# Expanded View Figures

**Figure EV1. Gene expression of astrocytes and ependymal cells in the diencephalon revealed by ACSA-2 MACS followed by scRNA-seq.**

A Scheme depicting dissected region (in turquoise green and dorsal part in red) from 2- to 3-mo-old Aldh1l1-eGFP mice (drawing left) and isolation by ACSA-2 magnetic-associated cell separation (schemes to the right) followed by 10×Genomics strategy.

B–E t-SNE projections of 4,651 single-cell transcriptomes with clusters colour coded and annotated post hoc based on their transcriptional profile identities (B) and showing the expression of representative astrocyte-specific marker genes (C–E; numbers reflect scaled log-normalized read counts for the specified gene for each cell, levels indicated on the right; purple: high expression, grey: low to no expression) grouped in three categories (C: relatively equal amongst clusters, D: higher in clusters 1–4, E: higher in clusters 0 and 5). Violin plot in E showing log count values of Atp1b2 expression for each cluster. Colour-coding corresponds to clusters shown in B. Cell numbers in the different clusters are: Cluster 0 = 1,042, Cluster 1 = 972, Cluster 2 = 779, Cluster 3 = 431, Cluster 4 = 414, Cluster 5 = 252, Cluster 6 = 219, Cluster 7 = 115, Cluster 8 = 85. For all differentially expressed genes per cluster of scRNA-seq data from diencephalic astrocytes after ACSA-2 MACS see Source Data for EV1B.

Source data are available online for this figure.

