Supplemental Experimental Procedures

Tissue samples

De-identified fetal cortical tissue samples were collected with previous patient consent in strict observance of the legal and institutional ethical regulations from elective pregnancy termination specimens at San Francisco General Hospital. Protocols were approved by the Human Gamete, Embryo and Stem Cell Research Committee (institutional review board) at the University of California, San Francisco. To examine regions of the germinal zone, samples were embedded in 3.5% low melting point agarose (Fisher) and sectioned perpendicular to the ventricle to 300 μm using a Leica VT1200S vibrating blade microtome in artificial cerebrospinal fluid containing 125 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1.25 mM NaH_2PO_4. The VZ and SVZ were microdissected from the same sections using a microsurgical blade. The VZ is very thin at these stages and microdissected VZ samples likely included some cells from the adjacent inner SVZ. To obtain single cell suspension, tissue samples were dissociated in a pre-warmed working solution of Papain and 2000 units/mL of DNase freshly diluted in Earl's Balanced Salt Solution according to manufacturer's instructions (Worthington Biochem. Corp.). The samples were incubated at 37° C for 20-30 minutes and centrifuged for 5 minutes at 300g. After removing the Papain/DNaseI supernatant, tissue was resuspended in 0.5 mL of sterile Dulbecco`s Phosphate Buffered Saline (DPBS) containing 3% FBS (Sigma) and 1000 units of DNAse and manually triturated by pipetting up and down approximately 10 times. The suspension was passed through a 40 μm strainer cap (BD Falcon) to yield a uniform single cell suspension. We compared eight paired regions of adjacent VZ and SVZ from three individuals (Brain 1 PFC and V1 - GW16.5, Brain 2 - GW16, Brain 3 - GW18) on separate C1 chips. Individual samples are listed in Figure S1E. In processing of Brain 3 cells we included ERCC

spike-in controls (Life Technologies) at 1:20,000 dilution as described before (Pollen et al., 2014). Recent work comparing the similarities of cells based on genes expression and separately based on RNA-spikes reveals uniform reaction conditions across chips and shows that biological variation between cells exceeds technical sources of variation (Zeisel et al., 2015), and we observed a similar pattern of biological variation across experiments, shown in Figure S1D.

Single Cell Capture and Library Preparation

The capture of single cells, generation of cDNA, and preparation of sequencing libraries was performed as recently described (Pollen et al., 2014). Briefly, cells were captured using the C_1^{TM} Single-Cell Auto Prep Integrated Fluidic Circuit (IFC) following the methods described in protocol PN 100-7168, http://www.fluidigm.com/. The PCR thermal protocol was adapted from a recent publication that optimized template-switching chemistry for single-cell mRNA Seq (Fan et al., 2012) using the SMARTer® Ultra Low RNA Kit (Cat. No. 63495, PT5163-1). The single cell cDNA reaction products were quantified using high sensitivity DNA chips (Agilent) and were then diluted to a final concentration of 0.15–0.30 ng/μL using C_1^{TM} Harvest Reagent. The Nextera® XT DNA Sample Preparation Kit (Illumina) was then used to convert diluted cDNA reaction products into sequencing libraries following manufacturer's instructions, with minor modifications. Reactions were run at one quarter of the recommended volume, the tagmentation step was extended to 10 minutes, and the extension time during PCR was increased from 30 seconds to 60 seconds. After the PCR step, samples were pooled, cleaned twice with 0.9X Agencourt AMPure XP SPRI beads (Beckman Coulter), eluted in DNA suspension buffer (Teknova) or EB buffer (Qiagen) buffer and quantified using High Sensitivity DNA Chip (Agilent).

