Supplementary Materials for

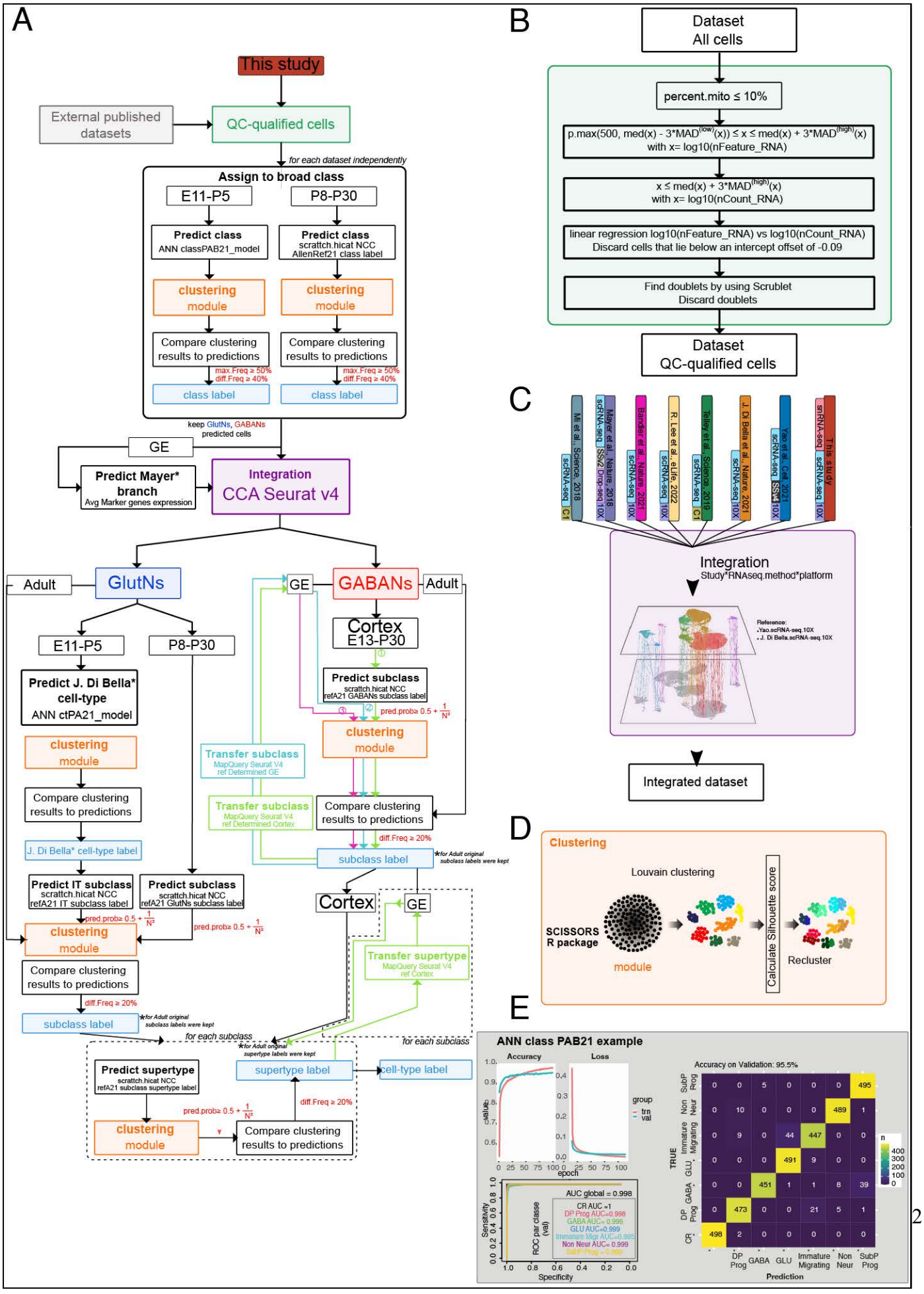
Title: Uncovering the Molecular Logic of Cortical Wiring Between Neuronal Subtypes Across Development via Ligand–Receptor Inference.

Authors: Rémi Mathieu[1], Tangra Draia-Nicolau[1], Léa Corbières[1], Annousha Govindan[1], Vianney Bensa[1], Emilie Pallesi[1], Alfonso Represa[1], Carlos Cardoso[1], Ludovic Telley[2]* & Antoine de Chevigny[1]*

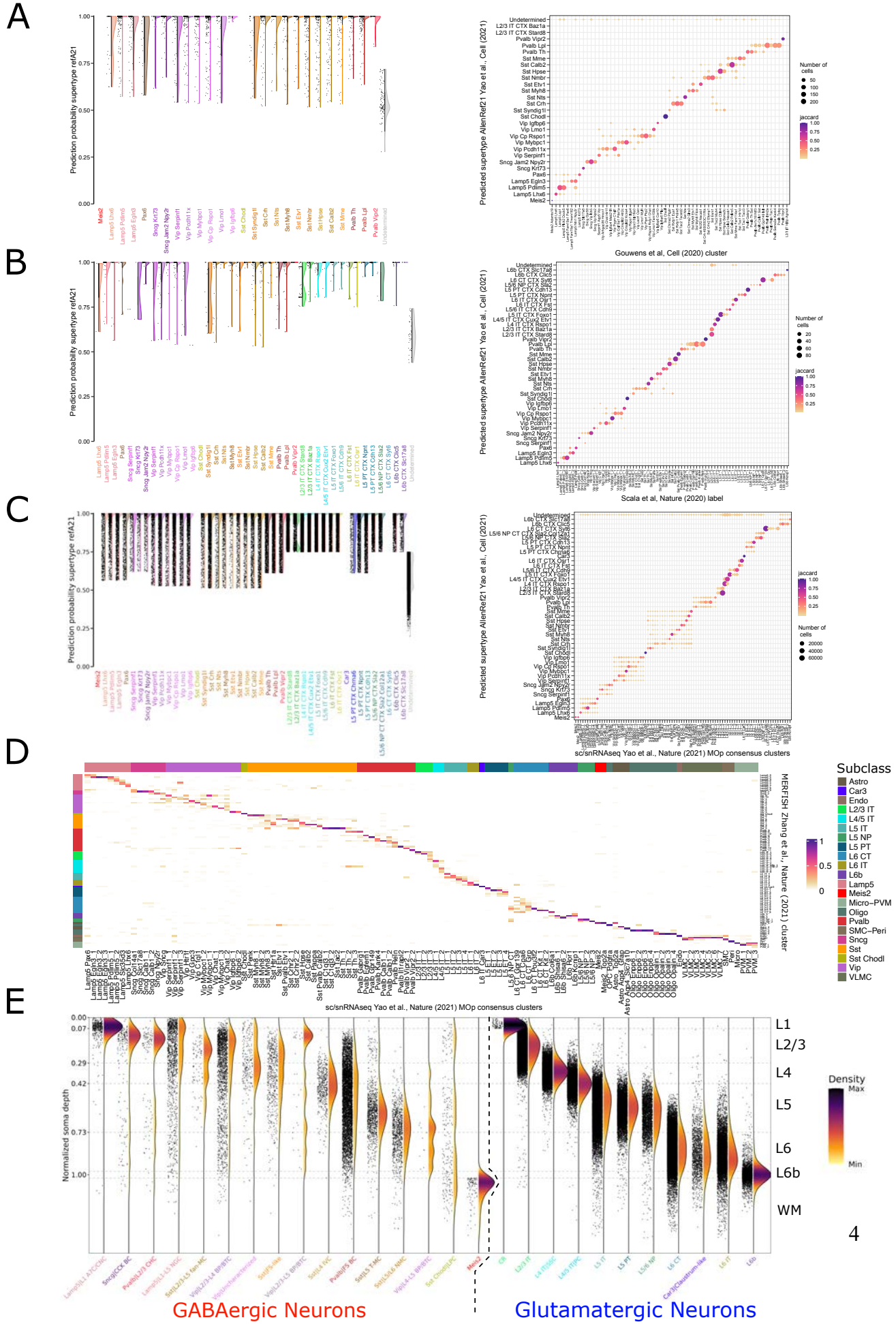
Corresponding authors: Antoine de Chevigny, antoine.de-chevigny@inserm.fr; Ludovic Telley, ludovic.telley@univ-lyon1.fr

The PDF file includes Figs. Supplementary 1 to 23

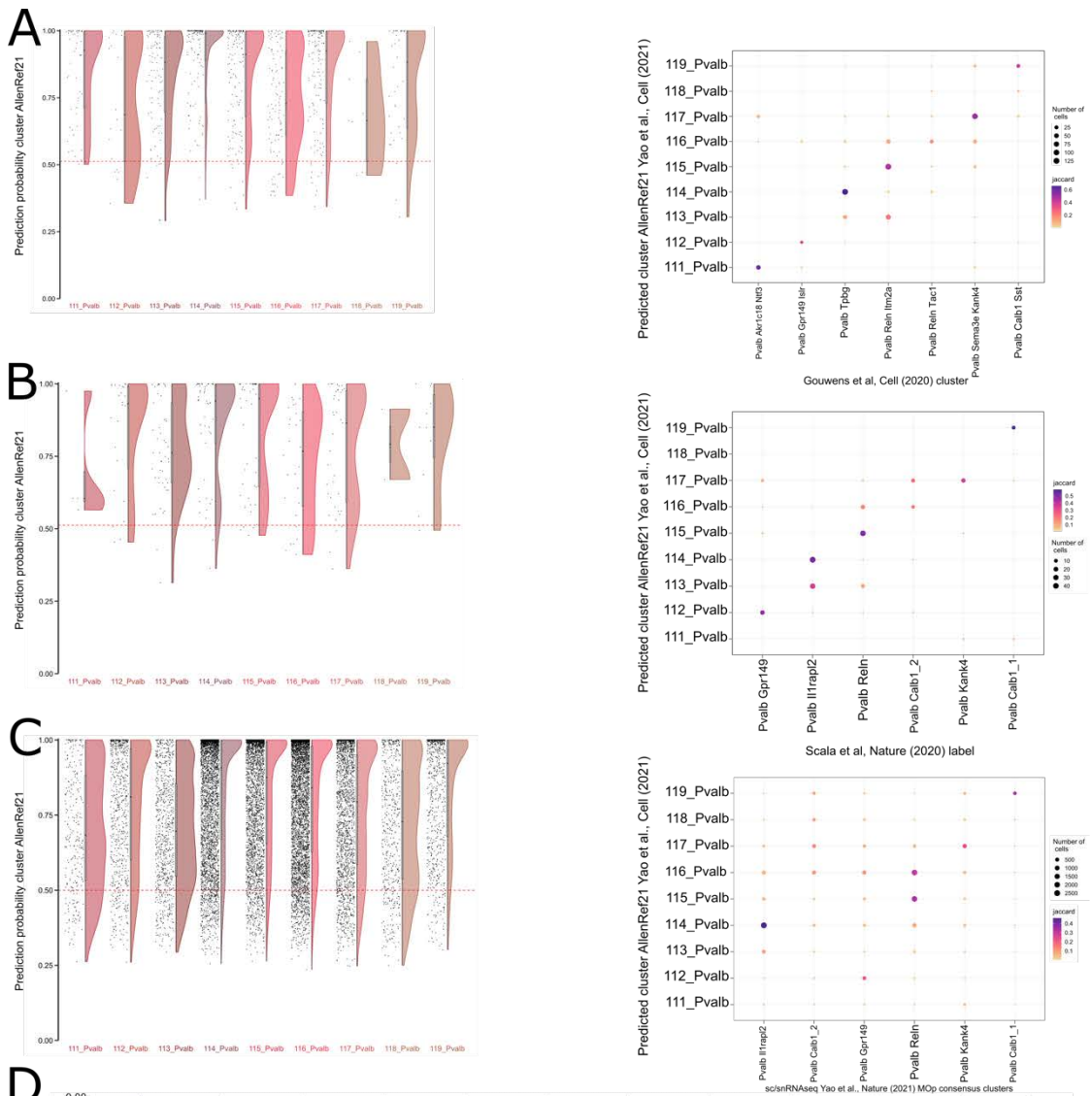
Other Supplementary Materials for this manuscript include the following: Supplementary Data 1 to 8


A
This study
External published datasets
QC-qualified cells
for each dataset independently
Assign to broad class
E11-P5
P8-P30
Predict class
ANN classPAB21_model
Predict class
scrattch.hicat NCC
AllenRef21 class label
clustering module
clustering module
Compare clustering results to predictions
Compare clustering results to predictions
max.Freq ≥ 50%
diff.Freq ≥ 40%
max.Freq ≥ 50%
diff.Freq ≥ 40%
class label
class label
keep GlutNs, GABANs predicted cells
GE
Predict Mayer* branch
Avg Marker genes expression
Integration
CCA Seurat v4
Adult
GlutNs
GE
GABANs
Adult
E11-P5
P8-P30
Cortex
E13-P30
Predict J. Di Bella* cell-type
ANN ctPA21_model
Predict subclass
scrattch.hicat NCC
refA21 GABANs subclass label
clustering module
clustering module
Transfer subclass
MapQuery Seurat V4
ref Determined GE
Transfer subclass
MapQuery Seurat V4
ref Determined Cortex
Compare clustering results to predictions
Compare clustering results to predictions
J. Di Bella* cell-type label
diff.Freq ≥ 20%
subclass label
*for Adult original subclass labels were kept
Predict IT subclass
scrattch.hicat NCC
refA21 IT subclass label
Predict subclass
scrattch.hicat NCC
refA21 GlutNs subclass label
Cortex
GE
clustering module
Transfer supertype
MapQuery Seurat V4
ref Cortex
Compare clustering results to predictions
diff.Freq ≥ 20%
subclass label
*for Adult original subclass labels were kept
for each subclass
for each subclass
*for Adult original supertype labels were kept
Predict supertype
scrattch.hicat NCC
refA21 subclass supertype label
supertype label
cell-type label
diff.Freq ≥ 20%
clustering module
Compare clustering results to predictions
B
Dataset
All cells
percent.mito ≤ 10%
p.max(500, med(x) - 3*MAD(low)(x)) ≤ x ≤ med(x) + 3*MAD(high)(x)
with x= log10(nFeature_RNA)
x ≤ med(x) + 3*MAD(high)(x)
with x= log10(nCount_RNA)
linear regression log10(nFeature_RNA) vs log10(nCount_RNA)
Discard cells that lie below an intercept offset of -0.09
Find doublets by using Scrublet
Discard doublets
Dataset
QC-qualified cells
C
Mi et al., Science, 2018
scRNA-seq C1
Mayer et al., Nature, 2018
scRNA-seq SSv2 Drop-seq 10X
Bander et al., Nature, 2021
scRNA-seq 10X
R. Lee et al. eLife, 2022
scRNA-seq 10X
Tolley et al. Science, 2019
scRNA-seq C1
J. Di Bella et al., Nature, 2021
scRNA-seq 10X
Yao et al., Cell, 2021
scRNA-seq SSv4 10X
This study
snRNA-seq 10X
Integration
Study*RNAseq.method*platform
Reference:
•Yao.scRNA-seq.10X
• J. Di Bella.scRNA-seq.10X
Integrated dataset
D
Clustering
Louvain clustering
SCISSORS
R package
module
Calculate Silhouette score
Recluster
E
ANN class PAB21 example
Accuracy
Loss
value
epoch
group
trn
val
AUC global = 0.998
CR AUC =1
DP Prog AUC=0.998
GABA AUC= 0.996
GLU AUC=0.999
Immature Migr AUC=0.995
Non Neur AUC= 0.999
SubP Prog = 0.999
Sensitivity
Specificity
ROC par classe (val)
Accuracy on Validation: 95.5%
TRUE
Prediction
DP Prog
GABA
GLU
Immature Migrating
Non Neur
SubP Prog
CR
n

