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Supplementary Materials for

Molecular atlas of the adult mouse brain

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The PDF file includes:

Figs. S1 to S13
Legends for movies S1 to S5

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/26/eabb3446/DC1)

Movies S1 to S5

Fig. S1. Registration of the 75 coronal brain sections.
The mouse brain reference atlas (ABA) in red was transformed during registration to align with the HE stained brain tissue images. Sections are sorted by decreasing Bregma coordinates along the anteroposterior axis. Adjacent sections have the same ID and can be differentiated by the appended letter. Sections 36, 39 and 40 were divided into two or three subregions as the atlas outline could not be fitted on the entire section.

Fig. S2. Quality control of sequencing data.
(**A**) Distribution of spots detected per gene in semi-log scale. Dashed line represents the cutoff set to discard genes expressed in less than 100 spots.
(**B**) Distribution of unique genes per spot. Dashed line represents the cutoff set to discard spots expressing less than 1000 genes.
(**C**) Boxplot of unique genes, each box representing a section. Box is the interquartile range, dash the median, star the mean, whiskers delimitate extreme values.
(**D**) Distribution of reads per gene in semi-log scale.
(**E**) Distribution of reads per spot.
(**F**) Boxplot of reads per spot, each box representing a section as in (C).
(**G**) View of a coronal section with segmented nuclei in black and segmented spot contours in red.
(**H**) Distribution of nuclei segmented per spot.
(**I**) Correlation between segmented nuclei and unique genes per spot. Pearson correlation coefficient: 0.348.
(**J**) Correlation between segmented nuclei and reads per spot. Pearson correlation coefficient: 0.296.

Fig. S3. Gene loads for the 45 biological independent components (ICs).
The gene loads representing the top 20 genes for each IC is shown.

Fig. S4. Spatial visualization of the 45 biological independent components (ICs)
The integer attributed to every IC corresponds to its rank among the first 80 components. Only spots with scores above the 95-th percentile in absolute value are displayed in three dimensional views.

Fig. S5. Detailed spatial composition of clusters.
Rows correspond to the 181 molecular clusters and are ordered according to the hierarchical clustering. Stacked bar plot represents the proportion of spots registered in each of the major brain region. The color columns show the two different color schemes: the similarity color scheme is unbiased and represents molecular similarity between clusters, the categorical color scheme is used to maximize visual contrast between adjacent clusters.

Fig. S6. Coronal views of molecular atlas color coded according to 3D t-SNE.
Left side shows the reference ABA, and the right side shows the molecular atlas with molecular similarity color scheme based on 3D t-SNE color code.

Fig. S7. Comparison of t-SNE and UMAP based color code to visualize molecular similarity of clusters.
(**A**) Visualization of clusters using UMAP or t-SNE.
(**B**) 3D UMAP plot to assign color code to clusters based on molecular similarity (top). Color code applied to spots in the brain atlas shows their distribution.

(**C**) Coloring of spots based on the 3D t-SNE color code in either the UMAP manifold or the t-SNE manifold (top row). Coloring of spots based on the 3D UMAP color code in either the UMAP manifold or the t-SNE manifold (bottom row).
(**D**) Coloring of clusters in the fan plot according to 3D UMAP color code (inner circle). Outer circle shows color code based on neuroanatomical definitions from the ABA.
(**E**) Comparison of 3D UMAP color code (left hemisphere) versus 3D t-SNE color code (right hemisphere) in coronal sections from the molecular atlas.

Fig. S8. Coronal views of molecular atlas color coded according to 3D UMAP.
Left side shows the reference ABA, and the right side shows the molecular atlas with molecular similarity color scheme based on 3D UMAP color code.

Fig. S9. Gene expression in molecular clusters.
Example images of coronal sections showing the expression of single genes as detected by ISH in the ABA database.
The left side shows ABA mouse brain reference atlas outline. The right side shows molecular atlas outline, including relevant molecular clusters with color-coded spots.
(**A**) Isocortex. Plate 4, +1.84 mm from bregma.
(**B**) Isocortex. Plate 14, -1.05 mm from bregma.
(**C**) Striatum. Plate 7, +0.94 mm from bregma.

Fig. S10. Single cell spatial mapping.
(**A**) Correlation in logarithmic space between the normalized gene counts in the molecular atlas (ST) and the single cell (SC) dataset after batch correction (see (B)). Pearson correlation coefficient: 0.81.
(**B**) Detailed pipeline for the single cell spatial mapping. We applied batch correction to homogenize the ST and SC datasets, trained the neural network with all the ST spots and used it to predict a molecular cluster for each cell in the SC dataset.
(**C**) Fine ground truth definition that accounts for layers. In addition to a region, the molecular clusters are also assigned to layers based on the registration in the ABA. Selected layers contain at least 30% of the spots and are colored in green. Circle area is proportional to the number of spots.
(**D**) Comparison of mapping accuracy for glutamatergic cells with coarse (not layer dependent) and fine (including layer definition) ground truth.

Fig. S11. Matrix of similarity between molecular clusters in the full molecular atlas and the brain palette atlas.
Rows correspond to the original clusters ordered by hierarchical clustering. Columns represent the clusters obtained with the reduced brain palette of 266 genes. Each tile is colored according to the ratio of spots from the column palette cluster that were initially assigned to the row original cluster. The sum of each column is therefore equal to one. Black rectangle delimitates the zoom-in visible in Fig. 5 (E), corresponding clusters have yellow background and can be visualized in Fig. 5 (G). Hippocampal clusters are highlighted with pink background and displayed in Fig. 5 (H) and (I).

Fig. S12. Expression of genes present in the brain palette.
Expression heatmap of the 266 genes in the 181 clusters in the full molecular atlas. Clusters are ordered by hierarchical clustering and genes by peak value. Expression is computed from the batch-corrected normalized matrix which is z-scored for every gene and averaged per cluster.

Fig. S13. GO identity for genes in the reduced brain palette.
(**A**) Top five significant enriched GO terms for genes in the brain palette (266 genes).
(**B**) Heatmap of genes and GO terms. Genes belonging to a GO term are represented by a red tile. Out of the 266 genes from the palette, 64 are associated with at least one of the top five enriched GO terms.
(**C**) Annotation of the 266 brain palette genes into neuronal versus non-neuronal based on the GO terminology.

VIDEOS

Video 1. Visualization of the spots in a reference atlas framework.
Left side: coronal section from the ABA.
Right side: coronal section showing spots colored by the categorical color scheme according to molecular cluster. Total number of sections: 40.

Video 2. Generating a molecular atlas.
Examples to visualize the process of transforming spots belonging to a molecular cluster into discrete volumes based on the SVM approach.

Video 3. Visualization of the molecular atlas.
Left side: coronal section from the ABA.
Right side: coronal section showing molecular clusters after SVM smoothing and colored by the molecular similarity color scheme. Top left shows updated coronal sectioning in the anteroposterior axis (red line). Total number of sections: 84.

Video 4. Visualization of selected isocortical clusters in the molecular atlas.
Visualization of selected isocortical layer- and region-specific molecular clusters in 3D (glass brain). ABA volume definitions are shown with transparent colors.

Video 5. Visualization of selected striatal clusters in the molecular atlas.
Visualization of selected striatal region-specific molecular clusters in 3D (glass brain). ABA volume definition of striatum is shown with transparent color.

