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Supplemental information

Novel human pluripotent stem cell-derived hypothalamus organoids demonstrate cellular diversity

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Supplemental Figures and Legends

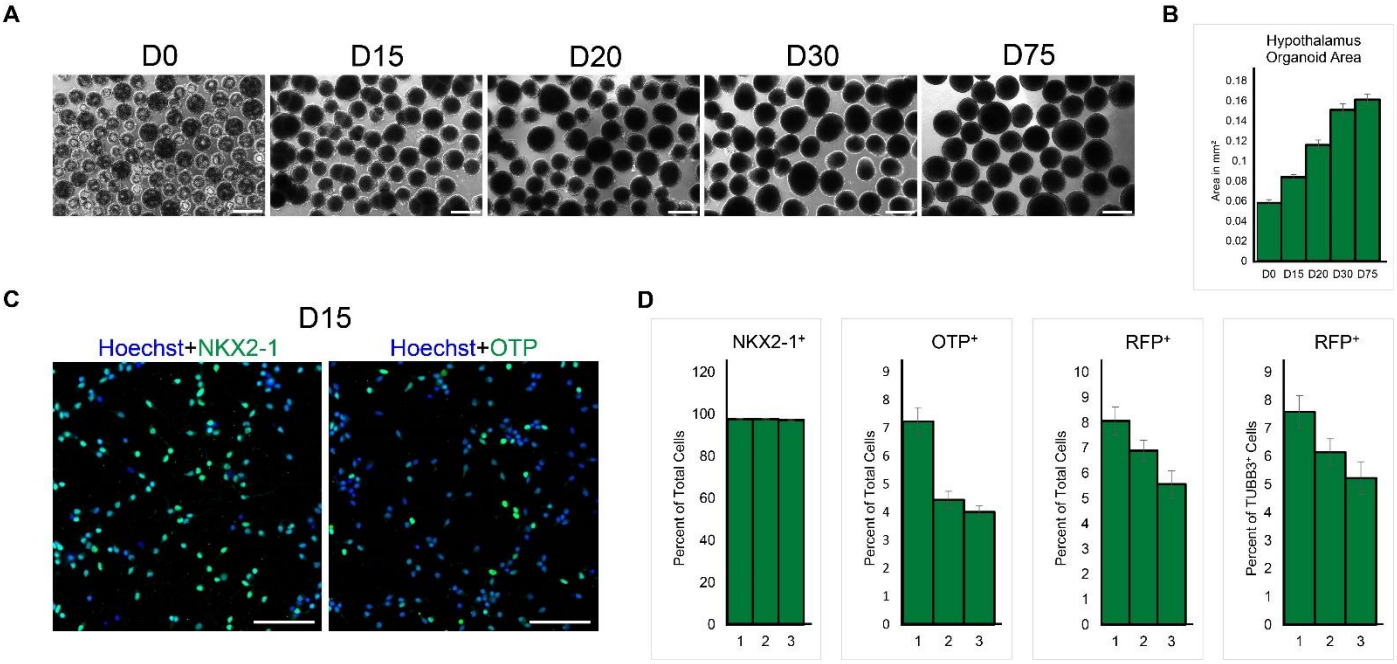

Figure S1 – Characterization of hypothalamus organoid size and patterning.
A) Brightfield images of organoids at several timepoints during differentiation. Scale bars: 500 μm.
B) Quantification of organoid area from A and similar images, showing an increase in size over time (n = 50; 25 organoids in duplicates were pooled together for quantification). Organoid area is shown in mm^2. Error bars represent SEM. SEM, standard error of the mean.
C) IF staining in D15 monolayer neurons for NKX2-1 and OTP. Hoechst was used as a nuclear marker. Scale bars: 100 μm.
D) Quantification of Figures 1B, S1C, and similar images for NKX2-1$^+$, OTP$^+$, and RFP$^+$ cells as a percentage of total cells, as well as for RFP$^+$ cells as a percentage of βIII Tubulin$^+$ cells (n = 30 per replicate). The numbers on the x-axis denote technical replicates (or well of a 96-well plate imaged). Error bars represent SEM.
Related to Figure 1.

