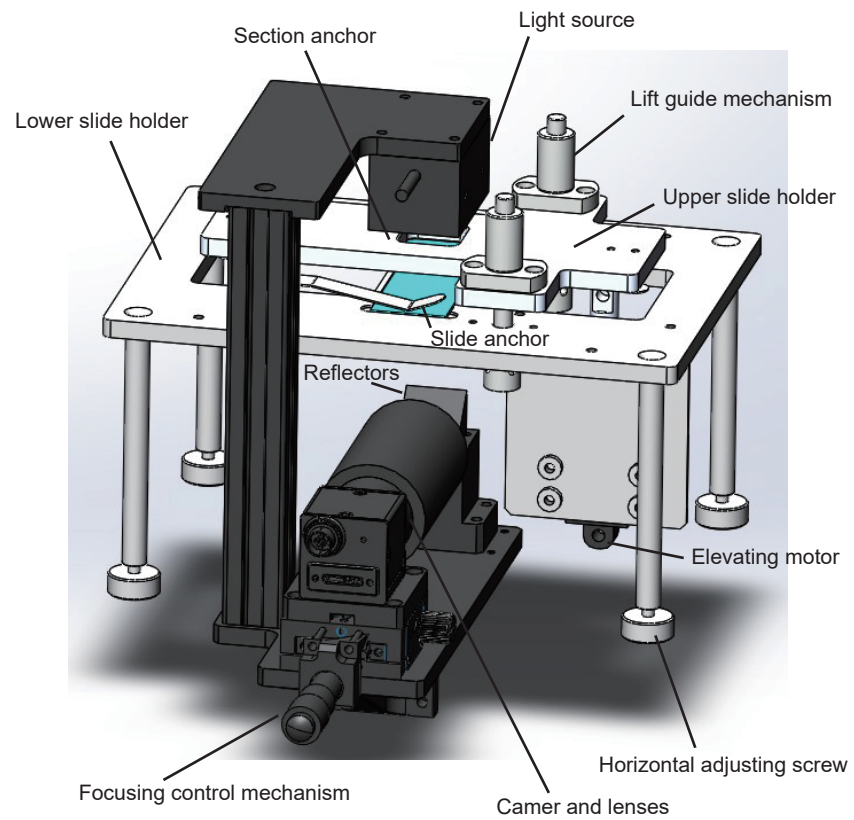
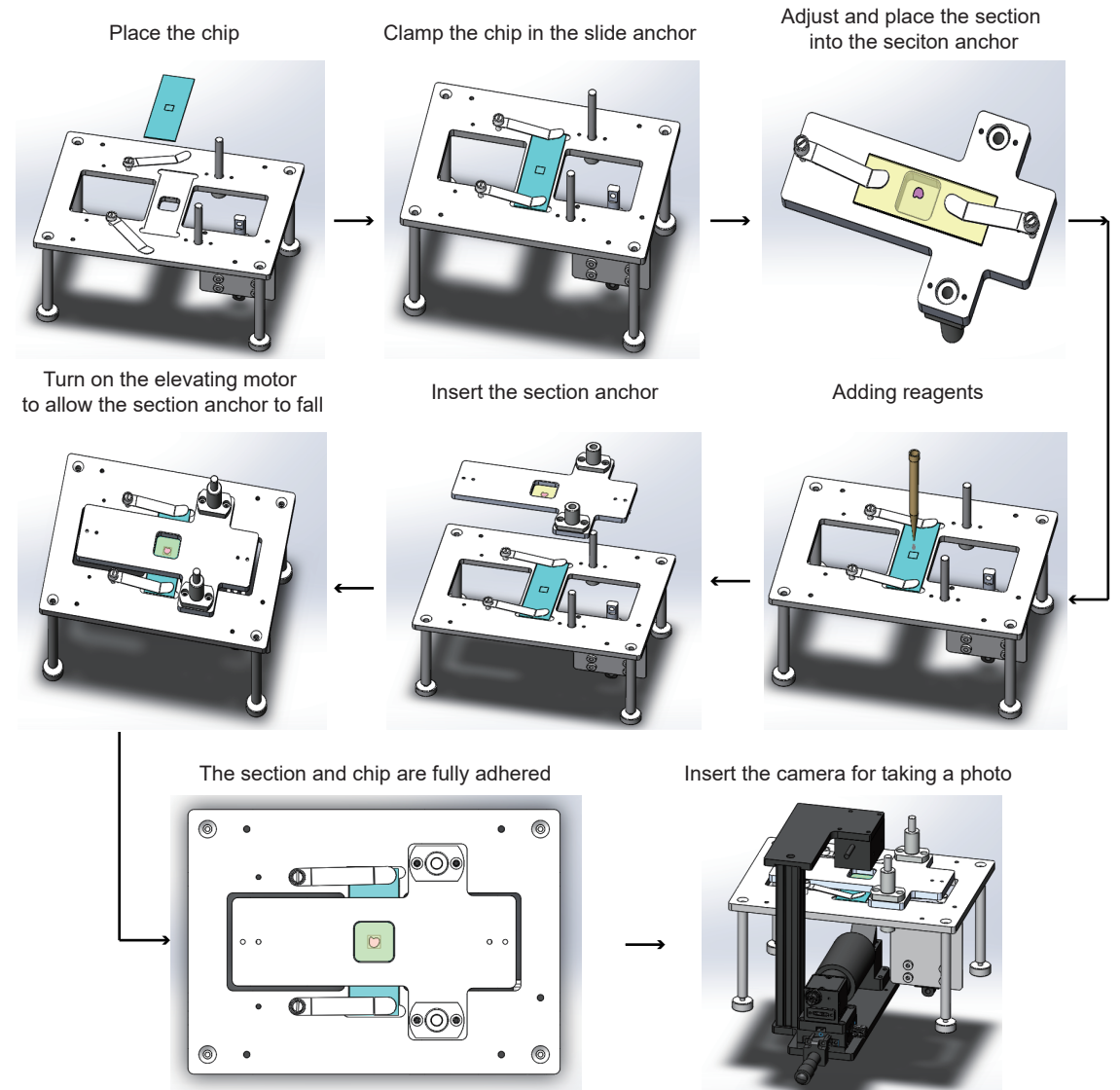


