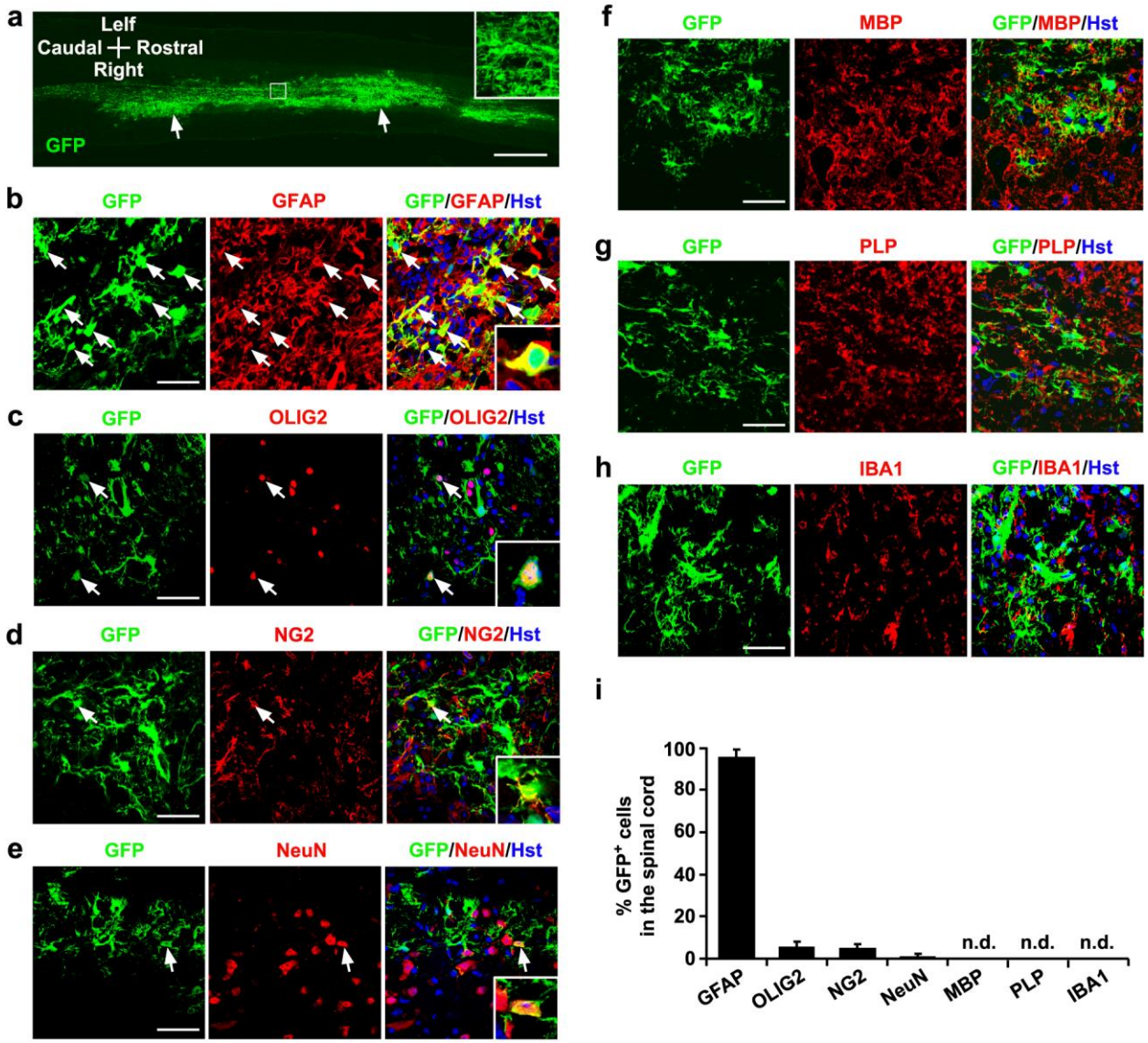
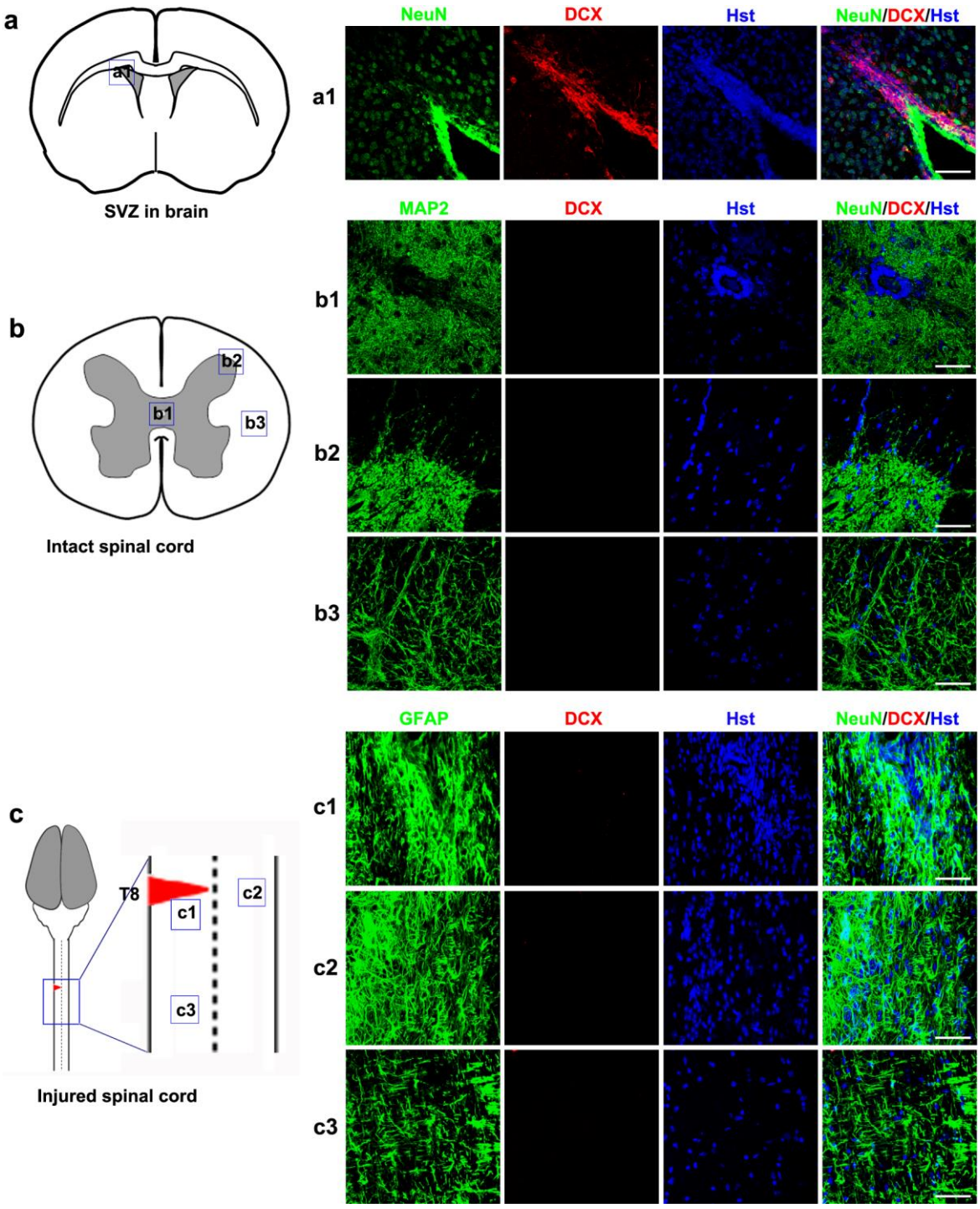
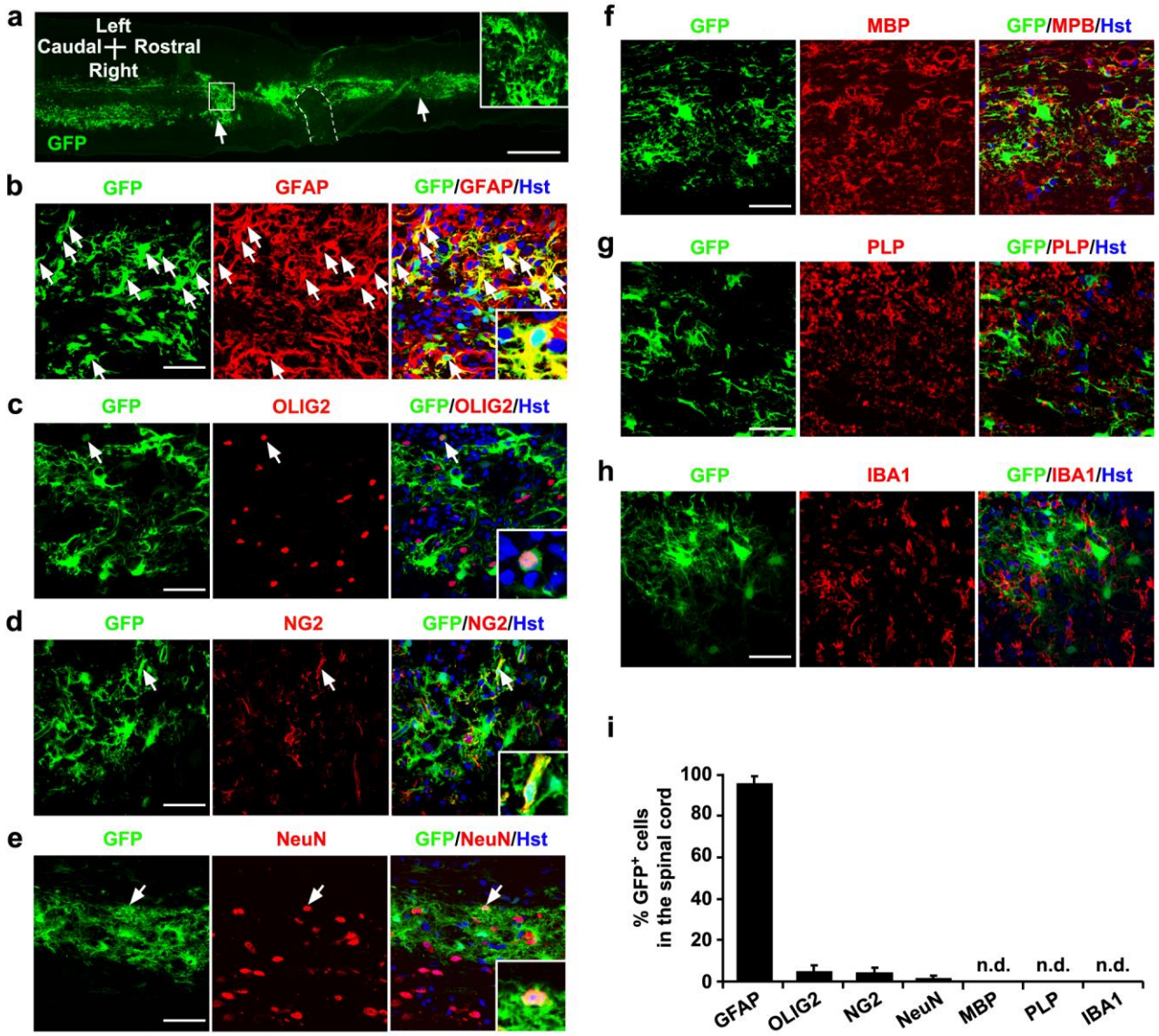


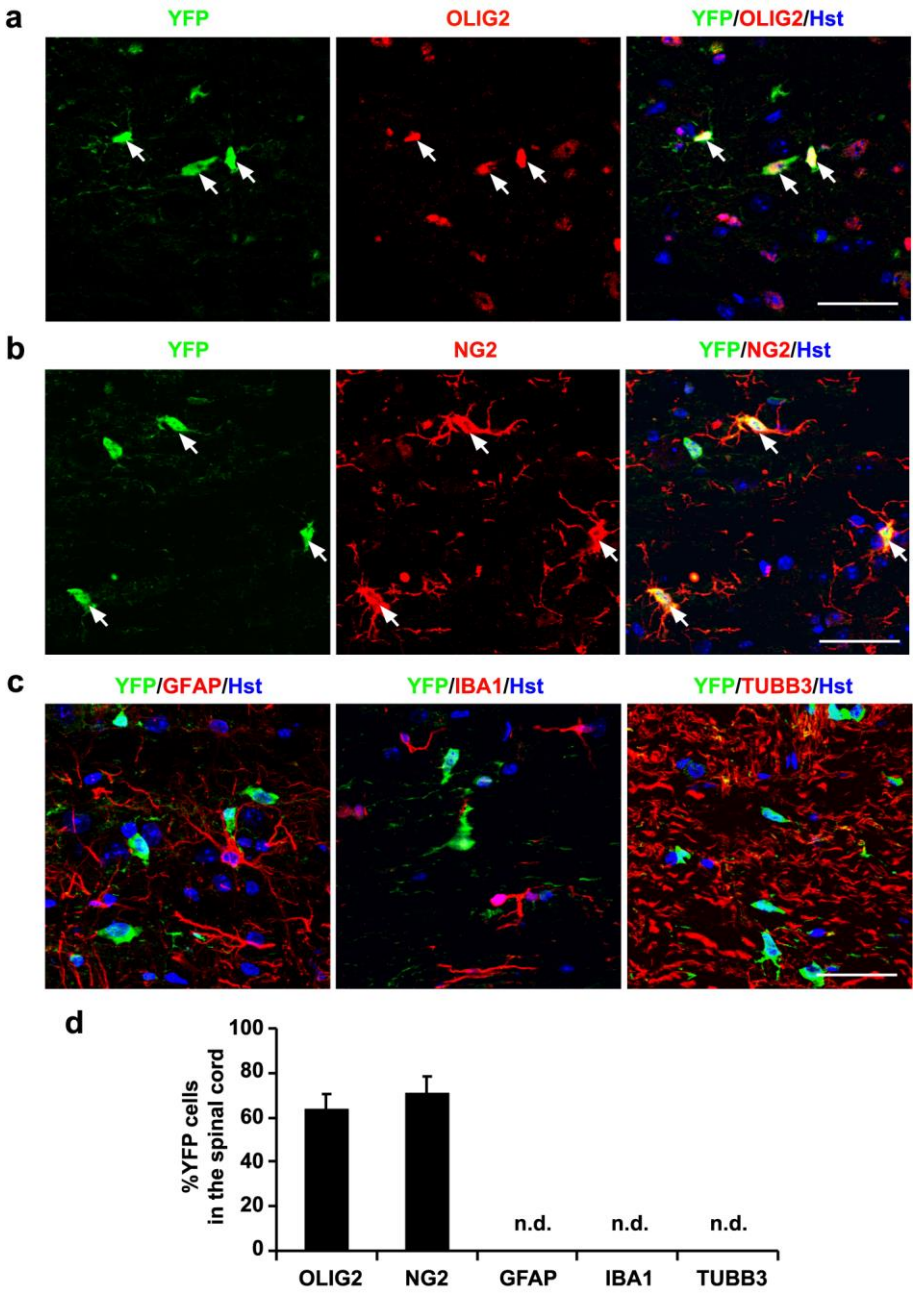
Supplementary Figure 1. Lentivirus under the *hGFAP* promoter drives gene expression mainly in spinal astrocytes. (**a**) A lower magnification view of a longitudinal section from the adult spinal cord injected with *hGFAP-GFP* at 1 wpi. Arrows show sites injected with