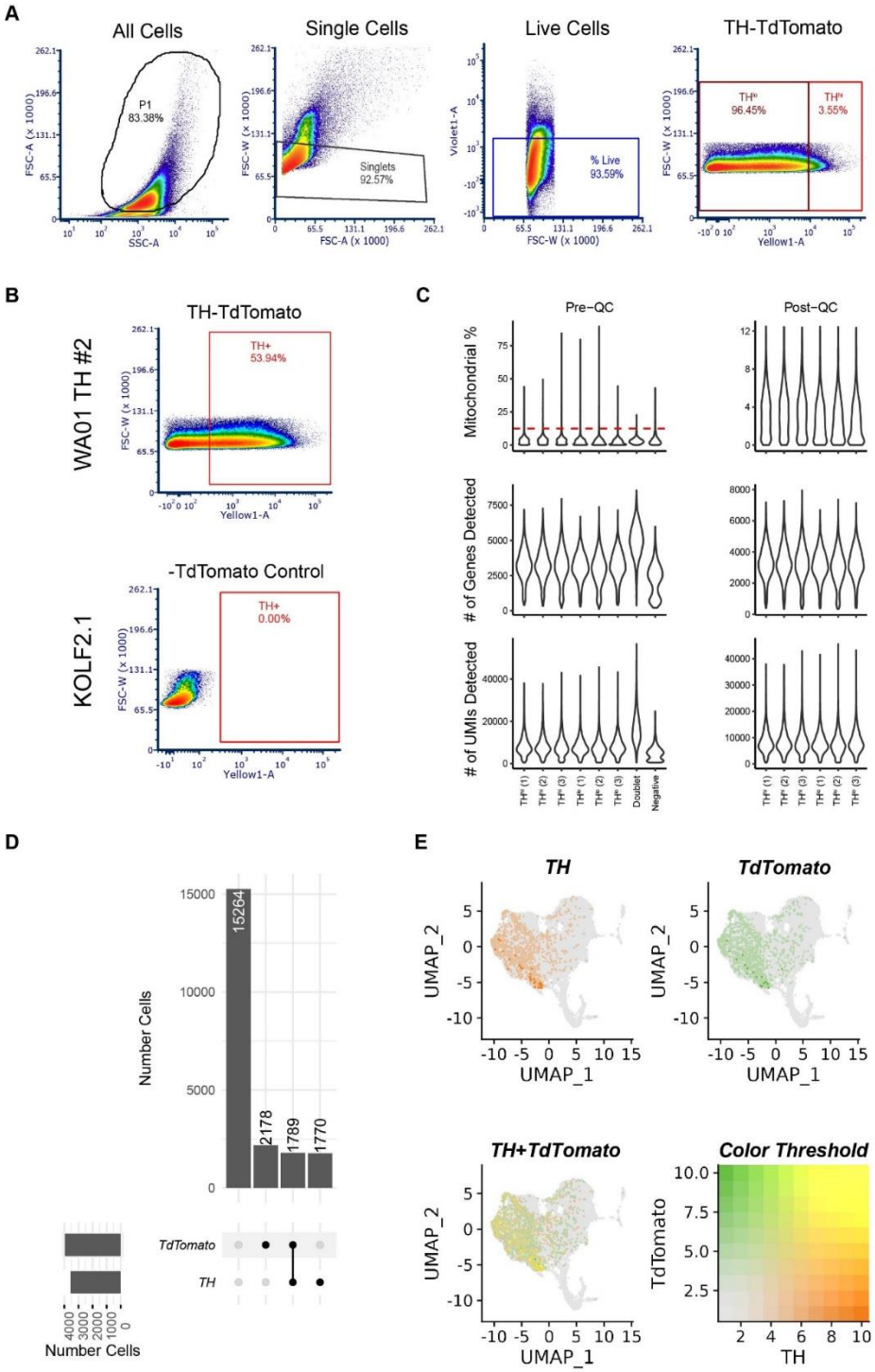

Figure S2 – Sorting and scRNA-seq QC plots for D85 hypothalamus organoids.
A) FACS gates for excluding cellular debris, doublets, and dead cells. The TH-TdTomato reporter was used to sort for two populations, TH^{hi} and TH^{lo}. FACS, fluorescence-activated cell sorting.
B) FACS gate showing the total TH^{+} population in D85 hypothalamus organoids (top) in comparison to organoids differentiated from a -TdTomato control hPSC line, KOLF2.1J (bottom).
C) Violin plots for mitochondrial percent, number of genes detected, and number of UMIs detected for each replicate before and after the QC steps. The doublets, negative droplets, droplets without detectable hashtags, and cells with high mitochondrial percent were removed at the QC stage. UMI, unique molecular identifier; QC, quality control.
D) Upset plot showing the number of cells expressing *TH*, *TdTomato*, or both in the organoid dataset.
E) UMAP plots showing the expression of *TH*, *TdTomato*, or both in the organoid dataset.
Related to Figure 3.

