Supporting Information

Kohyama *et al.* 10.1073/pnas.0808417105

SI Materials and Methods

Cell Culture and *in Vitro* Differentiation. Neural progenitor cells (AHPs) isolated from hippocampus of adult female Fischer 344 rats, used in this study, have been characterized previously (1). The methods for maintaining AHPs and inducing their differentiation into specific lineages have been reported (2). In brief, cells were cultured in N2-supplemented Dulbecco's modified Eagle's medium with F12 (DMEM/F12; Gibco) containing 20 ng/ml basic fibroblast growth factor (bFGF) (PeproTech) on culture dishes precoated with polyL-ornithine (Sigma) and laminin (BD Bioscience). AHPs were transferred to eight-well chamber slides (Nunc) at a density of 25,000 cells/well and were allowed to proliferate for 24 hours in DMEM/F12-N2 containing 20 ng/ml bFGF-2. The bFGF-2 was then withdrawn and the cells were subsequently cultured in DMEM/F12-N2 medium with 1 μmol/l retinoic acid (Sigma-Aldrich) and 5 μmol/l forskolin (Sigma-Aldrich) for 4 days (for neuronal differentiation). To induce oligodendrocyte differentiation, AHPs were trypsinized, washed with PBS, and directly plated into DMEM/F12-N2 medium containing 50 ng/ml IGF1 (PeproTech) and cultured for 4 days. AHP-derived neurons and oligodendrocytes were then cultured in DMEM/F12-N2 medium with 0.5% FBS in the presence or absence of 50 ng/ml LIF (Chemicon) for 2 or 4 days. AHPs were transfected by electroporation using a Nucleofector device (Amaxa), following the manufacturer's guidelines. MBP-Cre and CAG-CAT-EGFP vectors were kindly provided by Drs. M. Miura (University of Tokyo) and J. Miyazaki (Osaka University), respectively. Rat MeCP2 cDNA was inserted into a modified pCALNLw vector (3).

Reverse Transcription–PCR (RT-PCR). Total RNAs were isolated using Sepasol RNAI (nacalai tesque) and treated with DNase I (Promega). First-strand cDNAs were synthesized from 1 μg total RNA with SuperScript II (Invitrogen). The RT products (1 μl) were used as templates for PCR amplification (AmpliTaq Gold; Applied Biosystems) in 25-μl reactions that included 2 μmol/l gene-specific primers, as appropriate, from the following: rat GFAP: rGFAP-S, 5′-GGTGGAGAGGGACAATCTCA-3′; rGFAP-AS, 5′-CTCGAACTTCCTCCTCATGG-3′; rat aquaporin4 (Aqp4): rAqp4-S, 5′-CTCATCTCCCTCTGCTTTGG-3′; rAqp4-AS, 5′-TCAGTCCGTTTGGAATCACA-3′; rat G3PDH: rG3P-S, 5′-ACCACAGTCCATGCCATCAC-3′; rG3P-AS, 5′-TCCACCACCCTGTTGCTGTA -3′.

Western Blot Analysis. Western blot analysis was performed by an established method. Protein samples (20 μg) of each total cell extract were separated by 5–20% gradient SDS/PAGE, transferred to a nitrocellulose membrane, and probed with anti-GFAP (rabbit IgG, DAKO) and anti-β-actin (mouse IgG, Sigma) antibodies. Signals were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson Immunoresearch Laboratory) using an ECL kit (Amersham Biosciences). The amounts of proteins loaded in each slot were normalized to those of β-actin.

