Supporting Information

Pastrana et al. 10.1073/pnas.0810407106

SI Methods

In Vitro Binding of Fluorescent EGF Ligand to FACS-Purified Cells. Immediately after FACS, cells were plated onto 0.5 mg/mL poly-D-lysine (PDK) (Sigma) and 0.1 mg/mL laminin (Sigma)-coated 16-well LabTek chamber slides (Nunc) and allowed to settle for 2 h in a 37 °C incubator. Fluorescently complexed EGF ligand (2 μg/mL A647-EGF; Molecular Probes) was then added to each population for 30 min at 37 °C. Cells were fixed and stained for other markers or imaged as described below.

Flow Cytometry. SVZ cell populations were processed as for sorting and fixed in 3% paraformaldehyde on ice for 10 min, permeabilized, and immunostained with rabbit anti-GFAP (1:5000; Dako) and anti-rabbit Alexa700 (1:10,000; Molecular Probes) using a fixation/permeabilization kit (BD Biosciences). Cells were analyzed by using a Becton Dickinson LSRII Cell Analyzer and FlowJo data analysis software.

Immunocytochemistry. Cells were fixed for 10 min in a 1:1 mix of 3% paraformaldehyde and culture medium, followed by 10-min fixation in 3% paraformaldehyde. Cells were washed with PBS and blocked in 10% normal serum/0.1% Triton X-100. Cells were incubated with the primary antibodies overnight at 4 °C. Nuclear staining was performed by incubating with DAPI (1:1,000; Sigma) at room temperature. To analyze the purity of each population, cells were spun down onto 0.5 mg/mL PDK (Sigma)-coated 16-well LabTek chamber slides (Nunc) at 1,300 rpm for 2 min in a tabletop centrifuge (5804 Centrifuge, Eppendorf) and then fixed and immunostained.

Antibody dilutions: guinea pig anti-GLAST (1:1,000, Chemicon), mouse anti-Mash1 [1:10, gift from J. Johnson, (University of Texas Southwestern Medical Center, Dallas)], mouse anti-βIII tubulin (TuJ1; 1:500, Covance), rabbit anti-GFAP (1:1,000; Dako); and mouse IgM anti-O4 (1:500, Chemicon) were used. Secondary antibodies conjugated to fluorophores Cy2, Cy3, or Cy5 (1:1,000, Jackson ImmunoResearch) or Alexa488 or Alexa568 (1:5,000, Invitrogen) were used.

Immunohistochemistry. Whole mounts were prepared and stained as described by Doetsch and Alvarez-Buylla [Doetsch F, Alvarez-Buylla A (1996) Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc Natl Acad Sci USA* 93:14895–14890]. Samples were blocked in 10% normal serum/0.5% Triton X-100 for 2 h and incubated with primary antibodies diluted in the blocking solution overnight. Secondary antibodies conjugated to fluorophores were applied for 2 h at room temperature.

Antibody dilutions: sheep anti-EGFR (1:50; Upstate), rabbit anti-EGFR (1:200; Millipore), rabbit anti-GFP (1:1,000; Molecular Probes), sheep anti-GFP (1:250; Biogenesis), mouse anti-GFP (1:250; Invitrogen), goat anti-MCM2 (1:50; Santa Cruz), and mouse anti-βIII tubulin (TuJ1; 1:500, Covance) were used. Secondary antibodies conjugated to fluorophores Cy2, Cy3, or Cy5 (1:1,000, Jackson ImmunoResearch) were used.

RNA Extraction and Quantitative Real-Time Reverse Transcription PCR (qRT-PCR). Immediately after FACS, the cells were spun down and total RNA was isolated (RNA isolation kit, Ambion). qRT-PCR was performed in triplicates using SYBR green on a Stratagene MX3000 thermocycler (Applied Biosystems). The C_t threshold value is determined by using the automatic baseline determination feature on a Stratagene MX3000 and the relative expression of the genes was determined by using the expression $2^{-\Delta C_t}$.

