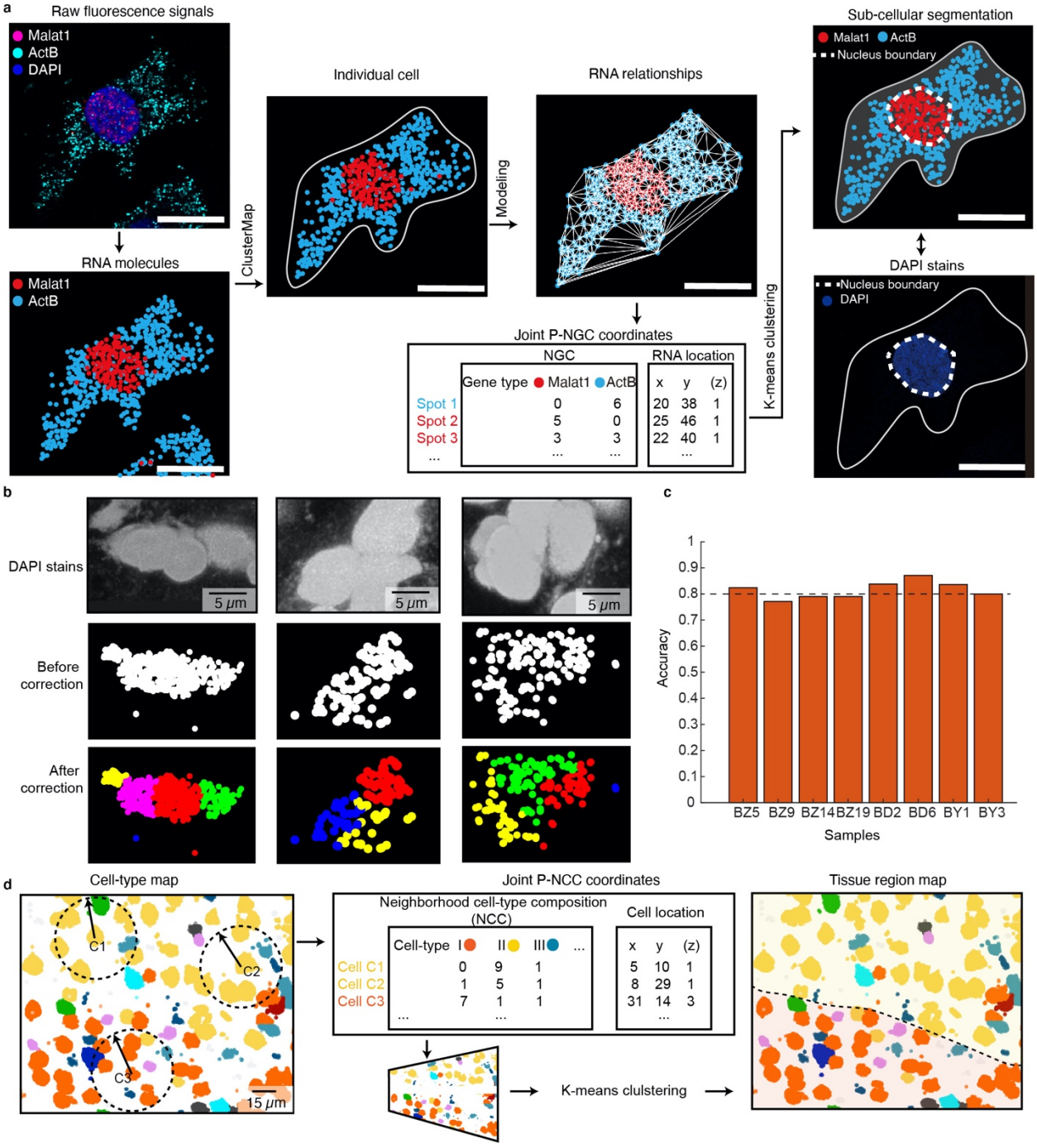
Supplementary information for ‘ClusterMap: multi-scale clustering analysis of spatial gene expression’