Immunostaining. Cells were fixed with 4% paraformaldehyde (PFA) and stained immunocytochemically as described previously (4), using the following antibodies: rabbit anti-MeCP2 (1:100, Santa Cruz), mouse anti-Map2ab (1:250, Sigma), rabbit anti-GFAP (1:1500, Chemicon), rabbit anti-cleaved caspase 3 (1:400, Cell Signaling), rabbit anti-phosphorylated STAT3 (1:500, Cell Signaling), rat anti-BrdU (1:400, OBT), mouse anti-RIP (1:100, Developmental Studies Hybridoma Bank), mouse anti-S100β (1:500, Sigma), rabbit anti-MBP (1:500, Chemicon), chicken anti-MBP (1:100, Aves Labs), guinea pig anti-GFAP (1:2500, Advanced Immunochemical) or rabbit anti-GFP (1:1000, MBL). The following secondary antibodies were used: Alexa488-conjugated goat anti-mouse IgG (Molecular Probes) or Cy3-conjugated goat anti-mouse IgG (Jackson Laboratory), Alexa488-conjugated goat anti-rabbit IgG (Molecular Probes) or Cy3-conjugated donkey anti-rabbit IgG (Jackson Laboratory), Cy3-conjugated goat anti-chicken IgY (Jackson Laboratory), or Cy5 conjugated goat anti-chicken IgY (Jackson Laboratory). All experiments were independently replicated at least three times. For immunohistochemical analysis, adult mouse brains were removed and postfixed with 4% PFA in PBS overnight at 4°C. Coronal sections (50 μm thick) were incubated at 4°C overnight with primary antibodies and then with secondary antibodies conjugated with Alexa Fluor (Molecular Probes).

Sections containing infarcted regions from three mice were selected to count the number of GFAP-positive cells in the EGFP-positive cells. The boundary or core infarcted areas were chosen randomly and captured by confocal microscopy (LSM510; Carl Zeiss).

Recombinant Retrovirus. Rat MeCP2 cDNA was cloned into the expression vector pMY, containing an internal ribosome entry site followed by the EGFP gene (5). The Plat-E packaging cell line was transiently transfected with retrovirus constructs using TransIT 293 (Muras) (4). On the following day, the medium was replaced with DMEM/F12-N2/bFGF and the cells were cultured in this medium for 1 day before virus was collected.

Bisulfite Sequencing. Sodium bisulfite treatment of genomic DNA was performed essentially as described previously (6). Briefly, 5 μg genomic DNA was digested with HindIII and EcoRI, denatured with 0.3 mol/l NaOH at 37°C for 15 minutes and incubated with 3.1 mol/l sodium bisulfite and 0.5 mmol/l hydroquinone at 55°C for 16 hours. The samples were purified using a desalting column (Promega) according to the manufacturer's instructions and eluted in 50 μl H_2O. A 3-mol/l quantity of NaOH (5.5 μl) was added and the samples were incubated at 37°C for 15 minutes. Samples were then neutralized by the addition of 3 mol/l ammonium acetate, ethanol-precipitated, and dissolved in H_2O. Specific DNA fragments were amplified by PCR using the following sets of primers: for the STAT3 recognition sequence-containing region in the *gfap* promoter, rGFmS (5′-GTAG-GATTTATTAAAGGAATTTTAGTAAAG-3′) and rGFmAS (5′-TCTACCCATACTTAAACTTCTAATATCTAC-3′); for the region around the transcriptional start site of the *gfap*, rGFmeEx1S (5′-GATAGGTGAGTAATTTATGGA-3′) and rGFmeEx1AS (5′-TTCAATCTCTCTACTCACTAACC-3′). The PCR products were cloned into pT7Blue vector (Novagen) and sequenced.

Chromatin Immunoprecipitation Assay. Chromatin immunoprecipitation (ChIP) was performed according to a protocol published by Upstate Biotechnologies. AHP-derived oligodendrocytes and the cells cultured with LIF for 2 days were exposed to formaldehyde, at a final concentration of 1%, added directly to the tissue culture medium. Co-immunoprecipitated DNA was used as a template for PCR with the following set of primers: Gfex1S (5′-TGACATCCCAGGAGCCAG-3′) and Gfex1AS (5′-CAGTCTCTCTGCTCACTAGCC-3′).

Animals. MBP-Cre Tg mice (7) were provided by Dr. M. Miura (University of Tokyo, Japan). CAG-CAT-EGFP Tg mice (8) were a gift from Dr. J. Miyazaki (Osaka University, Japan). All experimental procedures and protocols were approved by the Animal Care and Use Committee of Keio University.

