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## **Supplemental information**

### **Dendritic spine formation and synapse maturation in transcription factor-induced human iPSC-derived neurons**

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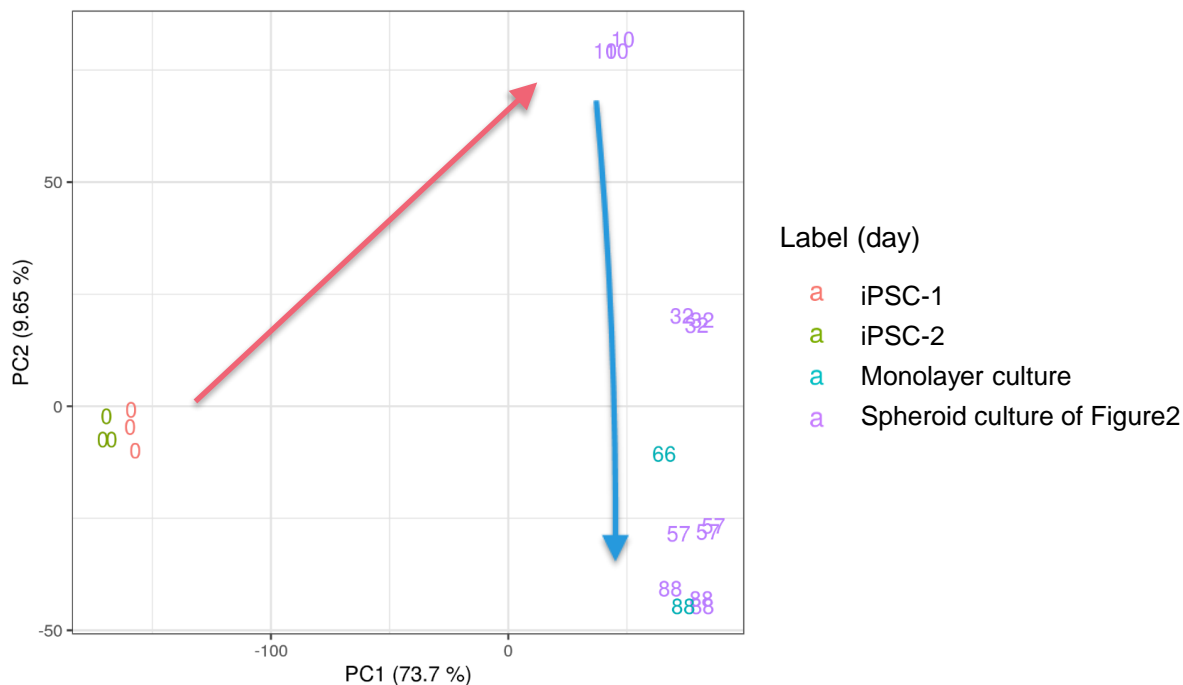


Figure S1. Reproducibility of the transcriptional profiles in monolayer cultures compared to spheroid cultures, related to Figure 2(A). PCA including additional RNA-Seq data from monolayer cultures of TF-induced iPSC neurons on day 66 and 88 following the induction of differentiation. The monolayer cultures reached a similar profile as the spheroid cultures on day 88.

