Cell Stem Cell

Supplemental Information

Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke

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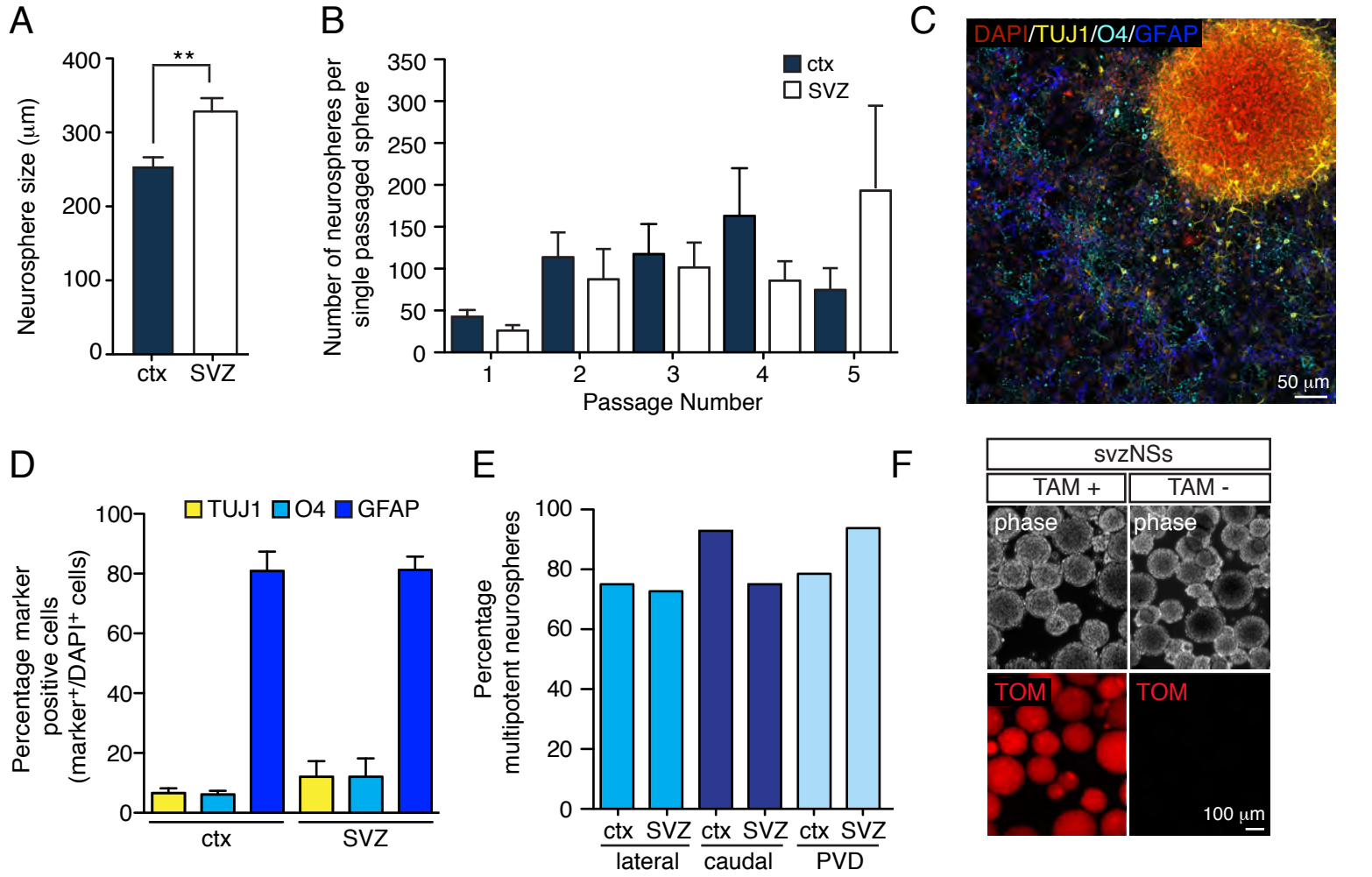
A
Neurosphere size (μm)
400
300
200
100
0
**
ctx
SVZ
B
Number of neurospheres per single passaged sphere
350
300
250
200
150
100
50
0
ctx
SVZ
1
2
3
4
5
Passage Number
C
DAPI/TUJ1/O4/GFAP
50 μm
D
Percentage marker positive cells (marker+/DAPI+ cells)
TUJ1
O4
GFAP
100
80
60
40
20
0
ctx
SVZ
E
Percentage multipotent neurospheres
100
80
60
40
20
0
ctx SVZ ctx SVZ ctx SVZ
lateral
caudal
PVD
F
svzNSs
TAM +
TAM -
phase
phase
TOM
TOM
100 μm

