SUPPORTING INFORMATION CAPTIONS

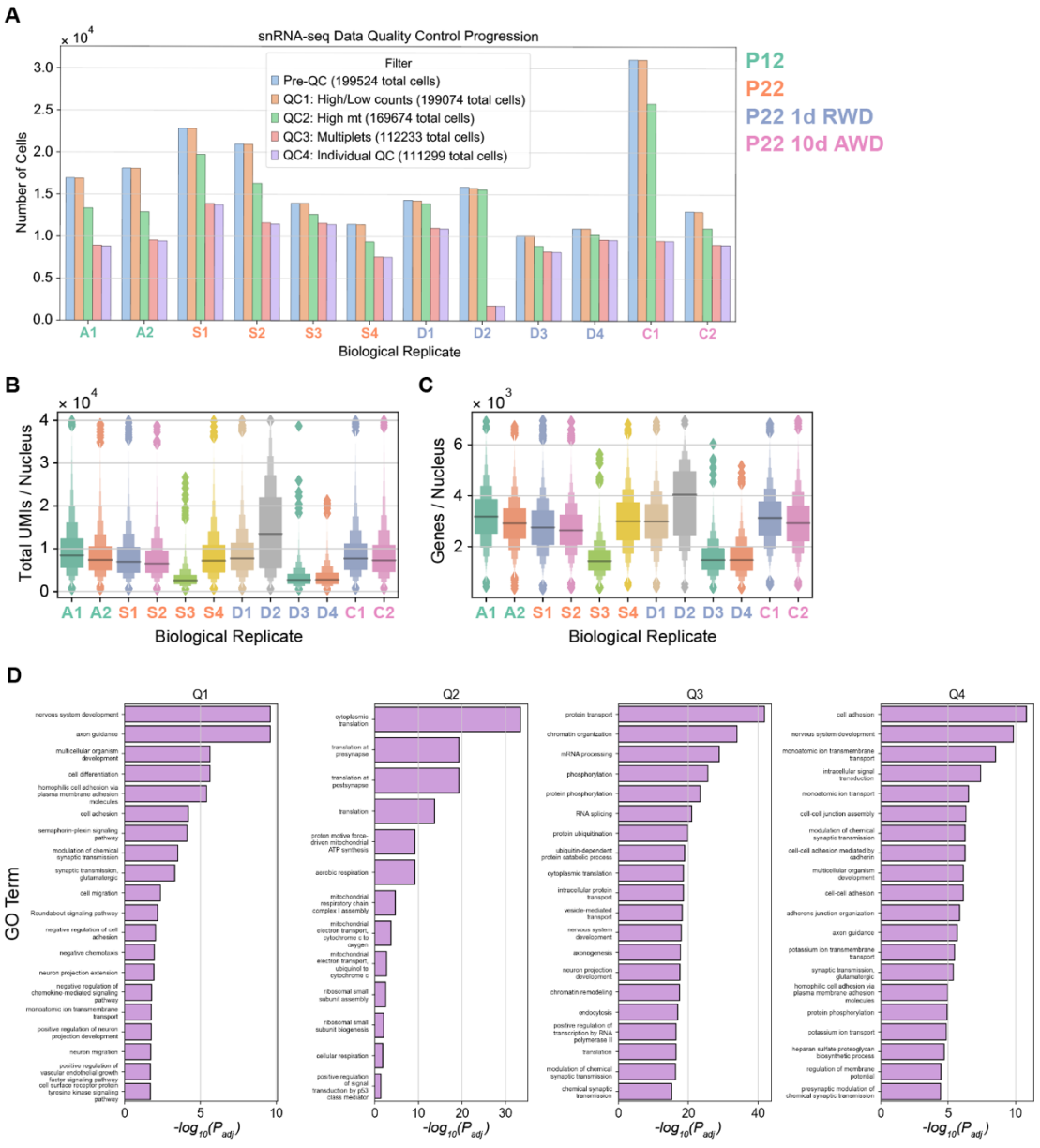

Figure S1. Data filtering steps, quality control, and gene ontology (GO) analysis of temporally regulated genes.

A. Bar plots showing the number of nuclei remaining in each biological replicate at the end of each filtering step (see **Methods** for details). Biological replicates (x-axis) are colored by their experimental condition (legend, right). "PreQC" represents the default number of nuclei the 10X CellRanger software provides. "QC4" represents the final set of nuclei used for downstream analyses.
B. Distribution of total RNA counts detected in each biological replicate from each condition.
C. Distribution of total number of genes detected in each biological replicate from each condition.
D. The top 20 "biological process" gene ontology terms for Q1-Q4 for glutamatergic subclasses as shown in **Fig 2D**.