Primer sequences:

gfap forward 5′-CAGGCAATCTGTTACACTTGG-3′
reverse 5′-TGTCTGCTCAATGTCTTCCCTA-3′
mash1 forward 5′-ATGCAGCTACTGTCCAAACG-3′
reverse 5′-AACAGTAAGGGGTGGGTGTG-3′
βIII tubulin forward 5′-GAGGAGGAGGGGGAGATGA-3′
reverse 5′-GAGCTGGTGAGAAGCAAAGC-3′
gapdh forward 5′-ACACATTGGGGGTAGGAACA-3′
reverse 5′-AACTTTGGCATTGTGGAAGG-3′
egfr forward 5′-GCTTGCAACGGTTCTCTCTC-3′
reverse 5′-CCGTTATCCATCCCTGACTC-3′
β-actin forward 5′-TGAGAGGGAAATCGTGCGTGACAT-3′
reverse 5′-ACCGCTCGTTGCCAATAGTGATGA-3′
gfp forward 5′-AAGTTCATCTGCACCACCG-3′
reverse 5′-TCCTTGAAGAAGATGGTGCG-3′

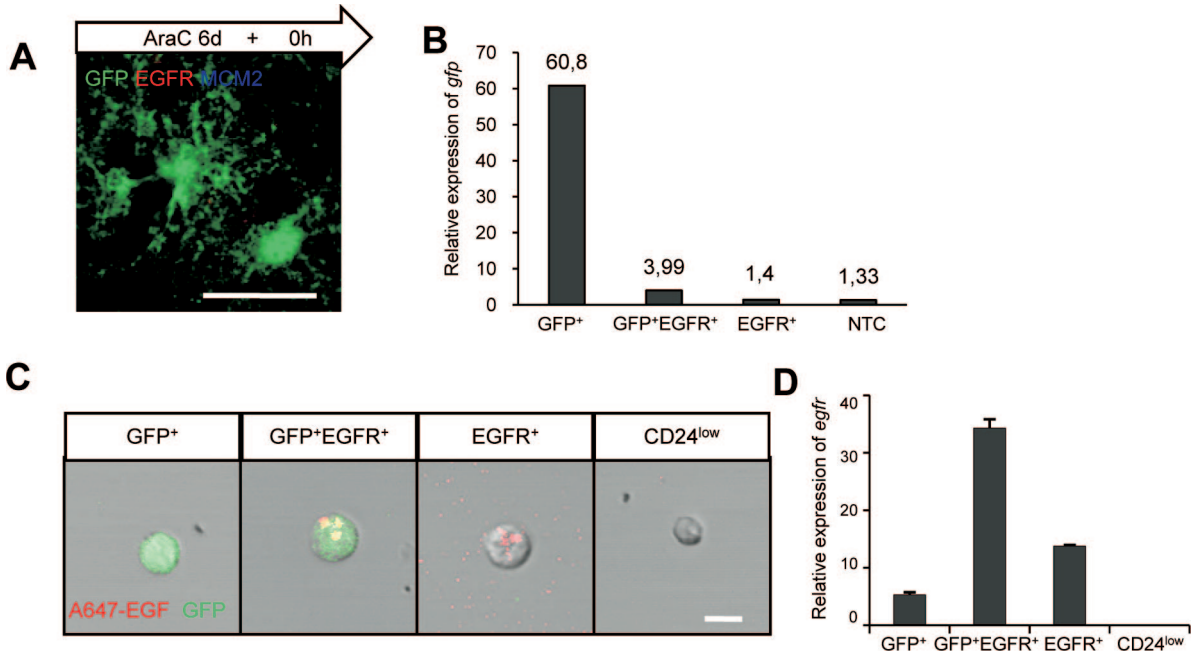

Fig. S1. (*A*) Quiescent astrocytes are present immediately after AraC treatment. A whole mount immunostained for GFP (green), EGFR (red), and MCM2 (blue) is shown. GFP$^+$ astrocytes remain after AraC treatment and are negative for EGFR and MCM2. (Scale bar, 20 μm.) (*B*) Relative expression of *gfp* mRNA detected by qRT-PCR in FACS-sorted populations. GFP$^+$ and GFP$^+$EGFR$^+$ SVZ populations expressed *gfp* mRNA, with GFP$^+$ cells expressing much higher levels than GFP$^+$EGFR$^+$ cells. *gfp* levels in EGFR+ cells were similar to the nontemplate control (NTC). Data represent relative expression normalized to βactin. (*C*) In vitro binding of the fluorescent EGF ligand to FACS-purified cell SVZ populations. Both GFP$^+$EGFR$^+$ and EGFR$^+$ cells bound fluorescent EGF ligand in vitro, but not the GFP$^+$ and CD24^{low} pools. (Scale bar, 10 μm.) (*D*) Relative quantity of *egfr* mRNA detected by qRT-PCR in each of the FACS-sorted populations. SVZ populations purified by FACS based on fluorescent EGF ligand (GFP$^+$EGFR$^+$ and EGFR$^+$) expressed high levels of *egfr*. Data represent relative quantity normalized to βactin.

