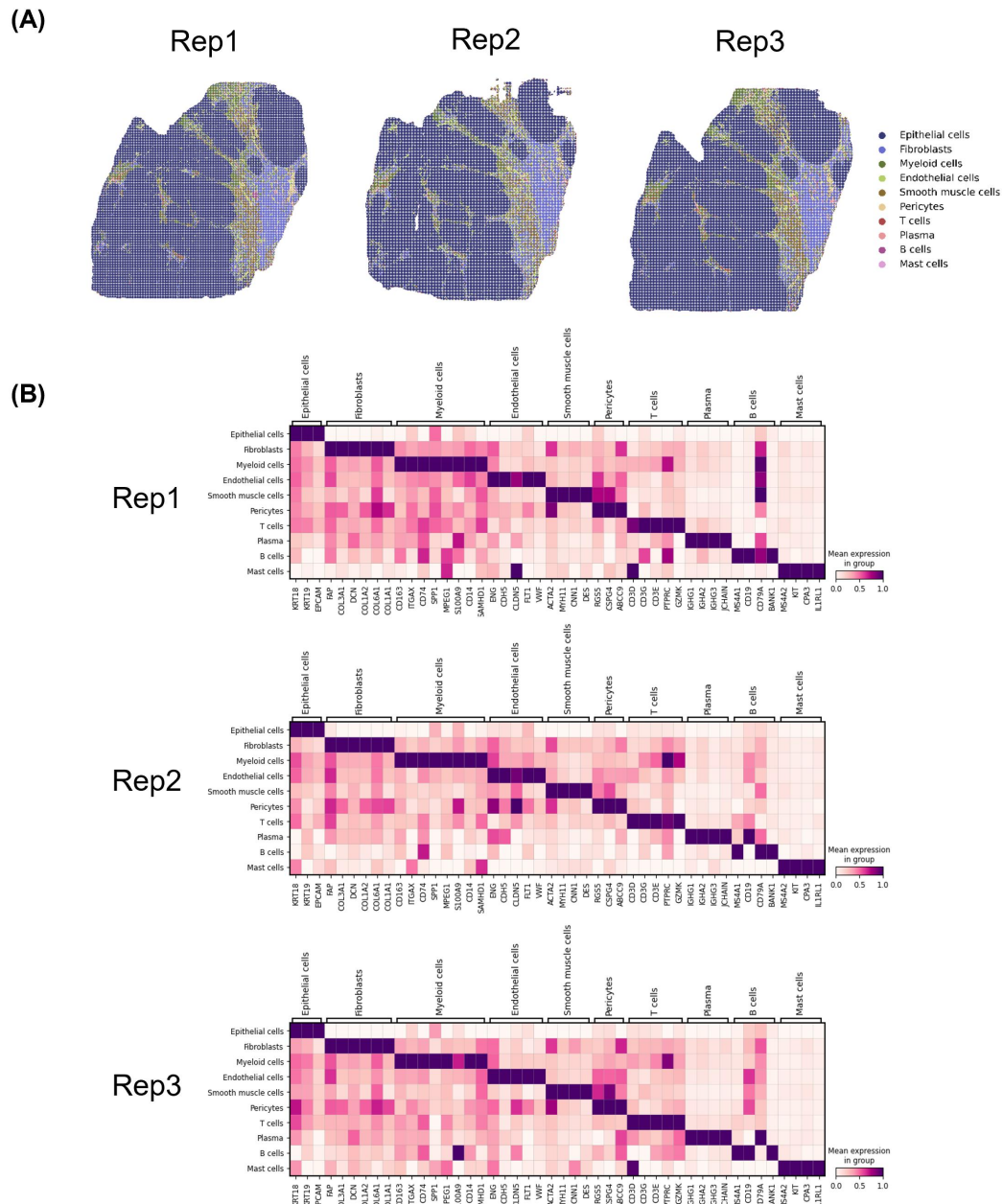


Fig. S2. The features of gene expression demonstrated technical reproducibility among three samples from ST-FFPE-mIF. (A) The heatmaps illustrate scaled expression of classic cell markers is similar in three replicates. **(B)** Spatial maps show spatial distribution of genes with high Moran's I score is similar among the three replicates.