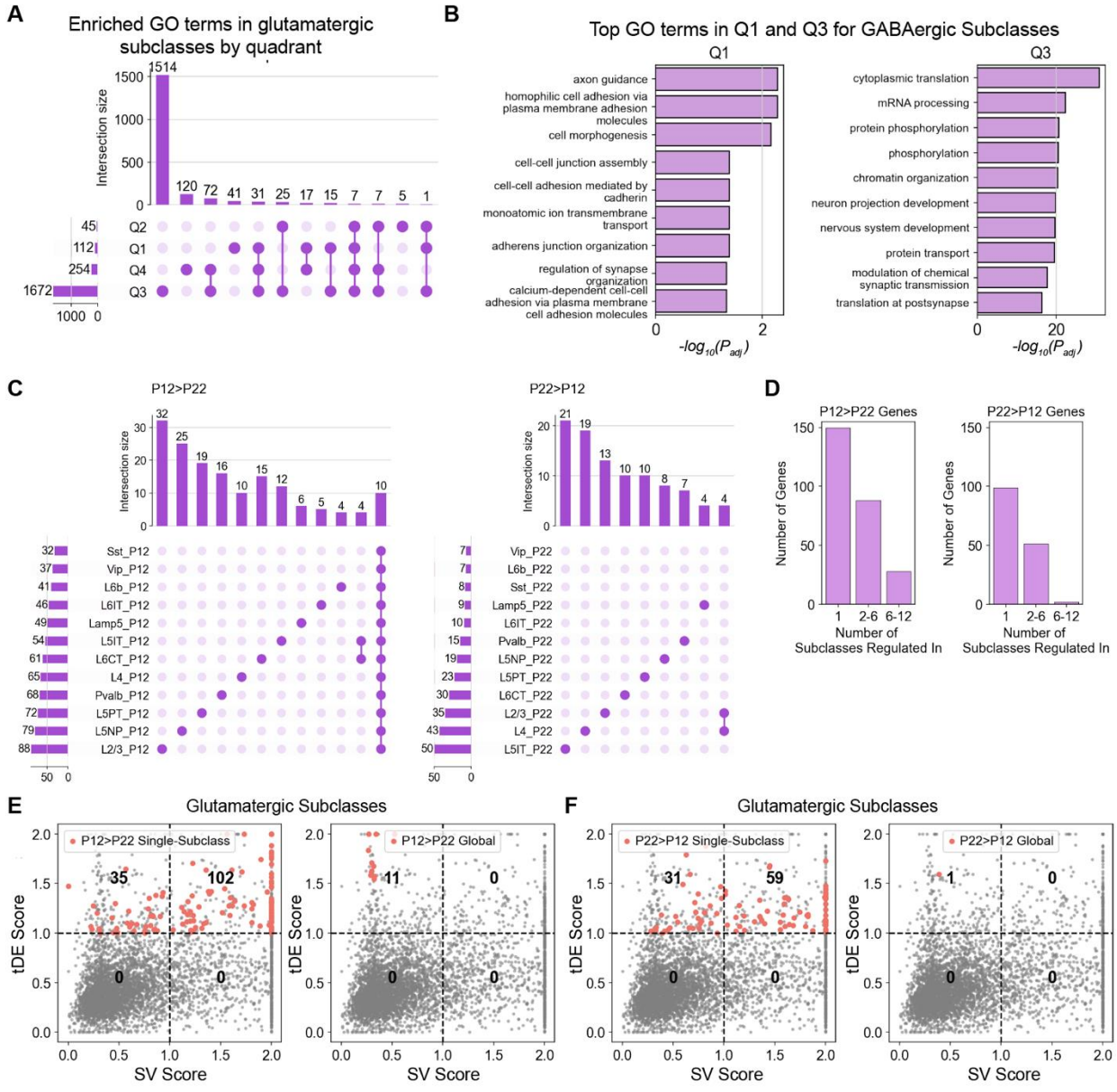

Figure S2. Developmental gene expression changes are subclass-specific.

A. UpSet plot (69) showing the overlap of GO terms associated with "biological process" (BP) across Q1-Q4 for glutamatergic neuronal subclasses. The lower panel indicates the set intersections corresponding to each column (e.g. the third column indicates the number of GO terms found in Q3 and Q4, but not in Q1 and Q2).
B. Top GO terms enriched in Q1 (*left*) and Q3 (*right*) for GABAergic neuronal subclasses.
C. UpSet plots showing that downregulated (*left*) and upregulated (*right*) tDE genes between P12 and P22 are primarily subclass-specific. Only set intersections containing at least four genes are shown. Note that, unlike panel A, the sets here correspond to groups of subclasses rather than groups of quadrants.
D. Bar plots summarizing that ~60% of genes are regulated in only one subclass and that the number of downregulated genes is ~1.6x that of upregulated genes.
E. Visualization of the subclass-specific and global P12>P22 genes from panel A in the quadrant analysis of **Fig 2B** for glutamatergic neurons.
F. Same as panel E for P22>P12 genes.