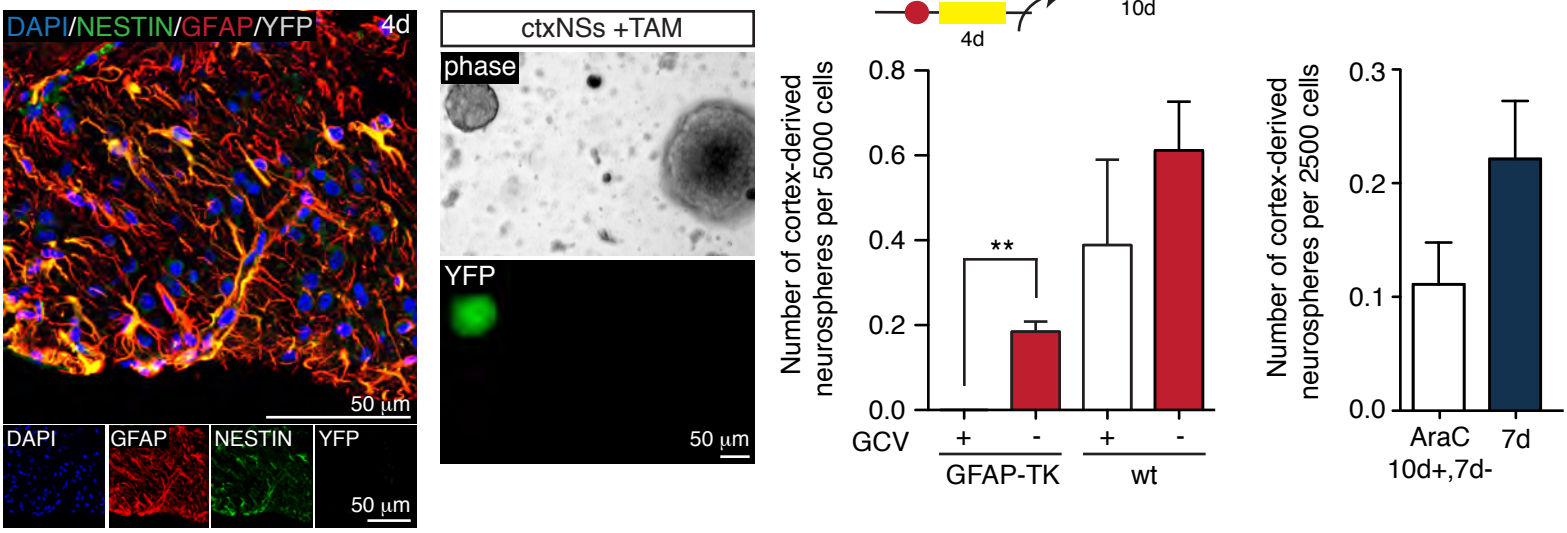
G
DAPI/NESTIN/GFAP/YFP
4d
50 μm
DAPI
GFAP
NESTIN
YFP
50 μm
H
ctxNSs +TAM
phase
YFP
50 μm
I
stroke
4d
neurosphere culture
GCV
10d
Number of cortex-derived neurospheres per 5000 cells
0.8
0.6
0.4
0.2
0.0
**
GCV
+
-
+
-
GFAP-TK
wt
J
Number of cortex-derived neurospheres per 2500 cells
0.3
0.2
0.1
0.0
AraC
7d
10d+,7d-

Figure S1

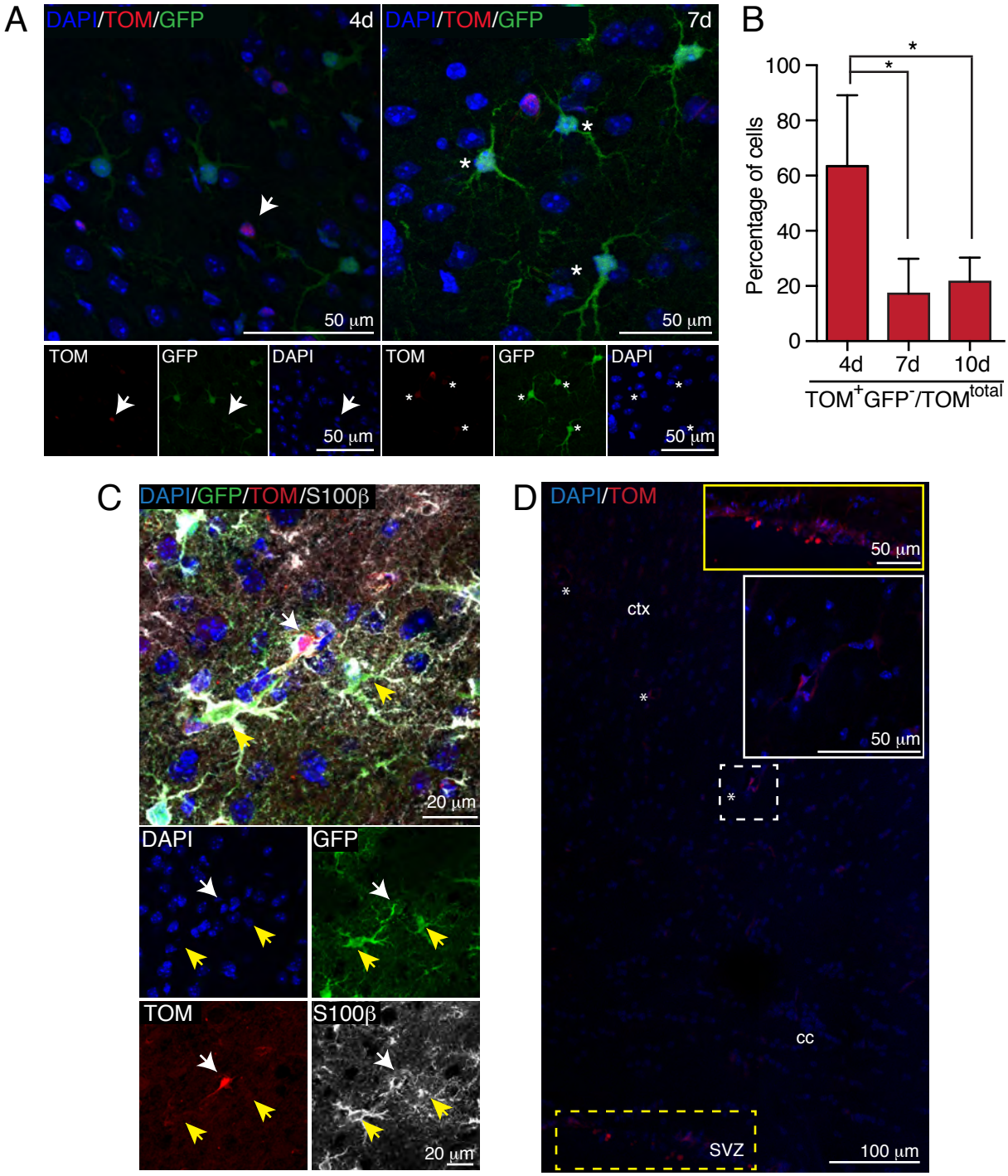
A
DAPI/TOM/GFP
4d
DAPI/TOM/GFP
7d
50 μm
50 μm
TOM
GFP
DAPI
50 μm
TOM
GFP
DAPI
50 μm
B
Percentage of cells
100
80
60
40
20
0
4d
7d
10d
TOM+GFP-/TOMtotal
C
DAPI/GFP/TOM/S100β
20 μm
DAPI
GFP
TOM
S100β
20 μm
D
DAPI/TOM
50 μm
ctx
50 μm
cc
SVZ
100 μm