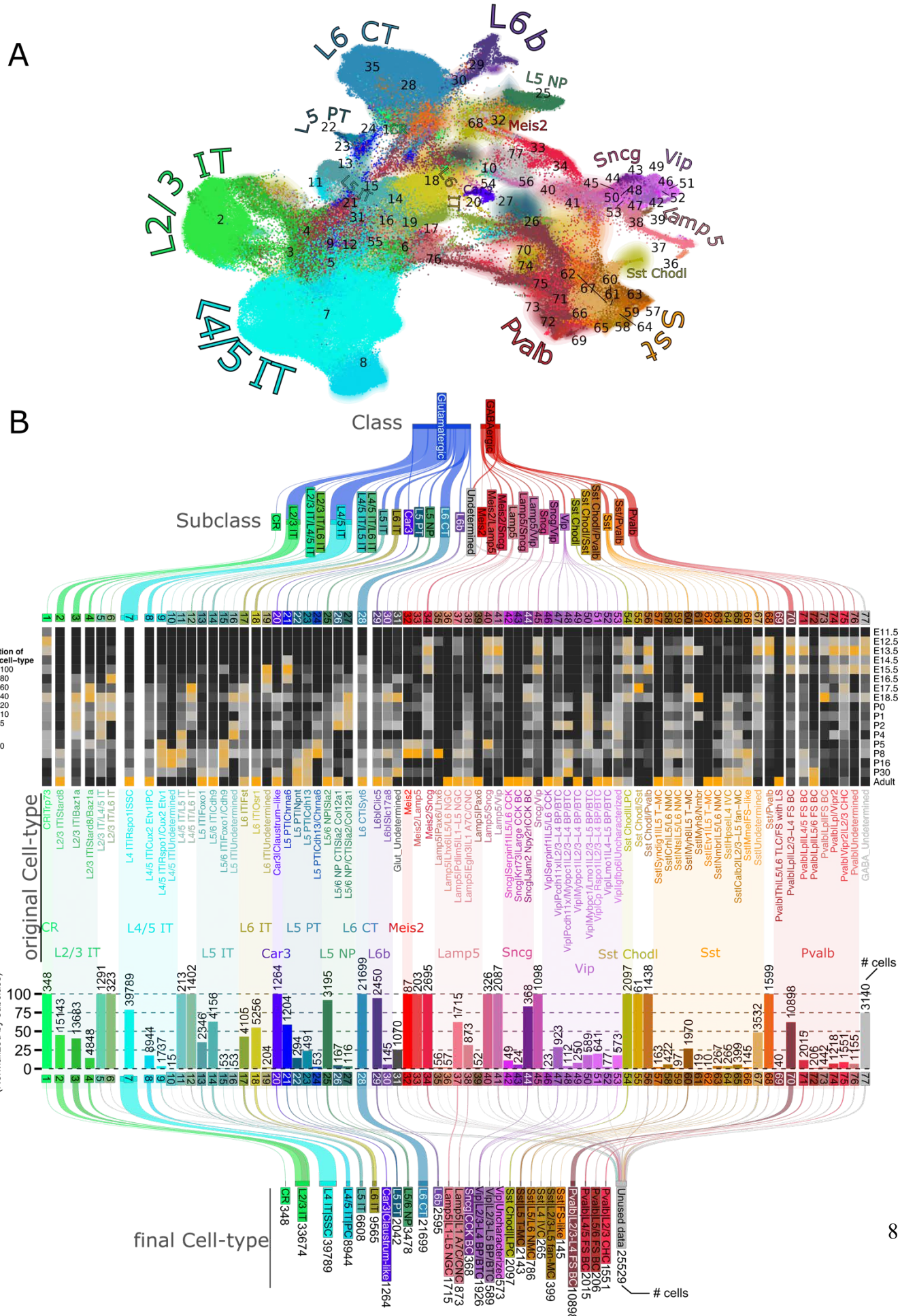
Supplementary fig. 1. scRNA-seq pipeline and analysis workflow. (a) Pipeline of the hierarchical procedure adopted to assign the identity of the cells. ANN: Artificial Neural Network, class PAB21_model: Di Bella et al., 2021 and Bandler et al., 2021 class model; NCC: Nearest centroid classifier; AllenRef21: reference dataset from Yao et al., 2021; ctPA21 model: Di Bella et al., 2021 cell-type model; IT: Intratelencephalic; CCA: Canonical correlation analysis. **(b)** Criteria used to keep cells passing quality control from individual experimental animals scRNA-seq data. **(c)** Integration of all the compiled datasets by using the SCT workflow of *Seurat* by taking the Yao et al, 2021 and J. Di Bella et al., 2021 datasets as reference. **(d)** Several rounds of Louvain clustering were performed by using the *SCISSOR* R package. **(e)** Example performance of the ANN classifier. Training and validation curves indicate high accuracy (~95%) with steadily decreasing loss. ROC analysis confirms excellent discrimination across all classes (global AUC = 0.998). The confusion matrix on the right illustrates that most predictions fall on the diagonal, with only minor misclassifications, supporting the overall validation accuracy of 95.5%.


A
B
C
D
E
Prediction probability supertype refA21
Predicted supertype AllenRef21 Yao et al., Cell (2021)
Gouwens et al, Cell (2020) cluster
Scala et al, Nature (2020) label
sc/snRNAseq Yao et al., Nature (2021) MOp consensus clusters
MERFISH Zhang et al., Nature (2021) cluster
Number of cells
jaccard
Subclass
Astro
Car3
Endo
L2/3 IT
L4/5 IT
L5 IT
L5 NP
L5 PT
L6 CT
L6 IT
L6b
Lamp5
Meis2
Micro−PVM
Oligo
Pvalb
SMC−Peri
Sncg
Sst
Sst Chodl
Vip
VLMC
Normalized soma depth
Density
Max
Min
L1
L2/3
L4
L5
L6
L6b
WM
GABAergic Neurons
Glutamatergic Neurons
4

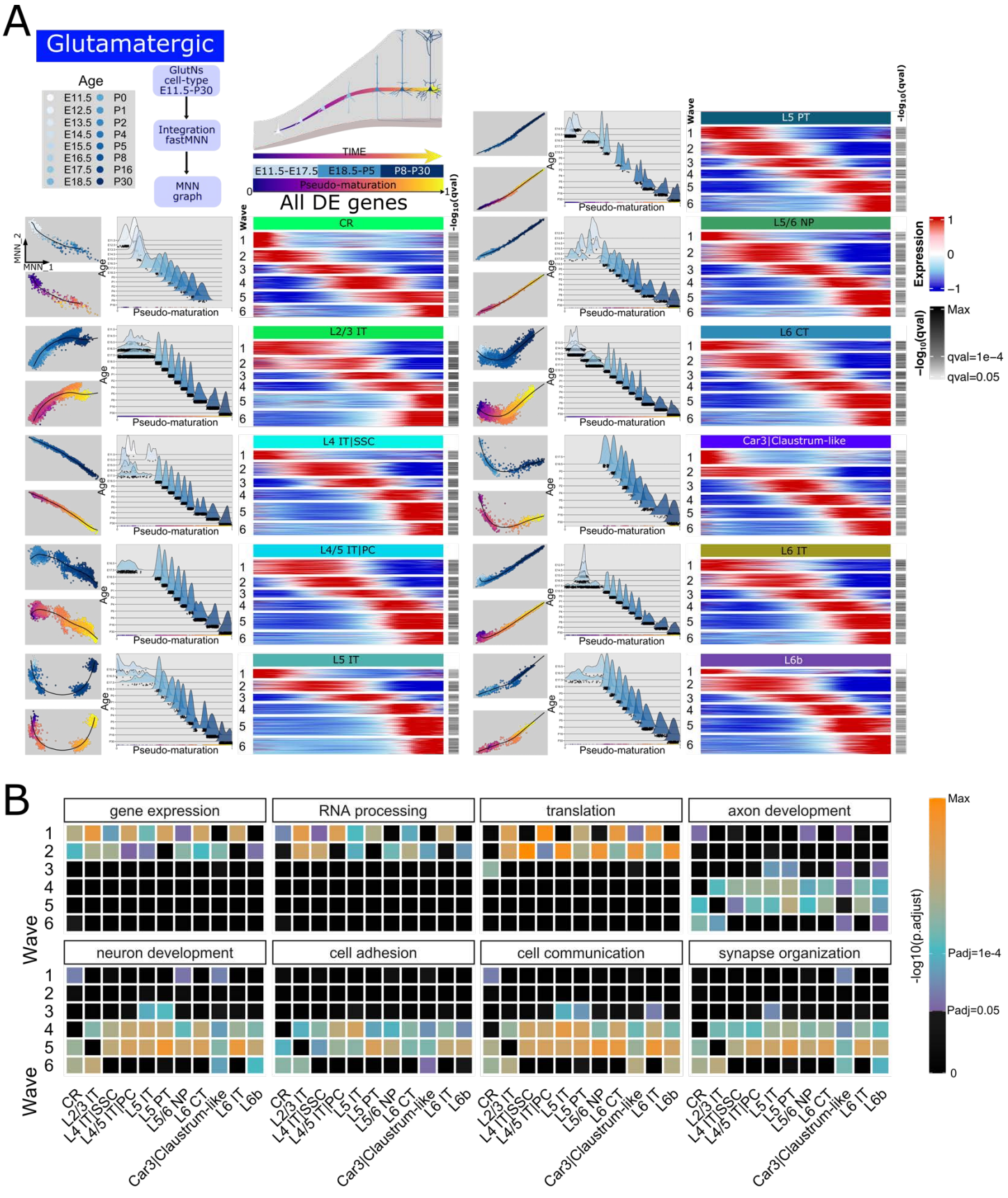
Supplementary fig. 2. Correspondence between supertype of the AllenRef21 reference and cell-types defined in patch-seq and MERFISH studies. Mapping results of Gouwens et al., 2020 patch-seq study (a), Scala et al., 2020 patch-seq study (b), and Yao et al., 2021 (c) on the AllenRef21 by using the nearest centroid classifier (NCC) of the *scrattch.hicat* R package. Left: Prediction probability resulting from the mapping performed with the NCC. Right: annotation comparisons resulting from the mapping of the different studies on the AllenRef21. The size of the dots indicates the number of overlapping cells, and the color indicates the Jaccard index (number of cells in intersection/number of cells in union). (d) Correlation matrix provided in BICCN study (supplementary fig. 5) that gives the correspondence between MOp consensus cluster taxonomy and MERFISH data. (e) Distribution of the different cell-types in the cortex extrapolating from data of normalized soma depth provided in Gouwens et al., 2020, Scala et al., 2020 and Zhang et al., 2021. Associated source data are provided


A
Prediction probability cluster AllenRef21
111_Pvalb 112_Pvalb 113_Pvalb 114_Pvalb 115_Pvalb 116_Pvalb 117_Pvalb 118_Pvalb 119_Pvalb
Predicted cluster AllenRef21 Yao et al., Cell (2021)
Gouwens et al, Cell (2020) cluster
Pvalb Ak1c18 Nfl3
Pvalb Gpr149 Isl1
Pvalb Tpbg
Pvalb Reln Itm2a
Pvalb Reln Tac1
Pvalb Sema3e Kank4
Pvalb Calb1 Sst
Number of cells
jaccard
B
Scala et al, Nature (2020) label
Pvalb Gpr149
Pvalb Il1rapl2
Pvalb Reln
Pvalb Calb1_2
Pvalb Kank4
Pvalb Calb1_1
C
D
sc/snRNAseq Yao et al., Nature (2021) MOp consensus clusters
Normalized soma depth
Density
Max
Min
E

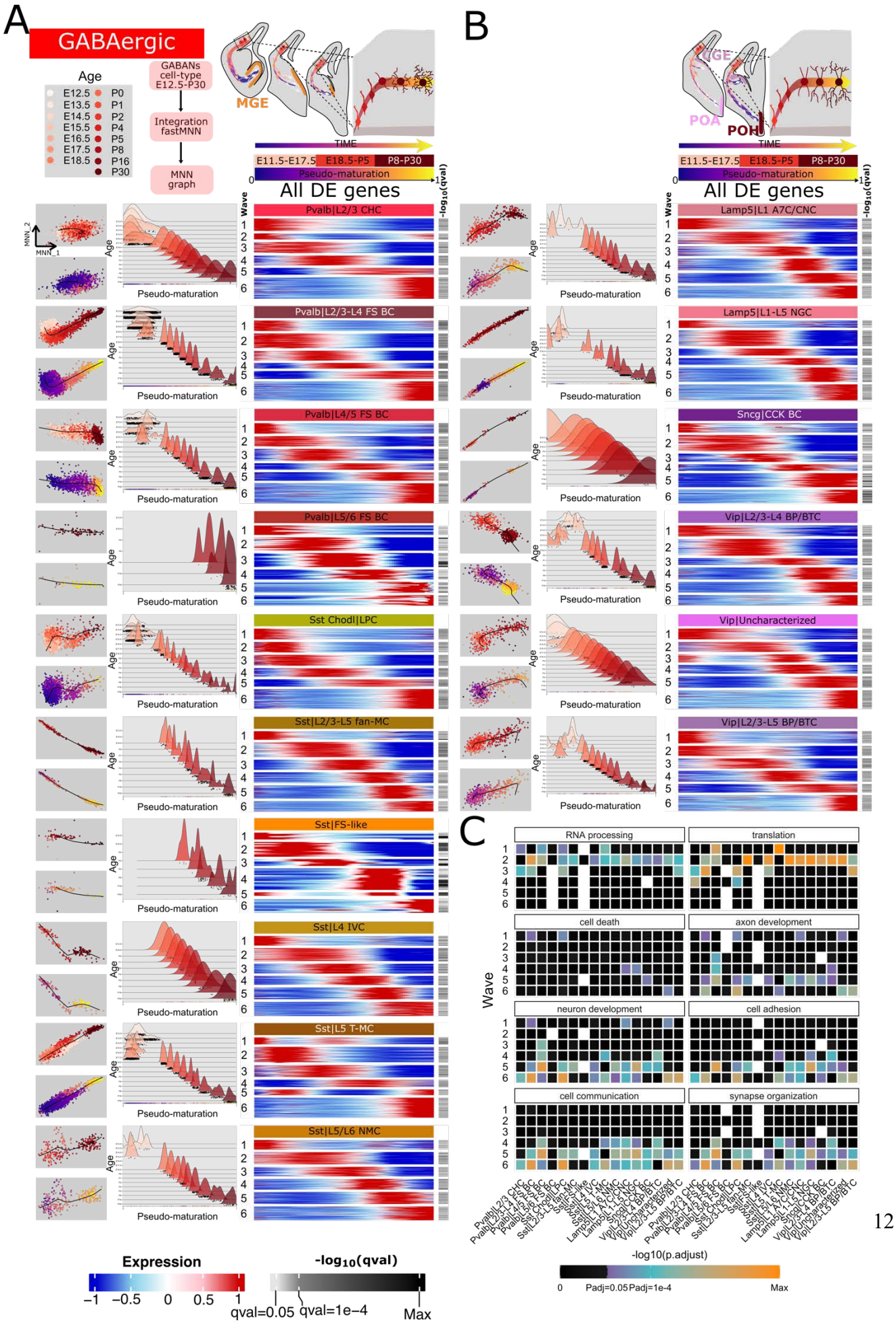
Supplementary fig. 3. Correspondence between Pvalb cluster of the AllenRef21 reference and ”Pvalb FS BC” cell- types defined in patch-seq and MERFISH studies. Mapping results of ”Pvalb FS BC” cell-types from Gouwens et al., 2020 patch-seq study (a), Scala et al., 2020 patch-seq study (b), and Yao et al.,Nature, 2021 (c) on the AllenRef21 by using the nearest centroid classifier (NCC) of the scrattch.hicat R package by taking the cluster labels as reference. Left: Prediction probability resulting from the mapping performed with the NCC. Right: annotations comparison resulting from the mapping of the different studies on the AllenRef21. The size of the dots indicates the number of overlapping cells, and the color indicates the Jaccard index (number of cells in intersection/number of cells in union). (d) Distribution of the different Pvalb clusters of the AllenRef21 in the cortex extrapolating from data of normalized soma depth provided in Gouwens et al., 2020, Scala et al., 2020 and Zhang et al., 2021. (e) Clusters in (d) were pooled in 3 cell-types: Pvalb L2/3-L4 FS BC, Pvalb L4/5 FS BC, Pvalb L5/6 FS BC. Distribution of the different defined Pvalb cell-types are plotted. Associated source data are provided


