Expanded View Figures

Figure EV1. Daam2 is expressed in astrocytes, and Daam2 loss regulates morphology specifically with no difference in astrocyte number.

A Co-labeling of Daam2 (red) and mature astrocyte marker GFAP (green) with fluorescent *in situ* hybridization and immunostaining in OB (a) and cortex (b). Filled triangles indicate co-labeled cells. Scale bar: 50 μm.

B Immunostaining from Daam2$^{LacZ/+}$ animals indicates that Daam2 (LacZ^{+}, Red) is not expressed in Nestin-positive (Green) neural progenitors. SVZ: subventricular zone, RMS: rostral migratory stream. Scale bar: 100 μm.

C–F Representative images (C) and quantification (D–F) of GLAST^{+}, NFIA^{+}, and BrdU^{+} cells in Daam2 cHet and Daam2 cKO mice. Data are presented as mean ± SEM. Student's *t*-test was used for statistical comparisons. $N = 4$–17 images from $N = 3$–5 mice. Scale bar: 100 μm.

G Representative images of astrocyte with Aldh1l1-GFP reporter in hippocampus and thalamus of Daam2 cHet and cKO mice at postnatal day 28. Scale bar: 50 μm.

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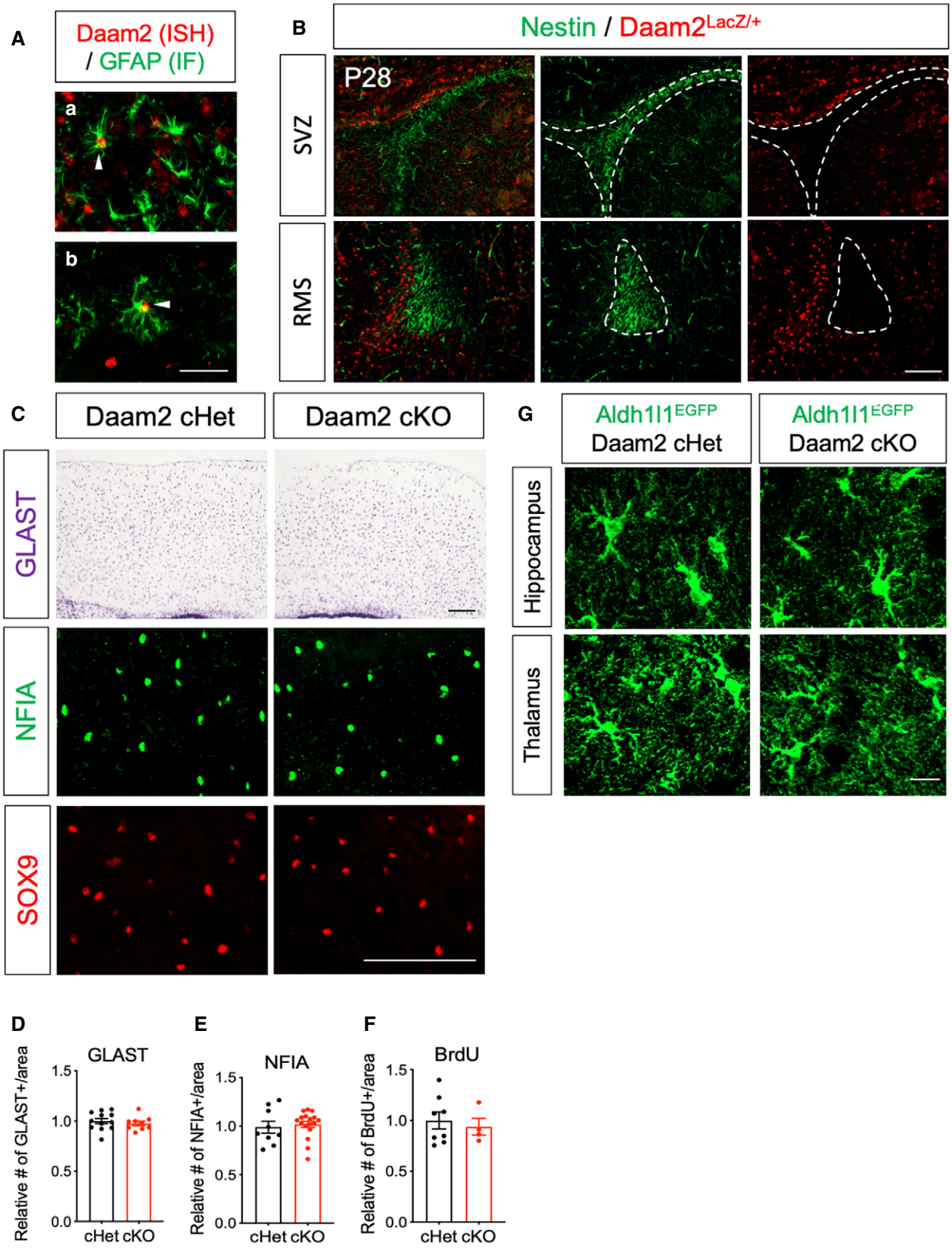
A
Daam2 (ISH)
/ GFAP (IF)
a
b
B
Nestin / Daam2LacZ/+
P28
SVZ
RMS
C
Daam2 cHet
Daam2 cKO
GLAST
NFIA
SOX9
G
Aldh1l1EGFP
Daam2 cHet
Aldh1l1EGFP
Daam2 cKO
Hippocampus
Thalamus
D
GLAST
Relative # of GLAST+/area
1.5
1.0
0.5
0.0
cHet cKO
E
NFIA
Relative # of NFIA+/area
1.5
1.0
0.5
0.0
cHet cKO
F
BrdU
Relative # of BrdU+/area
1.5
1.0
0.5
0.0
cHet cKO