Figure S2

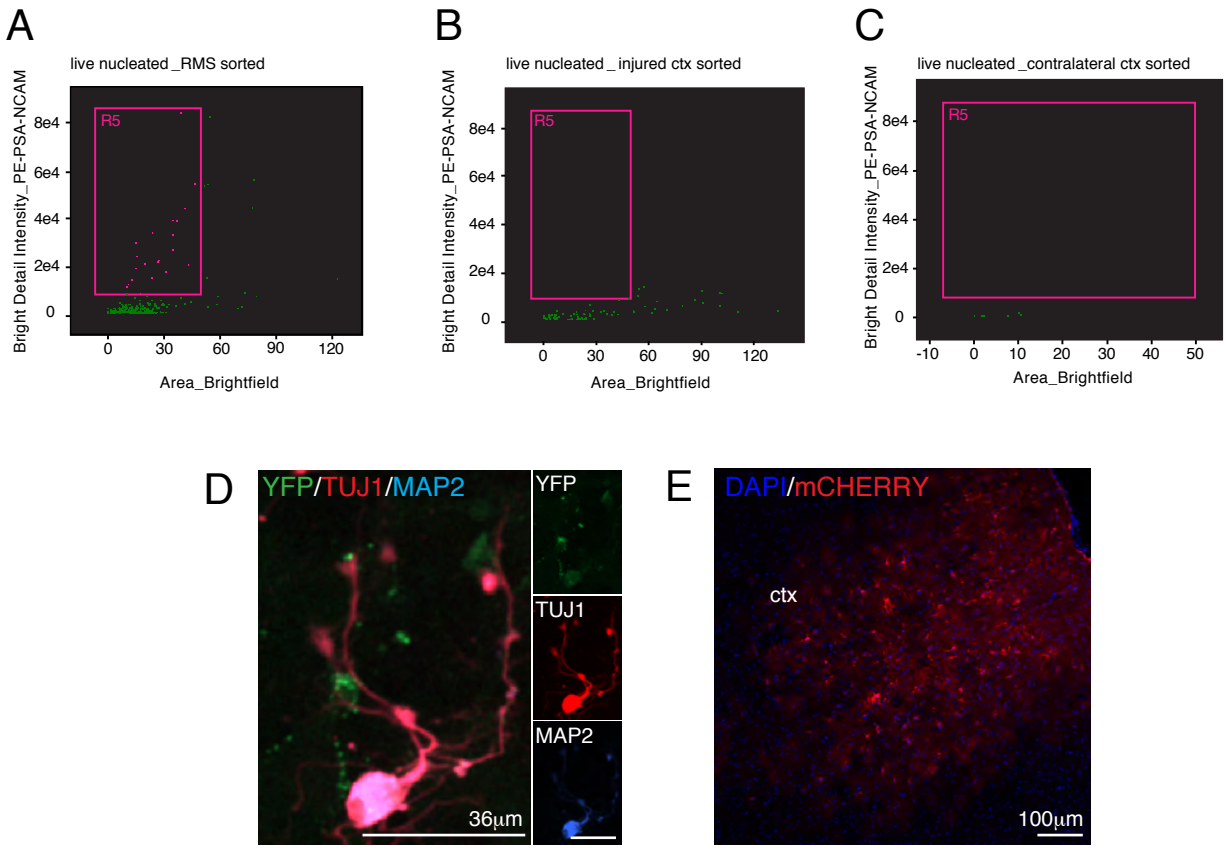
A
live nucleated _RMS sorted
Bright Detail Intensity_PE-PSA-NCAM
R5
8e4
6e4
4e4
2e4
0
0
30
60
90
120
Area_Brightfield
B
live nucleated _ injured ctx sorted
Bright Detail Intensity_PE-PSA-NCAM
R5
8e4
6e4
4e4
2e4
0
0
30
60
90
120
Area_Brightfield
C
live nucleated _contralateral ctx sorted
Bright Detail Intensity_PE-PSA-NCAM
R5
8e4
6e4
4e4
2e4
0
-10
0
10
20
30
40
50
Area_Brightfield
D
YFP/TUJ1/MAP2
YFP
TUJ1
MAP2
36μm
E
DAPI/mCHERRY
ctx
100μm

