Neuron

Supplemental Information

Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse

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Supplemental Figure and Table Legends

Figure S1, related to Figure 1
Morphology of human astrocytes *in vitro*. Human, n=20 cells from 5 patients. Rat, n=10 cells from one batch of astrocytes prepared from 6 rat brains combined. A. Total branch number of human and rat astrocytes grown in culture for 6 days. Two-tailed Wilcoxon rank sum test. *p<0.05. B. Total branch length of human and rat astrocytes grown in culture for 6 days. Two-tailed unpaired t-test. **p<0.01 C. Total territory size of human and rat astrocytes in culture. Territory size is defined as the 2-dimensional area that is delineated by the border of the astrocyte process (as labeled with GFAP). Two-tailed Wilcoxon rank sum test. ***p<0.001. D, E. Representative human (D) and rat (E) astrocytes in serum-free media at 6 div. Scale bar: 20μm. F. Bright field images of fetal (6 div) and adult (21 div) human astrocytes. Scale bar: 20 μm.

Figure S2, related to Figure 5
Only fetal human astrocytes proliferate *in vitro*. A. Percentage of EdU and Ki67 positive cells in cultures of human fetal and adult astrocytes. For the fetal condition, astrocytes were plated either directly after purification, or after freezing and thawing. n=6 coverslips per condition. Each coverslip has 87-347 cells. Two-tailed unpaired t-test. P<0.005. B. DAPI and EdU stain of fetal and adult human astrocytes in culture. Scale bar = 100 μm.

Figure S3, related to Figure 2
Survival of retinal ganglion cells in the presence and absence of human astrocytes in media with or without growth factors. A and B. Survival of retinal ganglion cells (RGCs) in growth medium without BDNF and CNTF. Astrocytes used in this experiment have been grown in culture for 21 days before being co-cultured with RGCs. Two tailed Wilconxon rank sum test. ***, p<0.001. C and D. Survival of RGCs in growth medium containing BDNF and CNTF. In this complete media, astrocytes do not increase RGC survival. Two tailed unpaired t-test. P>0.05. N.S., not significant.

Figure S4, related to Figure 4
A. Hierarchical clustering of gene expression of acutely purified postnatal human samples. Astro, astrocyte. Oligo, oligodendrocytes. Endo, endothelial cells. Mgl/Maco, microglia/macrophage. B. C. PVCLUST of human cell types. Red numbers represent approximately unbiased *p*-value and green values represent bootstrap probability value.

Figure S5, related to Figure 4
Volcano plots showing the number of differentially expressed genes under different conditions. X axis, ratio (fold change) of gene expression under the two different conditions. Log2 scale. Y axis, *p*-value of gene expression differences under the two conditions. –Log10 scale. Red dashed lines: 4 fold ratio and p=0.01. *p*-values are determined by two-tailed unpaired t-test. Gene symbols and expression levels of genes >4 fold differentially expressed (p<0.01) are listed in Table S6. GO terms enriched in these differentially expressed genes are listed in Table S5. A. Mouse astrocytes obtained by FACS and immunopanning. Only a small number of genes are differentially expressed by astrocytes obtained using the two methods. B. Comparison of mouse and human astrocytes obtained by the HepaCAM immunopanning method. C. Comparison of fetal (18gw) (APC) and mature (juvenile and adult, 8-63 yo) human astrocytes. D. Comparison of adult human astrocytes harvested from glioblastoma samples vs. healthy samples. E. Comparison of adult human astrocytes harvested from sclerotic hippocampus vs. healthy temporal lobe cortex.

Figure S6, related to Figure 4
PCR validation of a subset of genes differentially expressed by human and mouse glia cells. Fragments of 8 differentially expressed genes and house keeping gene Gapdh were amplified from human and mouse whole brain cDNA libraries. RNA-seq and PCR showed similar differential expression patterns for all of the 8 genes.

Table S1, related to Figure 1
Patient diagnosis and brain regions where tissue samples were taken.

Table S2, related to Figure 1
Gene expression of human brain cells before and after dissociation determined by Affymetrix Human Genome U133 Plus 2.0 microarray.

Table S3, related to Figure 4
Estimation of the percentage of contaminating cell types from each immunopanning purified mature cell types. The calculation is based on gene expression (determined by RNA-seq) of 5 classical cell type specific markers for each cell type (see Experimental Procedures).

Table S4, related to Figure 4
Complete gene expression data (in FPKM). Spreadsheet 1: Human and mouse. This spreadsheet contains expression of all genes with identical gene symbols in human and mouse. Spreadsheet 2: Human data only. This spreadsheet contains all human data, including the genes without corresponding identical gene symbols in mice. Spreadsheet 3: Mouse data only. This spreadsheet contains all mouse data, including genes without corresponding identical gene symbols in human. Mouse neuron, oligodendrocyte, microglia/macrophage, and endothelial cell data are from our previous publication (Zhang et al., 2014).

Table S5, related to Figure 4
GO terms enriched in different conditions and gene expression changes before and after dissociation. We compared gene expression of 5 different pairs of conditions in this table: Human vs mouse adult astrocytes purified with the HepaCAM immunopanning method; APC (18gw) vs mature (8-63yo) human astrocytes; astrocytes purified by HepaCAM immunopanning from glioblastoma vs healthy adult human brain tissue; astrocytes purified from sclerotic hippocampi vs healthy temporal lobe cortices; unpurified whole brain gene expression changes before and after dissociation. Genes >4 fold differentially expressed ($p<0.01$) were analyzed.

Table S6, related to Figure 4
Gene expression data (FPKM) of the same comparisons as in Table S5.

Table S7, related to Figure 4
Expression of genes differentially expressed in temporal lobe vs. other cortical areas by human (average expression from 12 patients in the age range of 8-63yo) and mouse astrocytes (average expression from 6 samples (each pooled from 2-3 mice) in the age range of 1-9 months). All genes expressed > 2 fold different ($P<0.05$) between temporal lobe cortex and other cortical areas (frontal lobe, occipital lobe, and parietal lobe) according to Allen Brain Atlas are included. P values were determined by two-tailed unpaired t-test. * indicates temporal lobe-depleted genes expressed at higher levels by mouse astrocytes than human astrocytes.

Table S8, related to Figure 4
Cell type-enriched expression pattern of human genes without mouse orthologs. Genes enriched >10 fold, expressed at FPKM>2 are listed. Absence of mouse orthologs were based on the Ensembl database and confirmed by BLAST search.

Table S9, related to Figure 4
Primer sequences for PCR and qPCR validation of differentially expressed genes in human vs mouse astrocytes and in fetal vs. mature human astrocytes.

Supplemental Experimental Procedures

Human tissue

Human brain tissue was obtained with informed consent under a Stanford University Institutional Review Board approved protocol. Juvenile and adult human brain tissue was obtained from surgeries for treating epilepsy and tumors. All experiments described in this study, except the reactive astrocyte gene induction studies (Figure 5 and 6, epilepsy, tumor, and tumor peripheral data), were performed with by-and-large normal temporal lobe cortex resected in order to access deeper hippocampus areas involved in epilepsy. The cortical tissue was determined as normal by electroencephalogram (EEG) and magnetic resonance imaging (MRI). For assessment of reactive astrocyte gene expression we used sclerotic hippocampus specimens involved in epileptic foci and cortical specimens from glioblastoma core and peripheral regions, defined as contrast enhancing and non-contrast enhancing regions, respectively. Tissue was immersed in 4°C Neurobasal medium (21103-049; Gibco) and transferred to the lab for tissue dissociation within 1 hour after resection. Fetal human brain tissue was obtained following elective pregnancy termination. Tissue was immersed in 4°C RPMI medium (12633-012; Life Technologies) and transferred to the lab for tissue dissociation within 5 hours after the procedure.

Vertebrate animals

All procedures involving animals were conducted in conformity with Stanford University guidelines that are in compliance with national and state laws and policies. We used mice for RNA-seq transcriptome profiling, in situ hybridization and PCR validation experiments of the RNA-seq data. We used rats for *in vitro* experiments including morphology measurement and calcium imaging because of higher yield and consistency of rat astrocyte culture compared to mouse astrocyte culture.

Purification of mature human astrocytes, neurons, oligodendrocytes, microglia/macrophages, and endothelial cells

We dissected out grey matter from mature human brain specimens and removed meninges and blood clots, and then chopped the tissue into pieces < $1mm^3$. We then incubated the tissue in 20 unit/ml papain at 34°C for 100 minutes and washed with a protease inhibitor stock solution. We then gently triturated the tissue with 5 ml serological pipettes in the protease inhibitor stock solution before spinning the cell suspension through a layer of protease inhibitor stock solution. We resuspended the cells in PBS with BSA and DNase and passed it through a Nitex filter to remove cell clumps. We then added the single cell suspension to a series of plastic petri dish pre-coated with cell type specific antibodies and incubated for 10 - 30 minutes each at room temperature. Unbound cells were transferred to the subsequent petri dish while the dish with bound cells was rinsed 8 times with about 20ml of PBS each time to wash away loosely bound contaminating cell types. The antibodies used include anti-CD45 to capture microglia/macrophages, anti-GalC

hybridoma supernatant to harvest oligodendrocytes, anti-O4 hybridoma to harvest oligodendrocytes precursor cells, anti-Thy1 (CD90) to harvest neurons, anti-HepaCAM to harvest astrocytes, and finally *Banderiaea simplicifolia* lectin 1 (BSL-1) to harvest endothelial cells. For RNA-seq, cell samples were scraped off the panning dish directly with Qiazol reagent (Qiagen). For cell culture and *in vitro* experiments, astrocytes bound to the anti-HepaCAM antibody coated dishes were incubated in a trypsin solution and incubated at 37°C for 5-10 minutes and gently squirted off the plate. We then spun down the astrocytes and plated them on poly-D-lysine coated plastic coverslips in a Neurobasal-DMEM based serum-free medium. We replaced half of the volume with fresh medium every 3-4 days to maintain the cultures.

