

Figure S1. Quantification of Astrocyte Markers. Percentage of astrocytes in each region expressing S100 β , S100 β +SOX9 or S100 β +SOX9+GFAP(A,C) or HEPACAM(B,D) of H9 ESC (A,B) or GM1 iPSC (C,D) derived astrocytes (n=8 biological replicates for each region and combination of antibodies). Total number of cells analyzed displayed in parenthesis. Error bars are standard deviation of S100 β +SOX9+GFAP(A) or HEPACAM(B). Percentage of cells expressing all three markers displayed.

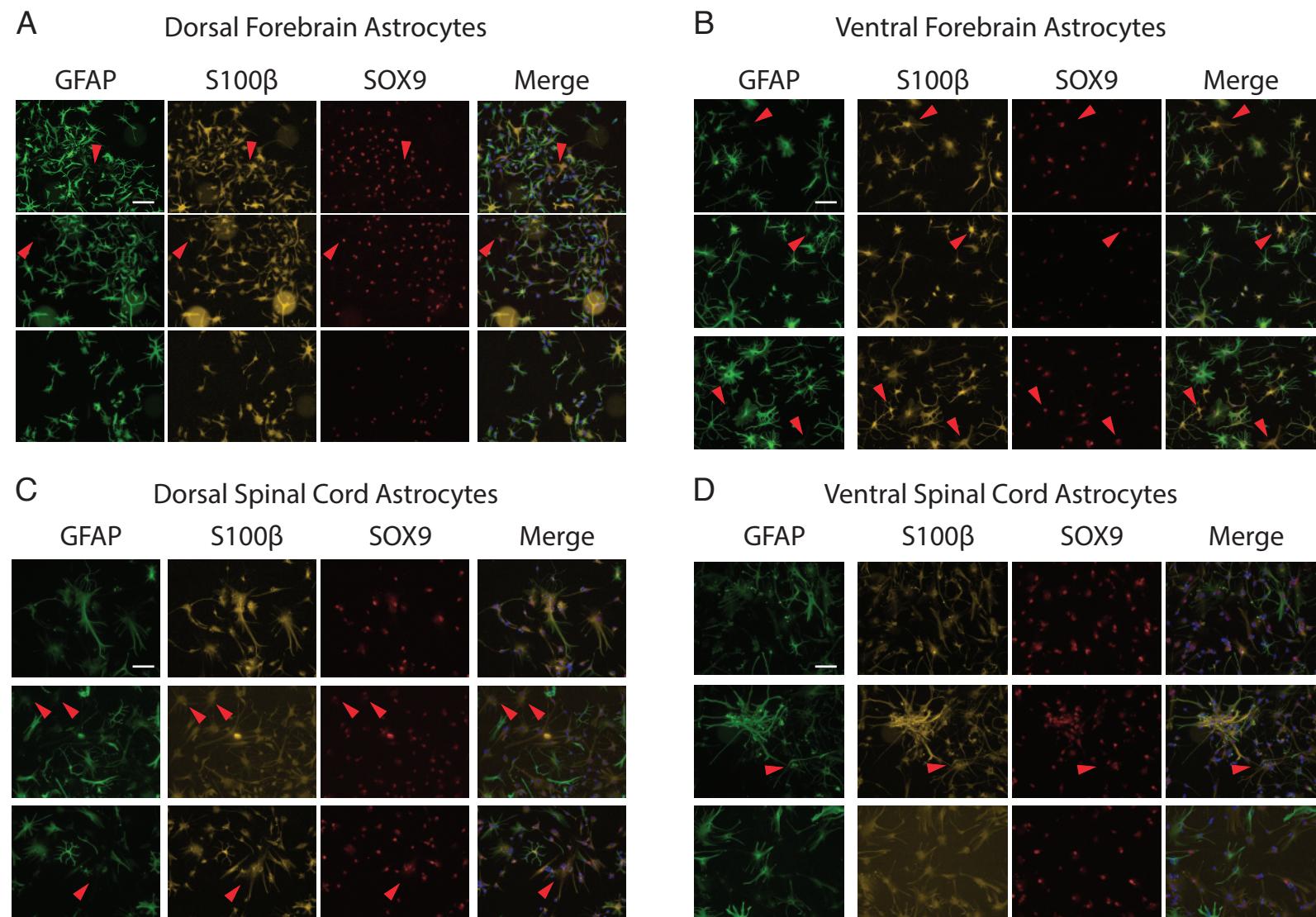


Figure S2. Representative images from high content image analysis of GFAP, S100 β and Sox9 expression in regional astrocytes. Regional astrocytes were immunostained for GFAP, S100 β and Sox9 and imaged automatically using a high-content imager. Representative images are shown in which astrocytes expressing S100 β and Sox9 but lacking GFAP expression can be seen (red arrowheads). Though these cells lack GFAP expression, they exhibit the characteristic morphology and gene expression (S100 β and Sox9) which mark cells of the astro-glial lineage. Scale bar = 200 μ M.