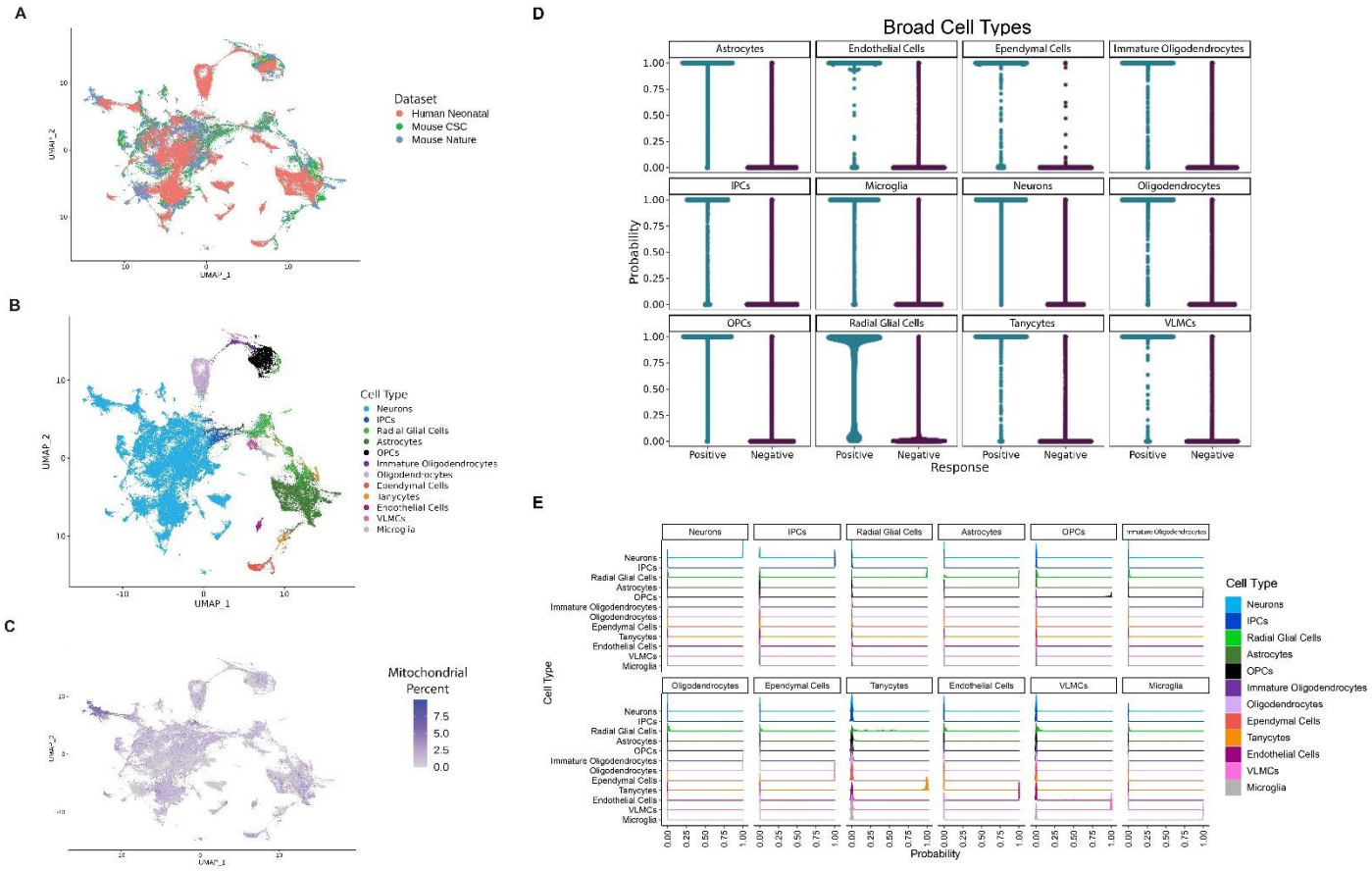

Figure S3 – Characterization and application of the reference datasets to D85 hypothalamus organoids.
A) UMAP plot showing the distribution the Human Neonatal[19], Mouse CSC[31], and Mouse Nature[30] references in the combined dataset.
B) UMAP plot of the 12 broad cell groups identified in the three references.
C) UMAP plot illustrating mitochondrial percent in the three datasets.
D) Training plots showing prediction probabilities for the 12 broad cell type categories that were applied from the three references to the hypothalamus organoid dataset to predict its cell types[19,30,31].
E) Probability density plots showing high classification confidence for each of the 12 cell types identified in hypothalamus organoids.
Related to Figure 3.