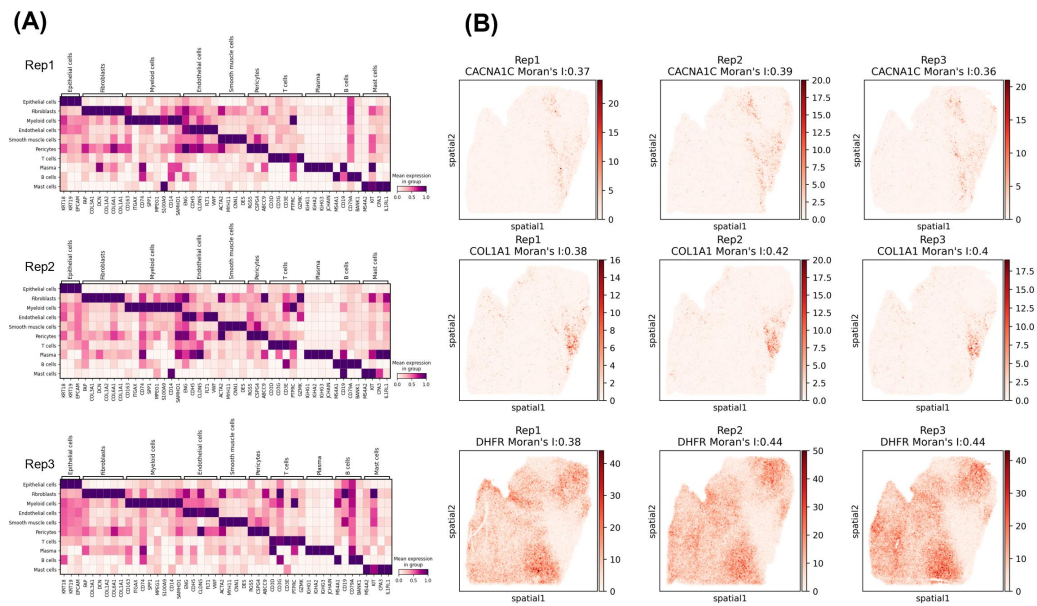


Fig. S3. Evaluation of antibody elution efficiency. (A) Evaluate the efficacy of antibody elution for the three markers following the first round of staining. A box plot illustrates the positive area from 20 FOVs before and after antibody elution, in comparison to the negative control.

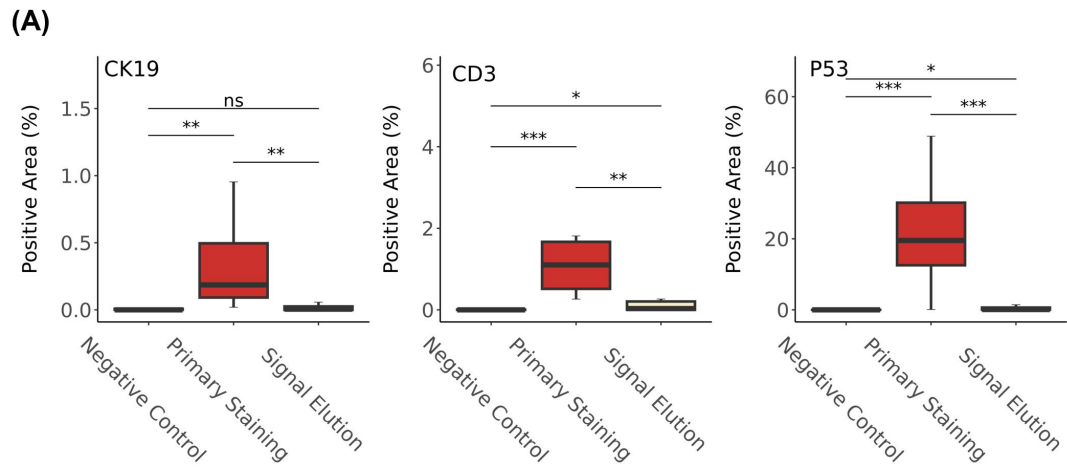


Fig. S4. Correlation of gene expression among three ST-FFPE-mIF samples. (A) These scatter plots illustrate a strong correlation in gene expression among three samples from ST-FFPE-mIF.