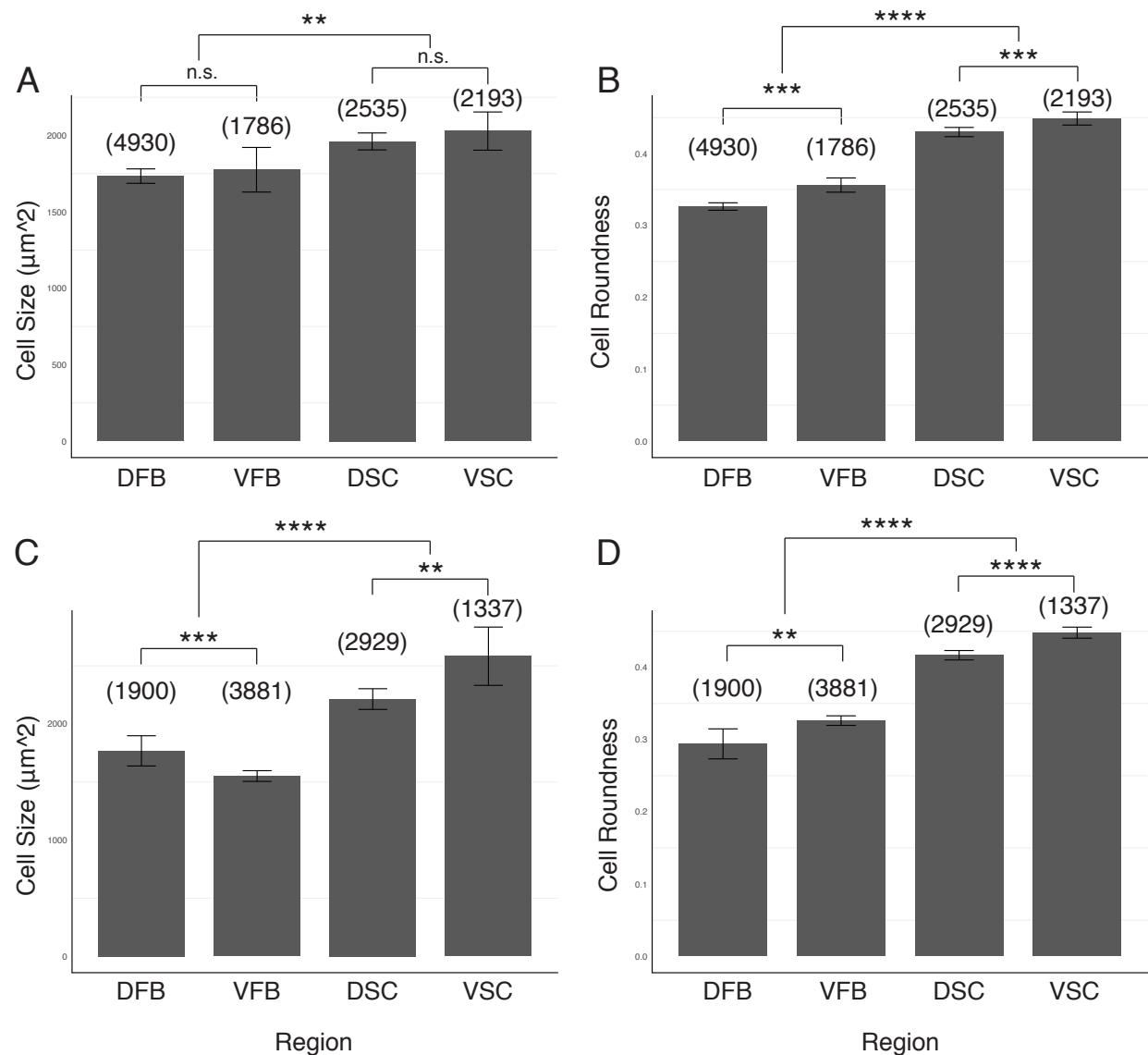


Figure S3. Quantification of Astrocyte Size and Morphology. Analysis of regional astrocyte size (A,C) and roundness (B,D) of H9 ESC (A,B) or GM1 iPSC (C,D) derived astrocytes (n=8 biological replicates for each region). Total number of cells analyzed displayed in parenthesis. Error bars are standard deviation. **= p<0.01, ***=p<0.001, ****=p<0.0001; student's t-test

