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Supplemental information

Generation of human cerebral organoids with a structured outer subventricular zone

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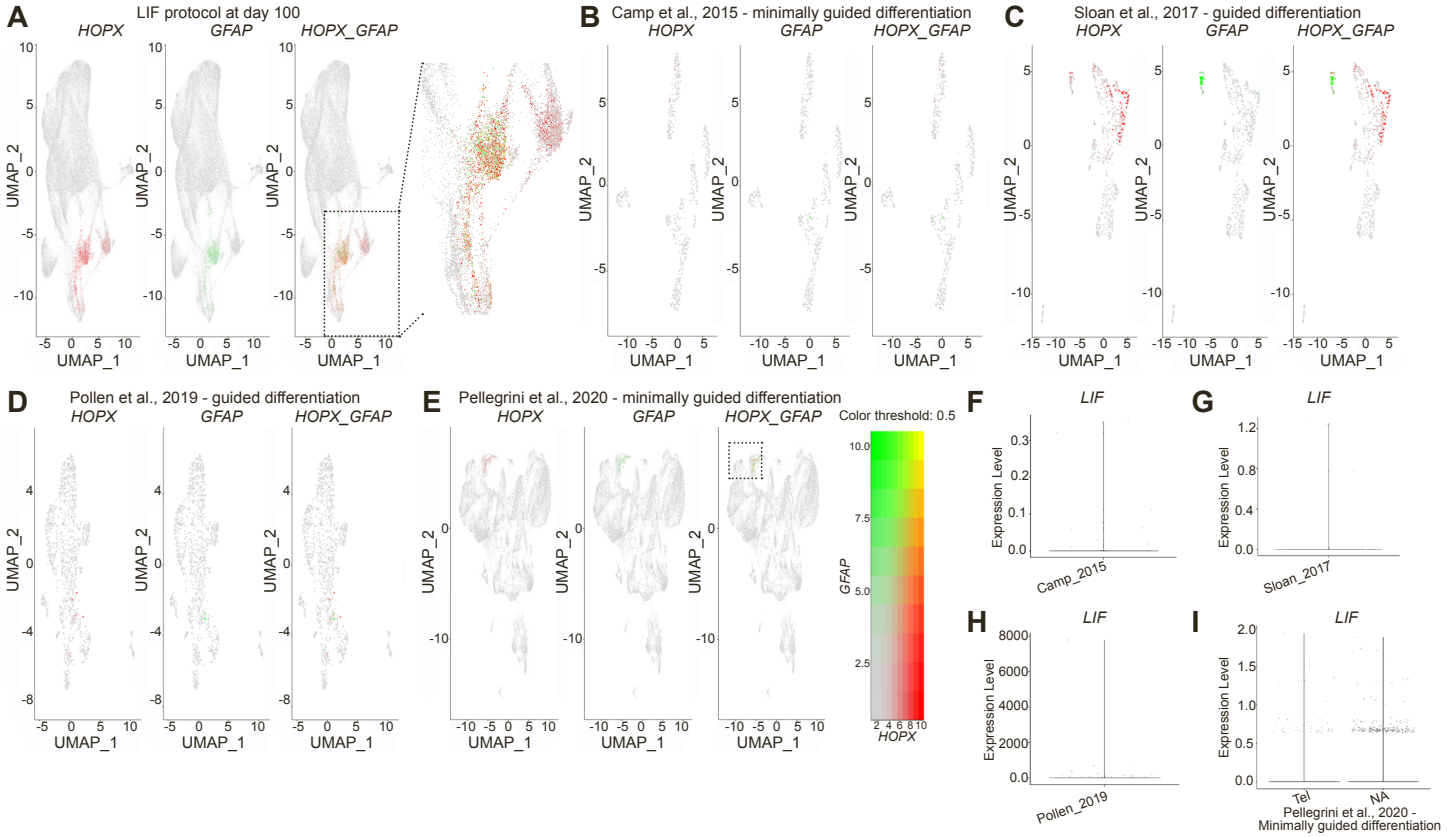
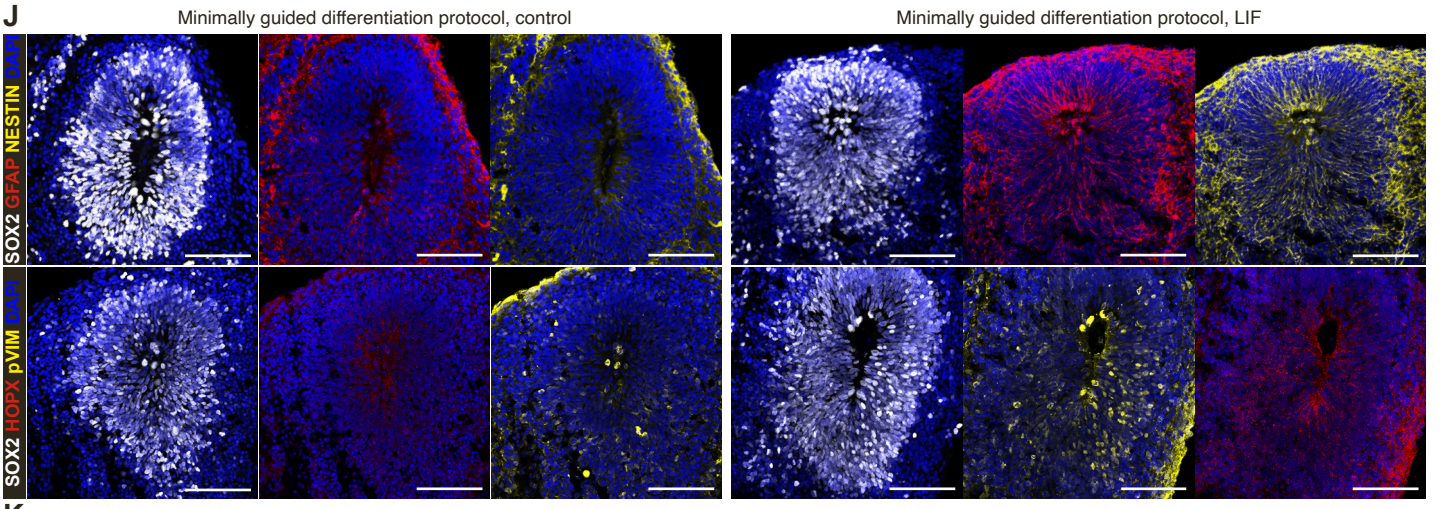
Supplemental Figure 1 related to Figure 1 and Figure 2