Purification of fetal human astrocytes and neurons

Fetal human astrocytes and neurons were purified in a similar protocol to the above-mentioned procedure with the following modifications: 7.5 unit/ml papain was used and the papain digestion time was shortened to 45 minutes. After obtaining a single cell suspension, the suspension was incubated in a 34°C waterbath for recovery of cell surface antigens digested by papain. Only the following three panning dishes were used: one coated with anti-CD45 to harvest and deplete microglia/macrophages, one coated with anti-Thy1 to harvest and deplete neurons, and one coated with anti-HepaCAM to harvest astrocytes. We used Bambanker reagent (BulldogBio BB01) to freeze and store fetal astrocytes and neurons according to manufacturor's instructions.

Purification of adult mouse astrocytes

For comparison of human and mouse astrocyte transcriptomes, we used 1, 4, 7, and 9 month-old C57BL6 mice, harvested grey matter from the whole cortex, and purified astrocytes with the identical protocol for mature human astrocytes.

Purification of fetal rat astrocytes

For comparison of calcium responses of fetal human and rat astrocytes, we purified fetal (E18.5) rat astrocytes by immunopanning. We dissected out cerebral cortices of a litter (8-12 embryos) of gestation day 18.5 (E18.5) Sprague-Dawley rat embryos, removed meninges, and dissociated the brain tissue into a single cell suspension using the same method to dissociate human fetal brain tissue. We then incubated the suspension at 34°C for one hour to recover cell surface antigens. We subsequently passed the suspension through the following panning dishes: two dishes coated only with secondary antibodies (150μg anti-rat IgG, Jackson ImmunoResearch 112-005-167) to deplete microglia and macrophages, one dish coated with anti-O4 antibodies (Bansal et al., 1989) (4ml of hybridoma supernatant diluted with 8ml DPBS/0.2% BSA) to deplete oligodendrocyte precursor cells, and one dish coated with anti-HepaCAM antibody (7.5 μg R&D systems, MAB4108) to harvest astrocytes.

Immunocytochemistry

Cultured cells were fixed with 4% PFA for 10 minutes at room temperature, permeablized and blocked with 10% goat serum with 0.2% Triton-X100. The following primary antibodies were used: chicken anti-GFAP (1:1000, Covance PCK-591P), rabbit anti-GFAP (1:1000, Dako Z0334), mouse anti-TuJ1 (1:1000, Sigma T8660), mouse anti-Ki67 (1:2000, BD 550609), mouse anti-bassoon (1:500, Enzo ADI-VAM-PS003) and rat anti-Homer (1:1000, Serotec AHP736). The appropriate secondary antibodies conjugated with Alexa fluorophores (Invitrogen) were used. The stained samples were mounted in VectorShield with DAPI (Vector Labs, H1200) to stain the nuclei of all cells. Images were acquired using a Zeiss AxioImager fluorescence microscope.

EdU incorporation assay

Cells were purified from human samples and cultured on PDL-coated coverslips for 1 day to recover. After 1 day of rest, EdU was added to the media according to manufacturer's guidelines (Life Technologies C10339). Cells were fixed at day 7 and imaged for EdU incorporation.

Neuron survival assay

We purified human fetal neurons with anti-Thy1 antibody and astrocytes with anti-HepaCAM antibody as described above. Neurons were plated at 10,000 cells per well on PDL-coated plastic coverslips in 24 well cell culture plates in serum-free medium containing BDNF and CNTF (see medium composition under Detailed protocol for the purification of human astrocytes). Astrocytes were plated on PDL-coated cell culture inserts with 1μm diameter pores (Corning, 08-771-9) in serum-free medium containing HBEGF. Neuron survival was determined according to manufacture's instructions with the Live/dead Viability/Cytotoxicity kit (Invitrogen, L3224) at 7 *div*.

Synapse formation assay

We purified RGCs by sequential immunopanning to greater than 99% purity from P5-P7 Sprague-Dawley rats (Charles River) and cultured in serum-free medium containing BDNF and CNTF on PDL-laminin coated glass coverslips at 75,000 cells per well as previously described (Winzeler and Wang, 2013). Human astrocytes were plated on inserts at 100,000-200,000 cells per insert and co-cultured with RGCs for 14-17 days. For quantification of structural synapses, RGCs were fixed and stained with antibodies against presynaptic marker Bassoon and postsynaptic marker Homer as described in the immunocytochemistry section above. Images were acquired with a 63x lense on a Zeiss AxioImager fluorescence microscope. Imaging fields were randomly selected by viewing with the DAPI channel to avoid biased selection of regions with dense or sparse synapses. Ten images each containing 1-6 cells were acquired from each coverslip and 2-3 coverslips per condition were imaged. Synapse number and size were quantified by a custom-written Matlab program (available upon request), which performs image thresholding, rolling ball background subtraction, puncta size selection, and recognizes colocalized signal from the Homer and Bassoon channels as synapses. The same thresholding settings were used for all images from each batch of experiment.

Electrophysiology

Whole-cell patch-clamp recordings from cultured RGC neurons were performed at room temperature in an isotonic saline solution (in mM: NaCl 125, $NaHCO_3$ 25, KCl 2.5, NaH_2PO_4 1.25, glucose 25, $MgCl_2$ 1, $CaCl_2$ 2). Patch electrodes with resistances of 2.5-3.5 MΩ were pulled from thick-walled borosilicate glass capillaries and were filled with an internal solution containing (in mM) potassium gluconate 130, NaCl 4, EGTA 5, $CaCl_2$ 0.5, 10 HEPES, MgATP 4, Na_2GTP 0.5 (pH 7.2 with KOH). Miniature excitatory postsynaptic currents (mEPSCs) were recorded in TTX (1μM, Alomone) from a holding potential of -70 mV. Series resistance was monitored throughout the recording and was <20 MΩ. Data were sampled at 50 kHz and filtered at 1 kHz using pClamp 9.2, and offline analysis of mEPSCs was performed using Clampfit 10.3 (Molecular Devices).

Synaptosome engulfment assay

Synaptosome purification and *in vitro* engulfment assays were performed as previously described (Chung et al., 2013). Briefly, synaptosomes were purified by percoll gradient from adult mouse brains and incubated with pHrodo Red, succinimidyl ester (Life Technologogies P36600). Human fetal and mature astrocytes were purified as above and grown for 7 days *in vitro*. After 7 days, the medium was replaced with either fresh medium supplemented with 5% serum (control), conditioned medium with synaptosomes, or fresh medium with

synaptosomes and 5% serum. After incubating the astrocytes at 37 °C for 3 hours, the cells were washed twice with PBS and lifted by trypsin digestion and gentle trituration. The cell suspension was then analyzed for pHrodoRed fluorescence with a BD Aria II sorter or LSR analyzer. For imaging of engulfed synaptosomes, astrocyte cytosol was labeled by a green fluorescent dye, Vibrant CFDA (Invitrogen V12883) per manufacturer's protocol after incubation with Phrodo Red labeled synaptosomes. Z-stacks of 0.38μm think optic sections were taken with a Zeiss LSM 710 inverted confocal microscope with environmental control chamber.

Morphology measurements

Human mature astrocytes were purified as above and rat mature astrocytes were purified as previously described (Foo et al., 2011) and plated to reach similar final density in the same growth medium. The cells were fixed and stained with anti-GFAP antibody at 5-6 div. Branches were traced with NIH ImageJ and the length and number of branches were quantified. To quantify territory size, we drew straight lines to connect the tips of neighboring branches and these straight lines form a polygon covering the territory of the cell. The area of the polygon was measured in ImageJ.

Calcium imaging

Purified human astrocytes were plated at 100,000 cells/imaging chamber (MatTek, P35GC-1.0-14-C) coated with poly-D-lysine. Cells were grown for at least 5 days before imaging. Cells were incubated for 15 minutes with 2μM Fluo 4 AM (Invitrogen, F-14201) and then washed 3 times with PBS and replaced with 1.5ml of growth medium per chamber. The cells were then allowed to recover from mechanical stimulation for 3 minutes and imaged in a humidified, temperature-controlled chamber with a 40x oil objective. Images were taken at 0.7s intervals. To quantify percentage of cells responding to ATP or glutamate stimulation, fluorescence levels were measured with ImageJ software. Cells with ΔF/F higher than 0.05 were counted as responsive. Intensity traces of cells were extracted using ImageJ and a custom Matlab script (available upon request) was used to determine the number of fluctuations during the duration of the imaging period.

RNA-seq library construction and sequencing

Total RNA was extracted using the miRNeasy kit (Qiagen) under the protocols of the manufacturer. The quality was assessed by Bioanalyzer. Samples with RNA integrity number higher than 8 were used for library construction. We used the Ovation® RNA-seq system V2 (Nugen 7102) to perform first and second-strand cDNA synthesis and SPIA amplification following the manufacturer's instructions, and fragmented cDNA with a sonicator (Covaris S2) using the following parameter: duty cycle 10%, intensity 5, cycles/burst 100, time 5 minutes. We then used the Next Ultra RNA-seq library prep kit for Illumina (NEB E7530) and NEBNext® multiplex oligos for Illumina® (NEB E7335 E7500) to perform end repair, adaptor ligation, and 5-6 cycles of PCR enrichment according to manufacturer's instructions. The quality of the libraries were then assessed by bioanalyzer and qPCR and high quality libraries were sequenced by the Illumina NextSeq sequencer to obtain 150bp pair-end reads.