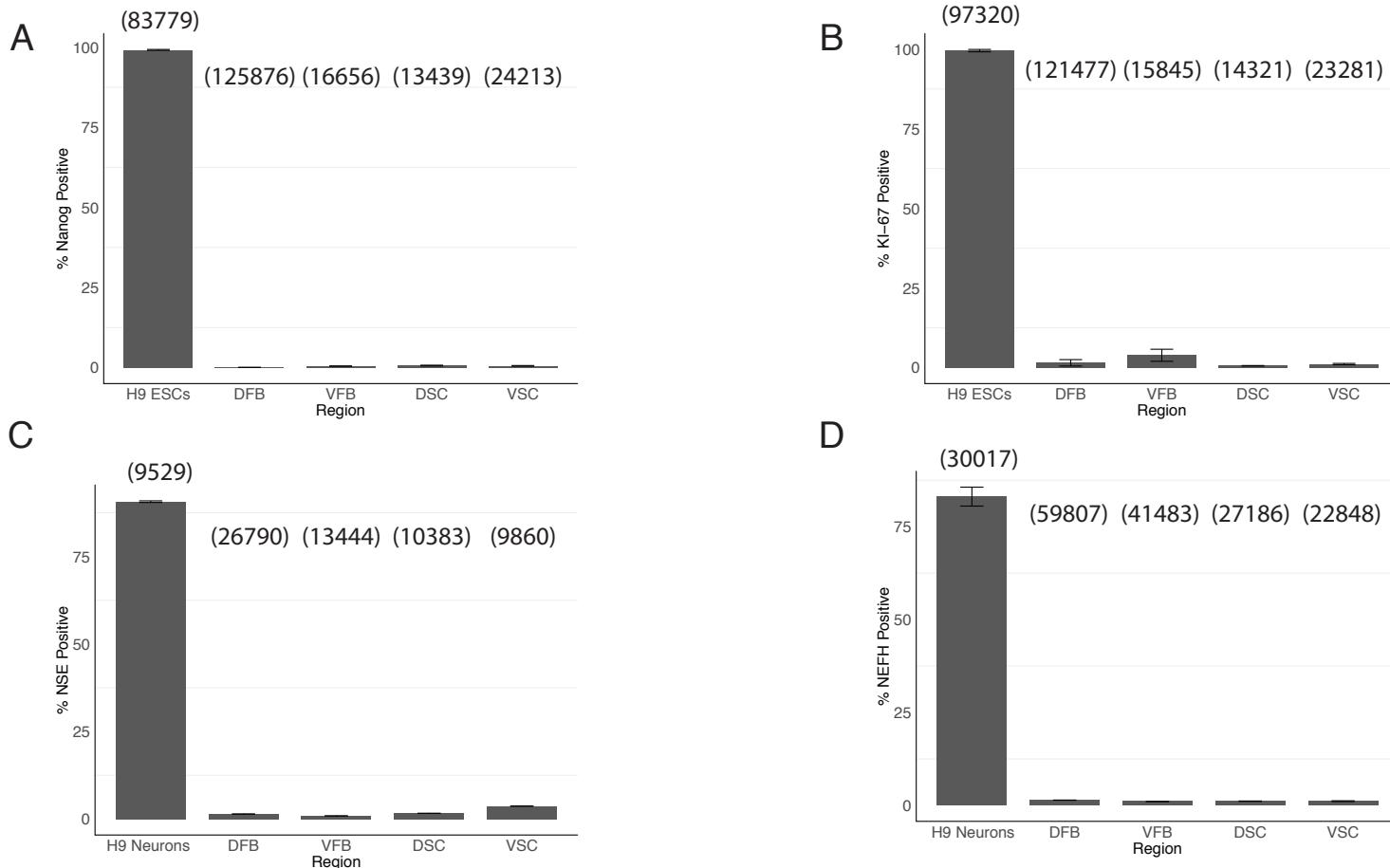


Figure S4. Quantification of Neuronal, Pluripotent and Proliferating Cell Markers. Analysis of non-astrocyte cell markers in regional astrocytes, including those for pluripotent cells (nanog) (A) and proliferating progenitors (ki-67) (B) via immunostaining in both H9 ESCs and regional astrocytes. The neuronal markers NFH and NSE and astrocyte marker GFAP were assayed via immunostaining in both cortical neurons and regional astrocytes (C-D). Numbers in parenthesis are numbers of cells analyzed

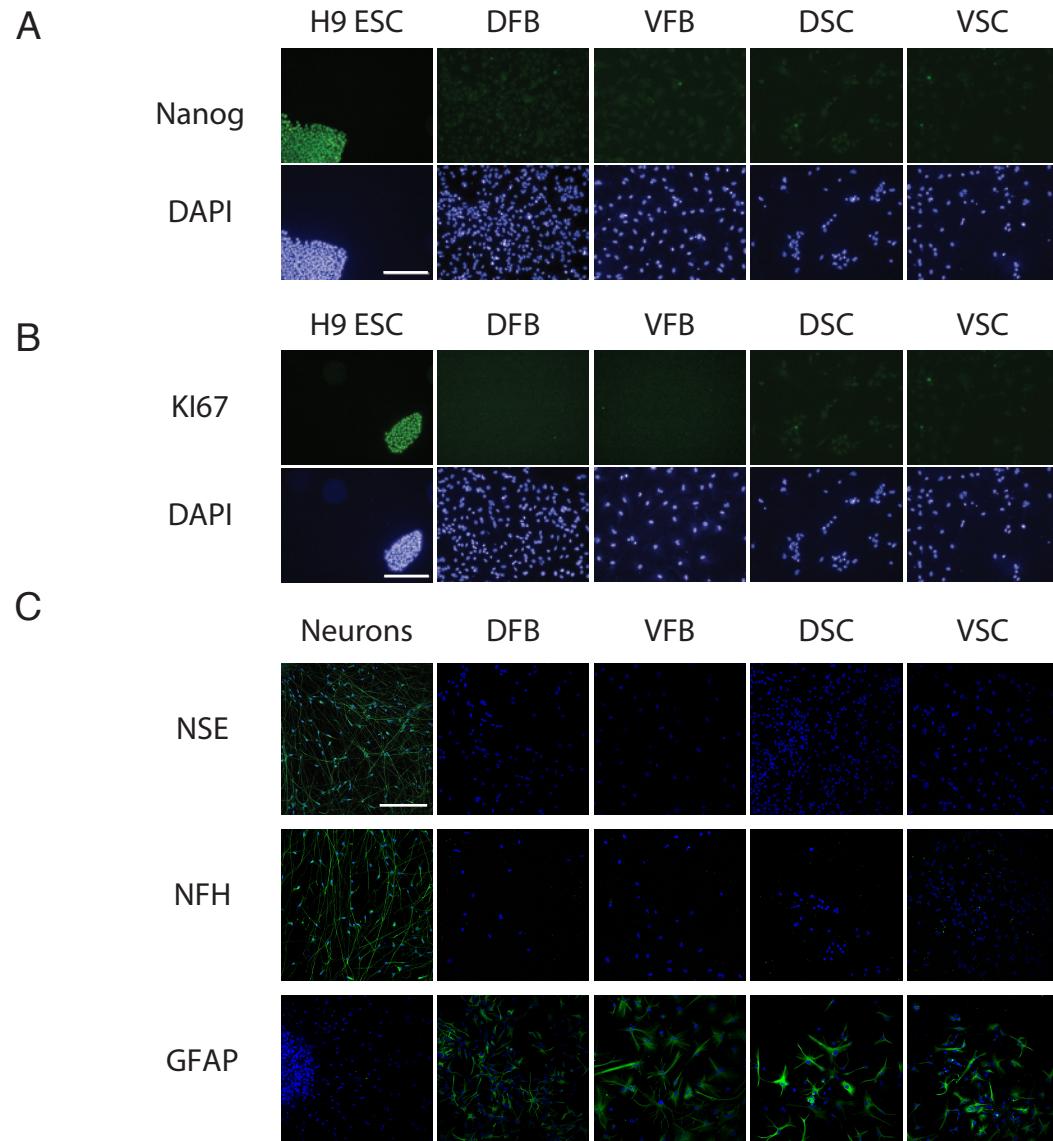


Figure S5. Expression of Pluripotent, Proliferation and Neuronal Markers in Astrocytes. (A) Expression of pluripotent marker (Nanog) in hESCs (positive control) and regional astrocytes. (B) Expression of proliferation marker (Ki67) in hESCs (positive control) and regional astrocytes. (C) Expression of neuronal markers (NFH, NSE) and the astrocyte marker (GFAP) in neurons (positive control for NFH and NSE and negative control for GFAP) and regional astrocytes. Scale bar = 200 μ m

