Figure S1
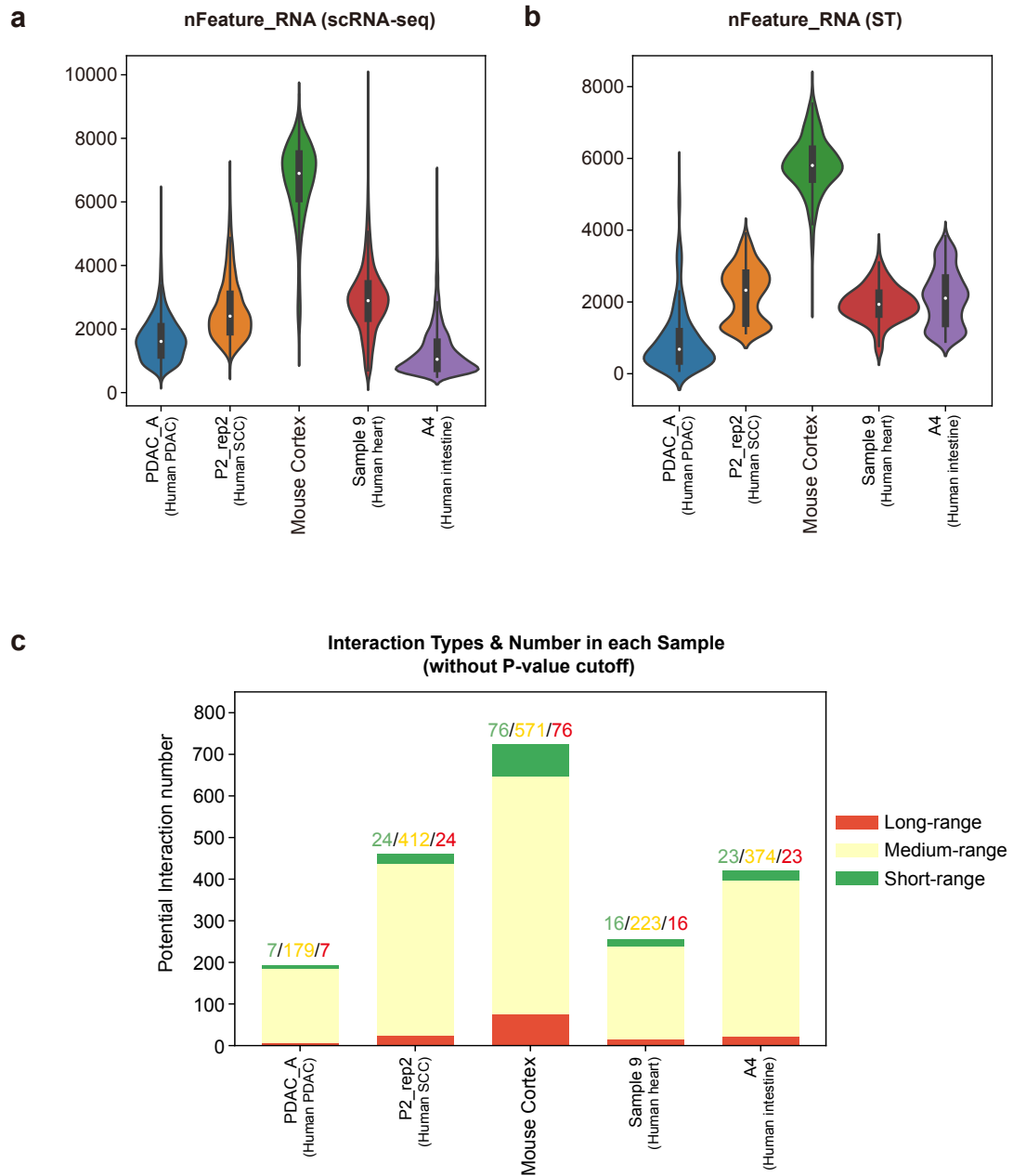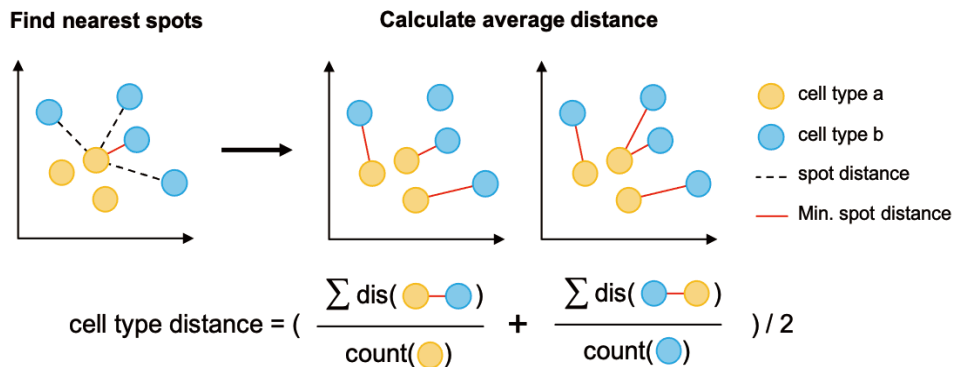