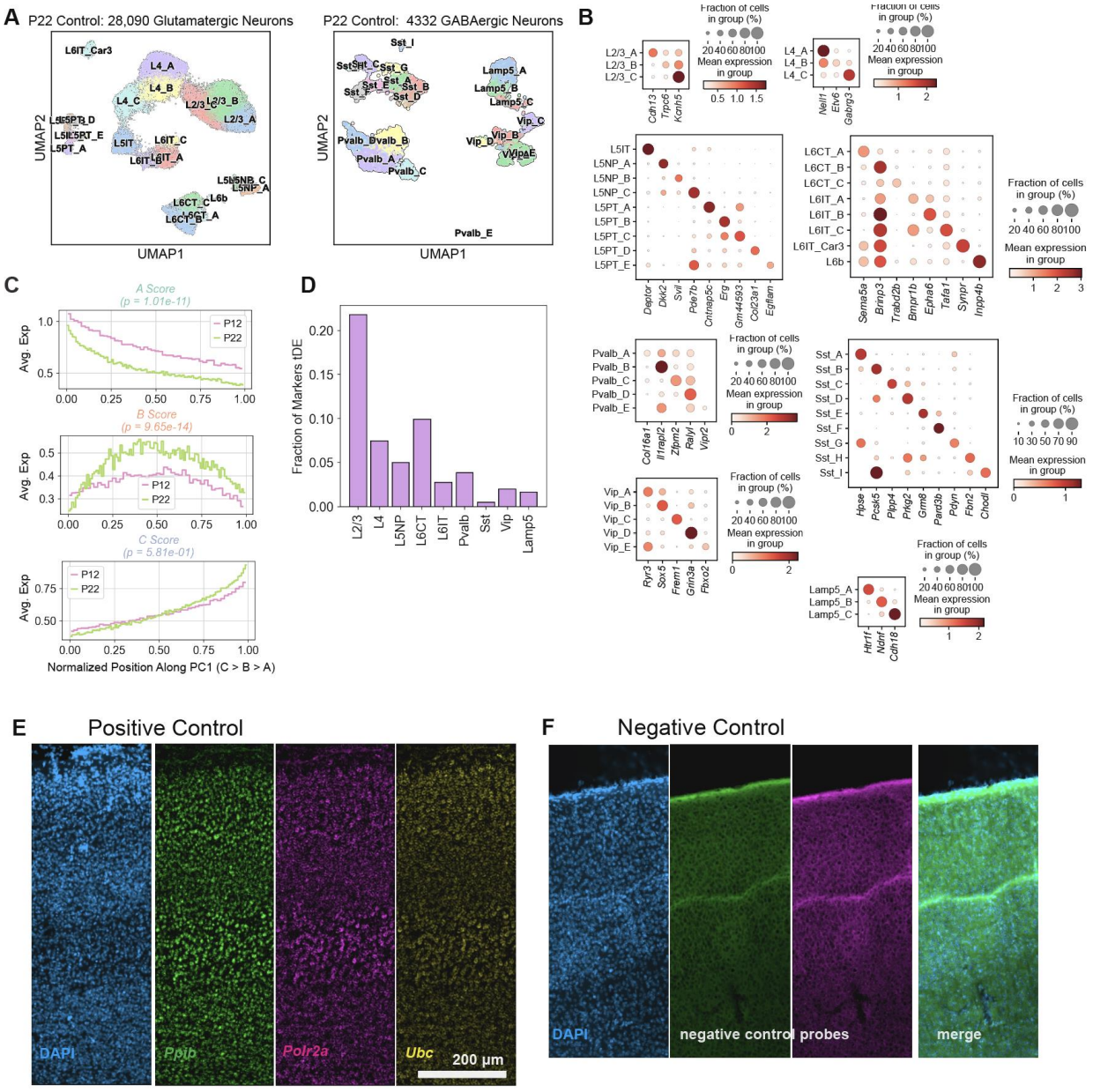

Figure S3. **Neuronal cell types at P22 and developmental changes.**

A. UMAP visualization of P22 wS1 cell types in glutamatergic (left) and GABAergic (right) neurons.
B. Dotplots showing top cell type markers within each subclass at P22. Within each dotplot panel, rows indicate cell types and columns indicate genes. The size of each circle corresponds to the % of cells with nonzero expression, and the color indicates average expression level.
C. Same as **Fig 4A**, but the y-axis now plots aggregate expression scores for_L2/3_A, L2/3_B, and L2/3_C along PC1. Curves correspond to P12 and P22. *P*-values are from a Kolmogorov–Smirnov test between the two ages.
D. Barplot showing that L2/3 has more markers that are tDE between P12 and P22 than the other subclasses.
E. Representative widefield images of RNAscope positive control showing expected labeling pattern.
F. Representative widefield images of RNAscope negative control using nontargeting probes showing no signal as expected.