Alignment of RNA sequencing data and outlier removal

An average of 2.9 million 100bp, paired-end reads were generated per library. Using cutadapt under the Trim Galore! wrapper with the default settings, reads were trimmed for quality, and Nextera transposase sequences were removed. Reads shorter than 20bp were discarded. Read level quality control was then assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were aligned to the NCBI human reference sequence GRCh37, by Tophat2 (Kim et al., 2013) using the --prefilter-multihits option and a guided alignment via the NCBI RefSeq transcriptome reference. Expression for RefSeq genes was quantified by the featureCounts routine, in the subRead library (Liao et al., 2014), using only uniquely mapping reads and discarding chimeric fragments and unpaired reads. Gene expression values were normalized based on library size as counts per million reads (CPM).

Low complexity libraries may result from chambers with debris, non-viable cells, incomplete lysis, or inefficient reverse-transcription and amplification. We explored three approaches to eliminate these outlier libraries that use a threshold for the number of genes detected, the median expression level across commonly detected genes, and the distribution of reads across genes. For the genes detected threshold, we required at least 1000 genes because many chambers below this threshold did not include viable cells. For the expression of commonly detected genes threshold, we selected a set of genes detected in at least half of the samples and removed samples with median expression values below the 15^{th} percentile for these genes. For the distribution of reads across genes threshold, we reasoned that low complexity libraries have a more even distribution of reads across genes, and evaluated libraries using

"Lorenz-like" curves analogous to an approach used for evaluating ChIP-sequencing data (Diaz et al. 2012). In this approach, libraries were compared to a reference library constructed by taking the geometric mean of each gene across samples, and the statistical significance of differences between individual samples and the reference was assessed via a score-test for binomial proportions. Results from all three methods largely converged, but the 1000 gene threshold excluded slightly more libraries and was selected for simplicity.

Principal Component Analysis and Expectation-Maximization Cluster Assignments

We reduced the dimensionality of our data by performing PCA on log-transformed CPM values, across all cells and also across cells interpreted to represent classically defined radial glia. We included all genes detected above 1 CPM in at least 2 cells and used the prcomp function in R (center=TRUE and scale.=FALSE). To focus on variation among cells within a sample, we performed PCA on the GW16.5 sample with most single cells and calculated PCA scores in the additional GW16 and GW18 samples using these eigengenes. To visualize the relationship between cells in two dimensions, we performed t-distributed stochastic neighbor embedding across PC1-10 sample scores using t-SNE package (http://lvdmaaten.github.io/software/).

After dimensionality reduction, we clustered cells using expectation-maximization (EM) algorithm based on PC 1-4 sample scores. This approach allowed us to take into account the relative contribution of all genes to variation across cells along the major axes of variation. We used the R package ConsensusClusterPlus (Wilkerson and Hayes, 2010) to estimate the optimal number of clusters (Figure S1). EM clustering was performed independently 1000 times using the R EMCluster package for k = 10 clusters. Because the EM clustering algorithm is based on a random initialization model, each iteration returns slightly variable cluster assignments. To

approximate the most likely solution, each iteration of the EM clustering algorithm was performed using the initialization method *exhaust.EM*, which calculates the model parameters *n* times with a different set of random initial samples, and only returns the best result. We set the number of initializations to *exhaust.iter=500*. After performing 1000 repetitions of the EM clustering algorithm (each repetition initialized 500 times and executed only on the best result), we obtained a matrix of cluster assignments for each cell (393 cells x 1000 EM cluster assignments). To evaluate how strongly each cluster was supported after 1000 iterations, we performed multiscale bootstrap resampling of cluster assignments using the R package pvClust (Suzuki and Shimodaira, 2006), with number of bootstrap = 5,000 and using complete linkage method and Euclidean distance for hierarchical clustering. This yielded approximately unbiased p-values (%) for each grouping reported in Figure S1E.