Figure EV1.

Figure EV2. High-resolution imaging reveals significant morphological alterations of cortical astrocytes.

A Schematic of postnatal electroporation for labeling single astrocytes in cortex. Representative image of GFP-labeled astrocytes in cortical barrel with DAPI staining and immunostaining with pan-astrocyte marker, NFIA confirmed GFP-labeled cells as astrocytes.

B Morphology of single cortical astrocytes was visualized and evaluated in each cortical layer using postnatal electroporation of a GFP transgene under control of astrocyte-specific GLAST promoter-driven pBase. Scale bar: 20 μm.

C, D Morphological alterations in single astrocytes of Daam2 cHet and cKO mice were assessed and quantified as in Fig 1H–K. Data are presented as mean ± SEM. Total $n = 13$–14 cells from $N = 5$ mice per genotype were used for analysis. Two-way ANOVA (C) and Student's t-test (D) are used for statistics. $^{*}P < 0.05$, $^{**}P < 0.01$.

E, F Representative images (E) and quantification (F) of 3D volume analysis in astrocytes of Daam2 cHet and cKO mice. Scale bar: 20 μm. (F) Data are presented as mean ± SEM. Total $n = 12$–23 cells from $N = 5$ mice per genotype were used for analysis. Two-way ANOVA is used for statistics. $^{*}P < 0.05$, $^{****}P < 0.0001$.

