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Supplementary information

Temporally divergent regulatory mechanisms govern neuronal diversification and maturation in the mouse and marmoset neocortex

In the format provided by the authors and unedited

Supplementary Information:

Supplementary Figures 1-12

Supplementary Table 1. (separate tab-delimited text file)

Dynamic genes in the Cux2/Tle4 bulk dataset. fClustId, final cluster assignment. clustLabel, cluster type description.

Supplementary Table 2. (separate tab-delimited text file)

Dynamic ATAC peaks in the Cux2/Tle4 bulk dataset. fClustId, final cluster assignment. clustLabel, cluster type description.

Supplementary Table 3. (separate tab-delimited text file)

Dynamic DMRs in the Cux2/Tle4 bulk dataset. fClustId, final cluster assignment. clustLabel, cluster type description.

Supplementary Table 4. (separate Excel file)

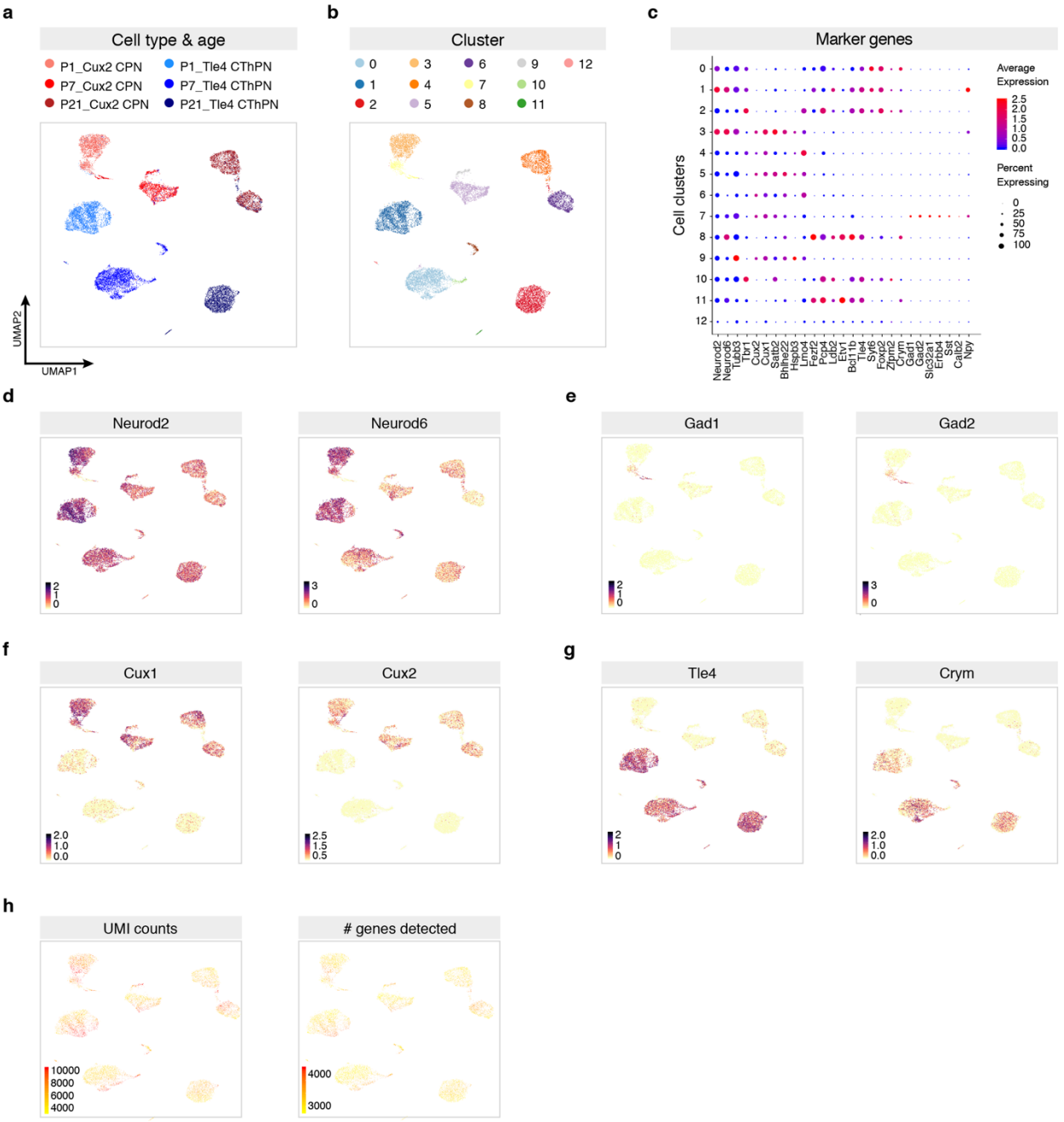
GO terms associated with shared-developmental Cux2/Tle4 gene expression clusters (tabs 1-5) and with shared-developmental Cux2/Tle4 ATAC peak clusters (tabs 6-9). Selected GO terms from the highest fold enrichment terms for each early and late developmental cluster. Test results of Fisher's exact test are reported as p-values and multiple comparison adjusted using the Benjamini-Hochberg correction.

Supplementary Table 5. (separate tab-delimited text file)

DNAse HS I peak tracks from the mouse ENCODE consortium used in this analysis.

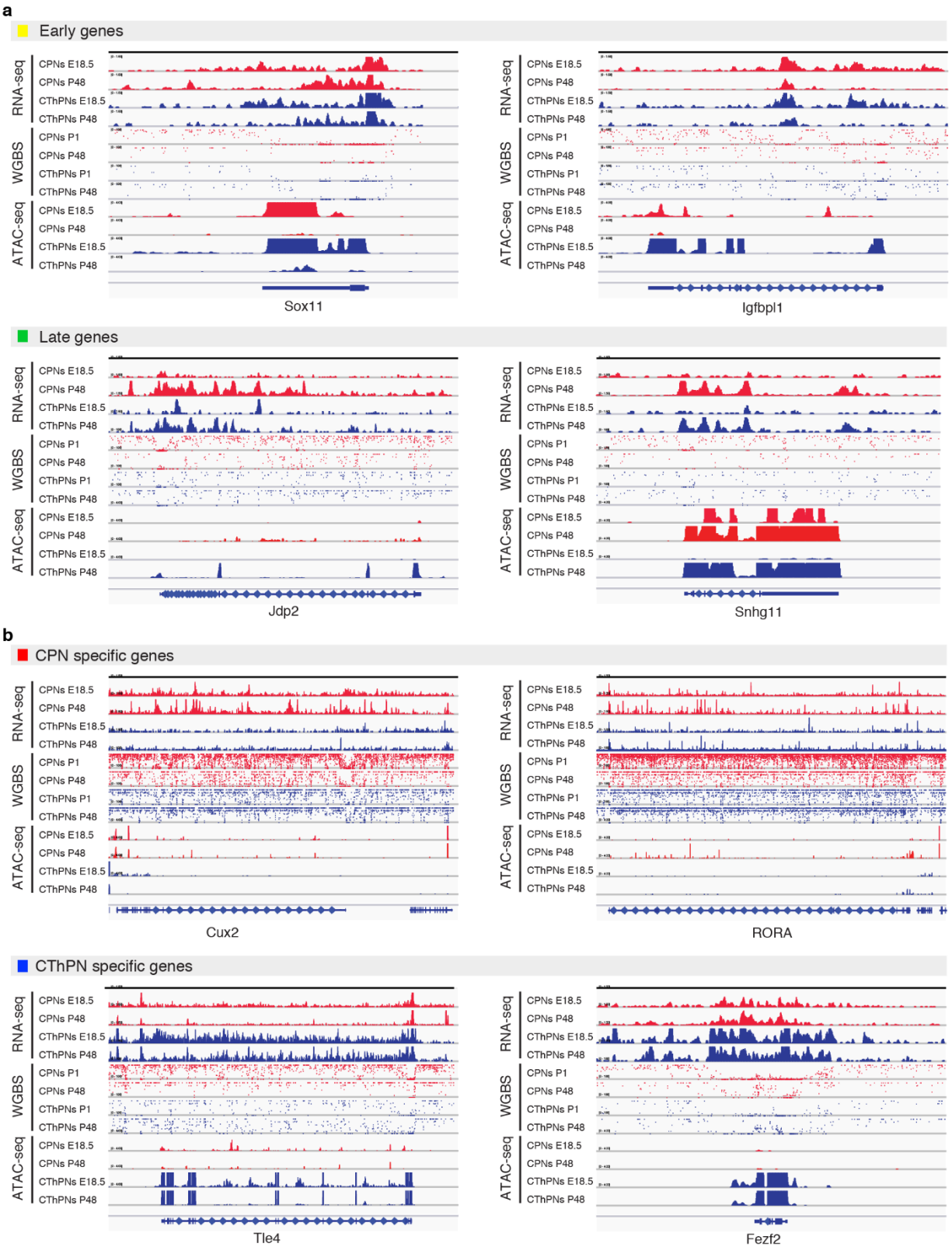
Supplementary Table 6. (separate tab-delimited text file)