Focal Cerebral Ischemia. Mice were anesthetized by nitrous oxide/oxygen/isoflurane (69/30/1%) administered through an inhalation mask during surgery. The right carotid bifurcation was exposed under a surgical microscope, and the external carotid artery was coagulated distal to the bifurcation. A silicone-coated 8–0 filament was then inserted through the stump of the external carotid artery and advanced (9–10 mm) to occlude the middle cerebral artery as described previously (9). After occlusion for 30 minutes, the filament was withdrawn and the incision closed. The animals were then allowed to recover from the anesthesia in a warm environment. Before performing the MCAO, a laser Doppler flowmeter probe (Model ALF21; Advance) was attached to the surface of the ipsilateral cortex to monitor regional cerebral blood flow. During surgery, rectal temperature was maintained at 37.0°C by placing the animals on a heating bed.

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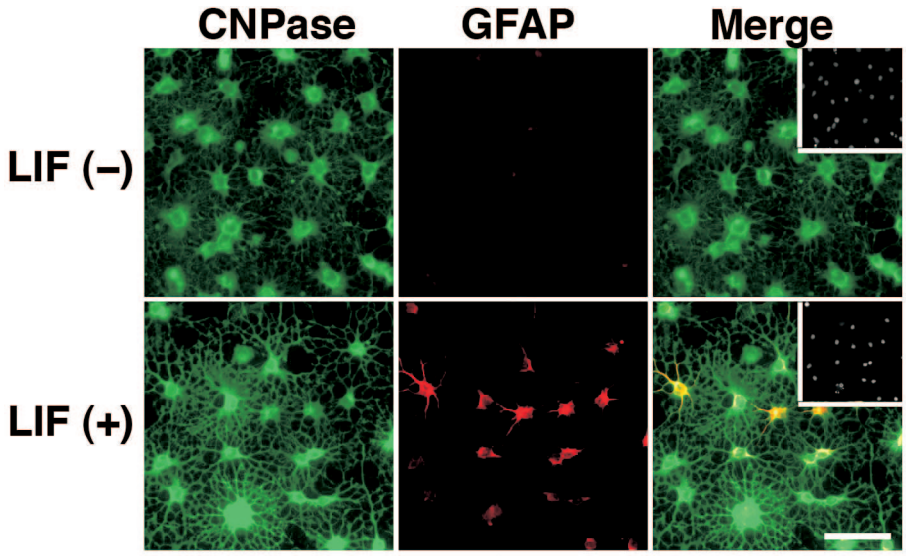

Fig. S1. Differentiation plasticity of oligodendrocytes. AHP-derived oligodendrocytes were cultured for 2 days with or without LIF (50 ng/ml). Cells were then stained with antibodies for CNPase (left, green) and GFAP (middle, red). *Insets:* Hoechst nuclear staining of each field. Scale bar = 50 μm.