Figure S3

Table S1, Related to Figure 1. Percentage YFP+ labeled neurospheres from the SVZ and cortex of NesCreER^{T2};YFP^{fl} mice

Animal #	% Labeled YFP+ svzNSs	% Labeled YFP+ ctxNSs
1	3%	7% (1 of 15)
2	3%	4% (1 of 16)
3	4%	9% (1 of 11)
4	6%	6% (1 of 17)
5	7%	7% (1 of 14)
6	7%	7% (3 of 44)
7	8%	8% (1 of 12)
8	8%	5% (1 of 19)
9	9%	16% (1 of 6)
10	22%	43% (3 of 7)
11	28%	33% (1 of 3)
12	50%	100% (1 of 1)

Supplemental Figure Legends

Figure S1, Related to Figure 1. Neurospheres derived from the stroke-injured cortex

(A) Size of ctxNSs (** =p=0.0011, unpaired *t* test with Welch's correction, t(135) = 3.343, data represent ± s.e.m., n_{cx} = 71, n_{SVZ} =72, 4 independent trials). (B) Number of neurospheres from individual clones at each passage (n.s., unpaired *t* test with Welch's correction (except passage 2), $t(75)_{passage1}$ = 1.528, $t(71)_{passage2}$ = 0.4922, $t(55)_{passage3}$ = 0.3393, $t(31)_{passage4}$ = 1.254, $t(19)_{passage5}$ = 1.161 data represent ± s.e.m., n_{P1cx} = 73, n_{P2cx} = 39, n_{P3cx} = 49, n_{P4cx} = 27, n_{P5cx} = 9, n_{P1SVZ} = 17, n_{P2SVZ} = 16, n_{P3SVZ} = 16, n_{P4SVZ} = 16, n_{P5SVZ} = 16, 4 independent trials). (C) Multipotent sphere with TUJ1+ neurons, O4+ oligodendrocytes and GFAP+ astrocytes. (D) Percentage of neurons, astrocytes and oligodendrocytes upon differentiation (n.s. unpaired *t* test, $t(18)_{Tuj1}$ = 1.325, $t(17)_{O4}$ = 1.4225, $t(8)_{GFAP}$ = 0.02691, data represent ± s.e.m. from n_{Tuj1_cx} = 16, n_{Tuj1_SVZ} = 4; n_{Tuj1_cx} = 13, n_{Tuj1_SVZ} = 6; n_{O4_cx} = 7, n_{O4_SVZ} = 3, 3 independent trials). (E) Percent multipotent neurospheres formed from the cortex after lesions at sites lateral and caudal to the original injury of the sensory-motor cortex and from the cortex after pial vessel disruption (PVD) (ns = p_{PVD} = 0.18684, $p_{lateral}$ = 0.90448 = p_{caudal} = 0.31732 Z-test for independent proportions, z_{PVD} = -1.3242 $z_{lateral}$ = 0.124, z_{caudal} = 1.0022, data represent $n_{lateral\_ctx\_}$ = 11, $n_{lateral\_SVZ}$ = 12, n_{caudal_ctx} = 4, , n_{caudal_SVZ} = 14, n_{PVD_ctx} = 16, n_{PVD_SVZ} = 28. (F) tdTOMATO expression in svzNSs from tamoxifen fed and unfed mice. (G) NESTIN expression in YFP-GFAP+ astrocytes 4d after

stroke. (H) YFP expression in ctxNSs from tamoxifen fed mice. (I) GCV ablation paradigm and number of cortex-derived neurospheres after GCV administration in wt and GFAP-TK mice (**=p= 0.0015, paired *t* test, t(4) = 7.770, data represent ± s.e.m. from n_{wt_+GCV} = 5, n_{wt_-GCV} = 5; n.s. = p= 0.3503, paired *t* test, t(5) = 1.030, data represent ± s.e.m. from $n_{GFAP\text{-}TK\_+GCV}$ = 6, $n_{GFAP\text{-}TK\_-GCV}$ = 6. (J) Number of ctxNSs at 7d and 7d after AraC ablation (n.s., unpaired *t* test, t (9) = 1.857, data represent ± s.e.m. from $n_{10dAraC\_7d}$ = 4, n_{7d} = 7. SVZ = subventricular zone, cc = corpus callosum, ctx = cortex, lv = lateral ventricle, PVD = pial vessel disruption, TAM = tamoxifen, TOM = tdTomato.

