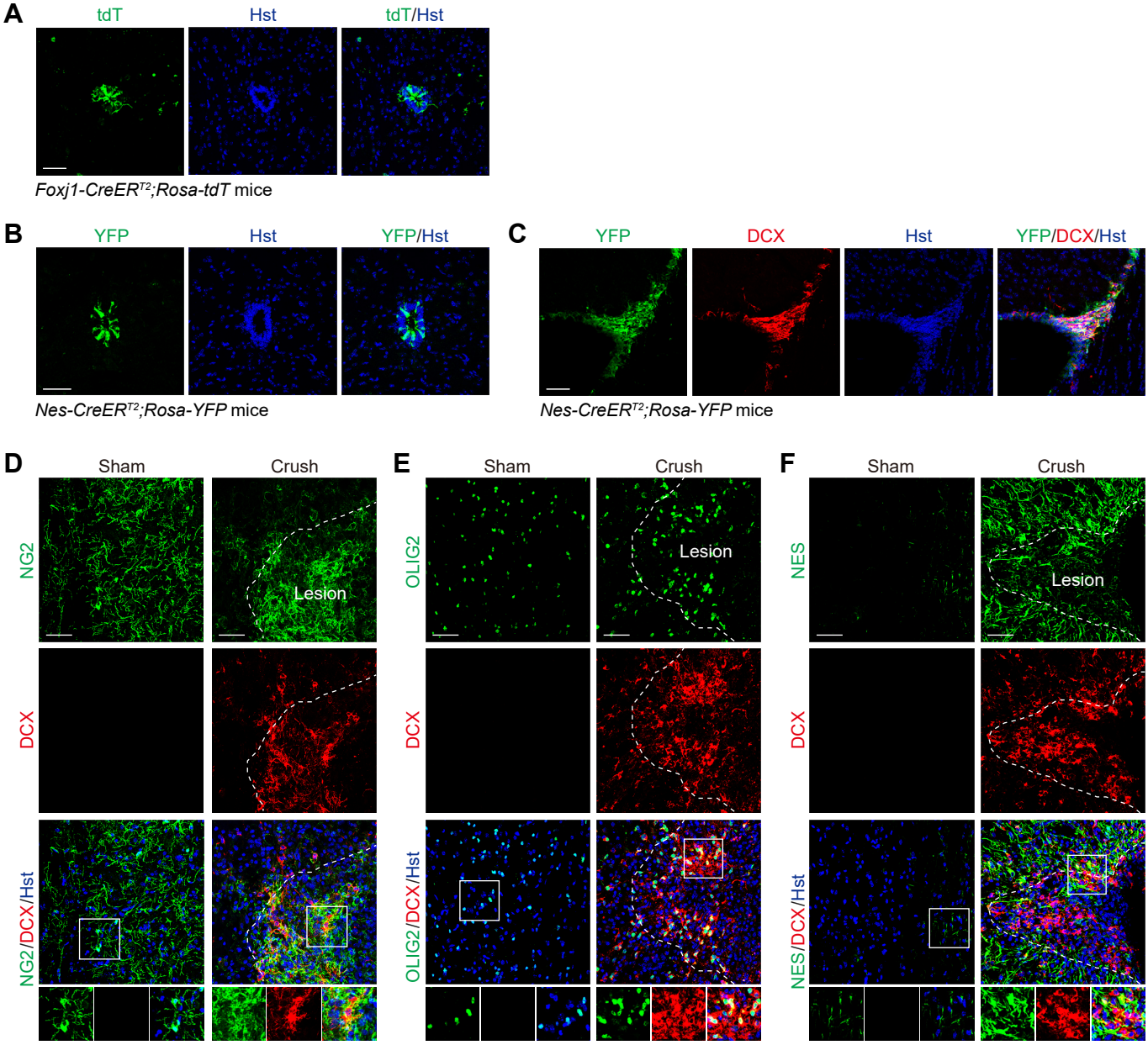


**Figure S1. Related to Figure 1. Genetic lineage-tracing and additional marker expression of SCI-induced DCX$^+$ cells.**

(A) Tracing ependymal cells lining the central canal in adult *Foxj1-CreER$^{T2}$;Rosa-tdT* mice. tdT is pseudocolored as green. Scale bar, 50 μm.

(B) Tracing spinal neural stem cells lining the central canal in adult *Nes-CreER$^{T2}$;Rosa-YFP* mice. Scale bar, 50 μm.