A
L6 CT
L6b
L5 NP
L5 PT
Meis2
Sncg
Vip
Lamp5
Sst Chodl
Sst
Pvalb
L4/5 IT
L2/3 IT
B
Class
Glutamatergic
GABAergic
Subclass
original Cell-type
final Cell-type
Fraction of Age/cell-type
Proportion of cell-type (%) (Normalized by subclass)
cells

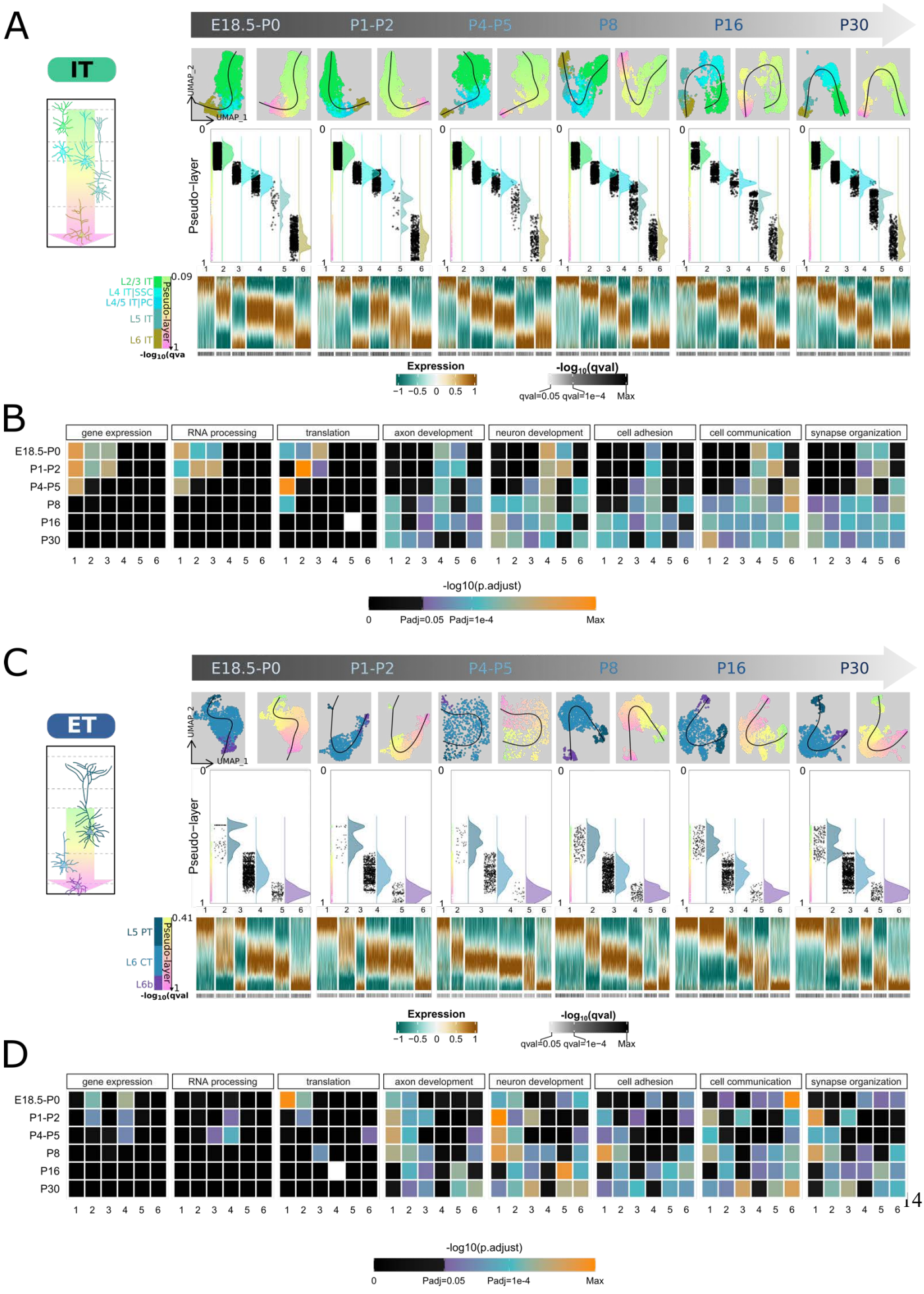
Supplementary fig. 4. Assignment of final cell-types from original cell-types assignment. (a) UMAP visualization of scRNA-seq and snRNA-seq data after integration. Cells are coloured by original cell-types assignment. **(b)** Simplification of the number of original cell-types found by pooling original cell-types sharing the same morphological, electrophysiological and connectivity type. From top to bottom: Sankey diagram depicting the different hierarchical levels of cell-type identities; heatmap representing the fraction of age per cell-type; bar plot representing the proportion of original cell-types in each subclass; Sankey diagram depicting the grouping of original cell-types in final cell-types that were used for downstream analyses.


A
Glutamatergic
Age
E11.5 P0
E12.5 P1
E13.5 P2
E14.5 P4
E15.5 P5
E16.5 P8
E17.5 P16
E18.5 P30
GlutNs cell-type E11.5-P30
Integration fastMNN
MNN graph
TIME
E11.5-E17.5
E18.5-P5
P8-P30
Pseudo-maturation
All DE genes
MNN_2
MNN_1
Age
Pseudo-maturation
Wave
-log10(qval)
CR
L2/3 IT
L4 IT|SSC
L4/5 IT|PC
L5 IT
L5 PT
L5/6 NP
L6 CT
Car3|Claustrum-like
L6 IT
L6b
Expression
1
0
−1
Max
qval=1e−4
qval=0.05
B
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
Wave
-log10(p.adjust)
Max
Padj=1e-4
Padj=0.05
0

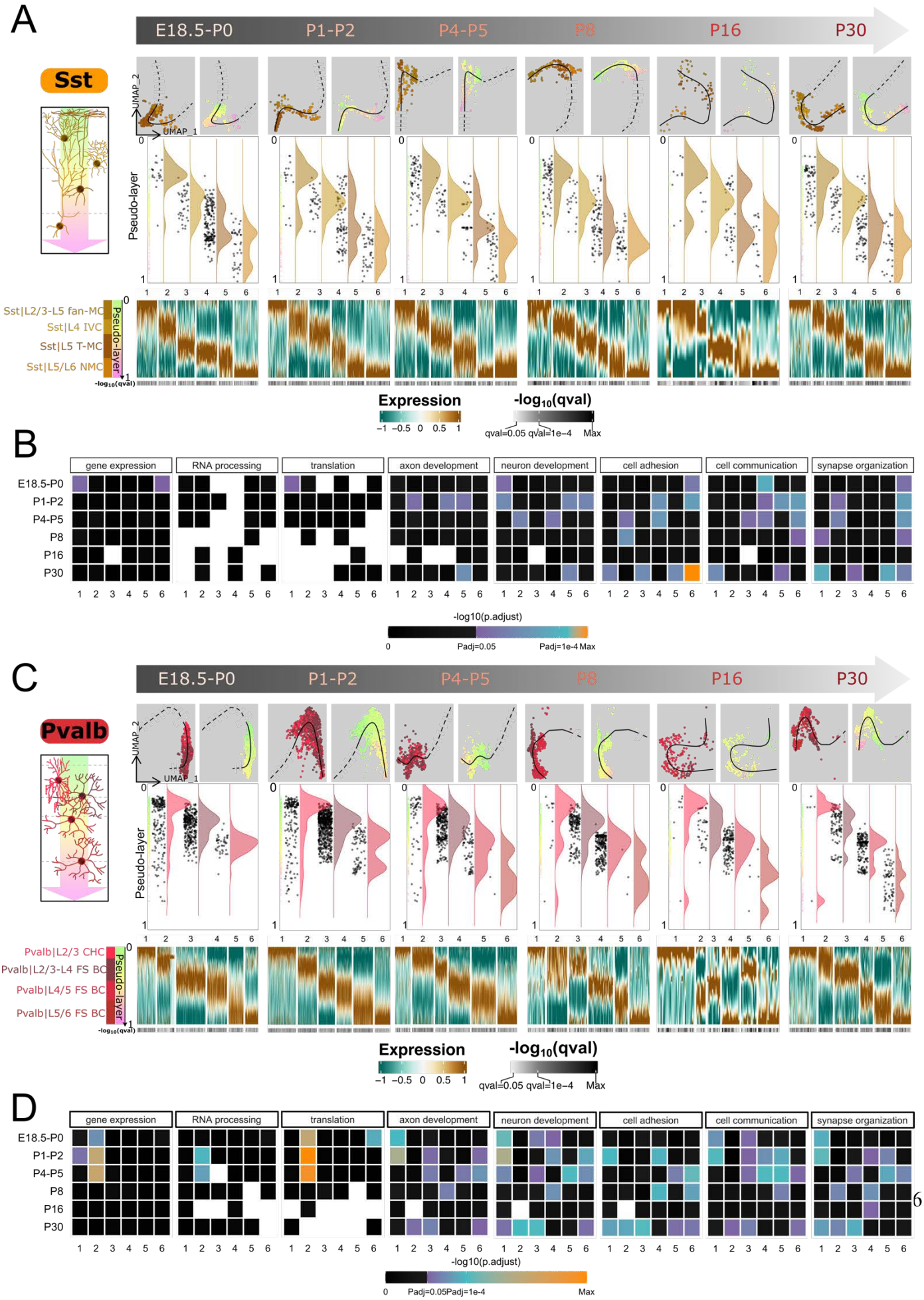
Supplementary fig. 5. Transcriptional dynamics of glutamatergic neuron cell-types during somatosensory cortex development. (a) Transcriptional dynamics of glutamatergic neuron cell-types along a pseudo-maturation axis. left: Mutual nearest-neighbor plot obtained after fastMNN integration showing chronotopic organization along a pseudo-maturation axis. Black line indicates pseudo-maturation axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-maturation axis. Genes with similar patterns were grouped in waves (numbers). **(b)** Examples of gene ontology processes associated with each of the expression waves for each glutamatergic neuron cell-types. Associated source data are provided


A
GABAergic
Age
E12.5
E13.5
E14.5
E15.5
E16.5
E17.5
E18.5
P0
P1
P2
P4
P5
P8
P16
P30
GABAN cell-type E12.5-P30
Integration fastMNN
MNN graph
MGE
TIME
E11.5-E17.5
E18.5-P5
P8-P30
Pseudo-maturation
All DE genes
Wave
-log10(qval)
Pvalb|L2/3 CHC
Pvalb|L2/3-L4 FS BC
Pvalb|L4/5 FS BC
Pvalb|L5/6 FS BC
Sst Chodl|LPC
Sst|L2/3-L5 fan-MC
Sst|FS-like
Sst|L4 IVC
Sst|L5 T-MC
Sst|L5/6 NMC
Pseudo-maturation
Age
B
LGE
POA
POH
Lamp5|L1 A7C/CNC
Lamp5|L1-L5 NGC
Sncg|CCK BC
Vip|L2/3-L4 BP/BTC
Vip|Uncharacterized
Vip|L2/3-L5 BP/BTC
C
RNA processing
translation
cell death
axon development
neuron development
cell adhesion
cell communication
synapse organization
Wave
-log10(p.adjust)
0
Padj=0.05
Padj=1e-4
Max
Expression
−1
−0.5
0
0.5
1
qval=0.05
qval=1e−4
Max