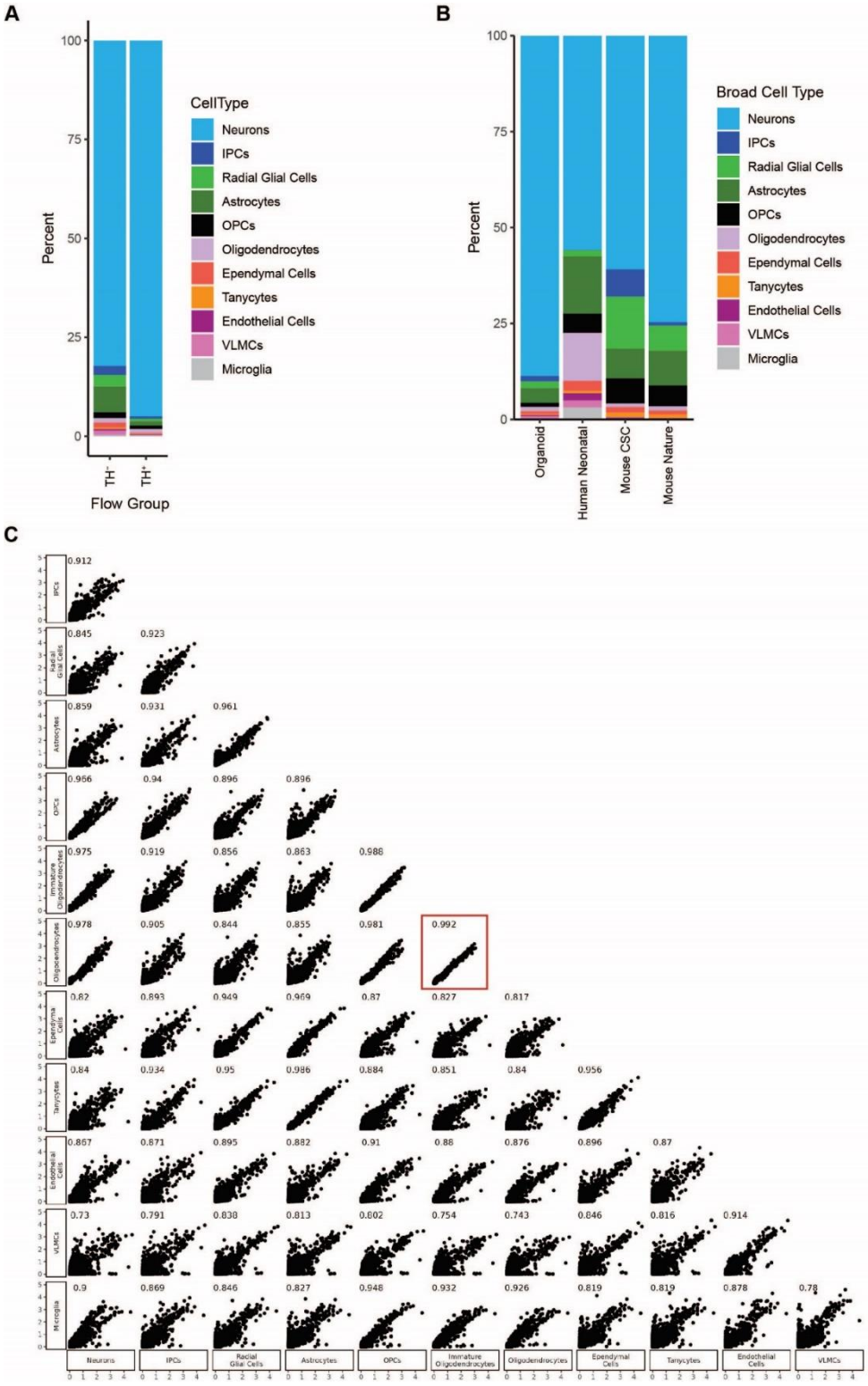

Figure S4 – Comparison of transcriptional profile and cell group distribution.
A) Bar plot comparing the percentage of each broad cell type in the hypothalamus organoids based on flow-sorted groups (TH^+ and TH^-).
B) Bar plot comparing the percentage of each broad cell type between the hypothalamus organoids and the three reference datasets used for classification[19,30,31].
C) Scatter plots illustrating the similarity correlation between different pairs of broad cell types. Red square marks the similarity between “Oligodendrocytes” and “Immature Oligodendrocytes” with a 0.992 correlation value.
Related to Figure 3.

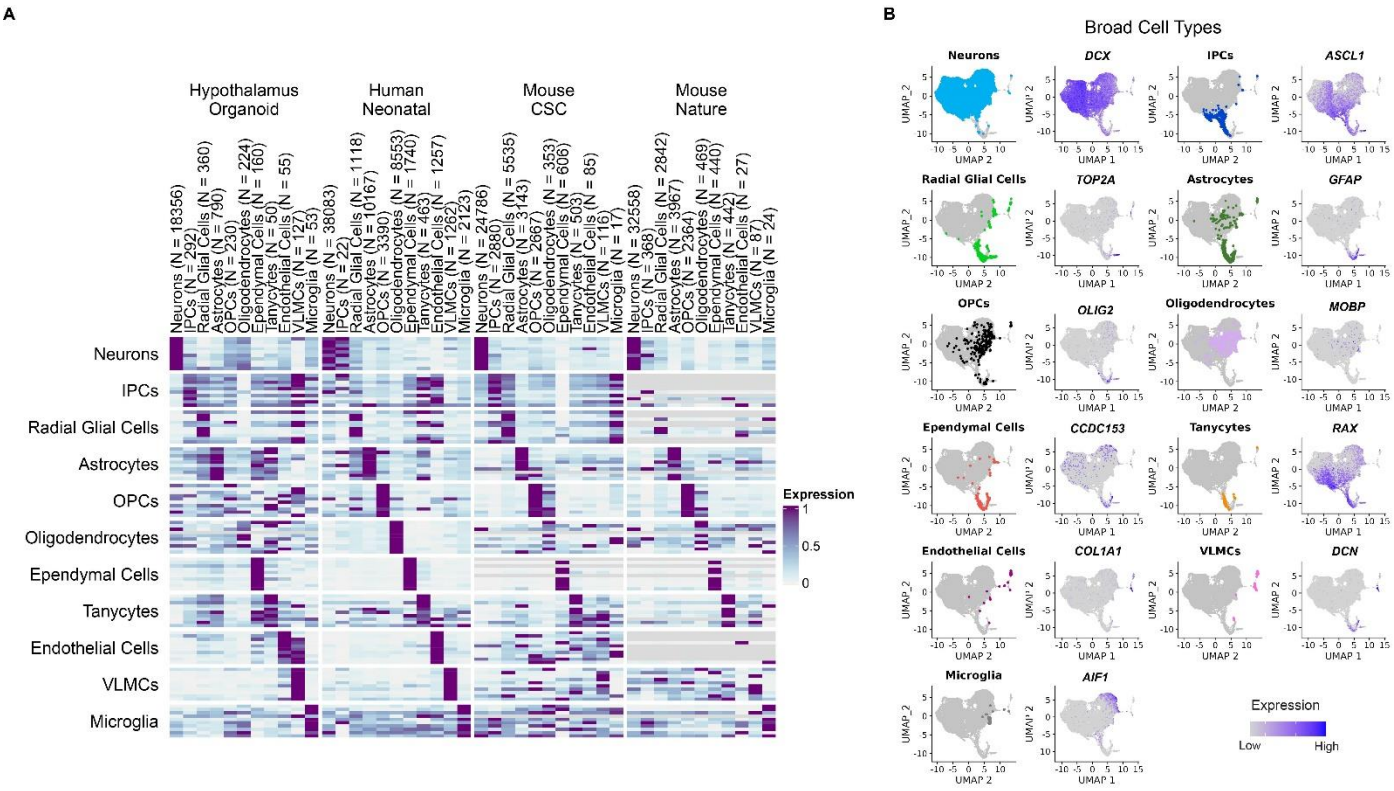

Figure S5 – Gene expression across the broad cell types.
A) The top 10 differentially expressed genes (DEGs) for each cell type were plotted for the hypothalamus organoids for comparison to the three references used for classification[19,30,31]. Grey coloring indicates genes that were not expressed in the dataset.
B) UMAP plots showing the annotated neuron subtype (columns 1 and 3) next to the expression of subtype representative markers (columns 2 and 4).
Related to Figure 3.