a
Raw fluorescence signals
Malat1
ActB
DAPI
RNA molecules
Malat1
ActB
ClusterMap
Individual cell
Modeling
RNA relationships
Joint P-NGC coordinates
NGC
RNA location
Gene type Malat1 ActB
x y (z)
Spot 1 0 6 20 38 1
Spot 2 5 0 25 46 1
Spot 3 3 3 22 40 1
K-means clustering
Sub-cellular segmentation
Malat1 ActB
Nucleus boundary
DAPI stains
Nucleus boundary
DAPI
b
DAPI stains
5 μm
Before correction
After correction
c
Accuracy
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BZ5 BZ9 BZ14 BZ19 BD2 BD6 BY1 BY3
Samples
d
Cell-type map
C1
C2
C3
15 μm
Joint P-NCC coordinates
Neighborhood cell-type composition (NCC)
Cell location
Cell-type I II III ...
Cell C1 0 9 1
Cell C2 1 5 1
Cell C3 7 1 1
x y (z)
5 10 1
8 29 1
31 14 3
K-means clustering
Tissue region map

Supplementary Figure 1

Sub-cellular analysis, validation of the cell identification method, and tissue region analysis.

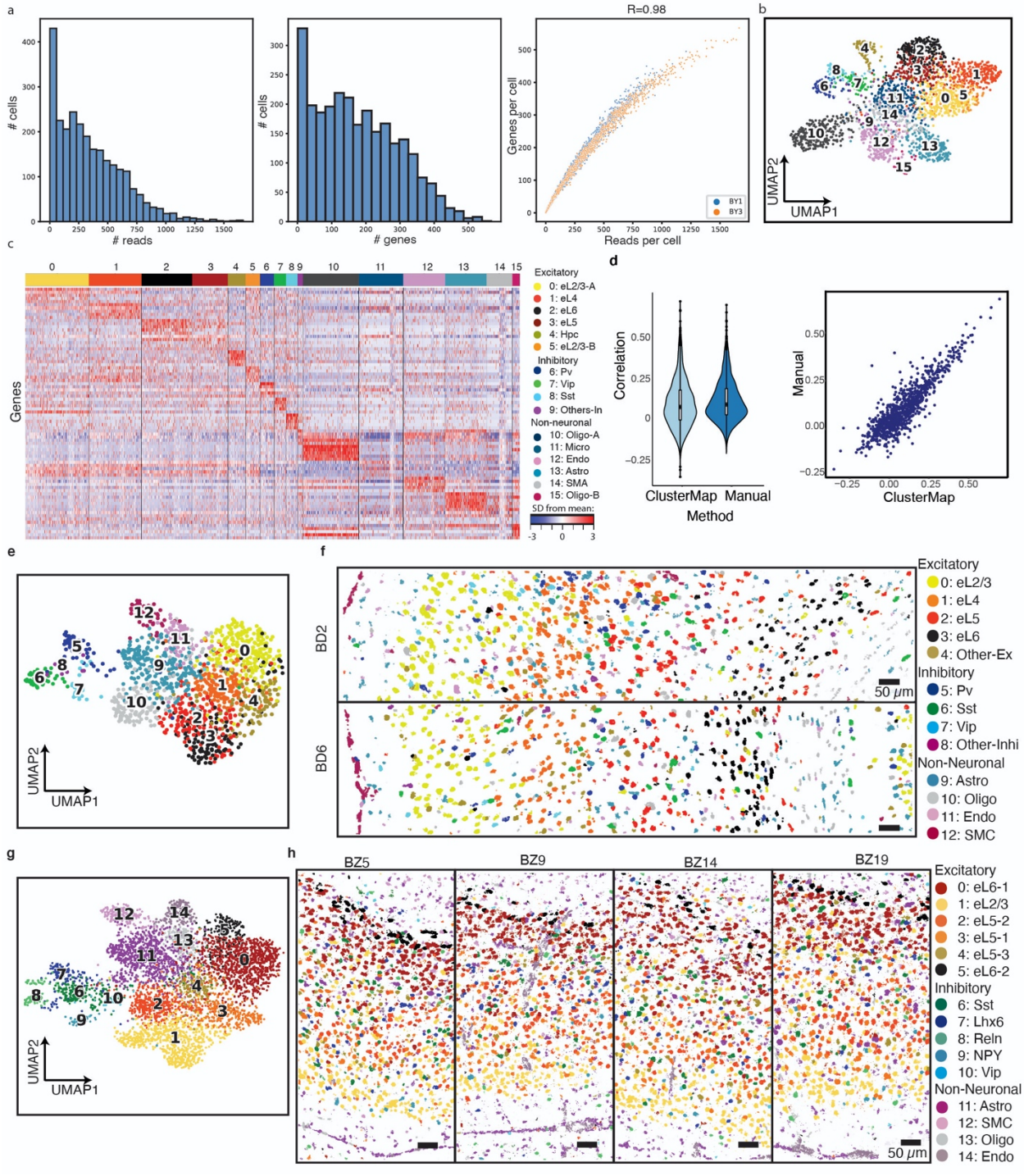
a, Subcellular analysis process the fourth panel IV in **Fig. 1d** by ClusterMap. A three-channel (magenta: *Malat1*; cyan: *ActB*; blue: DAPI) composite image shows raw fluorescent signals. After preprocessing mRNA molecules with specific genes are located, ClusterMap first performs cellular resolution and identifies individual cells. Then a mesh graph that models the relationships among mRNA spots in the cell is generated to compute the NGC coordinates and K-means clustering separate spots into two regions using joint physical and NGC coordinates. Finally a convex hull is constructed from the nucleus spots, denoting the nucleus boundary. The pattern of ClusterMap-constructed nucleus boundary is compared with the DAPI stains. Scale bar: 20μm.