List of CpG islands.

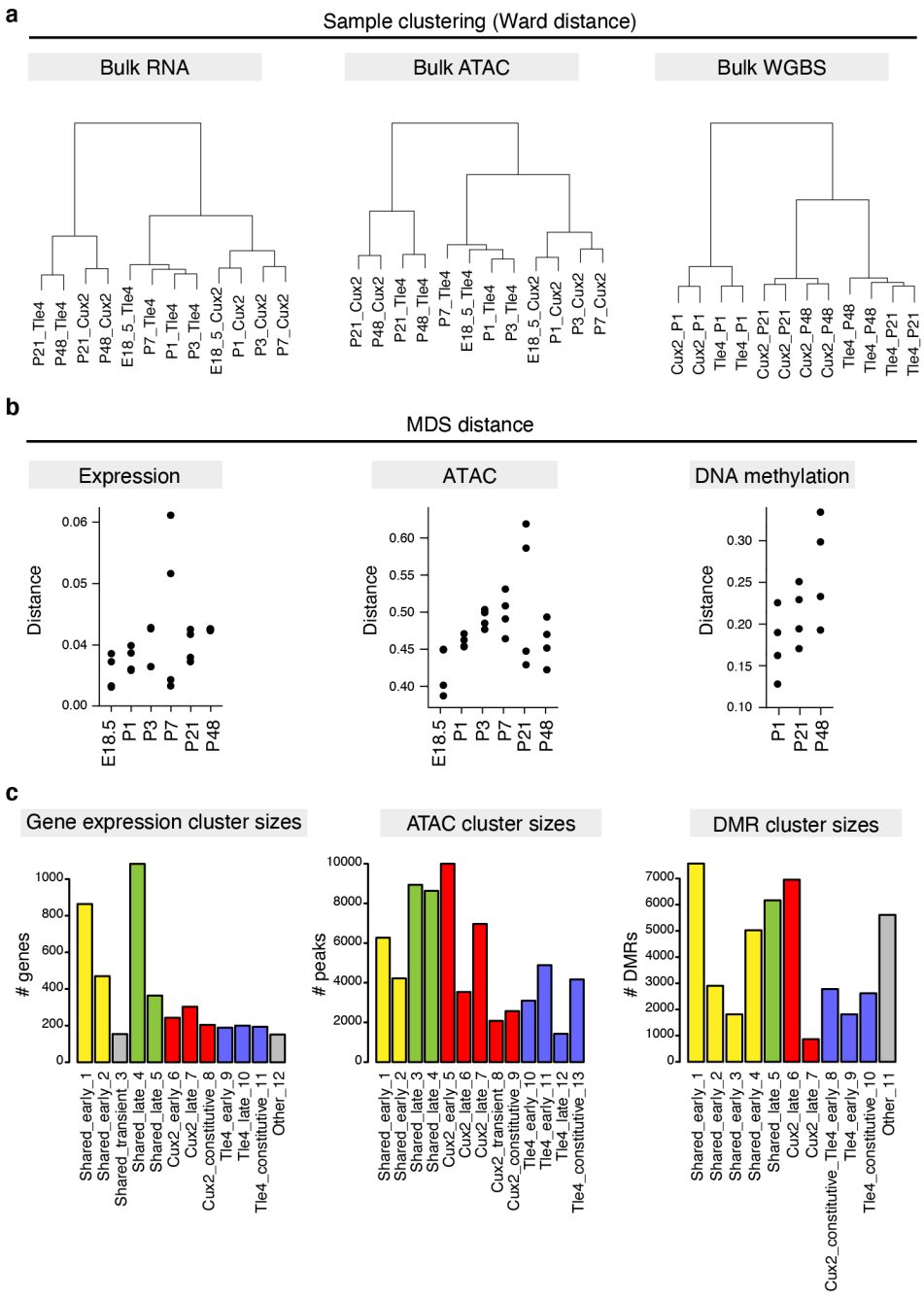


Supplementary Fig. 1. Verification of subtype identity of genetically-labelled mouse PN subtypes by single-cell RNA-seq of FACS-purified Cux2 CPN and Tle4 CThPN. a, UMAP showing distribution of cells by line and age. **b**, UMAP showing cell clusters after k-means clustering. **c**, Average expression of selected marker genes in each cluster. **d-g**, UMAPs showing expression of selected marker genes in each cell, including pan-neuronal markers (**d**) and markers for interneurons (**e**), CPN (**f**), and CThPN (**g**). The majority of cells express markers corresponding