lentivirus. Scale: 1 mm. (**b-h**) Confocal analyses of GFP^+ cells in the spinal cord. Nuclei were counterstained with Hoechst 33342 (Hst). Arrows show co-localization of GFP^+ cells with the indicated markers. Scales: 50 µm. (**i**) Quantification of marker expression in GFP^+ cells (mean ± SD, n=3). At least 500 GFP^+ cells were analyzed for each marker. n.d., not detected.



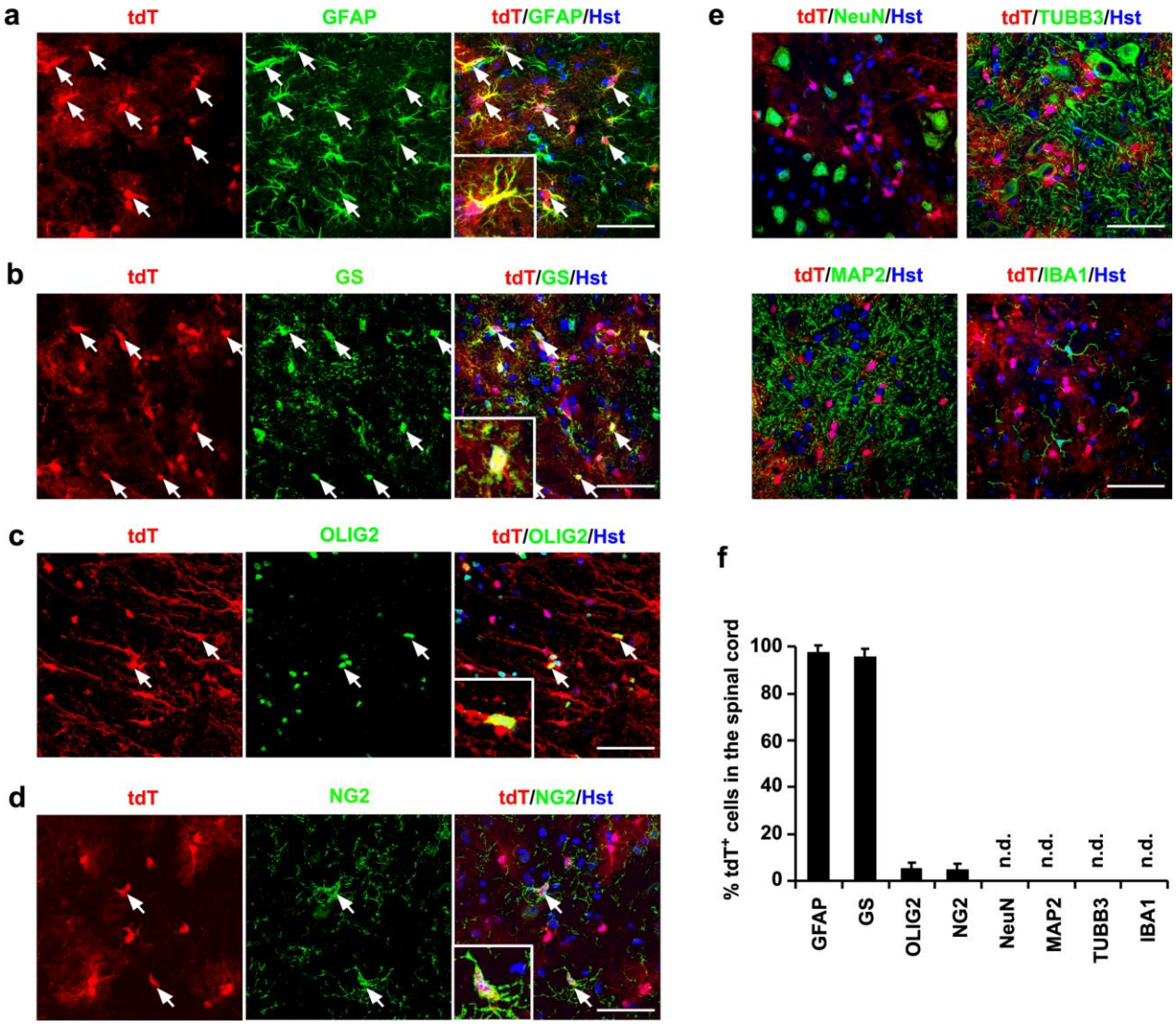