b, Examples of the cell identification correction using information from NGC space during ClusterMap procedures in **Fig. 2a**. Upper: DAPI stains showing the cell nuclei. Middle: Cell clustering results using only information in the physical space. Closely overlapping cells are not separated. Lower: With information from NGC space, the under-clustered cells are separated.

c, The accuracy of cell identification results from eight STARmap datasets compared with corresponding expert-annotated labels. BZ5, BZ9, BZ14, BZ19: four STARmap 166-gene sets in mouse medial prefrontal cortex (mPFC); BD2, BD6: two STARmap 160-gene sets in mouse V1. BY1, BY3: two STARmap 1020-gene sets in mouse V1. The horizontal line is at 80% accuracy.

d, ClusterMap constructs the tissue regions after cell-typing. First, the neighborhood cell-type composition (NCC) of each cell is computed by considering a sliding window over the cell-type map. Then both the NCC and physical locations of cells are combined for K-means clustering.

Cells with highly correlated neighboring cell-type composition and close spatial distances are merged into a single tissue region signature.


a
# cells
# reads
# genes
R=0.98
Genes per cell
Reads per cell
BY1
BY3
b
UMAP2
UMAP1
c
Genes
Excitatory
0: eL2/3-A
1: eL4
2: eL6
3: eL5
4: Hpc
5: eL2/3-B
Inhibitory
6: Pv
7: Vip
8: Sst
9: Others-In
Non-neuronal
10: Oligo-A
11: Micro
12: Endo
13: Astro
14: SMA
15: Oligo-B
SD from mean:
d
Correlation
ClusterMap
Manual
Method
Manual
ClusterMap
e
UMAP2
UMAP1
f
BD2
BD6
50 μm
Excitatory
0: eL2/3
1: eL4
2: eL5
3: eL6
4: Other-Ex
Inhibitory
5: Pv
6: Sst
7: Vip
8: Other-Inhi
Non-Neuronal
9: Astro
10: Oligo
11: Endo
12: SMC
g
UMAP2
UMAP1
h
BZ5
BZ9
BZ14
BZ19
50 μm
Excitatory
0: eL6-1
1: eL2/3
2: eL5-2
3: eL5-1
4: eL5-3
5: eL6-2
Inhibitory
6: Sst
7: Lhx6
8: Reln
9: NPY
10: Vip
Non-Neuronal
11: Astro
12: SMC
13: Oligo
14: Endo

Supplementary Figure 2

Identification of cell types in mouse V1 datasets.