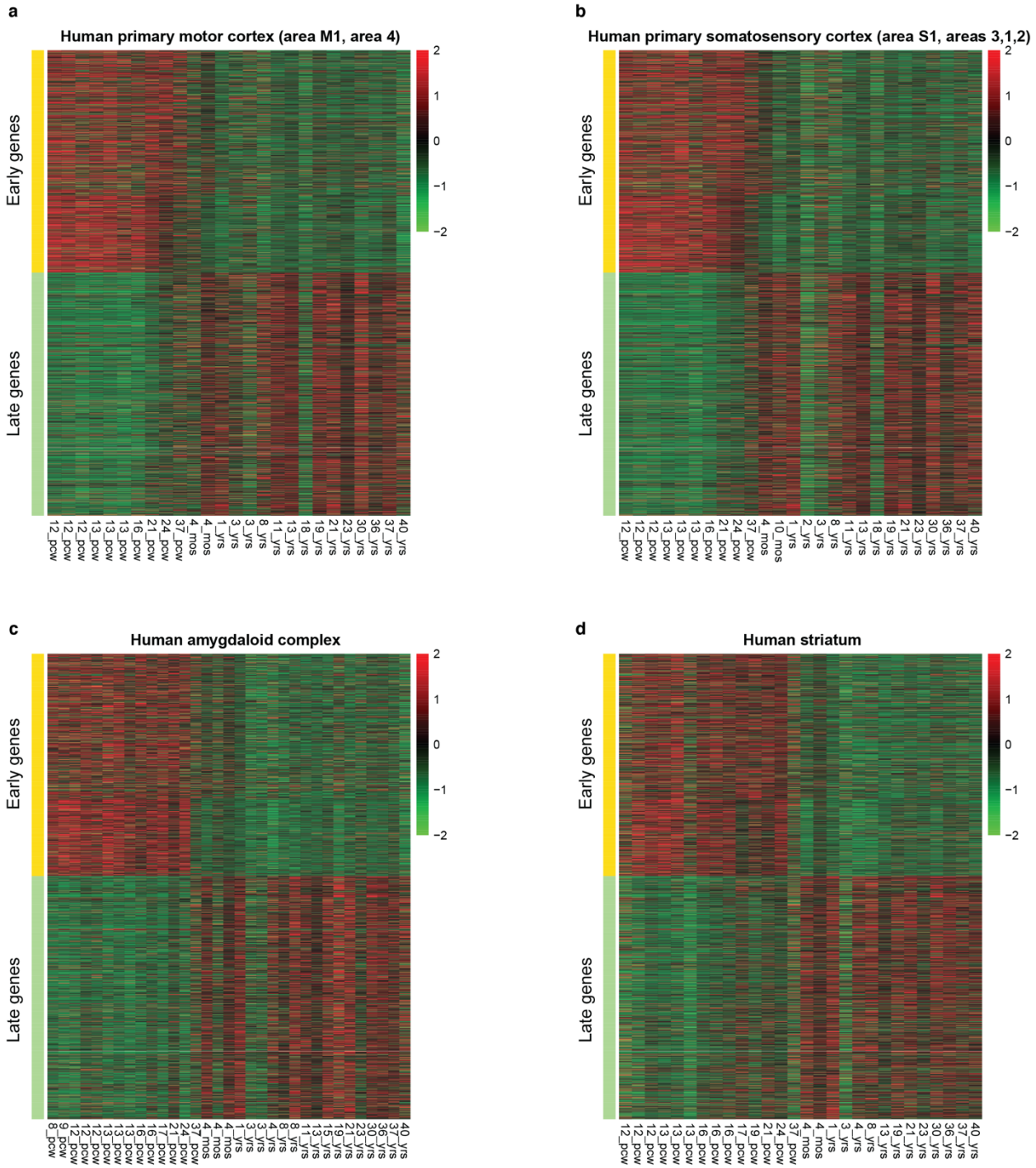
to the expected pyramidal neuron subtypes, with only minor contribution of interneurons. **h**, UMAPs showing number of UMIs and number of genes detected in each cell.


a
Early genes
RNA-seq
WGBS
ATAC-seq
CPNs E18.5
CPNs P48
CThPNs E18.5
CThPNs P48
CPNs P1
CPNs P48
CThPNs P1
CThPNs P48
CPNs E18.5
CPNs P48
CThPNs E18.5
CThPNs P48
Sox11
Igfbpl1
Late genes
Jdp2
Snhg11
b
CPN specific genes
Cux2
RORA
CThPN specific genes
Tle4
Fezf2

Supplementary Fig. 2. Example gene tracks for genes classified into different transcriptional categories. Examples showing Genome Browser views of RNA sequencing, ATAC-seq, and WGBS tracks, for examples of genes in (**a**) shared-developmental and (**b**) class-specific categories. Red: Cux2 CPNs; Blue: Tle4 CThPNs.

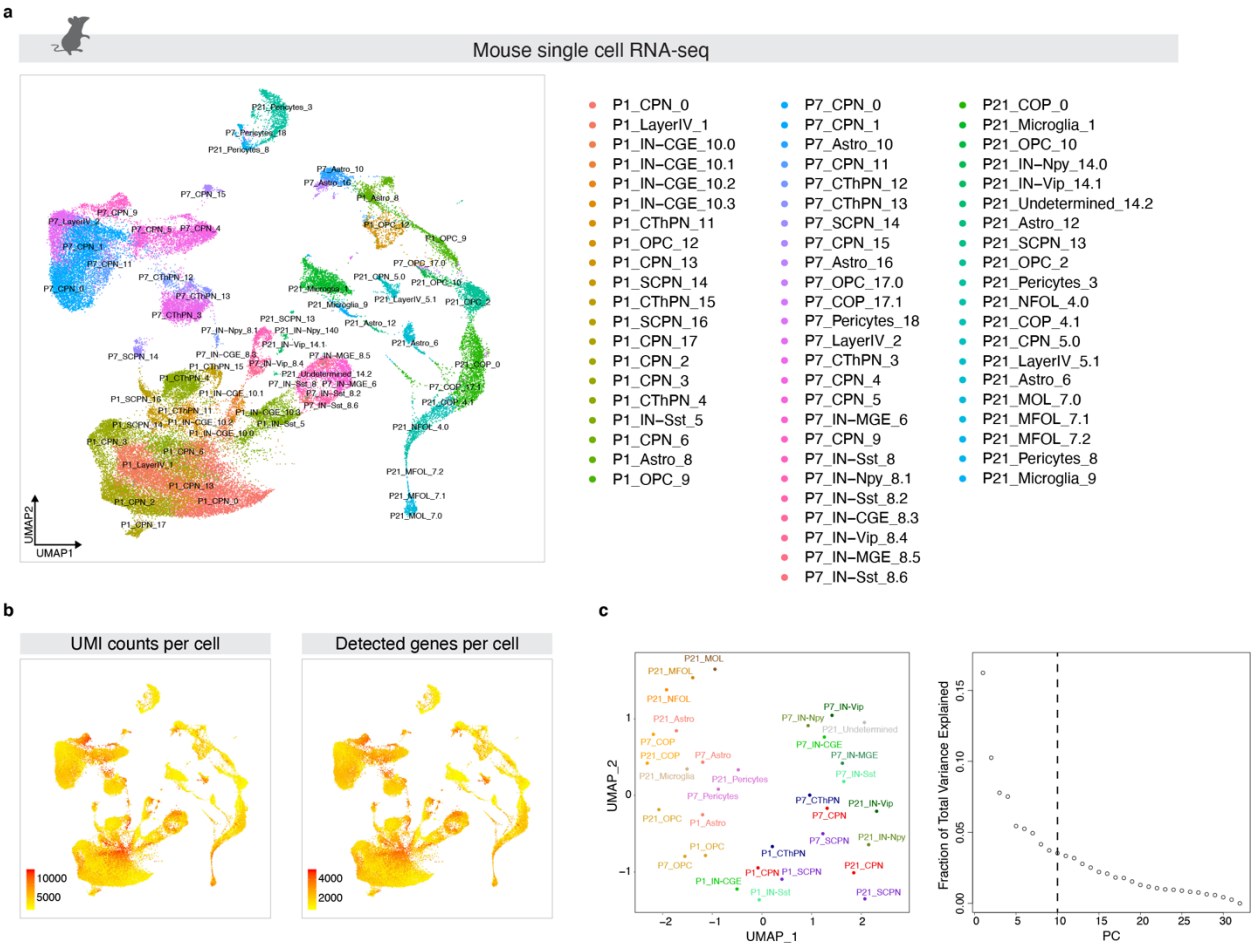


Supplementary Fig. 3. Characterizing bulk datasets. a, Dendrograms showing relationships between samples from different ages for each dataset. **b**, Distribution of pairwise sample distances (1-pearson correlation) between replicates at each time point (n = 4 independent biological samples per timepoint), for gene expression, open chromatin and DNAme. **c**, Number of features (genes, ATAC peaks, or DMRs) in each cluster for each dataset.