Specificity, differential expression, and enrichment analysis of gene sets

We examined the relationship between PC3 among radial glia and anatomical source using the Welch t-test. We then performed hierarchical clustering of 107 radial glia cells using the 1% of genes with the strongest positive and negative loading scores along PC3 and the pvClust package as above, and we interpreted the two significant groups as putative oRG and vRG cells. To identify genes with specific expression in putative oRG and vRG cells, we first measured the Pearson correlation of all genes to an ideal marker gene uniformly expressed in only one cell type across all 393 cells (Figures 3A, S2, and S3). We next filtered for genes with a correlation above 0.4 for one group and below 0.3 for the other group, or above 0.4 in both groups. To identify genes differentially expressed between oRG and vRG cells, we performed DESeq (Love et al., 2014) across single cells (n=55 oRG, n=52 vRG), treating each cell as an

independent replicate. Raw counts for each single cell were merged into a single matrix and normalized by size factors by DESeq2, choosing not to replace or exclude outlier counts (minReplicatesForReplace = Inf, cooksCutoff= Inf). To perform enrichment analysis on strongly loading PCA genes, specific genes, and differentially expressed genes, we used Enrichr (Chen et al., 2013) which uses the Fisher exact test and multiple hypothesis correction.

Evaluation of radial glia gene expression in human, macaque, mouse and glioblastoma datasets

We evaluated the average expression of predicted oRG, vRG, and pan-RG markers (from Figure 3A) across a range of datasets, including the Allen Institute Prenatal LMD Microarray Atlas (Miller et al., 2014) and NIH Blueprint (NHP) Atlas, as well as human and mouse gene coexpression networks (Lui et al., 2014) and single cell glioblastoma data (Patel et al., 2014). For the Allen Institute data, we downloaded microarray probes from two PCW21 human brain samples and E40 through E120 macaque samples. For genes evaluated with multiple microarray probes, we averaged log-transformed gene expression values. We then calculated the average expression of each set of genes across distinct laminae. For the gene coexpression data, we retrieved the differential percentile rank of radial glia gene network specificity in human and mouse (DS) and differential percentile rank of gene expression (DE) for each gene (Lui et al., 2014). We compared DS values between oRG, vRG, and pan-RG genes using the Wilcoxon rank-sum test. For the glioblastoma analysis, we downloaded the raw reads from single cells across five glioblastoma samples (Patel et al., 2014) and aligned reads, calculated gene expression levels, and removed outliers as described above, resulting in 598 cells. We then calculated the average expression of oRG, vRG, and pan-RG predicted genes in each tumor cell

and calculated the Pearson correlation between these values and a previously defined stemness signature of 52 genes for these tumors. To compare the correlations between these signatures across glioblastoma tumors, we used a test for assessing differences in correlation coefficients from samples of different sizes (Oldham et al., 2012). First, we used the Fisher transformation to convert correlation coefficients into z-scores and calculated a weighted average of the resulting z-scores based on the number of cells from each tumor; these weighted averages were then converted to the average correlation coefficients depicted in Figure 4 using the reverse Fisher transformation. Next, we divided the difference between resulting z-scores by the joint standard error, and then we calculated significance levels based on the standard normal distribution.

Immunohistochemistry

Tissue samples were fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose, and embedded in a 1:1 mixture of 30% sucrose and optimal cutting temperature (Thermo Scientific). Fixed macaque samples (Macaca mulatta) were collected by the laboratory of Dr. David Amaral for another study, and brain tissue was provided for this study as a generous gift. All procedures performed on macaques were approved by the Institutional Animal Care and Use Committee of the University of California, Davis, and strictly adhered to National Institutes of Health policies on primate animal subjects. Thin 20 μm cryosections were collected on superfrost slides (VWR) using Leica CM3050S cryostat. For immunohistochemistry, heat-induced antigen retrieval was performed in 10 mM sodium citrate buffer, pH 6. Primary antibodies: rat anti-BrdU [BU1/75 (ICR1)] (1:50, Abcam ab6326), mouse anti-CUX1 (1:250 Abcam, ab54583), chicken anti-EOMES (1:200, Millipore AB15894), rabbit anti-FAM107A (1:250, Sigma HPA055888), mouse anti-GFAP (1:500, Millipore, MAB3402), chicken anti-GFP