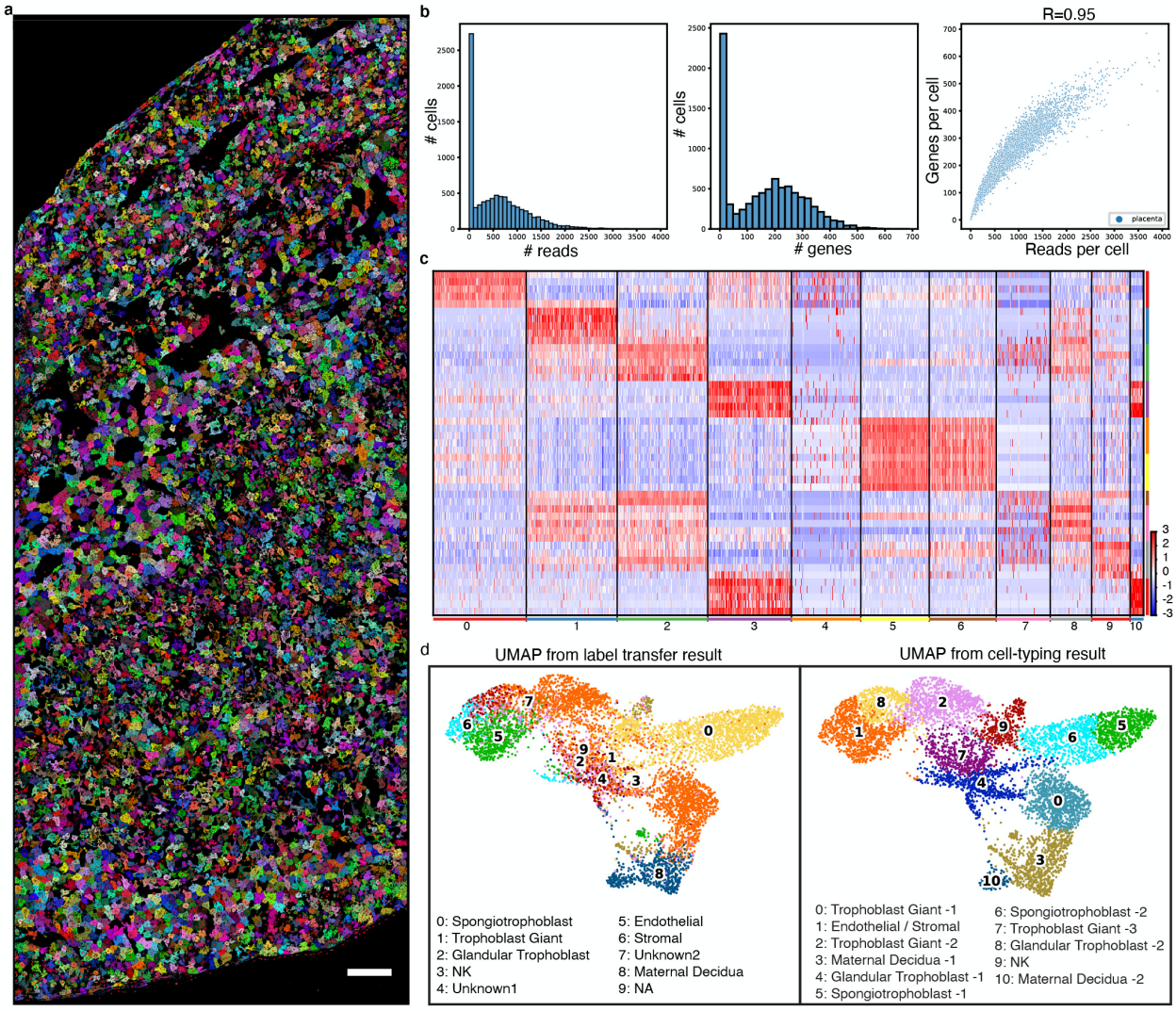
a, Statistics of ClusterMap-identified cells in STARmap mouse V1 1020-gene (two replicates: BY1 and BY3). Left: Histogram of detected reads (DNA amplicons) per cell. Middle: Histogram of genes per cell. Right: Correlation plot between genes per cell and reads per cell.