Supplementary Figure 1

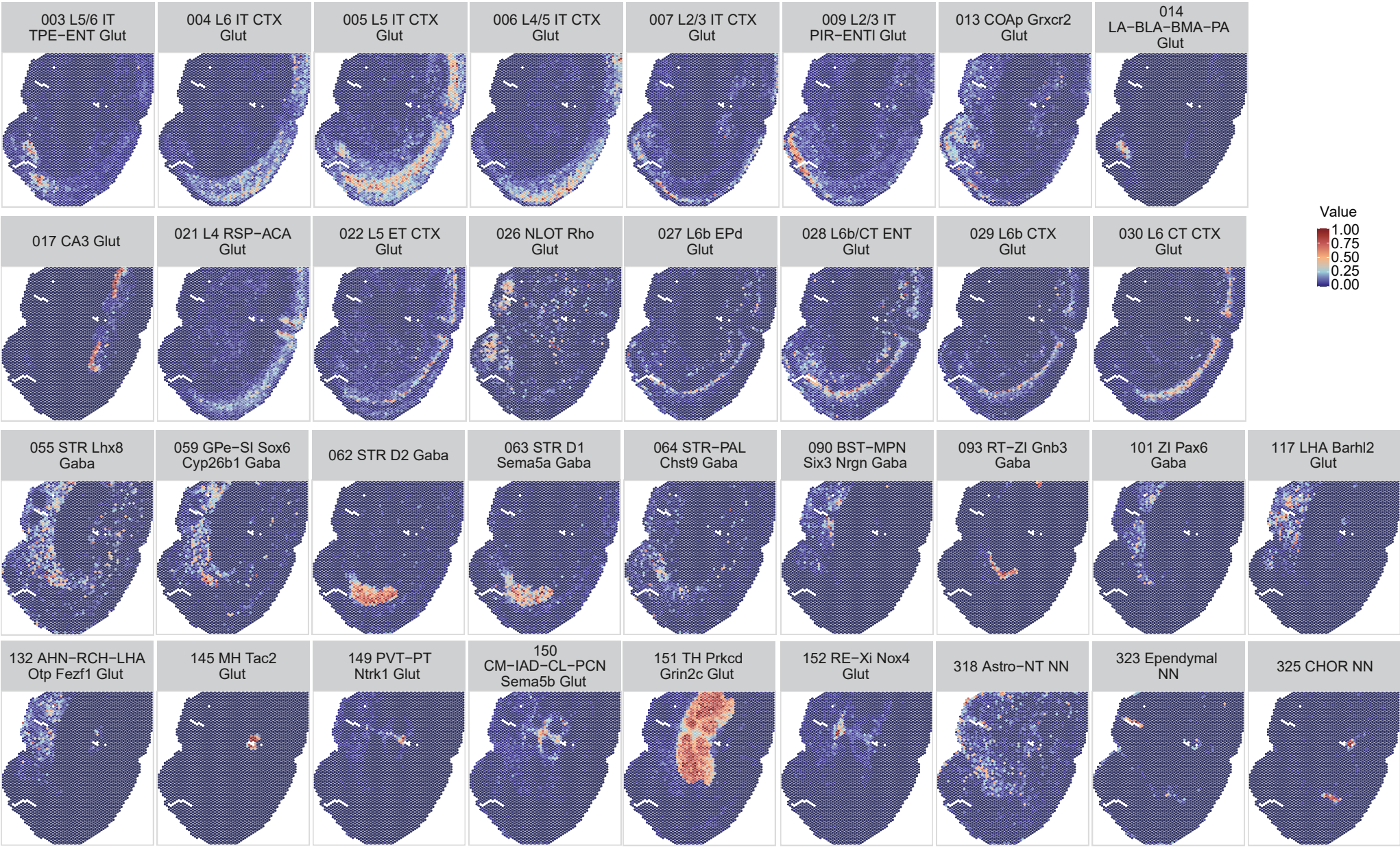
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b