FIGURE S1

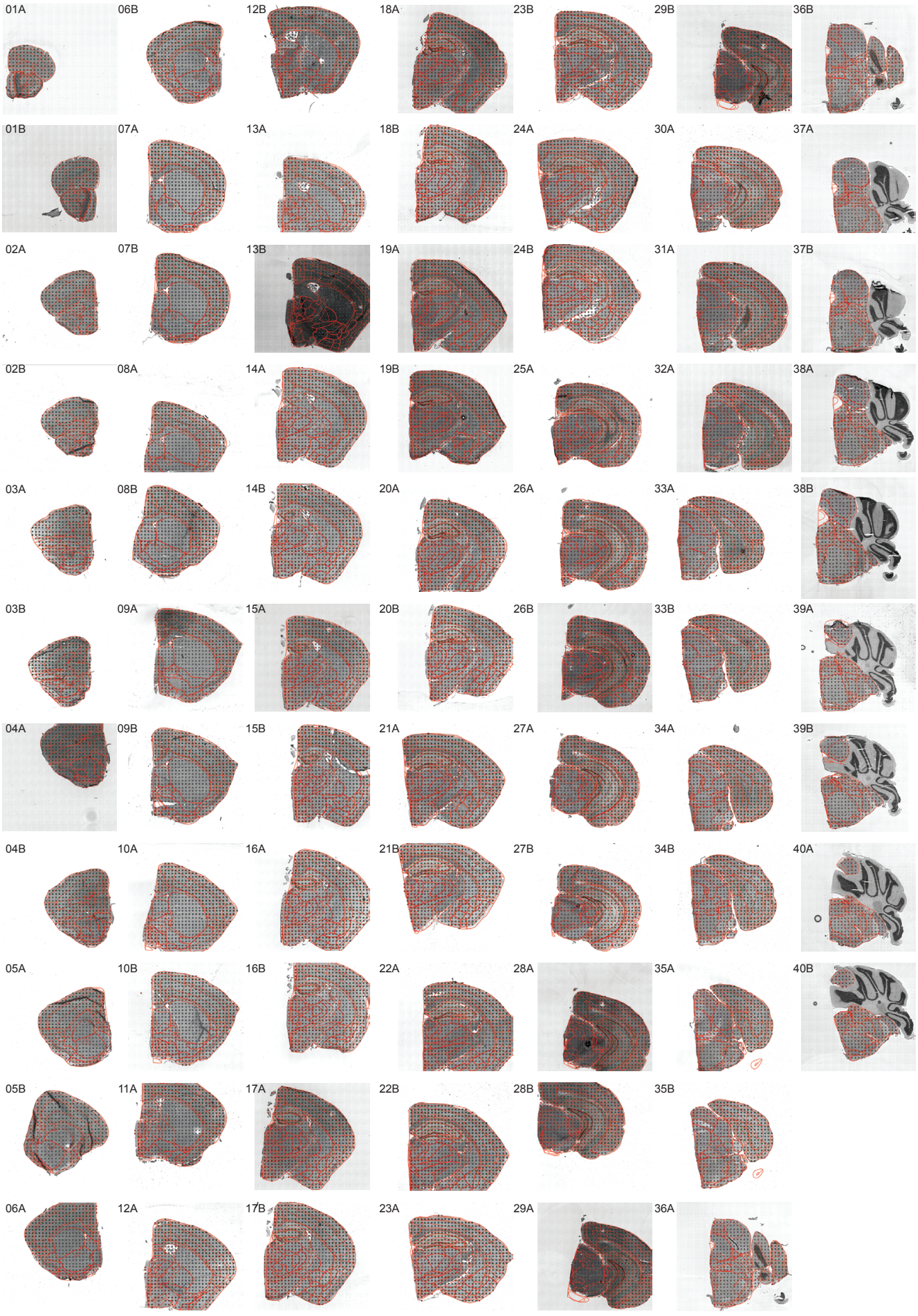
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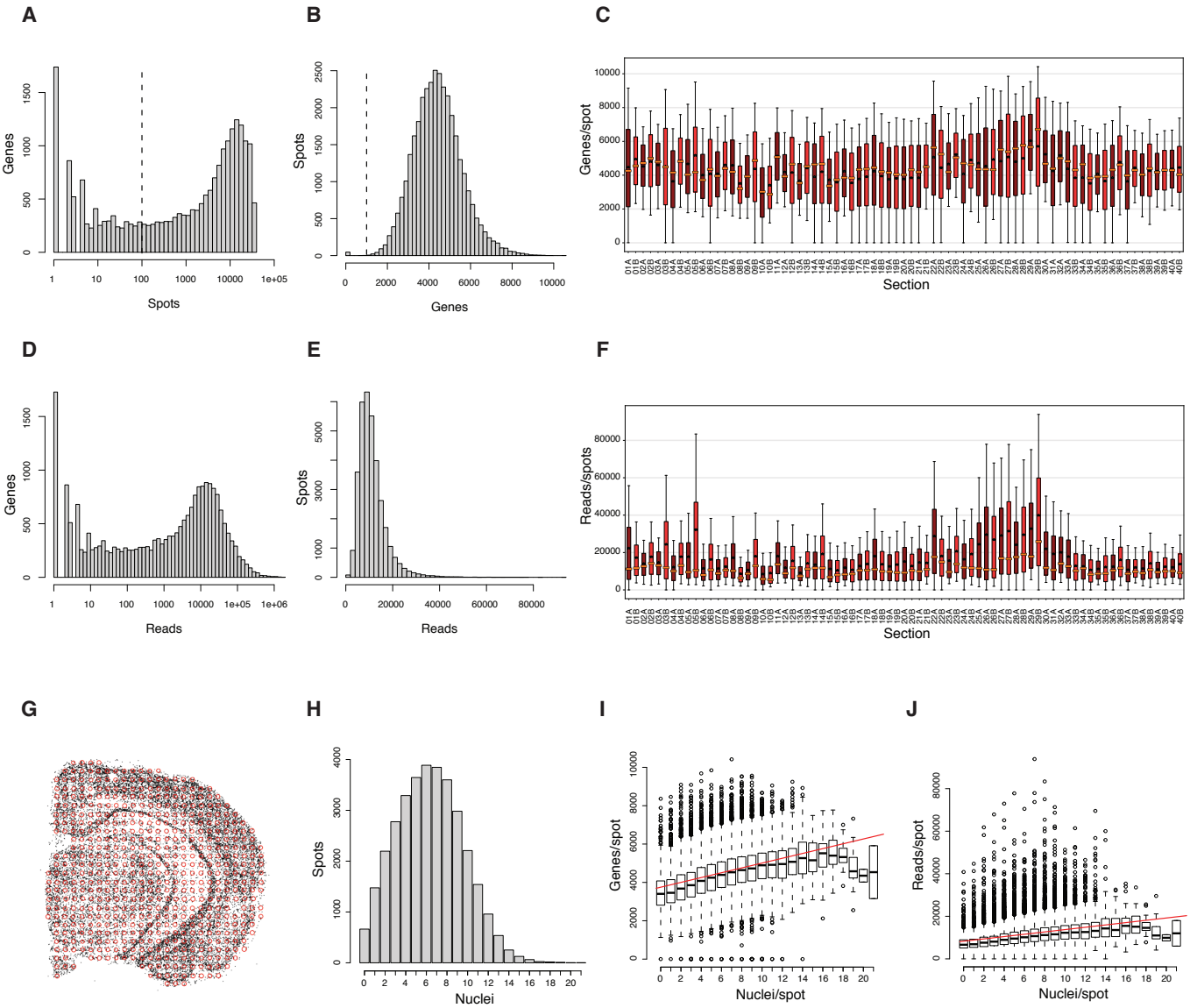
FIGURE S2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Spots
B
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Genes
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Reads
F
Reads/spots
Section
G
H
Spots
Nuclei
I
Genes/spot
Nuclei/spot
J
Reads/spot
Nuclei/spot

FIGURE S3

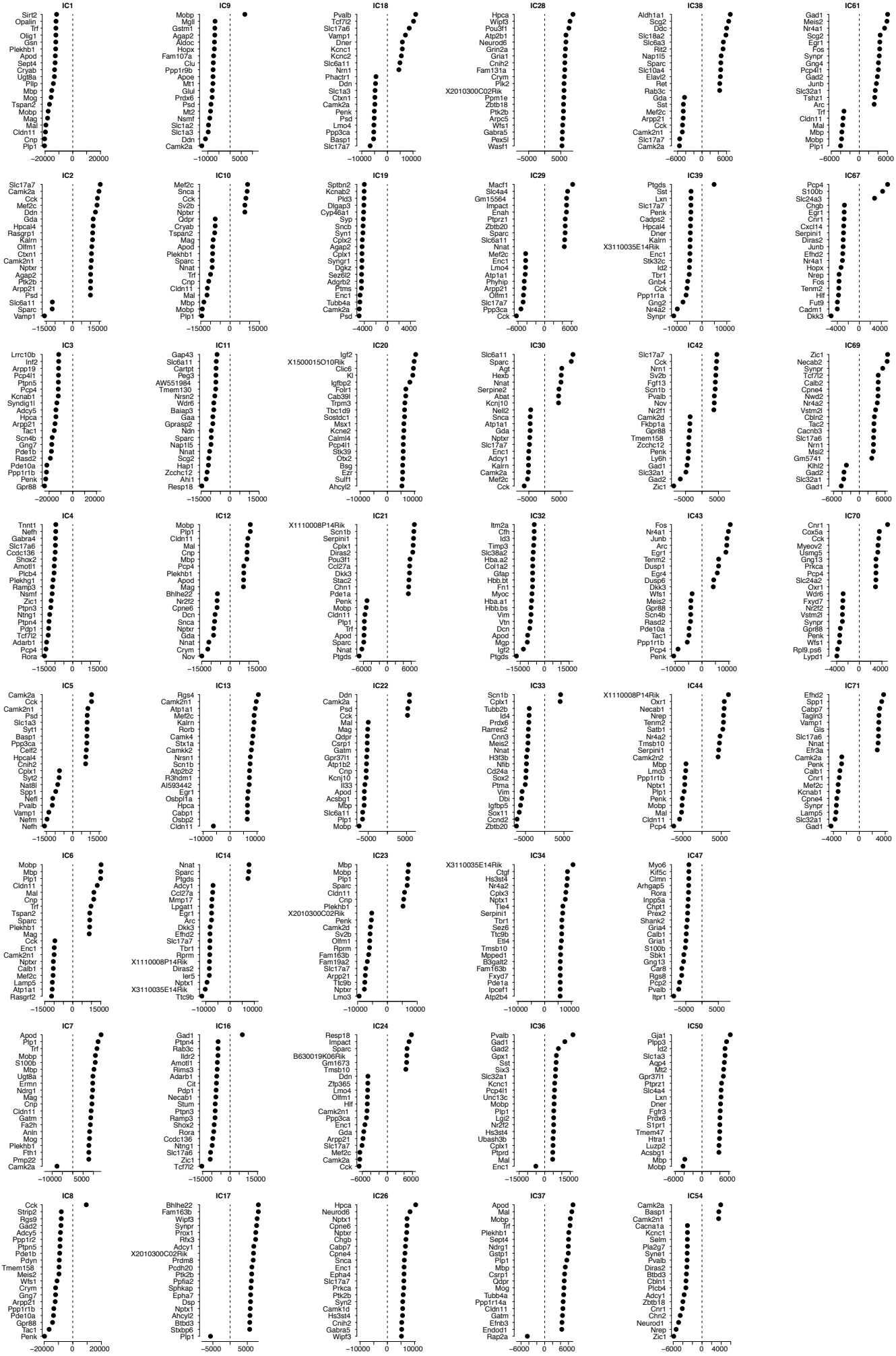
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FIGURE S4