b, **c**, UMAP and heatmap visualization of all excitatory, inhibitory and non-neuronal cell types in BY1 and BY3.

d, Correlation plots after integration with scRNA-seq atlas. Left: Violin plots of Pearson correlation between gene expression in scRNA-seq atlas and ClusterMap or manual. Right: Correlation plot between integration results of ClusterMap manual annotation.

e, **g**, UMAP visualization of all excitatory, inhibitory and non-neuronal cell types in STARmap 160-gene datasets in mouse V1 (two replicates: BD2, BD6, (**e**)), and STARmap 166-gene datasets in mPFC (four replicates, BZ5, BZ9, BZ14, BZ19, (**h**)).

f, **h**, Spatial organization map of cell types in BD2 and BD6 (**e**), and in BZ5, BZ9, BZ14 and BZ19 (**h**).


a
b
# cells
# reads
# genes
R=0.95
Genes per cell
Reads per cell
placenta
c
d
UMAP from label transfer result
UMAP from cell-typing result
0: Spongiotrophoblast
1: Trophoblast Giant
2: Glandular Trophoblast
3: NK
4: Unknown1
5: Endothelial
6: Stromal
7: Unknown2
8: Maternal Decidua
9: NA
0: Trophoblast Giant -1
1: Endothelial / Stromal
2: Trophoblast Giant -2
3: Maternal Decidua -1
4: Glandular Trophoblast -1
5: Spongiotrophoblast -1
6: Spongiotrophoblast -2
7: Trophoblast Giant -3
8: Glandular Trophoblast -2
9: NK
10: Maternal Decidua -2