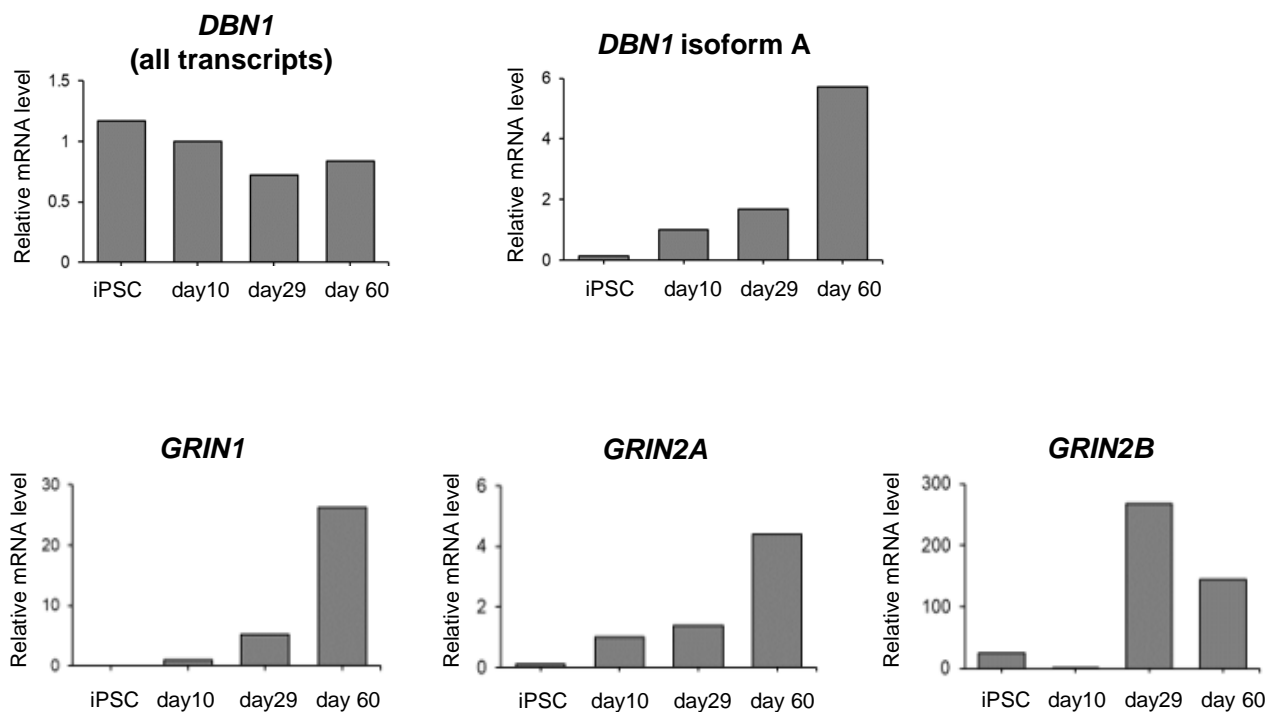


Figure S2. Reproducibility of transcriptional switches in monolayer cultures, related to Figure 3(C-E). RT-qPCR analysis of drebrin isoforms and of NMDA receptor subunits using an independent time-course series of monolayer cultures at day 10, 29 and 60 following the induction of differentiation (no statistical analysis was performed because we could not collect enough samples to make replicates at each time point).