Supplementary Fig. 4. Validation of temporal patterns of gene expression using external datasets. a-d, Expression of the human orthologs of the genes in the shared-early and shared-late gene clusters in human developing brain transcriptomic data from BrainSpan. Expression is shown at ages from 8 (amygdaloid complex) or 12 (all others) post-conceptional weeks to 40 years, for four brain regions: primary motor cortex (**a**), primary somatosensory cortex (**b**), amygdaloid

complex (**c**), and striatum (**d**). Color scale: row z-score. Abbreviations: pcw, post-conceptional weeks; mos, months; yrs, years.

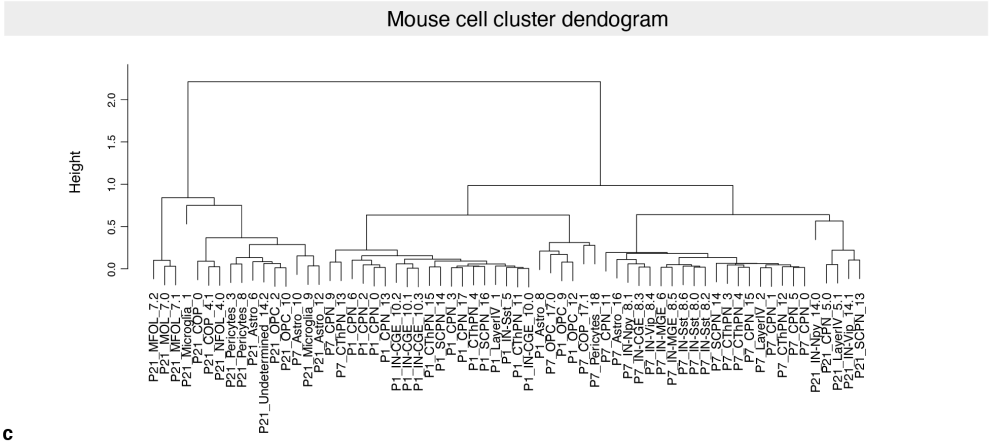


Supplementary Fig. 5. Characterization of mouse unfractionated single-cell RNA-seq dataset. a, UMAPs showing each cell cluster, broken down by age. **b**, UMAPs showing number of UMIs and number of genes detected in each cell. **c**, Similarity between cell clusters, as a UMAP representation of the top 10 principal components (PCs) after PCA computed on the average gene expression space.

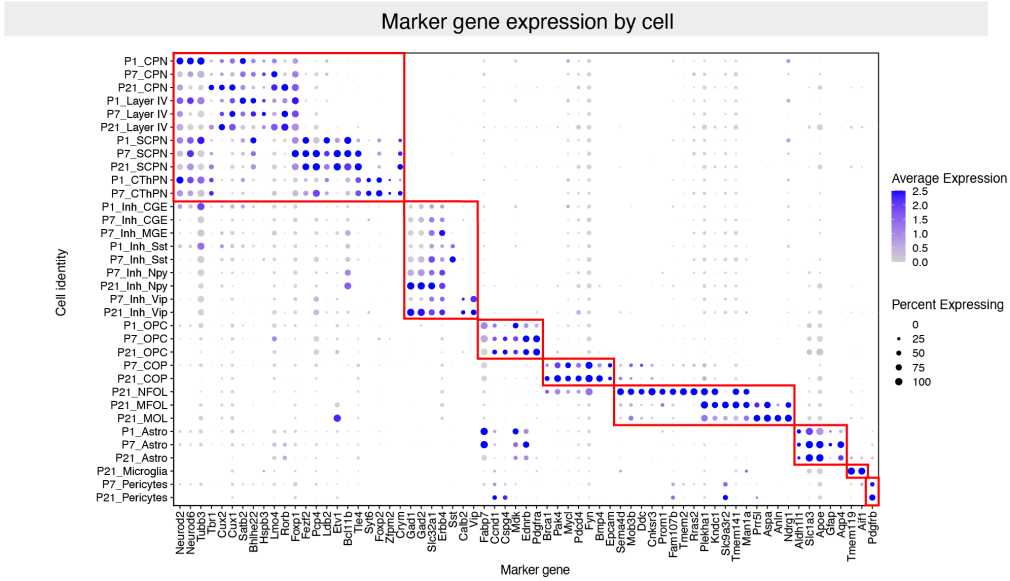
a


Marker gene set expression in mouse cell clusters
CellType markers
Endothelial
Pericytes
Microglia
Astro
OPC
NFOL
MOL
MFOL
COP
IN-Npy
IN-Vip
IN-Calb2
IN-Sst
IN-Pvalb
INs
Immature neurons
IP
Progenitors
Neurons
SP
CR
Layer IV
CPN_DL
CPN_UL
SCPN
CThPN
0.8
0.6
0.4
0.2
0.0