Supplementary Figure 3

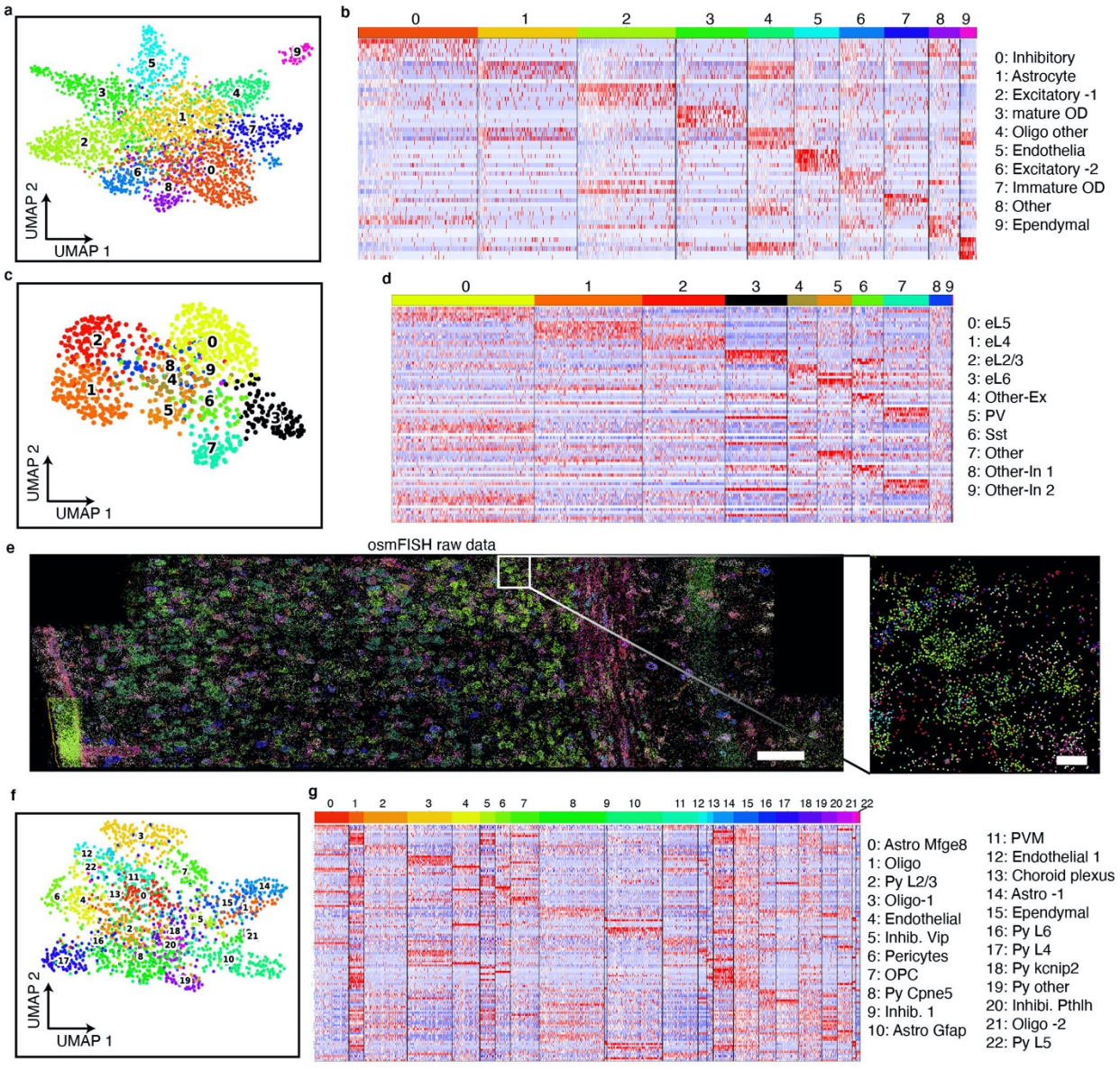
Analyses of the placental dataset.

a, ClusterMap generates the cell map of the STARmap mouse placenta 903-gene dataset, including 7,224 cells. Scale bar: 100 μm.

b, Statistics of ClusterMap identified placental cells as shown in (**a**). Left: Histogram of detected reads (DNA amplicons) per cell. Middle: Histogram of genes per cell. Right: Correlation plot between genes per cell and reads per cell.

c, Heatmap visualization of 11 cell types. Names are in the right panel of (**d**).

d, UMAP from label transfer results with scRNA-seq, compared with UMAP of the Louvain clustering in ClusterMap.


a
UMAP 2
UMAP 1
b
0: Inhibitory
1: Astrocyte
2: Excitatory -1
3: mature OD
4: Oligo other
5: Endothelia
6: Excitatory -2
7: Immature OD
8: Other
9: Ependymal
c
UMAP 2
UMAP 1
d
0: eL5
1: eL4
2: eL2/3
3: eL6
4: Other-Ex
5: PV
6: Sst
7: Other
8: Other-In 1
9: Other-In 2
e
osmFISH raw data
f
UMAP 2
UMAP 1
g
0: Astro Mfge8
1: Oligo
2: Py L2/3
3: Oligo-1
4: Endothelial
5: Inhib. Vip
6: Pericytes
7: OPC
8: Py Cpne5
9: Inhib. 1
10: Astro Gfap
11: PVM
12: Endothelial 1
13: Choroid plexus
14: Astro -1
15: Ependymal
16: Py L6
17: Py L4
18: Py kcnip2
19: Py other
20: Inhibi. Pthlh
21: Oligo -2
22: Py L5