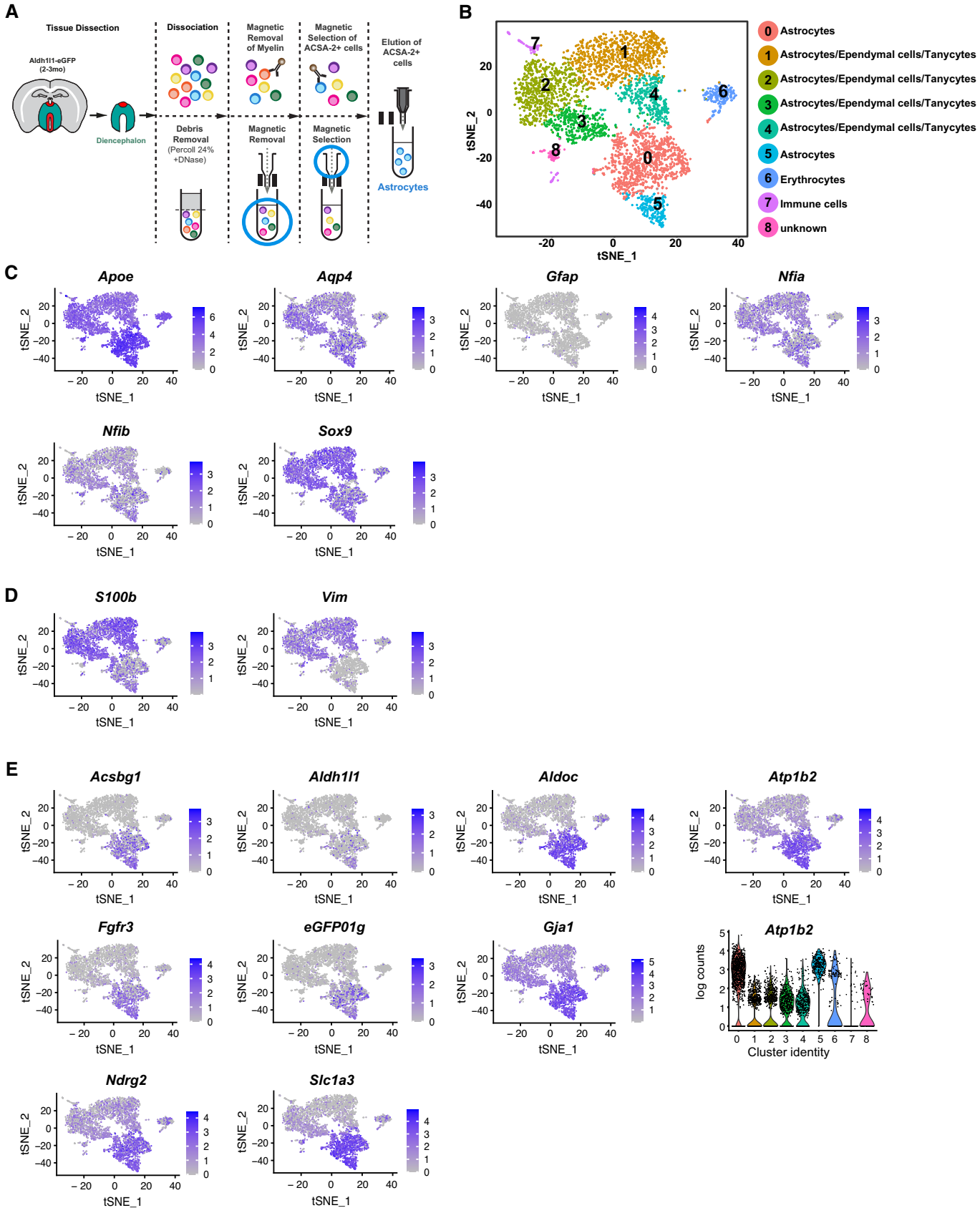


**Figure EV1.**

**Figure EV2. Expression of oligodendrocyte-, microglia-, neuron-, pericyte- and ependyma cell-specific genes in ACSA-2 sorted cells from the DIE.**

A–E t-SNE visualizations of all ACSA-2-isolated cells from the DIE (Fig EV1A) showing the expression of representative genes normally enriched in oligodendrocytes (A), microglia (B), neurons (C), pericytes (D) and ependymal cells (E). Purple represents high expression, grey low as in Fig EV1C–E.