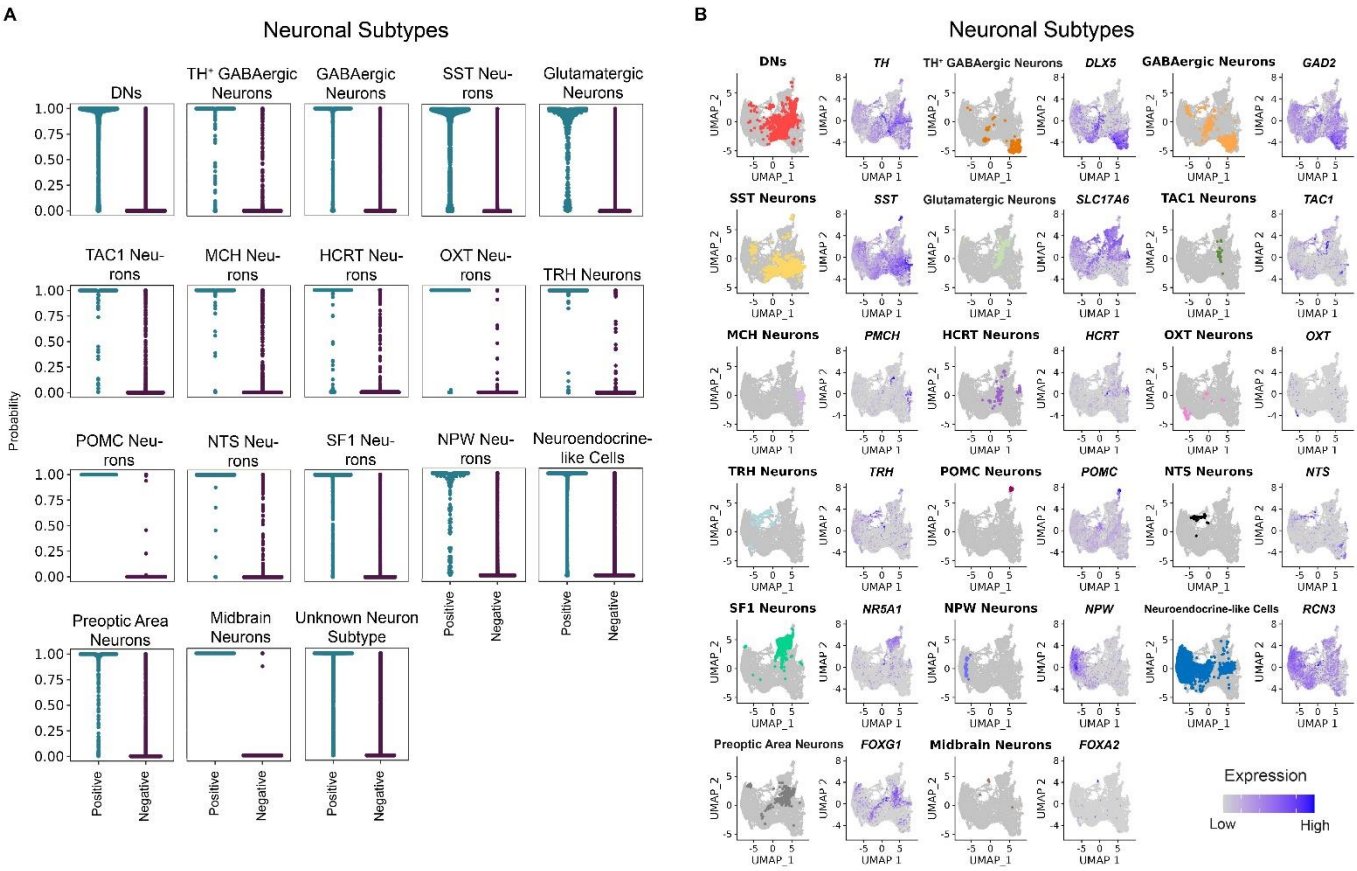

Figure S6 – Neuronal subtype categories.
A) Training plots showing prediction probabilities for the 17 neuronal subtype categories that were identified by unsupervised clustering.
B) UMAP plots showing the annotated neuron subtype (columns 1, 3, and 5) next to the expression of subtype representative markers for each subtype (columns 2, 4, and 6).
Related to Figure 3.