Supplementary Figure 4

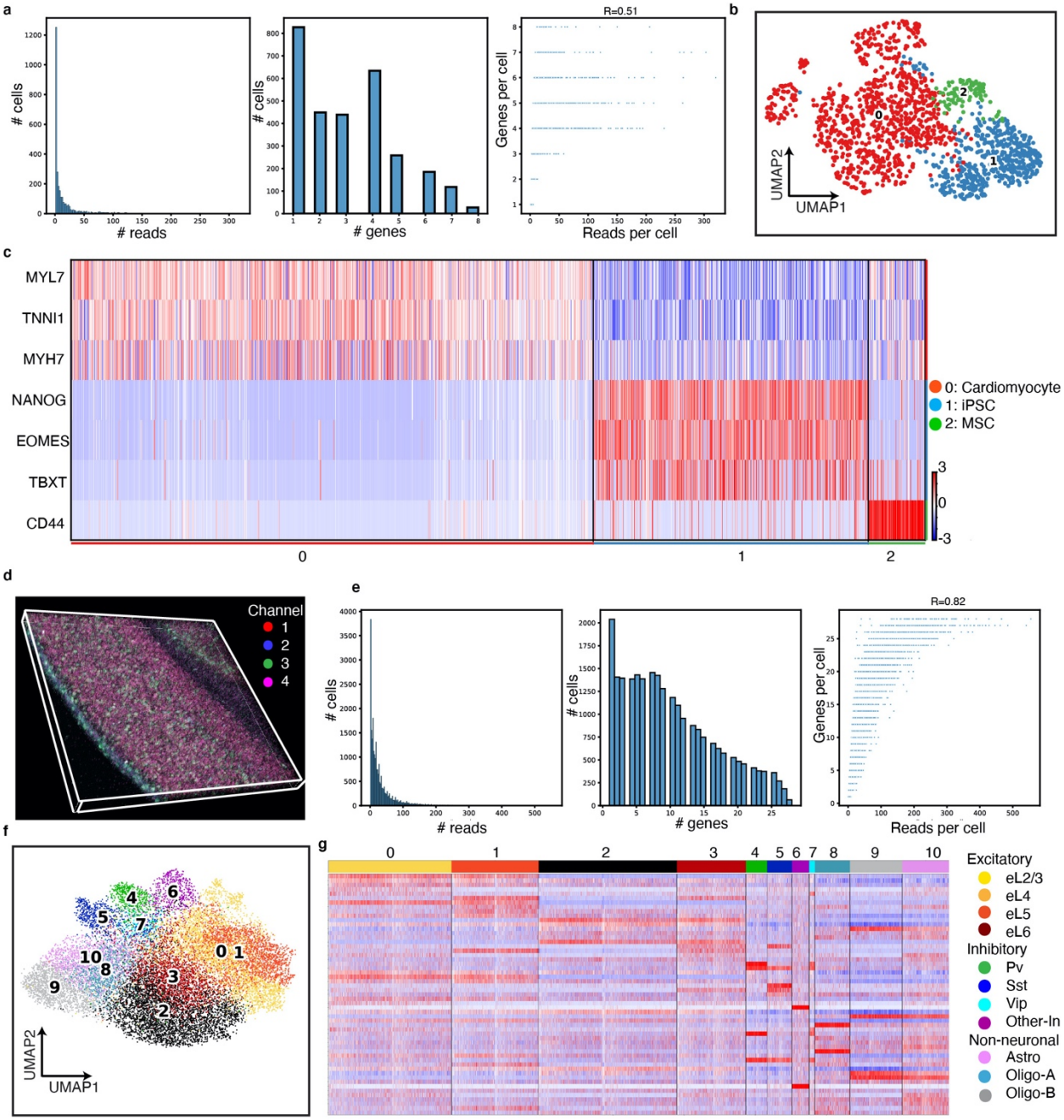
Analyses of datasets generated across various protocols.