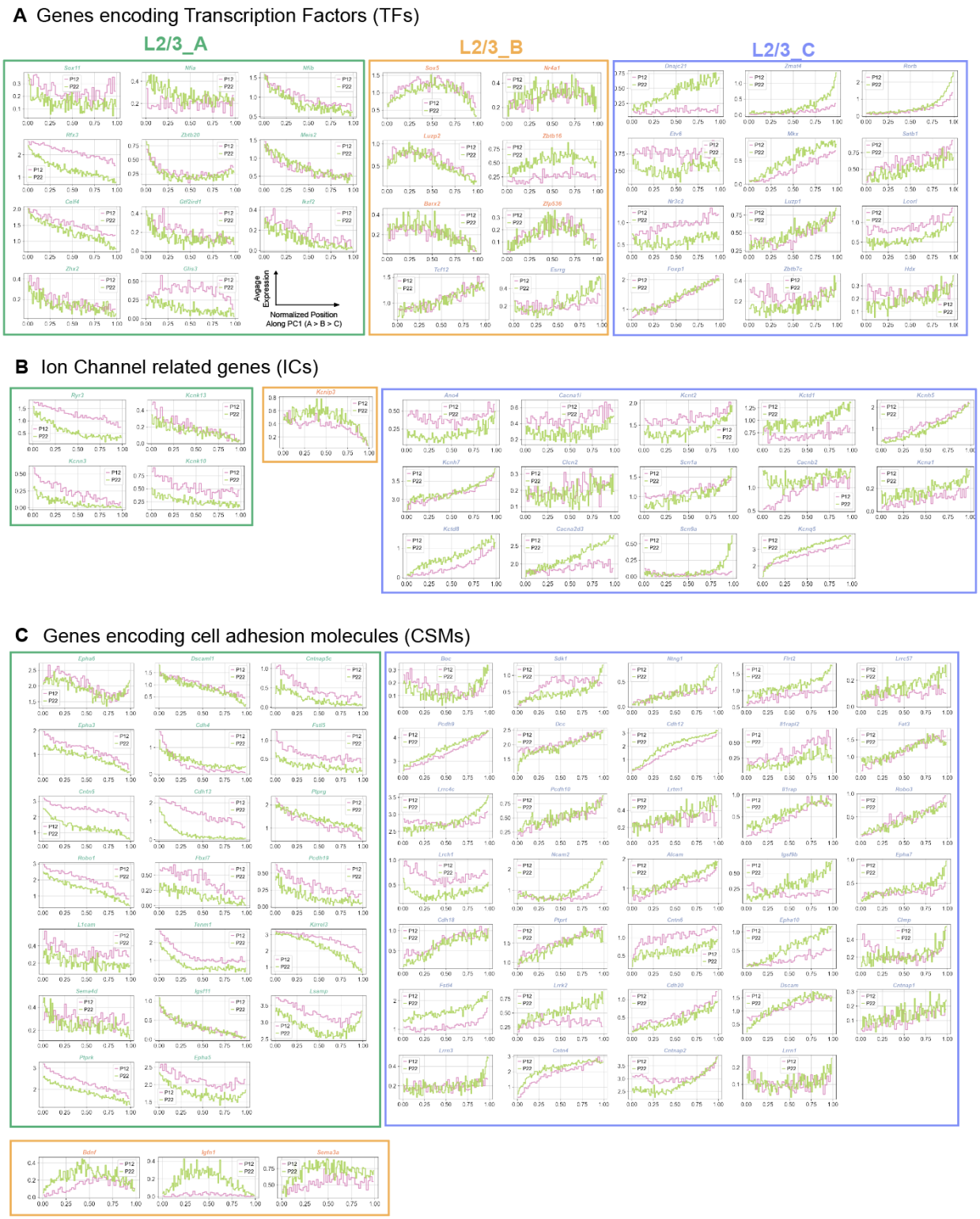

Figure S4. Expression patterns at P12 and P22 along PC1 of L2/3 type-enriched genes related to transcription factors (TFs), cell adhesion molecules (CAMs), and ion channels (ICs).