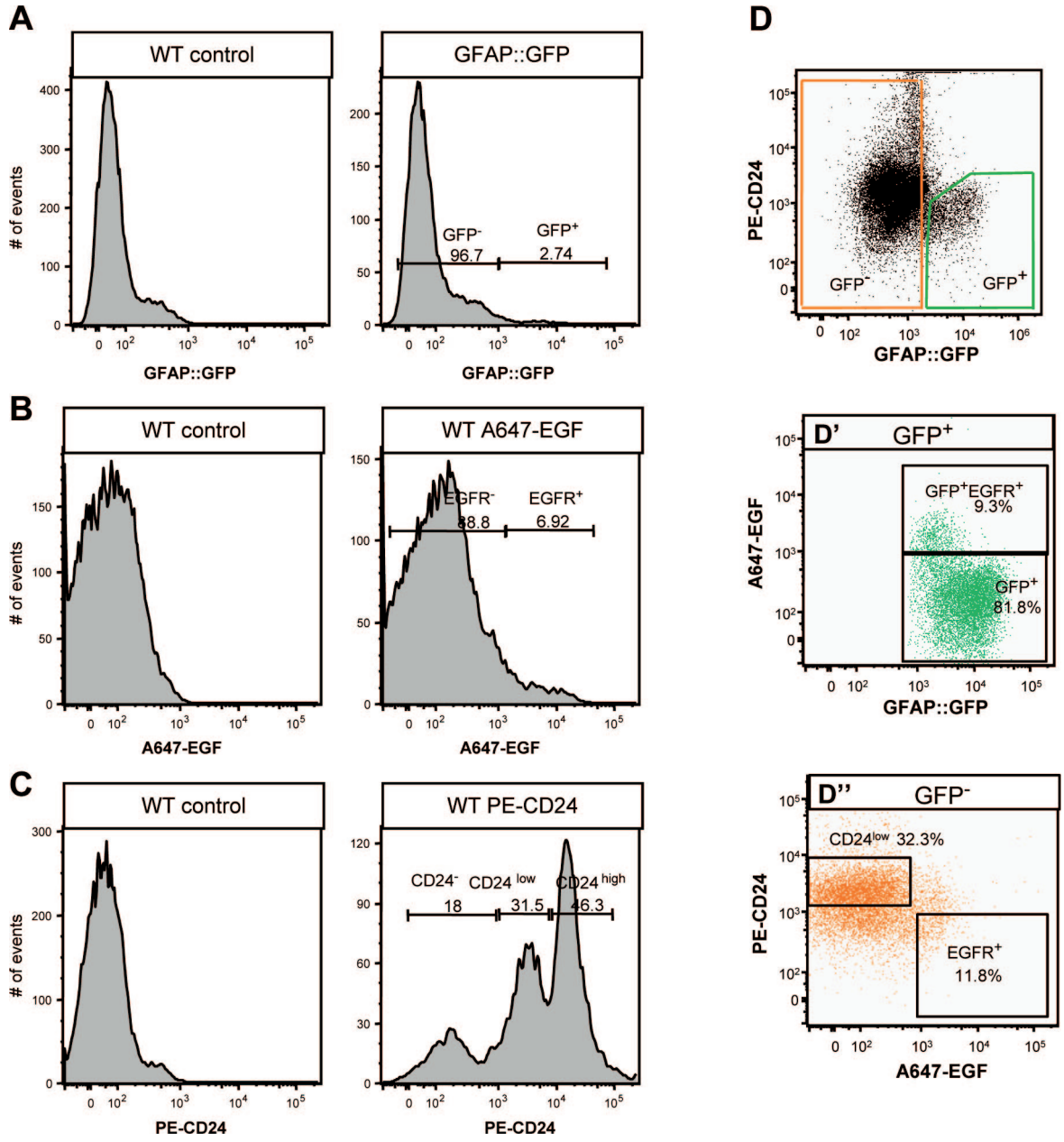

Fig. S2. Prospective isolation of stem cells and their progeny from the adult SVZ by using FACS. FACS plots of control samples used to set the gates for the purification of each SVZ cell type from adult *GFAP*::*GFP* transgenic mice are shown. (*A*) Analysis of FITC channel (*GFAP*::*GFP*) in SVZ from wild-type CD-1 mice (WT control, *left*) and SVZ from *GFAP*::*GFP* mice. Gates were determined using the WT control to establish the GFP^- and GFP^+ fractions shown. (*B*) Analysis of APC channel (A647-EGF) in SVZ from wt CD-1 mice (WT control, *Left*) and SVZ from WT CD-1 mice labeled with A647-EGF (WT A647-EGF). Gates were determined by using the WT control to establish the $EGFR^-$ and $EGFR^+$ fractions shown. (*C*) Analysis of PE channel (PE-CD24) in SVZ from WT CD-1 mice (WT control, *Left*) and SVZ from WT CD-1 mice labeled with PE-CD24 (WT PE-CD24). Gates were determined by using the WT control to establish the $CD24^-$ and $CD24^{low}$ and $CD24^{high}$ fractions. Numbers reflect the percentages of cells comprising each group. (*D*–*D*″) FACS plots of the purification of each SVZ cell type from adult *GFAP*::*GFP* transgenic mice using a live DNA dye (Vybrant