RNA-seq read mapping, transcript assembly, and expression level estimation

We analyzed RNA-seq reads with the Galaxy web-platform (http://usegalaxy.org). The FASTQ files were first groomed using the FASTQ groomer and then mapped using TopHat2, which invokes Bowtie as an internal read mapper. The paired end option was selected and human genome version 19 (hg19) and mouse genome version 9 (mm 9) was used as the reference genome for the human and mouse RNA-seq data, respectively. We then ran Cufflinks to assemble transcripts and estimate expression level as fragments per

kilobase of transcript sequence per million mapped fragments (FPKM).\. We deposited the RNA-seq data in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, accession number GSE73721).

In situ hybridization

Full-length human cDNA expression plasmids were obtained for *GPR98* and *LRRC3B* (Dharmocon). Digoxigenin (DIG) labeled single-stranded antisense riboprobes were prepared by linearizing plasmids and transcription with T7, Sp6, or T3 RNA polymerases and an RNA labeling kit (Roche) according to the instructions of the manufacturer. Fresh frozen 12 µm thick brain sections were processed as previously described (Zhang et al., 2014), with the following modifications: after developing with Cy3 Tyramide Signal Amplification Plus kit (Perkin Elmer Life and Analytical Sciences), the slides were blocked with 10% donkey serum and 0.2% Triton X-100 in PBS at room temperature for 1 hour, incubated with anti-SOX9 antibody (1:250 dilute, R&D systems AF3075) at 4°C over night, washed 3 times with PBS, incubated with donkey anti-goat Alexa488 secondary antibody (1:1000 dilute, Life Technologies A11-055) for 90 minutes at room temperature, washed 3 times with PBS. Lipofuscin fluorescence was quenched by incubating the slides with 0.03% Sudan Black B (Sigma 199664) for 90-120 seconds.

PCR and qPCR validation of genes differentially expressed by human and mouse cells

We used mouse 10 week old adult whole brain cDNA (Zyagen, MD201) and cDNA generated from human adult whole brain RNA (Clontech, 636561) for PCR reactions. We designed primers spanning exon-exon junctions to avoid amplification of genomic DNA. The primers used are listed in Table S9. We performed 32 cycles of amplification for all samples. Amplification of the house-keeping gene *GAPDH* from human and mouse had similar yields, indicating that similar amount of human and mouse cDNA were loaded in the reactions (Figure S6). For the qPCR data, astrocytes were purified from mouse and human brain tissue according to the above immunopanning protocols. The specificity and efficiency of all primers were first validated using gel electrophoresis and qPCR. The determination of each gene's C_T in qPCR was performed in triplicates. When determining fold changes in gene expression across tissue types, the C_T of each gene was normalized according to the C_T of the housekeeping gene GAPDH in the same tissue.

Analysis of genes differentially expressed by mouse and human astrocytes

Since ortholog information is variable in different databases, we only included human and mouse genes sharing the same gene symbols for our cross-species analysis. All processed human and mouse RNA-seq data are available in Table S4 and the raw data have been deposited in GEO and can be further analyzed for orthologous genes not included in our analysis. We compared FPKM levels of genes in human and mouse astrocytes without further normalization. We combined data obtained from human patients in the age range of 8-63 years and combined data from mice at 1, 4, 7, and 9 months of age. For comparison of astrocyte-enriched genes in human and mouse (Figure 4D, Venn diagram), we selected genes meeting the following two criteria: i) expressed at FPKM=5 or higher, and ii) 4 fold or higher enriched in astrocytes over the average expression of this gene in all other cell types. For human genes, we used human neuron, oligodendrocyte, microglia, and endothelial cell transcriptome data reported here. For mouse genes, we used data from other cell types that we have previously published (Zhang et al., 2014). We then determined the number of astrocyte-enriched genes shared by human and mouse and the number of astrocyte-enriched genes meeting the two criteria above in only one species.

To generate the list of top astrocyte-enriched genes shared by humans and mice, and those only present in human or mouse astrocytes (Table 2), we defined shared astrocyte genes as genes expressed at FPKM=0.5 or higher by both human and mouse astrocytes and enriched by at least 4 fold over the average expression in neurons, oligodendrocytes, microglia/macrophages, and endothelial cells. We ranked the shared astrocyte genes by average expression and listed the top 20 genes in Table 2. Species-specific astrocyte genes meet the same criteria but have FPKM values < 1 in astrocytes from the non-enriched species. We ranked species-specific astrocyte genes by fold enrichment and listed the top 20 genes in Table 2.

Analysis of genes differentially expressed by fetal and mature human astrocytes

To analyze genes expressed by both fetal and mature human astrocytes or those expressed only at one stage (Figure 5A), we counted the number of genes expressed at FPKM = 0.5 or higher at one or both of the two stages. To select genes differentially expressed by fetal and mature human astrocytes (Table 1), we ranked genes by the ratio of the average expression in mature astrocytes (8-63 years old) over the average expression in fetal astrocytes. We excluded genes with FPKM<5 in both fetal and mature stages to focus on highly expressed genes likely to be useful markers for mature or fetal astrocytes. We also excluded genes with >2 fold higher expression in another cell type other than astrocytes to remove genes from contaminating cell types.

Pre/Post dissociation transcriptome analysis

The pre-dissociation brain sample was immediately frozen in liquid nitrogen. Post-dissociation sample was obtained by performing the same papain dissociation as in an astrocyte purification experiment, letting it sit at room temperature (panning temperature) for the duration of a typical purification timeline (within about 5 hours from tissue resection), and freezing in liquid nitrogen. We prepared RNA with Invitrogen miRNeasy kit per manufacturer's instructions, and used Affymetrix Human Genome U133, 2.0 plus microarray to examine gene expression. The .cel files from the arrays were analyzed with Arraystar 4.0 software (DNAstar) using robust multichip average processing and quantile normalization. Genes up- or down-regulated more than 4-fold after dissociation were identified and flagged. Subsequently, each gene in a main and supplemental table was cross-checked and did not contain genes from this list.

Contamination estimation

To evaluate the type and level of contamination in each of the immunopanning purified cell types, we probed the RNA-seq data for classic cell type specific markers. For example, we estimated the level of neuron contamination in astrocyte samples by dividing the average FPKM of neuron marker gene expression in astrocytes by that in neurons. Cell type specific markers used in the analysis include: GFAP, ALDH1L1, AQP4, GJA1, and SOX9 for astrocytes, STMN2, SYN1, SYT1, GAD1, and CCK for neurons, MOG, MOBP, SOX10, PLP1, and GPR37 for oligodendrocytes, CCL2, CCL3, TLR2, CX3CR1, and IL1A for microglia/macrophage, and CLDN5, ELTD1, ESAM, ICAM2, and APOLD1 for endothelial cells.

Reactive astrocyte gene expression analysis

Reactive astrocyte genes were identified from the Zamanian mouse reactive astrocyte dataset (Zamanian et al., 2012) using 4-fold induction as the threshold. Genes induced by both ischemia and LPS, only by ischemia, and only by LPS were ranked by fold induction and the 30 genes with highest fold induction from each of the three categories were used as reactive astrocyte marker genes. To compare our RNA-seq data to existing transcriptome

data of reactive astrocytes and MD astrocytes obtained by microarray (Malik et al., 2014; Zamanian et al., 2012), we performed quantile normalization with the normalize.quantiles function in the preprocessCore Bioconductor package in R to make the distribution of the two datasets identical in statistical properties. We then used the normalized data from each sample to calculate the average expression of the top 30 reactive astrocyte genes induced by both LPS and MCAO or those induced only by one condition.

Construction of clustering dendrograms

Dendrograms were generated in Matlab using Spearman correlations, "Average" linkage", and no Optimal Leaf Order. Heatmap colors are based upon standardization across rows and scales represent standard deviations above or below mean. Dendrograms were created with all genes that have an average FPKM expression of 5 or higher across samples. Statistical significance of dendrograms was assessed using the PVCLUST package in R with a Spearman correlation and identical filtering settings.

Statistical Analysis.

To determine appropriate statistical tests for each experiment, we first assessed normality of each dataset by the Shapiro-Wilk test. For normally distributed datasets (p-values > 0.05 by Shapiro-Wilk test) including Figure 2 C, H, S1A, S2C, S3B, we used two-tailed unpaired t test. For non-normally distributed datasets (p-values <0.05 by Shapiro-Wilks test), we used the non-parametric one-tailed Wilcoxon rank sum test for Figure 2E and two-tailed Wilcoxon rank sum test for Figure 2K, 3D, S1A, C, S2A, S3A, S3C. We used Kolmogorov-Smirnov test for Figure 2J.

Detailed protocol for the purification of human astrocytes

Day Before

Prepare panning dishes:
Set up panning plates in 15cm petri dishes. 25ml of 50mM Tris-HCl pH 9.5 per dish.
1x CD45 plate: 60µl anti-rat IgG
3x GalC plate: 60µl anti-mouse IgG
2x O4 plate: 60ul anti-mouse IgM m-chain specific
1x Thy1 plate: 60µl anti-mouse IgG
1x HepaCAM plate: 60µl anti-mouse IgG
1x BSL plate: 50ug BSL-1

Prepare coverslips (this can also be done on the day of prep):
Wash coverslips once with sterile distilled water, transfer coverslips into 24 well plates, add 10ug/ml poly-D-lysine to each well, incubate at room temperature for 30 minutes, wash 3 times with water, and aspirate residual water to dry.

