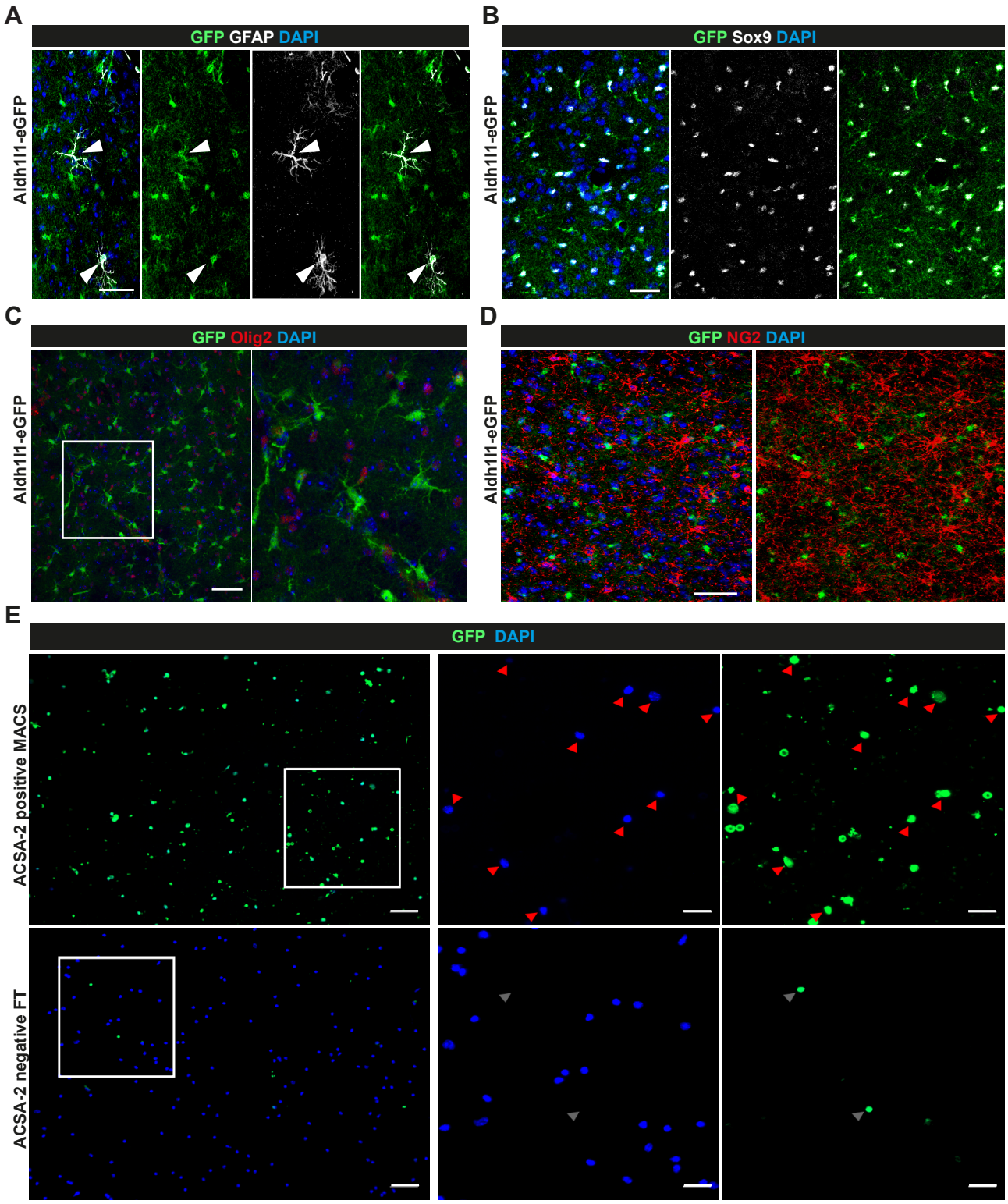
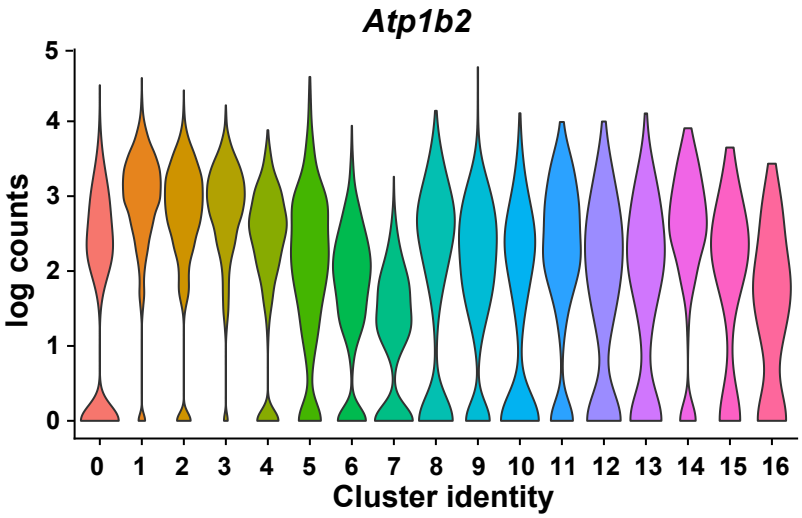