Figure S2, Related to Figure 3. SVZ-derived cells differentiate into reactive astrocytes in the cortex after stroke.

(A) tdTOMATO+ and GFP+ cells at 4d and 7d post stroke. White arrow shows a tdTomato+GFP- cell, white asterisks show tdTOMATO+GFP+ cells. (B) Ratio of SVZ-derived precursors ($TOM+GFP+/TOM^{total}$) in Nestin-$CreER^{T2}$;$tdTomato^{fl}$;hGFAP-GFP mice (* = p<0.05, One-way ANOVA with Tukey's post hoc, $F(3,10)_{Tom+GFP-\ /Tom^{total}+}$ = 8.442, F(3,10) data represent ± s.e.m., n_{4d} = 3, n_{7d} = 4, n_{10d} = 4). (C) S100β expression in tdTOMATO+GFP+ reactive astrocyte. White arrowheads show tdTOMATO+GFP+S100β+ positive cell, yellow arrowheads show tdTOMATO-GFP+S100β+ cells. (D) tdTOMATO expression in uninjured tamoxifen fed mice. White and yellow insets show higher magnification photos of regions in dashed white and yellow boxes, asterisks

show labeling of vasculature. ctx = cortex, cc = corpus callosum, DCX = doublecortin, SVZ = subventricular zone, TOM = tdTomato.

Figure S3, Related to Figure 4. Conversion of reactive astrocytes to neurons

(A) ImageStream plot of the MACS sorted RMS. Gated cells in R5 show punctate PSA-NCAM on the surface of cells in the RMS. (B) ImageStream plot of the MACS sorted injured cortex. (C) ImageStream plot of the MACS sorted uninjured contralateral cortex. (D) TUJ1+MAP2+ neuron converted from a YFP labeled astrocyte. (E) mCHERRY expression in the cortex after AAV5-Gfap-*Ascl1*-T2A–*mCherry* injection. ctx = cortex

Table S1, Related to Figure 1. Percentage YFP+ labeled neurospheres from the SVZ and cortex of NesCreER^{T2};YFP^{fl} mice.

Numbers represent the percentage of YFP labeled neurospheres in the SVZ (svzNSs) and cortex (ctxNSs) per animal.

Supplemental Experimental Procedures

Mice

For NS analysis of cortex-derived versus SVZ-derived NSs at various times post stroke, C57BL/6J or hGFAP^{promoter}-GFP (hGFAP-GFP; Jackson Labs: FVB/N-Tg(GFAPGFP)14Mes/J) transgenic mice (Zhuo et al., 1997) were used.

No differences were found between strains regarding neurosphere number, size, passaging and differentiation.

For selective ablation of GFAP+ neurosphere forming cells, GFAP^{promoter}-TK (GFAP-TK; Jackson Labs: B6.Cg-Tg(Gfap-TK)7.1Mvs/J) mice (Bush et al., 1998) were used.

For analysis of the origin of stem cells in the cortex post stroke, Nestin^{promoter}-CreER^{T2} (Nestin-CreER^{T2}) transgenic mice (Imayoshi et al., 2006) were crossed with Rosa26-loxP-stop-loxP-tdTomato (tdTomato^{fl}; Jackson Labs: B6;129S6-*Gt(ROSA)26Sortm9(CAG-tdTomato)Hze*/J) transgenic reporter mice (Madisen et al., 2010) and Nestin-CreER^{T2} mice (Lagace et al., 2007) were crossed with Rosa26-loxP-stop-loxP-YFP (YFP^{fl}; Jackson Labs: B6.129X1-*Gt(ROSA)26Sortm1(EYFP)Cos*/J) transgenic reporter mice (Srinivas et al., 2001), resulting in Nes-CreER^{T2};tdTomato^{fl} and Nestin-CreER^{T2};YFP^{fl} double transgenic mice. Nestin-CreER^{T2};tdTomato^{fl} mice were crossed to hGFAP-GFP mice, resulting Nestin-CreER^{T2};tdTomato^{fl};hGFAP-GFP triple transgenic mice. In addition, Cspg4^{promoter}-DsRed (Cspg4-DsRed; Jackson Labs: Tg(Cspg4-DsRed.T1)1Akik) mice (Zhu et al., 2008) were used.

To assess conversion of reactive astrocytes to neurons GFAP^{promoter}-CreER^{T2} (GFAP-CrER^{T2}; Jackson Labs: Tg(GFAP-cre/ERT2)13Kdmc) mice (Casper et al., 2007) were crossed to YFP^{fl} transgenic mice, resulting in GFAP-CreER^{T2};YFP^{fl} transgenic mice.

Both males and females aged 6 weeks – 5 months were included in the analysis. All mice were housed in a barrier facility with a 12 hour light/12 hour dark cycle and allowed free access to food and water with a maximum of 4 mice per cage. Experiments were conducted according to protocols approved by the Toronto Center for Phenogenomics and the Department of Comparative Medicine of the University of Toronto.