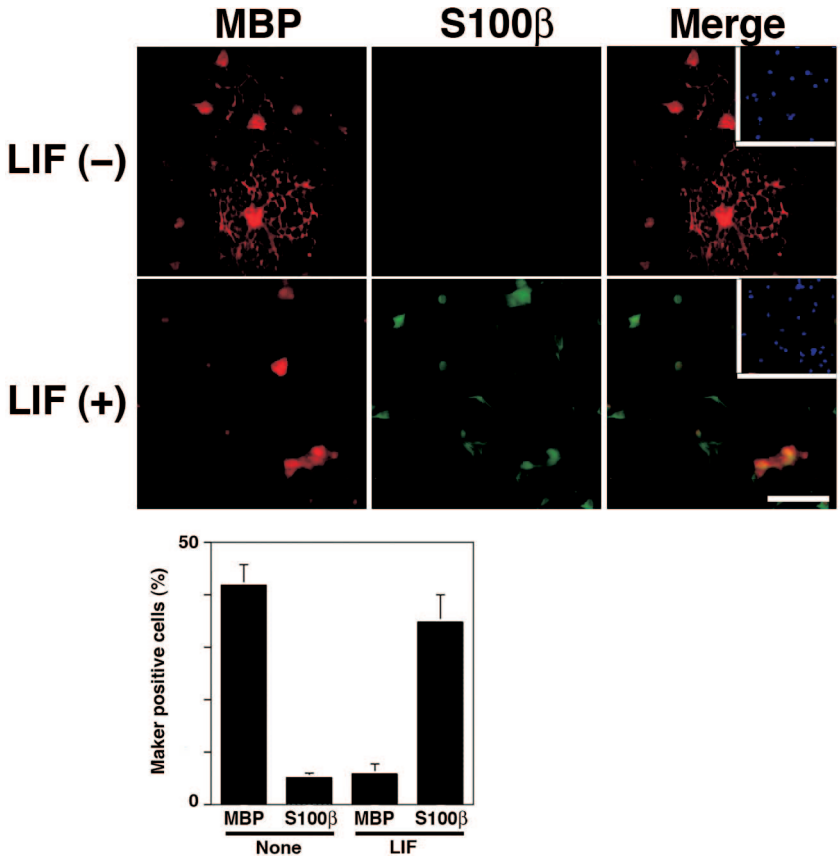

Fig. S2. S100β expression induced by LIF in oligodendrocytes. Oligodendrocytes were cultured with or without LIF (50 ng/ml) for 4 days and subsequently stained with antibodies for MBP (red) and S100β (green). *Insets:* Hoechst nuclear staining of each field. Scale bar = 50 μm. The percentages of S100β- and MBP-positive cells in total cells were quantified (graph). Data are mean ± SD.

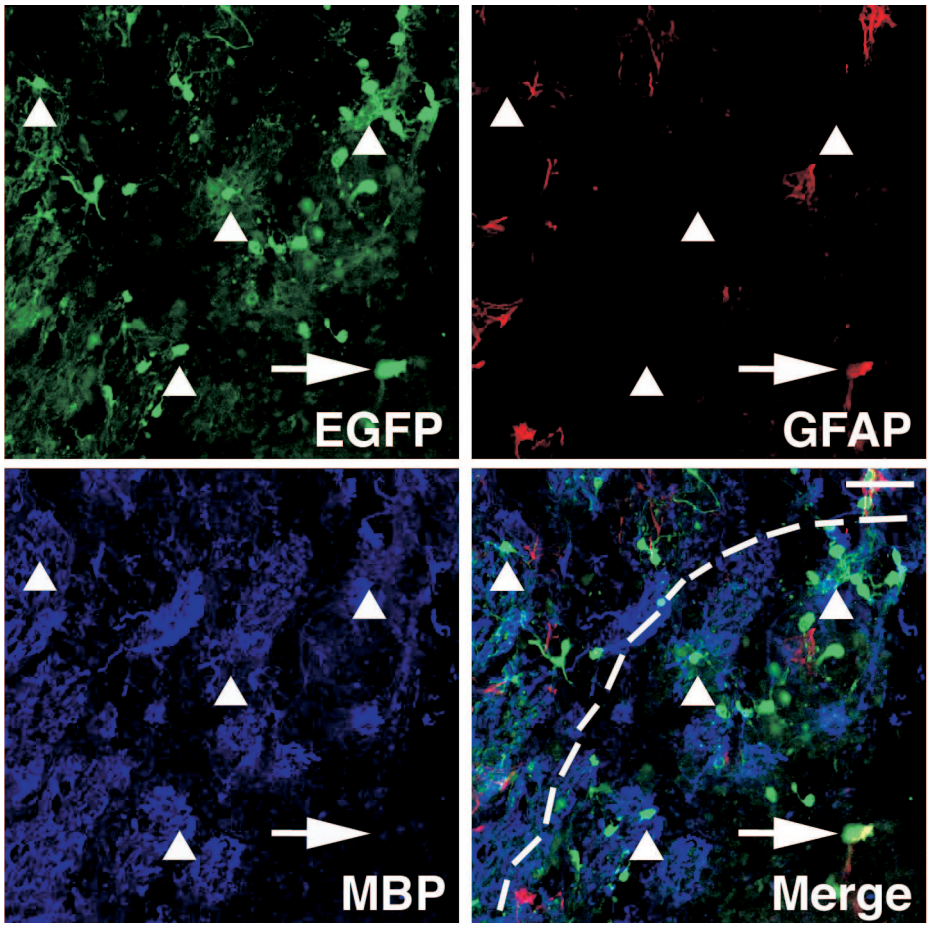

Fig. S3. Unconverted cells retain oligodendrocytic character. Cells expressing EGFP (green) but not GFAP (red) (arrowheads) were positive for the oligodendrocytic marker MBP (blue) 14 days after MCAO. Dotted line indicates boundary between peri-infarct (upper left region) and ischemic core (lower right region) areas.