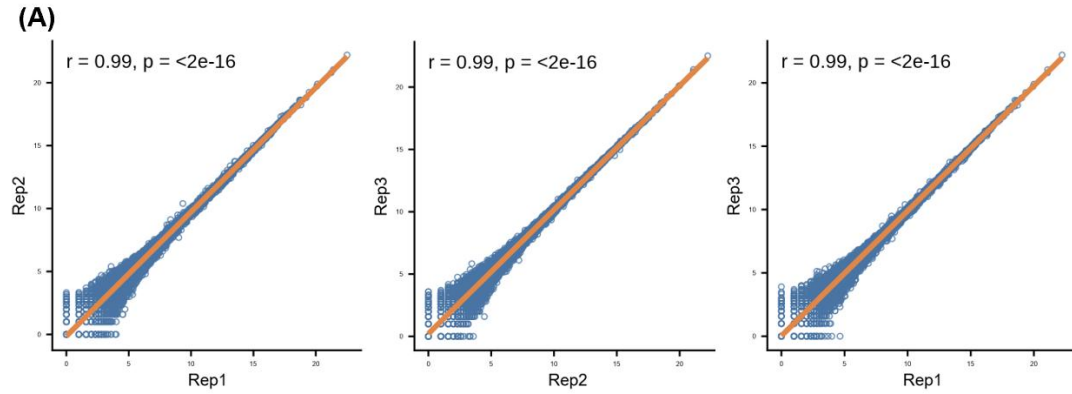


Fig. S5. Co-staining of mIF images and spatial data performance in different tumor tissues using ST-FFPE-mIF. (A) ST-FFPE-mIF profiling of a breast cancer FFPE section with high autofluorescence. Left: Autofluorescence images of adjacent section. Right: Co-detection of mIF image and enlarged representative region. Biomarkers and colors are shown on the bottom. The scale bars were 1000 μm and 100 μm respectively. (B) Respective mIF images of renal carcinoma and gastric cancer. Biomarkers and colors are shown at the bottom. The scale bars were 1000 μm and 100 μm respectively. (C) Violin plots show the number of captured UMIs and genes at bin50 resolution among three tumor sections.