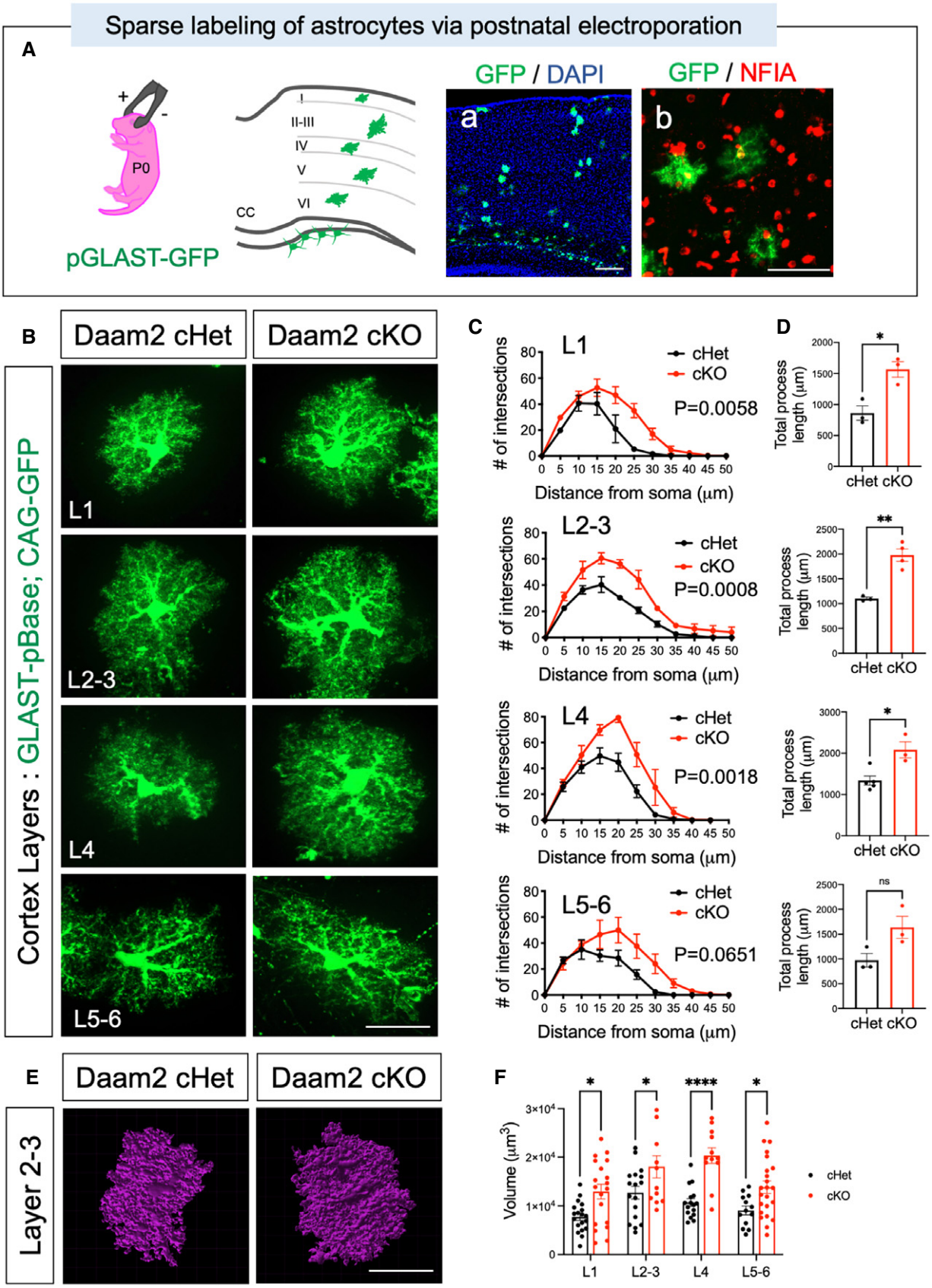
Sparse labeling of astrocytes via postnatal electroporation
A
P0
pGLAST-GFP
I
II-III
IV
V
VI
CC
GFP / DAPI
GFP / NFIA
a
b
B
Daam2 cHet
Daam2 cKO
Cortex Layers : GLAST-pBase; CAG-GFP
L1
L2-3
L4
L5-6
C
L1
L2-3
L4
L5-6
cHet
cKO
P=0.0058
P=0.0008
P=0.0018
P=0.0651
# of intersections
Distance from soma (μm)
D
Total process length (μm)
cHet cKO
ns
E
Daam2 cHet
Daam2 cKO
Layer 2-3
F
Volume (μm³)
cHet
cKO

Figure EV2.