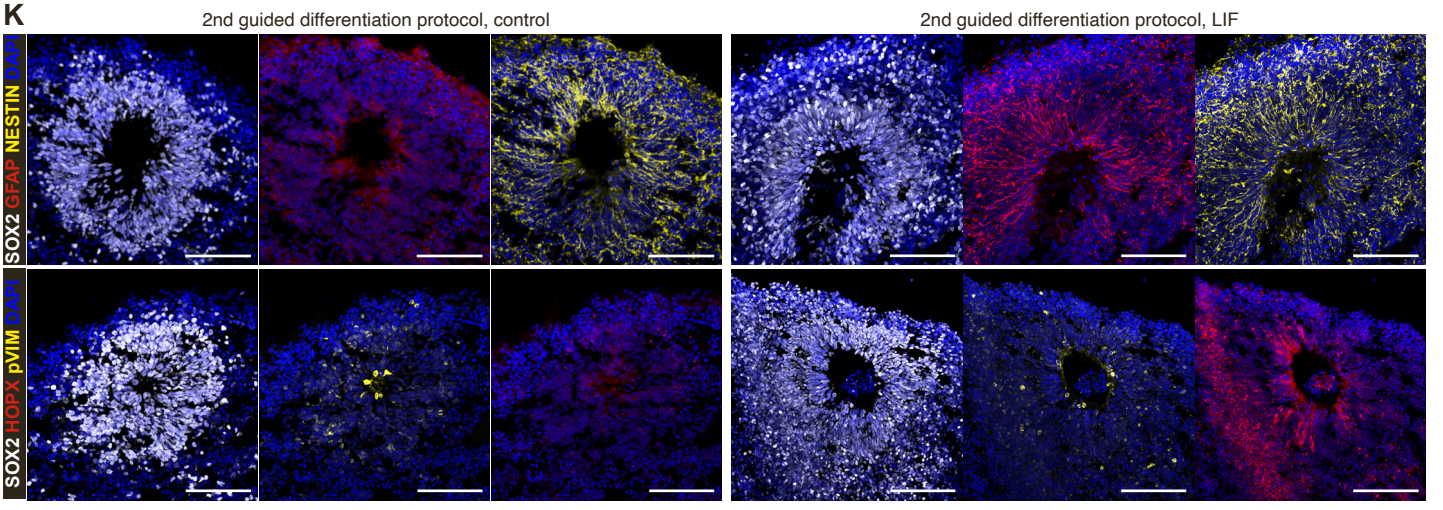


Figure S1. LIF in minimally guided and guided cortical differentiation protocols

(A-E) Feature plots from the scRNA-seq datasets depicting *HOXP, GFAP*, and co-expression of *HOXP* and *GFAP* in our study at day 100 of differentiation (**A**); their expression in other two guided differentiation protocols (**C** and **D**) and two minimally guided differentiation protocols (**B** and **E**).