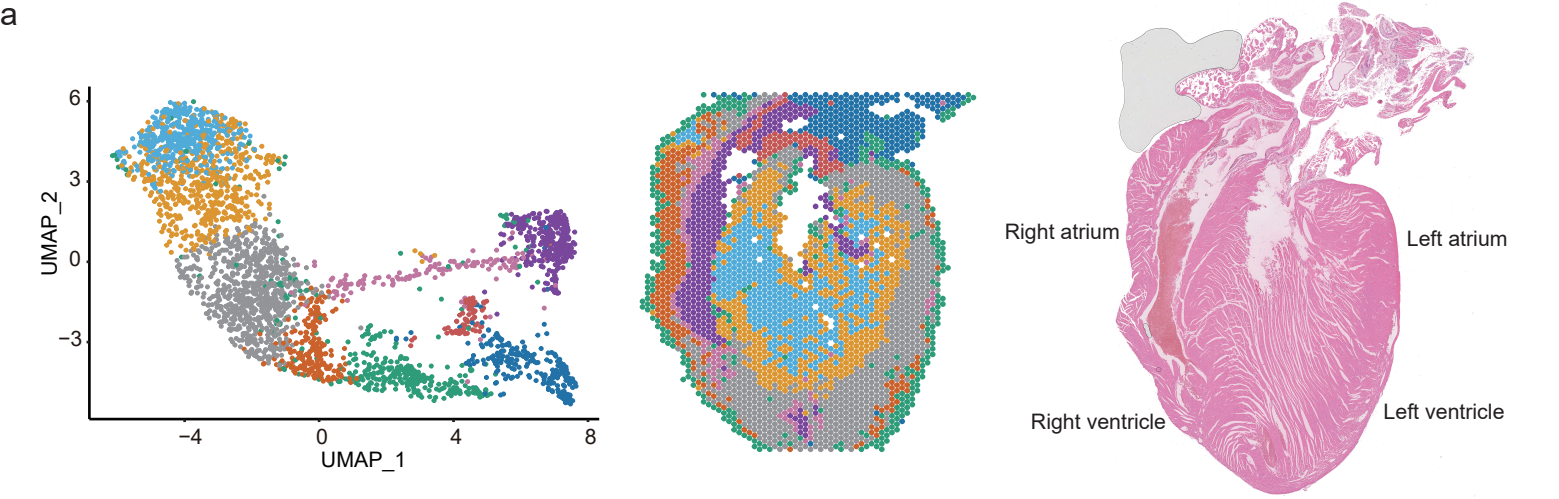


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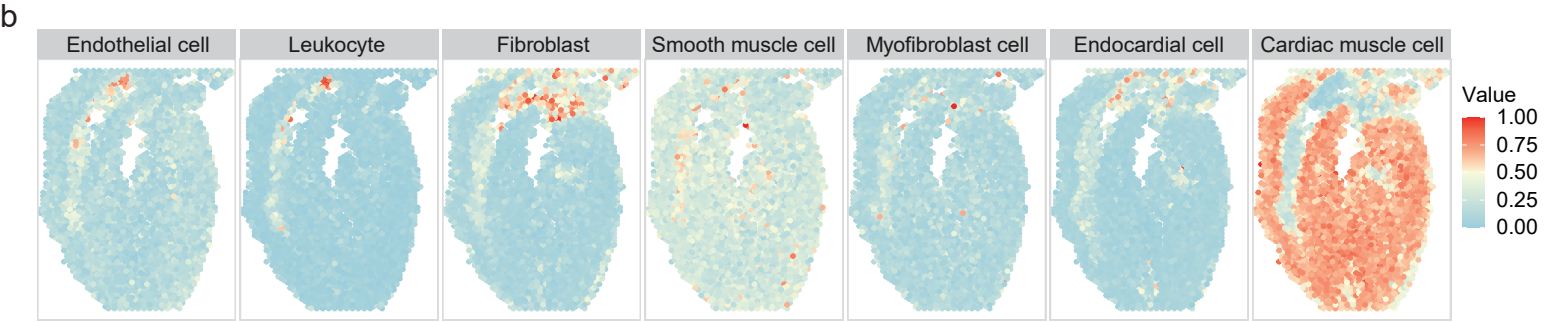


