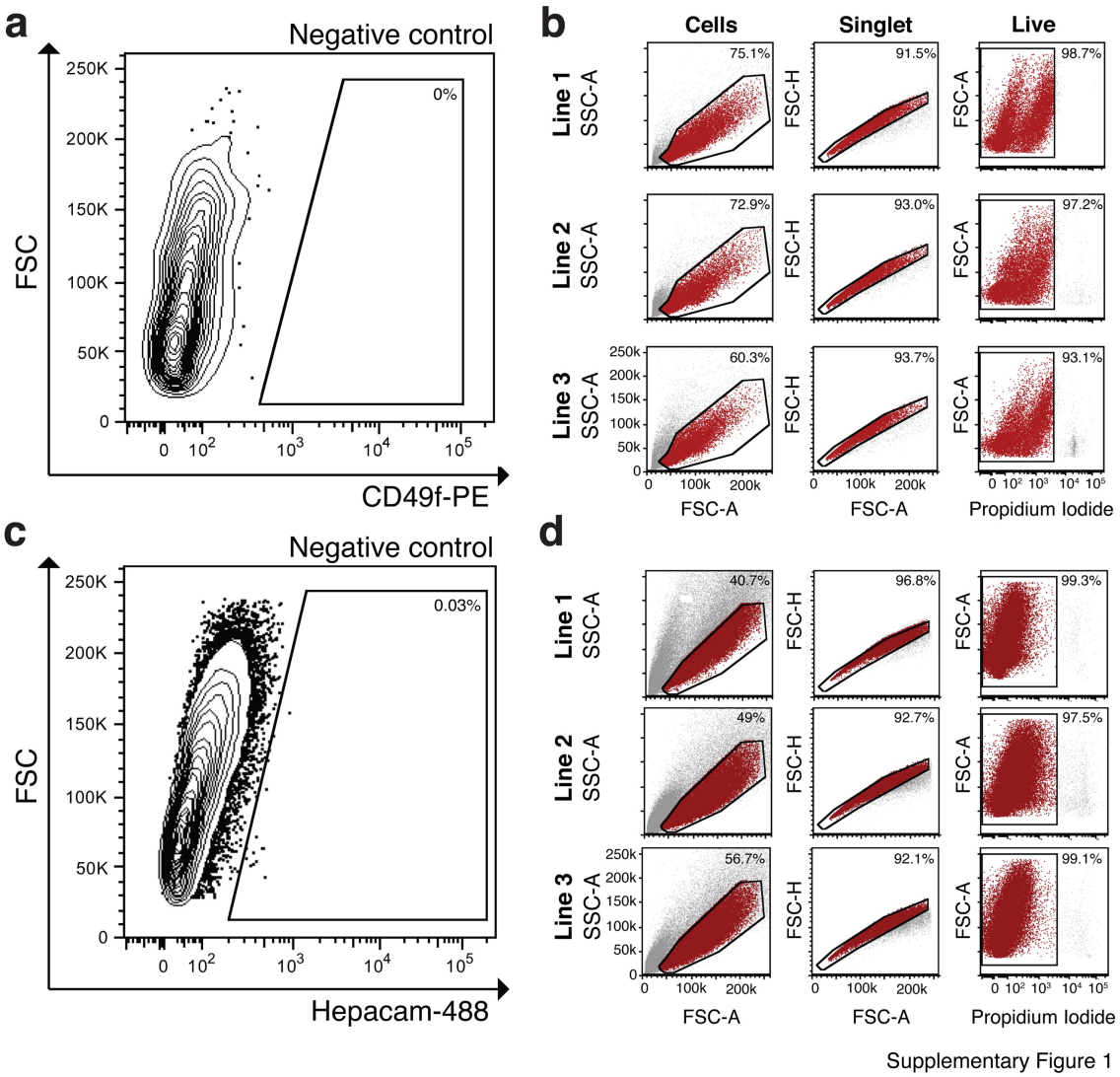


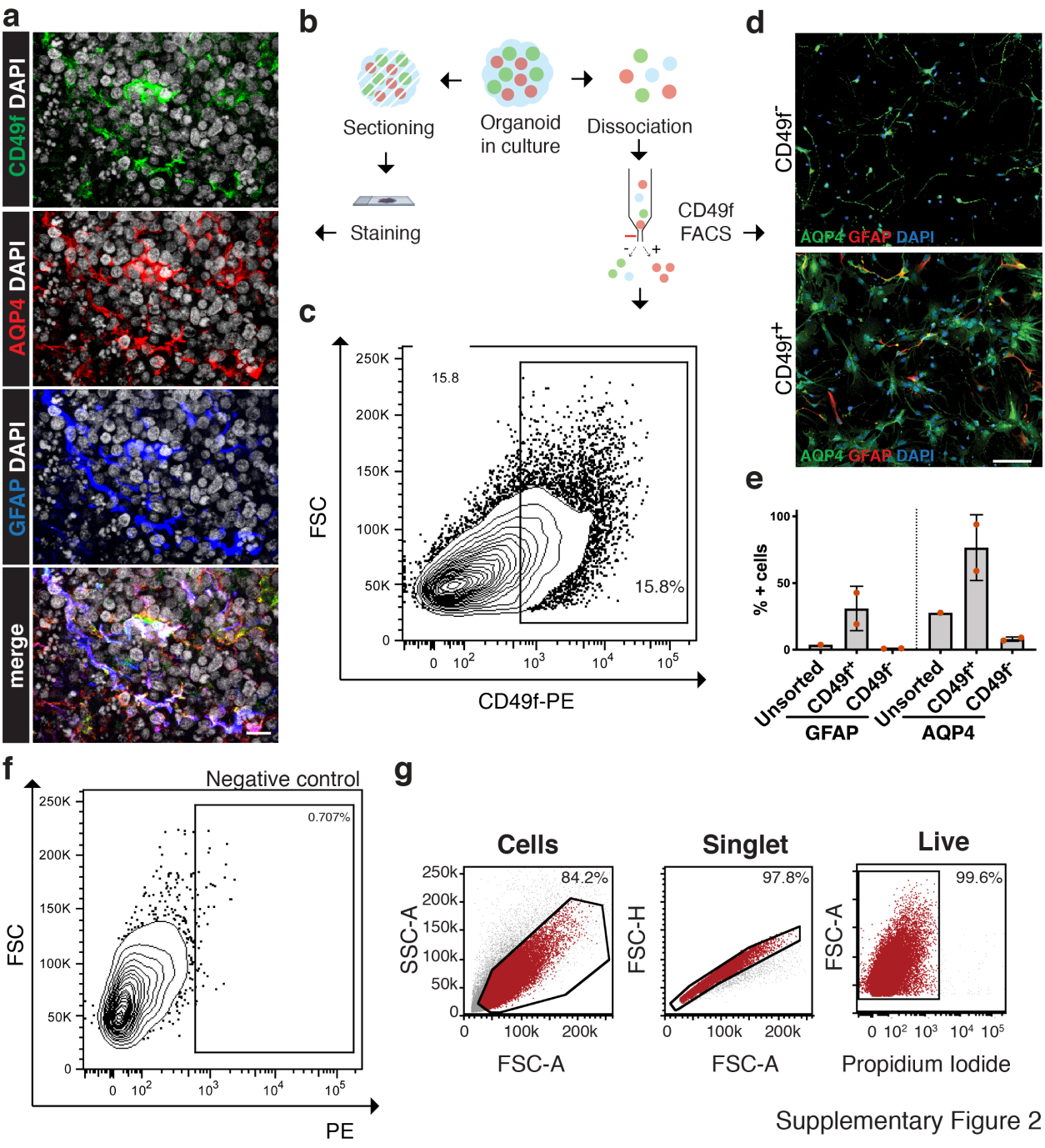
Figure S1: Gating strategies for CD49f FACS (Related to Fig. 1)

a) Representative flow-cytometry contour plot (with outliers shown) of the PI-stained negative control for the CD49f sort from mixed cultures.

b) Plots for side scatter and forward scatter, duplets exclusions and live gates depicting the gating strategy for the CD49f sort from mixed cultures for 3 lines.

c) Representative flow-cytometry contour plot (with outliers shown) of the PI-stained negative control for the HepaCAM sort.

d) Plots for side scatter and forward scatter, duplets exclusions and live gates depicting the gating strategy for the HepaCAM sort.



Supplementary Figure 2

Figure S2: CD49f allows astrocyte isolation from cortical organoids (Related to Fig. 1)

a) Representative immunofluorescence images of CD49f$^+$ (green), AQP4$^+$ (red), and GFAP$^+$ (blue) cells with DAPI nuclei (grey), showing that astrocytes are CD49f-positive in cryosections of hiPSC-derived oligocortical organoids. Scale bar, 10µm.

b) Schematic of experimental design depicting astrocyte immunostaining and isolation from oligocortical organoids.

c) Representative flow-cytometry contour plot (with outliers shown) of the CD49f sort from hiPSC-oligocortical organoids.

d) Representative immunofluorescence images of $GFAP^+$ (red), $AQP4^+$ (green), with DAPI nuclei (blue) in the $CD49f^+$ and $CD49f^-$ fractions of the sort from oligocortical organoids, showing that astrocytes are enriched in the $CD49f^+$ fraction. Scale bar, 100µm.

e) Percentages of $GFAP^+$ cells and $AQP4^+$ cells in the unsorted, $CD49f^+$, and $CD49f^-$ fractions of the sort, showing that $AQP4^+$ and $GFAP^+$ astrocytes are enriched in the $CD49f^+$ fraction of the sort. Error bars show mean ± standard deviation (n=1-2 independent differentiations).

f) Representative flow-cytometry contour plot (with outliers shown) of the PI-stained negative control for the CD49f sort from dissociated oligocortical organoids.

g) Plots for side scatter and forward scatter, duplets exclusions and live gates depicting the gating strategy for the CD49f sort dissociated oligocortical organoids.