(F-I) Expression levels of *LIF* in various cerebral organoid models. In a minimally guided differentiation protocol, telencephalon (Tel) cells do not express LIF, while not-assigned (NA) cell types do **(I)**.

(J) SOX2 (gray), GFAP (red), NESTIN (yellow), phospho-VIMENTIN (pVIM) (yellow) and HOPX (red) staining in day 60 brain organoids derived from a minimally guided differentiation protocol +/- LIF. Cell nuclei stained with DAPI. Scale bars 100 µm.

(K) SOX2 (gray), GFAP (red), NESTIN (yellow), phospho-VIMENTIN (pVIM) (yellow) and HOPX (red) staining in day 60 cortical organoids derived from a second guided differentiation protocol +/- LIF. Cell nuclei stained with DAPI. Scale bars 100 µm.

Supplemental Figure 2 related to Figure 1 and Figure 2

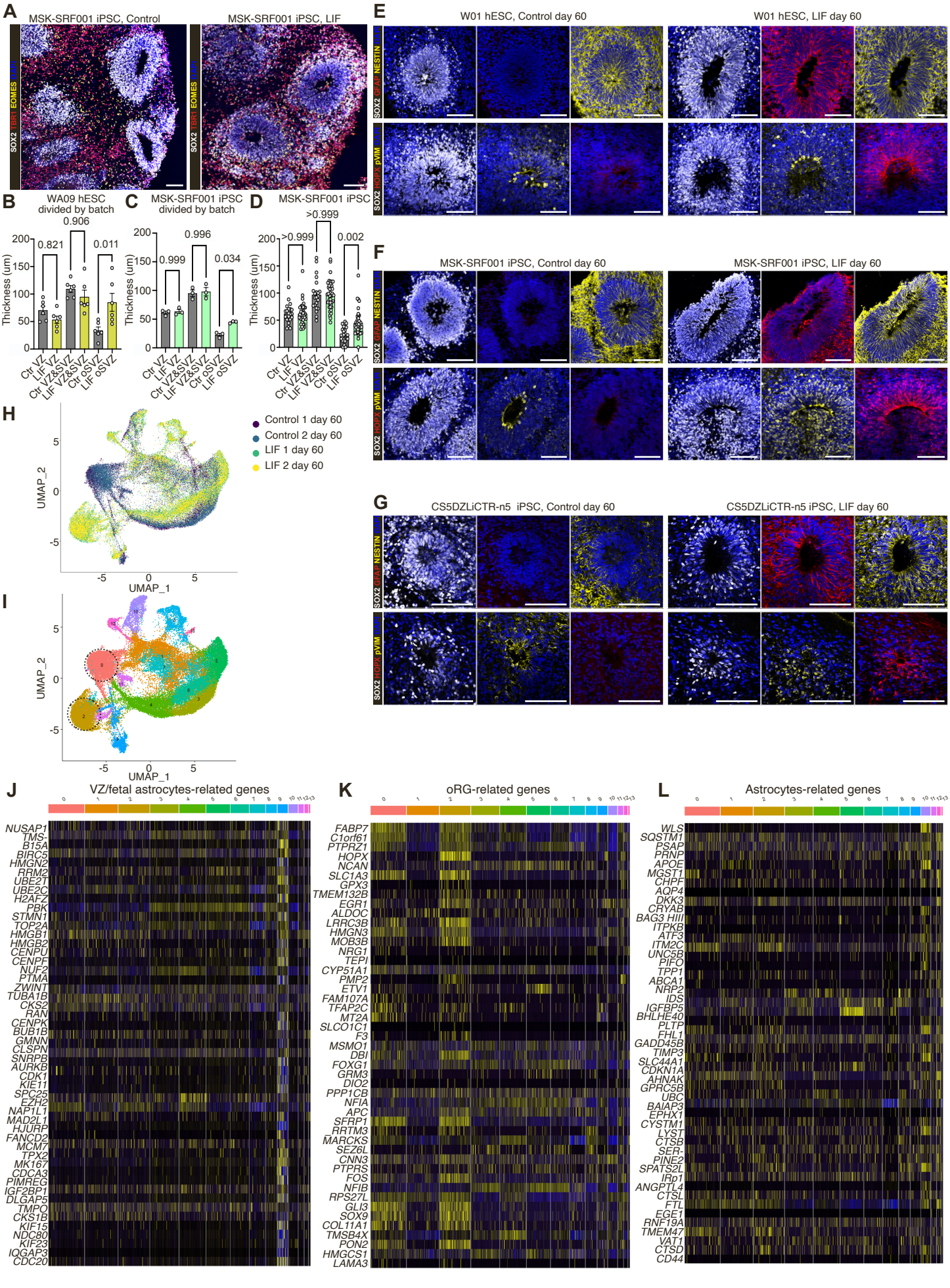


Figure S2. LIF treatment in various hPSC lines and transcriptional profiling

(A) SOX2 (gray), TBR1 (red) and EOMES/TBR2 (yellow) staining in day 60 MSK-SRF001 iPSC control and LIF-treated cortical organoids. Cell nuclei stained with DAPI. Scale bars 100 µm.

(B) WA09 hESC rosette area quantifications from Figure 1C, showing the inter-batch variability. One-way ANOVA with Tukey's test.

(C) MSK-SRF001 iPSC rosette area quantifications. One-way ANOVA with Tukey's test.

(D) MSK-SRF001 iPSC rosette area quantifications from **(C)** showing the inter-batch variability. One-way ANOVA with Tukey's test.

(E-G) SOX2 (gray), GFAP (red), NESTIN (yellow), HOPX (red), phospho-VIMENTIN (pVIM) (yellow) stainings in day 60 control and LIF-treated organoids from the WA01 hESC line **(D)**, the MSK-SRF001 iPSC line **(E)** and the CS5DZLiCTR iPSC line **(F)**. Cell nuclei stained with Dapi. Scale bars 100 µm.

(H) Single-cell RNA-seq (scRNA-seq) experiments at day 60 showing plots for original identity.

(I) Seurat clusters in control and LIF-treated organoids at day 60. The progenitor cluster (0) from control cortical organoids and the oRG cluster 2 from LIF-treated cortical organoids are highlighted.

(J-L) Heatmaps showing the transcriptional signatures of VZ/fetal astrocytes (**J**), oRG (**K**) and astrocytes (**L**) derived from Sloan et al.[10] and mapping of all the various cellular identities present at day 60 in control and LIF-treated organoids.