A. Expression patterns of type-enriched TFs at P12 and P22 in L2/3 cells ordered by PC1 value.
B. Same as A for ICs
C. Same as A for CAMs

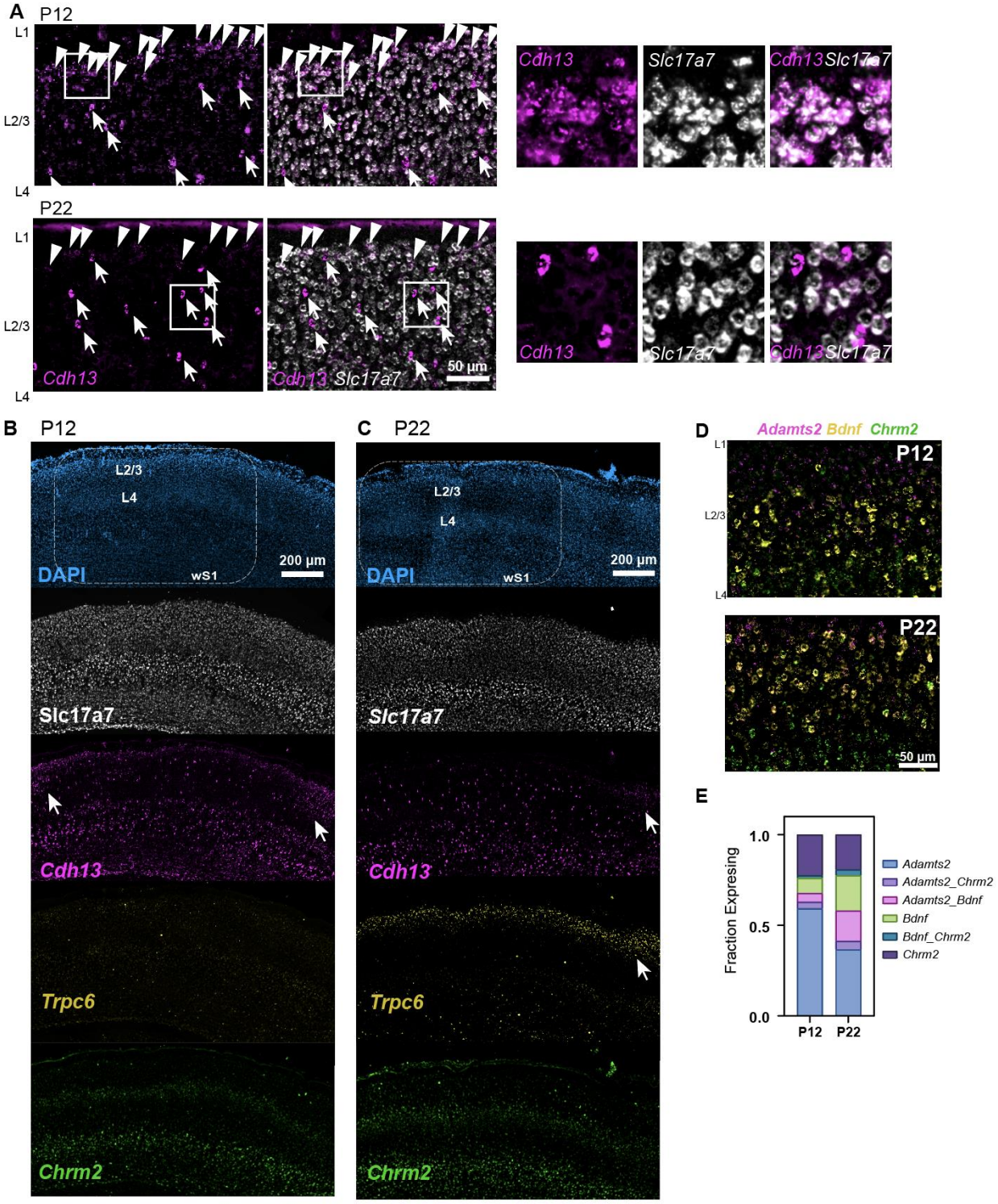

Figure S5. Representative FISH images of L2/3 cell type markers.

A. Representative images of *Cdh13* labeling at P12 (top row) and P22 (bottom row). Overlay with *Slc17a7* (vGlut1) shows that the majority of *Cdh13* expressing cells in the middle of L2/3 do not colocalize with *Slc17a7* (white arrows) whereas the *Cdh13*+ cells along the Layer 1/2 border do coexpress *Slc17a7* (white arrowheads). (right) Inset from area inside white squares.

B. Widefield images of 'across-row' section (see Methods for details) with wS1 and surrounding cortical areas at P12. Arrows indicate cortical regions outside of wS1 where labeling becomes denser.
C. Widefield images of 'across-row' section with wS1 and surrounding cortical areas at P22. Arrows indicate cortical regions outside of S1 where labeling becomes denser.
D. Representative FISH images of wS1 L2/3 labeling cell type markers *Adamts2, Bdnf,* and *Chrm2* at P12 and P22.
E. Quantification of the fraction of excitatory (*Slc17a7*+, not shown) L2/3 cells expressing one or more of markers *Adamts2*, *Bdnf*, and *Chrm2* at P12 and P22. N = 3-4 slices from 2 mice per time point.