Endothelin-1 stroke

Endothelin-1 (ET-1) strokes were preformed similar to as previously described (Tennant and Jones, 2009). One μL of 400 picomolar endothelin-1 (ET-1, Calbiochem) was injected streotaxically into the cortex at 0.6 AP, 2.25 ML, -1 DV (sensory-motor cortex coordinates), -1.7 AP, +4 ML, -3 DV (lateral coordinates) or -2.3 AP, +2 ML, -0.5 DV (caudal coordinates), relative to bregma. A 26 gauge Hamilton syringe with a 45 degree beveled tip was used to inject ET-1 at a rate of 0.1ml/min. The needle was left in place for 10 minutes after ET-1 injection to prevent backflow and then slowly withdrawn.

PVD stroke

PVD strokes were induced by removing the skull and dura in the region bound by 0.5 to +2.5 AP, +0.5 to 3 ML, relative to bregma. A saline-soaked cotton swab was used to remove pial vessels.

Cytosine b-D-arabinoside (AraC) infusion

For AraC infusion, a cannula was implanted to target the lateral ventricle (0.2 AP, 0.8 ML, and 2.5 DV, relative to bregma) and connected to a subcutaneous mini-osmotic pump (1007D, Alzet; 0.5μl/hour) overlying the shoulder blade and lateral to the spine. 2% AraC was delivered for 10-14 days to ablate cycling cells in the SVZ.

Tamoxifen induction

For tamoxifen induction *in vivo*, at or after 6 weeks of age, animals were fed tamoxifen food (Harlan) for 2 weeks and then switched to a high fat diet during a chase period of 2-3 weeks. Tamoxifen food was custom formulated to contain 0.5% TAM and 5% sucrose by weight and blue dye (to enable monitoring of consumption based on color of feces) in a high fat base (Harlan Diet 2019). For tamoxifen induction *in vitro*, 1mM OH-Tamoxifen (H7904, Sigma) was added every other day, for 7 days.

Adeno Associated Virus (AAV) Viral injections

AAV5-GFAP(0.7)^{promoter}-mCherry-T2A-mAscl1-WPRE (AAV5-Gfap-*Ascl1*-T2A-*mCherry*; 3.9 x 10^{13} GC/mL) and AAV5-GFAP(0.7)^{promoter}-mCherry-WPRE (AAV5-Gfap-*mCherry*; 2.9 x 10^{13} GC/mL) particles were purchased from VectorBioLabs. 1uL of AAV was injected into the injured cortex of NestinCreER^{T2};YFP^{fl} mice 7d post stroke at a rate of 0.1mL/min at the following

coordinates: 0.6 AP, 2.25 ML, -1 DV, 1.6 AP, 2.25ML -1 DV and -1 AP, 2.25 ML, -1 DV, relative to bregma. The needle was left in place for 10 minutes after AAV injection to prevent backflow and then slowly withdrawn.

5-ethynyl-2´-deoxyuridine (EdU) injections

250 mg/kg EdU (Life Technologies, E10187) was injected intraperitoneally once daily for either 2 or 4 days after stroke.

Tissue processing, immunohistochemistry and quantification.

Mice were anesthetized with Avertin, perfused transcardially and postfixed for 2 hours with 4% paraformaldehyde. Brains were cryopreserved in 30% sucrose, frozen and 18 μm coronal cryostat sections were cut.

Cryosections were blocked with 10% normal goat serum (NGS) and 0.3% triton in PBS and labeled with a primary antibody in PBS overnight at 4C, followed by incubation with a secondary antibody and a nuclear stain in PBS for 1h at room temperature (RT). For double and triple immunohistochemistry, sections were reblocked in 10% NGS and 0.3% triton in PBS, incubated with a primary antibody 4°C O/N and then with a secondary antibody at RT for 1h. Samples labeled with gt anti dcx primary antibody were blocked in 10% normal donkey serum and 0.3% triton in PBS and incubated O/N at RT. Samples labeled with anti Ch Alexa 488 secondary antibody were incubated for 2h at RT. For EdU

staining, a ClickiiT EdU imaging kit (Life Tech, C10337 and C103040) was used according to the manufacturer's instructions.

In vivo, overlapping expression of tdTOMATO and GFP or YFP and GFAP in cell bodies was quantified in three 40x confocal stacks in 3 coronal sections per animal. For quantification of EdU or dcx and YFP, three 20x confocal stacks in 3 coronal sections per animal were obtained. For analysis of mCherry, YFP and neuronal markers, at least 100 cells were counted in at least three 20x confocal stacks per animal. Imaging was performed using Zen 2011 software and a Zeiss two-photon microscope, Velocity software and a Zeiss spinning disc confocal or Axiovision software and a Zeiss Observer Z1 inverted fluorescence microscope. Linear adjustments of contrast and brightness were made to micrographs using the respective microscope software. Threshold intensity was set according to the background signal detected in controls.