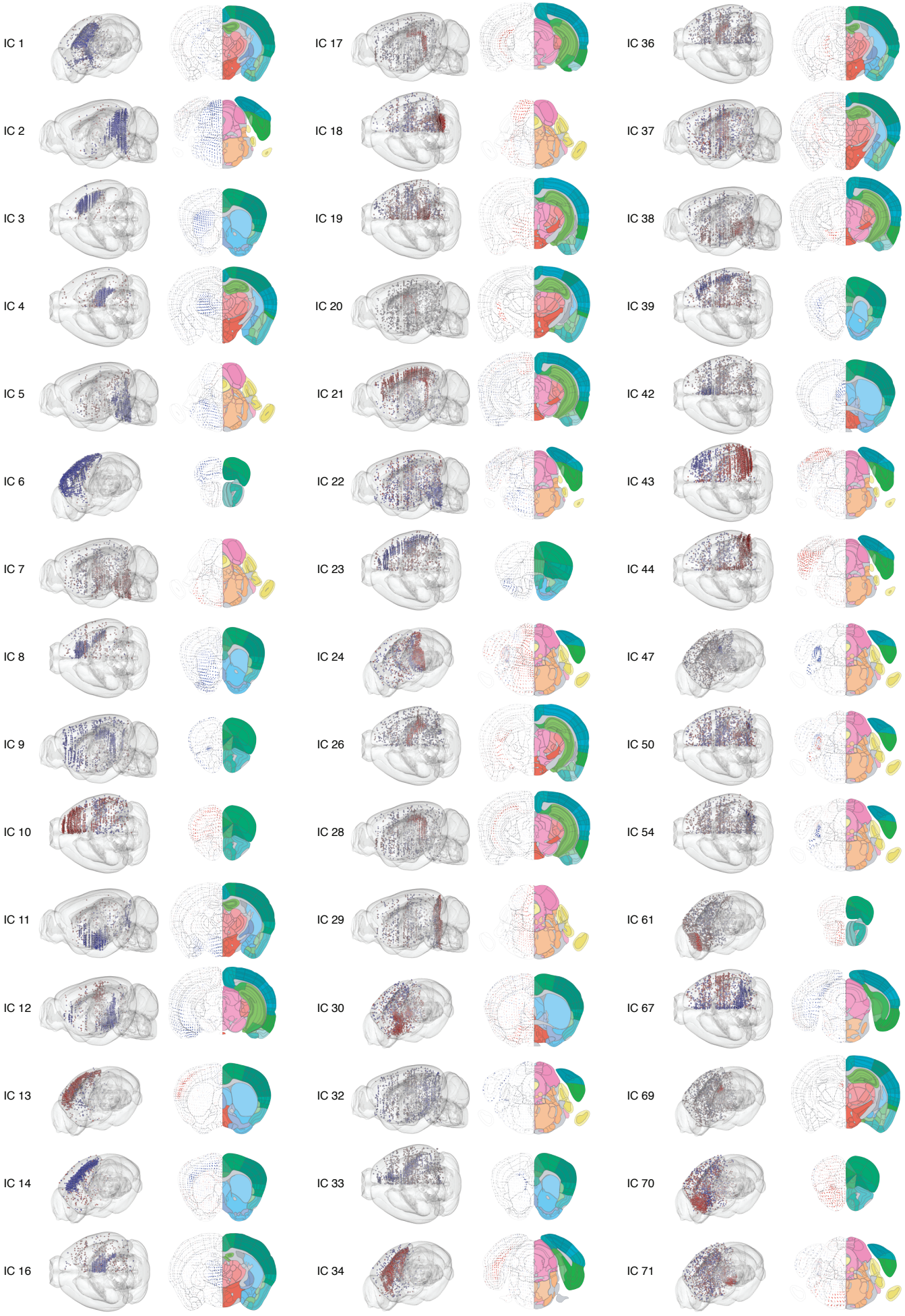
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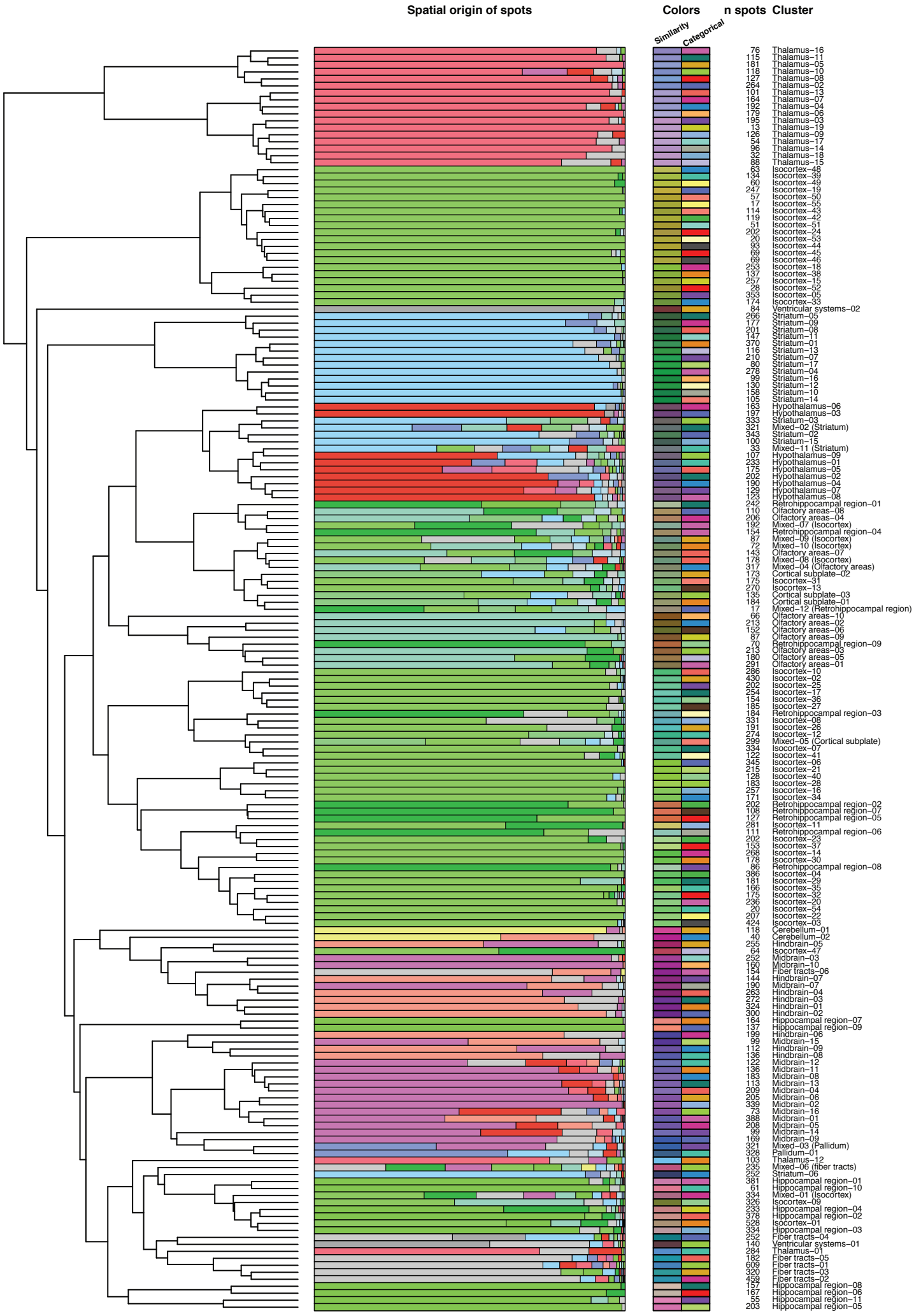
FIGURE S5