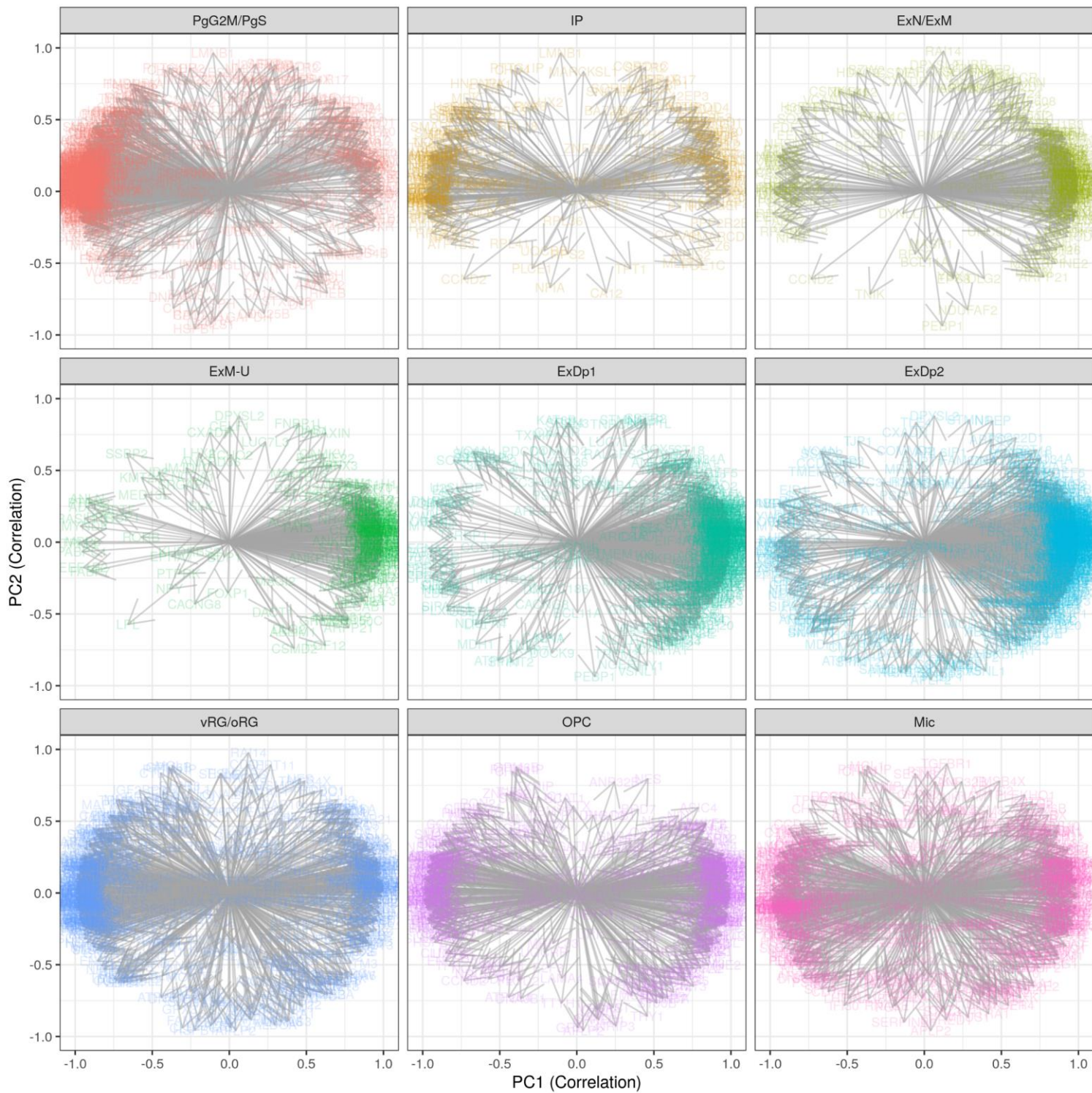
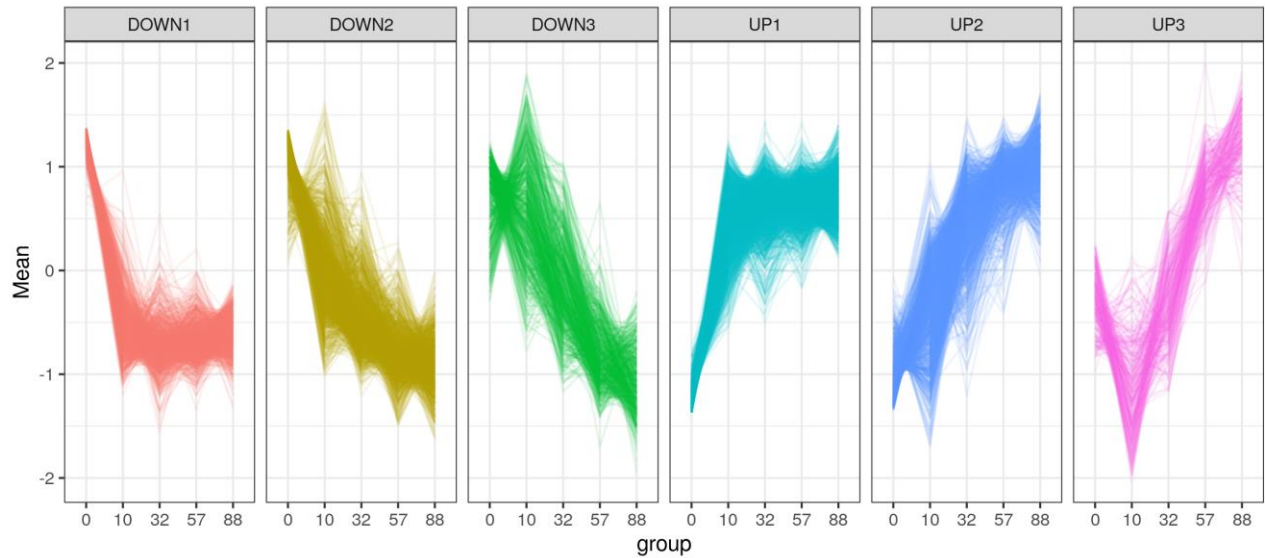


Figure S3. Additional graphs showing the factor loading vectors for each of the major cell types defined in the human developing cortex [S1], related to Figure 2 (B).