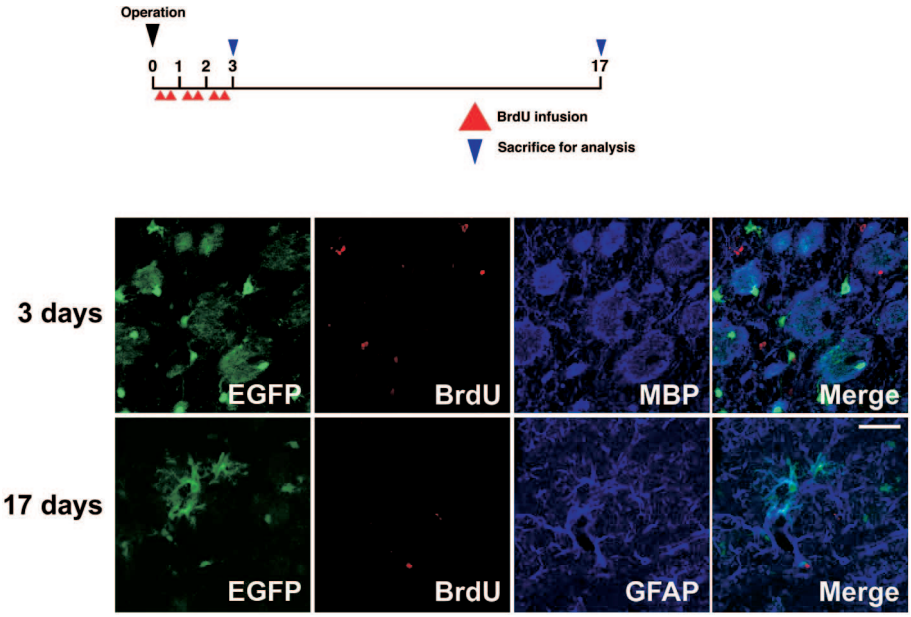

Fig. S4. O-A transition is independent of cell proliferation. Schematic diagram of BrdU labeling is shown in the upper. Mice received an i.p. injection of BrdU (50 mg/kg) twice a day for 3 days after ischemic injury. BrdU incorporation was assessed 3 and 17 days after MCAO surgery. EGFP-positive cells (green) were not positive for BrdU (red).