Summary of reagents used for immunohistochemistry

Primary Antibodies	**Dilution**	**Company**	**Catalogue Number**
Ms anti-β-III-tubulin (TUJ1)	1:500	Covance	MMS-435P-250
Rb anti-β-III-tubulin (TUJ1)	1:500	Covance	MRB-435P-100
Rb anti-GFAP	1:3000	DAKO	2016-04
Rb anti-S100β	1:200	DAKO	Z0311

Ms anti MAP2(a+b)	1:200	Sigma	M1406
Ms (IgM) anti O4	1:1000	R&D Systems	MAB1326
Rb anti KI-67	1:200	AbCam	Ab15580
Ch anti GFP	1:1000	Aves	GFP-1020
Rb anti RFP	1:2000	Rockland	600-401-379
Rb anti NeuN	1:100	Millipore	ABN78
Gt anti DCX	1:200	Santa Cruz	sc-8066
Secondary Antibodies	**Dilution**	**Company**	**Catalogue Number**
Anti ms IgM Alexa 568	1:400	Life Tech	A21043
Anti-ms IgG Alexa 568	1:400	Life Tech	A11031
Anti-ms IgG Alexa 488	1:400	Life Tech	A11001
Anti ms IgG Alexa 647	1:400	Life Tech	A21236
Anti gt IgG Alexa 647	1:400	Life Tech	A21447
Anti rb Alexa 568	1:400	Life Tech	A11036
Anti rb Alexa 647	1:400	Life Tech	A21245
Anti ch Alexa 488	1:400	Life Tech	A11039

Nuclear Stains	Dilution	Company	Catalogue Number
DAPI	50ng/mL	Life Tech	D3571
TOPRO	1:1000	Life Tech	T3605

Neurosphere Culture

Adult mice were anesthetized with isoflurane and cervically dislocated. Animals were decapitated and the meninges were removed. For cortical dissections, coronal slices were made using a scalpel blade and the cortex was collected, taking care to avoid the corpus callosum. The SVZ was dissected as previously described (Coles-Takabe et al., 2008; Tropepe et al., 1997). The tissue was enzymatically and mechanically dissociated into a single-cell suspension. Cells were plated at clonal density (5 cells/μL) in NS media (Neurobasal (Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech), epidermal growth factor (EGF; Sigma; 20ng/ml), fibroblast growth factor (FGF; Sigma; 10ng/ml), and heparin (2000ng/ml). For gangciclovir (GCV) ablation experiments, 20 μM GCV was added to the media before plating. After 10 days *in vitro*, the numbers of primary NSs was counted and the size of individual NSs was measured using Image J software (NIH). Primary NSs were grown for 10 days. For single sphere passaging, individual NSs were passaged every 7 days. For bulk sphere passaging, NSs were passaged every 7 days. NSs were

mechanically dissociated into a single-cell suspension, and replated under the same conditions as primary cultures. The number of ctxNSs per mouse was calculated as follows:

Number of cortical neurosphere forming cells per brain at 4d post stroke =

(number of cells after dissociation x number of neurospheres per well) / number of cells per well

Neurosphere differentiation immunocytochemistry and quantification.

One primary NS was plated per well of a 48-well plate coated with laminin (5 *µ*g/ml, Sigma) in differentiation medium (Neurobasal, Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech) and 1% fetal bovine serum (Life Tech). After 7 days of differentiation, cells were fixed with 4% paraformaldehyde.

For immunocytochemistry for cell phenotype markers, cell cultures were fixed in 4% PFA, blocked with 10% NGS in PBS and labeled with mouse anti-O4 overnight (O/N) at 4°C followed by an incubation with a secondary antibody. For double and triple immmunolabeling, cultures were re-blocked in 10% NGS and 0.3% triton in PBS and incubated with rabbit anti-GFAP and mouse anti-β-III-tubulin followed by incubation with a secondary antibody and DAPI. The number of phenotype marker positive cells and DAPI positive cells were quantified in three 10x photos per well. Images were captured using Zen 2011 software and a

Zeiss two-photon microscope or a Zeiss inverted microscope.

Reactive astrocyte culture

GFAP-CreER^{T2};YFP^{fl} adult mice were anesthetized with isoflurane and cervically dislocated at 4d post stroke. The meninges were removed and coronal slices were made using a scalpel blade. The cortex was collected, taking care to avoid the corpus callosum. The tissue was enzymatically and mechanically dissociated into a single-cell suspension. One cortical hemisphere was resuspended in 1mL of astrocyte media (DMEM/F12 (Life Tech) containing L-glutamine (2 mM, Life Tech), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Life Tech), B27 (1:50, Life Tech), 0.45% glucose (Sigma, G8769) and 10% fetal bovine serum (FBS, Life Tech)) and plated in 2 wells of a 24 well poly-d-lysine coated plate. Media was changed the next day. Cultures could only be established from the ispsilateral hemisphere and never from the hemisphere contralateral to the stroke site.

Astrocyte conversion in vitro

Two days after reactive astrocyte culture establishment from GFAP-CreER^{T2};YFP^{fl} mice, 4-Hydroxytamoxifen (4-OH-TAM) was added to astrocyte media. Media + 4-OH-TAM was changed every other day for 5d. After 7d, cells were transfected with PBase, PB-CAG-rtTA (Woltjen et al., 2009) and PB- tetO-*Ascl1*-T2A-*Bfp* or CAG-*Ascl1*-IRES-*Dsred* using HD Fugene (Roche) at a ratio of

2:1. For control experiments either PB-tet-O-*B*fp or CAG-*Dsred* was used. The media was changed to N2B27 (50% Neurobasal (Life Tech) and 50% DMEM/F12 (Life Tech) containing B27 (1:50, Life Tech) and N2 (1:100, Life Tech). For PB-tetO-*Ascl1*-T2A-*Bfp* expression, 1500 ng/mL dox was added to the media. Media was changed every other day for the duration of the experiment.