(1:1,000, Aves Labs, GFP-1020), rabbit anti-HOPX (1:1000, Sigma HPA030180), mouse anti-MKI67 (1:100, Dako M7240), rabbit anti-PTPRZ1 (1:1000, Sigma HPA015103), mouse anti-SATB2 (1:250, Santa Cruz SC81376), goat anti-SOX2 (1:200, Santa Cruz SC17320), rabbit anti-p-Y705 STAT3 (1:100, Cell Signaling 9145S), TBR1 (1:500, Abcam ab31940), rabbit anti-TNC (1:200, Abcam ab108930), were diluted in blocking buffer containing 10% Donkey Serum, 0.5 % Triton™-X100 and 0.2% gelatin diluted in PBS at pH=7.4. Binding was revealed using an appropriate Alexa Fluor™ 488, Alexa Fluor™ 546, and Alexa Fluor™ 647 fluorophore-conjugated secondary antibody (Life Technologies). Cell nuclei were counter-stained using DAPI (Life Technologies). Images were collected using a Leica TCS SP5 X Confocal microscope. For experiments involving incorporation of BrdU into organotypic slice of the human cortex, tissue samples were incubated with 50 μg/ml BrdU for 3 hours at 37°C in culture conditions described below, fixed in 4% Paraformaldehyde, cryopreserved in 30% sucrose, embedded and cryosectioned as described above. *In situ* hybridization for *CRYAB* is described below. After developing the *in situ* hybridization signal, sections were washed in PBS and antigen retrieval was performed as described above. Sections were permeabilized using PBS containing 0.5 % Triton™-X100 and blocked in blocking buffer containing 10% Donkey Serum, 0.5 % Triton™-X100 and 0.2% gelatin diluted in PBS at pH=7.4 for 1 hour, and incubated with antibody against phosphorylated histone H3 (Abcam, ab1791) diluted 1:500 blocking buffer overnight. Binding was revealed using a donkey anti-rabbit Alexa Fluor™ 488. Sections were fixed in 4% paraformaldehyde for 1 hour. To detect BrdU, sections were denatured with 2N HCl for 1 hr at 37°C followed by neutralization with 0.1M boric acid, and incubated with primary and secondary antibodies as described above. Slides were mounted with Aqua-mount (Lerner Laboratories).

***In situ* hybridization**

Probes complementary to target human mRNA used for RNA *in situ* hybridization were synthesized (Life Technologies) or cloned from primary human fetal cortical cDNA sample reverse-transcribed using SuperscriptIII Reverse Transcriptase (Invitrogen) with random hexamer primers, form a mixture of RNA samples isolated from GW16-21 human cortex using RNA Easy kit (Qiagen). *EOMES* probe was designed and synthesized to match the antisense bases 2467 to 3100 of NM_001278182.1, and *PPP1R17* probe was designed to be antisense to bases 199-848 of NM_006658.4. Primers specific to target genes of interest were designed using Primer3 and amplified by PCR using Phusion proofreading DNA polymerase (Thermo Scientific). Specific genes were amplified using the following primers: *CRYAB* forward - AAC CCC TGA CAT CAC CAT TC, reverse - CCA GAG ACC TGT TTC CTT GG, *CTNND2* forward - CCT GGG TGT GAG GAG CAG, reverse - GAA CCA TAG ATG AGG AAA CAC G, *FAM107A* forward - AGG CCT TCC CAC CAG GAA, reverse - AAT CCC TGG GGC ATC AGT*; FBXO32* forward - GGC TGC TGT GGA AGA AAC TC, reverse - CAC ATT GTA AAC AAA GCG TCT CC, *HOPX* forward - CAG CAA ACA CAG CTT CCA AA, reverse GGA AAT GCT AGC CAC ACC AT, *LIFR* forward - TCA AAG GGG CCT GAT ACT TGA AA, reverse- ATT TGG TGC AAC AAT GG, *MOXD1* forward - TTT TCC CTC CCT CCT TTT TC, reverse - TCC CAT AGC CTC TTT CTT TTC A, *NPY* forward - CTC GCC CGA CAG CAT AGT A, reverse - TGA TGA CAA AGG AAA ACA TTG C, *NRCAM* forward - TGG GTA TTC TTG ATT TCC TCA GA, reverse - TTT GGA TGA TTT GTT TTT GAT TC, *PALLD* forward - GCA GGT GAA CAA CCC TGA GT, reverse - TTT GGA TTT CCA GCC ACT CT, *PAX6* forward - ACT GTA ATC TTG GCC AGT ATT GAG A, reverse - GTT CCA ACG GAG AAG