Spatial origin of spots
Colors
Similarity
Categorical
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Cluster
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264 Thalamus–02
101 Thalamus–13
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192 Thalamus–04
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257 Isocortex–15
28 Isocortex–52
353 Isocortex–05
174 Isocortex–33
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147 Striatum–11
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116 Striatum–13
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158 Striatum–10
105 Striatum–14
163 Hypothalamus–06
197 Hypothalamus–03
333 Striatum–03
321 Mixed–02 (Striatum)
343 Striatum–02
100 Striatum–15
33 Mixed–11 (Striatum)
107 Hypothalamus–09
233 Hypothalamus–01
175 Hypothalamus–05
202 Hypothalamus–02
190 Hypothalamus–04
129 Hypothalamus–07
123 Hypothalamus–08
242 Retrohippocampal region–01
110 Olfactory areas–08
206 Olfactory areas–04
192 Mixed–07 (Isocortex)
154 Retrohippocampal region–04
87 Mixed–09 (Isocortex)
72 Mixed–10 (Isocortex)
143 Olfactory areas–07
178 Mixed–08 (Isocortex)
317 Mixed–04 (Olfactory areas)
173 Cortical subplate–02
175 Isocortex–31
270 Isocortex–13
135 Cortical subplate–03
184 Cortical subplate–01
17 Mixed–12 (Retrohippocampal region)
66 Olfactory areas–10
213 Olfactory areas–02
152 Olfactory areas–06
87 Olfactory areas–09
70 Retrohippocampal region–09
213 Olfactory areas–03
180 Olfactory areas–05
291 Olfactory areas–01
286 Isocortex–10
430 Isocortex–02
202 Isocortex–25
254 Isocortex–17
154 Isocortex–36
185 Isocortex–27
184 Retrohippocampal region–03
331 Isocortex–08
191 Isocortex–26
274 Isocortex–12
299 Mixed–05 (Cortical subplate)
334 Isocortex–07
122 Isocortex–41
345 Isocortex–06
215 Isocortex–21
128 Isocortex–40
183 Isocortex–28
257 Isocortex–16
171 Isocortex–34
202 Retrohippocampal region–02
108 Retrohippocampal region–07
127 Retrohippocampal region–05
281 Isocortex–11
111 Retrohippocampal region–06
202 Isocortex–23
153 Isocortex–37
268 Isocortex–14
178 Isocortex–30
86 Retrohippocampal region–08
386 Isocortex–04
181 Isocortex–29
166 Isocortex–35
175 Isocortex–32
236 Isocortex–20
20 Isocortex–54
207 Isocortex–22
424 Isocortex–03
118 Cerebellum–01
40 Cerebellum–02
255 Hindbrain–05
64 Isocortex–47
252 Midbrain–03
160 Midbrain–10
154 Fiber tracts–06
144 Hindbrain–07
190 Midbrain–07
263 Hindbrain–04
272 Hindbrain–03
324 Hindbrain–01
300 Hindbrain–02
164 Hippocampal region–07
137 Hippocampal region–09
199 Hindbrain–06
99 Midbrain–15
112 Hindbrain–09
136 Hindbrain–08
122 Midbrain–12
136 Midbrain–11
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339 Midbrain–02
73 Midbrain–16
388 Midbrain–01
208 Midbrain–05
99 Midbrain–14
169 Midbrain–09
321 Mixed–03 (Pallidum)
328 Pallidum–01
103 Thalamus–12
235 Mixed–06 (fiber tracts)
252 Striatum–06
381 Hippocampal region–01
61 Hippocampal region–10
334 Mixed–01 (Isocortex)
326 Isocortex–09
233 Hippocampal region–04
378 Hippocampal region–02
528 Isocortex–01
334 Hippocampal region–03
252 Fiber tracts–04
140 Ventricular systems–01
284 Thalamus–01
182 Fiber tracts–05
609 Fiber tracts–01
320 Fiber tracts–03
459 Fiber tracts–02
157 Hippocampal region–08
167 Hippocampal region–06
55 Hippocampal region–11
203 Hippocampal region–05

FIGURE S6

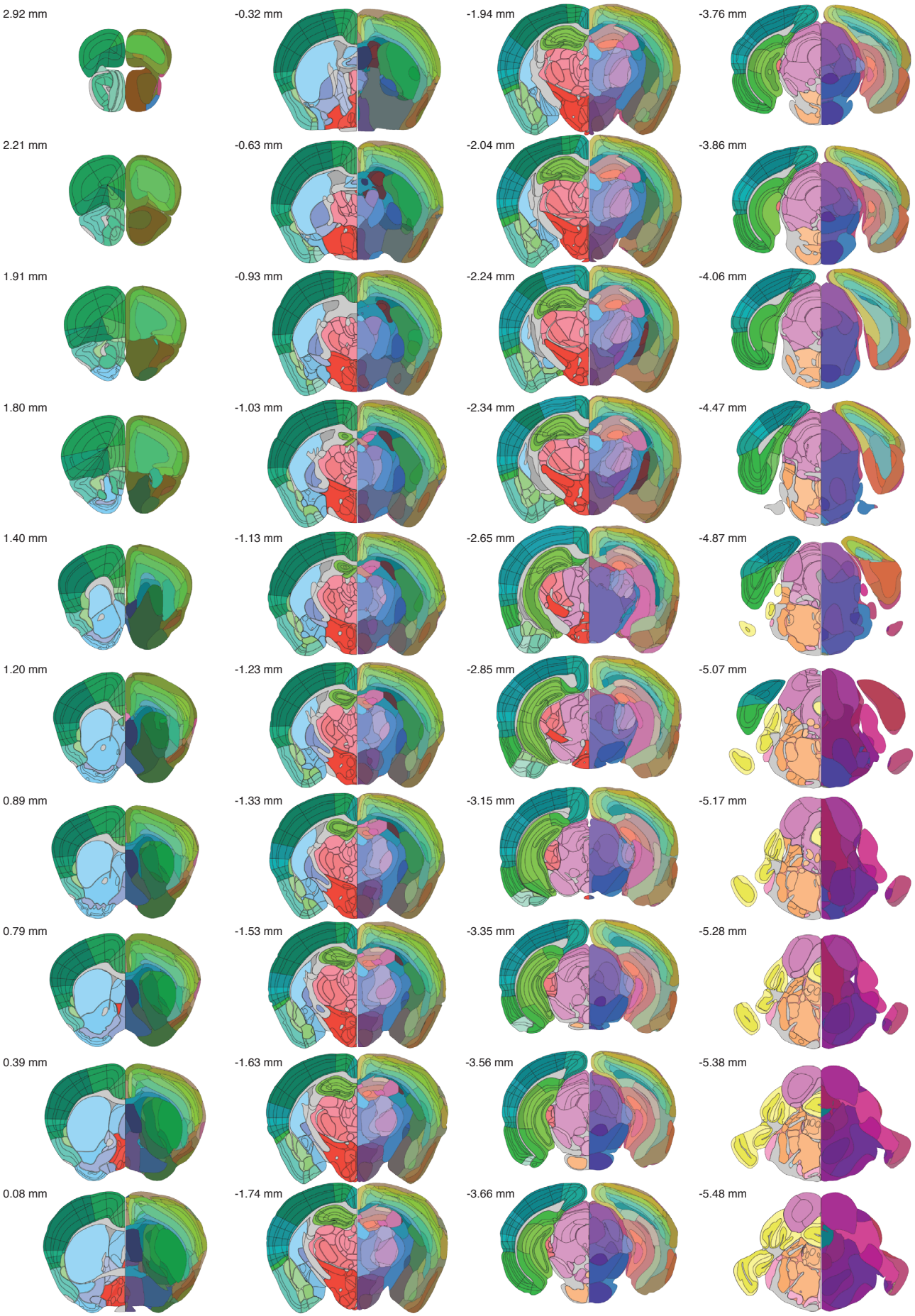

2.92 mm
-0.32 mm
-1.94 mm
-3.76 mm
2.21 mm
-0.63 mm
-2.04 mm
-3.86 mm
1.91 mm
-0.93 mm
-2.24 mm
-4.06 mm
1.80 mm
-1.03 mm
-2.34 mm
-4.47 mm
1.40 mm
-1.13 mm
-2.65 mm
-4.87 mm
1.20 mm
-1.23 mm
-2.85 mm
-5.07 mm
0.89 mm
-1.33 mm
-3.15 mm
-5.17 mm
0.79 mm
-1.53 mm
-3.35 mm
-5.28 mm
0.39 mm
-1.63 mm
-3.56 mm
-5.38 mm
0.08 mm
-1.74 mm
-3.66 mm
-5.48 mm