Gene coverages and the actual used short-range and long-range interaction numbers
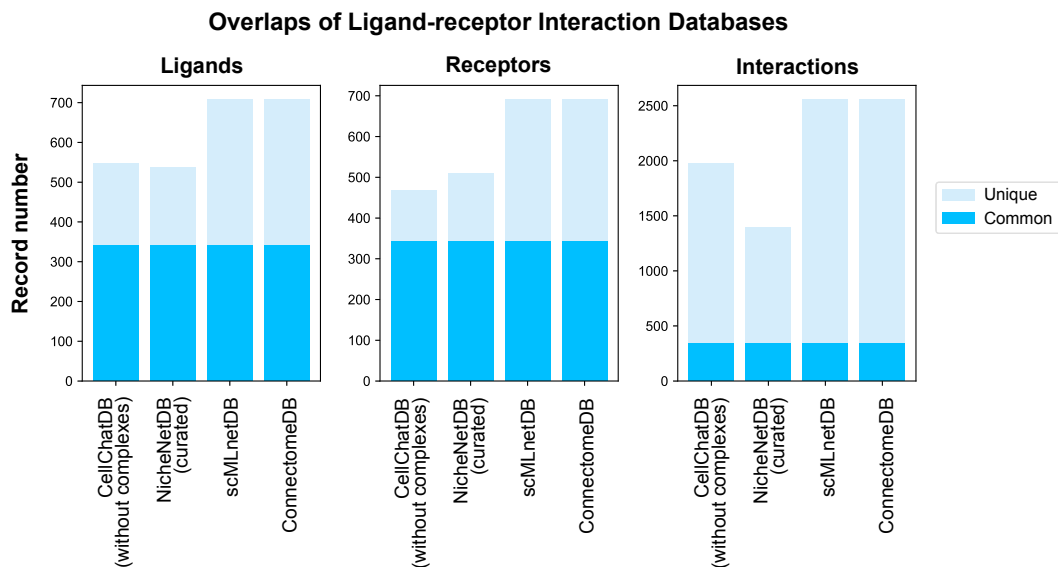
a. nFeature of exampled samples' scRNA-seq data.

b. nFeature of exampled samples' ST data.

c. The actual used short-range and long-range interaction numbers in calculating distance enrichment score in exampled samples.

Figure S2

a

Find nearest spots

Calculate average distance

- 🟡 cell type a
- 🔵 cell type b
- - - - spot distance
- —— Min. spot distance

$$\text{cell type distance} = \left(\frac{\sum \text{dis}(🟡\!-\!🔵)}{\text{count}(🟡)} + \frac{\sum \text{dis}(🔵\!-\!🟡)}{\text{count}(🔵)} \right) / 2$$