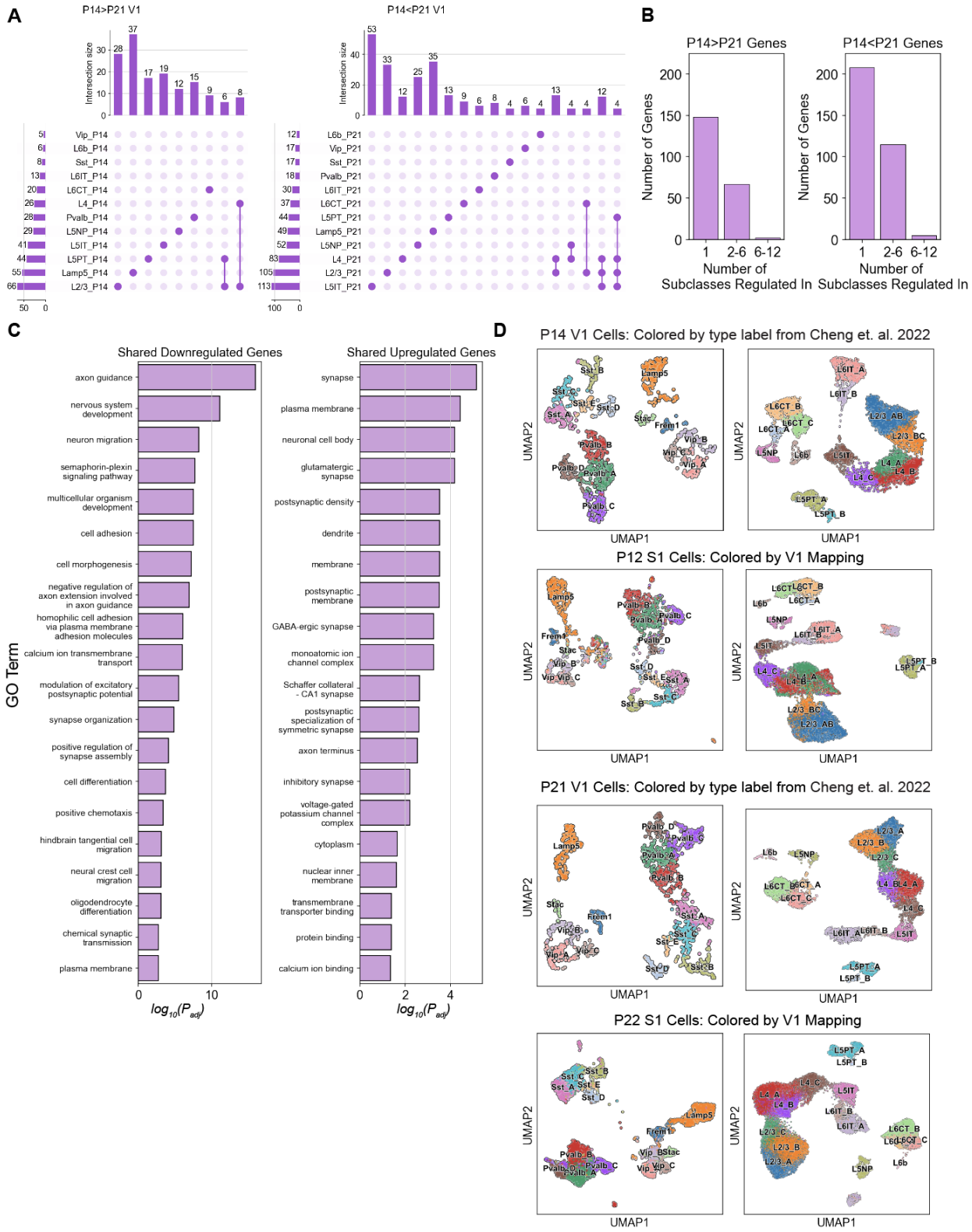

Figure S6. Temporal gene expression changes in V1, GO enrichment for shared and region-specific genes, and mapping analysis.

A. UpSet plot (as in **Fig S2C**) summarizing subclass-by-subclass tDE analysis of V1 data. Only combinations containing at least four genes are shown.
B. Barplots showing that as in the case of wS1 (**Fig S2D**), most downregulated (*left*) and upregulated (*right*) genes in V1 are subclass-specific.
C. Full list of GO terms enriched in shared downregulated (*left*) or upregulated (*right*) tDE genes between V1 and wS1.
D. UMAP plots of V1 (rows 1 and 3) and wS1 (rows 2 and 4) data colored by V1 labels. V1 neuron labels are based on the published clustering in Cheng et al. (10), while wS1 neurons were labeled using a supervised mapping analysis (see **Methods**).