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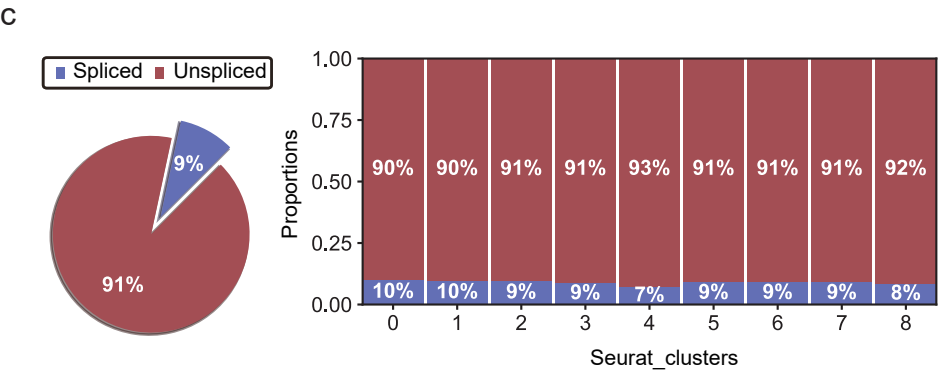
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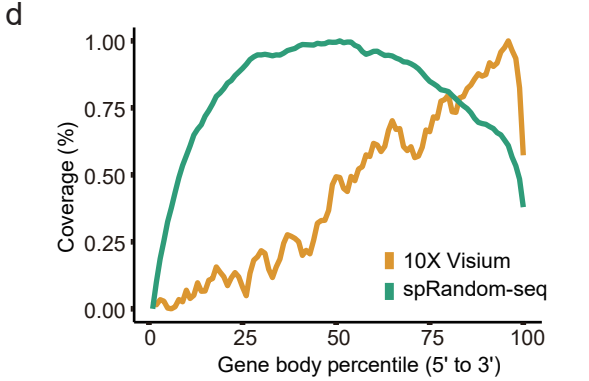
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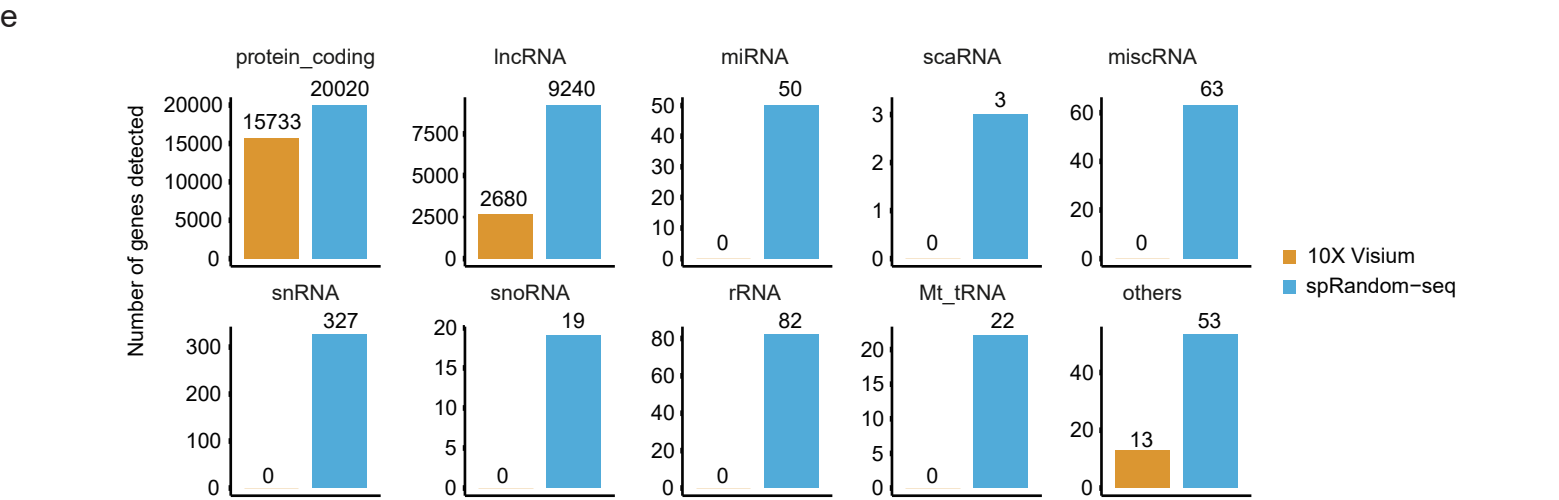
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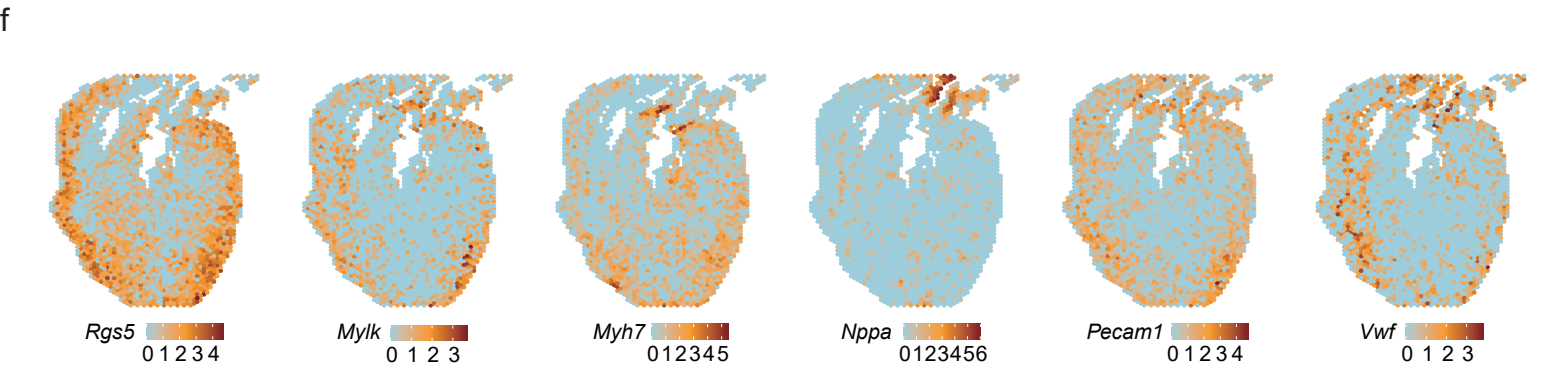
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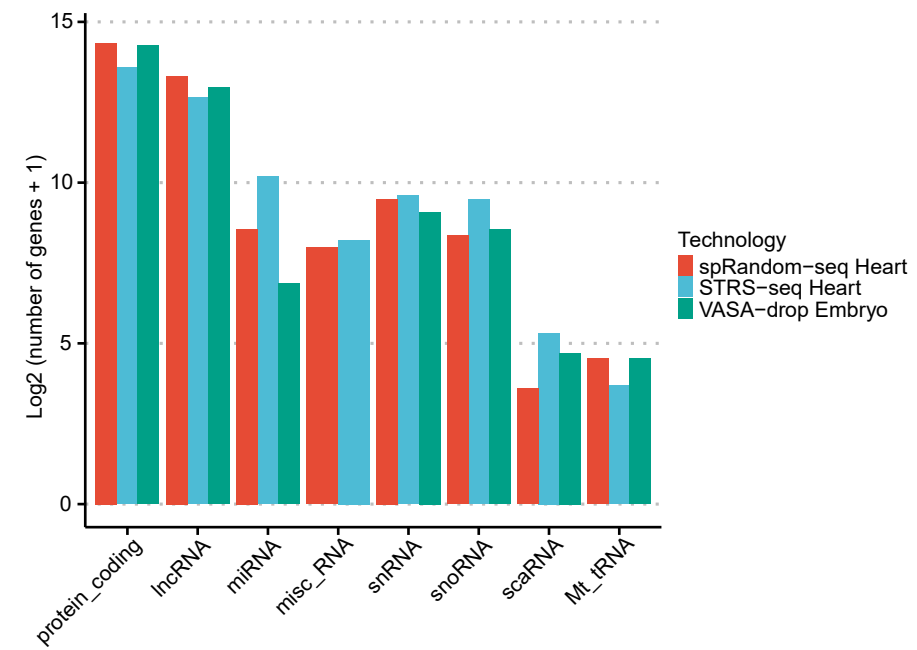
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Supplementary Figure 4