b

Overlaps of Ligand-receptor Interaction Databases

Ligands　　**Receptors**　　**Interactions**

Record number

Unique
Common

CellChatDB (without complexes)　NicheNetDB (curated)　scMLnetDB　ConnectomeDB

Schematic illustration of computing cell type distance and LR databases overlaps

a. The Schematic figure illustrates the procedure for computing cell type distance. Each point indicates a spot in the ST data, the color represents the cell type. The black dotted line shows the Euclidean distance between connected spots, while the red line indicates that the connected spots have the minimum distance than other black dotted line connected spots. The distance between cell types can be quantified through the mean of minimum Euclidean distances between spots.
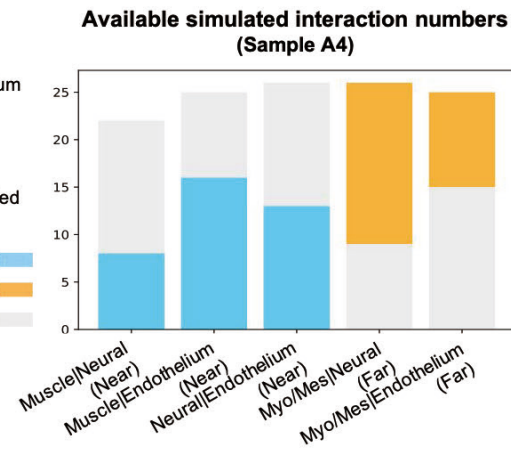
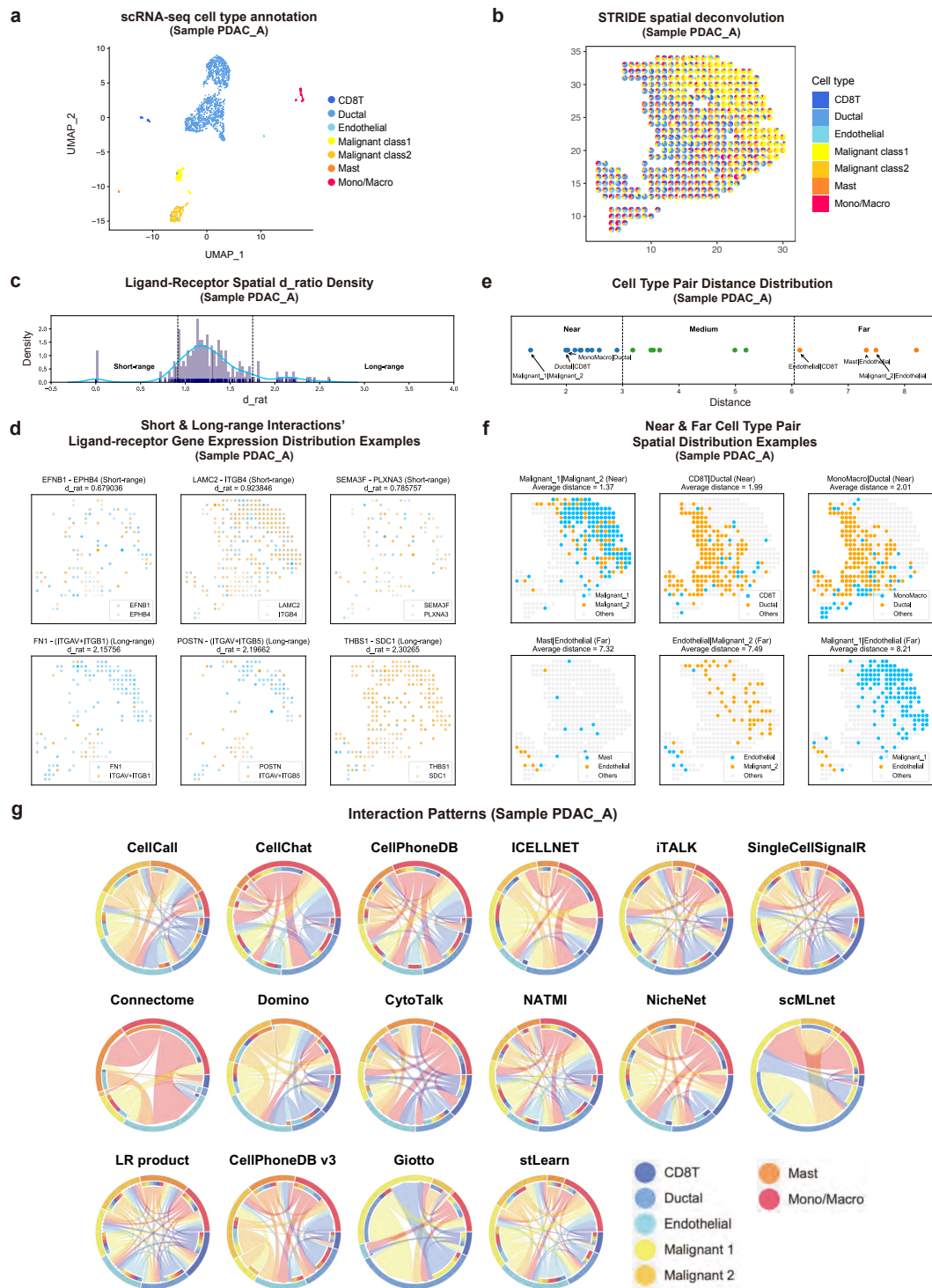b. The bar plots show the overlaps between interaction databases.

Figure S3
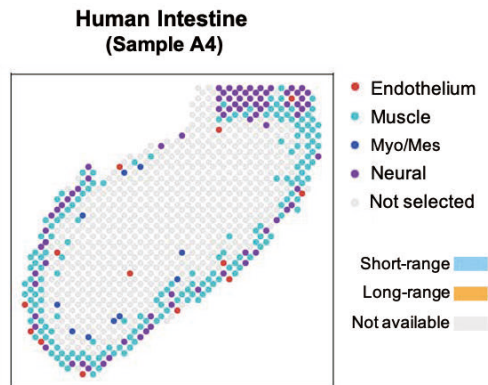
Example of selecting cell types and filtering simulated LR pairs

a. The scatter plot displays the example of randomly selecting 4 cell types in the data simulation procedure. Each point represents a spot in ST data, the selected spots are colored according to their cell types while the unselected spots colored in gray. Using sample A4 as an example.

b. the bar plot shows the number of simulated LR pairs after filtering. The kept LR pairs are colored according to their interaction types. The LR pairs which failed to pass the filtering are colored in gray.

Figure S4

a
scRNA-seq cell type annotation
(Sample PDAC_A)

b
STRIDE spatial deconvolution
(Sample PDAC_A)

c
Ligand-Receptor Spatial d_ratio Density
(Sample PDAC_A)

e
Cell Type Pair Distance Distribution
(Sample PDAC_A)

d
Short & Long-range Interactions'
Ligand-receptor Gene Expression Distribution Examples
(Sample PDAC_A)

f
Near & Far Cell Type Pair
Spatial Distribution Examples
(Sample PDAC_A)

g
Interaction Patterns (Sample PDAC_A)

Data processing results and examples of the sample PDAC_A

a. The UMAP of scRNA-seq data of the sample PDAC_A, colored by cell types.

b. The STRIDE deconvolution result of the sample PDAC_A. The pie plot in each spot represents the predicted cell type proportions.