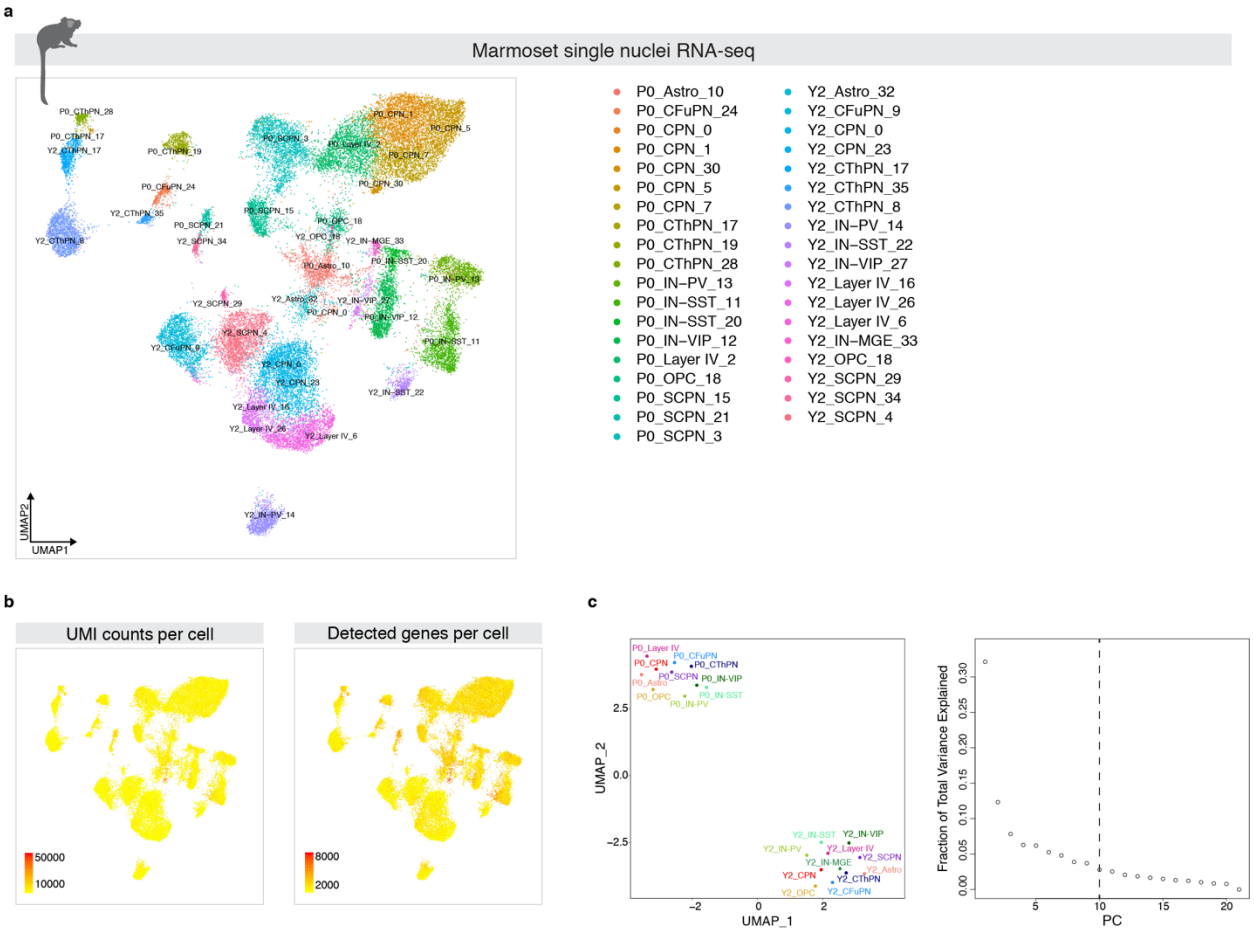
b


Mouse cell cluster dendogram
Height
2.0
1.5
1.0
0.5
0.0

c

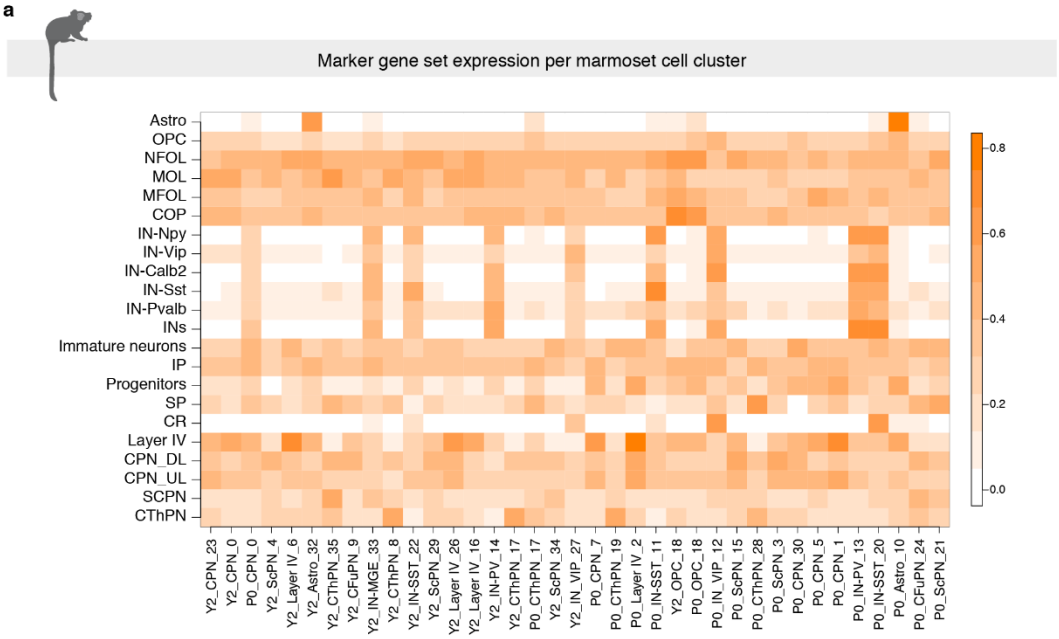

Marker gene expression by cell
Cell identity
Marker gene
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0
Percent Expressing
0
25
50
75
100

Supplementary Fig. 6. Characterization of mouse unfractionated single-cell RNA-seq dataset. a, Expression of gene sets representing combined expression of panels of known cell-type marker genes for each cell cluster. **b**, Dendrogram representing similarity between cell clusters. **c**, Average expression and percent of cells expressing for selected marker genes within cells of each assigned cell identity.

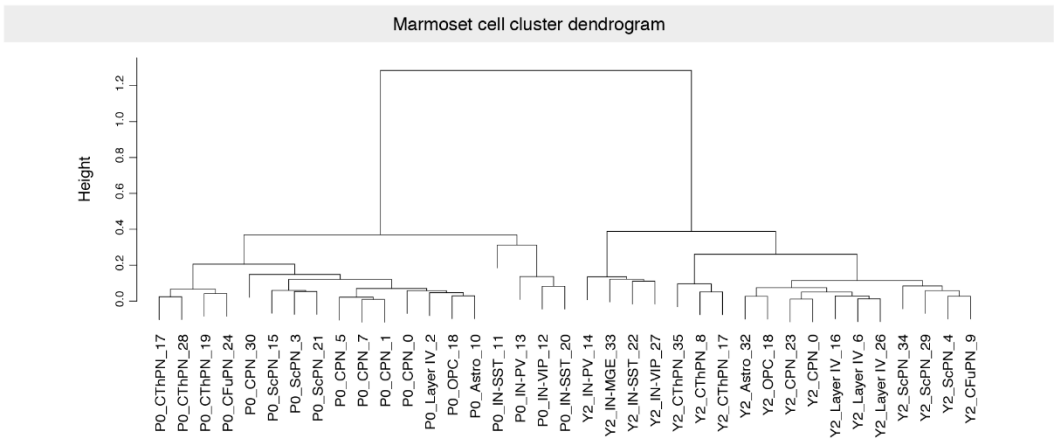


Supplementary Fig. 7. Characterization of marmoset single-cell RNA-seq dataset. a, UMAPs showing each cell cluster, broken down by age. **b**, UMAPs showing number of UMIs and number of genes detected in each cell. **c**, Similarity between cell clusters, as a UMAP representation of the top 10 principal components (PCs) after PCA computed on the average gene expression space.