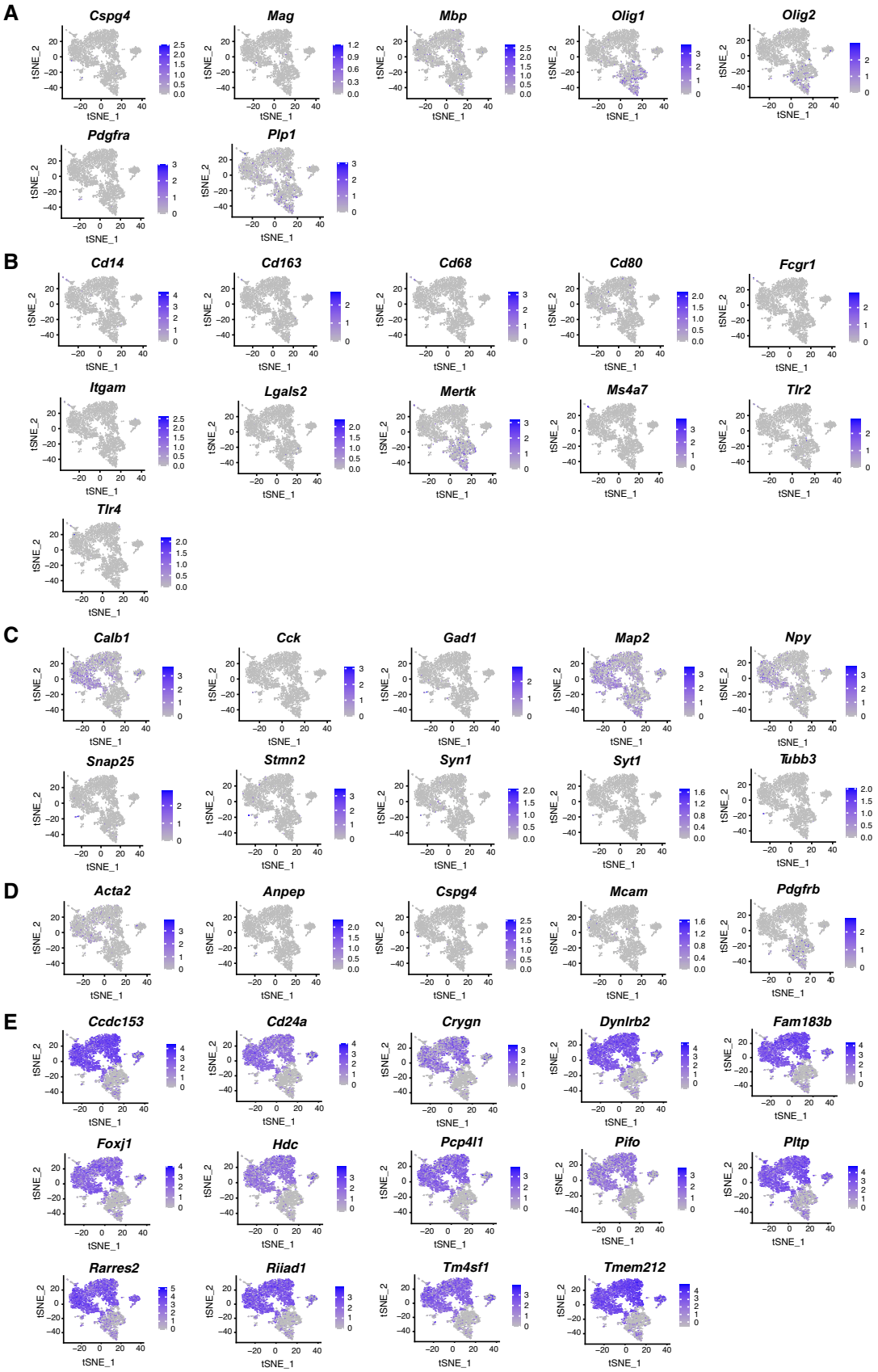


**Figure EV2.**

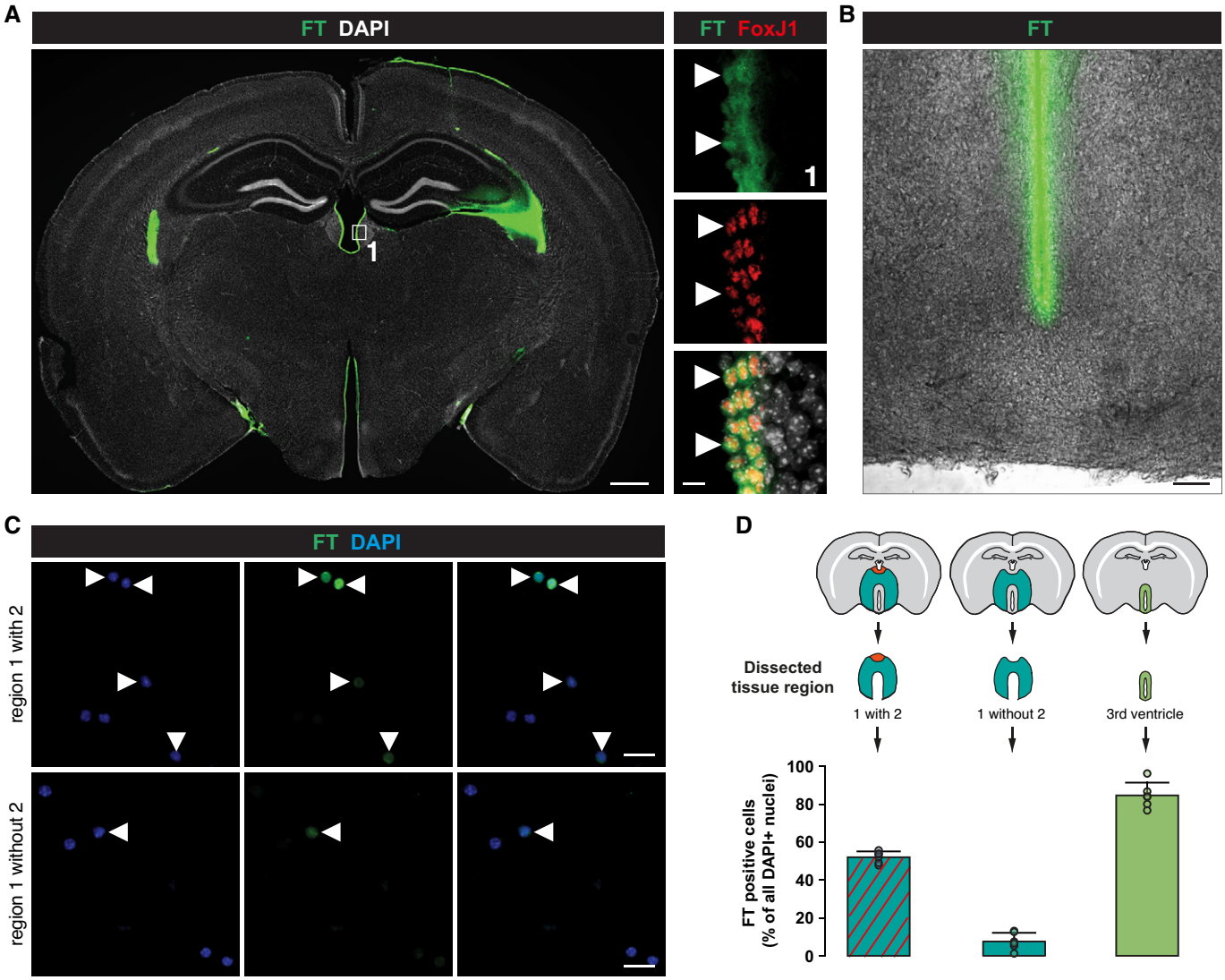


**Figure EV3. Optimization of ependymal cell removal using cell tracker/FlashTag.**

A Micrographs of coronal forebrain sections of 3-mo-old C57BL/6J mice stained as indicated showing that FlashTag (FT) labelled cells are all positive for the ependymal cell marker FoxJ1. Double-positive cells are indicated by white arrowheads. Scale bars: 500 μm (overview) and 20 μm (magnification).

B Brightfield micrograph of a coronal forebrain section of 3-mo-old C57BL/6J mice, showing that FT-positive cells are only lining around the lateral wall of the third ventricle and not penetrating within the parenchyma. Scale bar: 100 μm.

C Removing the dorsal part of the ventricle followed by ACSA-2 MACS from FT labelled 3-mo-old C57BL/6J mice resulted in very little remaining FT-positive cells (lower panel) compared to the tissue preparation where the dorsal part was included in the preparation (upper panel). FT labelled DAPI cells are highlighted by white arrowheads. Scale bars: 20 μm.

D Quantification of FT-positive cells after ACSA-2 MACS using different areas of dissection.

Data information: In D data are presented as mean ± SEM. Each dot represents one field per coverslip.