c. The density plot of the *d_ratio* distribution in the sample PDAC_A. The black dotted lines show the *d_ratio* boundaries of short-range and long-range interactions, respectively.
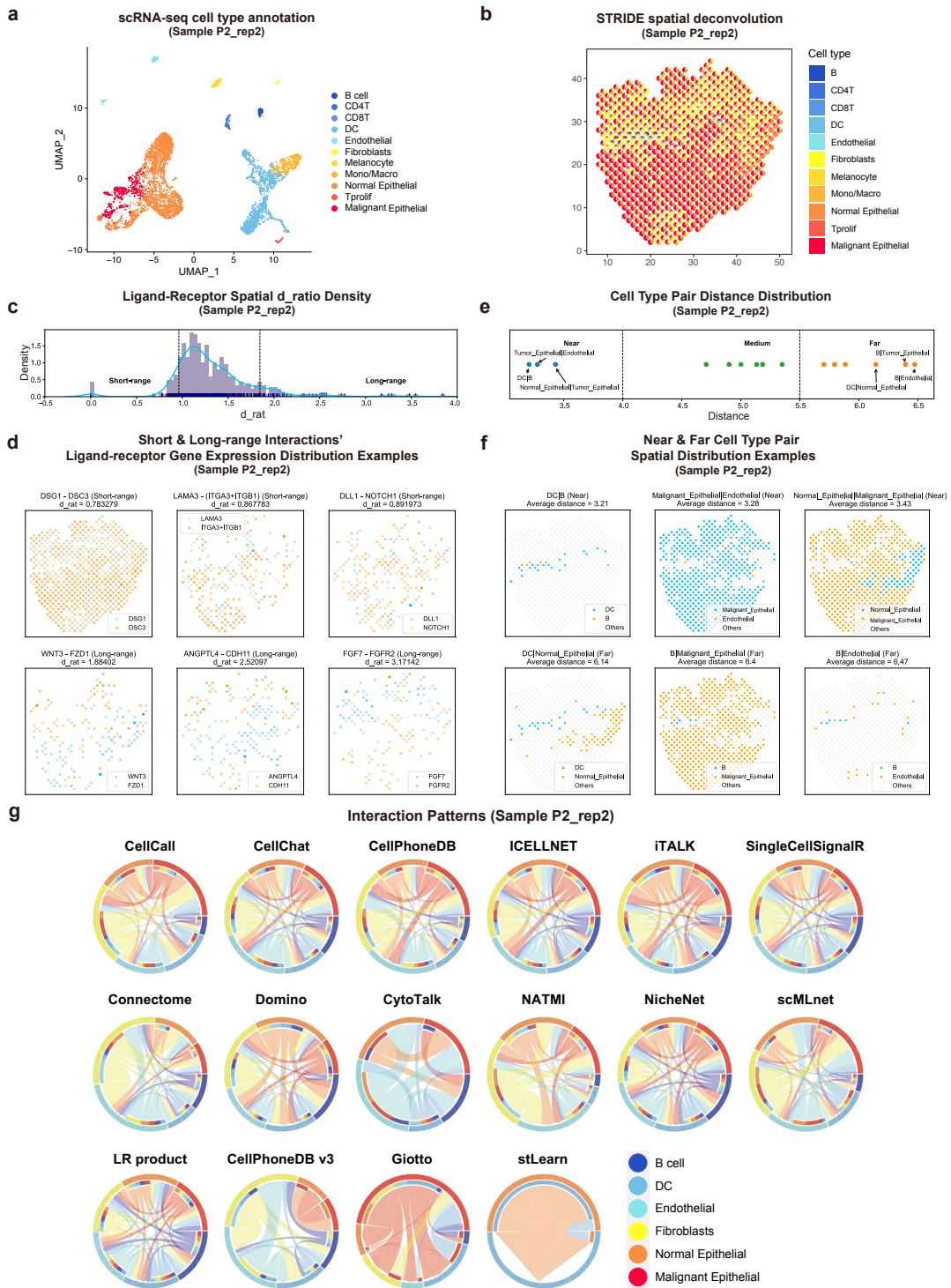
d. The examples of ligands (blue) and receptors' (orange) expression distributions in short-range (top) and long-range (bottom) interactions. The transparency of spot indicates the gene expression level.

e. The dot plot shows the distances of all cell type pairs in the sample PDAC_A, and the clustering results. The dot color stands for the type of cell type pair, blue for near cell type pair; green for medium cell type pair; orange for far cell type pair. The dotted lines are just for better displaying the different groups of cell type pairs and do not stand for the actual distance boundaries.

f. The examples of near (top) and far (bottom) cell type pairs' spatial distributions. The spot color is for dividing cell types.

g. The chord plot of the overall interaction pattern in the sample PDAC_A predicted by each tool. Color indicates cell types. For readability, only cell types in near and far cell type pairs are included in chord plots. The segment size reflects the relative number of interactions that the corresponding cell type participated in. The arrow indicates the interaction direction between cell types and its width shows the relative interaction number.

Figure S5

a scRNA-seq cell type annotation
(Sample P2_rep2)

- B cell
- CD4T
- CD8T
- DC
- Endothelial
- Fibroblasts
- Melanocyte
- Mono/Macro
- Normal Epithelial
- Tprolif
- Malignant Epithelial

b STRIDE spatial deconvolution
(Sample P2_rep2)

Cell type
- B
- CD4T
- CD8T
- DC
- Endothelial
- Fibroblasts
- Melanocyte
- Mono/Macro
- Normal Epithelial
- Tprolif
- Malignant Epithelial

c Ligand-Receptor Spatial d_ratio Density
(Sample P2_rep2)

e Cell Type Pair Distance Distribution
(Sample P2_rep2)

d Short & Long-range Interactions'
Ligand-receptor Gene Expression Distribution Examples
(Sample P2_rep2)

f Near & Far Cell Type Pair
Spatial Distribution Examples
(Sample P2_rep2)

g Interaction Patterns (Sample P2_rep2)

- B cell
- DC
- Endothelial
- Fibroblasts
- Normal Epithelial
- Malignant Epithelial