Day of prep

Solutions to Prepare:
*20ml x1 enzyme stock + Papain (400 units for postnatal and 150 units for fetal tissue) + 0.0032-0.0040g L-cysteine
**21ml x 2 inhibitor stock + 1.5ml Low Ovo + 100µl DNase
**10ml x1 inhibitor stock + 2ml High Ovo + 20µl DNase
***60ml x 1 0.2% BSA: 57ml dPBS + 3 ml 4% BSA + 60µl DNase
***50ml x 1 0.02% BSA: 45ml dPBS + 5ml of 0.2% BSA + 50µl DNase

1. Aliquot 20ml of enzyme stock* into a 50ml Falcon tube, break 2ml pipette, attach filter on

top, bubble CO_2 through until solution turns from red to orange, and put into 34°C water bath.

2. Aliquot and bubble 2x21ml and 1x10ml inhibitor stock** as in 1.
3. Wash each panning dish with PBS 3x then add the following antibodies: 20µl CD45 in 12ml of 0.2% BSA***, 4ml GalC in 8ml of 0.2% BSA, 4ml O4 in 8ml of 0.2% BSA, 20ul Thy1 in 12ml of 0.2% BSA, and 15ul HepaCAM in 12ml of 0.2% BSA. The BSL plate does not need additional antibodies.
4. Add papain to enzyme stock* bubbled with CO_2 and add 0.0036-0.0042g of L-cysteine. Warm up solution mixture in 34°C water bath at least 15 minutes before digestion.
5. Dissect brains in dPBS in 6cm petri dishes, cut out grey matter, remove meninges and blood clots, use No. 10 scalpel blade to chop brains into < $1mm^3$ pieces. Put ~0.5g of tissue into each 6cm petri dish and use multiple petri dishes for digestion if there are more than 0.5g of tissue.
6. Use 0.22mm filter to filter and discard 2 ml of enzyme stock, then filter 10ml into each petri dish containing finely chopped brain pieces. Add 100 µl DNase to each petri dish and swirl dish to mix.
7. Papain digestion: Put the petri dish on a 34 °C heat block, drill a ~0.5 cm diameter hole into the lid of the 6 cm petri dish with heated forceps, attach tubule from a CO_2 tank to a 0.22mm filter and put the filter tip into the hole so CO_2 flows over the enzyme stock solution with brain pieces. Shake the petri dish every 15 minutes. Digest for 100 minutes for postnatal tissue and 45 minutes for fetal tissue.
8. Equilibrate 20ml of 30% FCS and 8ml of EBSS in the incubator
9. After digestion, put digested brains into a Universal tube, wait for tissue to settle, aspirate supernatant, add 4.5ml of Low Ovo to cells to wash, wait for tissue to settle, repeat for a total of 4 washes.
10. Triturate. Add 4ml of Low Ovo into the Universal tube, suck up brain and Low Ovo solution with a 5ml serological pipette quickly and release quickly, repeat for 20-40 times. Be careful not to introduce bubbles. Do not lift 5ml pipette out of solution during solution to minimize introduction of air into the solution. Low Ovo will become cloudy, let brain chunks settle. Transfer single cells with a 1ml pipette to a Falcon tube, this is the cloudy solution on top of the chunks. Add to 4ml of Low Ovo to the Universal tube and repeat trituration. Grey matter will dissociate faster than white matter. Stop trituration when all the visible brain pieces left are white matter (white color).
11. Count cells by diluting it 1:1 with Trypan Blue. For postnatal human tissue, expect 2M brain cells from each gram of brain tissue. There will be lots of debris, plenty of red blood cells, and sparse brain cells. Do not count the red blood cells (they are small and bright). Expect much higher yield and no debris from fetal brain tissue.
12. Carefully use a 10ml pipette to layer 10ml of High Ovo under the single cell suspension. This should lead to a clear layer of liquid beneath a cloudy cell suspension.
13. Spin cells down through High Ovo at 100g for 5mins.
14. Aspirate liquid, one should see a pellet of cells at bottom of Falcon tube.
15. Resuspend cell pellet gently with 9ml of 0.02% BSA***
16. Filter cell suspension through Nitex mesh to remove chuncks.
17. Wash each panning dishes with 3x dPBS immediately before use.
18. Add cell suspension to CD45 plate and incubate at room temperature for 15 minutes. Examine the panning dish under a DIC microscope. If cells start to cluster, triturate gently with a 10ml serological pipette. Transfer the cells to the next panning plate either after the suggested time in this protocol or when visual examination of the plate indicates there are lots of cells stuck.
19. Shake the CD45 plate and transfer cell suspension to a GalC plate. Then use 1ml 0.02% BSA to wash the CD45 plate and collect the 1ml of solution from the plate and add to the

GalC plate. Incubate for 10 minutes.
20. Transfer to the second GalC plate.
21. Transfer to the third GalC plate.
22. Transfer to an O4 plate.
23. Transfer to the second O4 plate.
24. Transfer to the Thy1 plate.
25. Transfer to the HepaCAM plate.
26. Note: Postnatal tissue: for harvesting cell for RNA-seq or any other experiment that requires maximum purity, go through all the panning steps as described above. For harvesting cells for cell culture, perform a shortened panning procedure to ensure maximum survival. Pass the cells through 2 GalC plates and then to the HepaCAM plate. Expect to see myelin debris on the HepaCAM plate. However, myelin debris will not stick to PDL-coated coverslips. Change medium at 1div to wash away floating myelin debris. Fetal tissue: perform a shortened panning procedure. Pass the cells through CD45, Thy1, and HepaCAM plates. Due to predominantly neuronal cell type distribution at this age, neuronal contamination is more prevalent in these HepaCAM purified cells than those purified from adult brains.
27. Wash positive selection plate, ~8 times or until floating contaminating cells are gone with dPBS. For RNA-seq, scrape cells off with Qiazol reagent (Qiagen). For cell culture, go to the next step.
28. Add 200 units of trypsin to 8ml of equilibrated EBSS, incubate at 37°C for 3-15 minutes. Since the activity of different lots of trypsin can vary, it is important to determine the duration of trypsin digestion empirically. Take the plate out of the incubator after 3 minutes, tap side of the plate, and look under the microscope. Incubate for longer if most cells are still stuck and stop the digestion if about half of the cells are dislodged.
29. Squirt gently around the plate with 10ml of 30% FCS. Go through every part of the plate. Suck off dislodged cells and add to a 50ml Falcon tube.
30. Add another 10ml of 30%FCS to squirt if there are many cells left after the first round of squirting. Add cells to the Falcon tube.
31. Count cells.
32. Add 100 µl of DNase per 10ml of solution and spin cells down at 130g for 10 minutes.
33. Aspirate supernatant and resuspend cell pellet in growth media.
34. Pre-plate cells in 50 µl of media onto the center of coverslips. Gently transfer to the incubator, leave for 20 minutes, and carefully add on 450 µl growth medium per 24-well plate well.
35. To purify endothelial cells, add the flow through after the HepaCAM plate to the BSL plate. Incubate at room temperature for 30 minutes and harvest cell as described above.

Reagents

1x Earle's balanced salt solution (EBSS, Sigma E7510)
ACLAR plastic coverslips (Washed in 10% nitric acid over night on a shaker at room temperature and then in washed in water 5 times, 30 minute each, and in 75% ethanol once. Store in 75% ethanol.)
BDNF (Peprotech, 450-02)
Bovine serum albumin (Sigma, A4161)
BSL-1 (Banderiaea simplicifolia lectin 1; L-1100, Vector laboratories)
Ciliary neurotrophic factor (CNTF, Peprotech 450-13)
Dulbecco's modified eagle medium (DMEM, Invitrogen, 11960-044)
Dulbecco's PBS (dPBS) Gibco
0.4% DNAse, 12,500 units/ml (Worthington, LS002007)
Fetal calf serum (FCS, Gibco, 10437-028)
Foskolin (Sigma F6886)

GalC hybridoma supernatant
Goat anti-mouse IgG+IgM (H+L) (Jackson ImmunoResearch, 115-005-044)
Goat anti-rat IgG (H+L) chain (Jackson ImmunoResearch, 112-005-167)
Goat ant-mouse IgM m-chain (Jackson ImmunoResearch, 115-005-02)
HBEGF (Sigma E4643)
Insulin (Sigma I-6634)
L-cysteine hydrochloride monochloride (Sigma, C7880)
L-glutamine (Invitrogen, 25030-081)
Mouse anti HepaCAM (R&D systems, MAB4108)
Mouse anti human Thy1 (CD90) antibody (BD, 550402)
N-Acetyl-L-cysteine (NAC, Sigma, A8199)
Neurobasal (Gibco, 21103-049)
Nitex mesh (Tetko Inc, HC3-20)
NS21-MAX (R&D systems, AR008)
O4 hybridoma supernatant (mouse IgM)
Papain (Worthington, LS 03126)
Penicillin / streptomycin (Invitrogen, 15140-122)
Poly-D-Lysine (Sigma, P6407)
Rat anti-mouse CD45 (BD Pharmingen, 550539)
SATO (See below)
Sodium Pyruvate (Invitrogen, 11360-070)
3,3′,5-Triiodo-L-thyronine sodium salt (T3, Sigma T6397)
Trypsin 30,000 units/ml stock (Sigma, T9935)

Solutions Required

Enzyme Stock Solution

Final Volume = 200ml

Component	Volume	Final Concentration
10x EBSS	20ml	
30% D(+)-Glucose	2.4ml	0.46%
1M $NaHCO_3$	5.2ml	26mM
50mM EDTA	2ml	0.5mM
ddH_2O	170.4ml	

Bring to 200ml with ddH2O and filter through 0.22mm filter

Inhibitor Stock Solution

Final Volume = 500ml

Component	Volume	Final Concentration
10x EBSS	50ml	
30% D(+)-Glucose	6ml	0.46%
1M $NaHCO_3$	13ml	25mM
ddH_2O	431ml	

Bring to 500ml with ddH2O and filter through 0.22mm filter

Low Ovo (10X)

To 150 mL D-PBS, add 3g BSA (Sigma A8806). Mix well. Add 3g Trypsin inhibitor (Worthington LS003086) and mix to dissolve. Adjust pH to 7.4; requires the addition of approx. 1mL of 1N NaOH. When completely dissolved bring to 200mL with DPBS and filter through 0.22 µm filter. Make 1.0 mL aliquots and store at -20˚C.