Supplementary Figure 2. DCX$^+$ cells are absent in the adult spinal cord with or without lesions. Schematic diagrams on the left panels show positions in the brain or spinal cord in which cells were analyzed by IHC. (**a**) DCX$^+$ cells in the subventricular zone (SVZ) of the adult brain serve as positive controls. (**b-c**) DCX$^+$ cells are not detectable in either the intact (b) or hemisectioned (c) spinal cord. The hemisection site at the thoracic 8 (T8) level of the adult spinal cord is indicated by an arrowhead. Scales: 50 μm.



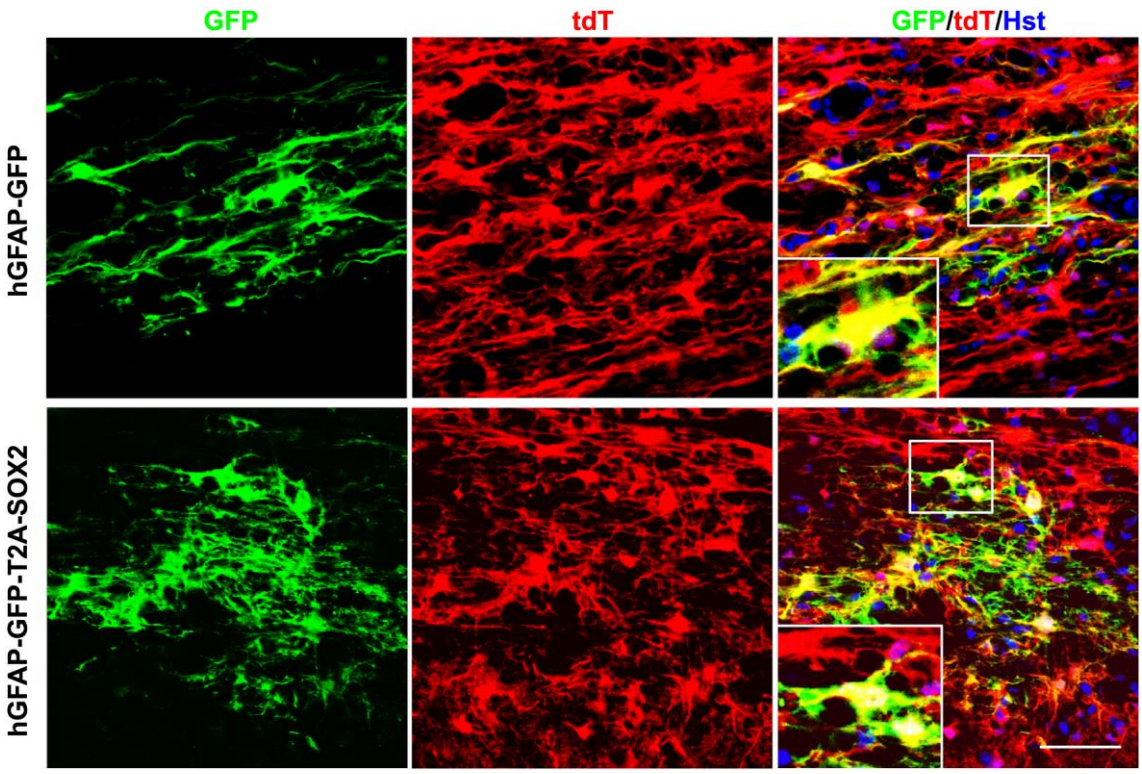
Supplementary Figure 3. Astrocytes are the main cell types targeted by *hGFAP* lentivirus in the injured adult spinal cord. (**a**) A lower magnification view of a longitudinal section from the injured adult spinal cord injected with *hGFAP-GFP* lentivirus at 1 wpi. Arrows show the viral injection sites. The area