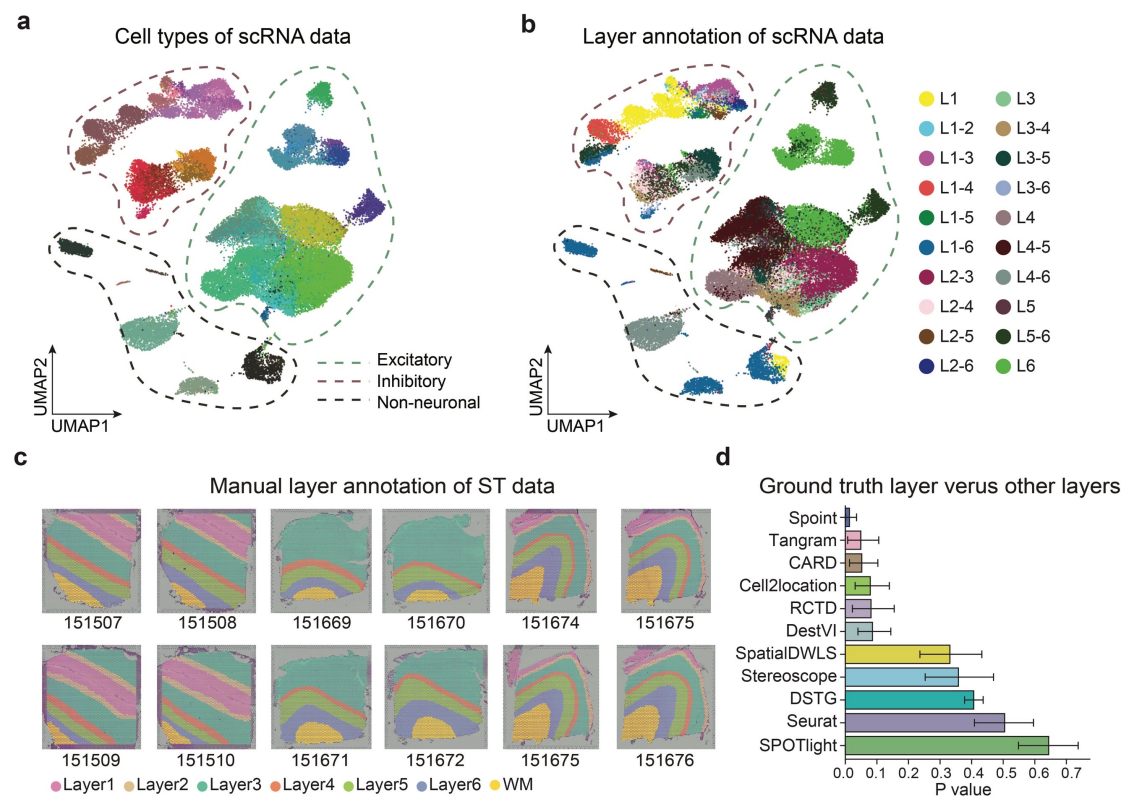


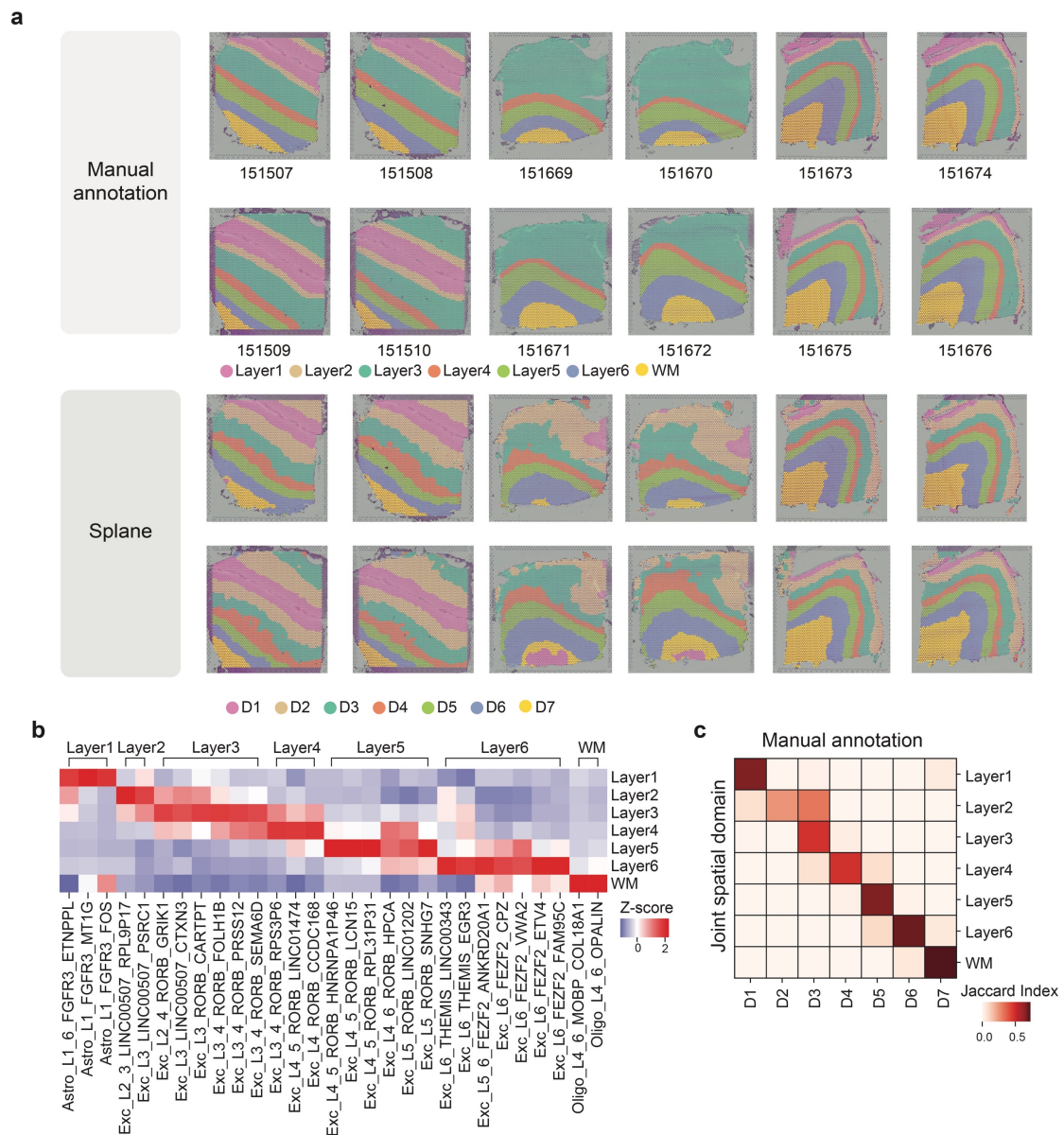
Supplementary Figures for “SPACEEL: characterizing
spatial transcriptome architectures by deep-learning”