ATT CAG ATG AGG C, *PDGFD* as previously described (25391964), *PTPRZ1* forward - GCC AAA ACA TGG CAG AAG AT, reverse - CCC CAC CCC TTT CTG TGT TA, *RTN1* forward - CCC CTC CCT CCA GTA CCA TA, reverse - TGA ATC CAT TAG GAA CTA CAG AGA AA, *SEZ6L* forward - AAC CCA TTT GGA GAG CTG TG, reverse - TCC TCT ATT TTG AGG GCT GAG A, *TAGLN2* forward - GAC GCG AGA ACT TCC AGA AC, reverse - CCC TGA CAG AAA GGA GCT TG, *TNC* forward - TCC CCA AAG GAA GTC ATT TTC, reverse - AGG TAA GGA GGG CAG TTT CC. PCR products of predicted band size were gel extracted and A-tailed using GoTaq® DNA Polymerase (Promega) and ligated into the pGEM®T-Easy Vector System (Promega). Ligation products were transfected into One Shot TOP10 Chemically Competent *E.coli* (Life Technologies). Cloned sequences were confirmed by sequencing. Digoxigenin labeled RNA probes for in situ hybridization were generated by linearizing the pGEM®T-Easy Vector and *in vitro* transcribing the probe using T7 or SP6 RNA Polymerase (Roche) in the presence of DIG-RNA Labeling Mix (Roche). *In situ* hybridization was performed blinded to the sense/antisense status for each probe and sense control probes gave no signal (data not shown). The *in situ* hybridization protocol has been described before (Wallace and Raff, 1999). For subsequent immunolabelling, slides were subjected to antigen retrieval as described above. Images were collected with a Leica DMI 4000B microscope using a Leica DFC295 camera.

Quantification of staining of human cortical sections.

Combined *in situ* hybridization and immunohistochemistry staining of the human cortical sections (20μm) were imaged as described above using epifluorescence microscopy at 20x magnification. For all candidate oRG markers shown in Figures 3, 5 and 6, *in situ* hybridization

was combined with immunostaining for SOX2, EOMES and SATB2. For clarity of the presentation, we only display SOX2 and EOMES channels. *In situ* hybridization signal was imaged under bright-field conditions with shading correction and white balance applied automatically through the LAS X Leica Software during acquisition. Overlay images were prepared using Photoshop (Adobe Systems). Counting ladders consisting of 10 boxes, each 500μm wide, were drawn based on an image of a graticule taken under the same magnification. For each biological replicate, two areas on non-adjacent sections were selected randomly. For each counting area, counting ladder was placed so that the 500μm wide edge was aligned with the ventricular surface and the ladder span the thickness of the germinal zone, which was determined based on SOX2 and EOMES expression domains. Cells positive for *in situ* signal were identified based on bright field image alone, but also in conjunction with the nuclear DAPI counterstain, and immunohistochemical detection of nuclear labeling for SOX2, EOMES and SATB2. Only distinct staining in the cell body associated with clearly identifiable nucleus was considered positive in this study, and examples of such cells are shown in Figure S4 and Figure S7. Cells positive for *in situ* signal were then scored for the nuclear expression SOX2, EOMES and SATB2. For samples from early stages, GW13.5 and GW14.5, results for counting boxes 1 and 2, 3 and 4, 5 and 6, 7 and 8, and 9 and 10 were combined. All quantification of *in situ* hybridization results are presented in Figure 6. For quantification of EOMES and PPP1R17 *in situ* staining, we used the same approach as described above but used 250μm counting ladder.