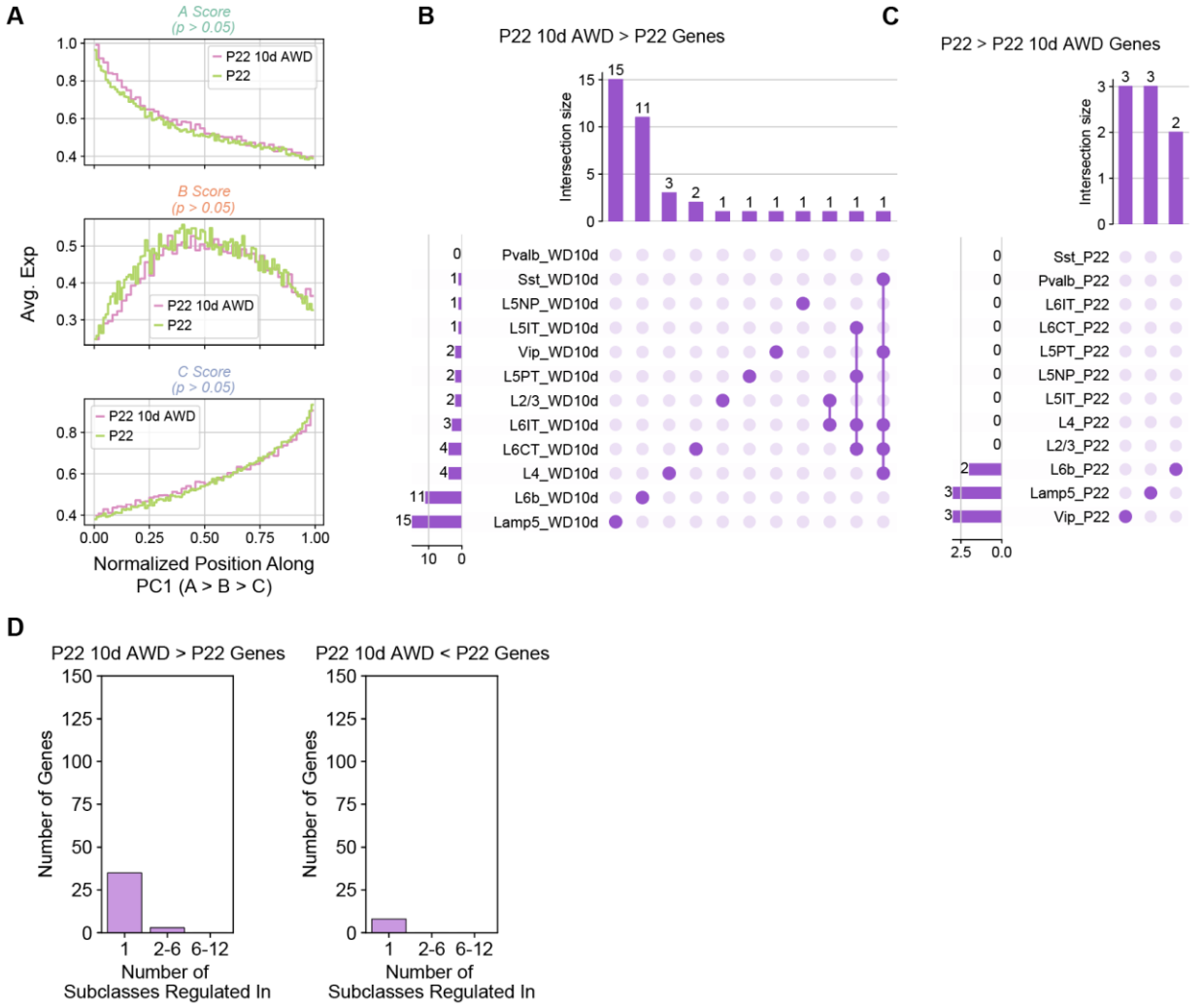

Figure S7. **Subclass-level gene expression changes between P22 10d AWD and P22 control.**

A. L2/3 type A, B, and C marker scores plotted as a function of a cell's position along PC1. *P* values are based on a Kolmogorov–Smirnov test comparing the two conditions.
B. UpSet plots showing that the few genes upregulated by 10d AWD are predominantly subclass-specific.
C. Same as B but for genes downregulated by 10d AWD.
D. Bar plots highlighting the small number of genes regulated by 10d AWD. Scale for y-axis is the same as for **Fig S2D** for comparison.