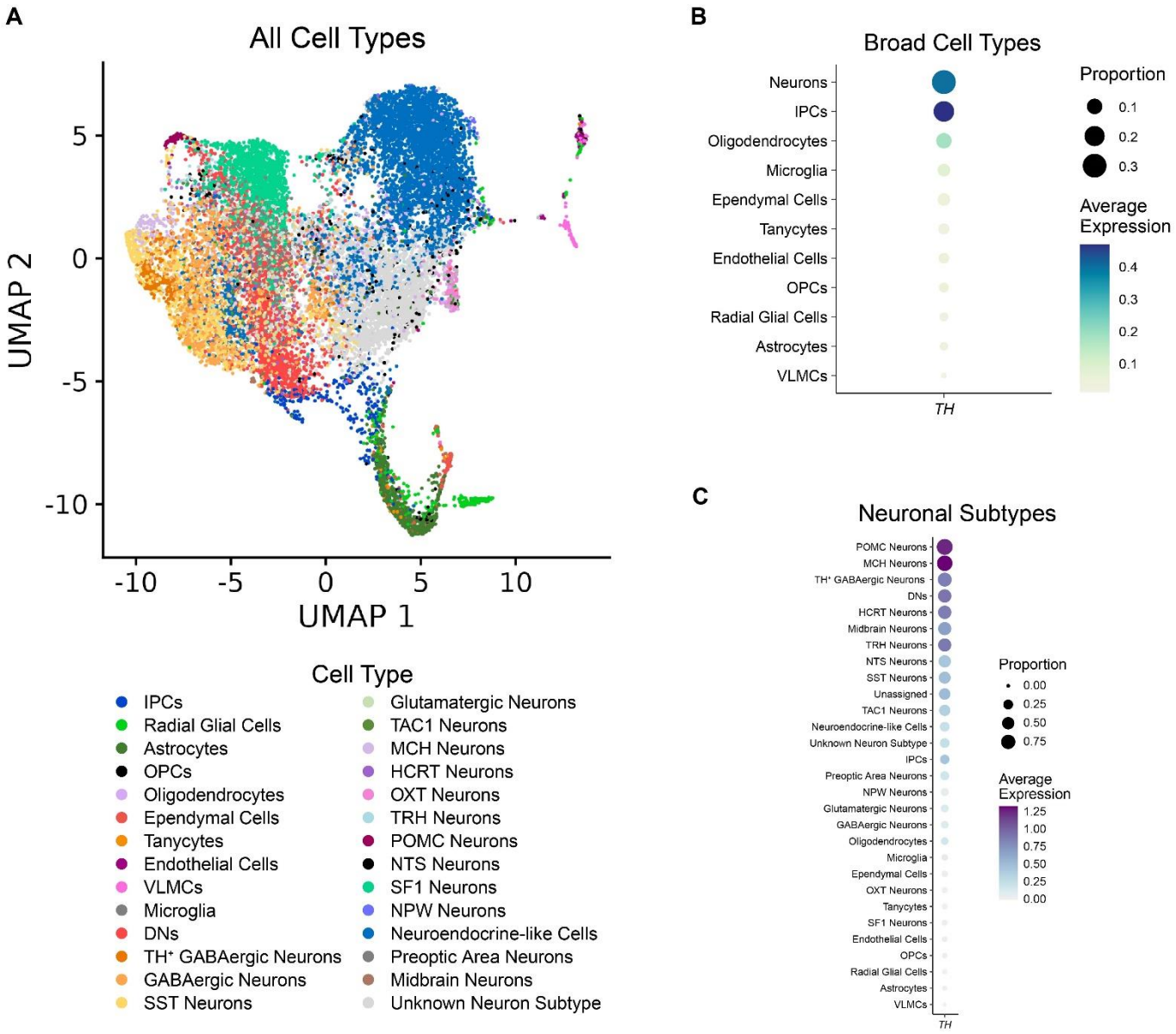

Figure S7 – Overview of all clusters and TH expression.
A) UMAP plot of all cell types consisting of the broad cell type and neuronal subtype categories in hypothalamus organoids.
B) Dot plot showing *TH* expression level and proportion of cells expressing *TH* in the broad cell types.
C) Dot plot showing *TH* expression level and proportion of cells expressing *TH* in the neuronal subtypes.
Related to Figure 3.