Quantification of TNC and STAT3 (p-Y705) immunohistochemical staining in human cortex sections was performed similarly to the quantification of *in situ* hybridization staining, with a few modifications. Images were collected using Leica TCS SP5 X Confocal microscope under constant laser power and exposure conditions. Optical sections were collected at 20x

magnification at constant separation to encompass staining through the depth of the section. Counting boxes were drawn in Photoshop based on scale bar information embedded in image properties of the image files. Strong nuclear immunostaining for STAT3 (p-Y705) of a DAPI, SOX2, EOMES or SATB2 positive nucleus was considered in this study and we did not detect appreciable non-nuclear staining in the sections analyzed. For TNC immunostaining, two distinct staining patterns can readily be identified. One, strong immunostaining closely surrounding a DAPI, SOX2, EOMES or SATB2 positive nucleus. High magnification examples of such staining are shown in Figure 3D, and this is the staining we considered in this study for quantification in Figure S7E. Two, fainter and diffuse staining in the extracellular space, not associated closely with cell nuclei, which we did not quantify. This diffuse staining first appears at around GW14.5 and becomes more intense at around GW18-GW20, and is usually strongest in the outer portions of the outer subventricular zone. Examples can be seen in Figure S7E. Quantification of PPP1R17 immunostaining combined with SOX2 and EOMES was performed as described for TNC immunostaining, but the width of the counting ladder was 300μm. Quantification of cells in the human OSVZ immunopositive for PPP1R17 and KI67, and cells immunopositive for ITGB5 in combination with TNC, PTPRZ1, KI67, PAX6 and EOMES, was performed using a single 250μm wide counting rectangle placed perpendicular to the edge of the lateral ventricle and spanning the thickness of the OSVZ. All quantification of tissue immunostaining was performed in two non-adjacent regions and on non-adjacent sections in every independent sample.

Organotypic slice culture experiments

Human fetal cortical slices were collected as described above. Slices were incubated in

the presence of Adenovirus expressing EGFP under the regulation of CMV promoter (Vector Biolabs, diluted 1:10,000 in culture media) transferred into slice culture inserts (Millicell) in 6-well culture plates (Corning) and cultured in media containing 66% Eagle's Basal Medium, 25% Hanks Balanced Salt Solution, 5% Fetal Bovine Serum, 1% N-2 supplement, 1% penicillin/streptomycin, and glutamine (Life Technologies). Slices were cultured in a 37 °C incubator at 5% CO_2, 8% O_2 overnight. Cultures were placed in a 37 °C incubator at 5% CO_2, 8% O_2. Slices were maintained for 36-48 hours in environment-controlled chamber. Timelapse images were collected using Leica TCS SP5 X Confocal microscope every 20 minutes. Immunostaining of tissue slices was performed as described above with the exception that the primary antibodies were diluted in blocking buffer containing 10% Donkey Serum, 2% Triton™-X100 and 0.2% gelatin, and all washes were performed using PBS containing 2% Triton™-X100. Primary antibodies were incubated for 48 hr. and secondary antibodies were incubated overnight at 4°C.

Perturbation of STAT3 signaling in human and mouse

To examine the function of decreased STAT3 signaling in oRG cells, we cultured fetal cortical slices as above in the presence of 10μM Nifuroxazide (Santa Cruz Biotechnology, sc-204128), 5μM Inhibitor III (Santa Cruz Biotechnology, sc-203282), or DMSO. After 48 hours slices were fixed for 12hr at 4°C. Immunostaining followed similar protocol to the BrdU and phospho-histone H3 immunostaining, except that Triton™-X100 was used at 2%, and primary antibody incubation was extended to 48 hrs. Images of the immunostaining were collected using Leica TCS SP5 X confocal microscope at constant exposure and separation of optical sections. We collected sections spanning the entire thickness of the slice, and throughout the germinal