FIGURE S7

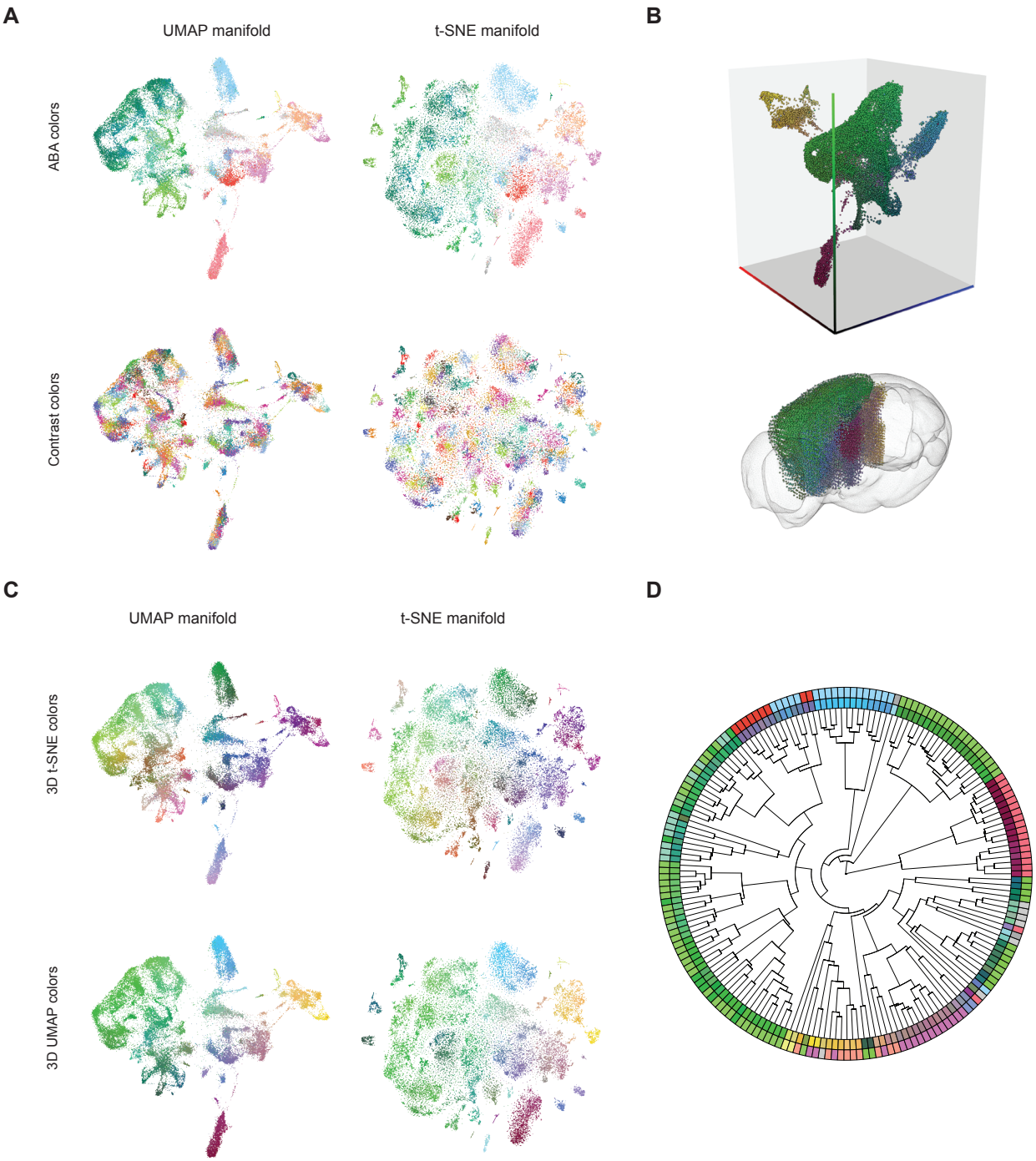

A
UMAP manifold
t-SNE manifold
ABA colors
Contrast colors
B
C
UMAP manifold
t-SNE manifold
3D t-SNE colors
3D UMAP colors
D
E
3D UMAP code 3D t-SNE code
Bregma +2.21 mm
Bregma +1.80 mm
Bregma +0.79 mm
Bregma +0.08 mm