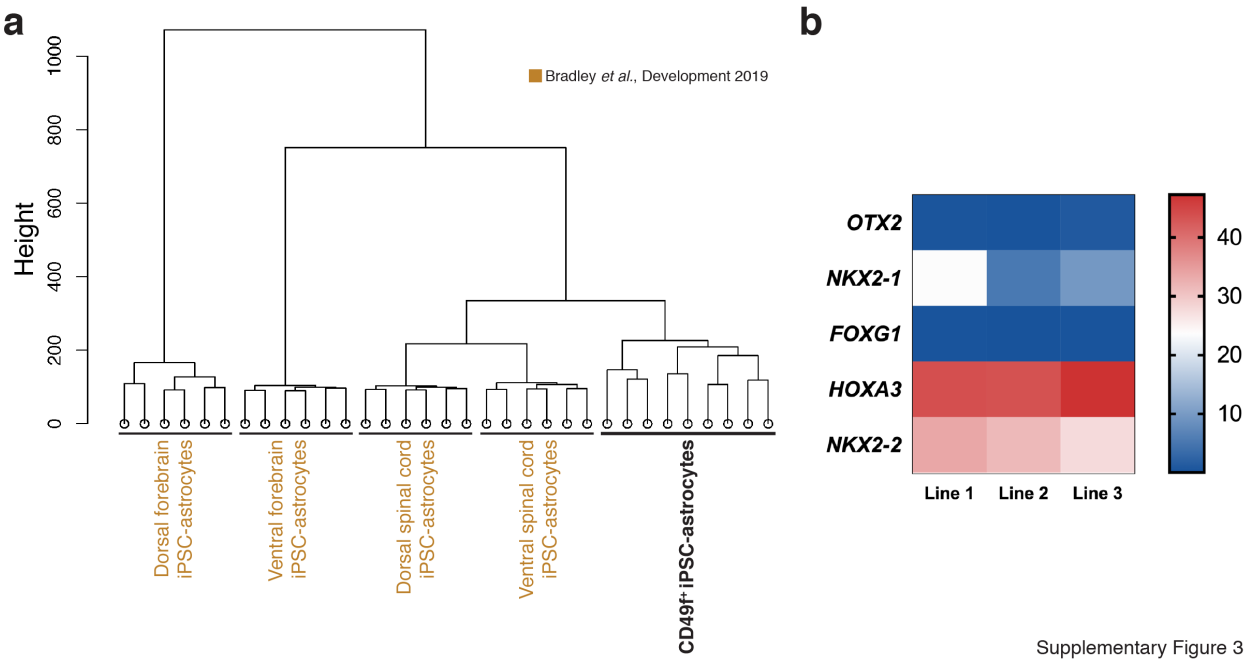
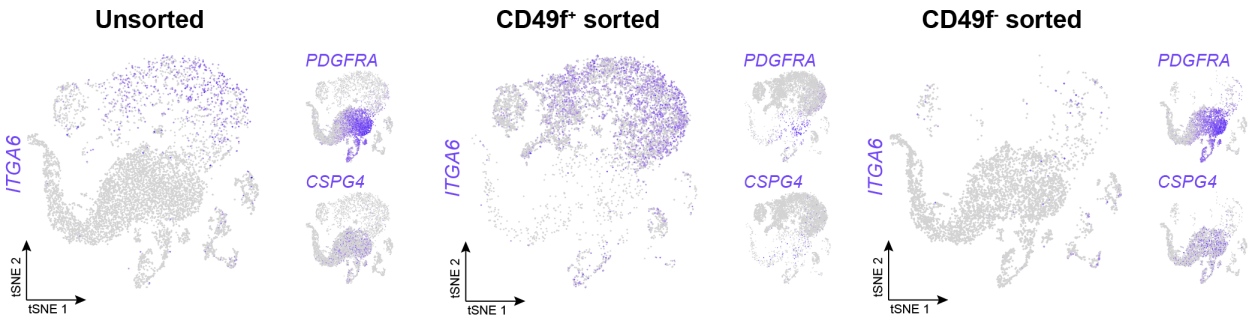


Figure S3: CD49f⁺ hiPSC-astrocytes transcriptionally resemble ventral spinal cord astrocytes (Related to Fig. 2)

a) Hierarchical clustering of RNA-Seq data showing that CD49f⁺ iPSC-astrocytes (black) cluster closely with spinal cord-patterned iPSC-derived astrocytes from an independent study and away from forebrain-patterned astrocytes (GEO: GSE133489 in brown). Analysis is based on transcriptome-wide expression. CD49f⁺ samples consist of 3 different lines and 3 technical replicates per line.

b) Heat map of regional transcripts from RNA-seq data (*OTX2,* forebrain; *NKX2-1*, ventral forebrain; *FOXG1*, dorsal forebrain; *HOXA3*, spinal cord; *NKX2-2*, ventral spinal cord), as transcripts per million in CD49f⁺ hiPSC-astrocytes. The expression of ventral spinal cord markers is consistent with sonic hedgehog and retinoic acid patterning, used in our differentiation protocol. Samples consist of 3 lines and three technical replicates per line.



Supplementary Figure 4

Figure S4: CD49f^{+} hiPSC-astrocytes do not express oligodendrocyte progenitor cell markers (Related to Fig. 3)

Cells expressing oligodendrocyte progenitor cell markers *PDGFRA* and *CSPG4* are enriched in the CD49f^{-} sorted fraction and do not overlap with *ITGA6* expression. tSNE feature plots of CD49f (*ITGA6*) and OPC (*PDGFRA, CSPG4*) transcripts from unsorted, CD49f^{+} sorted, and CD49f^{-} sorted cells. All data from one control line (line 3, n=1).