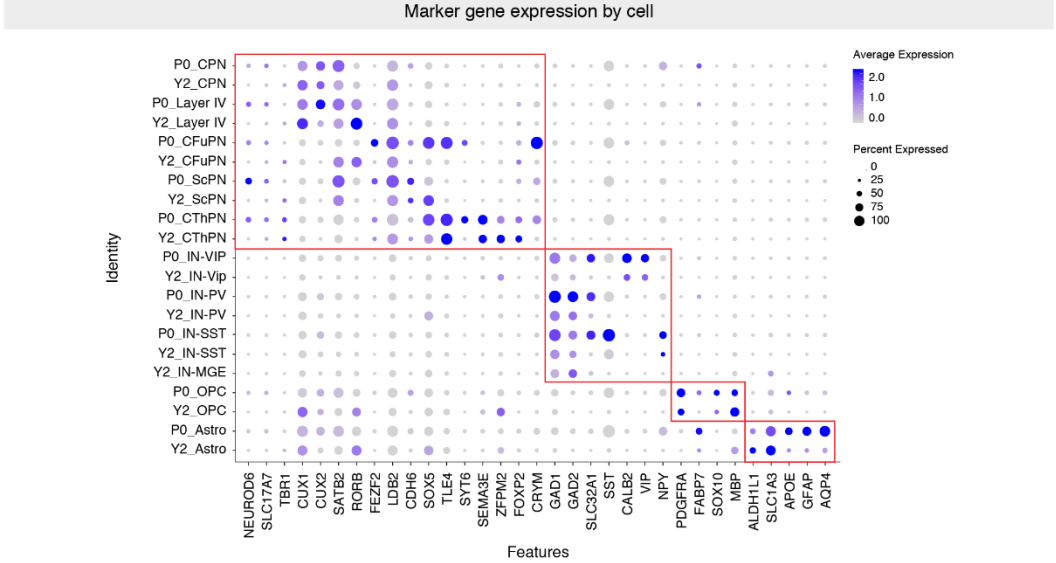
a


Marker gene set expression per marmoset cell cluster
Astro
OPC
NFOL
MOL
MFOL
COP
IN-Npy
IN-Vip
IN-Calb2
IN-Sst
IN-Pvalb
INs
Immature neurons
IP
Progenitors
SP
CR
Layer IV
CPN_DL
CPN_UL
SCPN
CThPN
0.8
0.6
0.4
0.2
0.0
Y2_CPN_23
Y2_CPN_0
P0_CPN_0
Y2_ScPN_4
Y2_Layer IV_6
Y2_Astro_32
Y2_CThPN_35
Y2_CFuPN_9
Y2_IN-MGE_33
Y2_CThPN_8
Y2_IN-SST_22
Y2_ScPN_29
Y2_Layer IV_26
Y2_Layer IV_16
Y2_IN-PV_14
Y2_CThPN_17
P0_CThPN_17
Y2_ScPN_34
Y2_IN_VIP_27
P0_CPN_7
P0_CThPN_19
P0_Layer IV_2
P0_IN-SST_11
Y2_OPC_18
P0_OPC_18
P0_IN_VIP_12
P0_ScPN_15
P0_CThPN_28
P0_ScPN_3
P0_CPN_30
P0_CPN_5
P0_CPN_1
P0_IN-PV_13
P0_IN-SST_20
P0_Astro_10
P0_CFuPN_24
P0_ScPN_21

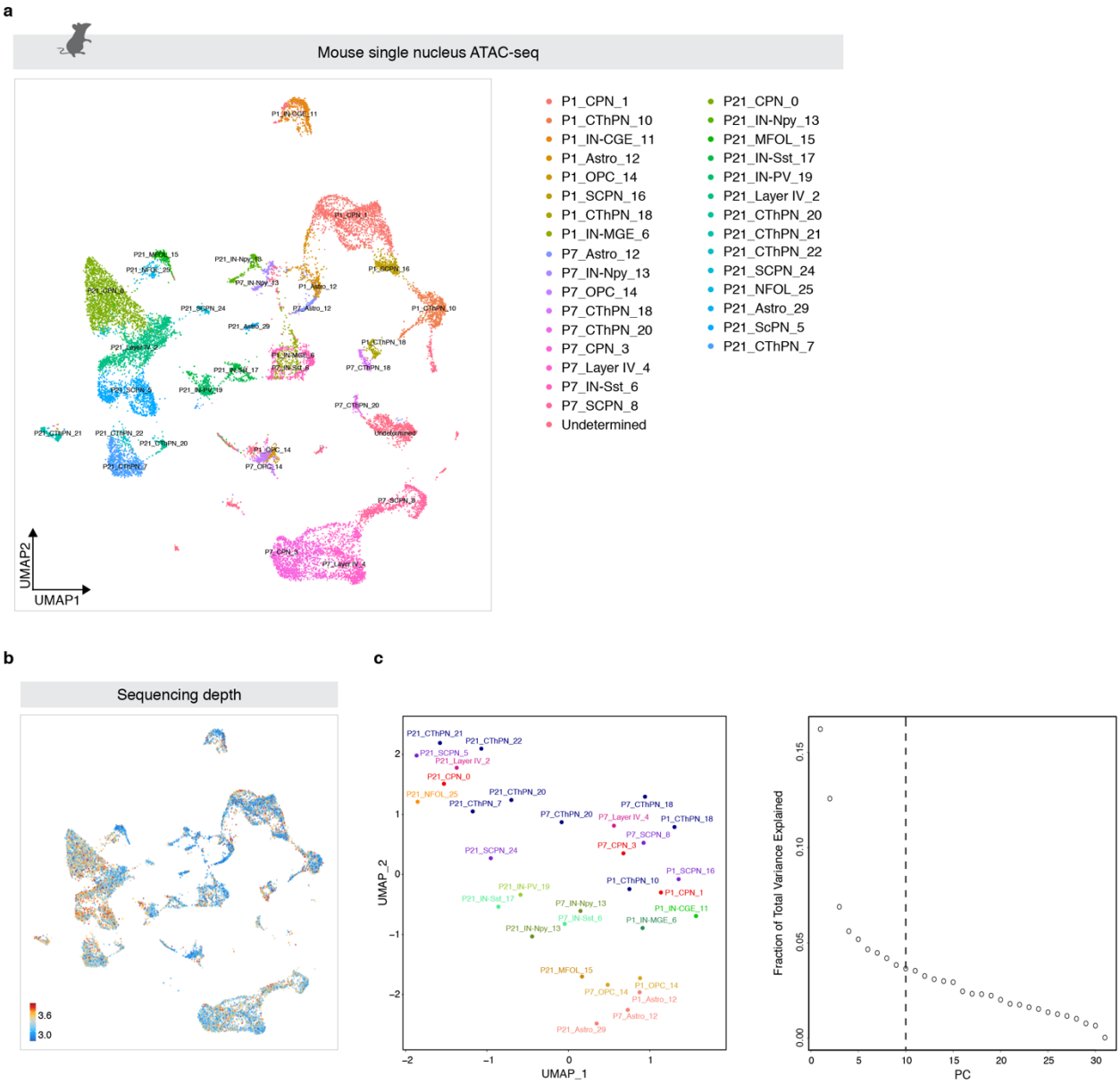
b


Marmoset cell cluster dendrogram
Height
0.0
0.2
0.4
0.6
0.8
1.0
1.2
P0_CThPN_17
P0_CThPN_28
P0_CThPN_19
P0_CFuPN_24
P0_CPN_30
P0_ScPN_15
P0_ScPN_3
P0_ScPN_21
P0_CPN_5
P0_CPN_7
P0_CPN_1
P0_CPN_0
P0_Layer IV_2
P0_OPC_18
P0_Astro_10
P0_IN-SST_11
P0_IN-PV_13
P0_IN-VIP_12
P0_IN-SST_20
Y2_IN-PV_14
Y2_IN-MGE_33
Y2_IN-SST_22
Y2_IN-VIP_27
Y2_CThPN_35
Y2_CThPN_8
Y2_CThPN_17
Y2_Astro_32
Y2_OPC_18
Y2_CPN_23
Y2_CPN_0
Y2_Layer IV_16
Y2_Layer IV_6
Y2_Layer IV_26
Y2_ScPN_34
Y2_ScPN_29
Y2_ScPN_4
Y2_CFuPN_9