Bregma -0.63 mm
Bregma -1.03 mm
Bregma -1.53 mm
Bregma -2.04 mm
Bregma -2.24 mm
Bregma -2.85 mm
Bregma -3.56 mm
Bregma -3.76 mm

FIGURE S8

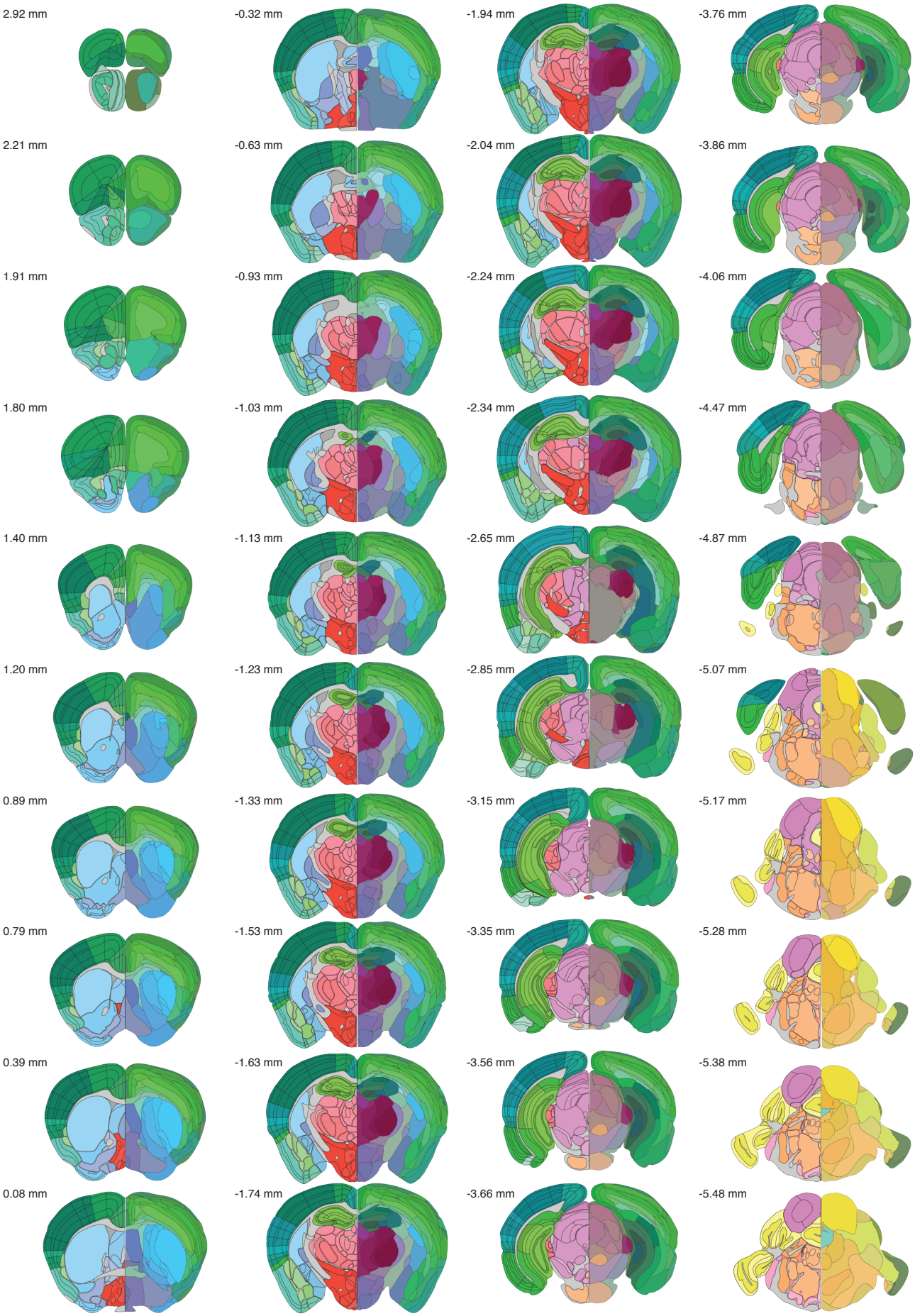
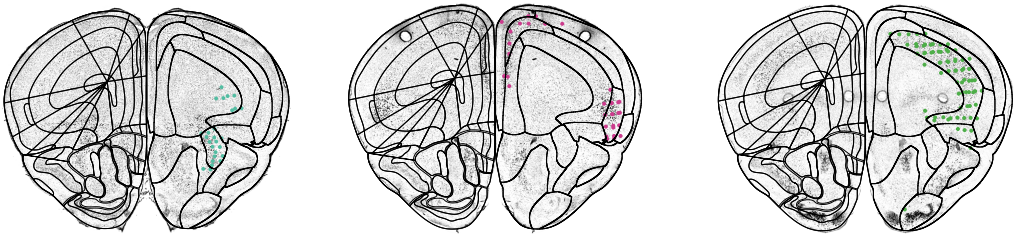
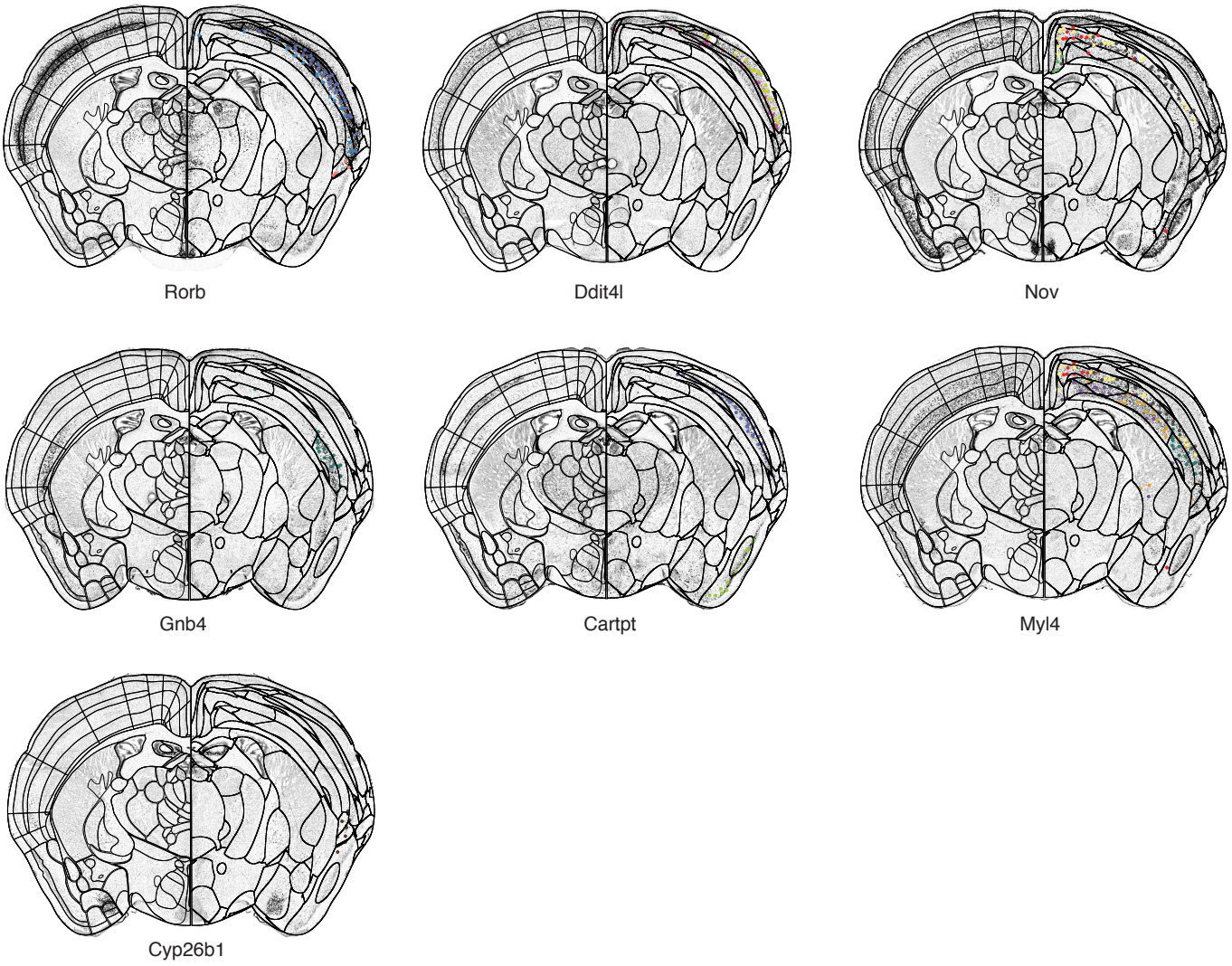