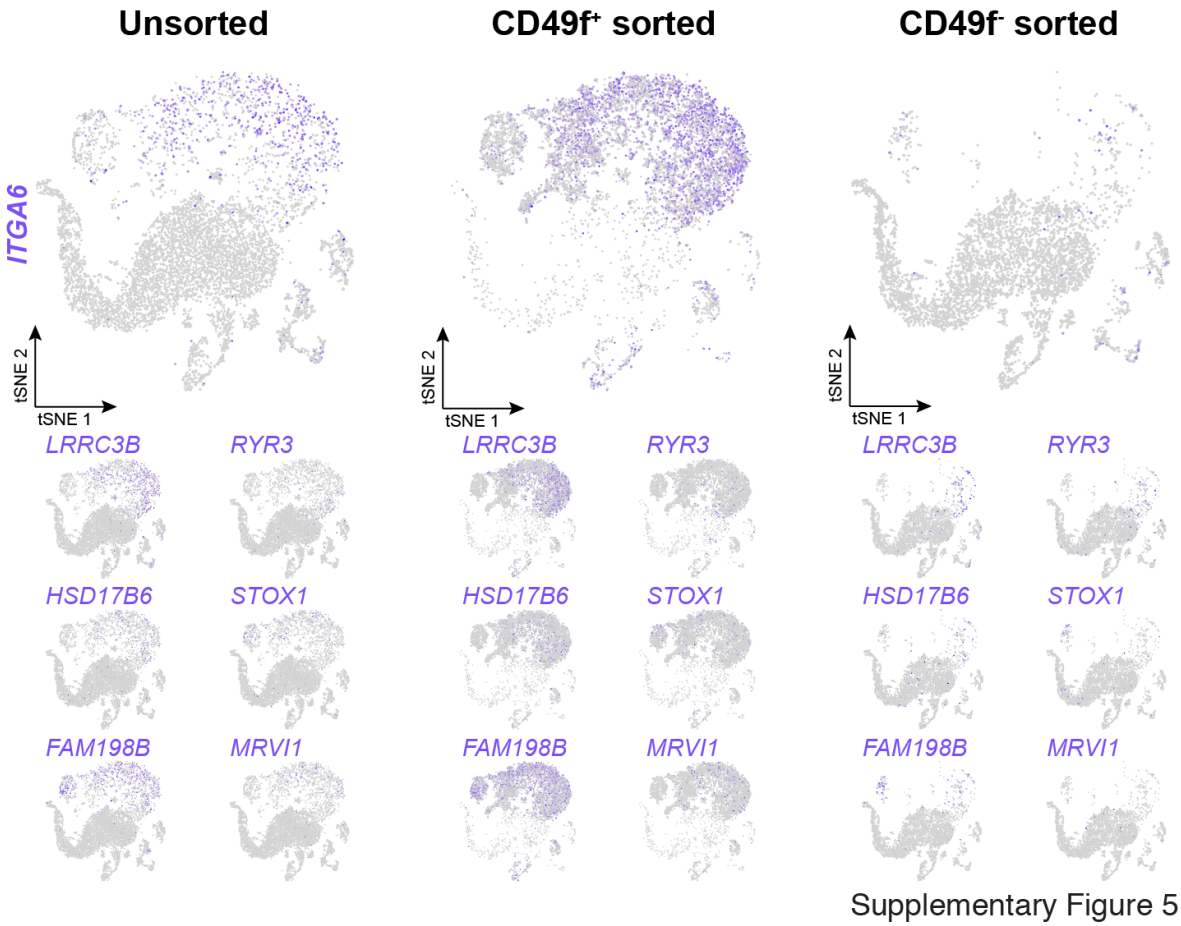
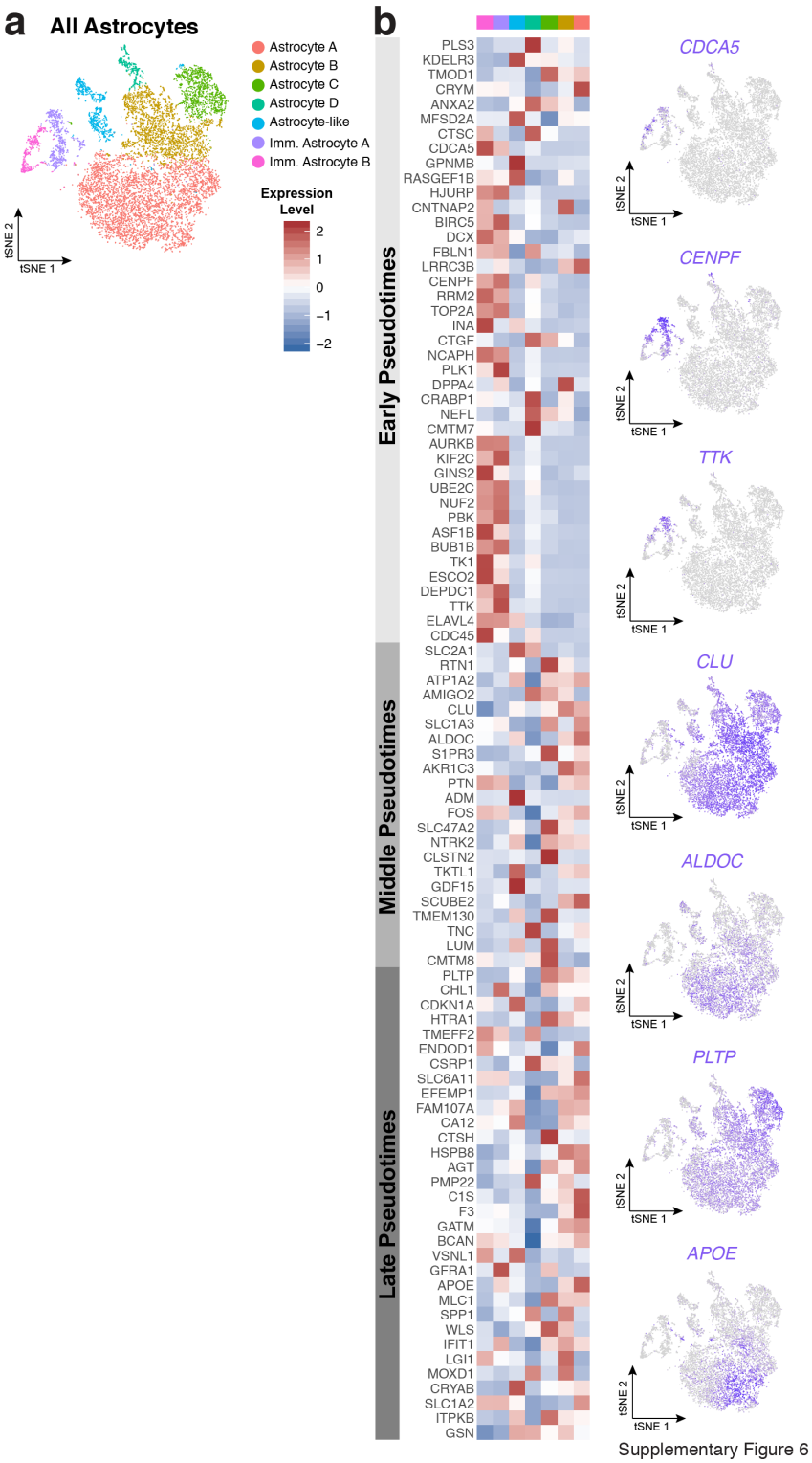