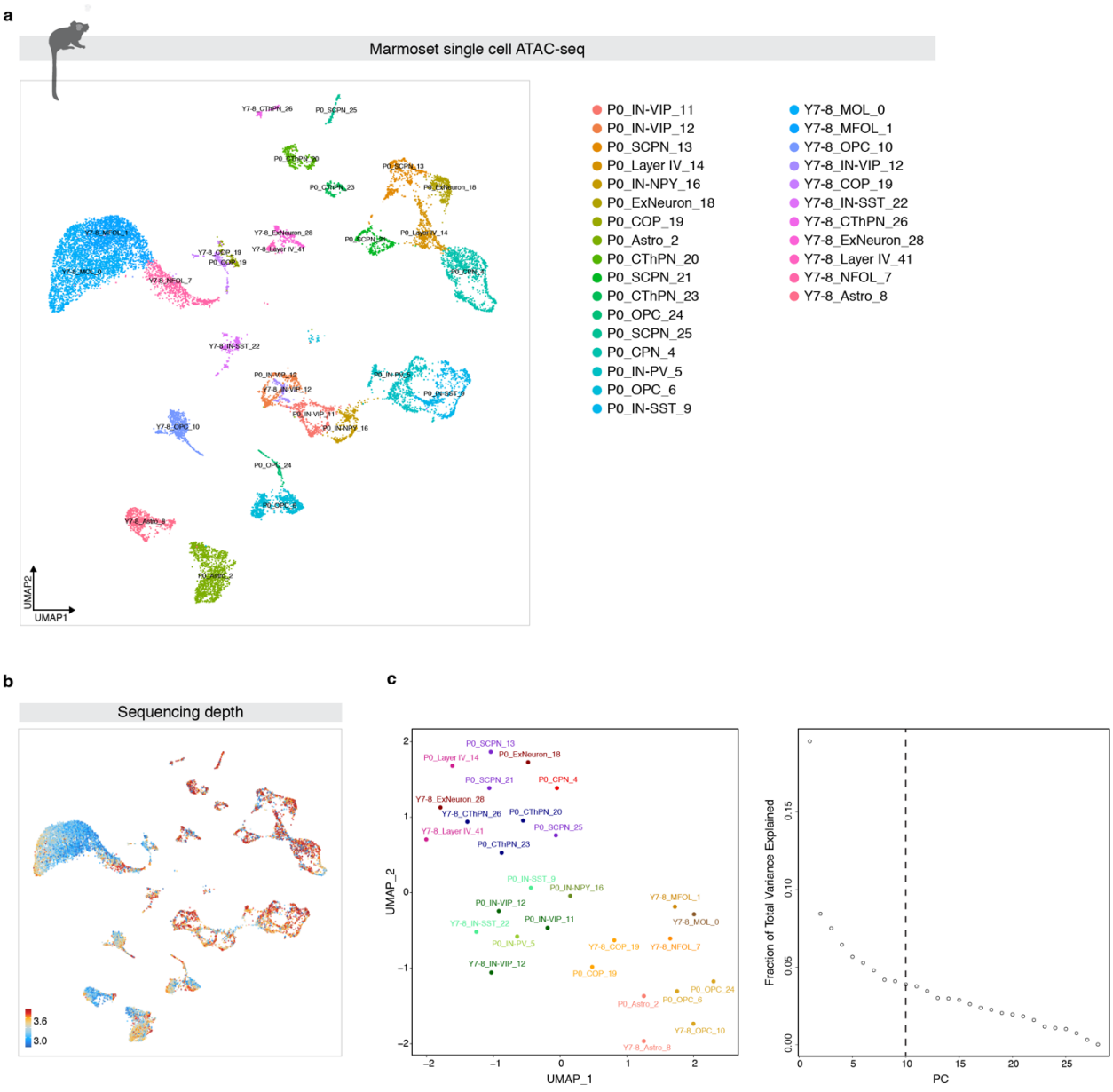
c


Marker gene expression by cell
Identity
P0_CPN
Y2_CPN
P0_Layer IV
Y2_Layer IV
P0_CFuPN
Y2_CFuPN
P0_ScPN
Y2_ScPN
P0_CThPN
Y2_CThPN
P0_IN-VIP
Y2_IN-Vip
P0_IN-PV
Y2_IN-PV
P0_IN-SST
Y2_IN-SST
Y2_IN-MGE
P0_OPC
Y2_OPC
P0_Astro
Y2_Astro
NEUROD6
SLC17A7
TBR1
CUX1
CUX2
SATB2
RORB
FEZF2
LDB2
CDH6
SOX5
TLE4
SYT6
SEMA3E
ZFPM2
FOXP2
CRYM
GAD1
GAD2
SLC32A1
SST
CALB2
VIP
NPY
PDGFRA
FABP7
SOX10
MBP
ALDH1L1
SLC1A3
APOE
GFAP
AQP4
Features
Average Expression
2.0
1.0
0.0
Percent Expressed
0
25
50
75
100