Data processing results and examples of the sample P2_rep2

a. The UMAP of scRNA-seq data of the sample P2_rep2, colored by cell types.

b. The STRIDE deconvolution result of the sample P2_rep2. The pie plot in each spot represents the predicted cell type proportions.

c. The density plot of the *d_ratio* distribution in the sample P2_rep2. The black dotted lines show the *d_ratio* boundaries of short-range and long-range interactions, respectively.
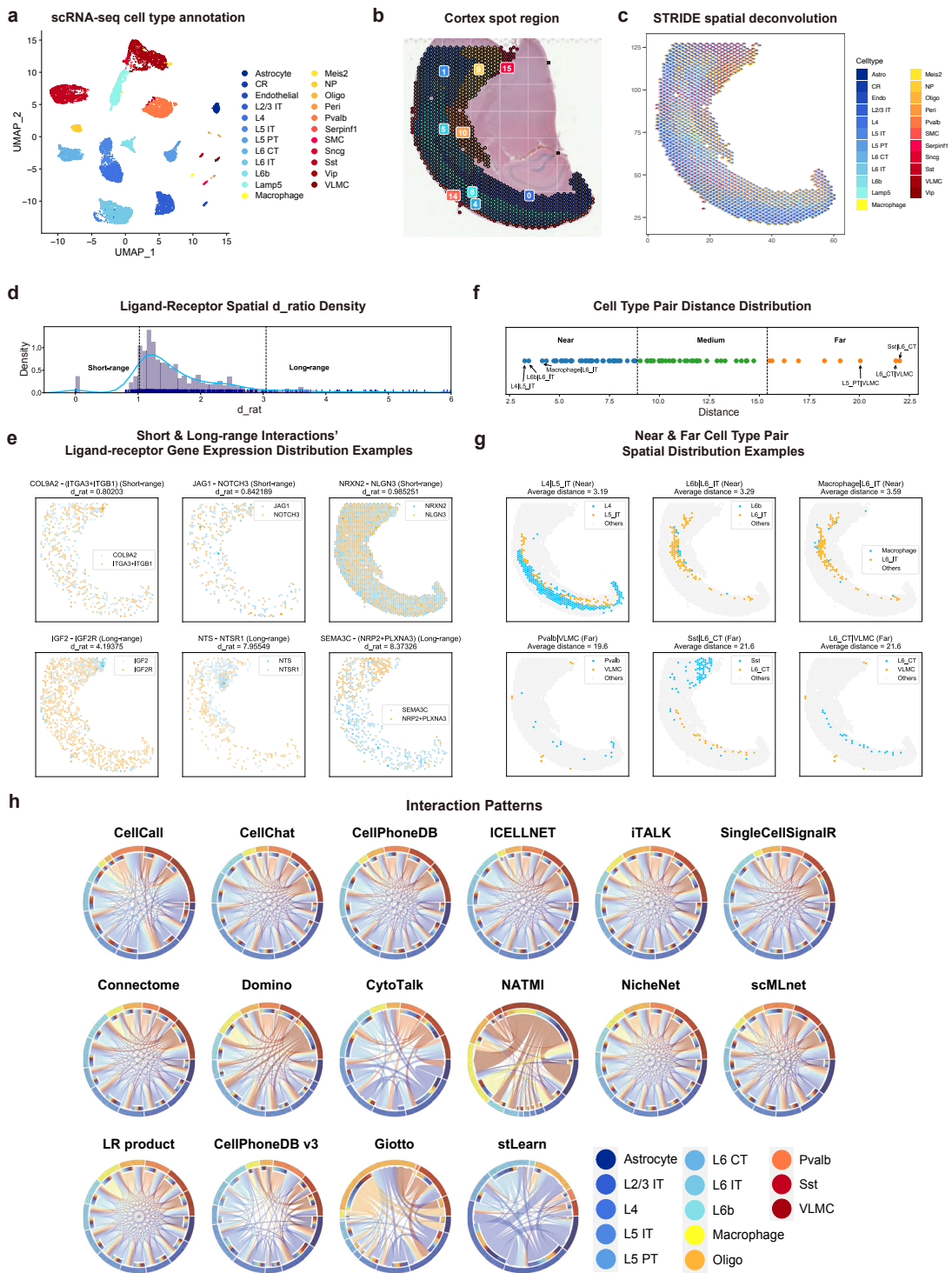
d. The examples of ligands (blue) and receptors' (orange) expression distributions in short-range (top) and long-range (bottom) interactions. The transparency of spot indicates the gene expression level.

e. The dot plot shows the distances of all cell type pairs in the sample P2_rep2, and the clustering results. The dot color stands for the type of cell type pair, blue for near cell type pair; green for medium cell type pair; orange for far cell type pair. The dotted lines are just for better displaying the different groups of cell type pairs and do not stand for the actual distance boundaries.

f. The examples of near (top) and far (bottom) cell type pairs' spatial distributions. The spot color is for dividing cell types.

g. The chord plot of the overall interaction pattern in the sample P2_rep2 predicted by each tool. Color indicates cell types. For readability, only cell types in near and far cell type pairs are included in chord plots. The segment size reflects the relative number of interactions that the corresponding cell type participated in. The arrow indicates the interaction direction between cell types and its width shows the relative interaction number.