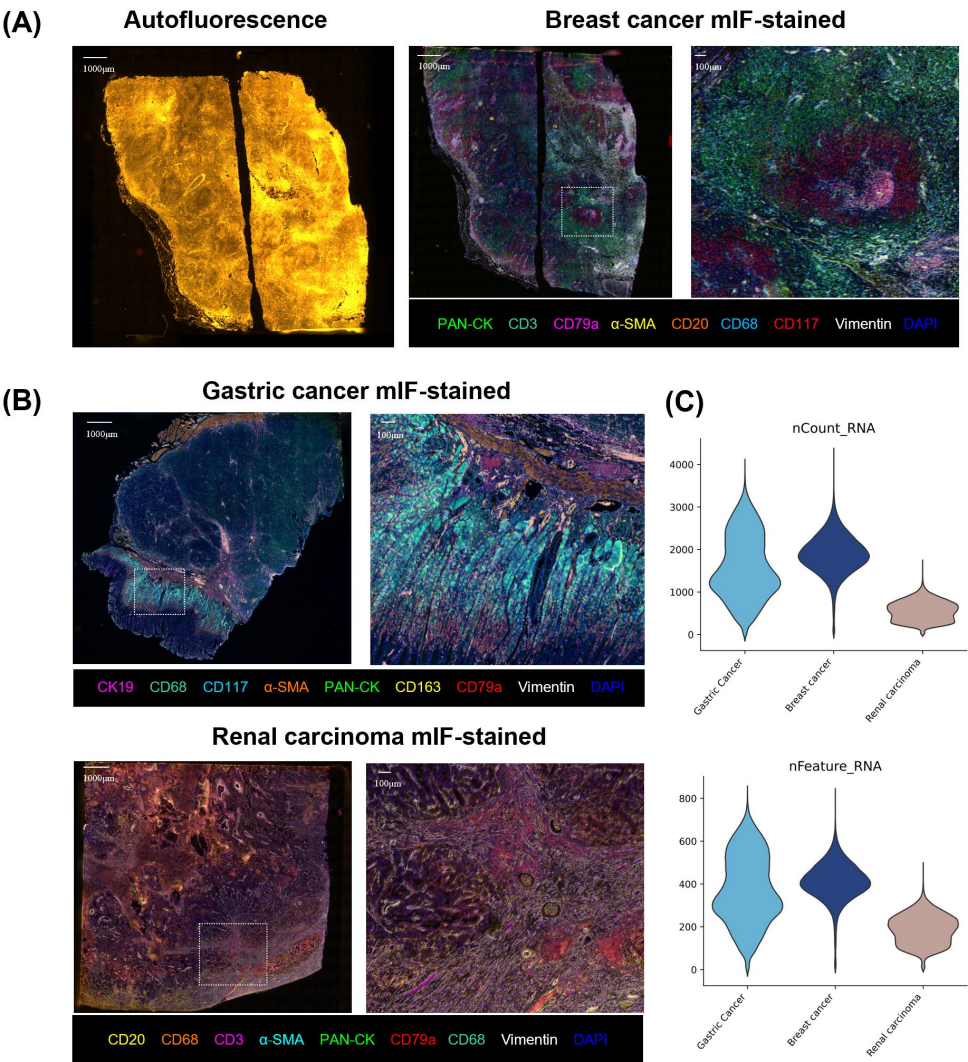


Fig. S6. Validation of cell segmentation based on DAPI. (A) Comparison of cell segmentation by Cellpose with manually corrected ground truth. The orange outlines in the figure denotes the corrected ground truth, while the red outlines indicate the boundaries predicted by Cellpose. The lower section of each image displays the IoU, AP, and recall for the respective FOV.

(A)

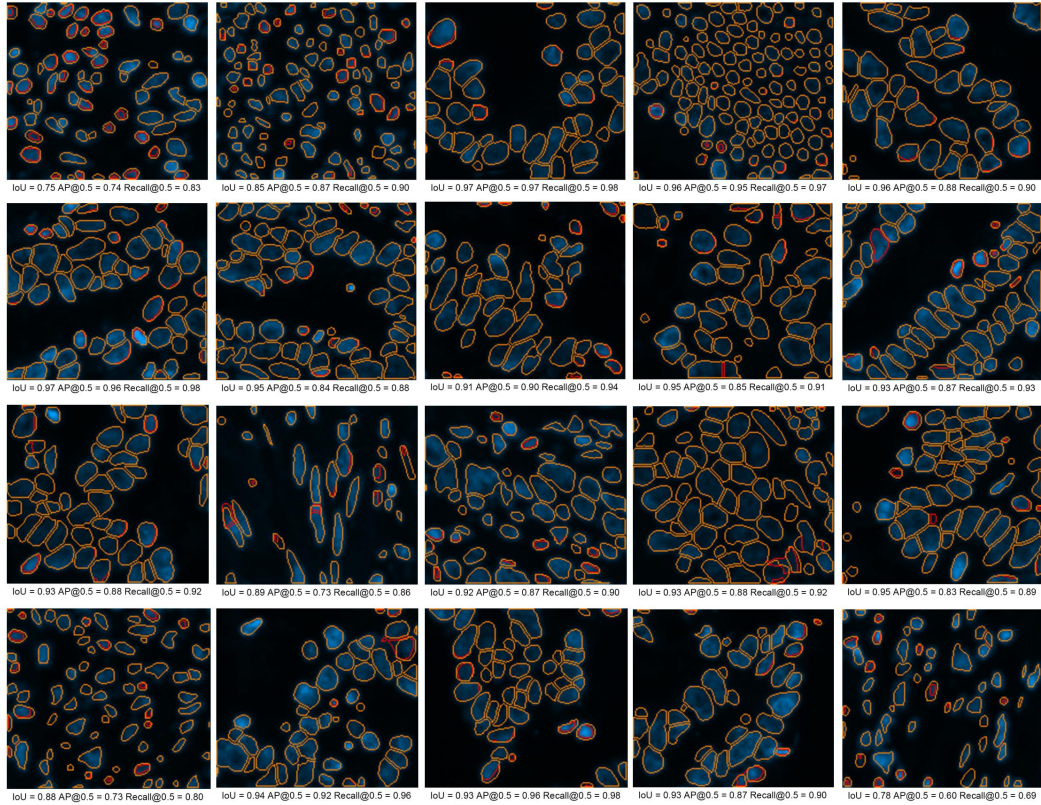


Fig. S7. Quality control intercellular communications among annotated clusters in CRC. (A) Circos plot shows cell-to-cell communications activities. (B) Inferred cell-to-cell communication activities associated with EGF signaling pathways explore detailed communications for distinct pathways. (C) The left shows that the inferred outgoing communication patterns of cells, which shows the correspondence between the inferred latent patterns and cell groups, as well as signaling pathways. The thickness of the flow indicates the contribution of the cell group or signaling pathway to each latent pattern. The right shows the inferred incoming communication patterns of target cells.