with hemisection-induced lesions is outlined with a dashed line. Scale: 1 mm. (**b-h**) Confocal analyses of GFP^+ cells in the spinal cord. Nuclei were counterstained with Hst. Arrows show co-localization of GFP^+ cells with the indicated markers. Scales: 50 µm. (**i**) Quantification of marker expression in GFP^+ cells (mean ± SD, n=3). At least 500 GFP^+ cells were analyzed for each marker. n.d., not detected.



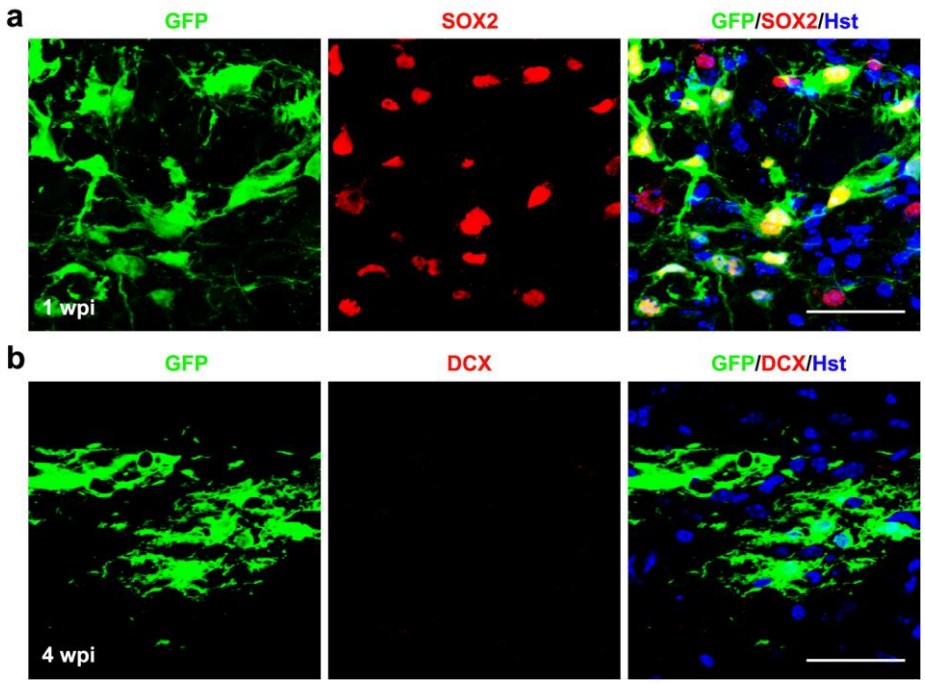
Supplementary Figure 4. Genetic tracing of NG2 cells in the adult spinal cord of *Ng2-Cre;Rosa-YFP* mice. (**a-c**) Confocal analyses of YFP^+ cells. Arrows show YFP^+ cells co-labeled with the indicated markers. Nuclei were counterstained with Hst. Scales: 40 µm. (**d**) Quantification of marker expression in YFP^+ cells (mean ± SD, n=3). At least 100 YFP^+ cells were analyzed for each marker. n.d., not detected.