2.92 mm
-0.32 mm
-1.94 mm
-3.76 mm
2.21 mm
-0.63 mm
-2.04 mm
-3.86 mm
1.91 mm
-0.93 mm
-2.24 mm
-4.06 mm
1.80 mm
-1.03 mm
-2.34 mm
-4.47 mm
1.40 mm
-1.13 mm
-2.65 mm
-4.87 mm
1.20 mm
-1.23 mm
-2.85 mm
-5.07 mm
0.89 mm
-1.33 mm
-3.15 mm
-5.17 mm
0.79 mm
-1.53 mm
-3.35 mm
-5.28 mm
0.39 mm
-1.63 mm
-3.56 mm
-5.38 mm
0.08 mm
-1.74 mm
-3.66 mm
-5.48 mm

FIGURE S9

A



B



C

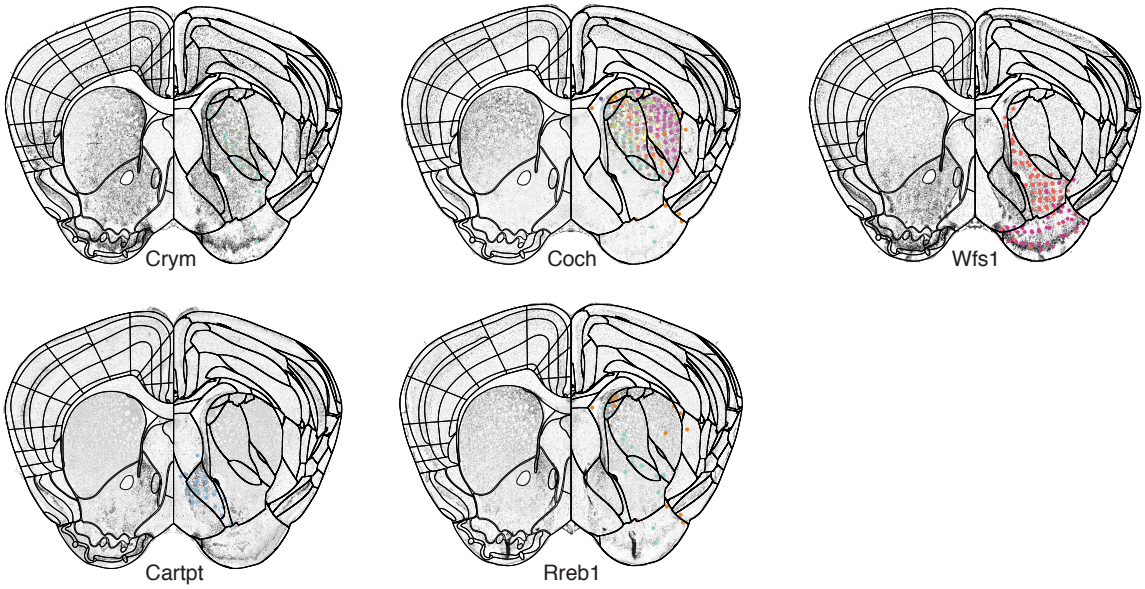


FIGURE S10

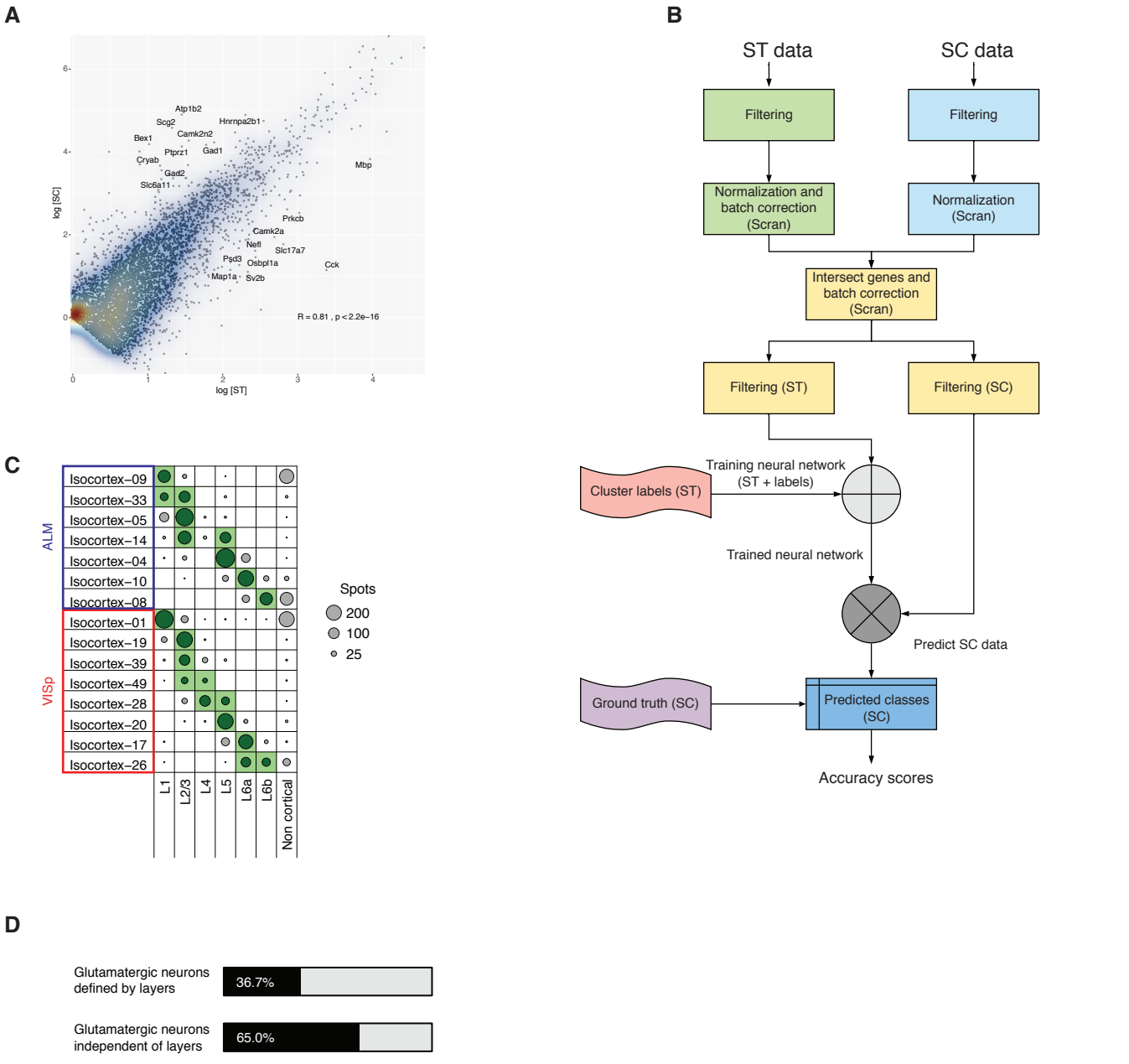

A
log [SC]
log [ST]
R = 0.81 , p < 2.2e−16
Atp1b2
Scg2
Hnrnpa2b1
Camk2n2
Bex1
Gad1
Ptprz1
Cryab
Mbp
Gad2
Slc6a1
Prkcb
Camk2a
Nefl
Slc17a7
Psd3
Osbpl1a
Cck
Map1a
Sv2b
B
ST data
SC data
Filtering
Filtering
Normalization and batch correction (Scran)
Normalization (Scran)
Intersect genes and batch correction (Scran)
Filtering (ST)
Filtering (SC)
Cluster labels (ST)
Training neural network (ST + labels)
Trained neural network
Predict SC data
Ground truth (SC)
Predicted classes (SC)
Accuracy scores
C
ALM
VISp
Isocortex−09
Isocortex−33
Isocortex−05
Isocortex−14
Isocortex−04
Isocortex−10
Isocortex−08
Isocortex−01
Isocortex−19
Isocortex−39
Isocortex−49
Isocortex−28
Isocortex−20
Isocortex−17
Isocortex−26
L1
L2/3
L4
L5
L6a
L6b
Non cortical
Spots
200
100
25
D
Glutamatergic neurons defined by layers
36.7%
Glutamatergic neurons independent of layers
65.0%

FIGURE S11

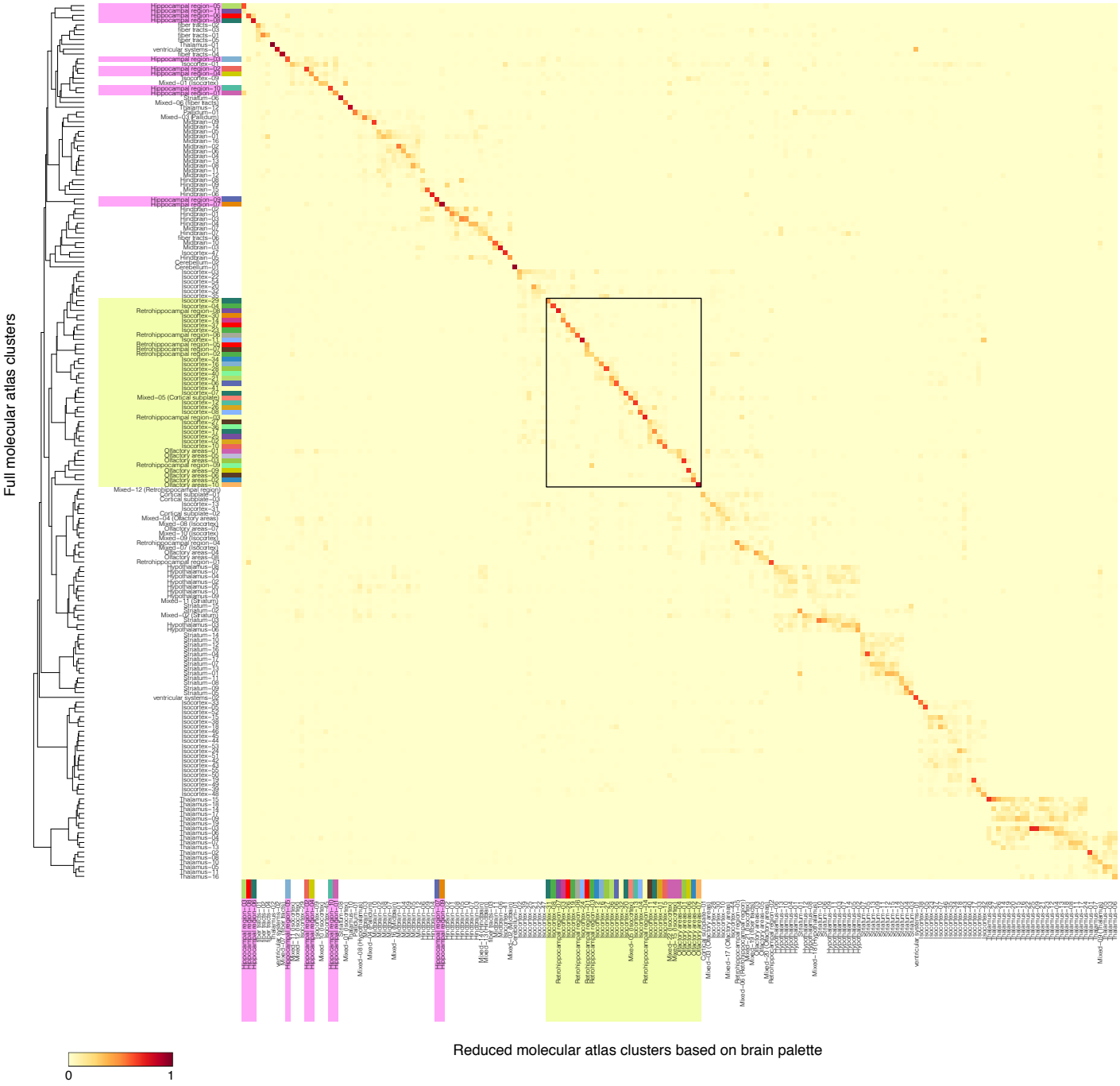

Full molecular atlas clusters
Reduced molecular atlas clusters based on brain palette
0
1