a, **b**, UMAP and heatmap visualization of ten cell types in the selected area from the MERFISH mouse POA dataset.

c, **d**, UMAP and heatmap visualization of ten cell types in the selected area from the pciSeq mouse isocortex dataset.

e, Raw spatial transcriptomics data of the selected area from the osmFISH mouse SSp dataset. Scale bar: 100μm. Left: zoomed in view of the highlighted square. Scale bar: 10 μm.

f, **g**, UMAP and heatmap visualization of seven main types and 22 subtypes of (**e**).


a
# cells
# reads
# genes
R=0.51
Genes per cell
Reads per cell
b
UMAP2
UMAP1
c
MYL7
TNNI1
MYH7
NANOG
EOMES
TBXT
CD44
0: Cardiomyocyte
1: iPSC
2: MSC
d
Channel
1
2
3
4
e
R=0.82
f
g
Excitatory
eL2/3
eL4
eL5
eL6
Inhibitory
Pv
Sst
Vip
Other-In
Non-neuronal
Astro
Oligo-A
Oligo-B

Supplementary Figure 5

Analyses in the 3D datasets.

a, Statistics of ClusterMap identified cells in the 3D STARmap cardiac organoid 8-gene dataset. Left: Histogram of detected reads (DNA amplicons) per cell. Middle: Histogram of genes per cell. Right: Correlation plot between genes per cell and reads per cell.

b, **c**, UMAP and heatmap visualization of three cell types in the STARmap cardiac organoid 8-gene dataset. The number of cells in each cell type is as follows: cardiomyocytes, 929; induced pluripotent stem cells (iPSCs), 489; mesenchymal stem cells (MSCs), 101.

d, 3D four-channel composite raw fluorescent image of the first sequencing round shows spatial arrangement of mRNA molecules in the STARmap mouse V1 28-gene dataset. Width 184 μm, height 194 μm, depth 100 μm.

e, Statistics of ClusterMap identified cells in (**d**). Left: Histogram of detected reads (DNA amplicons) per cell. Middle: Histogram of genes per cell. Right: Correlation plot between genes per cell and reads per cell.

f, **g**, UMAP and heatmap visualization of three cell types of (**d**).

Supplementary Tables

Supplementary Table 1

Dataset	Method	Tissue	# Gene	# Cell	# Cell type	Figure	Notes
STARmap mouse V1 1020-gene	STARmap	Mouse brain primary visual cortex	1,020	1,447	16	Fig. 1c, Fig. 2, Supplementary Fig. 2	Source: Ref. 6. 2D analysis.
STARmap mouse placenta 903-gene	STARmap	Mouse brain primary visual cortex	903	7224	11	Fig. 3, Fig. 4, Supplementary Fig. 3	New data. 2D analysis.
MERFISH mouse POA	MERFISH	Mouse brain hypothalamic preoptic region	140	3,113	10	Fig.5, Supplementary Fig. 4	Source: Ref. 3. 2D analysis.
pciSeq mouse isocortex	pciSeq	Mouse brain isocortex region	98	982	8	Fig.5, Supplementary Fig. 4	Source: Ref. 4. 2D analysis.
osmFISH mouse SSp	osmFISH	Mouse brain somatosensory cortex	33	1,962	19	Fig.5, Supplementary Fig. 4	Source: Ref. 5. 2D analysis.
STARmap cardiac organoid 8-gene	STARmap	Cardiac organoid	8	1,519	3	Fig. 6, Supplementary Fig. 5	New data, 3D analysis.
STARmap mouse V1 28-gene	STARmap	Mouse brain primary visual cortex	28	24,590	11	Fig. 6, Supplementary Fig. 5	Source: Ref. 6. 3D analysis.

Summary of the name, *in situ* sequencing protocol, tissue, number of genes, number of cells, number of cell types, corresponding figures and note of 7 datasets.