Supplementary Figure 1



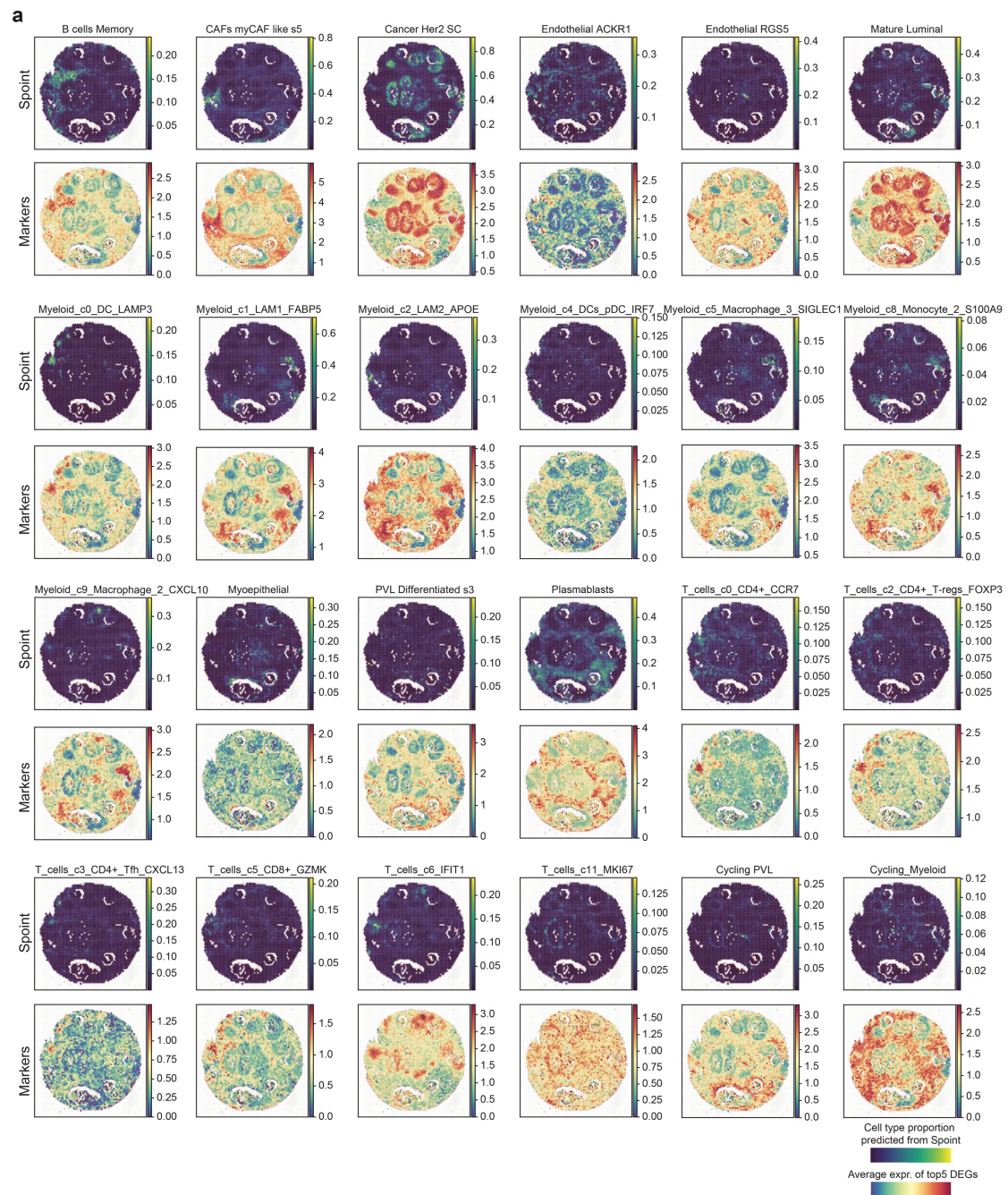
Supplementary Figure 1. a,b, Uniform manifold approximation and projection (UMAP) of all cells from the human brain scRNA-seq dataset downloaded from the Allen Brain Map, colored with the cell types (a) and the cortical layers (b) annotated by the original study (Yao *et al. Cell* 184, 3222, 2021). **c**, Cortical layer 1~6 and white matter (WM) for the 12 ST slices of the DLPFC dataset, annotated by the original study (Maynard *et al. Nat. Neurosci.* 24, 425, 2021). **d**, P-values of the deconvolution results generated by Spoint and other deconvolution methods for the DLPFC dataset. Bar heights, mean values; whiskers, 1.5× interquartile range; n=56 cell types.