FIGURE S12

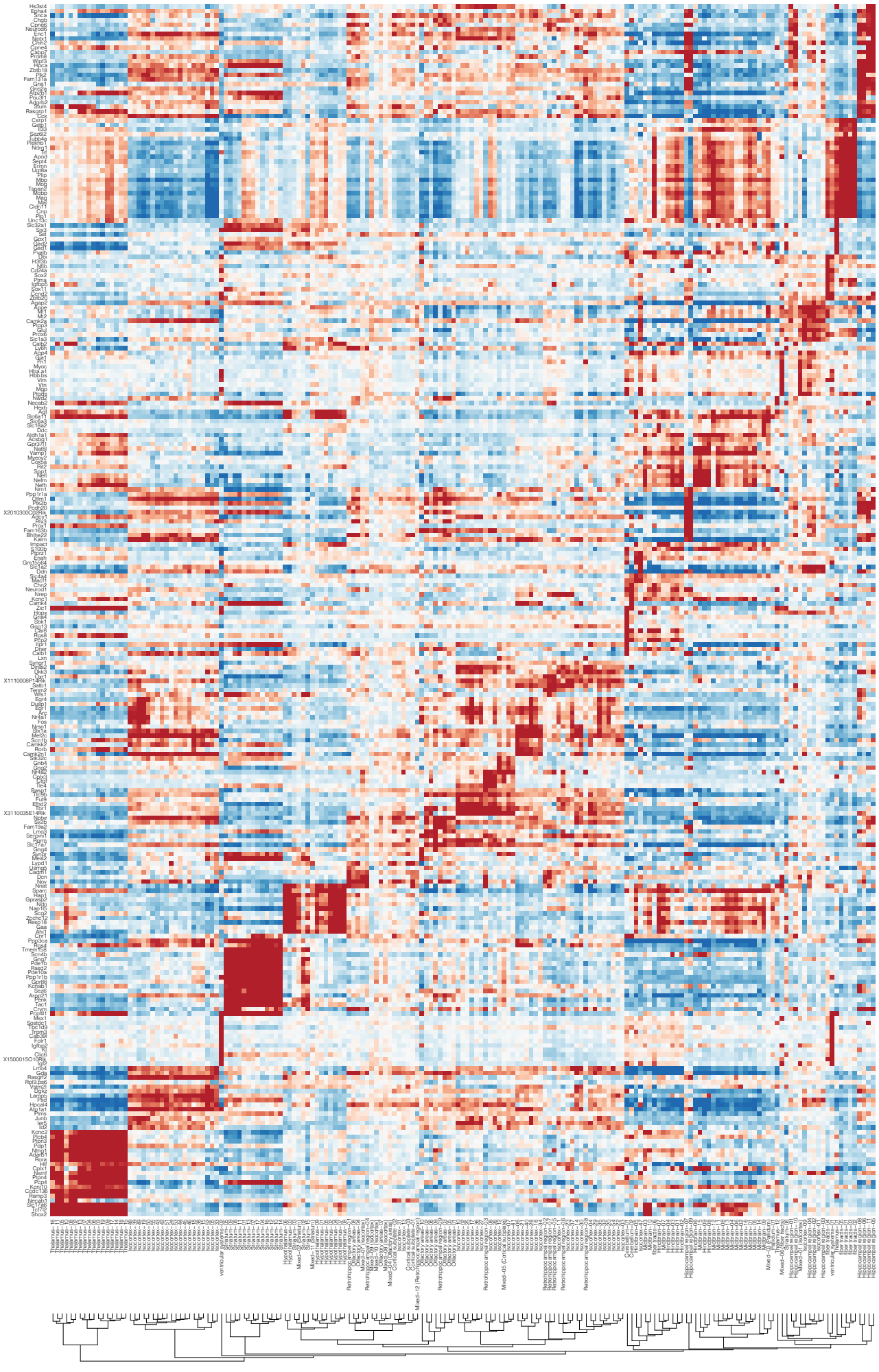
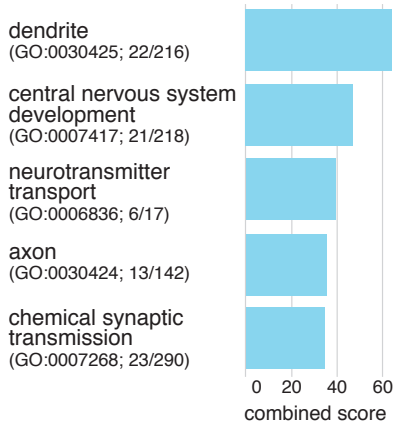
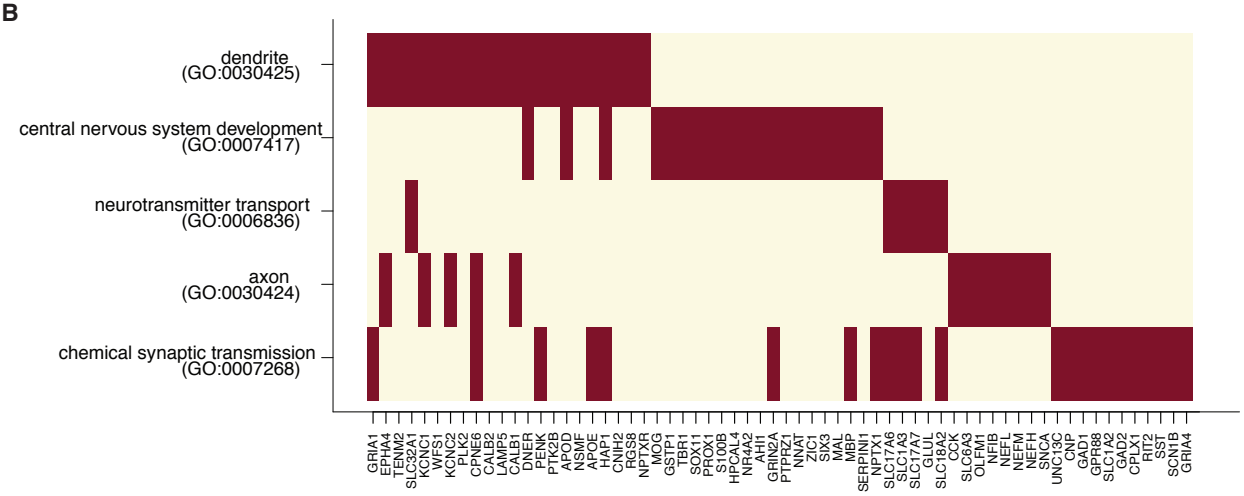


FIGURE S13

A



B



C

Neuronal genes			Non neuronal genes			
Adarb1	Adcy1	Ahi1	Acsbg1	Adgrb2	Agap2	Agt
Apod	Apoe	Arc	Aldh1a1	Aqp4	Arpp21	Basp1
Atp1a1	Atp2b1	Cadm1	Bhlhe22	Cab39l	Cabp7	Camk4
Calb1	Calb2	Camk2a	Camkk2	Car8	Ccdc136	Ccnd2
Camk2n1	Cck	Cd24a	Chgb	Chn2	Clic6	Cnp
Cldn11	Cnih2	Cnr1	Cox5a	Cpne4	Crym	Csrp1
Cplx1	Cplx3	Cpne6	Ctgf	Dcn	Diras2	Dkk3
Dbi	Ddc	Ddn	Dusp1	Efhd2	Egr1	Egr4
Dgkz	Dner	Enc1	Enah	Fam131a	Fam163b	Fam19a2
Epha4	Ermn	Fos	Fn1	Folr1	Fut9	Gaa
Gad1	Gad2	Glul	Gda	Gja1	Gm15564	Gnb4
Gng13	Gpr88	Gpx1	Gng2	Gng4	Gng7	Gpr37l1
Gria1	Gria4	Grin2a	Gprasp2	Gstp1	H3f3b	Hba.a1
Hap1	Hpca	Itpr1	Hbb.bs	Hexb	Hlf	Hopx
Kalrn	Kcnab1	Kcnc1	Hpcal4	Hs3st4	Id2	Ier5
Kcnc2	Kcnj10	Lamp5	Igf2	Igfbp2	Igfbp5	Il33
Macf1	Mag	Mbp	Impact	Junb	Kl	Lmo3
Myoc	Ndn	Nefh	Lmo4	Lxn	Ly6h	Lypd1
Nefl	Nefm	Nfib	Mal	Mef2c	Meis2	Mgp
Nptx1	Nrsn1	Nsmf	Mobp	Mog	Msx1	Mt1
Ntng1	Olfm1	Oxr1	Mt2	Myeov2	Nap1l5	Nat8l
Pcp2	Pcp4	Pde10a	Ndrg1	Necab1	Necab2	Neurod1
Pde1b	Penk	Plcb4	Neurod6	Nnat	Nov	Nptxr
Plekhb1	Plk2	Ppp1r1b	Nr4a1	Nr4a2	Nrep	Nrn1
Ppp3ca	Psd	Ptk2b	Nwd2	Pcdh20	Pcp4l1	Pdp1
Ptprz1	Pvalb	Resp18	Pllp	Plp1	Plpp3	Pou3f1
Rgs8	Rit2	S100b	Ppp1r1a	Prdm8	Prdx6	Prox1
Scg2	Scn1b	Serpini1	Ptgds	Ptma	Ptms	Ptpn3
Sez6	Sez6l2	Slc17a6	Ptpn4	Ramp3	Rasd2	Rasgrf2
Slc17a7	Slc18a2	Slc1a2	Rasgrp1	Rfx3	Rgs4	Rora
Slc1a3	Slc32a1	Slc6a11	Rorb	Rpl9.ps6	Rprm	Satb1
Slc6a3	Snca	Sst	Sbk1	Scn4b	Sept4	Shox2
Stx1a	Sv2b	Syngr1	Six3	Slc4a4	Sostdc1	Sox11
Synpr	Tac1	Tenm2	Sox2	Sparc	Spp1	Stk32c
Tubb4a	Unc13c	Vamp1	Stum	Tbc1d9	Tbr1	Tcf7l2
Wfs1	Vim	Vstm2l	Tle4	Tmem158	Trf	Trpm3
			Tspan2	Ttc9b	Ugt8a	Usmg5
			Wipf3	Vtn	X1110008P14Rik	X1500015O10Rik
			X2010300C02Rik	X3110035E14Rik	Zbtb18	Zbtb20
			Zcchc12	Zic1		