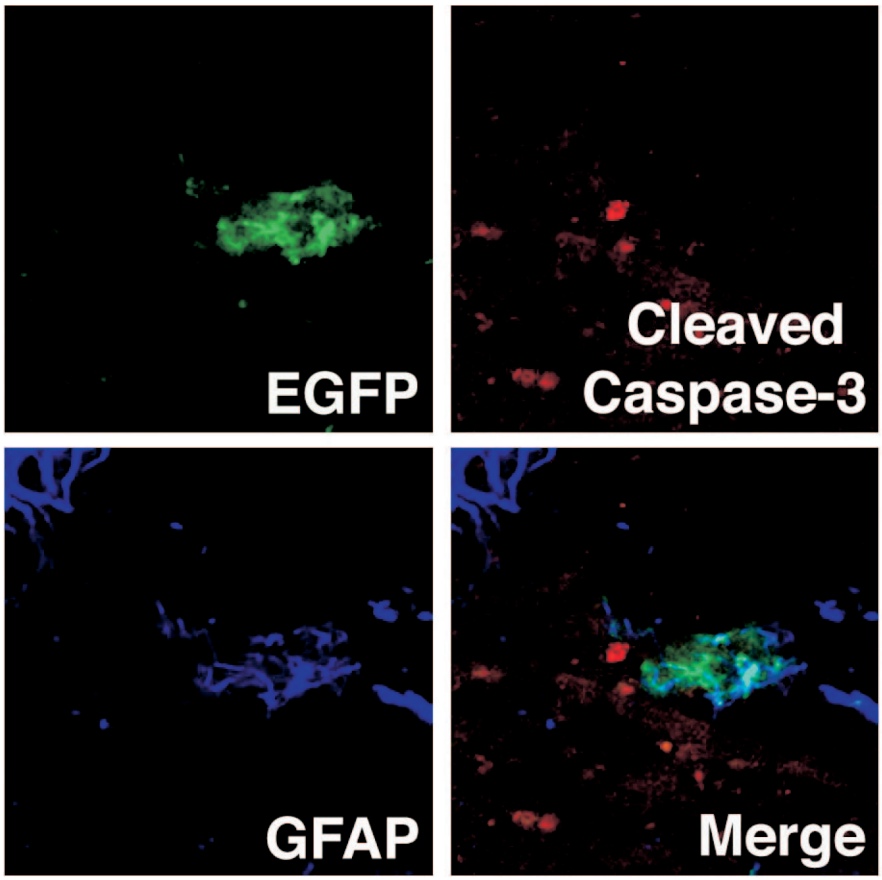

Fig. S5. EGFP and GFAP double-positive cells generated after MCAO injury are not apoptotic. Cells were examined 2 weeks after injury for immunoreactivity against cleaved caspase-3. EGFP-positive (green) and GFAP-positive (blue) astrocytes are not positive for cleaved caspase-3 (red).

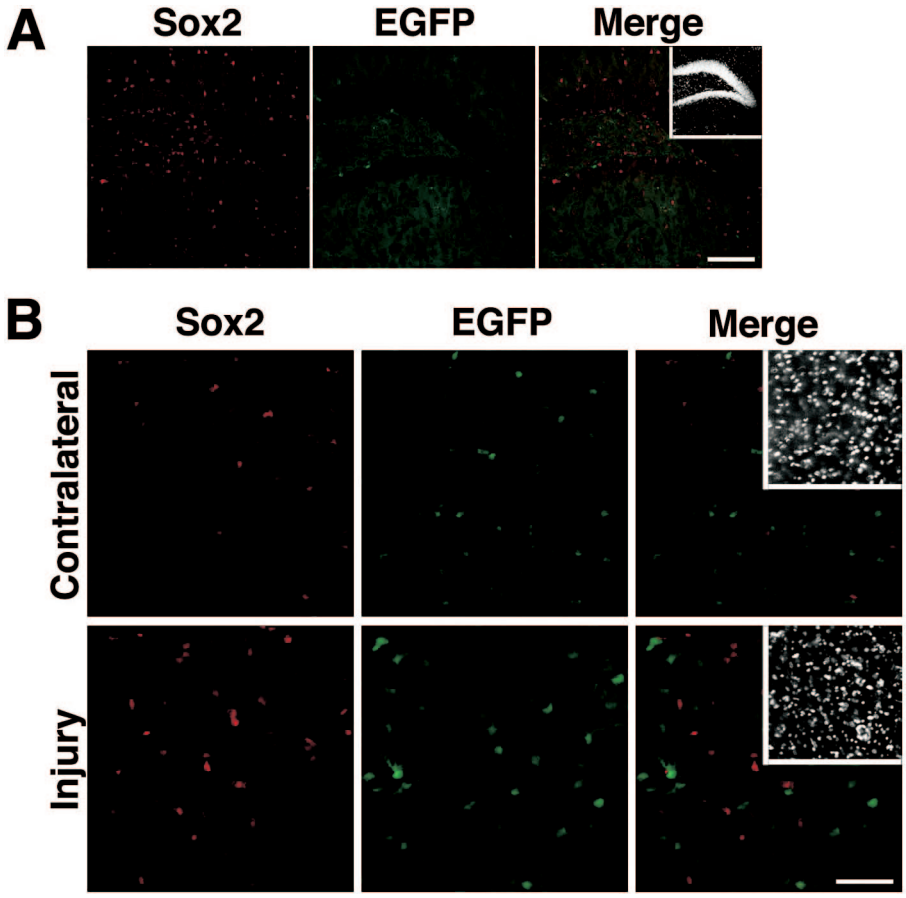

Fig. S6. Examination of Sox2 expression in injured MBP-Cre/EGFP transgenic mice. (*A*) Sox2 expression in the hippocampus. EGFP-positive cells (green) were not Sox2-positive (red). Scale bar = 100 μm. *Insets:* Hoechst nuclear staining of the field. (*B*) Sox2 expression in the striatum of injured MBP-Cre/EGFP mice was examined 3 days after ischemic insult. Sox2 expression (red) was not observed in EGFP-positive cells (green) on either the contralateral or the injured side. Scale bar = 50 μm. *Insets:* Hoechst nuclear staining of each field.