Supplementary Figure 2



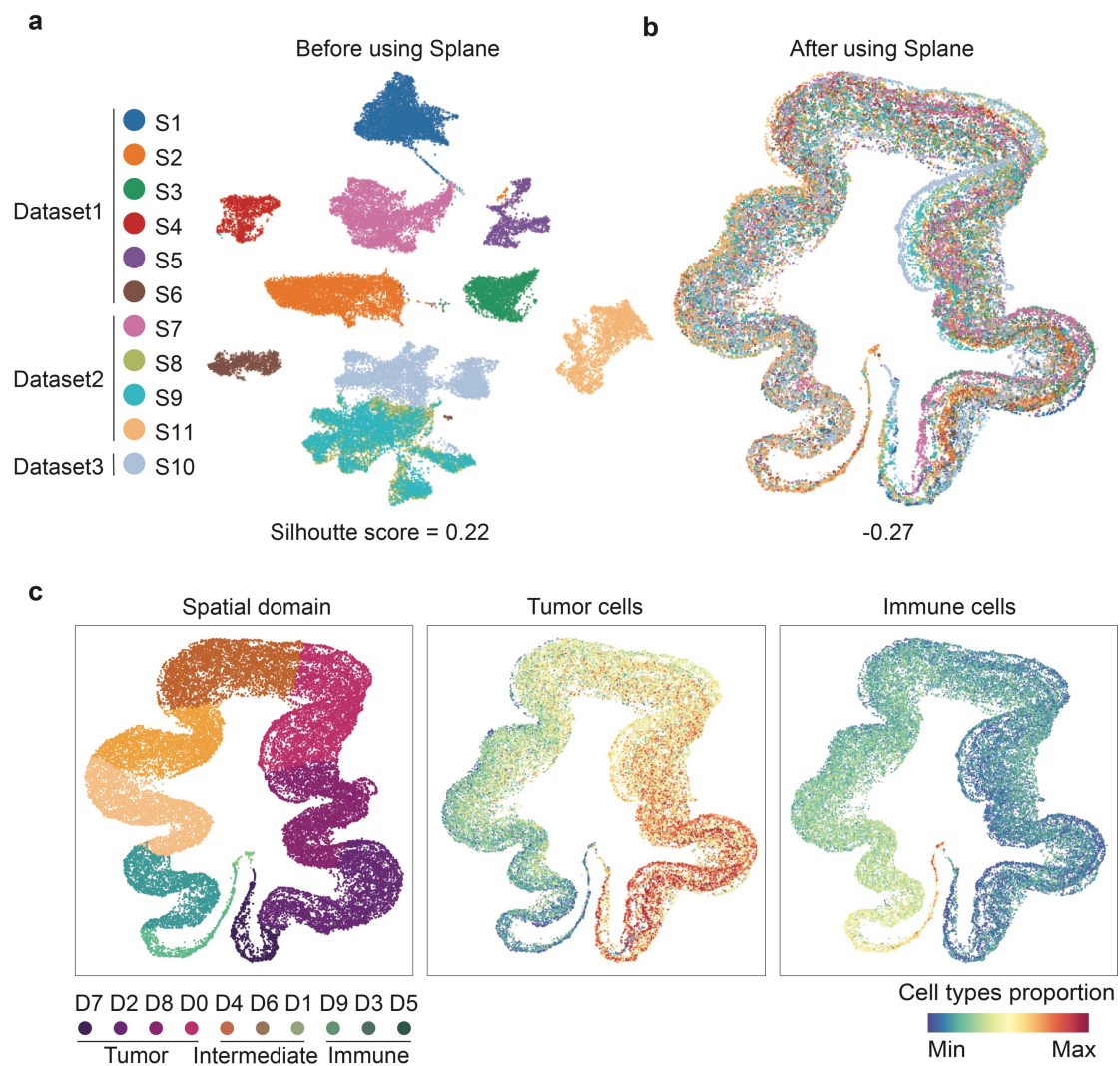
Supplementary Figure 2. a, Cortical layer 1~6/WM annotated by the original study (Maynard *et al. Nat. Neurosci.* 24, 425, 2021; upper panels) and spatial domains identified by Splane (lower panels) for the 12 ST slices of the DLPFC dataset. **b**, Proportion of each cell sub-type predicted by Spoint in cortical layers 1-6 and WM. **c**, Jaccard Index between the cortical layers/WM annotated by the original study and the spatial domains identified by Splane.