Figure S5: CD49f+ hiPSC-astrocytes express human -specific transcripts (Related to Fig. 3)

tSNE feature plots of CD49f (*ITGA6*) and human-specific astrocyte transcripts *LRRC3B, HSSD17B6, FAM198B, RYR3, STOX1, MRVI1* (identified by Zhang *et al.*) from unsorted, CD49f+ sorted, and CD49f- sorted cells. Cells expressing human-specific astrocyte transcripts are enriched in the CD49f+ fraction of the sort and overlap with *ITGA6* expression. All data from one control line (line 3, n=1).



Supplementary Figure 6

Figure S6: CD49f⁺ hiPSC-astrocytes express late pseudotime markers described in Sloan *et al.* 2017. (left) (Related to Fig. 3)

a) tSNE plot of all astrocytes from unsorted, CD49f⁺ sorted, and CD49f⁻ sorted cells (n = 12,061 astrocytes).

b) Heatmap and corresponding tSNE feature plots of markers described in Sloan *et al.* 2017, classified as early, middle, and late pseudotimes. Immature astrocytes (enriched in $CD49f^-$ fraction) have higher expression of early pseudotime transcripts, while mature astrocytes (enriched in $CD49f^+$ fraction) have higher expression of middle and late pseudotime transcripts. Abbreviations: Imm.= Immature.

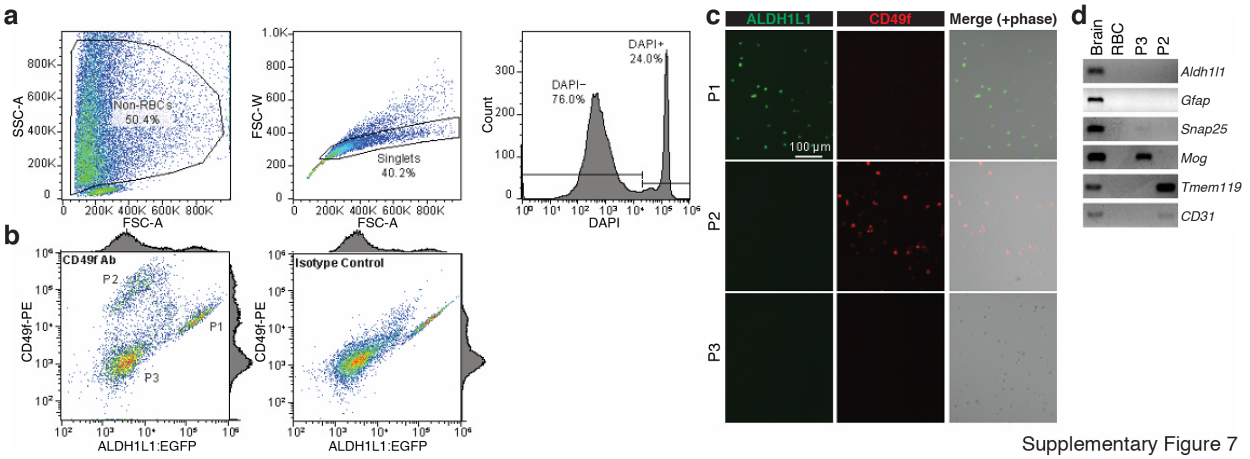


Figure S7: CD49f sorting strategy fails to purify astrocytes from *Aldh1l1*^{eGFP} mouse whole brain (Related to Fig. 4)

a) Representative FACS plots of isolated cells from *Aldh1l1*^{eGFP} mouse whole brain. Cells were stained with CD49f antibody, isotype control, or null-stained control, and sorted. The sorting strategy removed red blood cells (not shown in these plots), followed by doublets, debris, and live gating (DAPI).