Figure S3 related to Figure 1, 2 and 3

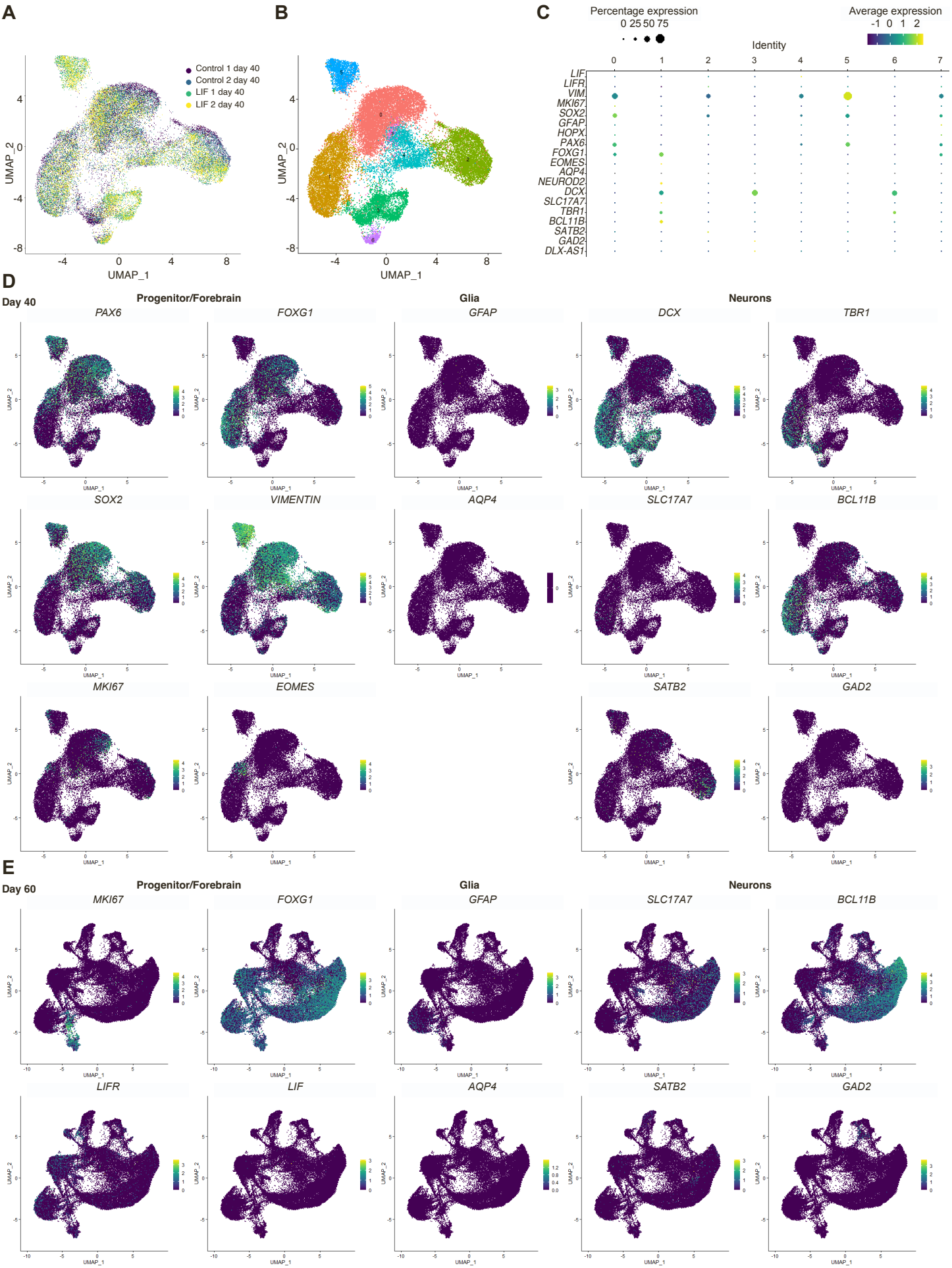


Figure S3. Day 40 and Day 60 scRNA-seq of control and LIF-treated cortical organoids

(A and B) scRNA-seq experiments showing plots for original identity **(A)** and Seurat clusters **(B)** in control and LIF-treated cortical organoids at day 40. Two independent batches of 10 organoids each were processed and analyzed by scRNA-seq for both control and LIF condition.

(C) Genes selected from B to mark selected populations of interest (dividing cells, neuronal cell types, neuronal progenitors, astrocytes, postmitotic neurons and radial glial cells) in control and LIF-treated organoid clusters at day 40.

(D) Feature plots depicting the distribution of the expression of key selected progenitor/forebrain, glia, and neuronal genes in control and LIF-treated organoids at day 40.

(E) Feature plots depicting the distribution of the expression of key selected progenitor/forebrain, glia, and neuronal genes in control and LIF-treated organoids at day 60.