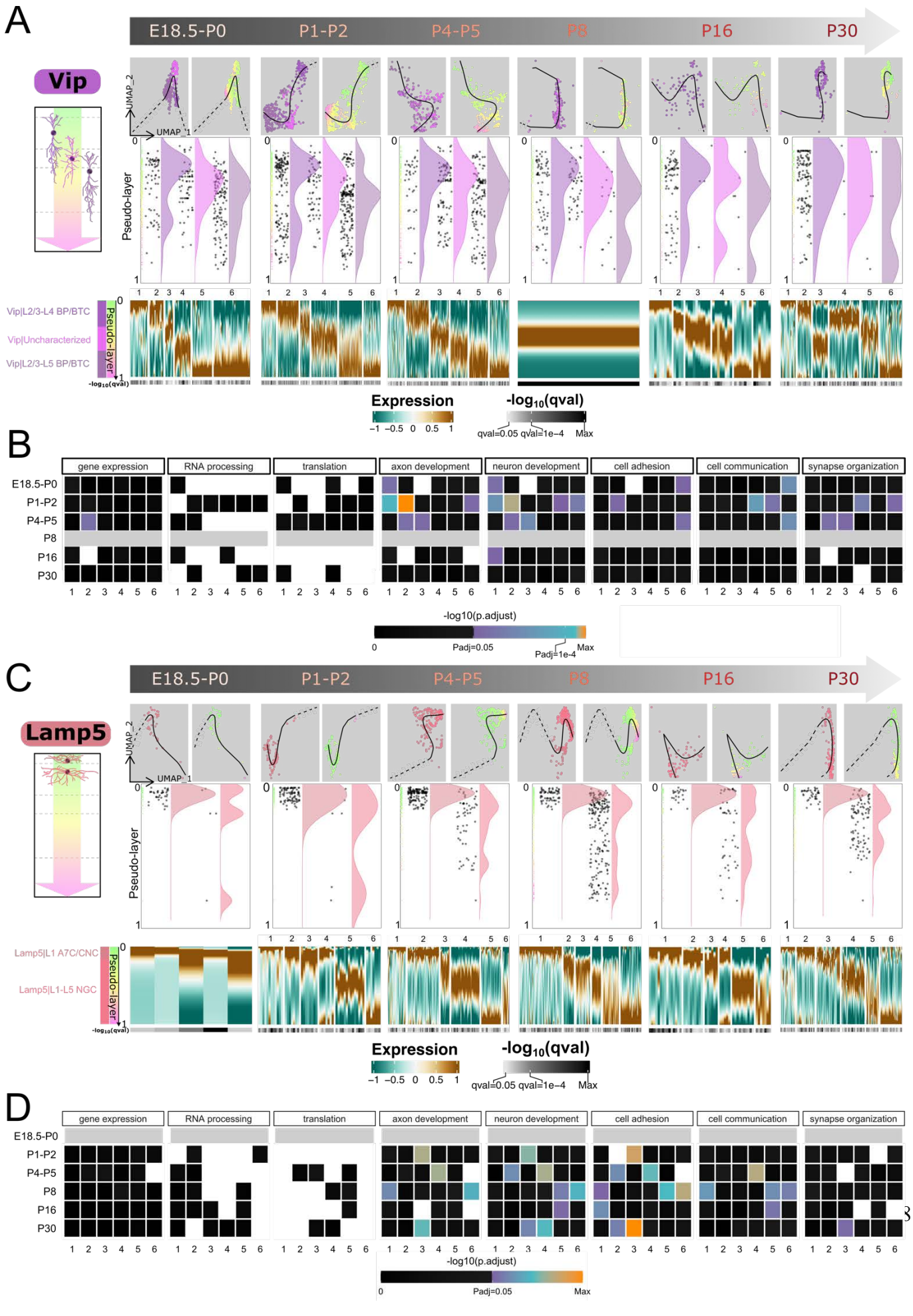
Supplementary fig. 6. Transcriptional dynamics of GABAergic neuron cell-types during somatosensory cortex development. (a) Transcriptional dynamics of MGE-derived GABAergic neuron cell-types along a pseudo-maturation axis. left: Mutual nearest-neighbor plot obtained after fastMNN integration showing chronotopic organization along a pseudo-maturation axis. Black line indicates pseudo-maturation axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-maturation axis. Genes with similar patterns were grouped in waves (numbers). **(b)** Same analysis than in **(a)** but for CGE/POA/POH-derived GABAergic neurons. **(c)** Examples of gene ontology processes associated with each of the expression waves for each GABAergic neuron cell-types. Associated source data are provided


A
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
IT
UMAP_2
UMAP_1
Pseudo−layer
L2/3 IT
L4 IT|SSC
L4/5 IT|PC
L5 IT
L6 IT
Pseudo-layer
0.09
-log10(qva
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e-4 Max
B
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
1 2 3 4 5 6
-log10(p.adjust)
0 Padj=0.05 Padj=1e-4 Max
C
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
ET
UMAP_2
UMAP_1
Pseudo−layer
L5 PT
L6 CT
L6b
Pseudo-layer
0.41
-log10(qval
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e-4 Max
D
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
1 2 3 4 5 6
-log10(p.adjust)
0 Padj=0.05 Padj=1e-4 Max

Supplementary fig. 7. Continuous distribution of IT and ET glutamatergic neurons along the cortical depth from E18.5 to P30. (a) Continuous distribution of IT glutamatergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(b)** Examples of gene ontology processes associated with each of the expression waves for each age. **(c)** Continuous distribution of ET glutamatergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(d)** Examples of gene ontology processes associated with each of the expression waves for each age. Associated source data are provided

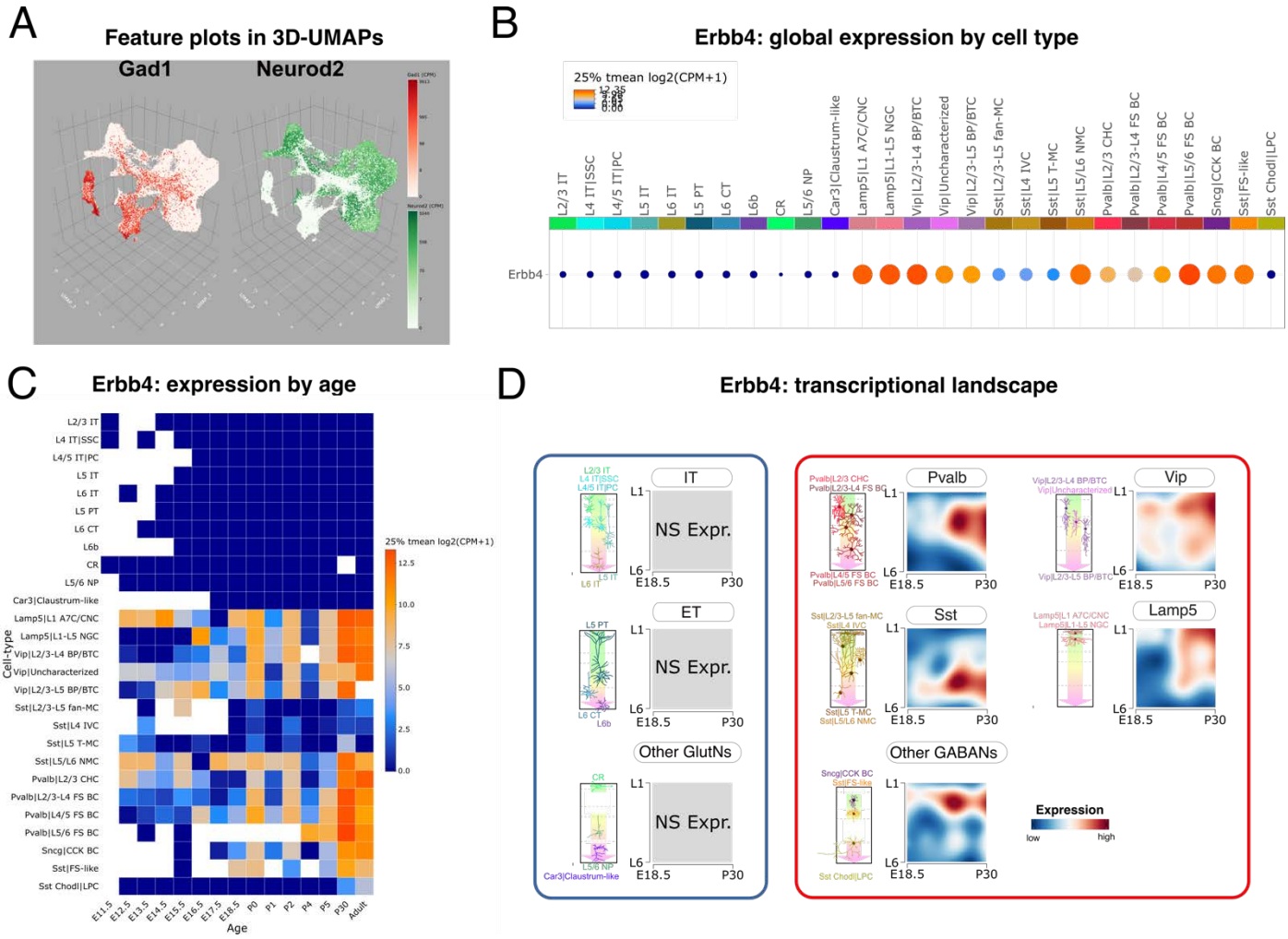

A
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Sst
UMAP_2
UMAP_1
Pseudo-layer
Sst|L2/3-L5 fan-MC
Sst|L4 IVC
Sst|L5 T-MC
Sst|L5/L6 NMC
-log10(qval)
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e−4 Max
B
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
-log10(p.adjust)
0 Padj=0.05 Padj=1e-4 Max
C
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Pvalb
UMAP_2
UMAP_1
Pseudo-layer
Pvalb|L2/3 CHC
Pvalb|L2/3-L4 FS BC
Pvalb|L4/5 FS BC
Pvalb|L5/6 FS BC
-log10(qval)
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e−4 Max
D
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
-log10(p.adjust)
0 Padj=0.05Padj=1e-4 Max

Supplementary fig. 8. Continuous distribution of Sst and Pvalb GABAergic neurons along the cortical depth from E18.5 to P30. (a) Continuous distribution of Sst GABAergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(b)** Examples of gene ontology processes associated with each of the expression waves for each age. **(c)** Continuous distribution of Pvalb GABAergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(d)** Examples of gene ontology processes associated with each of the expression waves for each age. Associated source data are provided

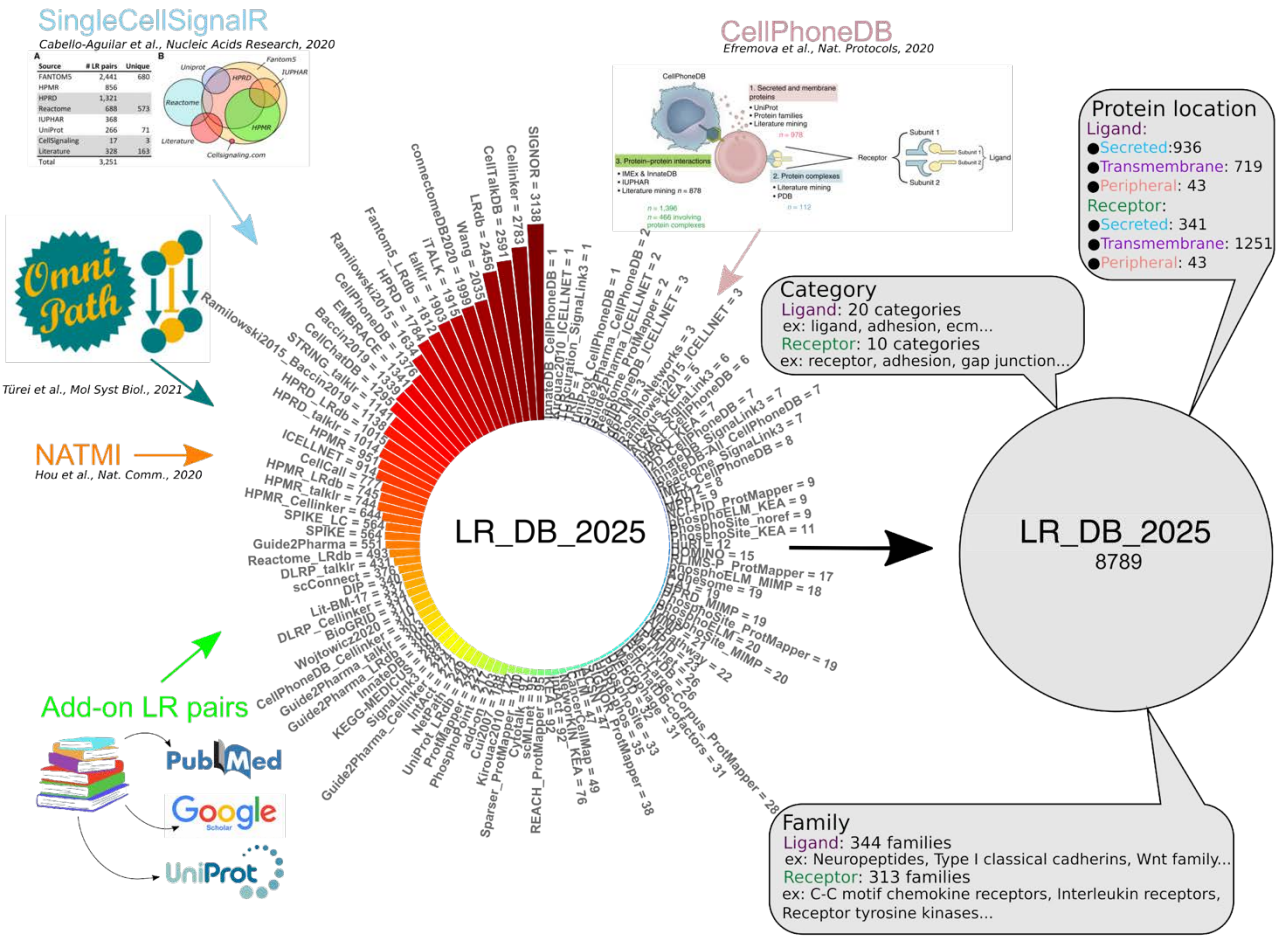

A
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Vip
UMAP_2
UMAP_1
Pseudo-layer
Vip|L2/3-L4 BP/BTC
Vip|Uncharacterized
Vip|L2/3-L5 BP/BTC
Pseudo-layer
-log10(qval)
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e−4 Max
B
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
-log10(p.adjust)
0
Padj=0.05
Padj=1e-4
Max
C
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Lamp5
UMAP_2
UMAP_1
Pseudo-layer
Lamp5|L1 A7C/CNC
Lamp5|L1-L5 NGC
Pseudo-layer
-log10(qval)
Expression
−1 −0.5 0 0.5 1
-log10(qval)
qval=0.05 qval=1e−4 Max
D
gene expression
RNA processing
translation
axon development
neuron development
cell adhesion
cell communication
synapse organization
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
-log10(p.adjust)
0
Padj=0.05
Max

Supplementary fig. 9. Continuous distribution of Vip and Lamp5 GABAergic neurons along the cortical depth from E18.5 to P30. (a) Continuous distribution of Vip GABAergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(b)** Examples of gene ontology processes associated with each of the expression waves for each age. **(c)** Continuous distribution of Lamp5 GABAergic neuron cell-types along a pseudo-layer axis. top: UMAP embedding obtained after *Seurat* SCT workflow integration showing spontaneous spatial organization along a pseudo-layer axis (middle). Black line indicates pseudo-layer axis onto which cells were aligned. right: Heatmap illustrating the expression of the differentially expressed (DE) genes along the pseudo-layer axis. Genes with similar patterns were grouped in waves (numbers). **(d)** Examples of gene ontology processes associated with each of the expression waves for each age. Associated source data are provided