A



B

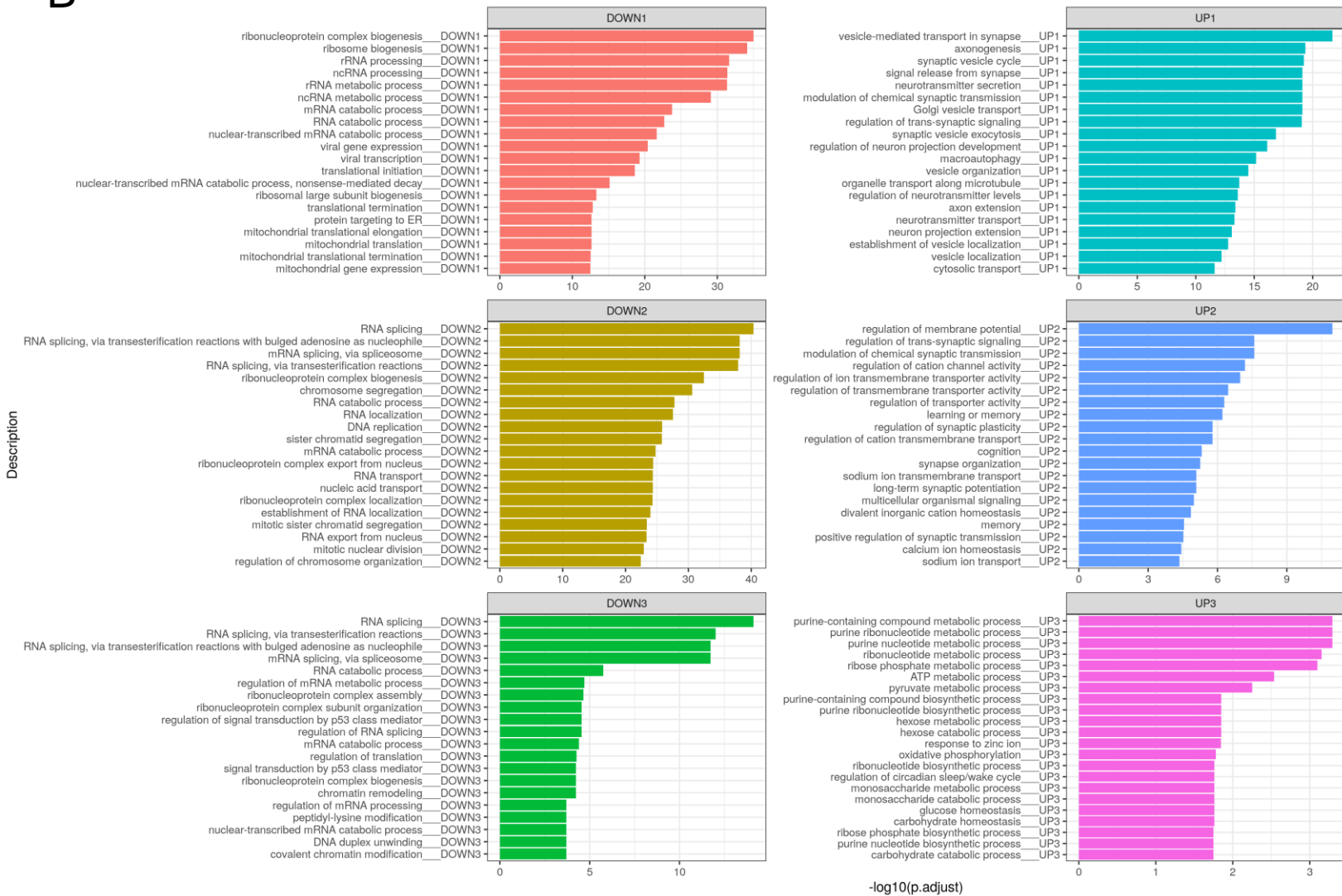


Figure S4. Results of the GO enrichment analysis for all 6 groups of genes categorized by hierarchical clustering, related to Figure 2 (D-F). (A) Gene expression profiles categorized into six patterns by hierarchical clustering. Each line is plotted from the mean expression levels of a gene with a significant variation across the time-course. (B) The leading 20 significantly enriched Gene Ontology (GO) terms for biological processes in each group.