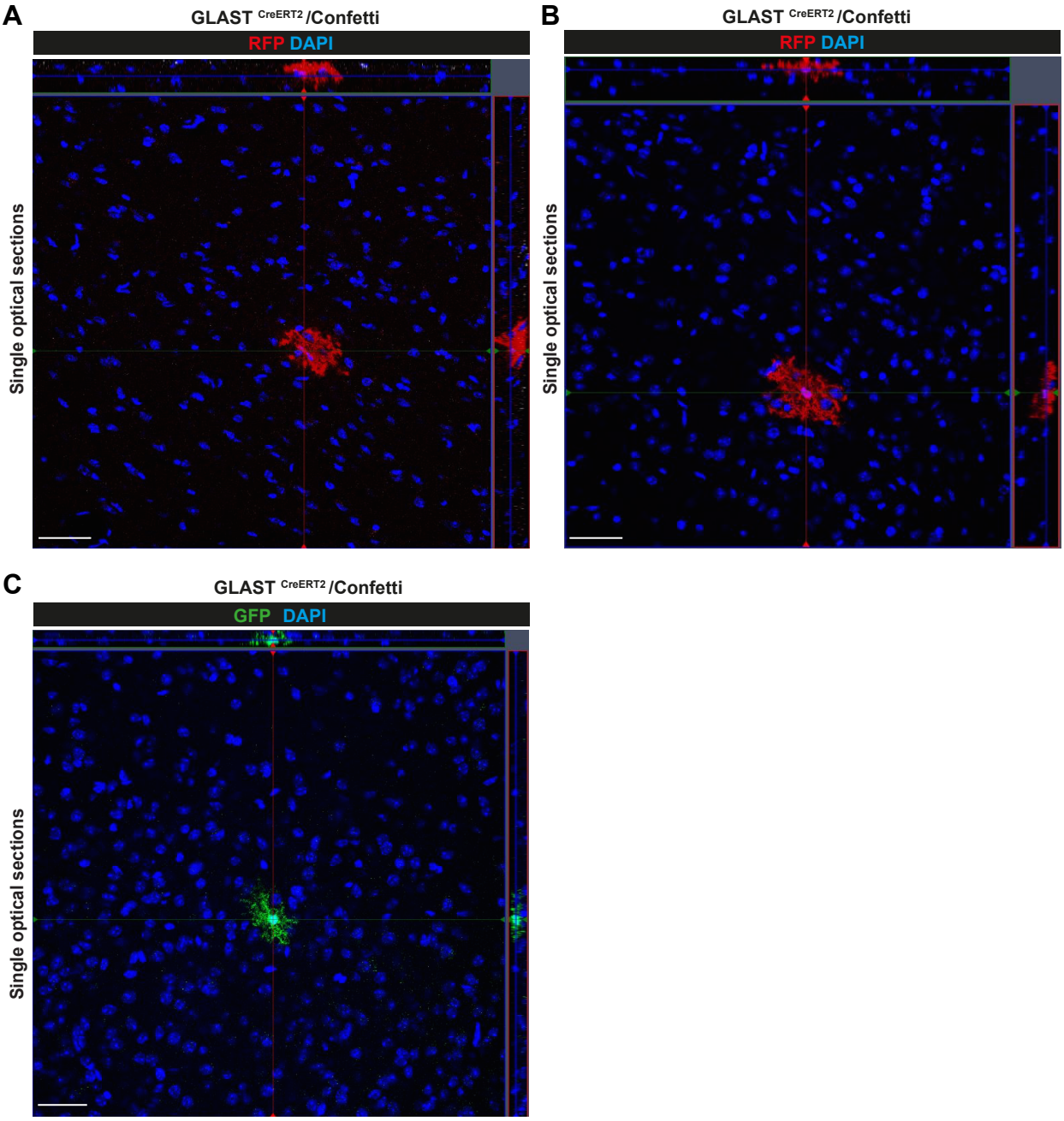
Table of contents