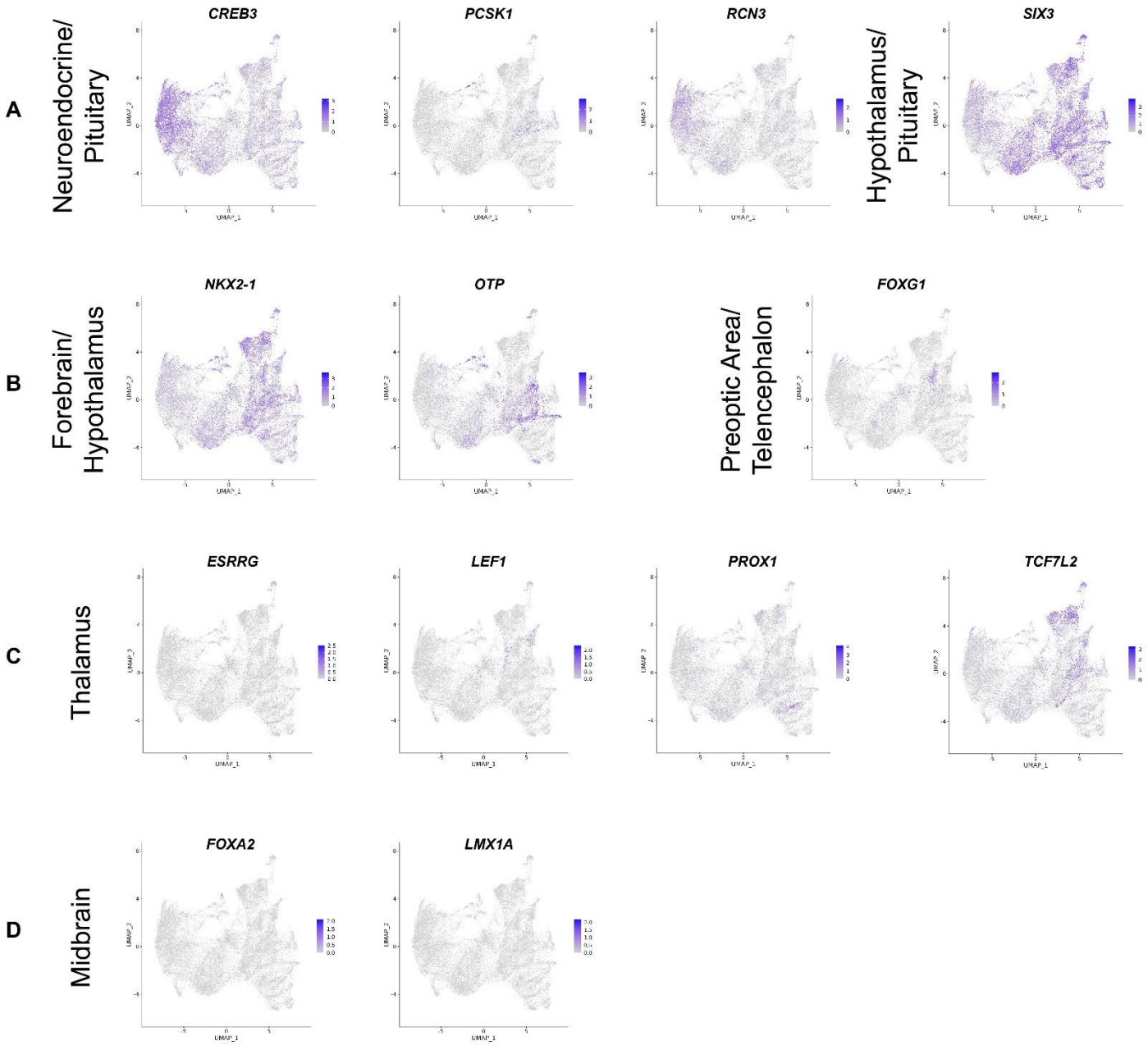

Figure S8 – Expression of cell type- and region-specific markers in hypothalamus organoids.
A) UMAP plots showing the expression of neuroendocrine and pituitary markers, as well as *SIX3* which is expressed in both the hypothalamus and pituitary cells.
B) UMAP plots showing the expression of forebrain, hypothalamus, and preoptic area/ventral telencephalon markers.
C) UMAP plots showing the expression of thalamus markers.
D) UMAP plots showing the expression of midbrain markers.
Related to Figure 3.