b) Compared to isotype control, CD49f^{+} cells yielded a distinct population of CD49f^{+} (population 2; P2), which were *Aldh1l1*^{eGFP}negative.

c) Immunofluorescent analysis of sorted cells. P1 (*Aldh1l1*$^{eGFP+}$ cells) were negative for CD49f, while P2 (CD49f^{+} cells) did not stain for the astrocyte reporter*Aldh1l1*^{eGFP}, suggesting that CD49f sorting strategy does not enrich for astrocytes from a mouse whole brain. P3 was negative for both markers (*Aldh1l1*$^{eGFP-}$CD49f^{-}). Fluorescent images are also merged with phase images.

d) PCR analysis for oligodendrocytes (*Mog*), neurons (*Snap25*), microglia (*Tmem119*) and endothelial (*Cd31*) markers, showing that sorted cells from the CD49f^{+} fraction express *Tmem119* and *Cd31,* but not *GFAP*.

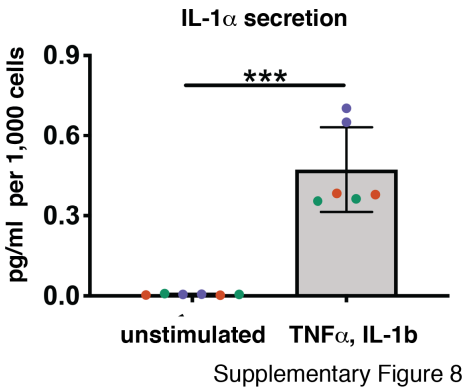
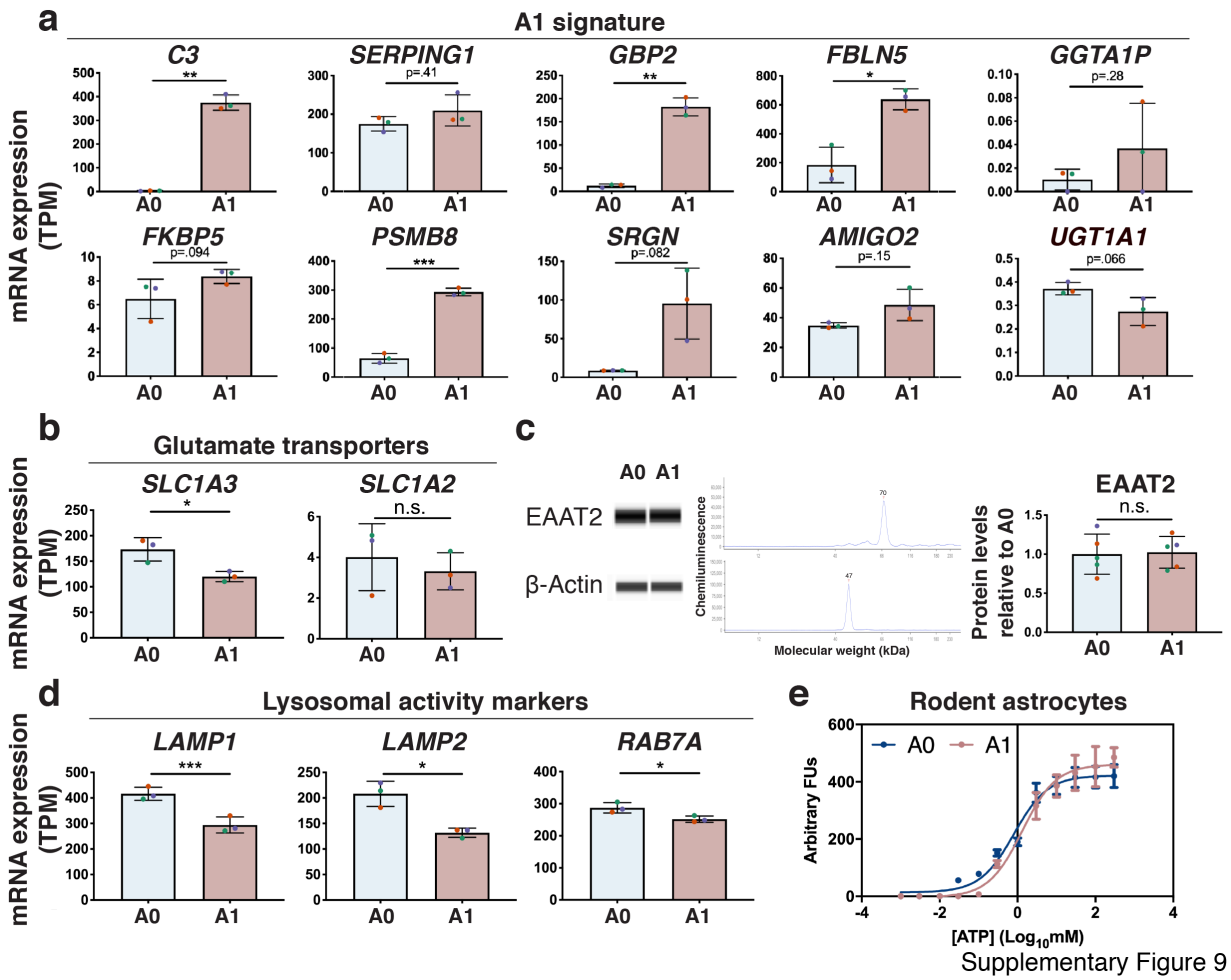