Supplementary Figure 3



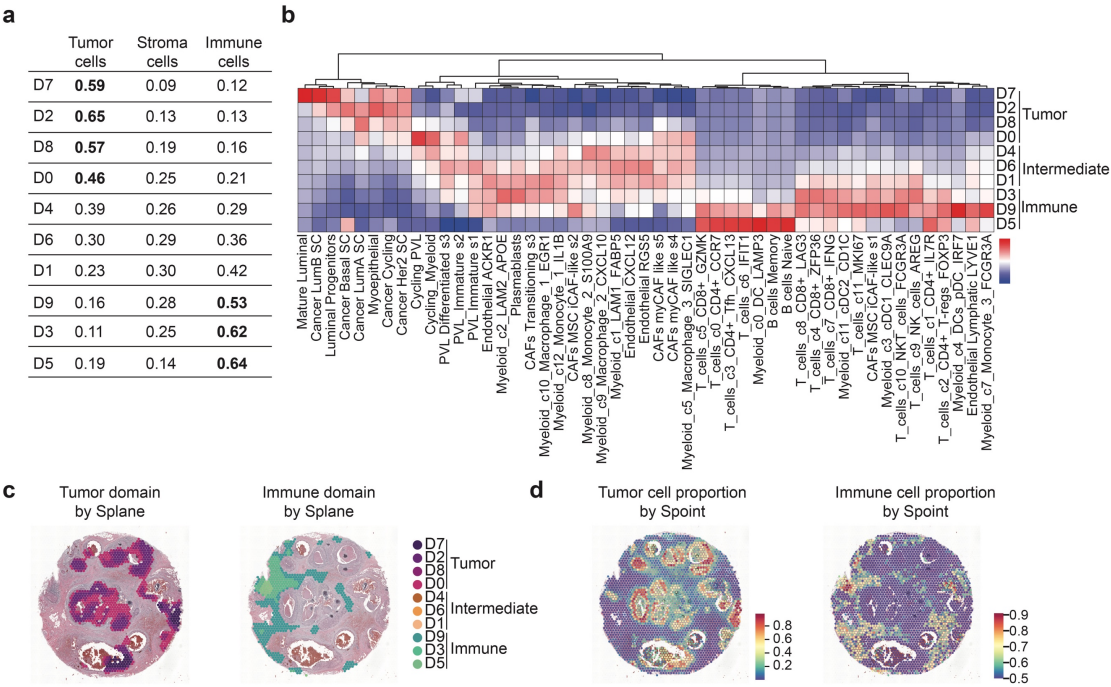
Supplementary Figure 3. Cell type compositions predicted by Spout for breast cancer ST slice S11 from the 10X Genomics dataset (<https://www.10xgenomics.com/resources/datasets>).