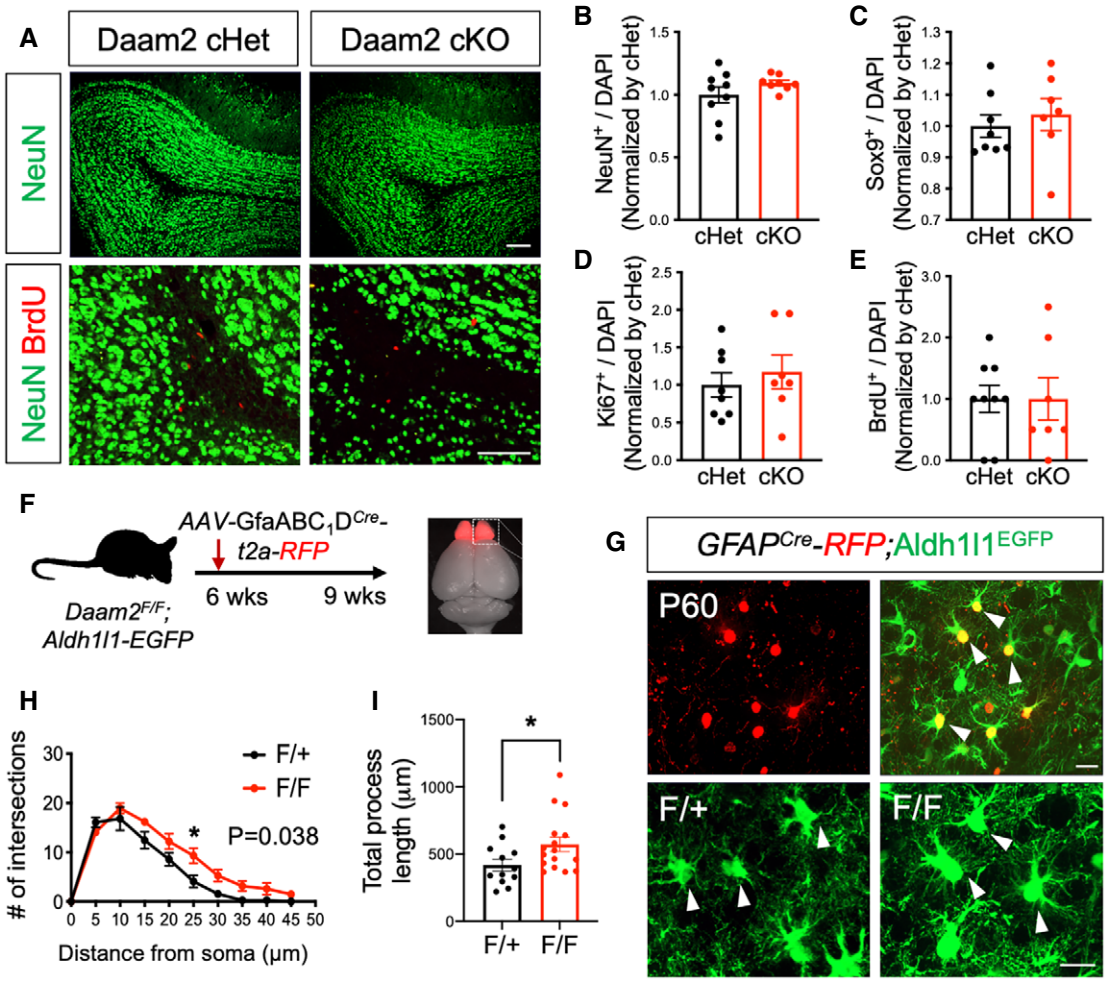

Figure EV3. Temporal loss of Daam2 in adult OB exhibits increased morphological complexity without altering neurogenesis.

A Representative images of NeuN and BrdU staining in OB of Daam2 cHet and cKO mice. Scale bar: 100 μm.

B–E No significant differences were found in numbers of $NeuN^+$, $Sox9^+$, $BrdU^+$, and $Ki67^+$ cells in OB of Daam2 cHet and Daam2 cKO mice. Data are presented as mean ± SEM. Student's *t*-test was used for statistical comparisons. $n = 7$–9 images from $N = 3$ mice per each genotype.

F To visualize morphology of OB astrocytes after development, AAV-GfaABC$_1$D-Cre-t2a-RFP was injected into 6-week-old $Daam2^{F/+}$ and $Daam2^{F/F}$ mice with Aldh1l1-EGFP reporter and analyzed three weeks later.

G–I Virus-infected, RFP-expressing, and Aldh1l1-EGFP co-labeled astrocytes (white triangles) were imaged and analyzed for their morphology as in Fig 1. Data are presented as mean ± SEM. $n = 12, 16$ cells, $N = 3$ mice per genotype. Two-way ANOVA (H) and Student's *t*-test (I) were used for statistical comparisons. $^*P < 0.05$. Scale bar: 50 μm.