Figure S8: IL-1α is secreted following IL-1β and TNFα stimulation (Related to Fig. 5)

CD49f+ hiPSC-derived astrocytes stimulated with TNFα and IL-1β secrete IL-1α. Concentration of IL-1α secreted in the supernatant of CD49f+ astrocytes that were left unstimulated or stimulated for 24 hours with TNFα and IL-1β. Concentrations are expressed in pg/ml and normalized to 1,000 cells. Different dot colors correspond to 3 different lines (n=6, 2 technical replicates per line). Error bars show mean ± standard deviation. p-values were calculated using a paired two-tailed t-test.



Supplementary Figure 9

Figure S9: CD49f⁺ hiPSC-astrocytes stimulated with TNFα, IL-1α, and C1q express A1 signature genes (Related to Fig. 6)

a) mRNA expression levels from RNA-Seq data as transcripts per million (TPM) of A1-specific transcripts (*C3, SERPING1, GBP2, FBLN5, GGTA1P, FKBP5, PSMB8, SRGN, AMIGO2, UGT1A1*) are upregulated in astrocytes stimulated with TNFα, IL-1α, and C1q (A1) compared to unstimulated astrocytes (A0). Different dot colors correspond to 3 different lines. Error bars show mean ± standard deviation (n=3 independent lines). p-values were calculated using a two-tailed, paired t-test.

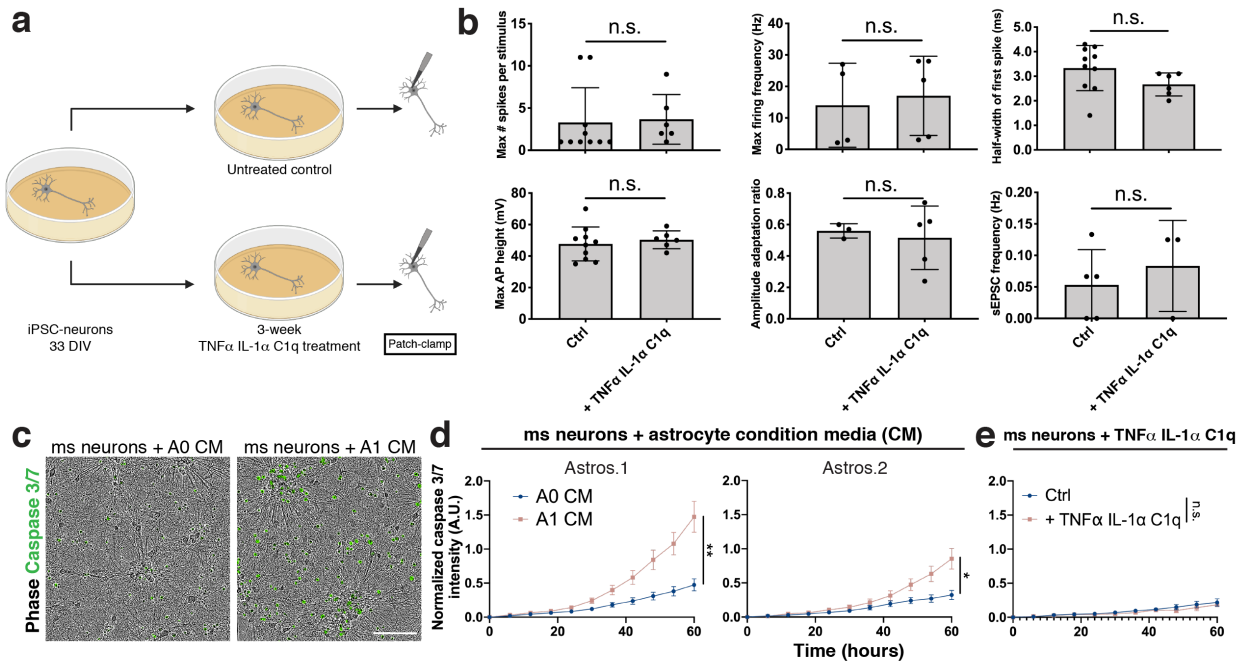
b) mRNA expression levels from RNA-Seq data as transcripts per million (TPM) of known astrocyte glutamate transporters (*SLC1A3, SLC1A2*) in unstimulated astrocytes (A0) and astrocytes stimulated with TNFα, IL-1α, and C1q (A1). Different dot colors correspond to 3 lines. Error bars show mean ± standard deviation (n=3 independent lines). p-values were calculated using a two-tailed, paired t-test.

c) Western blots and electropherograms for EAAT2 and β-actin, and quantification of protein levels for EAAT2 in A0 and A1-like astrocytes, normalized to β-actin, from two

independent experiments. EAAT2 protein levels are unchanged in A1-like astrocytes vs. A0 astrocytes. Different dot colors correspond to 3 lines. Error bars show mean ± standard deviation (n=5, 1-2 replicates per line). p-values were calculated using a two-tailed, paired t-test.