Supplementary Figure 4



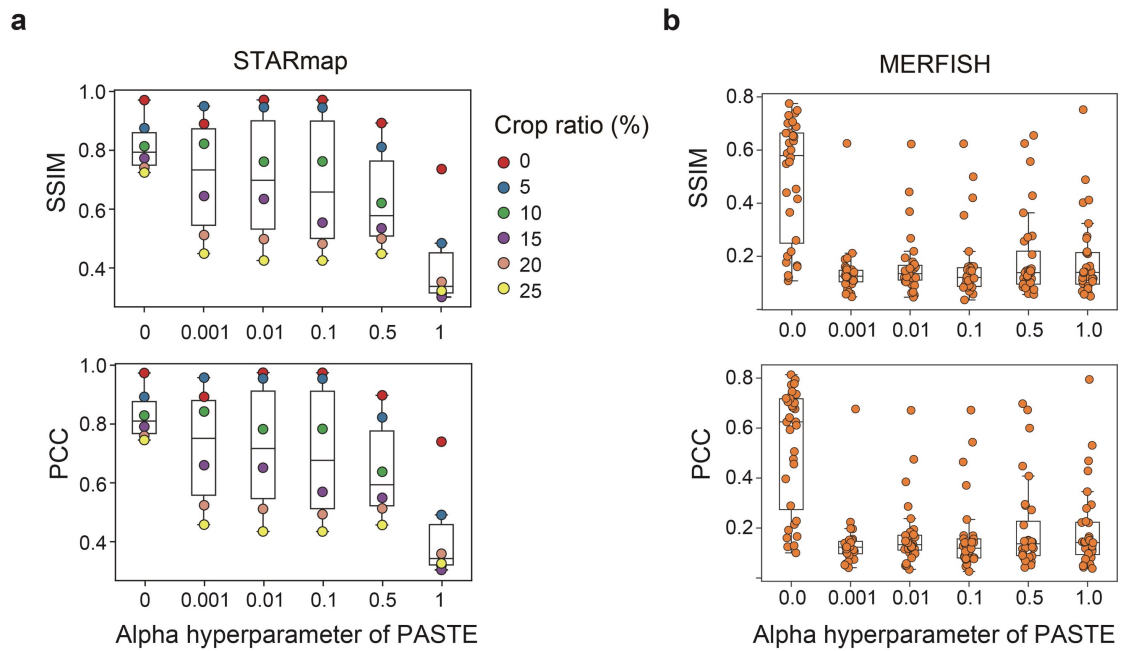
Supplementary Figure 4. a,b, UMAP of all spots from the 11 breast cancer slices of three different datasets, calculated from the raw expression matrix (a) and the shared latent features generated by Splane (b). The silhouette coefficient represents the ratio of within-cluster to inter-cluster dissimilarity (Liu et al. *Nat. Comm.* 11, 3155, 2020). A higher Silhouette score indicates a strong batch effect between slices from different experimental batches. **c,** Distributions of spatial domains, tumor cells, and immune cells in the UMAP of all spots.

Supplementary Figure 5



Supplementary Figure 5. a, Proportions of tumor cells, stroma cells, and immune cells in spatial domains identified by Splane for the 11 breast cancer slices. **b**, Proportion of each cell sub-type in spatial domains identified by Splane. **c**, Distributions of tumor domains and immune domains in slice S11. **d**, Proportions of tumor cells and immune cells in slice S11.

Supplementary Figure 6



Supplementary Figure 6. Optimization of the α hyperparameter of PASTE when processing the STARmap (a) and MERFISH (b) datasets. α is set to 0, as it generated the best results for both datasets.

a

Slice10 Slice15 Slice21 Slice26 Slice32

Cell-type

Spatial domain

Legend for Cell-type:

- L2/3 IT
- L4/5 IT
- L5 IT
- L5 ET
- L5/6 NP
- L6 IT
- L6 CT
- L6b
- Lamp5
- Pvalb
- Sncg
- Sst
- Vip
- Other

Legend for Spatial domain:

- Domain1
- Domain2
- Domain3
- Domain4
- Domain5
- Domain6
- Domain7

b

L2/3 IT L4/5 IT L5 IT L5 ET L5/6 NP L6 IT L6 CT L6b

1 2 3 4 5 SSn1 SS12 1 2 3 4 5 1 2 3 4 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 6 7 8 9 10 1 2 3

Cortical depth

Domain1

Domain2

Domain3

Domain4

Domain5

Domain6

Domain7

c

Jaccard Index between spatial domains and cortical layers

Legend for Jaccard Index:

- 0.6
- 0.3
- 0.0

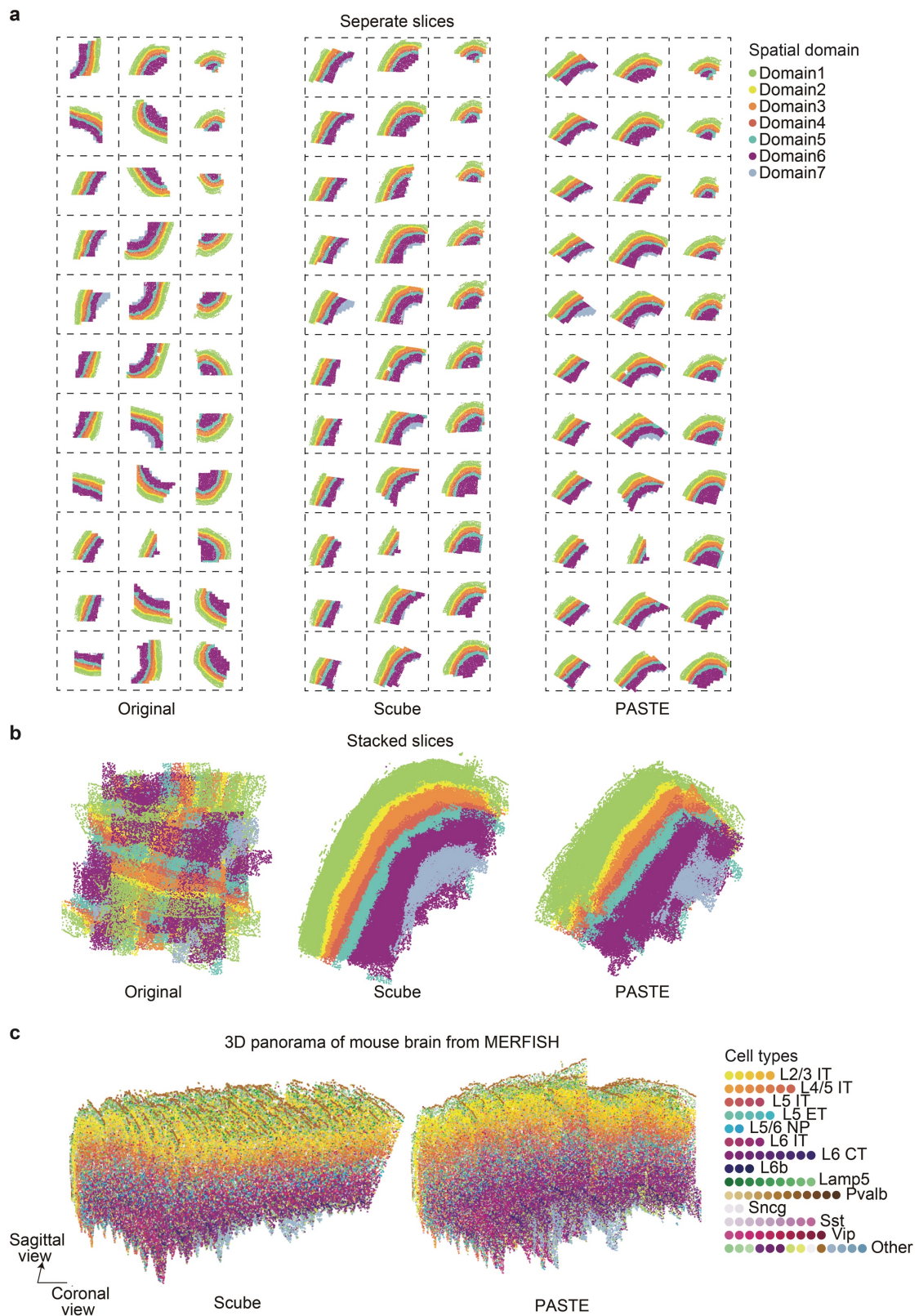
Legend for Jaccard Index (Spatial domains):