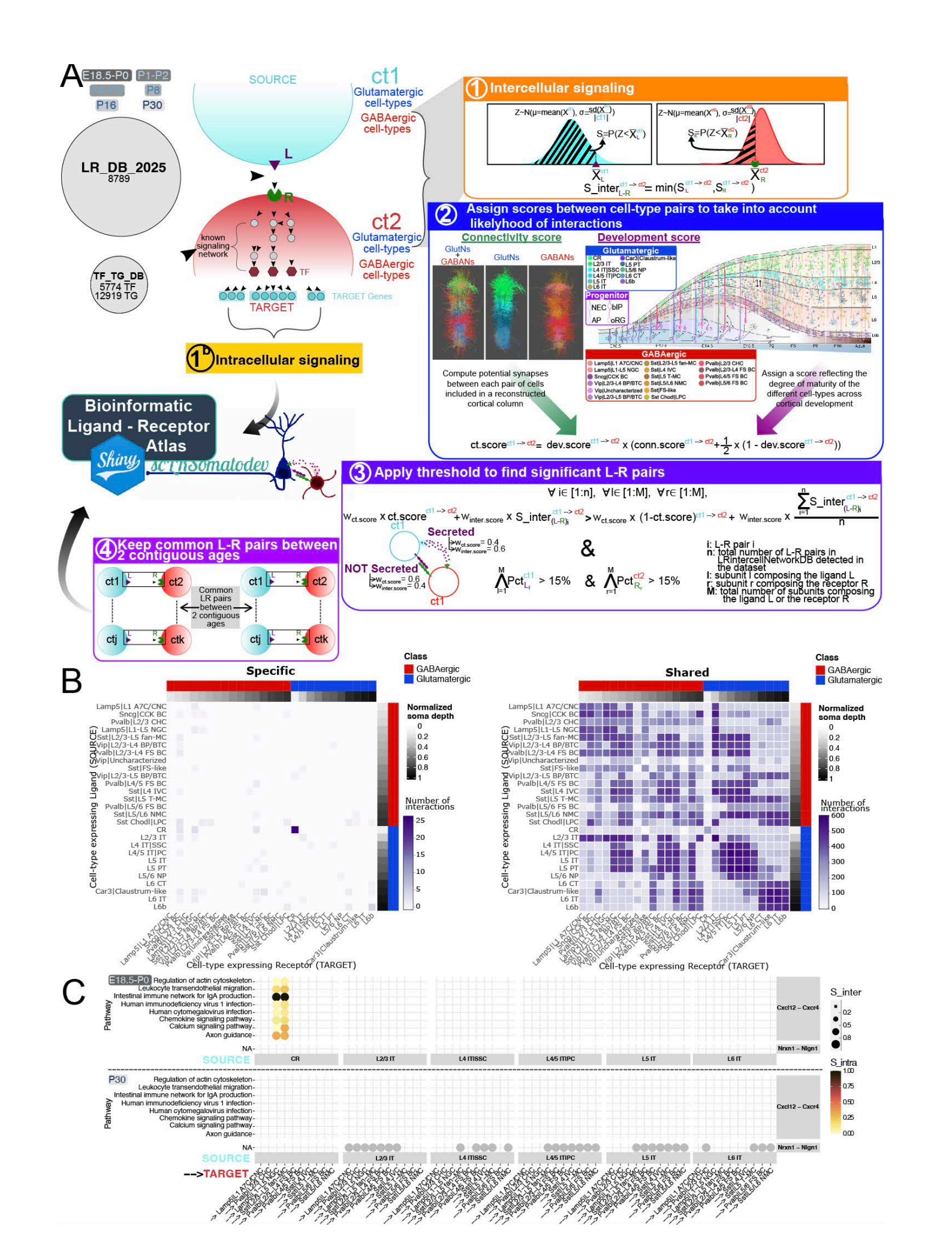
Supplementary fig. 10. Spatiotemporal transcriptional dynamics. Spatiotemporal transcriptional dynamics. Spatiotemporal transcriptional landscape of the IT (A), ET (B), Other GlutN (C), Pvalb (D), Sst (E), Vip (F), Lamp5 (G) and Other GABAN (H) family are represented. For each panel, top: 2D map in which each cell of a given family is embedded according to its pseudo-maturation (x-axis) and pseudo-layer (y-axis) scores. Bottom: A generalized additive model (GAM) was applied to generate, for each gene, a 2D spatio-temporal transcriptional map covering somatosensory cortex development.



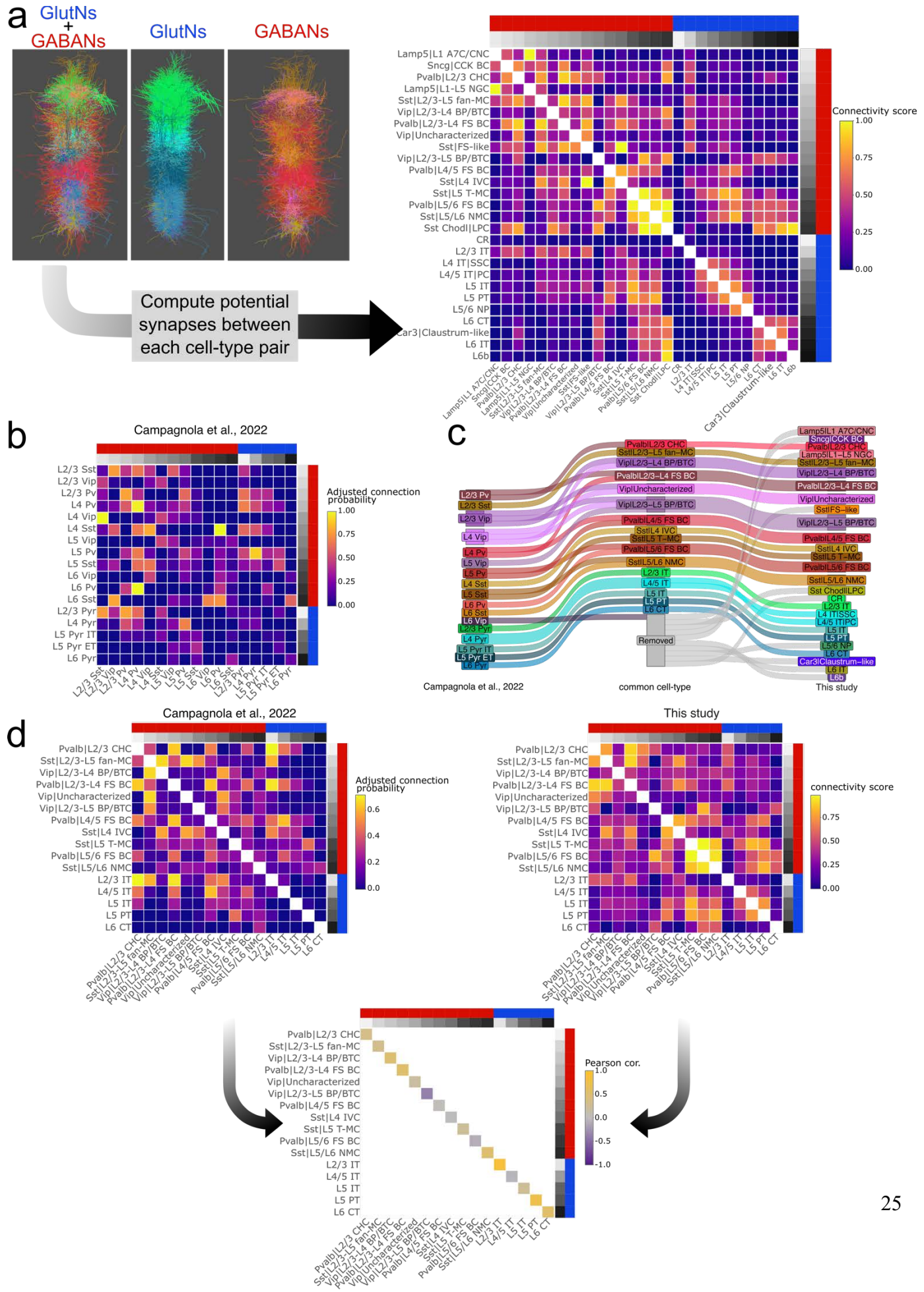
Supplementary fig. 11: Gene expression explorations with the shiny app scLRSomatodev. (A) Feature plot for two example genes (Gad1 and Neurod2) on 3D-UMAP of all cells from 17 ages. (B) Dot plot of absolute gene expression per cell-type (grouping of all ages). Circle size reflects percentage of cells expressing Erbb4 per cell-type. (C) Heat map of Erbb4 absolute expression (25 % trimmed mean of log2(CPM +1)) over mouse age in all 27 neuronal cell-types. (D) Transcriptional landscapes of Erbb4 expression in each GlutN (left, blue contour) and GABAN (right, red contour) families.



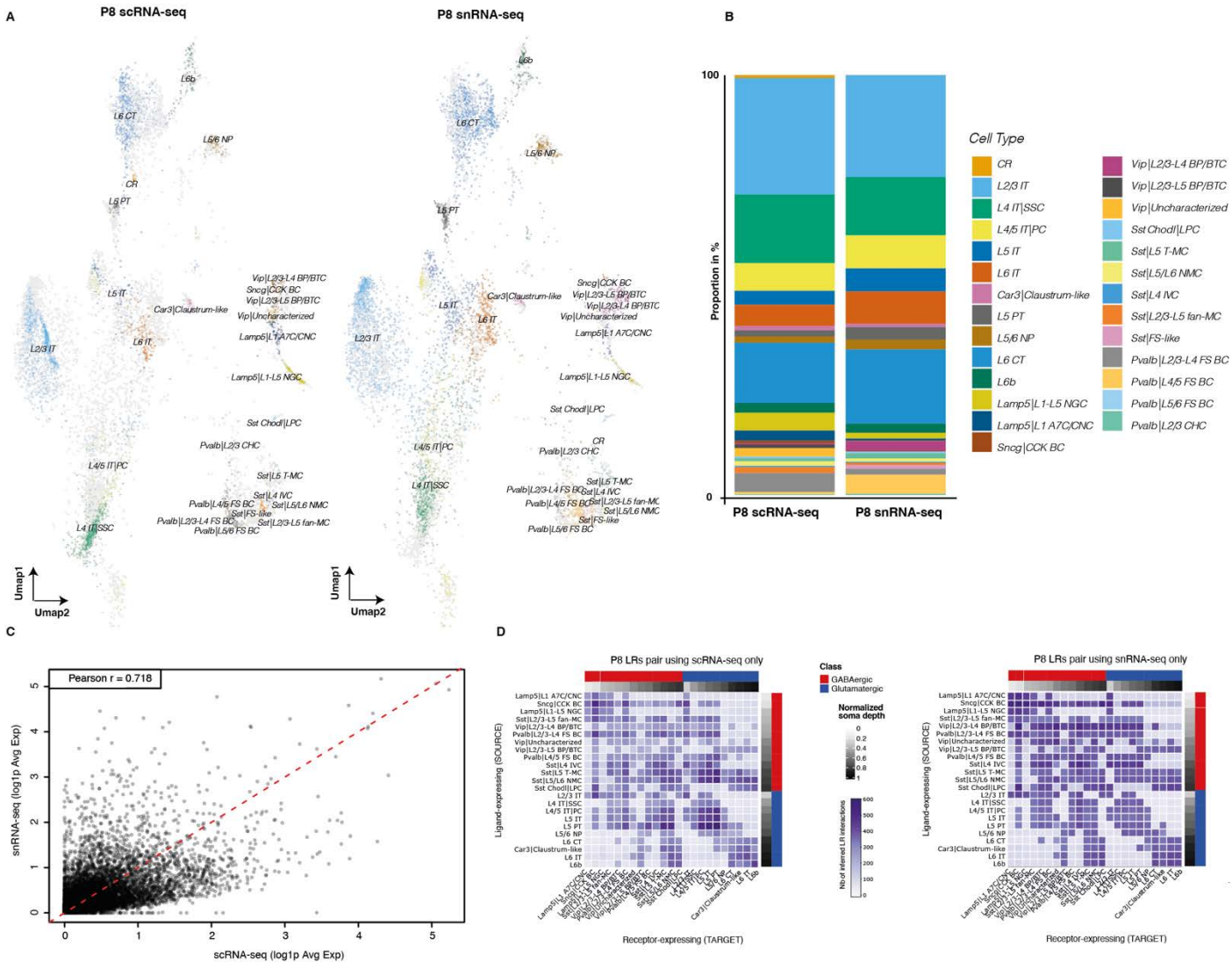
Supplementary fig. 12. The ligand-receptor database. LR_DB_2025 was obtained by integrating and curating 109 existing public databases, to which we manually added 203 LR pairs based on literature. Each ligand and receptor was assigned a category and a family based on Omnipath annotations and manual curation, as well as HGNC and UniProt databases, respectively. In total, LR_DB_2025 includes 8,789 LR pairs, with 20 distinct ligand categories, 10 distinct receptor categories, 344 distinct ligand families, and 313 distinct receptor families. Associated source data are provided


A
E18.5-P0
P1-P2
P8
P16
P30
LR_DB_2025
8789
TF_TG_DB
5774 TF
12919 TG
SOURCE
L
R
known signaling network
TF
TARGET
TARGET Genes
ct1
Glutamatergic cell-types
GABAergic cell-types
ct2
Glutamatergic cell-types
GABAergic cell-types
1 Intercellular signaling
S_inter
1b Intracellular signaling
Bioinformatic Ligand - Receptor Atlas
Shiny
scTensomatodev
2 Assign scores between cell-type pairs to take into account likelyhood of interactions
Connectivity score
Development score
GlutNs + GABANs
GlutNs
GABANs
Glutamatergic
Progenitor
NEC
bIP
AP
oRG
GABAergic
Compute potential synapses between each pair of cells included in a reconstructed cortical column
Assign a score reflecting the degree of maturity of the different cell-types across cortical development
3 Apply threshold to find significant L-R pairs
Secreted
NOT Secreted
&
i: L-R pair i
n: total number of L-R pairs in LRintercellNetworkDB detected in the dataset
l: subunit l composing the ligand L
r: subunit r composing the receptor R
M: total number of subunits composing the ligand L or the receptor R
4 Keep common L-R pairs between 2 contiguous ages
Common LR pairs between 2 contiguous ages
B
Specific
Shared
Class
GABAergic
Glutamatergic
Normalized soma depth
Number of interactions
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
C
E18.5-P0
P30
Pathway
Regulation of actin cytoskeleton
Leukocyte transendothelial migration
Intestinal immune network for IgA production
Human immunodeficiency virus 1 infection
Human cytomegalovirus infection
Chemokine signaling pathway
Calcium signaling pathway
Axon guidance
NA
Cxcl12 – Cxcr4
Nrxn1 – Nlgn1
S_inter
S_intra
SOURCE
-->TARGET
CR
L2/3 IT
L4 ITISSC
L4/5 ITIPC
L5 IT
L6 IT