High Ovo (10x)

To 150 mL D-PBS add 6g BSA (Sigma A8806). Add 6g Trypsin inhibitor (Worthington LS003083) and mix to dissolve. Adjust pH to 7.4; requires the addition of at least 1.5mL of 1N NaOH. If necessary, add NaOH until solution no longer too acidic. Bring to 200mL with

DPBS. When completely dissolved, filter through 0.22 µm filter. Make 1.0 mL aliquots and store at -20˚C.

Sato (100X)

To prepare:

Add the following to 80 mL Neurobasal medium:	Final conc.
800 mg transferrin (Sigma T-1147)	100 µg/mL
800 mg BSA	100 µg/mL
128 mg putrescine (Sigma P5780)	16 µg/mL
20 µl progesterone (Sigma P8783) (from stock: 2.5 mg in 100 µl EtOH)	60 ng/mL (0.2 µM)
800 µl sodium selenite (Sigma S5261) (4.0 mg+10 µl 1N NaOH in 10 mL NB)	40 ng/mL

*Do not reuse progesterone and Na selenite stocks; make fresh each time.

Mix well and filter through pre-rinsed 0.22 µm filter.

Make 200µl or 800µl aliquots; store at -20˚C

Astrocyte growth media

50% Neurobasal
50% DMEM
100 units/ml of penicillin
100 µg/ml streptomycin
1mM Sodium Pyruvate
2mM L-glutamine
1x SATO
5µg/ml NAC
5ng/ml HBEGF

Neuron growth medium for synapse formation experiments

For rat retinal ganglion cells and human fetal neurons
16mL DMEM
4mL dH20
200µL 0.5mg/ml Insulin
200µL 100mM Pyruvate
200µL 100x Penicillin / Streptomycin
200µL 200mM L-Glutamine
200µL 100x Sato
200µL 4µg/ml Thyroxine (T3)
400µL NS21-Max
20µL 5mg/ml NAC
Filter sterilize
Immediately before use add 20µL per 20mL each: 4.2mg/ml forskolin, 50µg/ml BDNF, 10µg/ml CNTF

Neuron growth medium for neuron survival experiments

Omit NS21-Max, BDNF, and CNTF from the neuron growth medium for synapse formation experiments.

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FIGURE S1

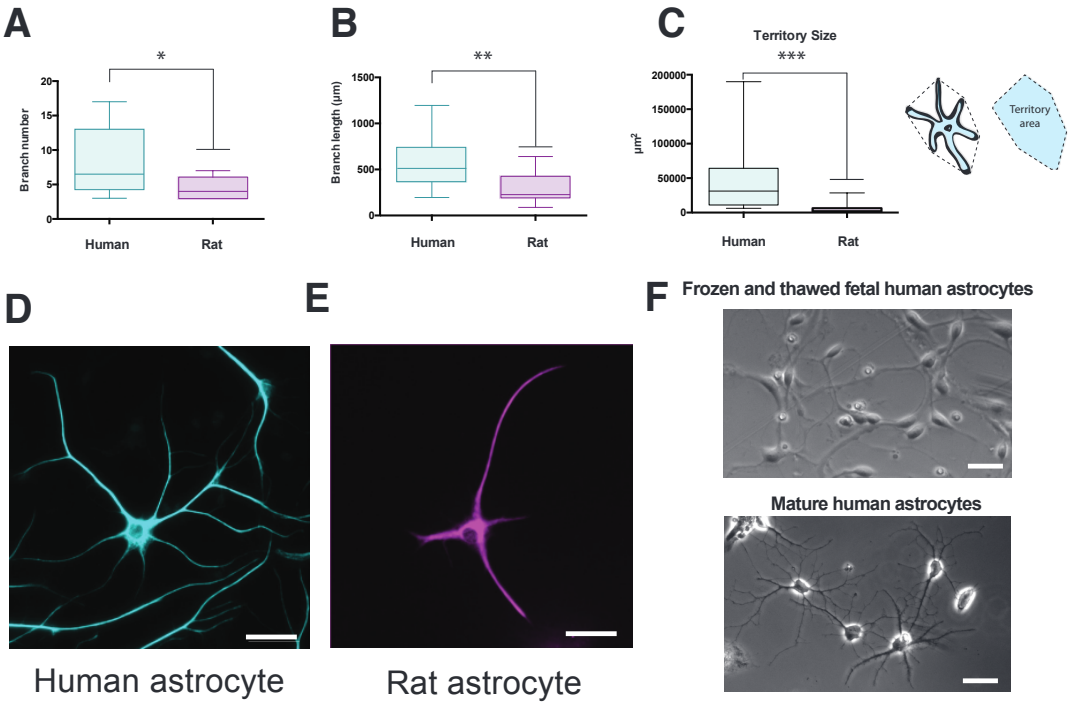

A
*
Branch number
20
15
10
5
0
Human
Rat
B
**
Branch length (µm)
1500
1000
500
0
Human
Rat
C
Territory Size
***
µm²
200000
150000
100000
50000
0
Human
Rat
Territory area
D
Human astrocyte
E
Rat astrocyte
F
Frozen and thawed fetal human astrocytes
Mature human astrocytes

FIGURE S2

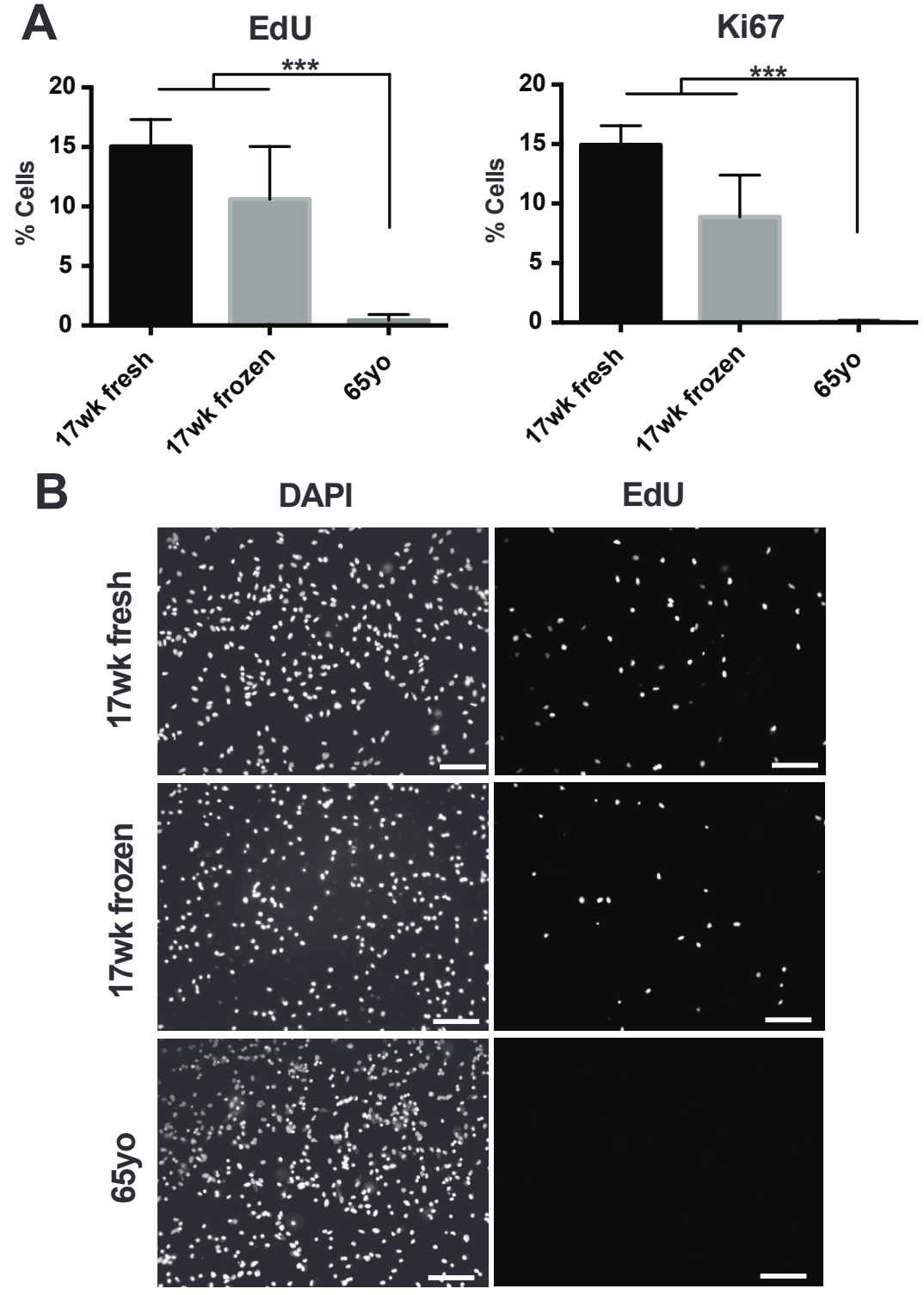

A
EdU
Ki67
***
***
% Cells
20
15
10
5
0
17wk fresh
17wk frozen
65yo
B
DAPI
EdU
17wk fresh
17wk frozen
65yo