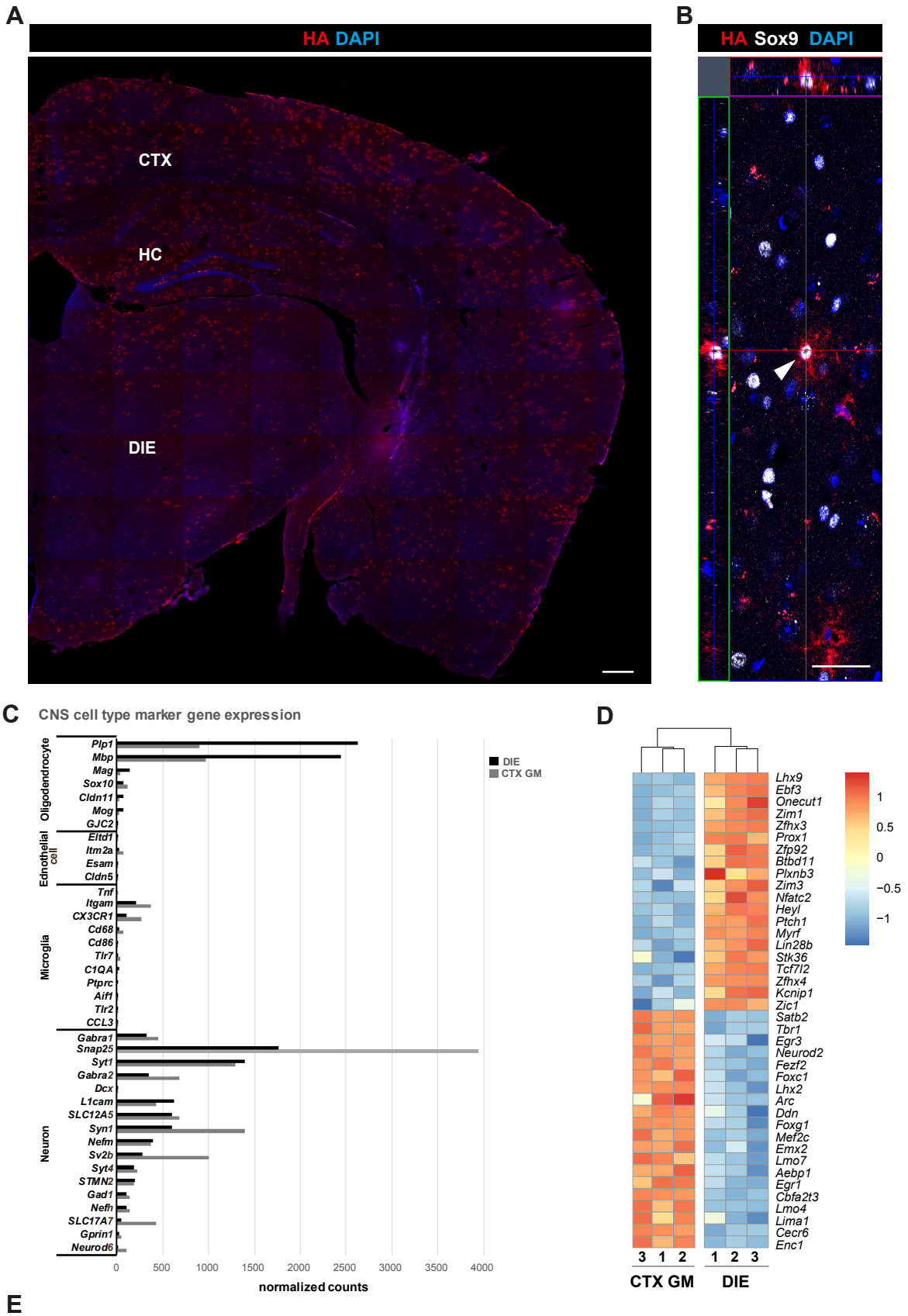
Appendix Figure S1



Appendix Figure S2



Appendix Figure S3



Appendix Figure S4

Appendix Figure Legends

Appendix Figure S1 - Specificity of astrocyte labelling in Aldh1l1-eGFP mice and by ACSA2 MACS

A-D Micrographs of forebrain sections of 3-mo-old Aldh1l1-eGFP mice stained as indicated showing that GFP^+ cells are double labeled with astrocyte markers, such as GFAP (A) and Sox9 (B), but negative for oligodendrocyte progenitor markers such as Olig2 (C) and NG2 (D). Inset in left panel C shown enlarged in the right panel. Scale bars: 50 µm (A), 75 µm (B, C, D). $GFAP^+$ GFP cells are highlighted by white arrowheads.

E MACS with ACSA-2 of DIE cells from 3-mo-old Aldh1l1-eGFP mice (Figure EV1A) resulting in virtually only GFP^+ cells amongst the ACSA-2 selected fraction (top), while virtually no GFP^+ cells were contained in the ACSA-2 negative flow through (bottom) when cells were plated after isolation and stained as indicated. Inset in left panel shown enlarged in the middle and right panels. GFP^+ DAPI cells are highlighted by red arrowheads (ACSA-2 selected fraction) or grey arrowheads (ACSA-2 negative flow through). FT = flow-through. Scale bars: 50 µm (left panels), 20 µm (middle and right panels).

Appendix Figure S2 - Distribution of Atp1b2 gene across all cell clusters

Violin plot showing scaled log normalized read counts of the Atp1b2 expression for each cluster. Color-coding corresponds to clusters shown in Figure 1B.