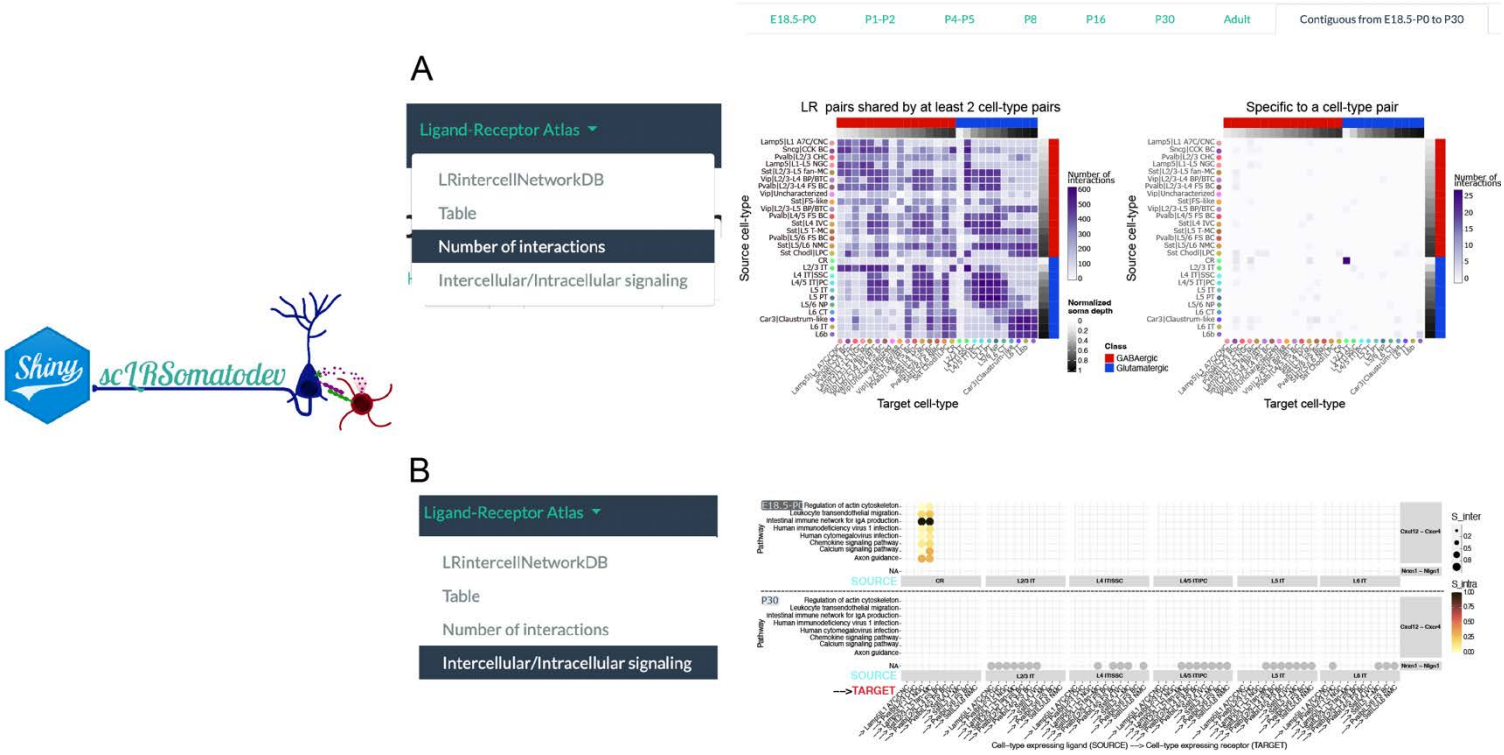
Supplementary fig. 13. Establishment of a ligand-receptor atlas between glutamatergic and GABAergic cell-types. (A) scSeqComm detailed pipeline. A Shiny app called scLRSomatoDev was constructed to allow the neuroscience community to view the ligand-receptor atlas and to follow gene expression throughout the somatosensory cortex development. (B) Specific and share interactions between all cell pairs that are contiguous between at least 2 stages. (C) Example dot plot for inter and intracellular communications. Associated source data are provided


a
GlutNs + GABANs
GlutNs
GABANs
Compute potential synapses between each cell-type pair
Connectivity score
b
Campagnola et al., 2022
Adjusted connection probability
c
Campagnola et al., 2022
common cell-type
This study
Removed
d
Campagnola et al., 2022
Adjusted connection probability
This study
connectivity score
Pearson cor.

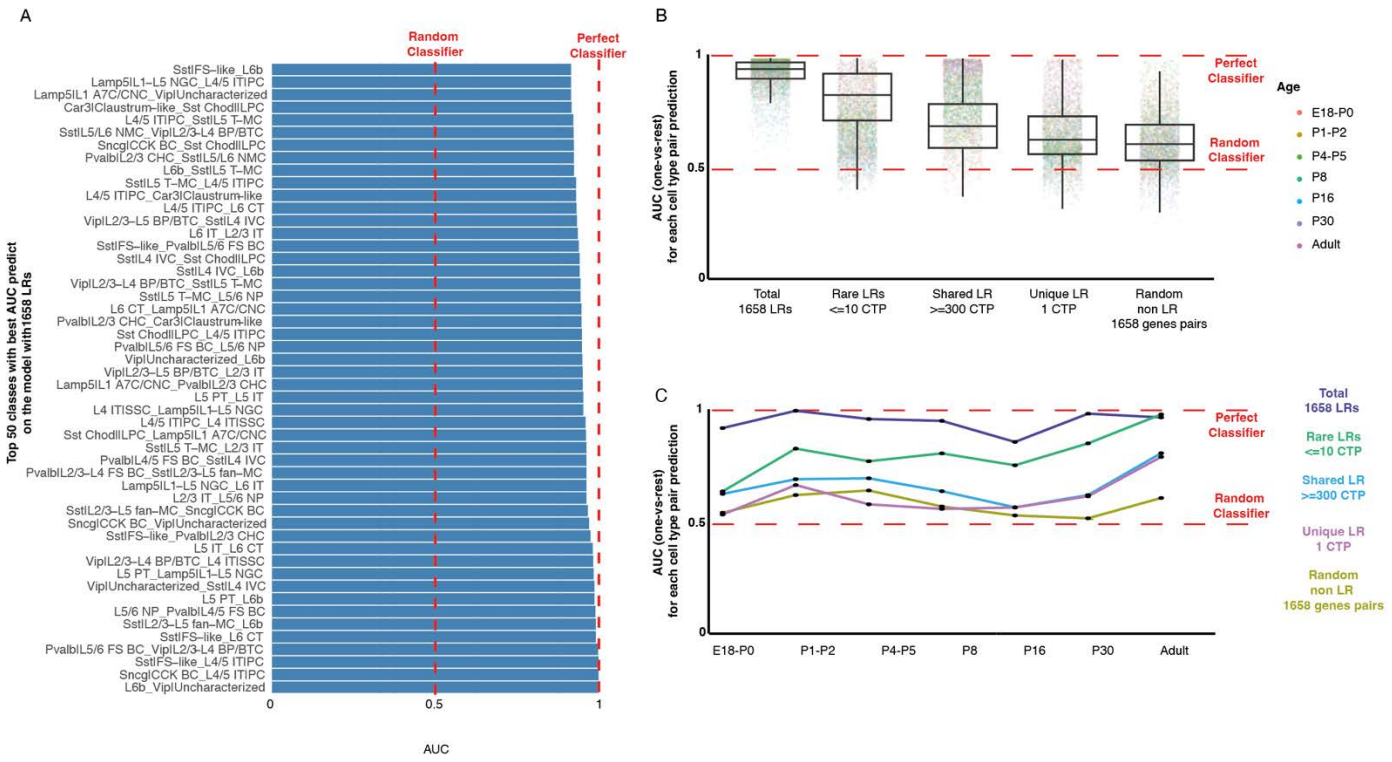
Supplementary fig. 14. Comparison between the connectivity score obtained from the reconstructed cortical column and the connection probability from Campagnola et al., 2022. (a) Connectivity score between cell-type pairs obtained by computing potential synapses between each cell pairs from the reconstructed cortical column. (b) Adjusted connection probability between each cell-type pairs from Campagnola et al.,2022. (c) Sankey plot representing the common nomenclature used to compare cell-types from Campagnola et al., 2022 and this study. (d) Correlation between each common cell-type from Campagnola et al., 2022 and this study. As the adjusted connection probability between each cell-type pair was directed in Campagnola et al, 2022, the mean of the 2 directions was taken to make the comparison between Campagnola et al, 2022 and this study. Associated source data are provided



Supplementary fig. 15. scRNA-seq and snRNA-seq datasets comparaison. (A) UMAP projections of single-cell (left) and single-nucleus (right) RNA-seq datasets from postnatal day 8 (P8) mouse cortex. Cells are colored and annotated at cell type level. **(B)** Stacked bar plots showing the proportional representation of cell types detected by scRNA-seq (left) and snRNA-seq (right). While the overall diversity of populations is preserved, relative subtitle abundances differ between methods. **(C)** Correlation of average gene expression between scRNA-seq and snRNA-seq across all cell types. Each dot represents a gene, with the red dashed line indicating the perfect correlation. Pearson correlation coefficient is r = 0.718, demonstrating strong concordance between the two modalities. **(D)** Ligand–receptor (LR) interaction analysis at P8. Heatmaps show predicted LR pairs identified using scRNA-seq only (left) or snRNA-seq only (right). Rows represent ligand-expressing clusters and columns represent receptor-expressing clusters, with signal strength indicated by color intensity. GABAergic and glutamatergic classes are highlighted, and normalized soma depth is provided on the side bars. No major differences are observed between scRNA-seq and snRNA-seq predictions. Associated source data are provided:



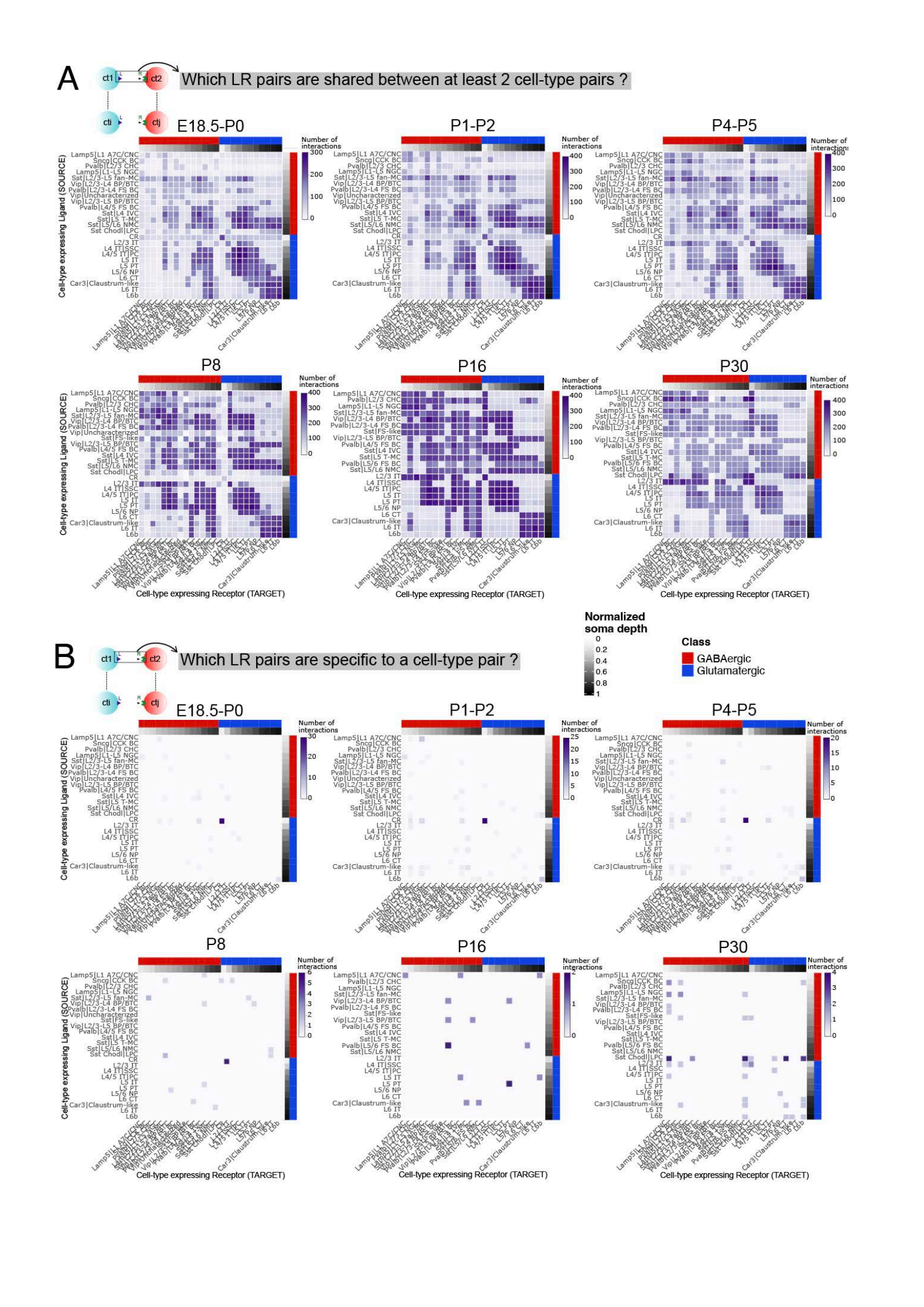
Supplementary fig. 16. LR prediction panel of the Shiny app scLRSomatodev. Top, function Number of interactions. Bottom, function Intercellular/Intracellular signaling. (A) Heatmaps representing the number of interactions between all cell-types pairs that are specific to a cell-type pair (left) or shared by at least 2 cell-type pairs (right) and that are common between 2 contiguous ages. The blue color represents glutamatergic cell-types and the red color the GABAergic cell-types. The white to black color gradient represents the median of the normalized soma depth of each cell-type. 0 representing the pial surface and 1 the white matter. (B) Examples of intercellular (S_inter) and intracellular (S_intra) signaling for two ligand-receptor pairs (Cxcl12 - Cxcr4 and Nrxn1 - Nlgn1) between IT family, CR cell-type and Sst, Pvalb and Lamp5 familes at E18.5-P0 and P30. Pathways are shown on the left. LR pairs are represented on the right. Grey circles: LR pairs having no S_intra score values (i.e., LR pairs having no annotated receptor downstream transcription factors).