FIGURE S3

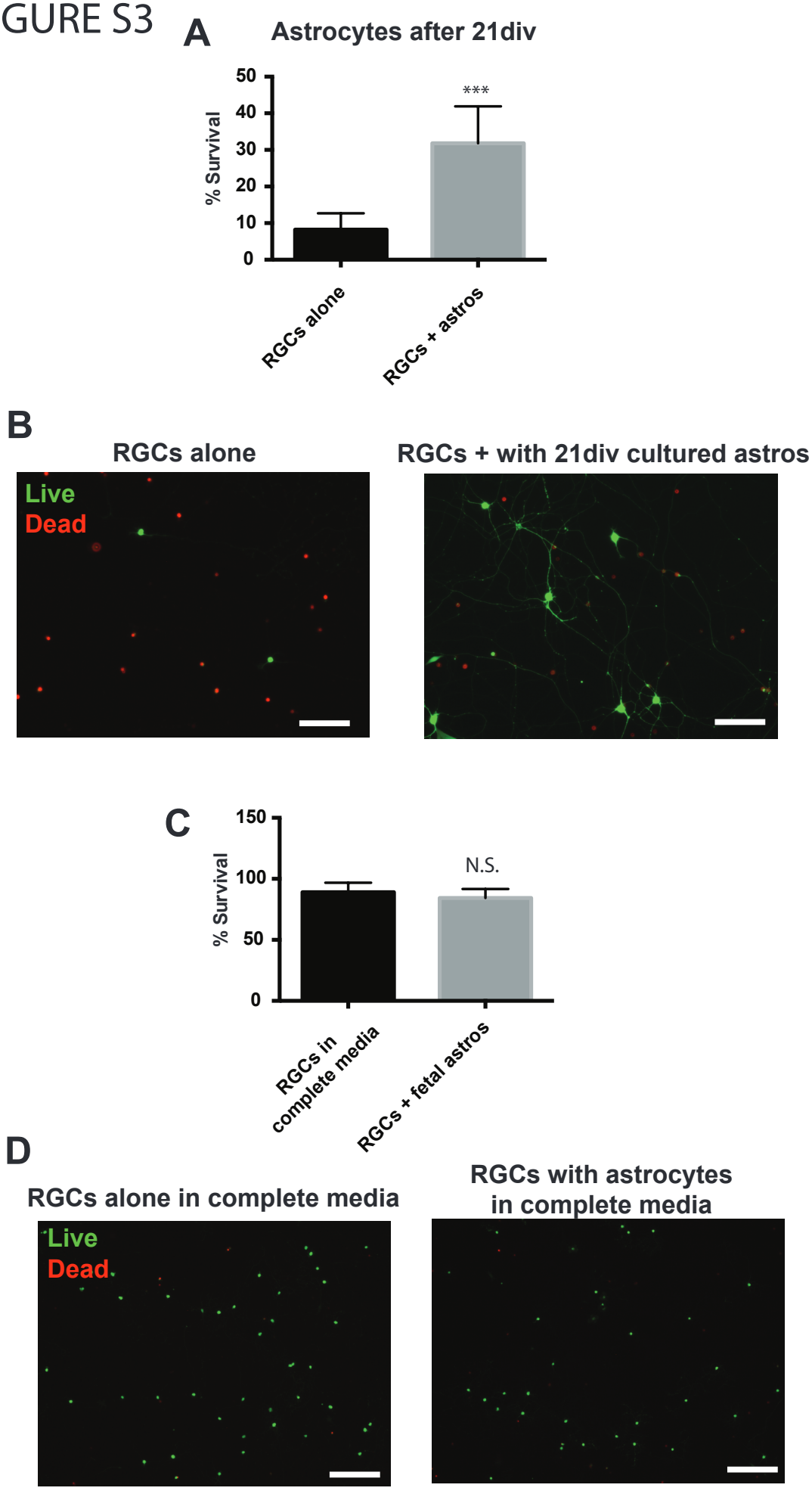
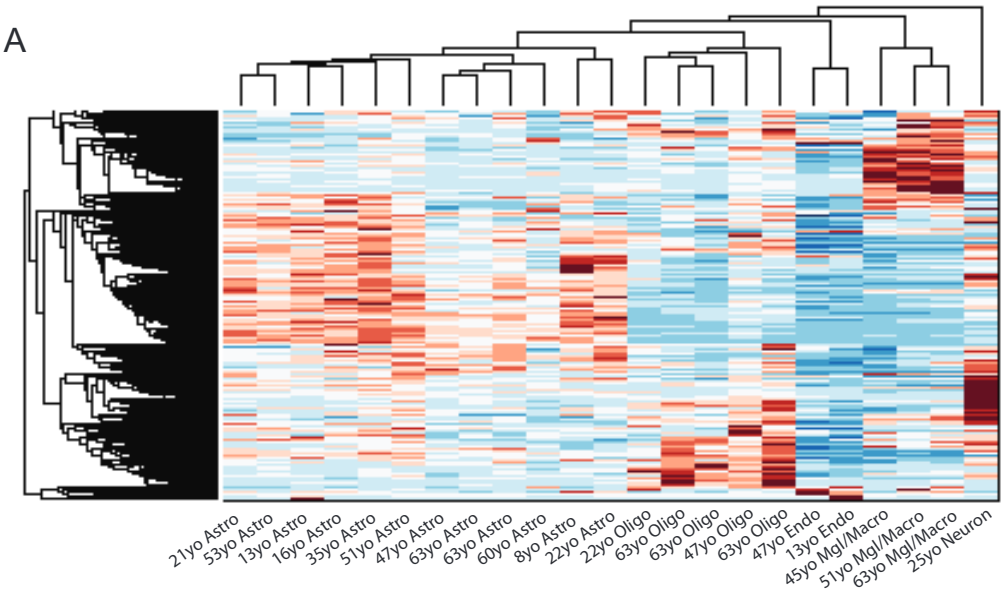
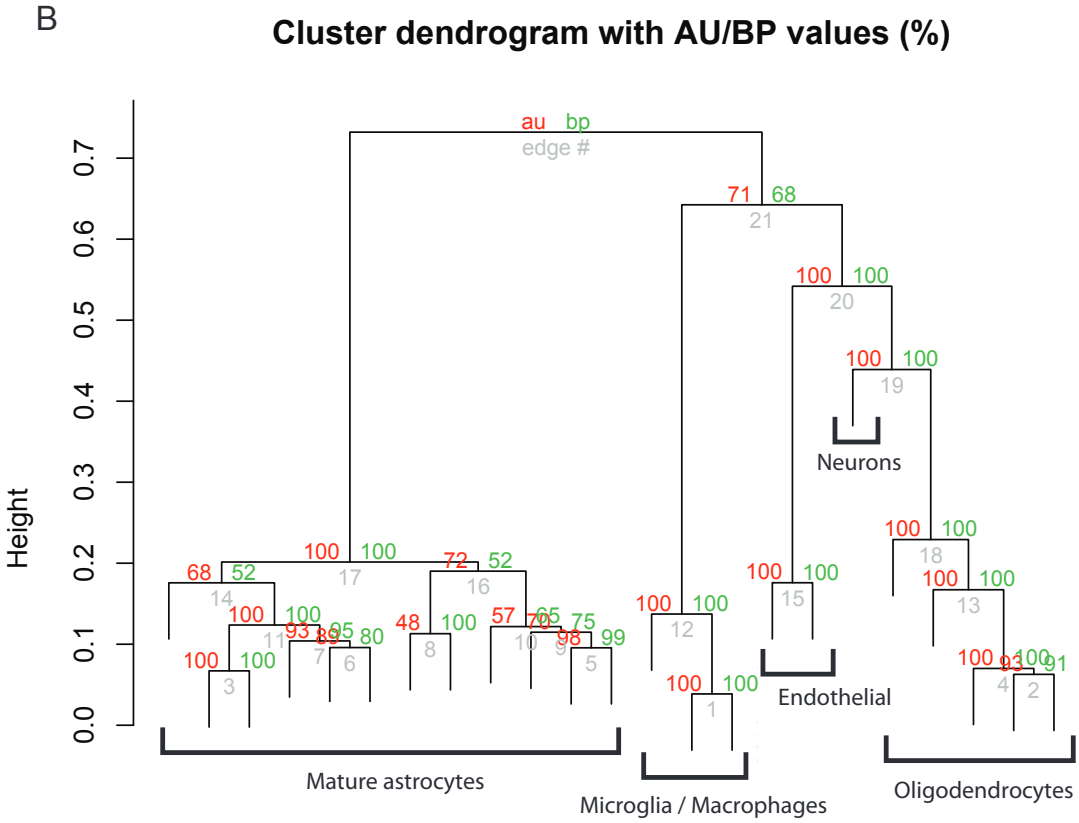