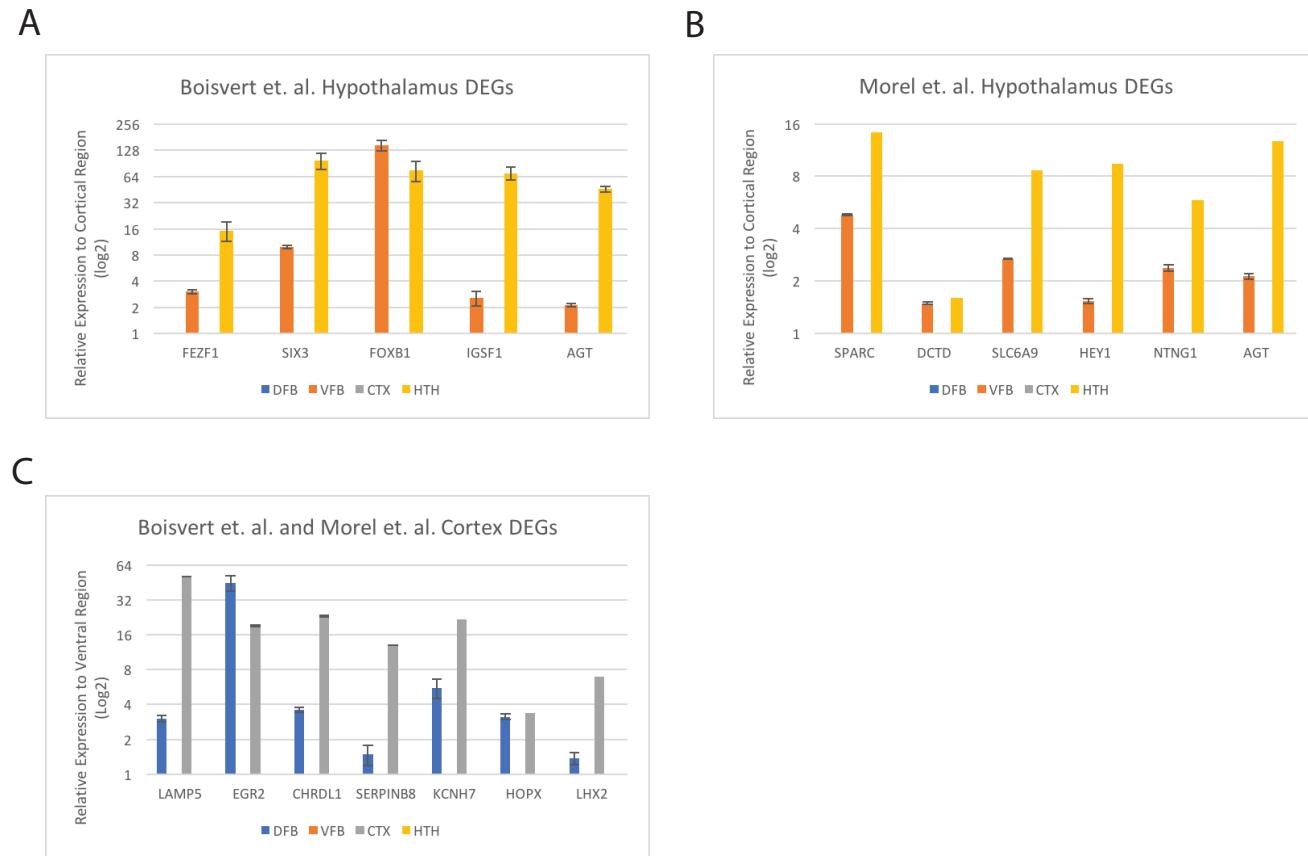


Figure S6. Comparison of human and mouse regional astrocyte DEGs. DEGs of cortical and hypothalamic astrocytes identified in Morel et al 2017 and Boisvert et al 2018 compared to ESC-derived DFB and VFB astrocyte groups. RNA-Seq of human ESC-derived regional astrocytes were selected for confirmation and compared to normalized expression of mouse DEGs. (n=3 biological replicates for human RNA-Seq data. Error bars are standard deviation where available).

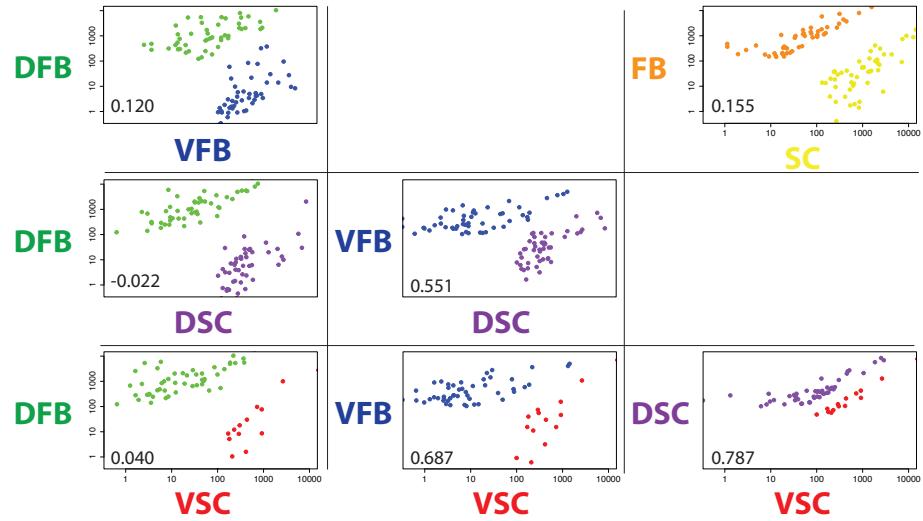


Figure S7. Scatter plot of the expression in TPM of the top 30 DEGs (14 for VSC) compared pairwise to each group (axis is represented in Log10 TPM). Number in lower left displays the Pearson correlation coefficient of the gene expression between the groups.