Figure S4 related to Figure 3 and 4

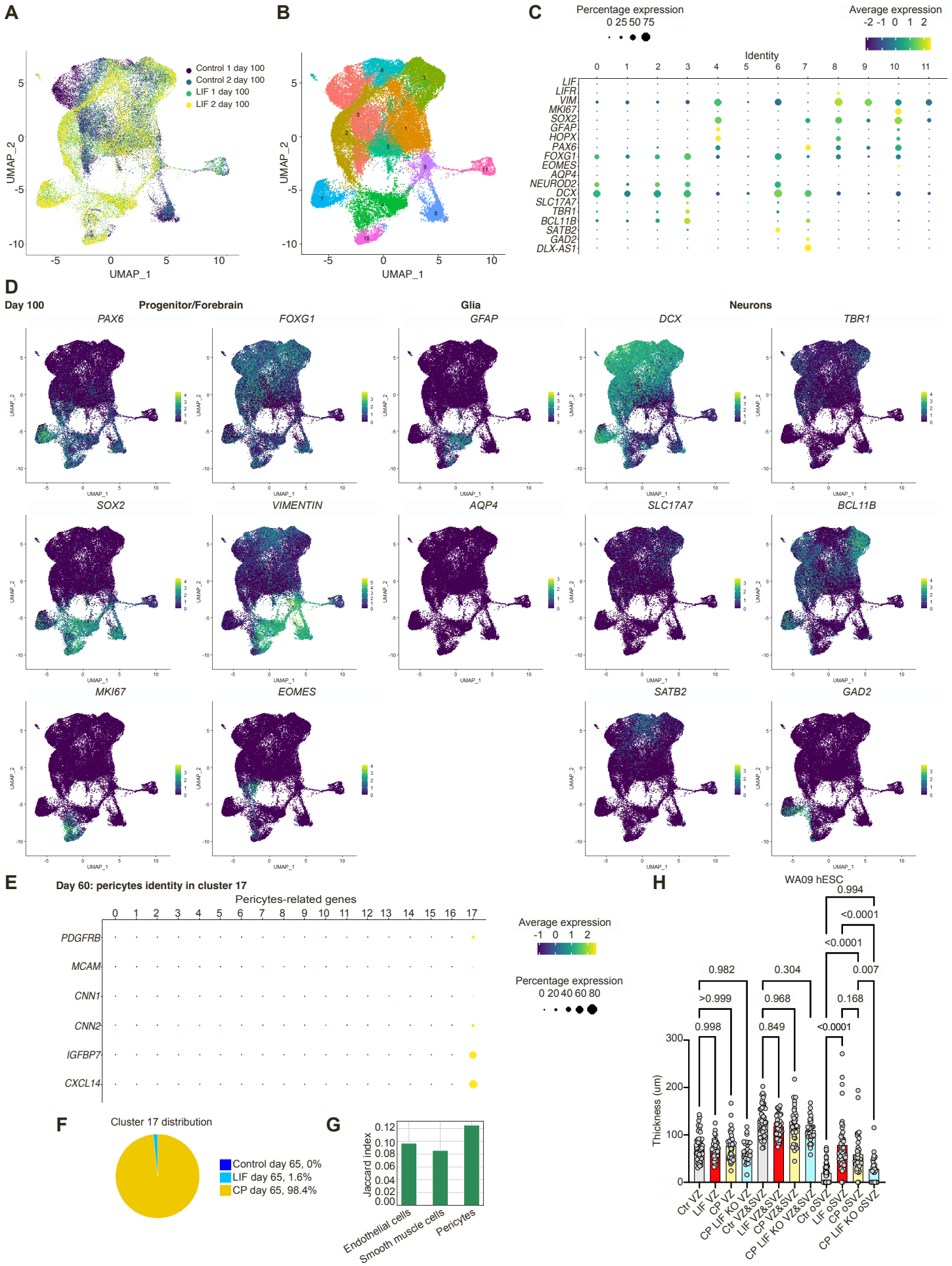


Figure S4. Day 100 scRNA-seq of control and LIF-treated cortical organoids, and identification and impact of LIF-secreting pericytes in CP assembloids

(A and B) scRNA-seq experiments showing plots for original identity **(A)** and Seurat clusters **(B)** in control and LIF-treated cortical organoids at day 100. Two independent batches of 10 organoids each were processed and analyzed by scRNA-seq for both control and LIF conditions.

(C) Genes selected from **(B)** to mark selected populations of interest (dividing cells, neuronal cell types, neuronal progenitors, astrocytes, postmitotic neurons, and radial glial cells) in control and LIF-treated cortical organoid identities at day 100.

(D) Feature plots depicting the distribution of the expression of key selected progenitor/forebrain, glia, and cortical neuron genes in control and LIF-treated cortical organoids at day 100.

(E) Genes selected from Figure 4L to mark selected key pericyte markers in cluster 17 in CP assembloids at day 65.

(F) Pie chart showing cluster 17 distribution in control organoids, LIF-treated organoids and CP assembloids at day 65. Identity 17 is almost exclusively enriched in CP assembloids and represents the pericytes population.

(G) Jaccard index for identity 17, specific for the CP assembloids, shows a stronger overlap with fetal brain pericytes compared to endothelial cells or smooth muscle cells.

(H) Rosette quantifications of WA09 control and LIF-treated cortical organoids, CP assembloids and CP LIF KO assembloids at day 60, based on the separation of the regions as shown in Figure 1B. One-way ANOVA with Tukey's test. Adjusted p values are shown on the graph.