- L1
- L2/3
- L4
- L5
- L6
- L6b
- WM

Legend for Jaccard Index (Cortical layers):

- Domain1
- Domain2
- Domain3
- Domain4
- Domain5
- Domain6
- Domain7

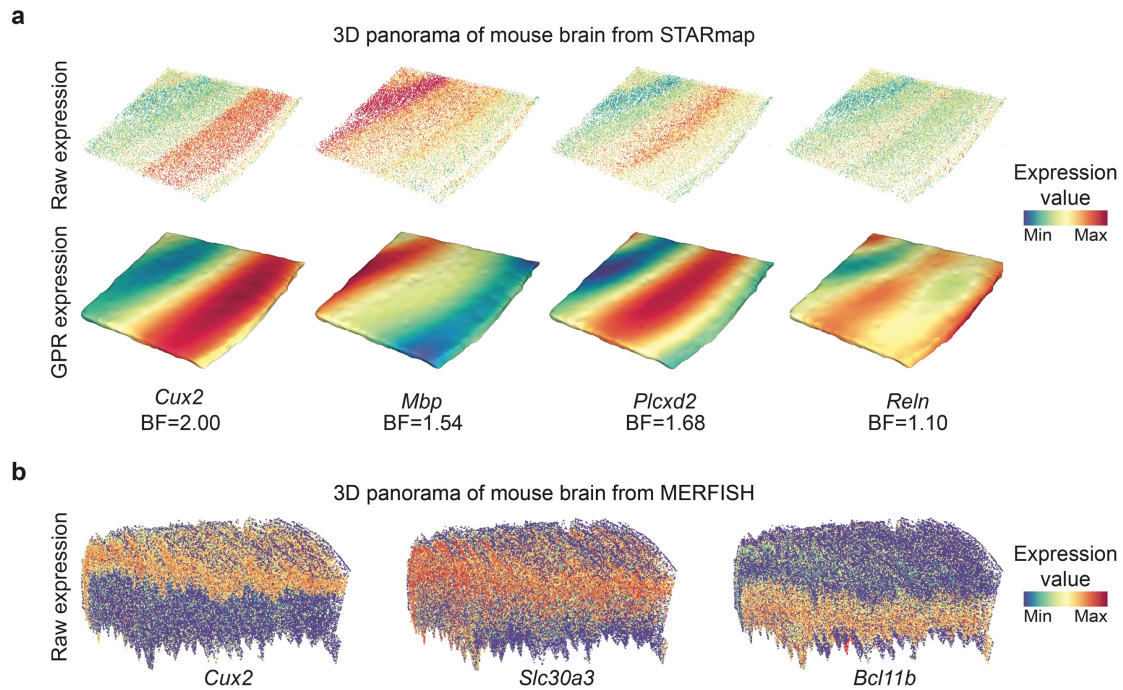
Supplementary Figure 8



Supplementary Figure 8. a, From left to right are the original positions, Scube-transformed positions, and PASTE-transformed positions of the 33 ST slices from the mouse brain MERFISH dataset. **b**, From left to right are the slices stacked according to

the original positions, the Scube-transformed positions, and the PASTE-transformed positions. **c**, The 3D alignment constructed by Scube and PASTE. Cells are labelled by cell type.

Supplementary Figure 9



Supplementary Figure 9. a, Raw expression distributions (upper panels) and GPR model-predicted expression distributions (lower panels) of *Cux2*, *Mbp*, *Plcxd2*, and *Reln* on the coronal plane of the 3D alignment constructed by Scube for the MOp MERFISH dataset. **b**, The expression distribution of *Cux2*, *Slc30a3*, and *Bcl11b* in the 3D alignment constructed by Scube.