DyeCycle). (*D*) GFAP-positive SVZ astrocytes were separated based on GFP expression from other GFP^- SVZ cell types. (*D*′) From the GFP^+ pool, we isolated SVZ stem cell astrocytes (GFP^+EGFR^+) from other SVZ astrocytes (GFP^+EGFR^-) based on EGFR expression. (*D*″) From the GFP-negative cell fraction, we isolated transit amplifying cells ($EGFR^+$) and neuroblasts ($CD24^{low}$). Percentages for a representative experiment are shown next to each gate. All FACS data are presented using ''logical'' or biexponential display for 100,000 cells.

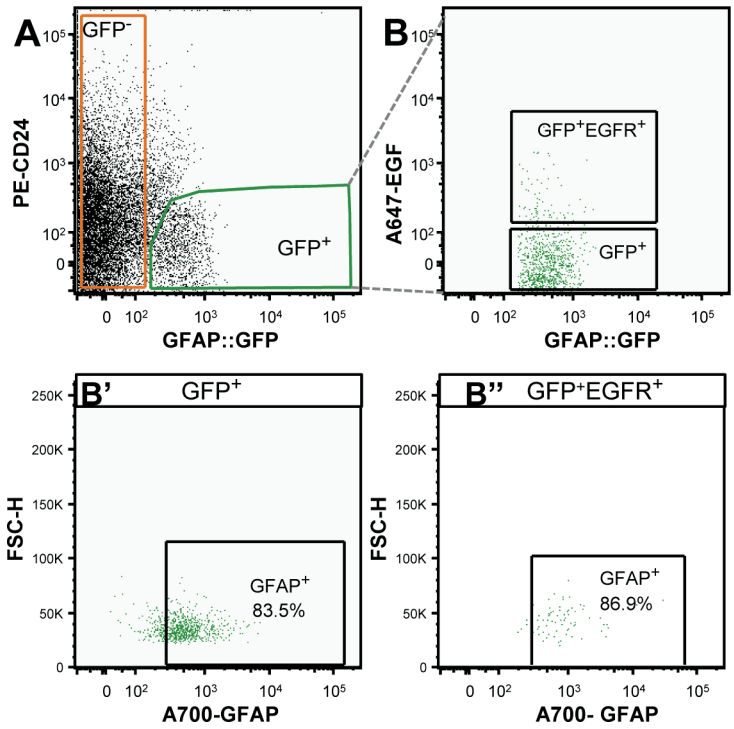

Fig. S3. Flow cytometric analysis of GFAP expression in GFP$^+$ and GFP$^+$EGFR$^+$ astrocyte populations. (*A*) SVZ astrocytes were separated based on GFP expression from GFP$^-$ SVZ cells. (*B*) From the GFP$^+$ pool, we isolated activated SVZ stem cell astrocytes (GFP$^+$EGFR$^+$) from other SVZ astrocytes (GFP$^+$) based on EGFR expression. GFAP (A700-GFAP) was analyzed in these populations. 83.5% of the GFP$^+$ (*B'*) and 87% of GFP$^+$EGFR$^+$ (*B''*) populations were GFAP positive. Percentages represented by each population are shown next to the gates and are averages of three independent experiments. FSC-H, forward scattered light.