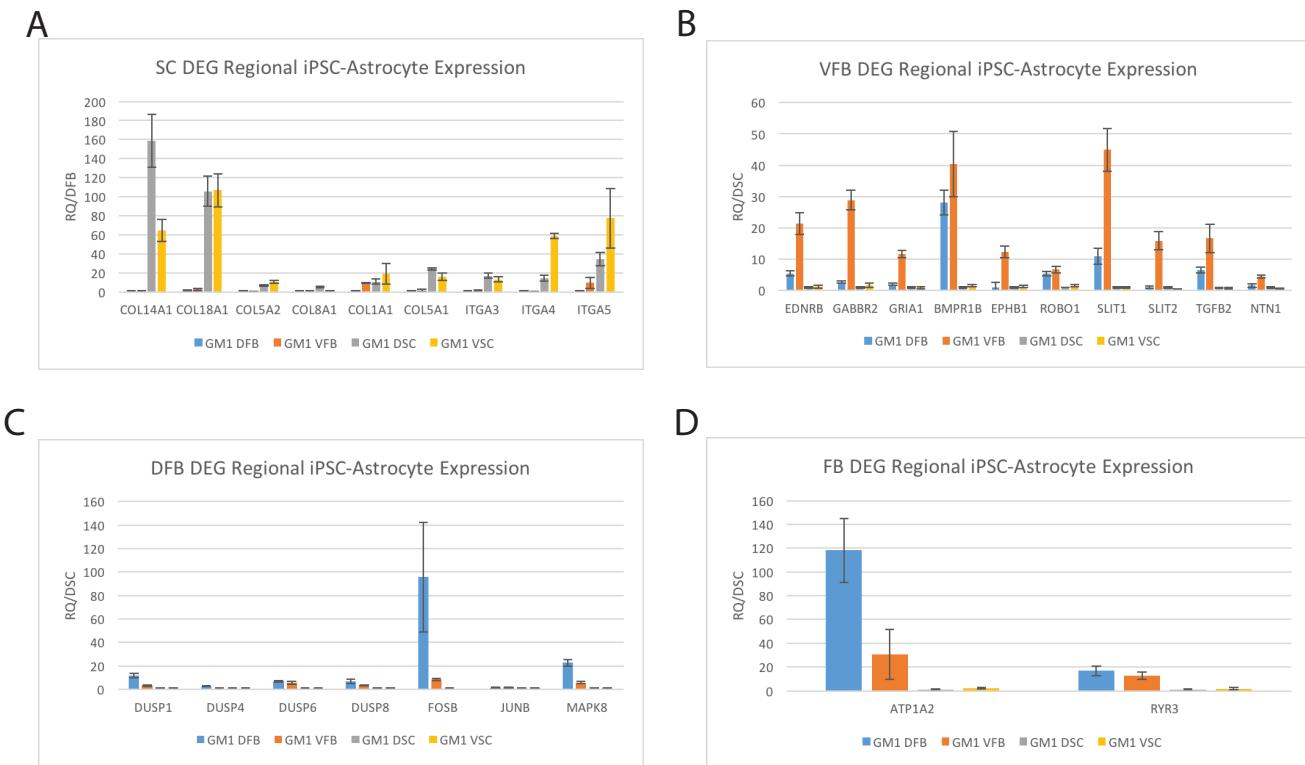


Figure S8. qPCR analysis of iPSC-derived regional astrocytes. DEGs identified by RNA-Seq of ESC-derived regional astrocytes were selected for confirmation. (n=3 technical replicates. Error bars are standard deviation of RQ)