A
Astrocytes after 21div
% Survival
50
40
30
20
10
0
***
RGCs alone
RGCs + astros
B
RGCs alone
RGCs + with 21div cultured astros
Live
Dead
C
% Survival
150
100
50
0
N.S.
RGCs in complete media
RGCs + fetal astros
D
RGCs alone in complete media
RGCs with astrocytes in complete media
Live
Dead

Figure S4

A



B



C

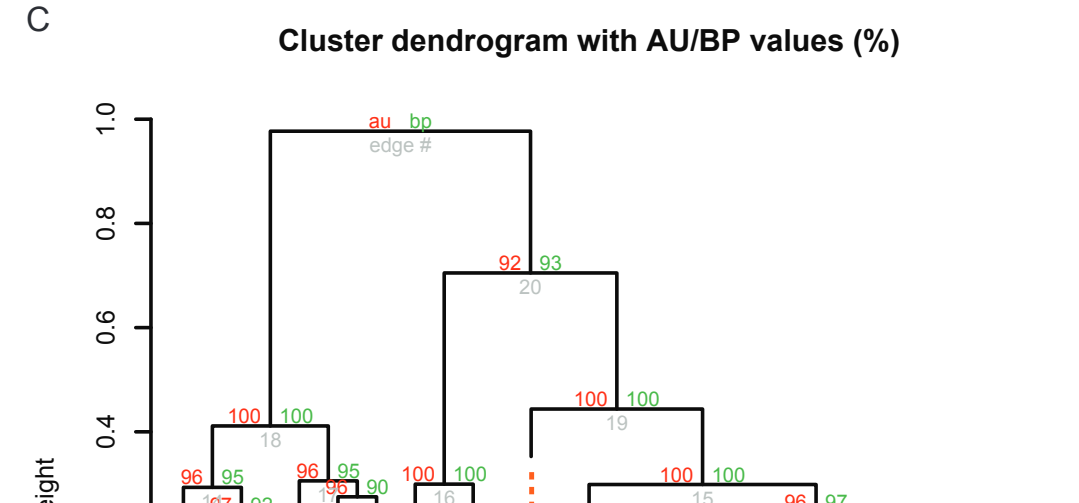
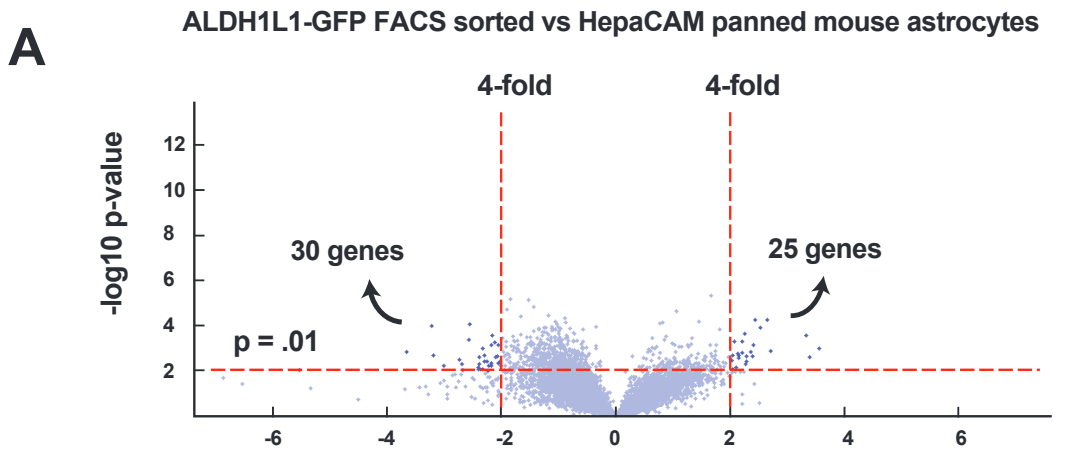
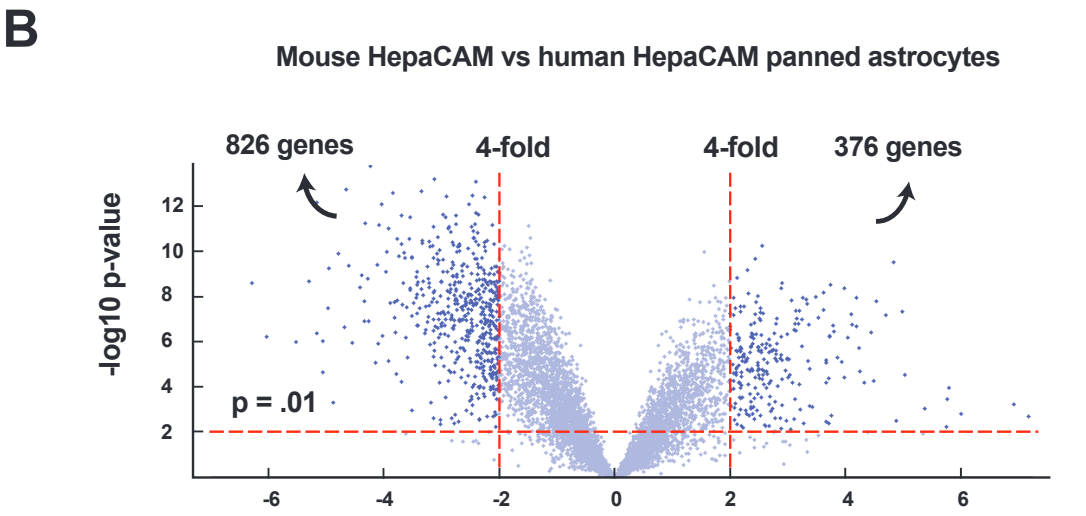
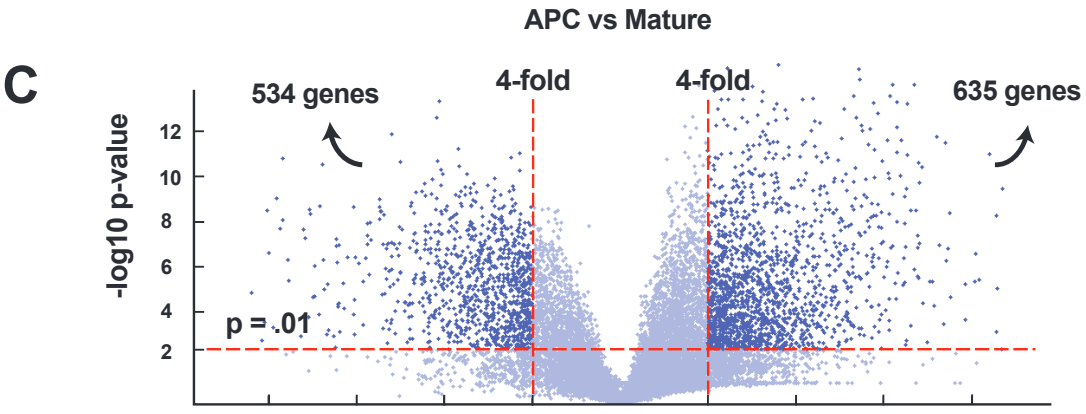
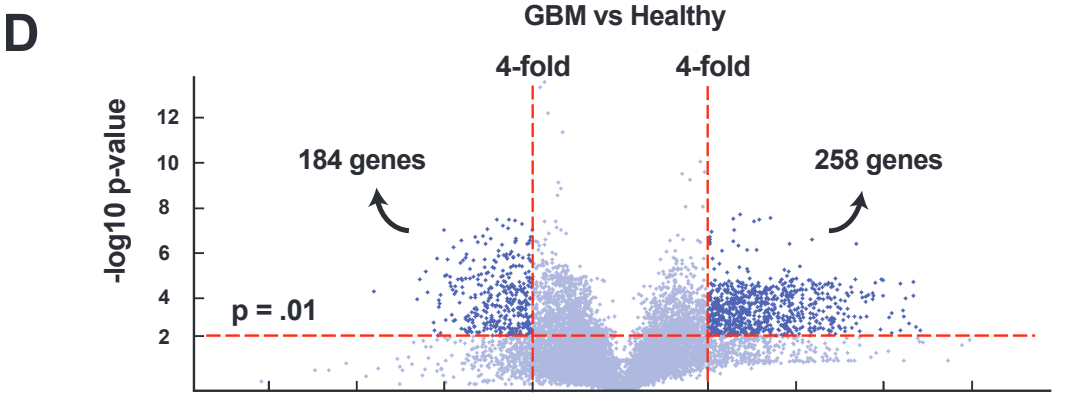


FIGURE S5


A
ALDH1L1-GFP FACS sorted vs HepaCAM panned mouse astrocytes
4-fold
4-fold
-log10 p-value
30 genes
25 genes
p = .01


B
Mouse HepaCAM vs human HepaCAM panned astrocytes
826 genes
4-fold
4-fold
376 genes
-log10 p-value
p = .01