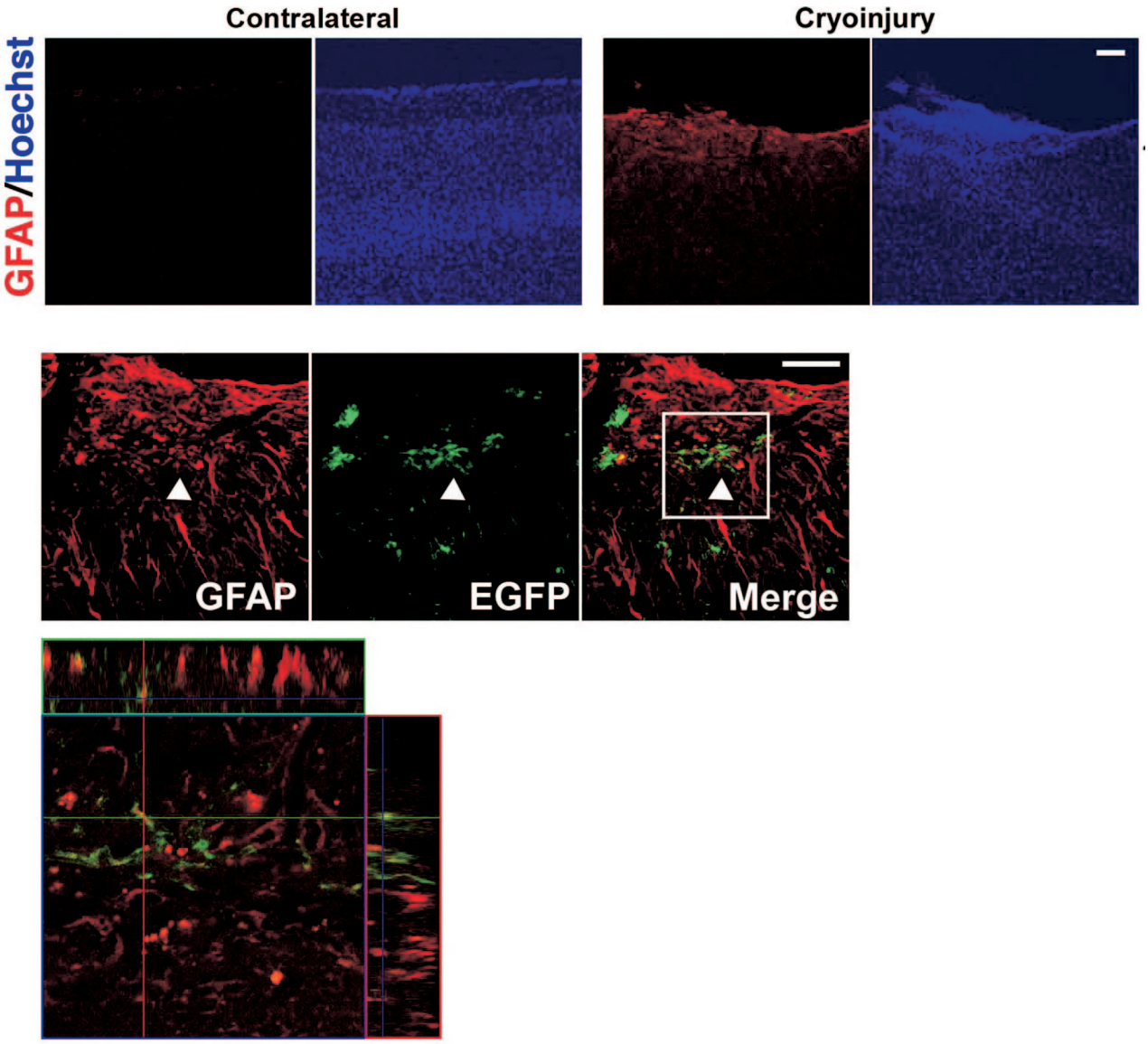

Fig. S7. Representative image of MBP-Cre/EGFP transgenic mice 2 weeks after cold injury. Cold injury was effected by placing a metal probe that had been equilibrated in liquid nitrogen for 15 minutes against the right parietal bone for 60 seconds. GFAP expression increased after cold injury (top panel). Colocalization of EGFP and GFAP was observed (arrowhead, middle panel). Bottom panel shows three-dimensional digital image of square area in middle panel.