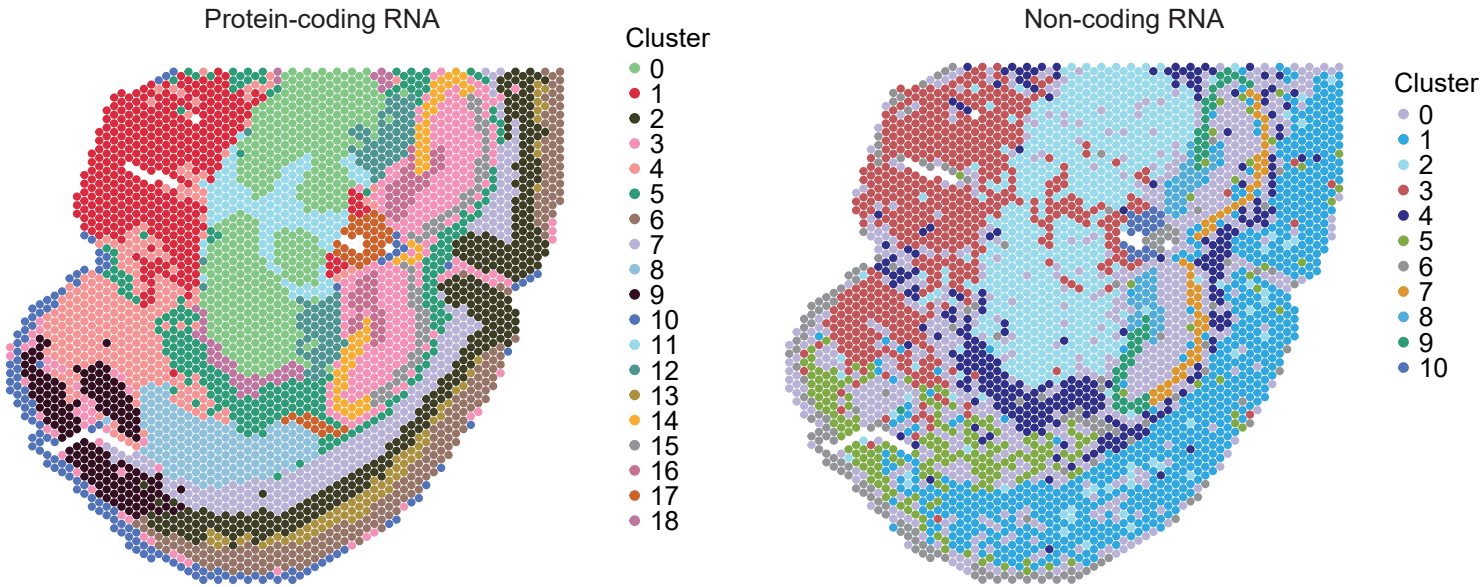


Supplementary Figure 5

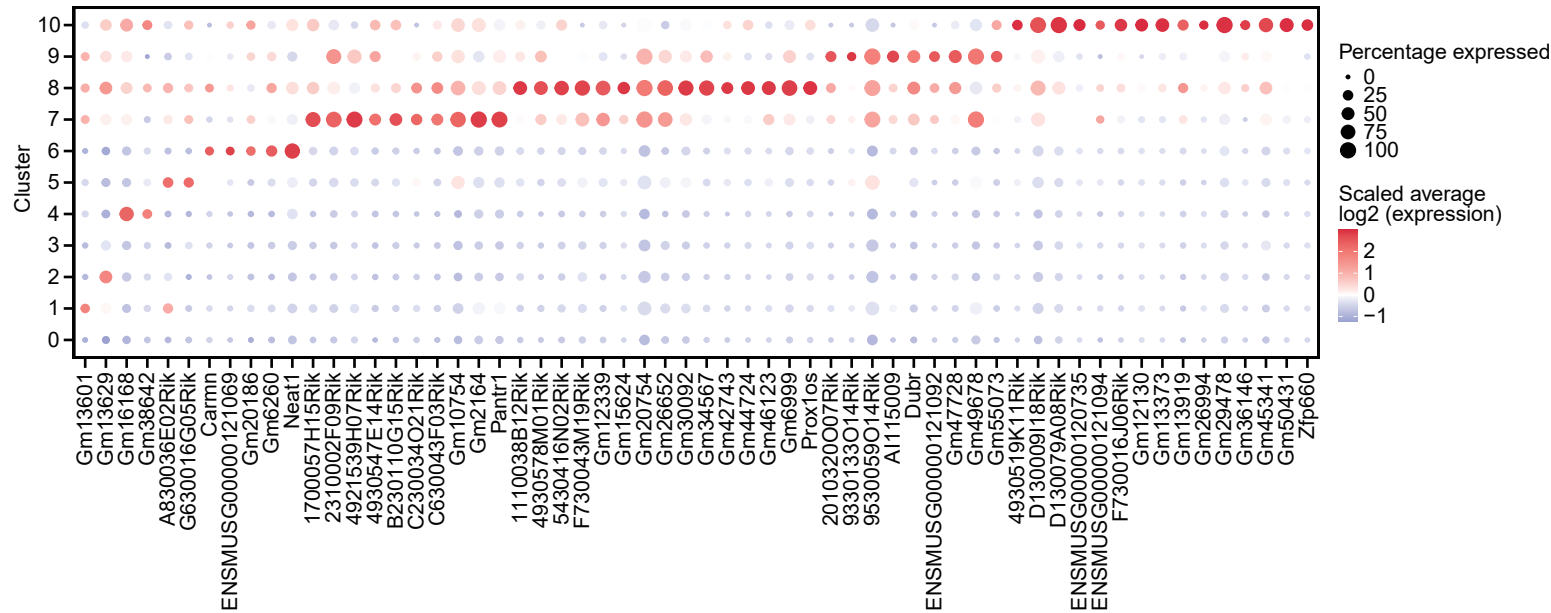


Supplementary Figure 6