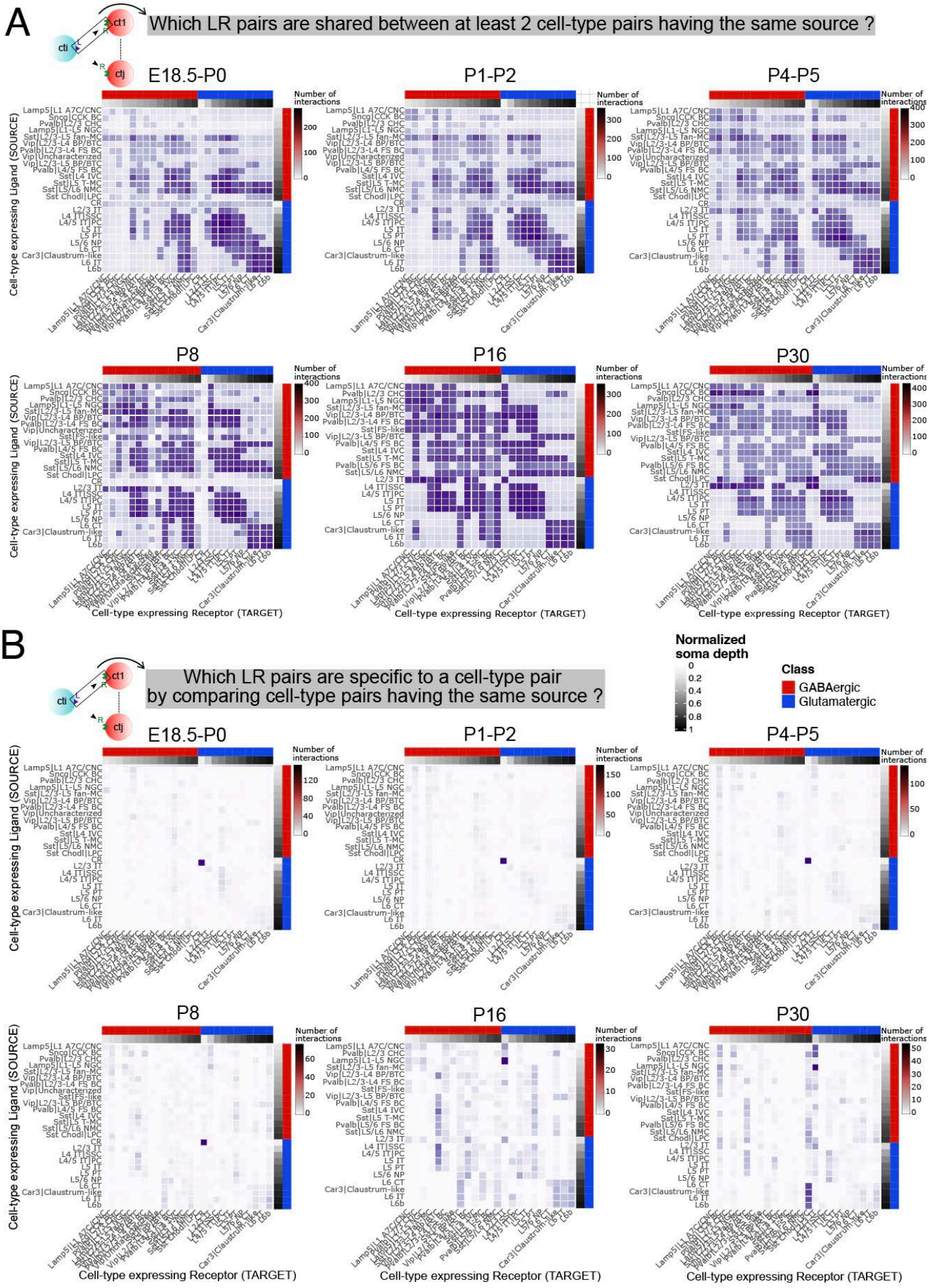
Supplementary fig. 17. Predicting source–target cell-type pairs with machine learning reveals specificity encoded by combinatorial ligand–receptor patterns.

(A) Classification performance for the top 50 cell-type pairs (CTPs) predicted using all significant LR pairs. Bars show ROC–AUC values (one-vs-rest), with most CTPs reaching well above chance (red dashed line, random classifier) and approaching high separability (perfect classifier, AUC = 1).

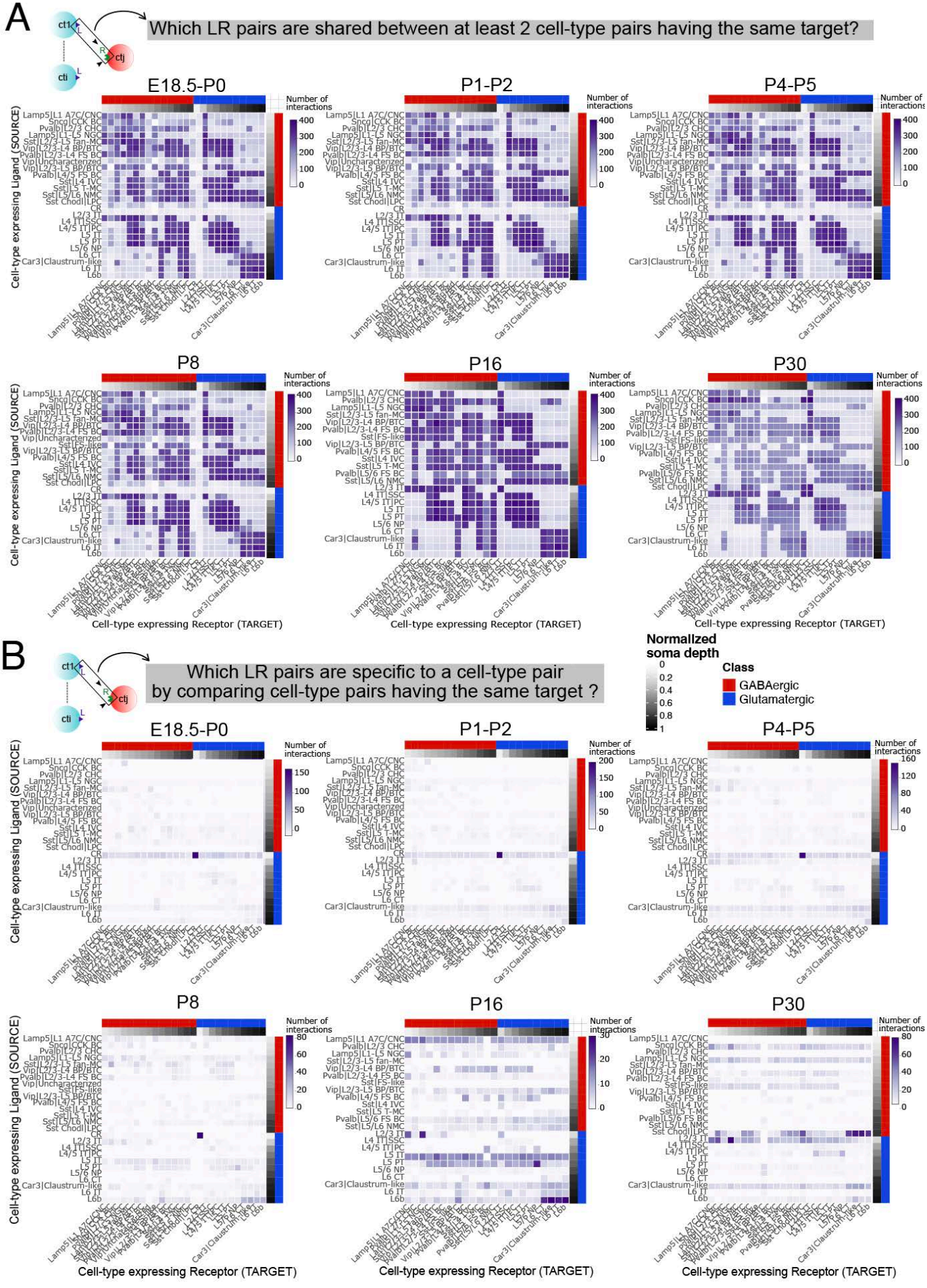
(B) Comparison of predictive performance across different LR feature sets. Models trained on all significant pairs perform best, while models restricted to shared or unique LR pairs perform at or near chance. Models trained only on rare LR pairs (≤10 CTPs) consistently exceed chance and contribute meaningful signal. Randomized non-LR gene–gene pairs do not provide predictive power. **(C)** Developmental trajectory of classifier performance across ages (E18–P0 to Adult). Rare-only LR pairs show reproducible predictive signal above chance across stages, while shared or unique pairs alone remain near chance. Models trained on the full LR set (shared + rare + occasional unique) achieve the highest performance, consistent with specificity arising from the joint usage of different LR subsets.


A
Which LR pairs are shared between at least 2 cell-type pairs ?
ct1
ct2
cti
ctj
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Number of interactions
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
B
Which LR pairs are specific to a cell-type pair ?
Normalized soma depth
Class
GABAergic
Glutamatergic

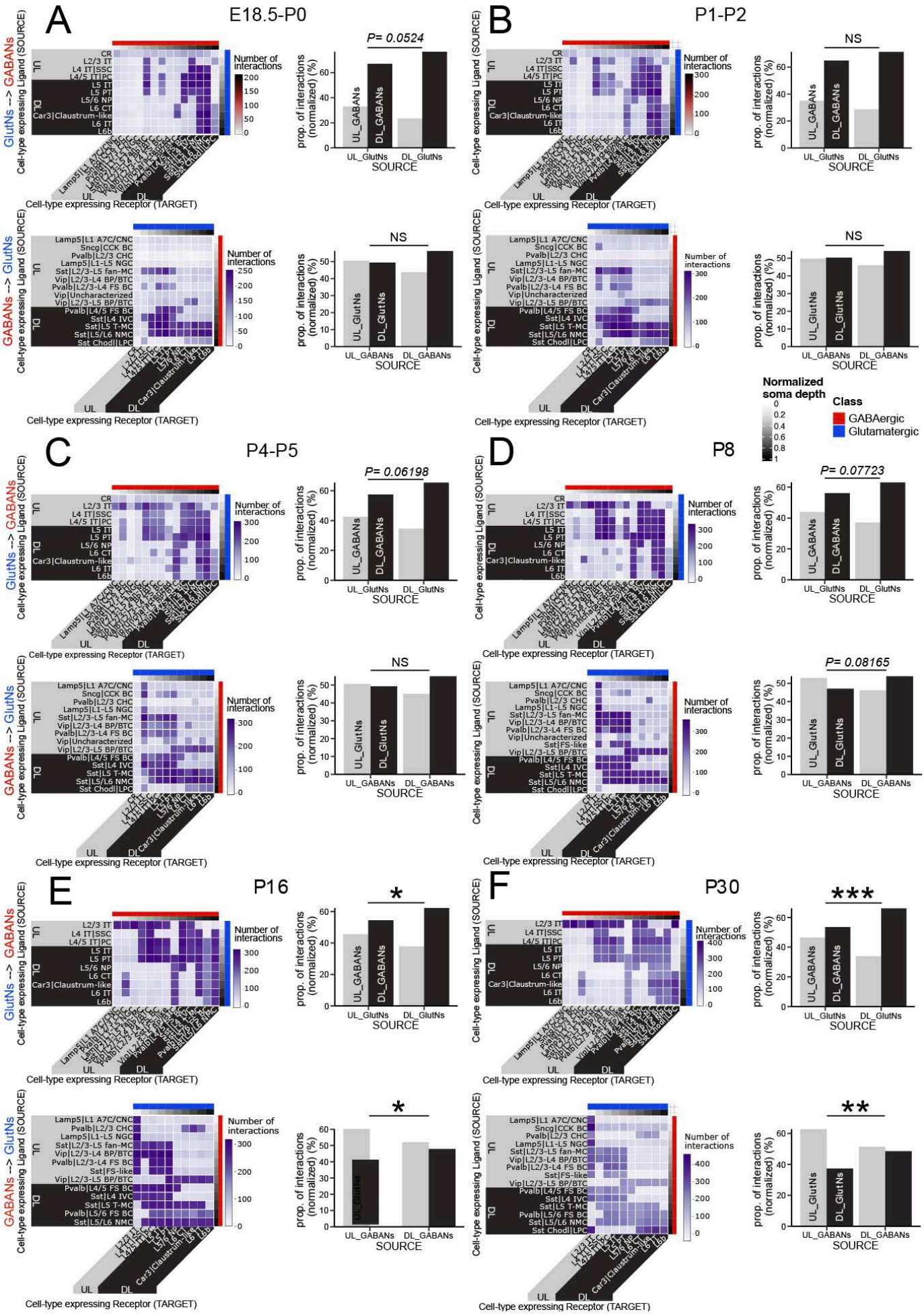
Supplementary fig. 18. Number of shared and specific inferred LR interactions between cell-type pairs from E18.5-P0 to P30. (A) Heatmap representing the number of interactions between all cell-type pairs that are at least shared by 2 cell-type pairs. **(B)** Heatmap representing the number of interactions between all cell-types pairs that are specific to a cell-type pair (cell-type expressing ligand). The blue color represents glutamatergic cell-types and the red color the GABAergic cell-types. The white to black color gradient represents the median of the normalized soma depth of each cell-type. 0 representing the pial surface and 1 the white matter.


A
Which LR pairs are shared between at least 2 cell-type pairs having the same source ?
cti
ct1
ctj
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Number of interactions
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
B
Which LR pairs are specific to a cell-type pair
by comparing cell-type pairs having the same source ?
Normalized soma depth
Class
GABAergic
Glutamatergic

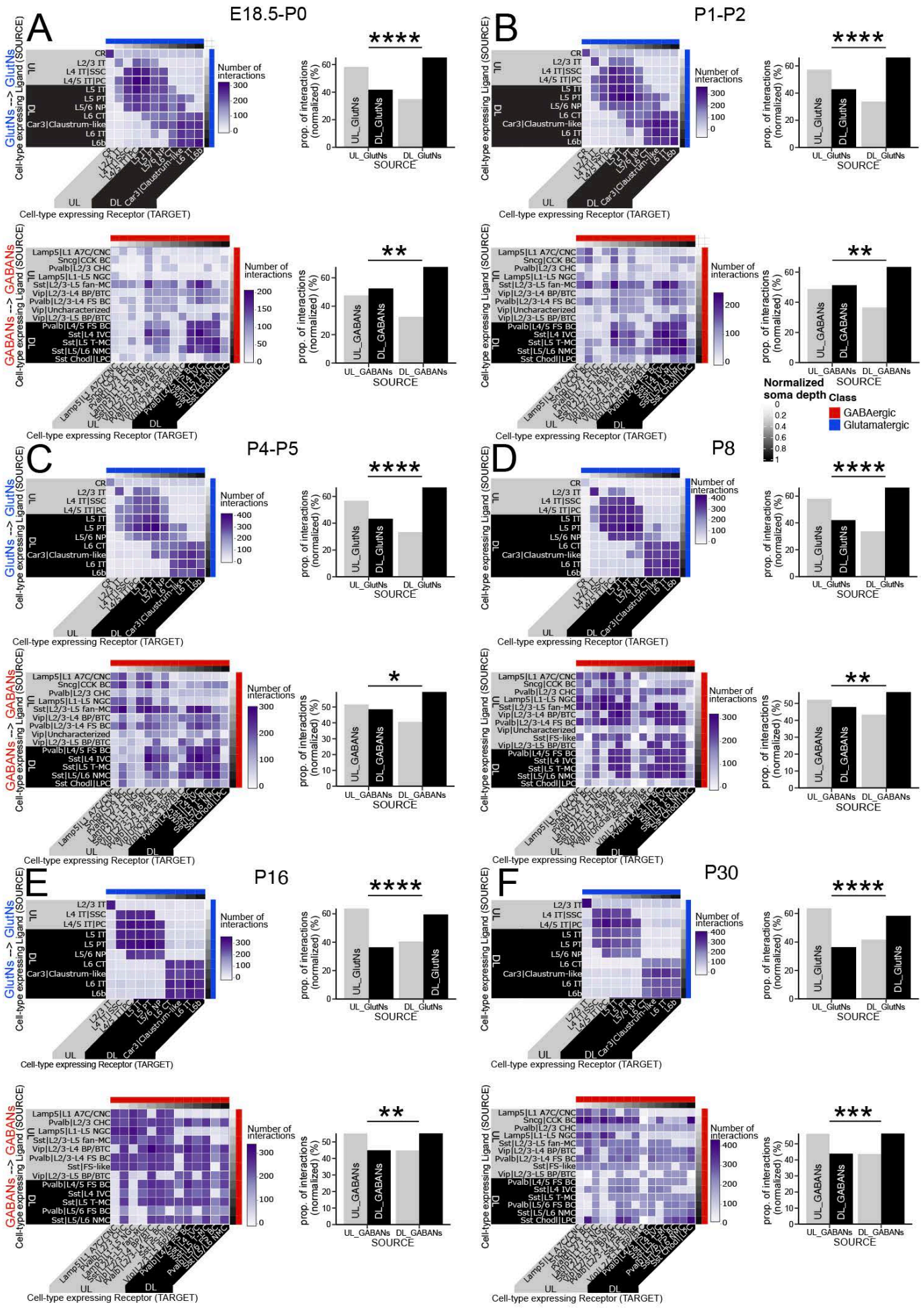
Supplementary fig. 19. Number of shared and specific inferred LR interactions between cell-type pairs having the same source from E18.5-P0 to P30. (A) Heatmap representing the number of interactions between all cell-type pairs that are at least shared by 2 cell-type pairs having the same source. (B) Heatmap representing the number of interactions between all cell-type pairs that are specific to a cell-type pair by comparing cell- type pairs having the same source (cell-type expressing ligand). The blue color represents glutamatergic cell-types and the red color the GABAergic cell-types. The white to black color gradient represents the median of the normalized soma depth of each cell-type. 0 representing the pial surface and 1 the white matter.