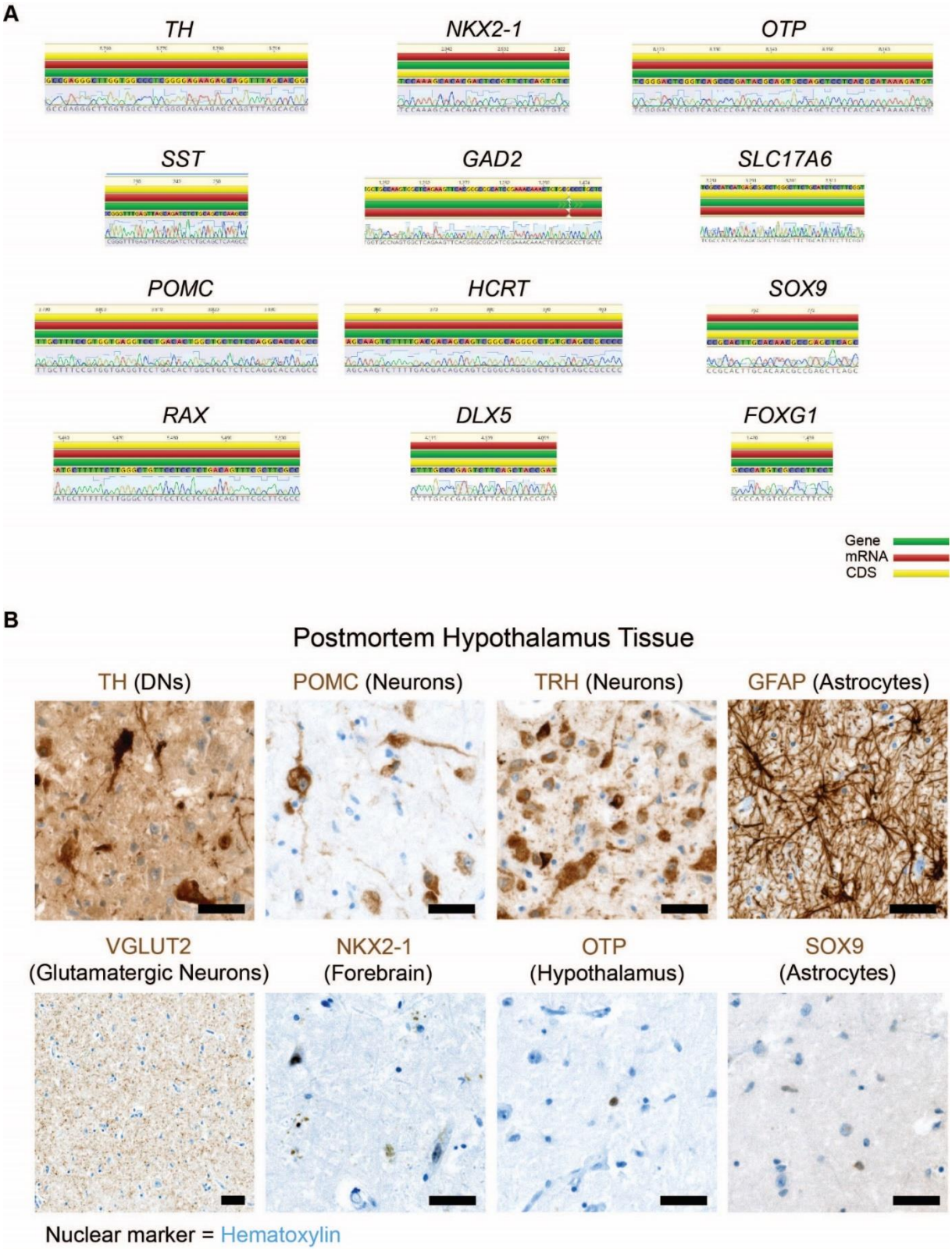

Figure S9 – Validation of qRT-PCR primers and IHC antibodies used in this study.
A) Sequencing alignments of qRT-PCR primer PCR products with the gene, mRNA, and coding sequence (CDS) of their corresponding genes, validating the primers for each marker.
B) IHC stains for cell type-specific markers in postmortem hypothalamus tissue. Hematoxylin was used as a nuclear marker in all images. Scale bars: 50 μm.
Related to Figure 4.

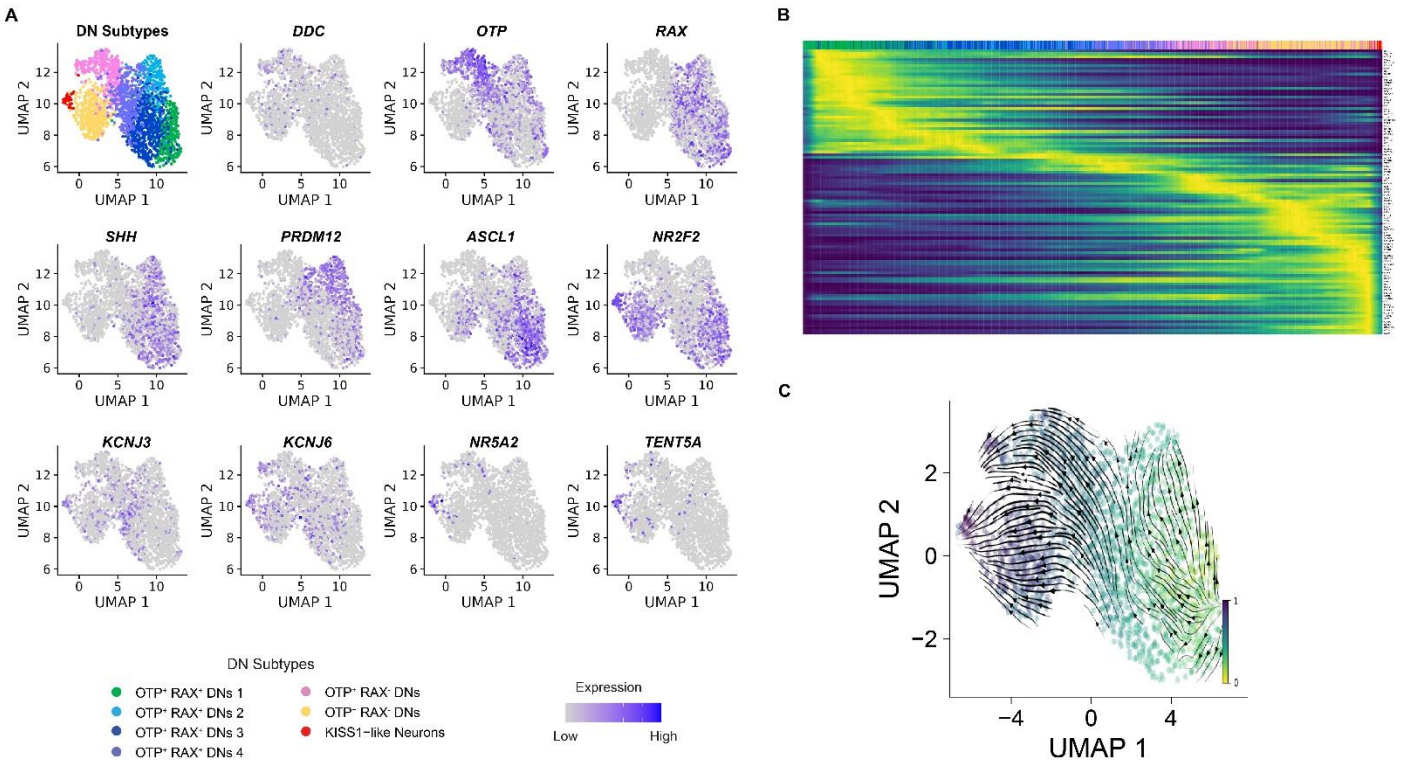

Figure S10 – Markers used for DN subtype clustering and RNA velocity analysis.
A) UMAP plots of DNs showing the expression of markers that were used to annotate the DN subtypes.
B) Top dynamic genes that were used to determine the RNA velocity pseudotime.
C) UMAP of DNs colored by latent time with pseudotime trajectory arrows overlayed.
Related to Figure 5.

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