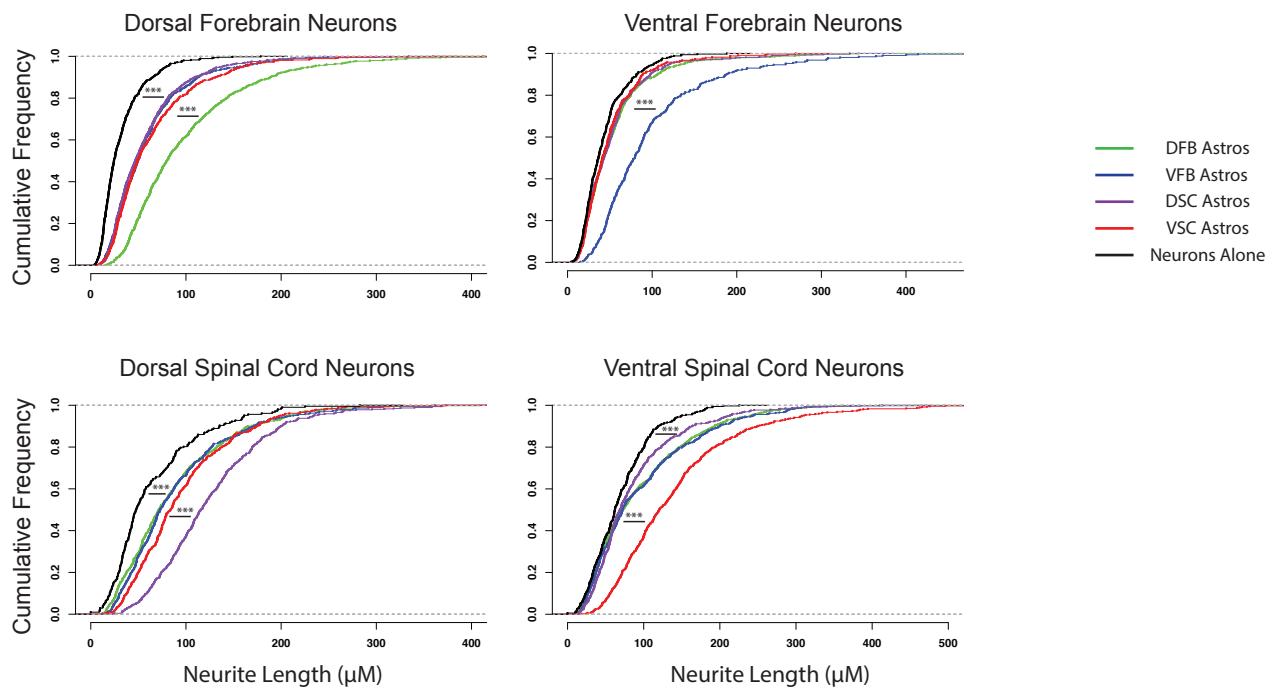


Figure S9. Cumulative frequency of regional neuronal neurite growth following 3 days in co-culture with regional astrocytes or alone. *** = $p < 0.001$ Kruskal-Wallis test.

Table S1. Expression of cell-type specific marker genes. Average expression in TPM of astrocyte, neuronal and oligodendrocyte marker genes. Genes associated with astrocytes are in bold and genes associated with neurons are in italic.

GENE	DFB	VFB	DSC	VSC
GFAP	54879	254113	219286	178595
AQP4	14733	21459	1679	806
S100B	1347	4882	3731	3744
SOX9	17955	11580	6434	6233
CD44	23382	35500	37842	40821
VIM	84193	99417	121871	89875
SLC1A3	40528	5603	9313	5065
SLC1A2	5805	1618	1059	843
NOTCH1	13620	11548	8795	9775
GJA1	19175	24082	31141	17927
NFIA	8843	4809	1673	1791
NFIB	11967	8258	9535	8723
NFIX	3658	8268	11426	12527
<i>RBFOX3 (NEUN)</i>	2	2	9	8
NEUROD1	0	0	21	13
NEUROD2	1	1	3	4
NEFH	30	49	148	150
MAG	0	0	0	0
MBP	29	52	219	284
SOX10	3	22	16	25
GJC2 (CXN47)	29	16	17	24
PDGFA	290	626	833	866
CSPG4	497	960	1073	1202

Table S2. Anatomical regions associated with dorsal forebrain astrocytes by Allen Brain Atlas transcriptome database.

TERM	OVERLAP	ADJUSTED P-VALUE	COMBINED SCORE
TELENCEPHALIC VESICLE	39/301	3.55E-11	45.28
ALAR PLATE OF EVAGINATED TELENCEPHALIC VESICLE	39/301	3.55E-11	43.72
CEREBRUM	39/301	3.55E-11	42.09
CAUDAL SECONDARY PROSENCEPHALON	39/301	3.55E-11	37.66
SECONDARY PROSENCEPHALON	37/301	4.56E-10	39.19
LATERAL SEPTAL NUCLEUS, ROSTRAL (ROSTROVENTRAL) PART	37/301	4.56E-10	33.66
LATERAL SEPTAL NUCLEUS	36/301	1.28E-09	41.19
LATERAL SEPTAL COMPLEX	36/301	1.28E-09	36.22
LATERAL SEPTAL NUCLEUS, INTERMEDIODORSAL PART	36/301	1.28E-09	36.07
LAYER 3 OF AOV CORTEX	35/301	4.75E-09	33.33
INTERMEDIATE PART OF STRSE	34/301	1.36E-08	33.46
BASIC CELL GROUPS AND REGIONS	34/301	1.36E-08	33.04
STRIATAL SEPTUM	34/301	1.36E-08	32.34
ROOT	34/301	1.36E-08	32.32
FOREBRAIN	33/301	4.62E-08	28.81
TELENCEPHALIC VESICLE	39/301	3.55E-11	45.28

Comparison of DFB RNA-Seq gene expression with gene expression from the Allen Brain Atlas upregulated database. Analysis was done via ENRICHR (<http://amp.pharm.mssm.edu/Enrichr>)

Table S3. Anatomical regions associated with ventral forebrain astrocytes by Allen Brain Atlas transcriptome database.

TERM	OVERLAP	ADJUSTED P-VALUE	COMBINED SCORE
PRETHALAMIC EMINENCE	28/301	1.15E-06	25.80
PERIVENTRICULAR STRATUM OF PCPL	27/301	2.88E-06	25.21
DORSAL PART OF ALAR P3	28/301	1.15E-06	25.00
INTERMEDIATE STRATUM OF PTHE	28/301	1.15E-06	23.72
PCPL PART OF THE PERIAQUEDUCTAL GRAY	27/301	2.88E-06	23.20
PRECOMMISSURAL NUCLEUS	27/301	2.88E-06	21.98
MANTLE ZONE OF PTHE	28/301	1.15E-06	21.94
MANTLE ZONE OF JCPD	26/301	6.81E-06	21.88
NUCLEUS OF THE STRIA MEDULLARIS (PRETHALAMIC)	28/301	1.15E-06	21.72
DORSAL JUXTACOMMISSURAL PRETECTAL NUCLEUS	26/301	6.81E-06	21.71
INTERMEDIATE STRATUM OF JCPD	26/301	6.81E-06	21.24
DORSAL PART OF JCP	26/301	6.81E-06	21.16
PARVICELLULAR INTERSTITIAL NUCLEUS OF THE POSTERIOR COMMISSURE	26/301	6.81E-06	17.81

Comparison of VFB RNA-Seq gene expression with gene expression from the Allen Brain Atlas upregulated database. Analysis was done via ENRICHHR (<http://amp.pharm.mssm.edu/Enrichr>)

Table S4. Genes with disease relevance not previously associated with astrocytes.