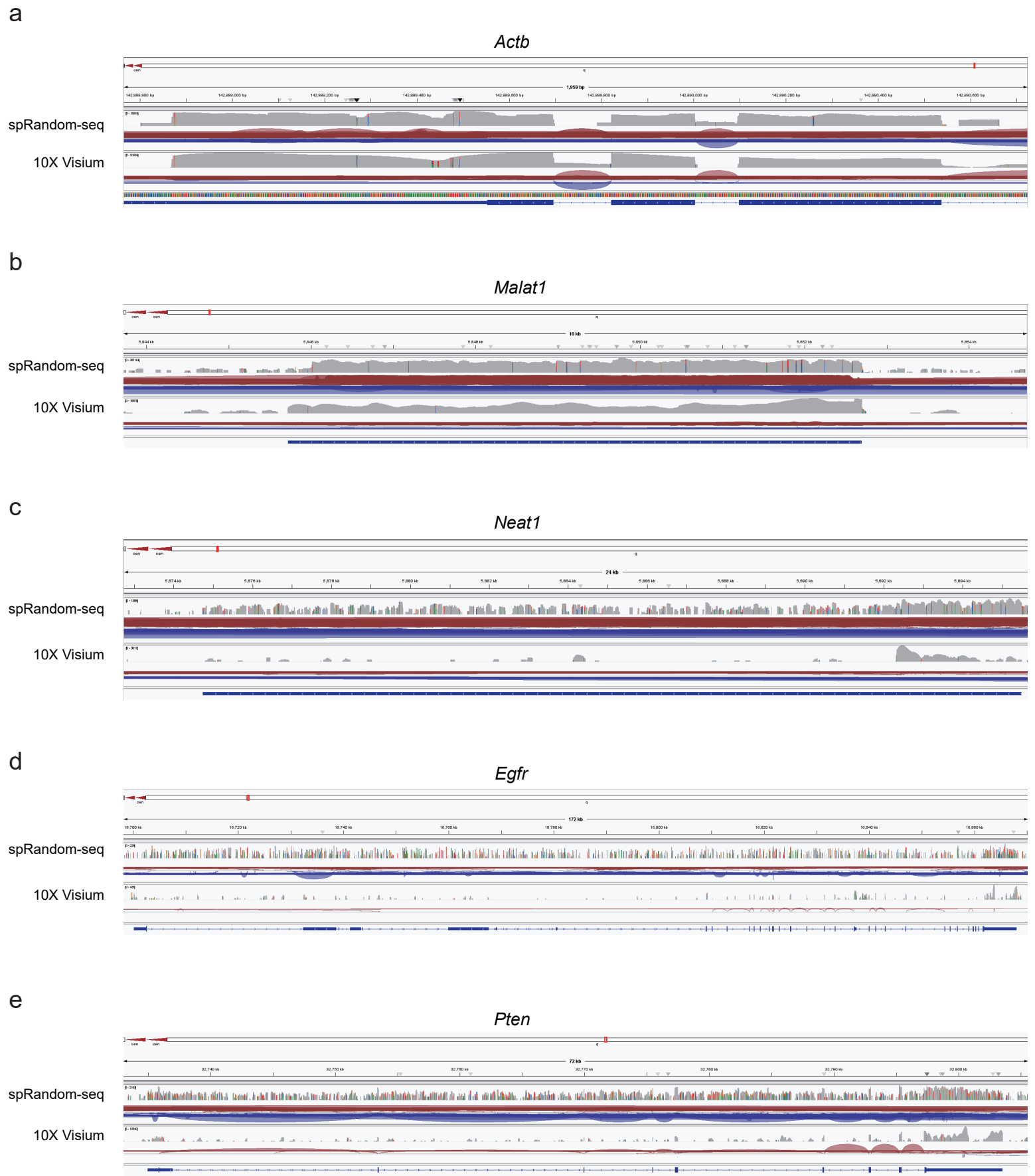
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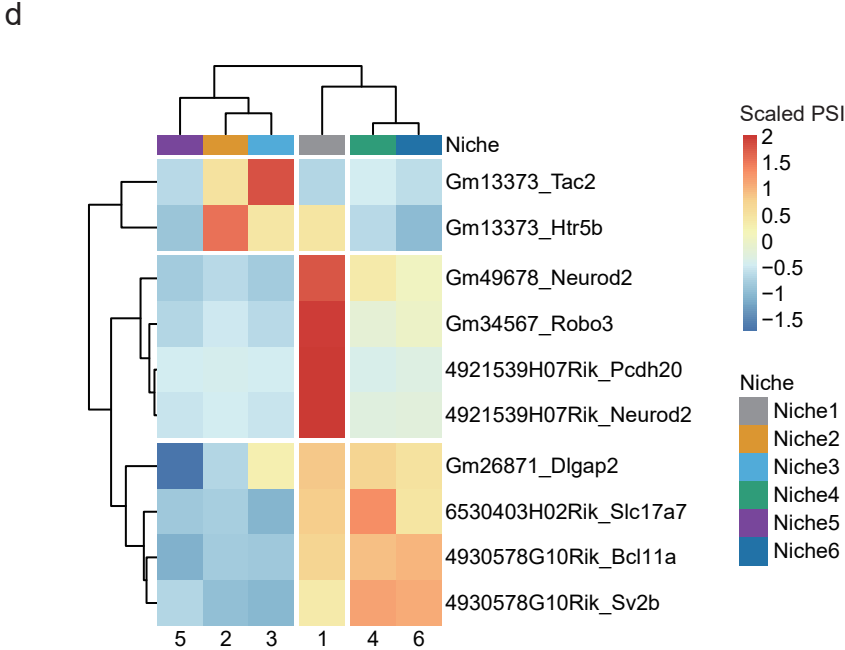