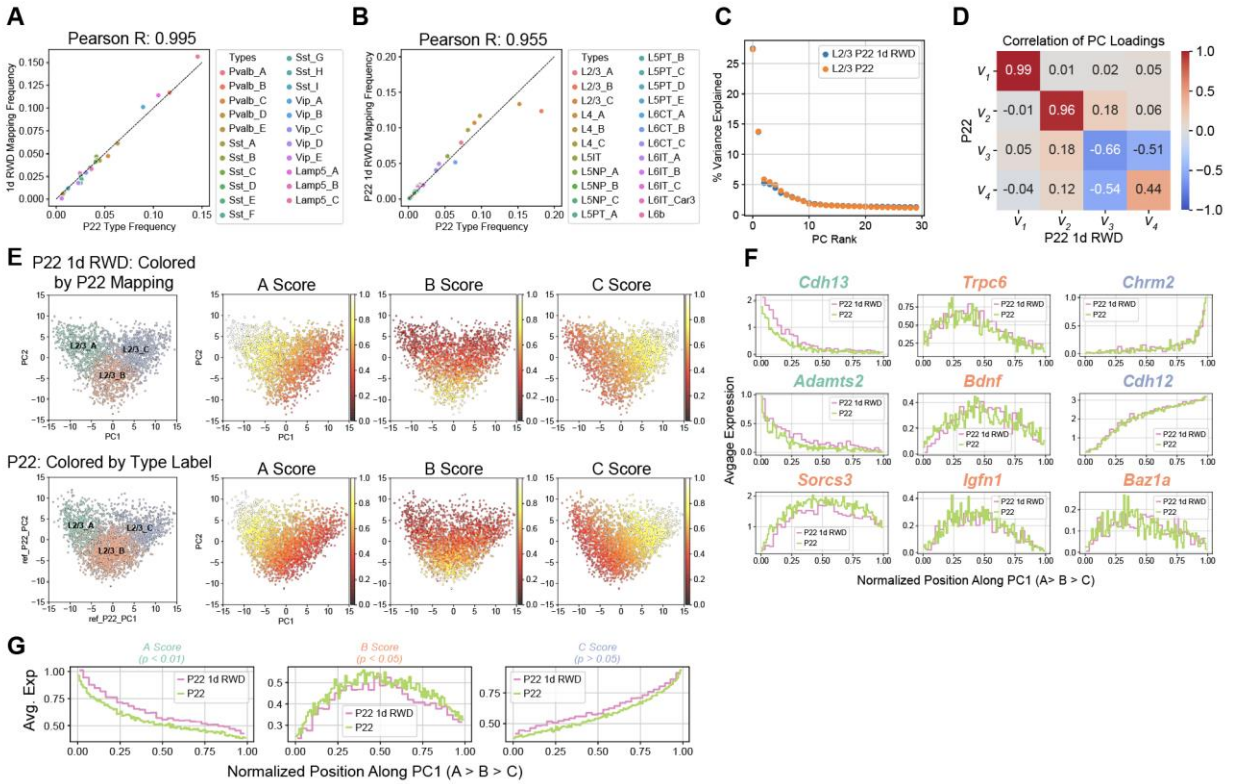

Figure S8. **1d RWD has little effect on L2/3 cell type identity.**

A. GABAergic cell types have approximately the same relative frequency at P22 1d RWD and P22 normal whisker experience. Representation as in **Fig 6A**.
B. Same as panel A, for glutamatergic cell types.
C. PC1 and PC2 are sufficient to describe transcriptional variance within L2/3 in the normal P22 and P22 10d AWD datasets
D. Similar to **Fig 6D,** comparing principal eigenvectors between the P22 1d RWD and normal P22 datasets. The first two principal eigenvectors map 1:1.
E. Similar to **Fig 6E** comparing the PC1 vs. PC2 distribution and type-specific scores between P22 1d RWD and normal P22 L2/3 datasets.
F. L2/3 markers genes, as in **Fig 4A**, are shown as a function of cells' position along PC1 comparing patterns between normal P22 and P22 1d RWD.
G. L2/3 type A, B, and C marker scores plotted as a function of a cell's position along PC1. *P* values are from a Kolmogorov–Smirnov test between the two conditions.

Table Legends

Table S1. tDE and SV scores for every tested gene in glutamatergic and GABAergic neurons.

Table S2. Subclass-by-subclass differential expression testing results between P12 and P22.

Table S3. Cell type markers from each subclass at P22.

Table S4. Subclass-by-subclass differential expression testing results between P14 and P21 V1 data.

Table S5. Subclass-by-subclass differential expression testing results between P22 10d AWD and P22

Table S6. Subclass-by-subclass differential expression testing results between P22 1d RWD and P22