zone outlined by the SOX2 and EOMES expression domains at the basal side and by the edge of the ventricular surface at the apical side. Rectangular counting boxes were 500μm wide and were placed perpendicular to the outer edge of the OSVZ and span the thickness of the SVZ. Quantification of immunopositive cells and co-localization of immunostaining was performed using Imaris (Bitplane) and was aided by the automatic "spots" in Imaris, followed by manual curation. Quantification was performed in two slices per biological replicate (total of four biological replicates), and in two randomly selected areas per slice. Proportions of SOX2 or EOMES cells that incorporated BrdU were averaged across counting areas and slices for each biological replicate. To test whether either inhibitor had an effect on the incorporation of BrdU in radial glia (SOX2+ cells) or intermediate progenitors (EOMES+ cells) we first performed a two-way analysis of variance using Matlab (Mathworks). Both Nifuroxazide and Inhibitor III had statistically significant effects on the proportion of radial glia that incorporated BrdU ($p=0.014$ and $p=0.0055$, respectively, two-way ANOVA, $n=4$). Proportions of intermediate progenitor cells incorporating BrdU were not significantly different. We next performed post-hoc paired Student's t-test on all regions, inhibitors, and treatments, and those p-values are displayed in Figure 4J legend.

To examine the function of p-Y705 STAT3 phosphorylation in mouse radial glia, we performed *in utero* electroporation as described previously (Saito, 2006) at E13.5 of timed-pregnant Swiss Webster mice. Timed-pregnant mice were obtained from Simonsen Laboratories and maintained according to protocols approved by the UCSF Institutional Animal Care and Use Committee. Pregnant dams were deeply anesthetized with inhaled isoflurane and euthanized by cervical dislocation. Embryos were decapitated and dissected brains were fixed in 4% paraformaldehyde overnight. We transfected either control Ub-GFP plasmid (Addgene, 11155)

or a mutant form of STAT3 which mimics the Y705 phosphorylation state (Bromberg et al., 1998) driven by the EF1a promoter (Addgene, 24983). After 48 hours, embryos were collected, fixed, immunostained, and quantified as described above. Immunostained mouse cortex sections were imaged at 20x magnification using Leica TCS SP5 X confocal microscope at constant exposure and separation of optical sections. Only embryos with clear expression of the fluorescent reporter in the lateral pallium across the rostro-caudal extent of the lateral ventricle were considered in this study. Quantification of GFP, SOX2, and EOMES positive cells was performed in three non-adjacent coronal sections through central telencephalon. In each section, a 200μm wide counting ladder with 3 counting boxes was positioned with the 200μm side aligned at the edge of ventricular surface and spanning the thickness of the germinal zone (VZ and SVZ).

Single cell clonal analysis of outer radial glia

Cortical germinal zones were microdissected, and cells dissociated using Papain Dissociation Kit (Worthington). Dissociated cells were infected for 2-4 hrs with pNIT-GFP retrovirus (a kind gift from F.Gage, Salk Institute) at 37°C. Cells were resuspended in culture medium containing DMEM (Invitrogen, 11965), 1% B-27 supplement (Invitrogen, 12587-010), 1% N-2 supplement (17502-048), and recombinant human FGF-basic (10 ng/ml, Peprotech, AF-100-18B). Cells were plated at ~100,000 cells/cm^2 on matrigel (BD Biosciences). Three days later, cultures were trypsinized, and single GFP positive cells were FACS (ARIA, BD Biosciences) sorted at 1 cell/well into 96 well plates containing the same media pre-seeded with confluent mouse cortical astrocyte feeder layer (Clones 1 and 2), or human cortical feeder cells (clone 3). Dead cells were excluded by Sytox Blue (LifeTech) uptake, doublets were excluded by

forward and side scatter sorting, and GFP positive gating was established by comparison to cultures not transduced with retrovirus. Post-sorting, 96 well plates were examined by fluorescence microscopy to confirm the presence of only 1 cell per well. The following day, single cells were imaged for one week at 20 minute intervals by continuous live time lapse fluorescence microscopy to identify the mitotic behavior of the initial cell divisions for each clone. The plates containing oRG cell clones shown in Figure S6 were then returned to the cell culture incubator for six weeks without FGF and fixed for immunostaining to determine the fate of the daughters in each clone.

Supplemental References

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