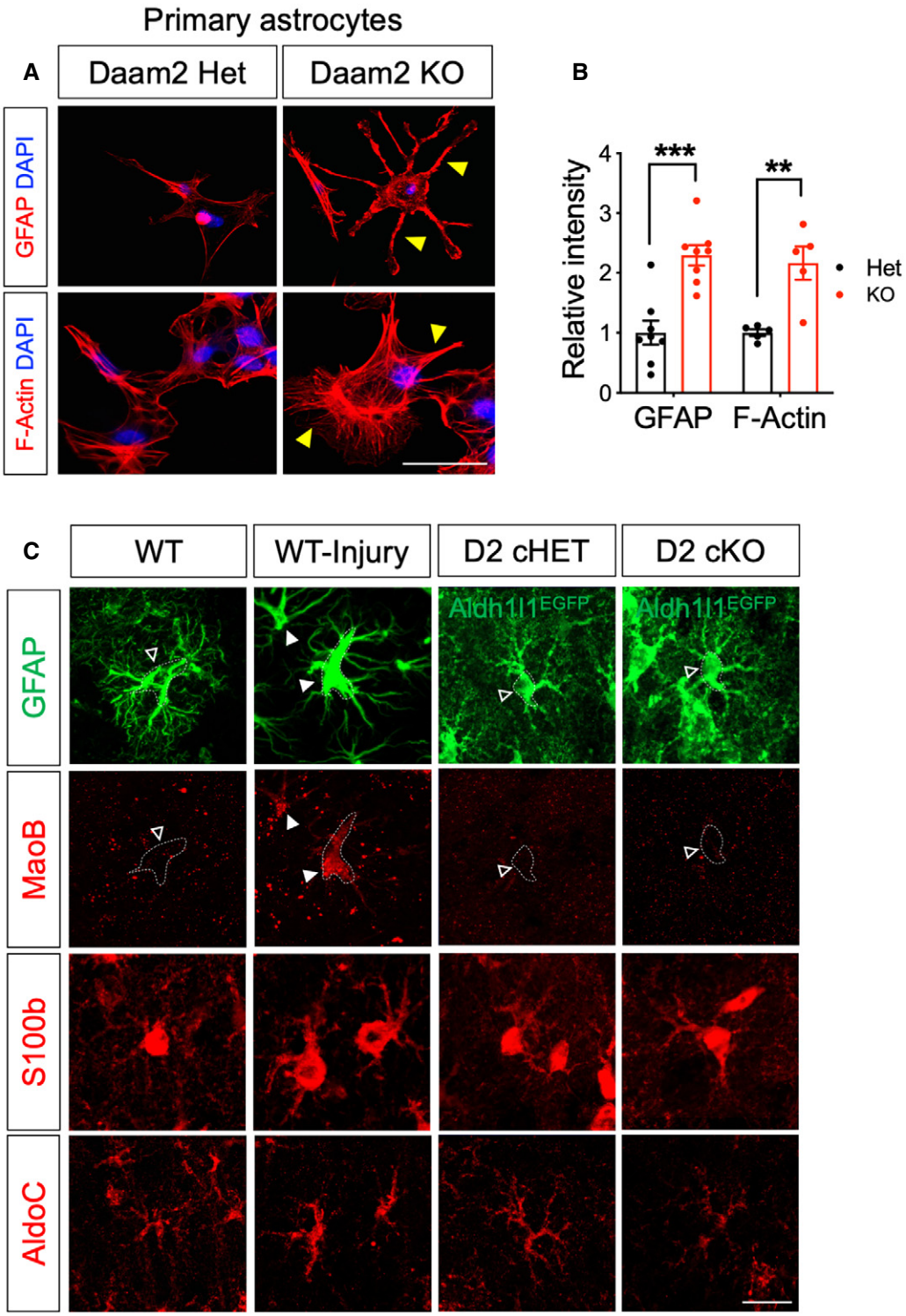

Figure EV4. Daam2-deficient astrocytes display increased GFAP *in vitro* and normal level of MaoB, S100b, and AldoC expression *in vivo*.

A, B Representative images and quantification of immunostaining of GFAP and F-actin in cultured primary astrocyte of Daam2 Het and KO mice. Yellow triangles indicate increased astrocytic processes in Daam2 KO astrocytes. Scale bar: 20 μm. Experiments are repeated as triplicate, and data are presented as mean ± SEM. Student's *t*-test was used, $^{**}P < 0.01$, $^{***}P < 0.001$.

C Representative images of immunostaining with reactive astrocyte markers. Photothrombotic stroke was induced in the ipsilateral cortex of wild-type mice and used as a positive control for reactive gliosis. Dotted outline indicates astrocyte cell body labeled with GFAP or Aldh1l1-EGFP. Filled triangles and empty triangles indicate increased expression and baseline level of expression or no changes in MaoB staining, respectively. Scale bar: 20 μm.