C
APC vs Mature
534 genes
4-fold
4-fold
635 genes
-log10 p-value
p = .01


D
GBM vs Healthy
4-fold
4-fold
184 genes
258 genes
-log10 p-value
p = .01


E
Epilepsy vs Healthy

Figure S6

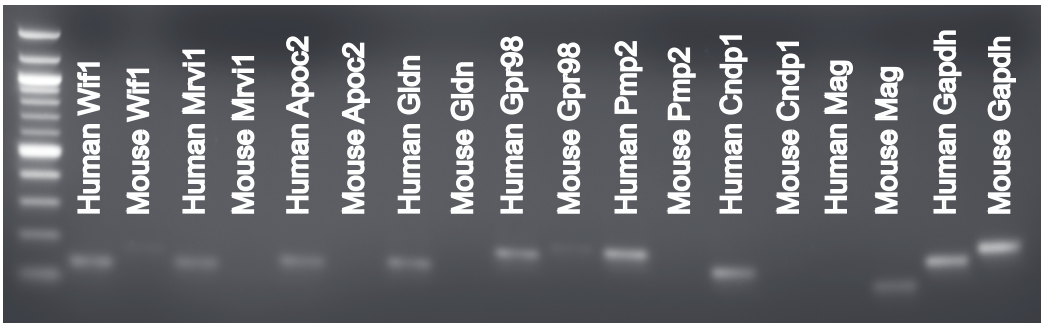

Human Wif1
Mouse Wif1
Human Mrvi1
Mouse Mrvi1
Human Apoc2
Mouse Apoc2
Human Gldn
Mouse Gldn
Human Gpr98
Mouse Gpr98
Human Pmp2
Mouse Pmp2
Human Cndp1
Mouse Cndp1
Human Mag
Mouse Mag
Human Gapdh
Mouse Gapdh

Table S1. Patient information

Specimen ID	Age (year)	Diagnosis	Brain region
1	8	Epilepsy	Anterior temporal lobe cortex
2	13	Epilepsy	Anterior temporal lobe cortex
3	16	Epilepsy	Anterior temporal lobe cortex
4	21	Epilepsy	Anterior temporal lobe cortex
5	22	Epilepsy	Anterior temporal lobe cortex
6	25	Epilepsy	Anterior temporal lobe cortex
7	35	Epilepsy	Anterior temporal lobe cortex
8	45	Epilepsy	Anterior temporal lobe cortex
9	47	Epilepsy	Anterior temporal lobe cortex
10	51	Epilepsy	Anterior temporal lobe cortex
11	53	Epilepsy	Anterior temporal lobe cortex
12	60	Epilepsy	Anterior temporal lobe cortex
13	63	Epilepsy	Anterior temporal lobe cortex
14	63	Epilepsy	Anterior temporal lobe cortex
15	21	Epilepsy	Hippocampus
16	22	Epilepsy	Hippocampus
17	53	Epilepsy	Hippocampus
18	53	Epilepsy	Hippocampus
19	59	Glioblastoma	Cortex, tumor core
20	59	Glioblastoma	Cortex, tumor peripheral region
21	64	Glioblastoma	Cortex, tumor core
22	65	Glioblastoma	Cortex, tumor core

Table S3. Estimation of contamination

Estimation of contamination		Purified cell type				
		Astrocyte	Neuron	Oligodendrocyte	Microglia	Endothelial
Contaminating cell type	Astrocyte		5.7%	2.4%	0.3%	2.0%
	Neuron	0.6%		1.3%	0.1%	2.9%
	Oligodendrocyte	3.1%	1.6%		0.6%	0.9%
	Microglia	0.8%	1.0%	5.1%		0.5%
	Endothelial	0.5%	1.5%	0.4%	0.8%	

Table S7 Comparison of the expression of temporal lobe enriched or depleted genes by human and mouse astrocytes

Gene symbol	Temporal lobe vs. other cortical areas		Human vs. Mouse	
	Fold change	P value	Fold change	P value
Klk8	2.670	0.000	0.260	0.016
Ptger3	2.514	0.000	1.000	1.000
Mgp	2.436	0.000	1.212	0.689
Kctd4	2.422	0.000	0.407	0.001
Pdyn	2.307	0.000	1.368	0.250
Loc100129291	2.303	0.000	NA	NA
Capsl	2.166	0.000	0.202	0.025
Dusp13	2.123	0.000	1.008	0.496
C1orf187	2.027	0.000	NA	NA
Met	2.015	0.000	0.921	0.425
Akap14	2.011	0.000	1.133	0.493
Ctxn3	0.350	0.000	0.524	0.122
Onecut2	0.414	0.000	1.452	0.035
Pvalb*	0.465	0.000	0.240	0.006
Hapln4*	0.481	0.000	0.342	0.007

Table S8 Cell type-enriched expression of some human genes without mouse orthologs

Gene	Expression (FPKM)					Fold enrichment	Enriched cel
	Astrocyte	Neuron	Oligodendrocyte	Microglia/macrophage	Endothelial cells		
A2ML1	3.7	0.1	0.1	0.1	0.2	26.7	Astrocyt
LOC100507206	5.3	0.9	0.1	0.1	0.3	15.5	Astrocyt
LOC100130894	2.7	0.1	0.1	0.1	0.1	22.8	Astrocyt
UG0898H09	18.6	3.6	1.4	0.1	0.5	13.4	Astrocyt
LOC100506795	2.1	0.3	0.1	0.2	0.1	11.5	Astrocyt
LOC100499183	2.6	0.3	0.4	0.1	0.1	11.8	Astrocyt
ZNF204P	0.3	10.6	0.8	0.4	0.4	22.3	Neuron
RFPL1-AS1	0.4	7.3	0.2	0.5	0.2	21.5	Neuron
LOC728730	0.4	4.2	0.2	0.1	0.2	17.9	Neuron
PAR5	7.3	74.6	6.6	1.8	2.8	16.1	Neuron
TCEAL2	23.2	147.8	25.7	1.2	4.0	10.9	Neuron
FAM106CP	0.6	4.9	0.5	0.6	0.2	10.6	Neuron
GHRLOS2	1.0	0.6	0.4	9.4	0.8	13.5	Microglia/macr
LOC100130357	0.3	0.4	0.4	3.9	0.2	12.2	Microglia/macr
GGTA1P	3.2	1.1	2.5	21.4	0.9	11.2	Microglia/macr
CECR1	5.1	0.8	5.2	36.2	3.1	10.2	Microglia/macr

Table S9. Primer sequences for validation of differentially expressed genes in human and mouse.

Gene		Human Primer	Mouse primer
WIF1	Forward	CCAGGACTAGAGGGAGAGCA	GTGAACTCAGCAAATGCCCC
	Reverse	TCGCAGACAGGCTTTGAACA	CTCTCGACACTGGCACTTGT
MRVI1	Forward	CCATCAGCTGTTTCCCCGAA	TCGCCTGATCTCTGGTGTTG
	Reverse	TTCTGCTGTGGTGGTGCTC	CTGGGGAGATGCCAAAAGGA
APOC2	Forward	TTGGGATTTGAGGTCCAGGG	CATGGGGTCTCGGTTCTTCC
	Reverse	CTACAGCGGGCAGGTATGTC	CTTAAGAGGGAGCCCTGCAC
GLDN	Forward	GCTCAGCTCGCAACAAGCG	CGCCTTTCTGGCCGAATTGA
	Forward	CAGAAGGTCCTGTGAGGCAGA	ATCACTCGAATCGGCACCAT
GPR98	Forward	GGGATGCCCTCTGCATCTTT	TAGTCTGCAGGGACTTTATGTTT
	Reverse	CGTCCTCTCCATACAGCGAT	TTTCCCAGTCAGATGGCACC
PMP2	Forward	ATGAAAGCTCTGGGTGTGGG	AAGTGCAGAAGTGGGATGGG
	Reverse	TCCTCTCTGCAGGGTTACGA	CCAGACAAGCCAGCTACAGA
CNDP1	Forward	GACGGAGGTAGACGGGAAAC	TCAGCAGATGCCTGATGGTC
	Reverse	AAGTTCCTCCAGGGCAACAG	CCCCGTCCATACAGTTTCCC
MAG	Forward	ACTGAGTCCAAGTTGTCTGGC	CACCAAGGACAGCTACACCC
	Reverse	AAGCCTTTAGGTCCCCAGCA	AAGAGAAAGGGAGCCCACAG
WIF1	Forward	TGTCAACAAGCTGAGTGCCC	ACTGTGAGAAAGCCCTGTGC
	Reverse	CAGGTGGTTGAGCAGTTTGC	TTCCCGGGTAAAAGCAGGTC
AGXT2L1	Forward	TGCCCATGGAGTGGTCAAAG	CAGGCGTAAGGCTAAGTCGT
	Reverse	TGCTGACTTGCGTCCCAG	TGCAGTCTAAGTACCGTTCGC
HSD17B	Forward	AGCAAGTCCGAGAAGGAAGC	AGACTGAAGGAAGACCCGAG
	Reverse	CACTCTCAAGCCTCGTGCAT	ATGAAGAAATGTCTGTCTGTGATG
GPRC5B	Forward	GCGTTGGTGACTTCGAGAGG	CACGAACATTCGAGGGAGGTAT
	Forward	CGGGCCTACATGGAGAACAA	CAGCCATTTCAGTCCCTCCAT
LRRC3B	Forward	CTAAGCATACGCACCCAACG	TTTCCTTGCTTGGGCACCTTG
	Reverse	CGGGTCAGGGAGGGTAGTC	CCTTGATTGGGAACCTTGCG
GSTM2	Forward	TGATGTCCTTGAGAGAAACCAAG	CCTGGACTTTCCCAATCTGCC
	Reverse	TTCTCCAAGCCCTCAAATCGG	TCATCTTCTCAGGGAGACCCTC
RANBP3L	Forward	TTAATTCAGGCCAGTGCCCA	
	Reverse	TCATGAACAGGCAACCGACT	