Figure S6

a scRNA-seq cell type annotation

b Cortex spot region

c STRIDE spatial deconvolution

d Ligand-Receptor Spatial d_ratio Density

f Cell Type Pair Distance Distribution

e Short & Long-range Interactions'
Ligand-receptor Gene Expression Distribution Examples

g Near & Far Cell Type Pair
Spatial Distribution Examples

h Interaction Patterns

Data processing results and examples of the mouse cortex dataset

a. The UMAP of scRNA-seq data of the mouse cortex dataset, colored by cell types.

b. The spot clusters of the cortex region in the ST data. Numbers and colors for distinguishing different clusters. Two outliers (masked by black cross signs) in the cluster 15 are removed since they are absolutely not in the cortex region.

c. The STRIDE deconvolution result of mouse cortex dataset's ST data. The pie plot in

each spot represents the predicted cell type proportions.

d. The density plot of the *d_ratio* distribution in the mouse cortex dataset. The black dotted lines show the *d_ratio* boundaries of short-range and long-range interactions, respectively.
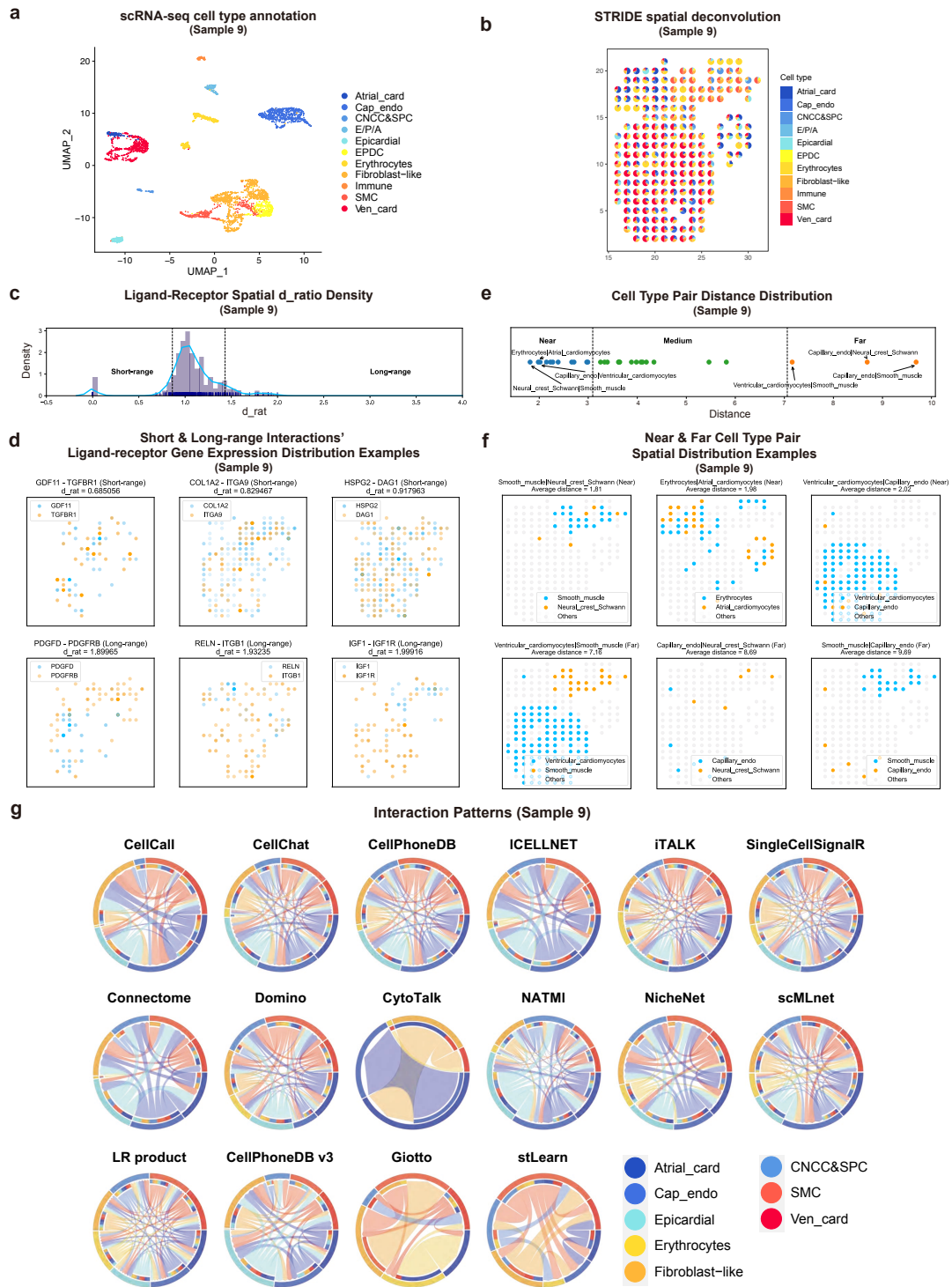
e. The examples of ligands (blue) and receptors' (orange) expression distributions in short-range (top) and long-range (bottom) interactions. The transparency of spot indicates the gene expression level.

f. The dot plot shows the distances of all cell type pairs in the mouse cortex dataset, and the clustering results. The dot color stands for the type of cell type pair, blue for near cell type pair; green for medium cell type pair; orange for far cell type pair. The dotted lines are just for better displaying the different groups of cell type pairs and do not stand for the actual distance boundaries.

g. The examples of near (top) and far (bottom) cell type pairs' spatial distributions. The spot color is for dividing cell types.

h. The chord plot of the overall interaction pattern in the mouse cortex dataset predicted by each tool. Color indicates cell types. For readability, only cell types in near and far cell type pairs are included in chord plots. The segment size reflects the relative number of interactions that the corresponding cell type participated in. The arrow indicates the interaction direction between cell types and its width shows the relative interaction number.