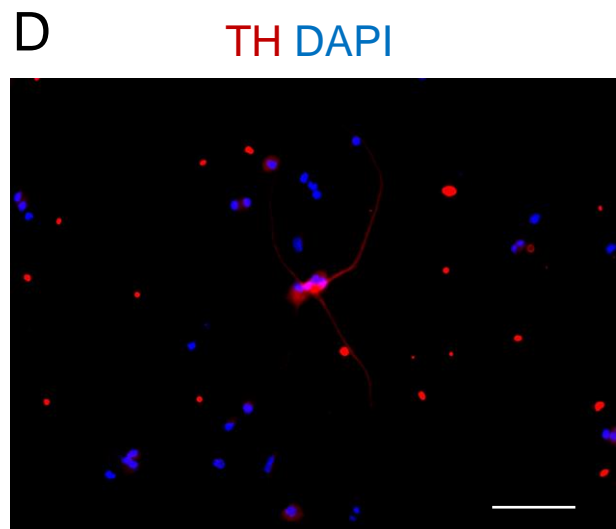
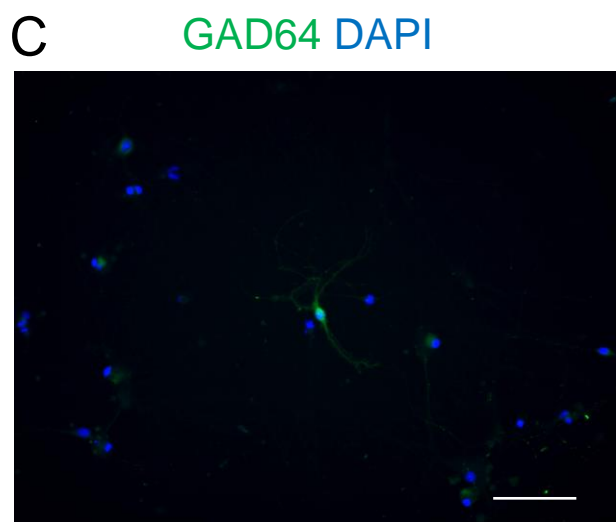
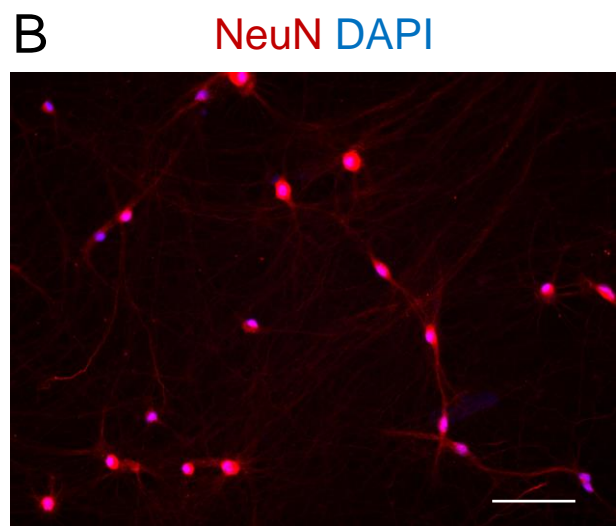
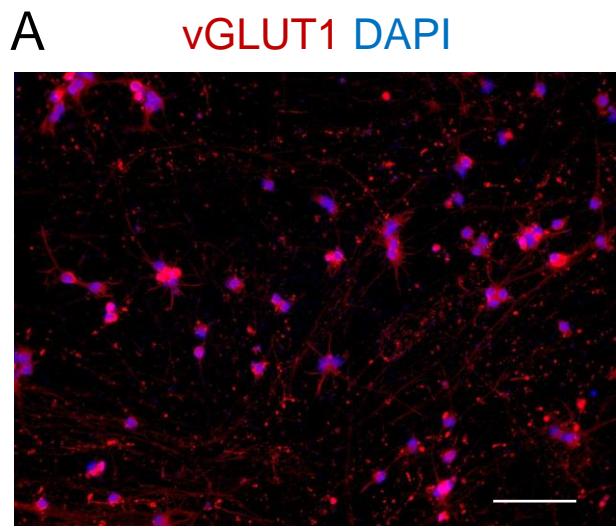


Figure S5. Representative immunofluorescence images ( $20 \times$  objective) showing the expression of known neuronal markers, related to Figure 3(A). The early expression of (A) the glutamatergic marker vGLUT1 (red) was shown on day 28, and the expression of (B) NeuN (red), (C) GAD64 (green), and (D) TH (red) in late cultures on day 85. GAD64 and TH-expressing cells were rarely detected, so the images were centered on the one cell that was found. TH staining also shows artifact particles of unknown origin. Nuclei have been stained with DAPI. White scale bar: 100  $\mu$ m.

Supplemental Reference:

[S1] Polioudakis, D., de la Torre-Ubieta, L., Langerman, J., Elkins, A.G., Shi, X., Stein, J.L., Vuong, C.K., Nichterwitz, S., Gevorgian, M., Opland, C.K., et al. (2019). A Single-Cell Transcriptomic Atlas of Human Neocortical Development during Mid-gestation. *Neuron* 103, 785-801.e8. [10.1016/j.neuron.2019.06.011](https://doi.org/10.1016/j.neuron.2019.06.011).