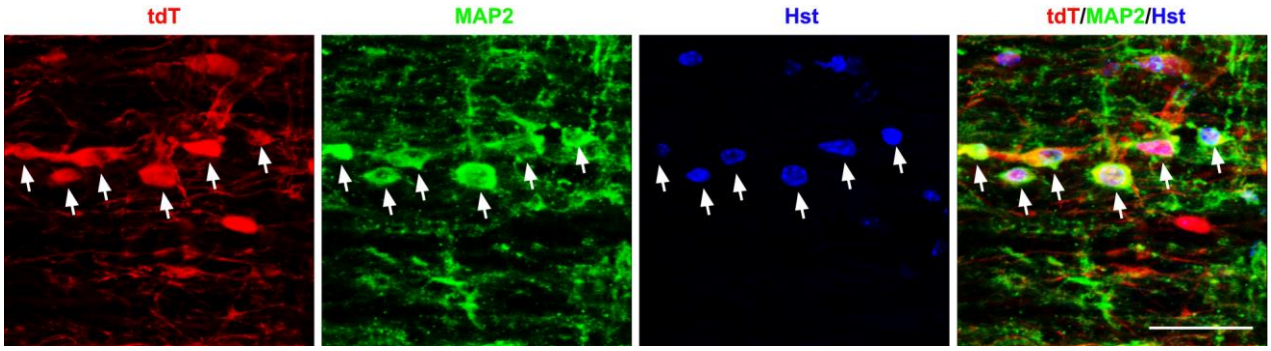
Supplementary Figure 5. Glial cells but not neurons are traced in the spinal cord of *mGfap-Cre line 77.6;Rosa-tdT mice*. (**a-e**) Confocal analyses of tdT-positive cells. Nuclei were counterstained with Hst. Arrows show tdT^+ cells co-labeled with the indicated markers. Scales: 50 µm. (**f**) Quantification of marker expression in tdT^+ cells (mean ± SD, n=4). At least 400 tdT^+ cells were analyzed for each marker. n.d., not detected.



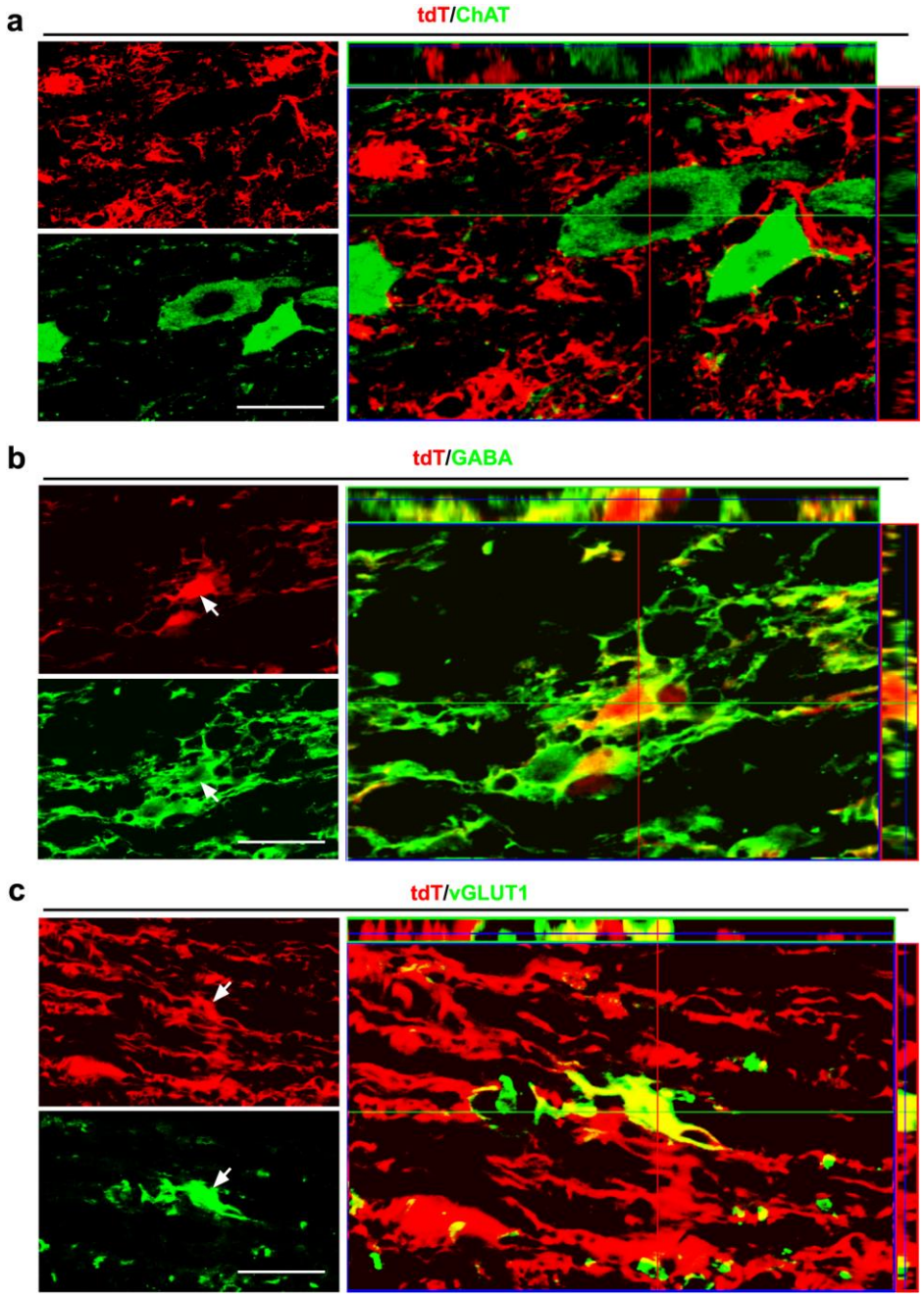
Supplementary Figure 6. Targeting tdT-traced astrocytes by lentivirus under the hGFAP promoter in the spinal cord of *mGfap-Cre line 77.6;Rosa-tdT* mice. Mice were injected with lentivirus expressing *hGFAP-GFP* or *hGFAP-GFP-T2A-SOX2* and analyzed at 1 wpi. Scales: 50 µm.