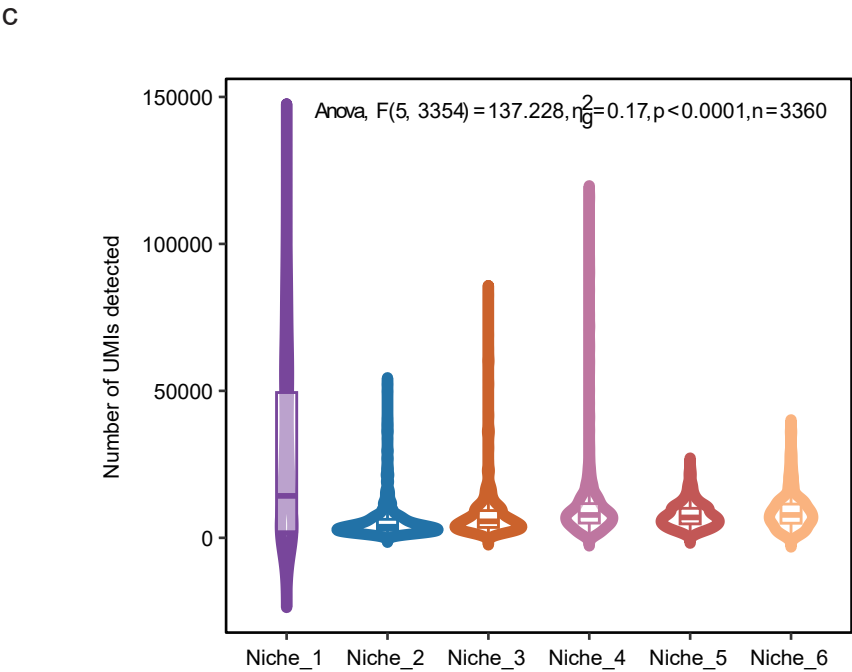
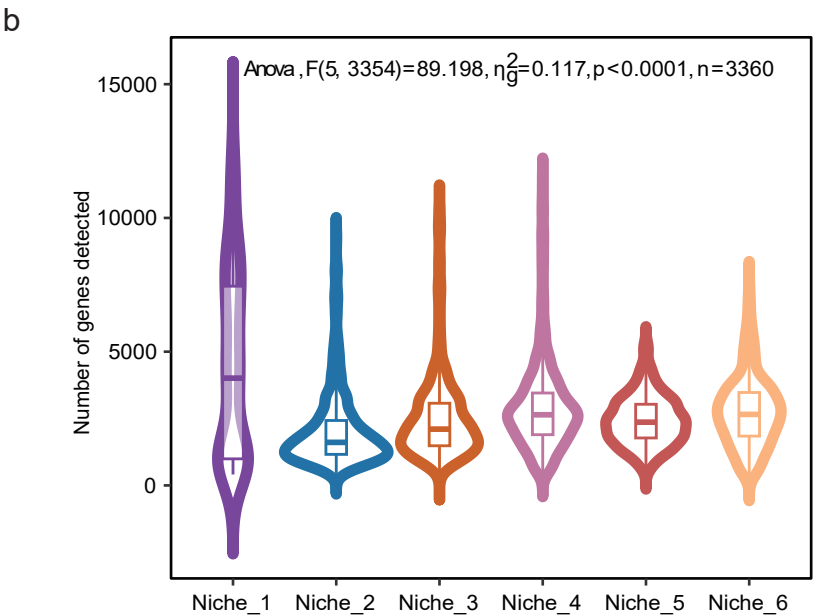
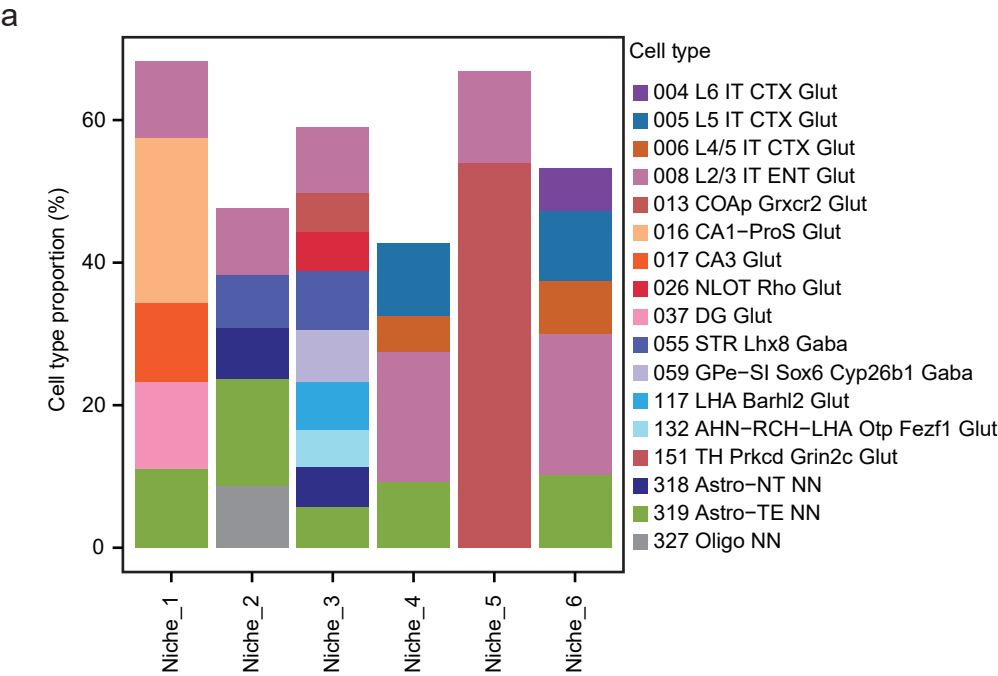
b