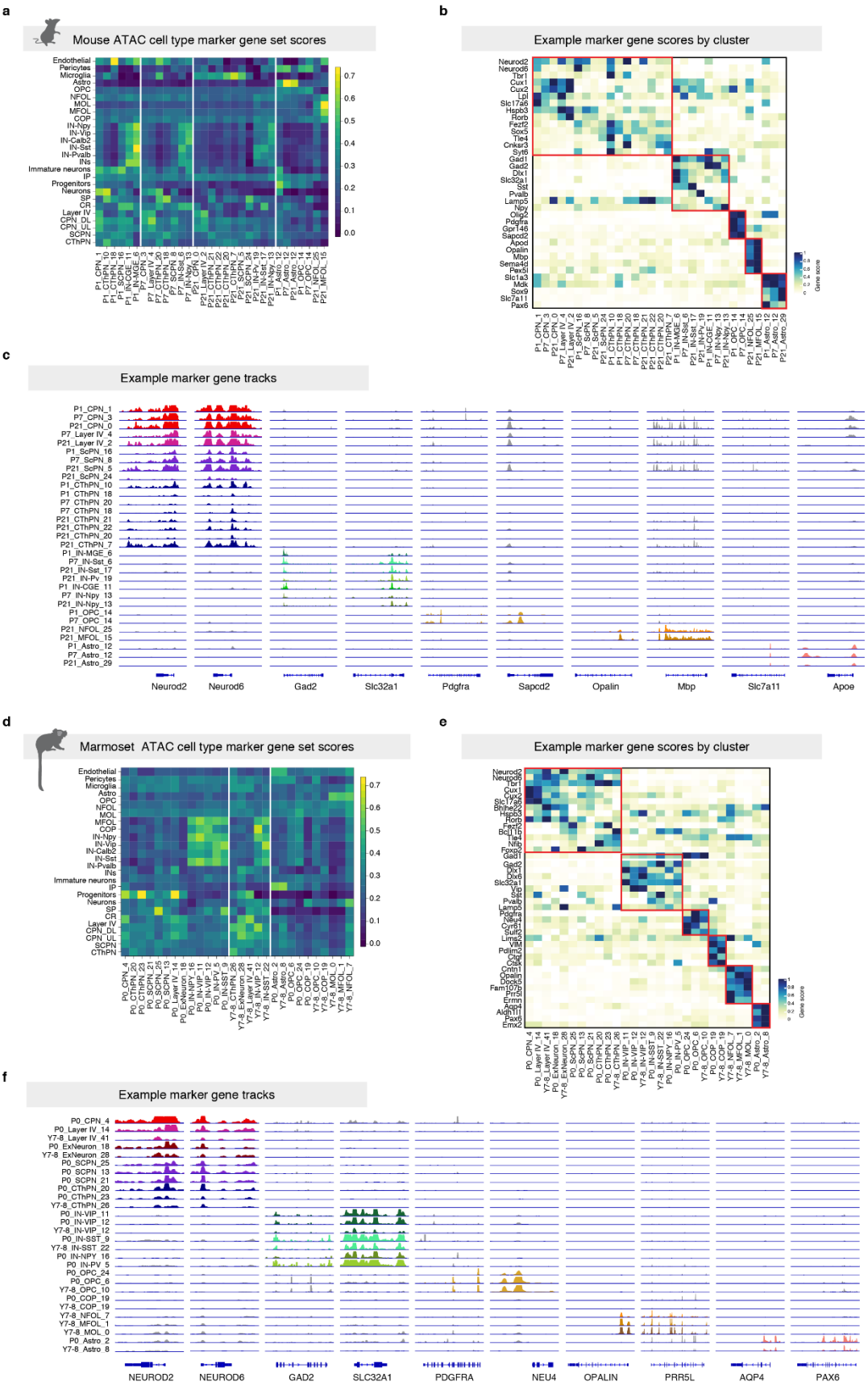
Supplementary Fig. 8. Characterization of marmoset single-cell RNA-seq dataset. a, Expression of gene sets representing combined expression of panels of known cell-type marker genes for each cell cluster. **b**, Dendrogram representing similarity between cell clusters. **c**, Average expression and percent of cells expressing for selected marker genes within cells of each assigned cell identity.



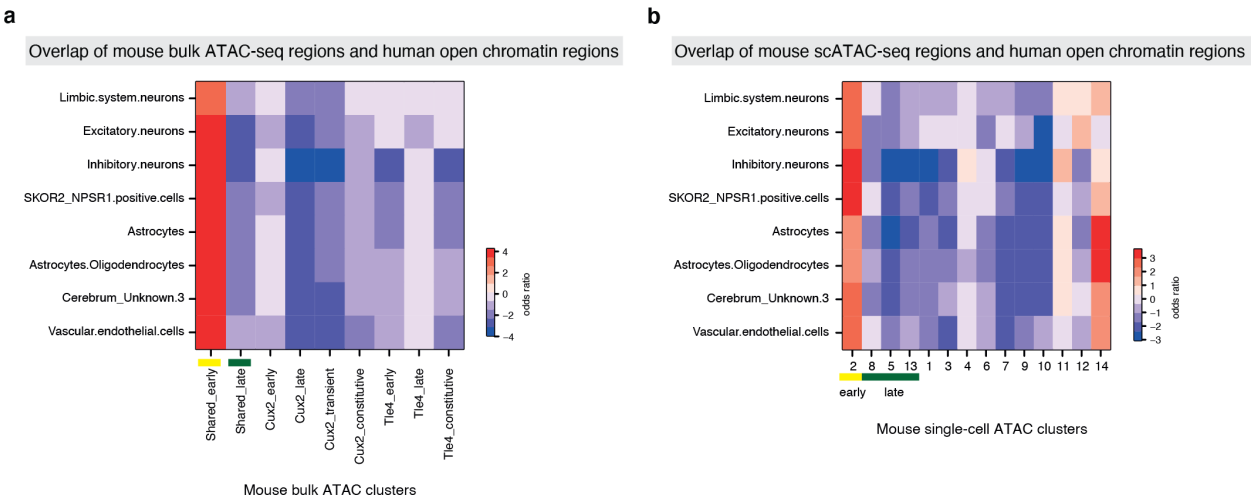
Supplementary Fig. 9. Characterization of mouse single-cell ATAC-seq dataset. a, UMAPs showing each cell cluster, broken down by age. **b**, UMAP showing depth of sequencing for each cell. **c**, Similarity between cell clusters, as a UMAP representation of the top 10 principal components (PCs) after PCA computed on the average gene expression space.



Supplementary Fig. 10. Characterization of marmoset single-cell ATAC-seq dataset. a, UMAPs showing each cell cluster, broken down by age. **b**, UMAP showing depth of sequencing for each cell. **c**, Similarity between cell clusters, as a UMAP representation of the top 10 principal components (PCs) after PCA computed on the average gene expression space.


a
Mouse ATAC cell type marker gene set scores
b
Example marker gene scores by cluster
c
Example marker gene tracks
Neurod2
Neurod6
Gad2
Slc32a1
Pdgfra
Sapcd2
Opalin
Mbp
Slc7a11
Apoe
d
Marmoset ATAC cell type marker gene set scores
e
Example marker gene scores by cluster
f
Example marker gene tracks
NEUROD2
NEUROD6
GAD2
SLC32A1
PDGFRA
NEU4
OPALIN
PRR5L
AQP4
PAX6

Supplementary Fig. 11. Cell-type assignments for scATAC datasets. a, Average gene score for mouse assigned cell identities using the same panel of cell-type marker gene sets as in Supplementary Fig. 5. **b**, Gene score for example individual marker genes. **c**, Mouse gene tracks for example marker genes across each assigned cell identity. **d**, Average gene score for marmoset assigned cell identities using the same panels of cell-type marker gene sets as in Supplementary Fig. 7. **e**, Gene score for example individual marker genes. **f**, Marmoset gene tracks for example marker genes across each assigned cell identity.



Supplementary Fig. 12. Correlation of mouse versus human chromatin open regions. a, Odds ratio for significant (BH corrected p-value <=0.05) overlaps of mouse bulk ATAC-seq clusters for PN (x-axis) and open chromatin regions in human fetal cerebrum. **b**, Odds ratio for significant (BH corrected p-value <=0.05) overlaps of mouse neuronal scATAC-seq-based open chromatin clusters (shown in e) (x-axis) and open chromatin regions in human fetal cerebrum.