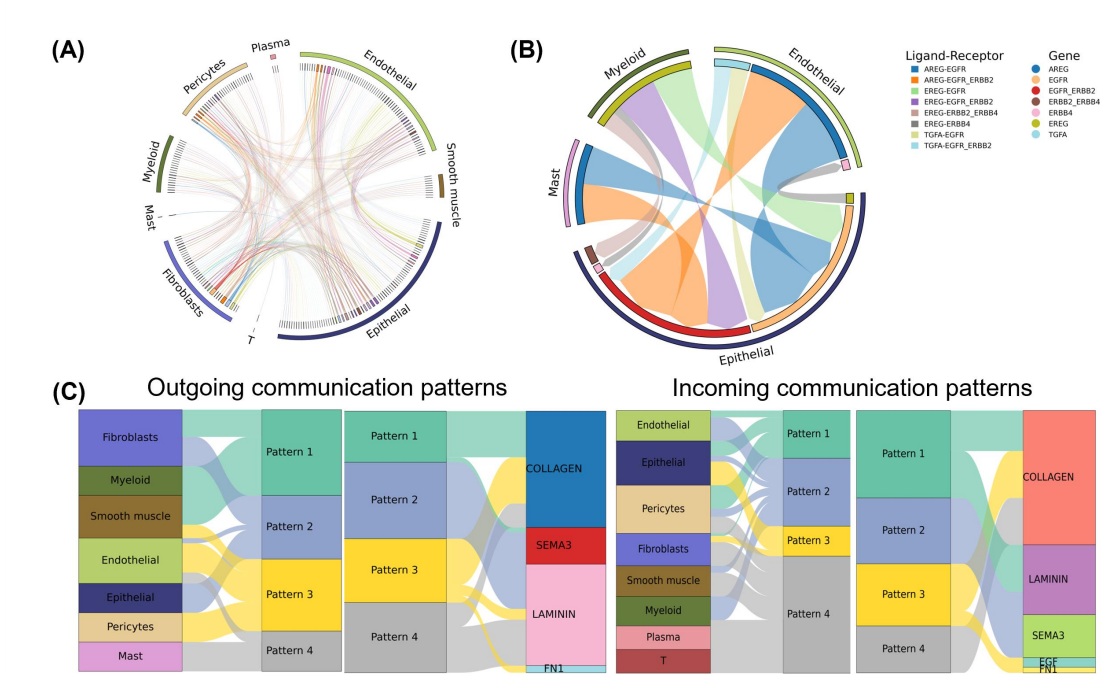


Fig. S8. Cell segmentation based on membrane fluorescence signals has potential to infer more accurate cell boundaries. (A) On the left, the representative FOV represents cell segmentation based on CD20 staining. The orange circles indicate the inferred cell boundaries based on this signal. On the right, red circles indicate the inferred cell boundaries based on DAPI signal. (B) Comparison of the predicted boundaries derived from the three methods (CD20-based, DAPI-based and manual correction). The upper right displays the IoU of DAPI-based and ground truth, as well as CD20-based and ground truth.

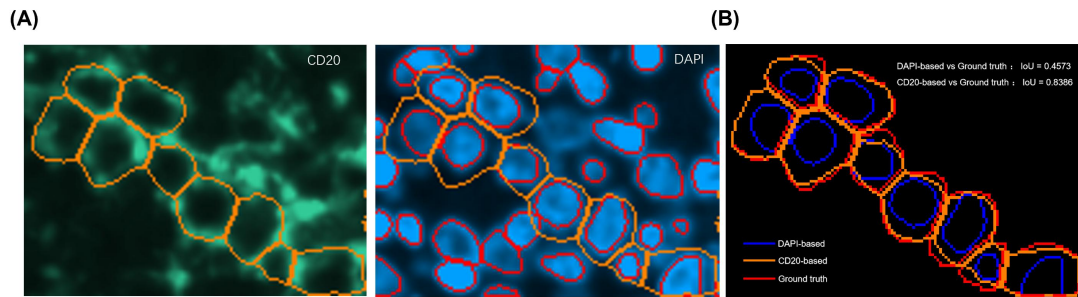


Fig. S9. Comparison between the two staining methods utilizing ST-FFPE-mIF in lymphoma. (A) Fluorescence images of two distinct mIF staining methods in lymphoma. Biomarkers and colors are shown on the bottom. The scale bars were 1000 μm and 100 μm respectively. (B) Violin plots show the number of captured UMIs and genes at bin50 resolution between two mIF stained method in lymphoma.