Appendix Figure S3 - FP labelled cells in $GLAST^{CreERT2}$/Confetti mice are mostly single astrocytes distant from each other

A-C Orthogonal projections of single optical forebrain sections stained as indicated for fluorescent proteins (FP) in astrocytes labelled in 3-mo-old $GLAST^{CreERT2}$/Confetti mice 21dpt.

Appendix Figure S4 - Characterization of Ribotag expression in $GLAST^{CreERT2}$/$RPL22^{HA}$/eGFP and RNA-seq data

A, B Micrographs of forebrain sections of 3-mo-old $GLAST^{CreERT2}$/$RPL22^{HA}$/eGFP mice 21dpt as overview stained for HA (A, B) and Sox9 (B) showing the specificity of the mouse line with HA detected only $Sox9^+$ astrocytes. Scale bars: 250 µm (A), 30 µm (B). CTX: cerebral cortex; DIE: diencephalon; HC: hippocampus.

C Gene expression profile of marker genes for cell types depicted on the left in the Ribotag-astrocyte RNA-seq data. Grey bars CTX GM samples; black bars DIE samples.

D Heatmap shows row scaled, log transformed values of normalized expression of 40 transcription factors with greatest change between conditions and mean normalized expression of at least 60. Red indicates higher and blue lower expression.

E qRT-PCR of Smad4 gene expression in ACSA-2 MACS sorted cells of the DIE vs CTX GM of 2-mo-old C57BL/6J mice (n = 3, each n consists of 3 technical replicates, ns).

Data information: In E data are presented as mean ± SEM. Each dot represents one n. (nonparametric Mann-Whitney test).