Figure S7

a scRNA-seq cell type annotation (Sample 9)

b STRIDE spatial deconvolution (Sample 9)

c Ligand-Receptor Spatial d_ratio Density (Sample 9)

e Cell Type Pair Distance Distribution (Sample 9)

d Short & Long-range Interactions' Ligand-receptor Gene Expression Distribution Examples (Sample 9)

f Near & Far Cell Type Pair Spatial Distribution Examples (Sample 9)

g Interaction Patterns (Sample 9)

Data processing results and examples of the sample 9

a. The UMAP of scRNA-seq data of the sample 9, colored by cell types.

b. The STRIDE deconvolution result of the sample 9. The pie plot in each spot represents the predicted cell type proportions.

c. The density plot of the *d_ratio* distribution in the sample 9. The black dotted lines show the *d_ratio* boundaries of short-range and long-range interactions, respectively.
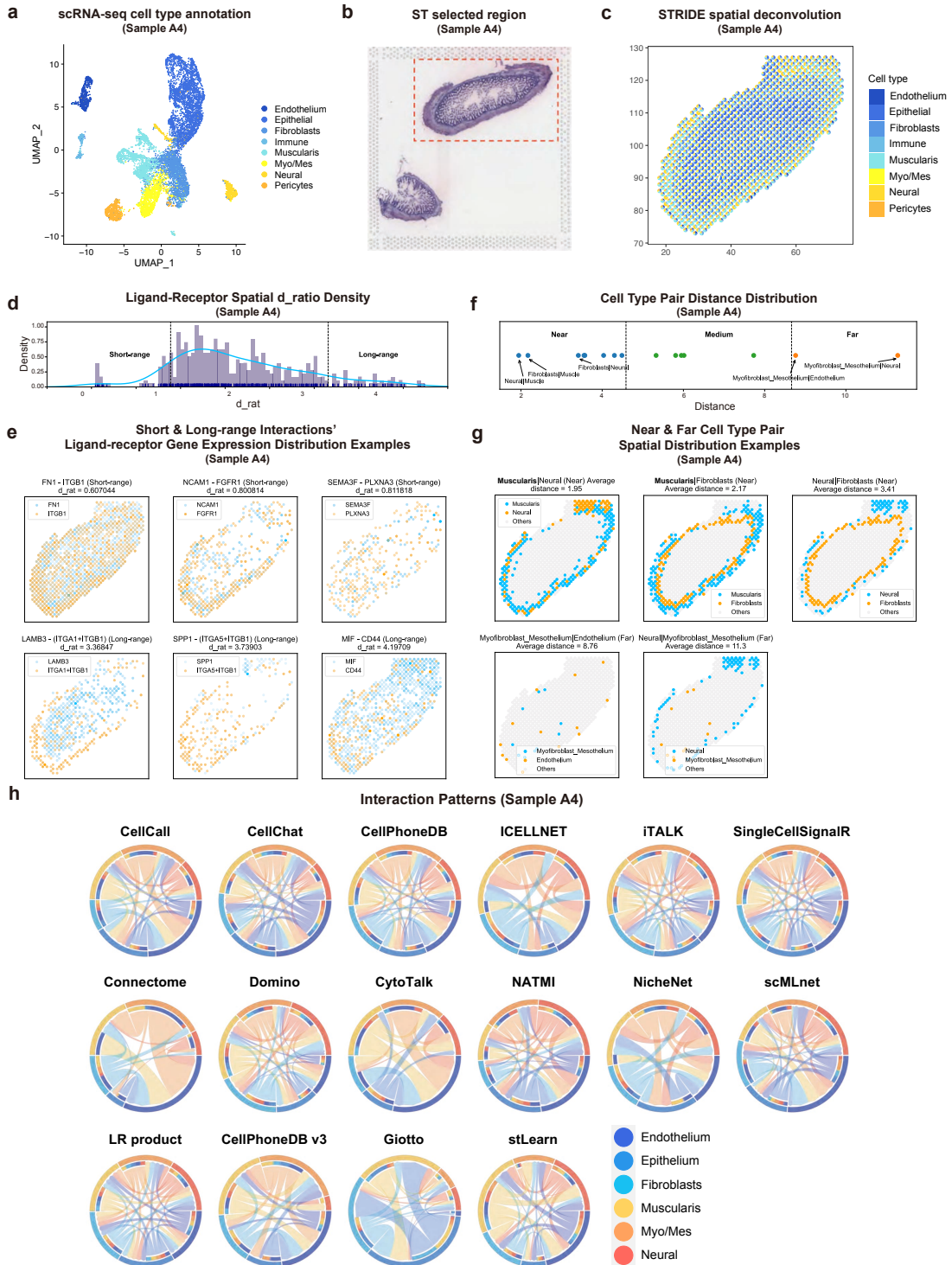
d. The examples of ligands (blue) and receptors' (orange) expression distributions in short-range (top) and long-range (bottom) interactions. The transparency of spot indicates the gene expression level.