Gene Symbol	Gene Name	Average TPM ± SD	Gene Function	Disease Relevance
DPYSL3	Dihydropyrimidine like 3	52558 ± 6765	Expressed in mouse astrocytes <i>in-vivo</i> (Oldham et al., 2008), Involved with axonal growth in motor neurons(Blasco et al., 2013)	Mutations associated with ALS(Blasco et al., 2013). Disruption of microRNA targeting DPYSL3 found in schizophrenic subjects(Miller et al., 2012)
HSPA8	Heat Shock 70kDa Protein 8	39387 ± 5012	General chaperone	Potential biomarker for suicidal persons(Kékesi et al., 2012). Associated with schizophrenic patients(Bozidis et al., 2014).
QKI	QKI, KH domain containing, RNA binding	35563 ± 8876	RNA-binding protein involved in mRNA production. Regulates GFAP protein expression(Radomska et al., 2013).	Loss in oligodendrocytes associated with schizophrenia(Aberg, Saetre, Jareborg, & Jazin, 2006).
YWHAE	Tyrosine 3-Monoxygenase/Tryptophan 5-Monoxygenase Activation Protein Epsilon	25049 ± 2514	Member of the 14-3-3 family. Epsilon regulates neuronal migration.	YWHAE is a potential biomarkers in suicidal patients(Kékesi et al., 2012). Part of a proposed mechanism of Miller-Dieker syndrome(Toyo-oka et al., 2003).
YWHAZ	Tyrosine 3-Monoxygenase/Tryptophan 5-Monoxygenase Activation Protein Zeta	21185 ± 2052	Member of the 14-3-3 family. Epsilon regulates neuronal migration.	YWHAZ is a potential biomarkers in suicidal patients(Kékesi et al., 2012). Part of a proposed mechanism of Miller-Dieker syndrome(Toyo-oka et al., 2003).
RTN4	Reticulon 4	24489 ± 3092	Neurite outgrowth inhibitor	Inhibition of RTN4 improves neuro-regeneration in spinal cord injury models(Wang, Xiong, Ren, & Sun, 2012). Upregulated in hypothalamus astrocytes of sleep-deprived mice(Kim et al., 2014).
TTC3	Tetratricopeptide Repeat Domain 3	23839 ± 1584	E3 ubiquitin ligase targeting members of the AKT family.	Interaction of TTC3 with Citron-K and Citron N alters neuronal development in Down Syndrome(Berto et al., 2007).
SRRM2	Serine/Arginine Repetitive Matrix 2	19019 ± 1271	Member of the spliceosome and involved in pre-mRNA splicing.	Upregulated in the Substantia Nigra of PD patients. May be an effective biomarker(Shehadeh et al., 2010).
PSAP	Prosaposin	52850 ± 19072	Non-enzymatic glycoprotein. Involved in beta-galactosidase hydrolysis. PSAP is cleaved into four functions Saposins.	Deficiency in PSAP causes different leukodystrophies based on how the mutations affect PSAP proteolysis to SAP A-D.
A2M	Alpha-2-Macroglobulin	40142 ± 22228 VFB Avg: 70792 ± 1321	Protease inhibitor and cytokine transporter. Mediates the clearance of Aβ.	Due to its role in the removal of Aβ, it has been implicated in AD(Kovacs, 2000)
HNRNPA1	Heterogeneous Nuclear Ribonucleoprotein A1	39578 ± 14511	RNA-binding proteins that associate with pre-mRNAs in the nucleus and influence pre-mRNA processing, as well as other aspects of mRNA metabolism and transport.	Loss of hnRNP A1 in ALS spinal cord neurons with TDP inclusions(Honda et al., 2015).
GPM6A	Neuronal Membrane Glycoprotein M6-A	31198 ± 4522	Involved in neuronal plasticity and neurite outgrowth.	Mutations correlate with claustrophobic patients. Mutant mice display claustrophobic-like behavior(EI-Kordi et al., 2013).

List of genes not previously associated with human astrocytes which are highly expressed in all astrocyte subtypes. Average expression and Standard Deviation (SD) are in TPM.

Table S5. Differentially expressed genes of interest in human regional astrocytes.

Region Expressed	Gene symbol	Gene Name	Regional Expression (Average TPM) ± SD	Gene Function	Disease Relevance
Forebrain	ATP1A2	ATPase Na+/K+ Transporting Subunit Alpha 2	FB: 5857.66 ± 190.69	Maintains the K+ and Na+ gradient across the membrane	Mutations linked to epilepsy and migraines(Carreño et al., 2013; Deprez et al., 2008; Gritz & Radcliffe, 2013)
			SC: 129.61 ± 49.60		
Forebrain	ATP13A4	ATPase Type 13A4	FB: 161.60 ± 59.53	P-Type ATPase subfamily. Localized to ER and regulates calcium in COS7 cells	Mutations linked to Autism and speech impairment(Vallipuram, Grenville, & Crawford, 2010).
			SC: 12.88 ± 3.90		
Forebrain	RYR3	Ryanodine Receptor 3	FB: 986.97 ± 208.92	Ryanodine receptor. Releases calcium from ER following stimulus.	Mutations in patients with epileptic encephalopathies(Appenzeller et al., 2014). Up-regulation protects against neurodegeneration in AD mouse models(Supnet, Noonan, Richard, Bradley, & Mayne, 2010).
			SC: 55.20 ± 10.32		
Forebrain	LUZP2	Leucine Zipper Protein 2	FB: 332.27 ± 40.42	Leucine zipper transcription factor.	Deleted in patients with Wilms tumor-Aniridia-Genitourinary anomalies-mental Retardation though mice with LUZP2 KO had no phenotype(Wu, Michaud, & Johnson, 2003).
			SC: 18.69 ± 8.53		
Spinal Cord	GDF6	Growth differentiation factor 6	FB: 7.33 ± 4.26	TGFβ family member. Required for normal axial skeleton development	Mutations cause Klippel-Feil Syndrome, a congenital disorder of spinal segmentation(Chacón-Camacho, Camarillo-Blancarte, Peláez-González, Mendiola, & Zenteno, 2012).
			SC: 530.50 ± 151.21		
Spinal Cord	CACNA1A	Calcium voltage-gated channel subunit alpha 1a	FB: 70.89 ± 60.05	Voltage-dependent calcium channel predominantly expressed in the CNS	Missense mutations found in patients with hemiplegic migraines (Carreño et al., 2013).
			SC: 2309.89 ± 383.06		
Spinal Cord	POPDC3	Popeye Domain-Containing Protein 3	FB: 10.55 ± 7.93	Popeye-domain proteins are important for skeletal and cardiac muscle development.	POPDC3 deleted in a patient with a Prader-Willi like disease(Bonaglia et al., 2008)
			SC: 621.45 ± 50.29		
Spinal Cord	POPDC1 (BVES)	Blood Vessel Epicardial Substance	FB: 440.4 ± 36.2	Popeye-domain proteins are important for skeletal and cardiac muscle development.	POPDC3 deleted in a patient with a Prader-Willi like disease(Bonaglia et al., 2008)
			SC: 552.0 ± 29.4		
Dorsal Forebrain	FOSB	FBJ Murine Osteosarcoma Viral Oncogene Homolog B	DFB: 483.5 ± 101.9	Leucine zipper transcription factor that interacts with members of the JUN family.	Upregulated with cocaine and amphetamine use in the nucleus accumbens(Ehrlich, Sommer, Canas, & Unterwald, 2002; Murphy, Russig, Pezze, Ferger, & Feldon, 2003).
			Non-DFB: 5.6 ± 3.6		
Dorsal Forebrain	ARC	Activity-regulated Cytoskeleton-associated Protein	DFB: 2870.9 ± 306.6	Required for consolidation of long-term memory. Regulates cytoskeletal organization.	Disruption in ARC expression leads to schizophrenic behaviors in mice(Managò et al., 2016).
			Non-DFB: 52.5 ± 39.9		