A
Which LR pairs are shared between at least 2 cell-type pairs having the same target?
ct1
ctj
cti
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Number of interactions
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
B
Which LR pairs are specific to a cell-type pair
by comparing cell-type pairs having the same target ?
Normalized soma depth
Class
GABAergic
Glutamatergic

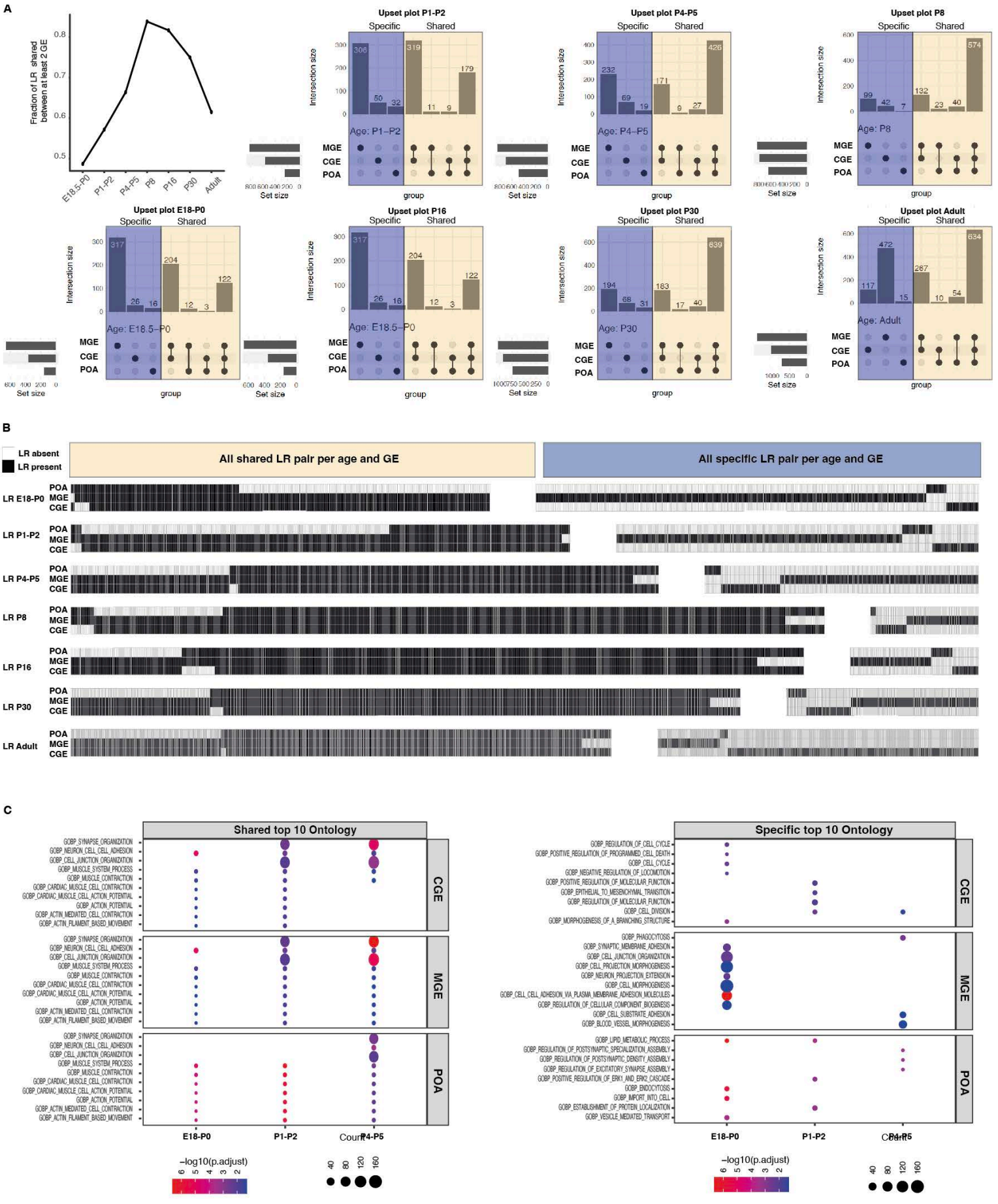
Supplementary fig. 20. Number of shared and specific interactions between cell-type pairs having the same target from E18.5-P0 to P30. (A) Heatmap representing the number of interactions between all cell-type pairs that are at least shared by 2 cell-type pairs having the same target. (B) Heatmap representing the number of interactions between all cell-type pairs that are specific to a cell-type pair by comparing cell-type pairs having the same target (cell-type expressing receptor).The blue color represents glutamatergic cell-types and the red color the GABAergic cell-types. The white to black color gradient represents the median of the normalized soma depth of each cell-type. 0 representing the pial surface and 1 the white matter.


A
E18.5-P0
B
P1-P2
C
P4-P5
D
P8
E
P16
F
P30
GlutNs --> GABANs
GABANs --> GlutNs
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
Number of interactions
prop. of interactions (normalized) (%)
SOURCE
UL_GABANs
DL_GABANs
UL_GlutNs
DL_GlutNs
P= 0.0524
NS
P= 0.06198
P= 0.07723
P= 0.08165
*
***
**
Normalized soma depth
Class
GABAergic
Glutamatergic

Supplementary fig. 21. Number of interactions between GlutNs SOURCE (or TARGET) cell-types and GABANs TARGET (or SOURCE) cell-types. Top: heatmap representing the number of interactions between GlutNs expressing ligand (SOURCE) and GABANs expressing receptor (TARGET) along with the total percentage of interactions (normalized) between the UL GlutNs and the UL and DL GABANs versus between the DL GlutNs and the UL and DL GABANs at E18.5-P0 **(A)**, P1-P2 **(B)**, P4-P5 **(C)**, P8 **D)**, P16 **(E)** and P30 **(F)**. Bottom: heatmap representing the number of interactions between GABANs expressing ligand (SOURCE) and GlutNs expressing receptors (TARGET) along with the total percentage of interactions (normalized) between the UL GABANs and the UL and DL GlutNs versus between the DL GABANs and the UL and DL GlutNs at E18.5-P0 **(A)**, P1-P2 **(B)**, P4-P5 **(C)**, P8 **(D)**, P16 **(E)** and P30 **(F)**. The number of interactions was normalized by dividing by the number of cell-type couples involved before achieving the χ^2 test so that the comparison does not suffer from the unbalanced number of cell-types between UL and DL. *$p<0.05$, **$p<0.01$, ***$p<0.001$, χ^2 test. Associated source data are provided.


A
E18.5-P0
B
P1-P2
C
P4-P5
D
P8
E
P16
F
P30
GlutNs --> GlutNs
GABANs --> GABANs
Cell-type expressing Ligand (SOURCE)
Cell-type expressing Receptor (TARGET)
UL
DL
CR
L2/3 IT
L4 IT|SSC
L4/5 IT|PC
L5 IT
L5 PT
L5/6 NP
L6 CT
Car3|Claustrum-like
L6 IT
L6b
Lamp5|L1 A7C/CNC
Sncg|CCK BC
Pvalb|L2/3 CHC
Lamp5|L1-L5 NGC
Sst|L2/3-L5 fan-MC
Vip|L2/3-L4 BP/BTC
Pvalb|L2/3-L4 FS BC
Vip|Uncharacterized
Sst|FS-like
Vip|L2/3-L5 BP/BTC
Pvalb|L4/5 FS BC
Sst|L4 IVC
Sst|L5 T-MC
Pvalb|L5/6 FS BC
Sst|L5/L6 NMC
Sst Chodl|LPC
Number of interactions
prop. of interactions (normalized) (%)
UL_GlutNs
DL_GlutNs
UL_GABANs
DL_GABANs
SOURCE
****
**
*
***
Normalized soma depth
Class
GABAergic
Glutamatergic

Supplementary fig. 22. Number of interactions between cell-types of the same class. Top: heatmap representing the number of interactions between GlutNs expressing ligand (SOURCE) and GlutNs expressing receptor (TARGET) along with the total percentage of interactions (normalized) between the UL GlutNs and the UL and DL GlutNs versus between the DL GlutNs and the UL and DL GlutNs at E18.5-P0 **(A)**, P1-P2 **(B)**, P4-P5 **(C)**, P8 **(D)**, P16 **(E)** and P30 **(F)**. Bottom: heatmap representing the number of interactions between GABANs expressing ligand (SOURCE) and GABANs expressing receptors (TARGET) along with the total percentage of interactions (normalized) between the UL GABANs and the UL and DL GABANs versus between the DL GABANs and the UL and DL GABANs at E18.5-P0 **(A)**, P1-P2 **(B)**, P4-P5 **(C)**, P8 **(D)**, P16 **(E)** and P30 **(F)**. The number of interactions was normalized by dividing by the number of cell-type couples involved before achieving the χ^2 test so that the comparison does not suffer from the unbalanced number of cell-types between UL and DL. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, χ^2 test. Associated source data are provided.


A
Fraction of LR shared between at least 2 GE
E18.5-P0
P1-P2
P4-P5
P8
P16
P30
Adult
Upset plot P1-P2
Upset plot P4-P5
Upset plot P8
Upset plot E18-P0
Upset plot P16
Upset plot P30
Upset plot Adult
Specific
Shared
Intersection size
Set size
group
MGE
CGE
POA
B
LR absent
LR present
All shared LR pair per age and GE
All specific LR pair per age and GE
LR E18-P0
LR P1-P2
LR P4-P5
LR P8
LR P16
LR P30
LR Adult
C
Shared top 10 Ontology
Specific top 10 Ontology
GOBP_SYNAPSE_ORGANIZATION
GOBP_NEURON_CELL_CELL_ADHESION
GOBP_CELL_JUNCTION_ORGANIZATION
GOBP_MUSCLE_SYSTEM_PROCESS
GOBP_MUSCLE_CONTRACTION
GOBP_CARDIAC_MUSCLE_CELL_CONTRACTION
GOBP_CARDIAC_MUSCLE_CELL_ACTION_POTENTIAL
GOBP_ACTION_POTENTIAL
GOBP_ACTIN_MEDIATED_CELL_CONTRACTION
GOBP_ACTIN_FILAMENT_BASED_MOVEMENT
GOBP_REGULATION_OF_CELL_CYCLE
GOBP_POSITIVE_REGULATION_OF_PROGRAMMED_CELL_DEATH
GOBP_CELL_CYCLE
GOBP_NEGATIVE_REGULATION_OF_LOCOMOTION
GOBP_POSITIVE_REGULATION_OF_MOLECULAR_FUNCTION
GOBP_EPITHELIAL_TO_MESENCHYMAL_TRANSITION
GOBP_REGULATION_OF_MOLECULAR_FUNCTION
GOBP_CELL_DIVISION
GOBP_MORPHOGENESIS_OF_A_BRANCHING_STRUCTURE
GOBP_PHAGOCYTOSIS
GOBP_SYNAPTIC_MEMBRANE_ADHESION
GOBP_CELL_JUNCTION_ORGANIZATION
GOBP_CELL_PROJECTION_MORPHOGENESIS
GOBP_NEURON_PROJECTION_EXTENSION
GOBP_CELL_MORPHOGENESIS
GOBP_CELL_CELL_ADHESION_VIA_PLASMA_MEMBRANE_ADHESION_MOLECULES
GOBP_REGULATION_OF_CELLULAR_COMPONENT_BIOGENESIS
GOBP_CELL_SUBSTRATE_ADHESION
GOBP_BLOOD_VESSEL_MORPHOGENESIS
GOBP_LIPID_METABOLIC_PROCESS
GOBP_REGULATION_OF_POSTSYNAPTIC_SPECIALIZATION_ASSEMBLY
GOBP_REGULATION_OF_POSTSYNAPTIC_DENSITY_ASSEMBLY
GOBP_REGULATION_OF_EXCITATORY_SYNAPSE_ASSEMBLY
GOBP_POSITIVE_REGULATION_OF_ERK1_AND_ERK2_CASCADE
GOBP_ENDOCYTOSIS
GOBP_IMPORT_INTO_CELL
GOBP_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION
GOBP_VESICLE_MEDIATED_TRANSPORT
E18-P0
P1-P2
P4-P5
Count
−log10(p.adjust)

Supplementary fig. 23. Ganglionic Eminence show specific and shared ligand–receptor modules across cortical development. (A) Temporal dynamics of sharing versus specificity of predicted ligand–receptor (LR) interactions, (left) line plot showing the fraction of LR pairs that are shared by at least 2 ganglionic eminence (GE) lineages (MGE, CGE, POA) at each developmental stage (E18.5–Adult) when Target cell type is Glutamatergic pallial cells. Right, for each stage, UpSet plots partition significant LR pairs into GE-specific (left panels) and shared (right panels). Bars on the left of each UpSet plot indicate the size of the LR sets for Source: MGE, CGE, and POA and Target (Glutamatergic pallial cells); intersection bars show the number of LR pairs common to the indicated GE combinations. **(B)** Presence/absence "barcode" maps of LR pairs across development. Left block: all shared LR pairs (columns) with rows for POA, MGE, and CGE at each stage; black = present, white = absent. Right block: all GE-specific LR pairs, displayed in the same format. This panel highlights the switch from early GE-specific modules to a more shared program in mid-development, followed by a re-emergence of GE specificity in adulthood. **(C)** Gene Ontology enrichment for LR modules. Left, shared LR modules; right, GE-specific LR modules. For each GE and stage, the top 10 GO Biological Process terms are shown. Dot color indicates –log10(adjusted p value) and dot size indicates the number of LR genes contributing to the term. Enrichments emphasize synapse- and adhesion-related processes among shared modules and GE-biased functional programs among specific modules**.** Associated source data are provided.

Supplementary Data 1. The LR database LR_DB_2025.

Supplementary Data 2. Categories of LRs belonging to 5 main neurodevelopmental processes

Supplementary Data 3. Development scores

Supplementary Data 4. Connectivity scores

Supplementary Data 5. Ganglionic Eminence ontology enriched

Supplementary Data 6. Categories of LRs belonging to neurodevelopmental diseases

Supplementary Data 7. Oligonucleotide sequences for shRNA.

Supplementary Data 8. Spatiotemporal_Kegg_analysis.

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