e. The dot plot shows the distances of all cell type pairs in the sample 9, and the clustering results. The dot color stands for the type of cell type pair, blue for near cell type pair; green for medium cell type pair; orange for far cell type pair. The dotted lines are just for better displaying the different groups of cell type pairs and do not stand for the actual distance boundaries.

f. The examples of near (top) and far (bottom) cell type pairs' spatial distributions. The spot color is for dividing cell types.

g. The chord plot of the overall interaction pattern in the sample 9 predicted by each tool. Color indicates cell types. For readability, only cell types in near and far cell type pairs are included in chord plots. The segment size reflects the relative number of interactions that the corresponding cell type participated in. The arrow indicates the interaction direction between cell types and its width shows the relative interaction number.

Figure S8

a scRNA-seq cell type annotation (Sample A4)

b ST selected region (Sample A4)

c STRIDE spatial deconvolution (Sample A4)

d Ligand-Receptor Spatial d_ratio Density (Sample A4)

f Cell Type Pair Distance Distribution (Sample A4)

e Short & Long-range Interactions'
Ligand-receptor Gene Expression Distribution Examples (Sample A4)

g Near & Far Cell Type Pair
Spatial Distribution Examples (Sample A4)

h Interaction Patterns (Sample A4)

Data processing results and examples of the sample A4

a. The UMAP of scRNA-seq data of the sample A4, colored by cell types.

b. The actually used ST sample region in this study (marked by red dotted line).

c. The STRIDE deconvolution result of the sample A4. The pie plot in each spot represents the predicted cell type proportions.

d. The density plot of the *d_ratio* distribution in the sample A4. The black dotted lines show the *d_ratio* boundaries of short-range and long-range interactions, respectively.

e. The examples of ligands (blue) and receptors' (orange) expression distributions in short-range (top) and long-range (bottom) interactions. The transparency of spot indicates the gene expression level.

f. The dot plot shows the distances of all cell type pairs in the sample A4, and the clustering results. The dot color stands for the type of cell type pair, blue for near cell type pair; green for medium cell type pair; orange for far cell type pair. The dotted lines are just for better displaying the different groups of cell type pairs and do not stand for the actual distance boundaries.

g. The examples of near (top) and far (bottom) cell type pairs' spatial distributions. The spot color is for dividing cell types.

h. The chord plot of the overall interaction pattern in the sample A4 predicted by each tool. Color indicates cell types. For readability, only cell types in near and far cell type pairs are included in chord plots. The segment size reflects the relative number of interactions that the corresponding cell type participated in. The arrow indicates the interaction direction between cell types and its width shows the relative interaction number.

Figure S9
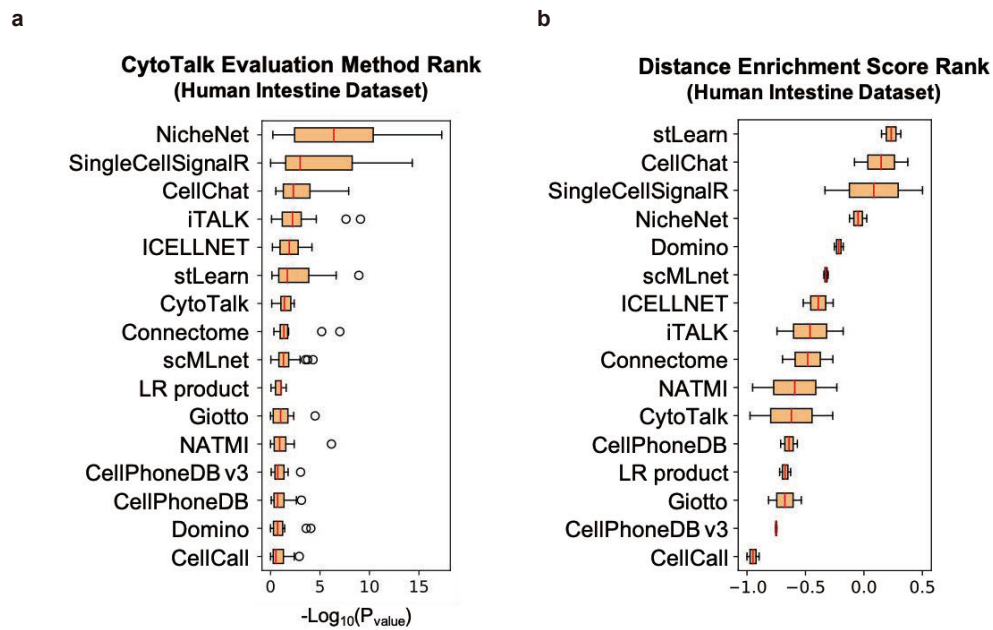
Evaluation results of the CytoTalk and DES metrics are similar.

a. Evaluation rank in human intestinal dataset using CytoTalk evaluation method. The box reflects the overall statistical significance across all samples and cell types. Tools are sorted by the median statistical significance. The 'LR product' represents the baseline method based on the product of ligand and receptor expressions.

b. DES rank in the human intestinal dataset. The box plot reflects the overall distribution of DES across all samples. Tools are sorted by the median DES. The 'LR product' represents the baseline methxsod based on the product of ligand and receptor expressions.