The number of neurons was quantified by assessing converted neurons (TUJ1+YFP+BFP+ or TUJ1+YFP+dsred+ cells)/transfected astrocytes (YFP+BFP+ or YFP+DSRED+ cells).

Plasmids

PB-tetO-*Ascl1*-T2A-*Bfp*

Fusion PCR was used to amplify *Ascl1* and *Bfp* from Tet-O-FUW-*Ascl1* (Addgene) and pENTR-BFP and generate an attb PCR product with a T2A linker between *Ascl1* and *Bfp* and gateway overhangs using the following primers:

Attb_Ascl1_Fwd

5'GGGGACAAGTTTGTACAAAAAAGCAGGGTTCGCCACCATGGAGCAAAAGC TCATTT**3'**

Ascl1_T2A_Rev

5'CCTCTGCCCTCTCCGGATCCGAACCAGTTGGTAAAGTCC**3'**

T2A_BFP_Fwd

5'GGATCCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGG AGAATCCTGGCCCCATGGTGAGCAAGGGCGAG**3'**

BFP_Attb_Rev

5'GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACTTGTACAGCTGCTC**3'**

The attb PCR product was cloned using Gateway into pRetroX (Life Tech) and Piggybac transposon destination vectors. Vectors were sequenced for verification.

CAG-*Ascl1*-IRES-*Dsred* and CAG-*Dsred* were kind gifts from Magdalena Götz.

MACS and Image Stream FACS Analysis

Adult mice were anesthetized with isoflurane and cervically dislocated. Animals were decapitated at 4d post stroke. The meninges were removed and coronal slices were made using a scalpel blade. The cortex was collected, taking care to avoid the corpus callosum. The RMS was also dissected. The tissue was enzymatically and mechanically dissociated into a single-cell suspension. Cells were resuspended in MACS sorting buffer (Ca^{++} and Mg^{++} free PBS with 2% FBS and 1mM EDTA), stained with anti-PSA-NCAM-PE (1:100, Miltenyi Biotech, 30-093-274) and MACS sorted using a PE selection kit (Stem Cell Technologies, 18554) and an EasySep Magnet (Stem Cell Technologies, 18000), according to the manufacturer's instructions. For ImageStream analysis, dissociated cells were resuspended in 500uL of PBS (azide and serum/protein free) and stained with a fixable cell viability dye (FVD eFluor 780; eBioscience, 65-0865-14) according to the manufacturer's instructions. Cells were then washed with 10%

FBS in PBS, resuspended in 500 μL of MACS sorting buffer, stained with anti-PSA-NCAM-PE (1:100, Miltenyi Biotech, 30-093-274) or a mouse IgM-PE control (1:100, Miltenyi Biotech, 130-099-127), MACS sorted using a PE selection kit (Stem Cell Technologies, 18554) and an EasySep Magnet (Stem Cell Technologies, 18000), according to the manufacturer's instructions, stained with Hoescht (1 μg/mL, BD Biosciences, 33342) for 30 min at RT and resuspended in MACS sorting buffer. Cells from the MACS sorted fractions, column flow through (supernatant) fractions and unsorted cells from the injured cortex, uninjured (contralateral) cortex and RMS were analysed with an Amnis ImageStreamTM Mark II imaging flow cytometer (AMNIS). Single stained controls were collected for the compensation matrix. The resulting compensated image files were analysed using IDEAS analysis software (AMNIS). Focused, single cells were selected based on viability (exclusion of FVD e780 dye) and the presence of a nucleus (positive for Hoescht staining). The live nucleated cells were examined for PE-PSA-NCAM signal and the specific and punctate PSA-NCAM-PE binding to cells was described using the Bright Detail Intensity feature of the IDEAS software. The increased Bright Detail Intensity scores differentiated PSA-NCAM-PE binding from non-specific, diffuse binding of the IgM-PE isotype control. The antibody binding patterns were confirmed in composite cell images of bright field and PE fluorescence generated from the ImageStream data.

Analysis of the RMS supernatant showed high purity (only one cell escaped the column).

Data Acquisition and Statistical Analysis

All experiments were conducted blind, using a numbering system. F tests were used to compare variance between groups. Unpaired two-tailed *t* tests or unpaired two-tailed *t* tests with Welch's correction (for unequal variance) were used for comparisons between two groups. One-factor ANOVA was used followed by post hoc analysis (Tukey's or Dunnet's test) for comparisons between three or more groups. Comparison of percentages between groups was done using two sample z-tests for individual proportions. Differences were considered significant at $p < 0.05$. Values are presented as mean ± SEM.

Supplemental References

Tennant, K.A., and Jones, T.A. (2009). Sensorimotor behavioral effects of endothelin-1 induced small cortical infarcts in C57BL/6 mice. Journal of neuroscience methods *181*, 18-26.