Supplementary Figure 7



Supplementary Figure 8



Supplementary Fig. 1 | A physical photograph of the home-made instrument. a, Schematic diagram of the home-made instrument for cDNA transferred. **b,** Flow diagram of the home-made instrument for cDNA transferred step by step.

Supplementary Fig. 2 | Spatial maps of the rest cell-type for spRandom-seq in FFPE mouse brain section.

Supplementary Fig. 3 | Validation and benchmark of spRandom-seq using a FFPE mouse heart section. a, Spatial visualization of FFPE mouse heart section analyzed by spRandom-seq at 55 μm resolution. **b,** Spatial maps of classic cell-type for spRandom-seq in FFPE mouse heart section. **c,** Percents of spliced and unspliced transcripts in different cell clusters. **d,** Read coverage along the gene body by spRandom-seq in FFPE mouse heart section. **e,** Counts of different RNA biotypes detected in FFPE mouse brain section. **f,** Spatial maps of classic cell-type markers genes for spRandom-seq in the FFPE mouse heart section. Spots in which the transcript was not detected are shown as yellow. Green scale indicates log-normalized expression.

Supplementary Fig. 4 | Spatial visualization of six selected genes expressions of the FFPE mouse brain section and ISH images of the adult mouse brain taken from ABA.

Supplementary Fig. 5 | The RNA biotype compositions of three total RNA seq methods (spRandom-seq, VASA-drop and STRS-seq). The selected sections for the three methods were: FFPE mouse heart, fresh frozen mouse embryo and fresh frozen mouse heart respectively.

Supplementary Fig. 6 | Characterization of non-coding RNA in mouse brain using spRandom-seq. a, UMAP of clustering results of coronal FFPE mouse brain section using only protein-coding or non-coding RNAs respectively. b, Dotplot of top differentially expressed ncRNAs in each cluster based on the unsupervised clustering with only ncRNAs

Supplementary Fig. 7 | The IGV track for five genes from 10X Visium (fresh frozen mouse coronal brain) and spRandom-seq (FFPE mouse coronal brain): *Actb*, *Malat1*, *Neat1*, *Egfr* and *Pten*.

Supplementary Fig. 8 | Characterization of lncRNA across niches in mouse brain using spRandom-seq. a, The major cell

type proportion of each niche. **b**, The number of genes detected for each niche. **c**, The number of UMI detected for each niche. **d**, Heatmap showing the correlation between niches and lncRNA-mRNA pairs.