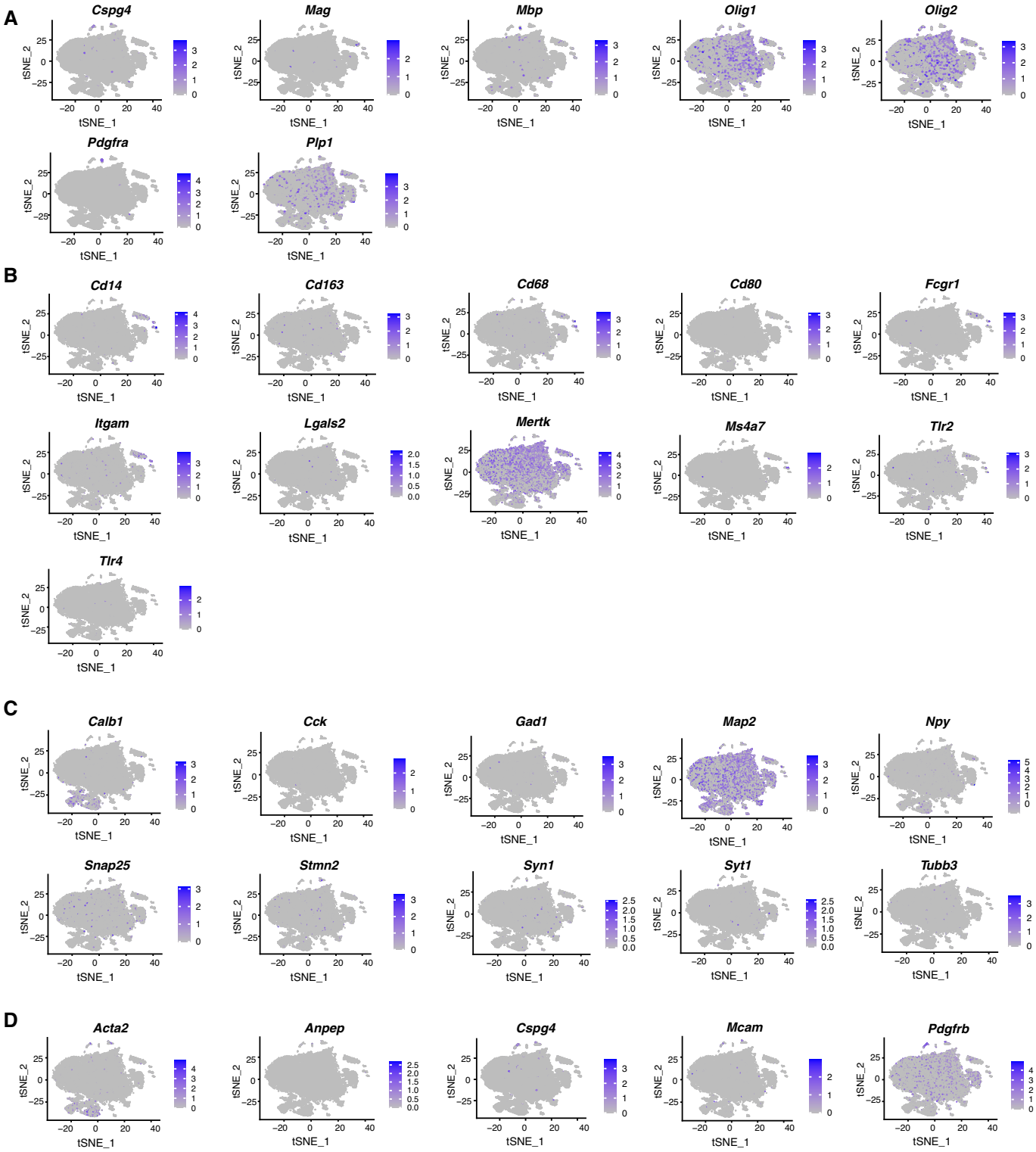


**Figure EV4. Expression of oligodendrocyte-, microglia-, neuron-, and pericyte cell-specific genes in ACSA-2 sorted DIE astrocytes using an optimized dissection.**

A–D t-SNE visualizations of all ACSA-2-isolated cells from the DIE (Fig 1A) showing the expression of representative genes normally enriched in oligodendrocytes (A), neurons (B), microglia (C), and pericytes (D). Purple represents high expression, grey low as in Fig 1C and D.