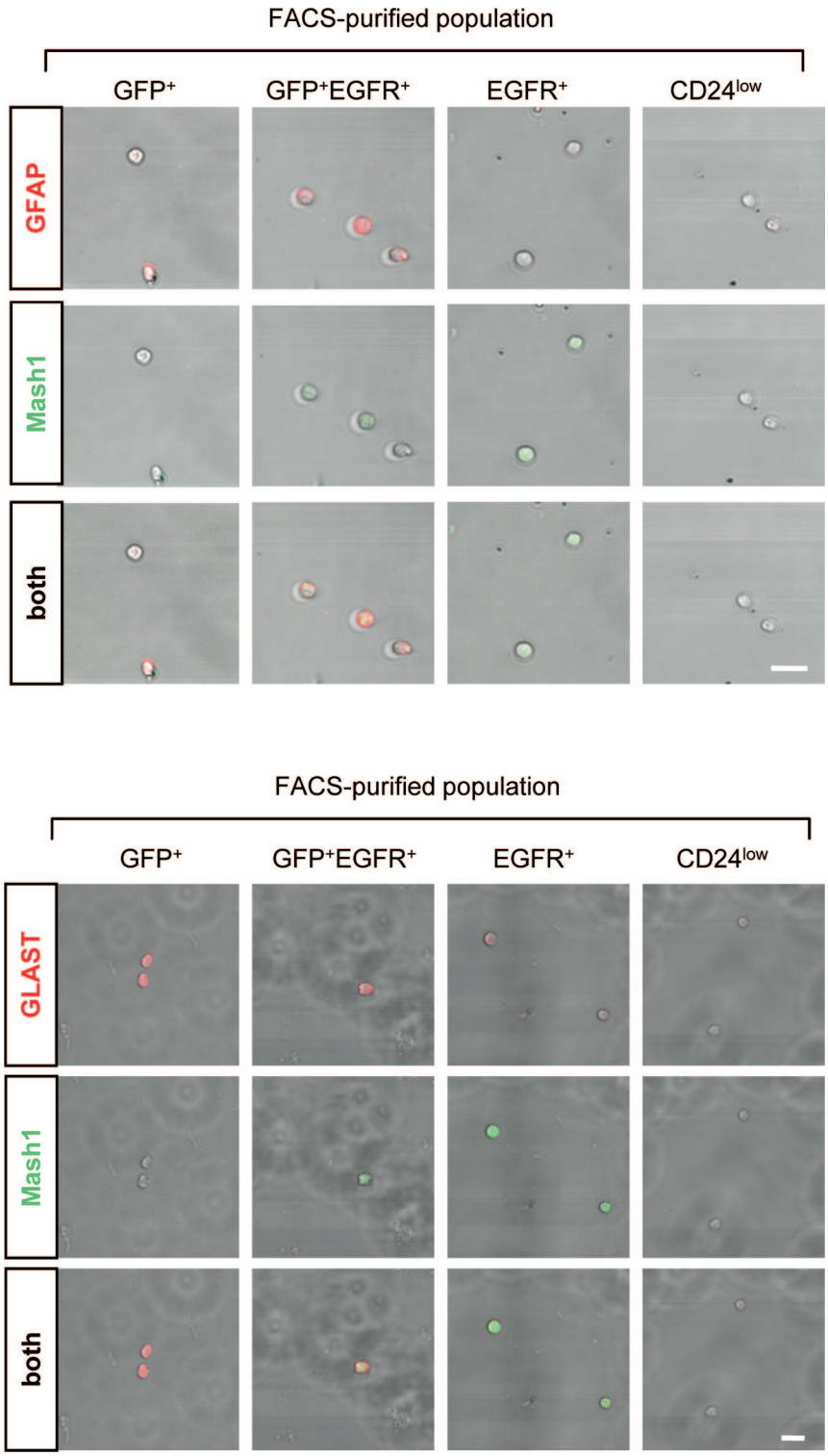

Fig. S4. Analysis of GFAP/Mash1 and GLAST/Mash1 expression in FACS-purified populations. Acute immunostaining of SVZ populations purified by FACS from the adult mouse brain is shown. FACS-purified SVZ astrocytes (GFP^+), activated stem cell astrocytes (GFP^+EGFR^+), transit amplifying cells ($EGFR^+$), and neuroblasts ($CD24^{low}$) were stained with combinations of antibodies against glial fibrillary acidic protein (GFAP, red) and the transcription factor Mash1 (green) (*Upper*) or glutamate transporter GLAST (red) and Mash1 (green) (*Lower*). (Scale bars, 20 μm.)

Table S1. Neurosphere-forming capacity of purified SVZ populations in response to different growth factors

NS-forming capacity	GFP^+	GFP^+EGFR^+	EGR^+	$CD24^{low}$	Unsorted SVZ
EGF	−	+	+	−	+
bFGF	−	+	+	−	+
EGF + bFGF	−	+	+	−	+
EGF + PDGF	−	+	+	−	+
bFGF + PDGF	−	+	+	−	+
PDGF	−	−	−	−	−
2ary-3ary NS formation		+	+		+

EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; NS, neurosphere; SVZ, subventricular zone.