d) mRNA expression levels from RNA-Seq data as transcripts per million (TPM) of lysosomal activity markers (*LAMP1, LAMP2, RAB7A*) in unstimulated astrocytes (A0) and astrocytes stimulated with TNFα, IL-1α, and C1q (A1). All markers are significantly downregulated upon A1 stimulation. Different dot colors correspond to 3 lines. Error bars show mean ± standard deviation (n=3 independent lines). p-values were calculated using a two-tailed, paired t-test.

e) Dose-response curve of rodent astrocytes to ATP treatment. Error bars show mean ± standard error of the mean. There was no alteration in response (no shift in curve) in response to ATP in rodent A1-reactive astrocytes when compared to control.



Supplementary figure 10

Figure S10: TNFα, IL-1α, and C1q are not toxic to neurons, while conditioned medium from A1-like CD49f⁺ hiPSC-astrocytes is toxic to mouse neurons (Related to Fig. 7)

a) Schematic of neuronal cultures stimulated with inflammatory cytokines.

b) Direct treatment with TNFα, IL-1α, and C1q, in the absence of astrocytes, has no effect on the electrophysiological properties of hiPSC-neurons. Bar graphs showing the maximum number of evoked spikes per 1 second stimulus (n=10;6), the maximum firing frequency in hertz (Hz) (n=4;5), the half-width of the first spike (ms) (n=10;6), the maximum action potential height (mV) (n=10;6), the amplitude adaptation ratio between first and last action potential (n=3;5), and the sEPSC frequency (Hz) (n=5;3) in hiPSC-neurons at day 53 when cultured with or without TNFα, IL-1α, and C1q during days 33-53. Each dot represents an independent cell from which we recorded. n=neurons co-cultured without cytokine cocktail; neurons co-cultured with cytokine cocktail. Error bars show mean ± standard deviation. p-values were calculated using a two-tailed, unpaired t-test.

c) Representative images of mouse neurons treated with A0 or A1 astrocyte conditioned media (CM) for 60 hours, showing caspase 3/7 (green) and phase. A1 conditioned medium increases apoptosis in mouse neuronal cultures. Scale bar, 100µm. Abbreviations: CM= conditioned medium

d) Time course analysis and quantification (60 hours) of apoptotic caspase 3/7⁺ cells in mouse neuronal cultures after stimulation with A0 or A1 CD49f⁺ hiPSC astrocyte conditioned media. Error bars represent the standard error of the mean (n=6-12 replicates

per line), indicating a neurotoxic effect of A1 conditioned medium. p-values were calculated using a two-way ANOVA.

e) Time course analysis and quantification (60 hours) of the caspase 3/7 integrated intensity (normalized to confluence) in mouse neurons during treatment with TNFα, IL-1α, and C1q. Error bars represent the standard error of the mean (n=6 replicates per line), demonstrating no direct effect of the cytokine cocktail on neuronal apoptosis. p-values were calculated using a two-way ANOVA.

NYSCF The New York Stem Cell Foundation Research Institute

NYSCF ID: 051121-01-MR-017 Lot# E051-3E

Certificate of Analysis

Product Description	iPS Cell Line	
Publication(s) describing iPSC establishment	NA	
Parent cell line and cell type	051121-01-FB-001	Fibroblasts
Unique Parent Cell Line ID	10-005_1121	
Method of Reprogramming	mRNA	
Media	Freedom	
Cell Culture Matrix	Geltrex	
Passage method	Accutase	
Split ratio	1:10-1:20 every 5-7 days	
Reported Sex (Demographics)	Female	
Calculated Sex (DNA)	Female	

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	Cryotube thaw to single well of 12 well plate	>50% Confluency reached within 10 days	Pass
Sterility	SteriTEQ	Negative	Pass
Mycoplasma	Lonza MycoAlert Plus	Negative	Pass
Karyotype	Illumina CoreExome24	Normal Karyotype (No Autosomal CNVs >2.5 Mb)	Pass
Identity Match	Fluidigm SNPTrace Analysis	Match parent line	Pass
Pluripotency Expression Profile	Nanostring Pluripotency Scorecard Analysis	Express markers of pluripotency with absence of early differentiation markers	Pass
Differentiation Capacity	Nanostring 3 Germ Layer Scorecard Analysis	Ectoderm Analysis	-0.18
Differentiation Capacity	Nanostring 3 Germ Layer Scorecard Analysis	Mesoderm Analysis	-0.09
Differentiation Capacity	Nanostring 3 Germ Layer Scorecard Analysis	Endoderm Analysis	-0.06

Notes
A negative score in the 3 germ layer analysis indicates a potentially reduced ability to differentiate into the corresponding germ layer. An asterisk indicates the line performed outside of the range of reference lines used in this analysis. For more information see Bock et al., 2011 (DOI: 10.1016/j.cell.2010.12.032).

☒Pass
☐Fail
☐Other:

Daniel Paull, PhD
Vice President, Stem Cell Technology Platforms
Date: 03/02/2020

Figure S11: iPSC lines undergo a rigorous quality check (Related to STAR Methods) CoA for iPSC line 051121-01-MR-017 describing results of quality check including a sterility check, mycoplasma testing, karyotyping, and a pluripotency check.