Supplementary Figure 7. Constitutive expression of SOX2 prohibits induction of DCX$^+$ cells. (**a**) Robust expression of SOX2 under the constitutively active CAG promoter. (**b**) No DCX$^+$ cells were detected in the spinal cord 4 weeks post viral injection. Scales: 40 µm.



Supplementary Figure 8. Long-term survival of astrocyte-derived neurons in the adult spinal cord. *mGfap-Cre;Rosa-tdT* mice were injected with SOX2-expressing lentivirus, treated with VPA, and analyzed at 30 wpi. Arrows indicate astrocytes-converted neurons. Scale: 40 µm.



Supplementary Figure 9. Subtype analysis of SOX2-induced neurons from astrocytes. The spinal cords of adult *mGfap-Cre line 77.6;Rosa-tdT* mice were injected with *hGFAP-SOX2* or a control virus and examined at 8 wpi. (**a**) ChAT$^+$ motoneurons are not traced by tdT. (**b-c**) Ectopic SOX2 induces tdT-traced astrocytes to become GABA$^+$ (**b**) or vGLUT1$^+$ (**c**) neurons. Marker expression is also shown by orthogonal views in the right panels. Scales: 40 µm.

Supplementary Table 1. Primary antibodies used for immunohistochemistry

Antibody	Host	Dilution	Source
GFP (Green fluorescent protein)	Chick	1:600	Aves Labs
GFAP (Glial fibrillary acidic protein)	Mouse	1:500	Sigma
GS (Glutamine synthetase)	Mouse	1:500	Chemicon
Olig2	Rabbit	1:200	Millipore
NG2	Rabbit	1:500	Millipore
NeuN	Mouse	1:500	Chemicon
Map-2	Mouse	1:250	Sigma
TUBB3 (betaIII-tubulin, also known as Tuj-1)	Mouse	1:1000	Covance
TUBB3 (betaIII-tubulin, also known as Tuj-1)	Rabbit	1:500	Covance
MBP (Myelin basic protein)	Mouse	1:1000	Millipore
PLP (Myelin proteolipid protein)	Mouse	1:100	Millipore
Iba-1	Rabbit	1:1000	Wako
SSEA1 (stage-specific embryonic antigen 1)	Mouse	1:100	Lowa
Klf4 (Kruppel-like factor 4)	Goat	1:100	R&D
Sox1 (Sex determining region Y-box 1)	Rabbit	1:500	Cell Signaling
Sox2 (Sex determining region Y-box 2)	Rabbit	1:500	Millipore
Sox3 (Sex determining region Y-box 3)	Rabbit	1:500	A gift of Dr. Klymkowsky
DCX (Doublecortin)	Goat	1:150	Santa Cruz Biotechnology
BrdU (5-bromo-2-deoxyuridine)	Rat	1:500	Accurate Chemical
Ki67	Rabbit	1:500	Novocastra
GABA (Gamma aminobutyric acid)	Rabbit	1:1000	Sigma
vGlut1 (Vesicular glutamate transporter 1)	Mouse	1:100	UC Davis
GAD-6 (Glutamic acid decarboxylase 65)	Mouse	1:100	Hybridoma Bank
ChAT (Choline acetyltransferase)	Goat	1:200	Chemicon
Synapsin-1	Rabbit	1:100	Cell Signaling