Dorsal Forebrain	GABBR2	GABA Type A Receptor β 2 Subunit	DFB: 183.8 \pm 18.1 Non-DFB: 25.6 \pm 36.1	GABA receptor related to circadian rhythms.	Associated with childhood disintegrative disease and childhood electroclinical syndrome(Appenzeller et al., 2014).
Ventral Forebrain	SFTA3	Surfactant Associated 3	VFB: 428.3 \pm 21.3 Non-VFB: 0.7 \pm 0.5	Expressed in the lung. Has putative immunological roles.	Deleted in a patient with choreoathetosis, congenital hypothyroidism and neonatal respiratory distress syndrome(Schicht et al., 2014).
Ventral Forebrain	SULF1	Sulfatase 1	VFB: 1438.3 \pm 80.8 Non-VFB 21.3 \pm 11.9	HSPG modifying enzyme	Upregulated in astrocytes in Costello Syndrome(Krencik et al., 2015).
Ventral Forebrain	HSPB8	Heat Shock Protein Family B Member 8	VFB: 4981.8 \pm 178.3 Non-VFB: 903.7 \pm 678.3	Heat shock protein containing an α B-crystallin domain.	May improve ability of astrocytes to clear proteinaceous aggregates. Upregulated in astrocytes in AD patients(Seidel et al., 2012)
Dorsal Spinal Cord	TACR1	Tachykinin Receptor 1	DSC: 587.7 \pm 26.2 Non-DSC: 108.9 \pm 109.5	Endogenous receptor for Substance P	Mediates behavioral stress response in alcohol dependence(Blaine, Claus, Harlaar, & Hutchison, 2013).
Dorsal Spinal Cord	PRDM8	PR Domain 8	DSC: 424.1 \pm 14.4 Non-DSC: 31.8 \pm 21.1	Histone methyltransferase	Knockout mistargets neurons of the dorsal telencephalon(Inoue et al., 2015).
Dorsal Spinal Cord	SLIT3	Slit Guidance Ligand 3	DSC: 2723.3 \pm 43.9 Non-DSC: 204.2 \pm 223.9	Ligand for ROBO receptors. Plays role in axon guidance and development of sensory neurons in the spinal cord.	--
Ventral Spinal Cord	HAND2	Heart and Neural Crest Derivatives Expressed 2	VSC: 208.7 \pm 24.2 Non-VSC: 19.6 \pm 28.2	bHLH transcription factor important for cardiac muscle formation.	--
Ventral Spinal Cord	Serpini1	Serpin Family Member I Member 1	VSC: 921.0 \pm 56.2 Non-VSC: 91.9 \pm 98.1	Serine protease inhibitor expressed in the brain.	--
Ventral Spinal Cord	OLFML3	Olfactomedin Like 3	VSC: 290.5 \pm 30.6 Non-VSC: 68.8 \pm 50.9	Secreted scaffold protein. Important for dorso-ventral axis patterning. Highly expressed in skeletal muscle.	--

List of differentially expressed genes with disease implications or not previously described in astrocytes. Gene Expression and Standard Deviation (SD) are in TPM.

Table S6. Primary antibodies used in this study.

Gene	Manufacturer	Catalog #	Dilution	RRID
GFAP	Dako	Z0334	1:5000	AB_10013382
S100β	Millipore	MAB079-1	1:1000	AB_571112
SOX9	R&D Systems	AF3075	1:1000	AB_2194160
OTX2	R&D Systems	AF1979	1:500	AB_2157172
NKX2.2	Abcam	Ab191077	1:100	NA
NKX2.1	Chemicon	MAB5460	1:250	AB_571072
Hepacam	Proteintech	18177-1-AP	1:100	AB_2279680
Occludin	Sigma	33-1500	1:50	AB_2533101
HOXA3	Sigma	H3791	1:200	AB_10621818
FOGX1	Abcam	ab18259	1:200	AB_732415
Nanog	Stemgent	09-0020	1:500	AB_2298294
NF200	Santa Cruz	SC-5279	1:500	AB_628051
NSE	Abcam	Ab53025	1:500	AB_881756
Ki-67	Abcam	Ab16667	1:500	AB_302549

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