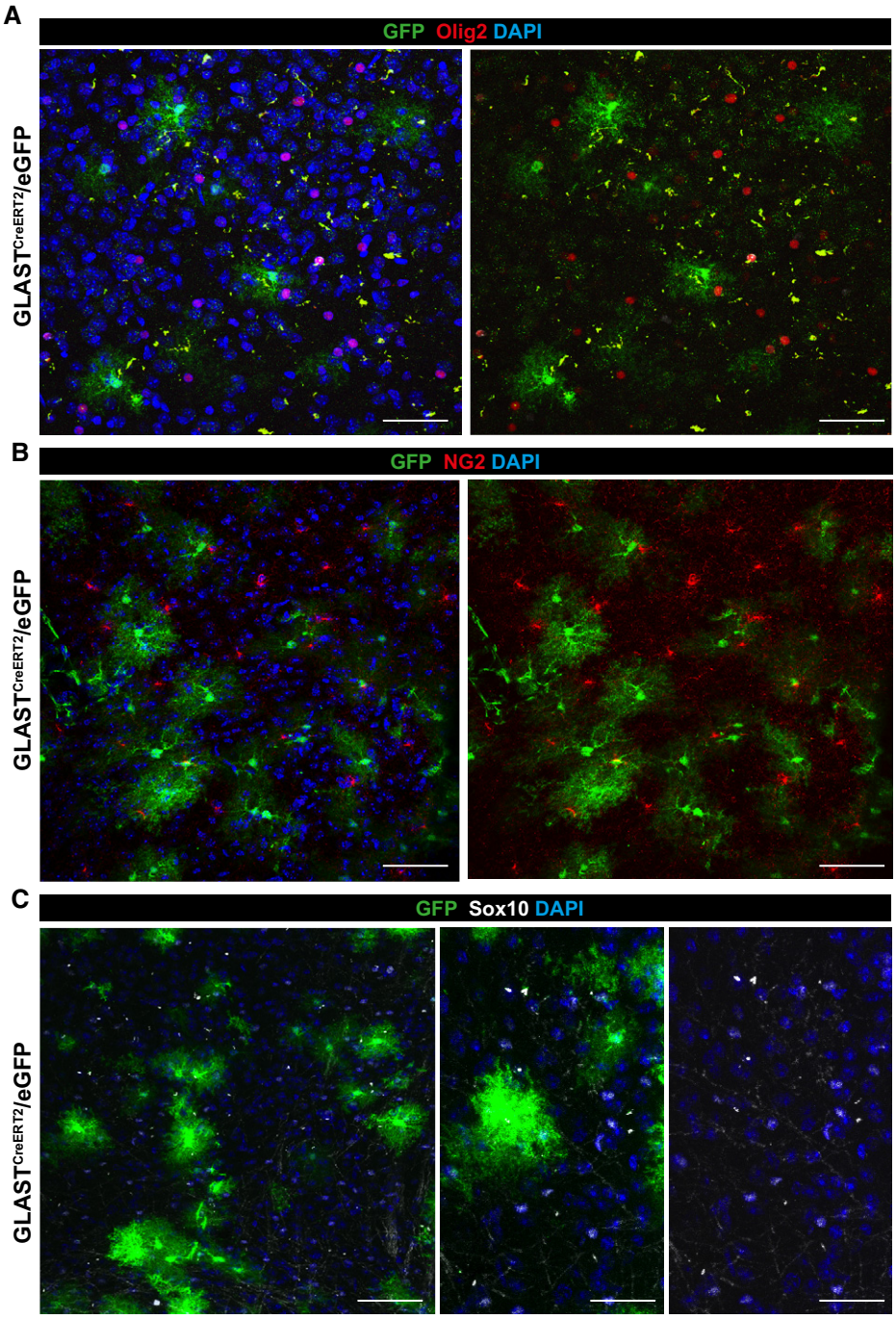


**Figure EV5. Specificity for astrocyte labelling in GLAST$^{CreERT2}$/eGFP mice.**

A–C Maximum intensity projections showing immunostainings of brain sections of 3-mo-old GLAST$^{CreERT2}$/eGFP mice for GFP and Olig2 (A) or NG2 (B) or Sox10 (C). Note that GFP$^{+}$ cell population do not comprise cells of the oligodendroglial lineage. Scale bars: 50 μm (A, middle and right panels C), 75 μm (B and left panel C).