(C) Robust labeling of neural stem cells and DCX$^+$ cells in the lateral ventricle of adult *Nes-CreER$^{T2}$;Rosa-YFP* mice. Scale bar, 50 μm.

(D-F) Confocal images of the indicated markers surrounding the injured spinal cord regions. Mice with crush injury were analyzed at 1 week post injury (wpi). Enlarged views of the boxed regions are shown on the bottom panels. Scale bars, 50 μm.

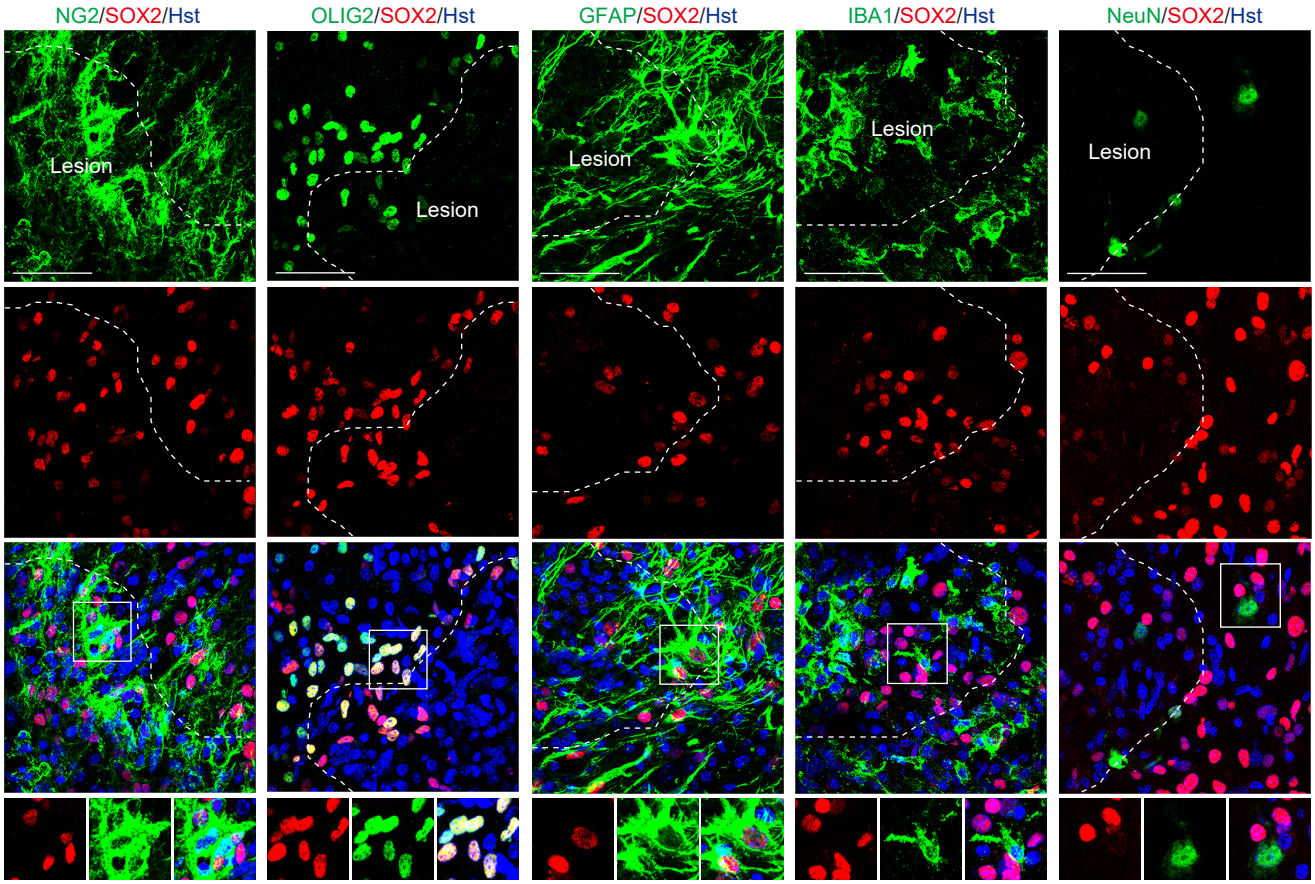


**Figure S2. Related to Figure 2. Predominant macroglial expression of SOX2 in the injured spinal cord.**

Confocal images of the indicated markers surrounding the contusion-injured spinal cord. Immunostaining were performed at 1 wpi. Enlarged views of the boxed regions are shown on the bottom panels. Scale bars, 50 μm.

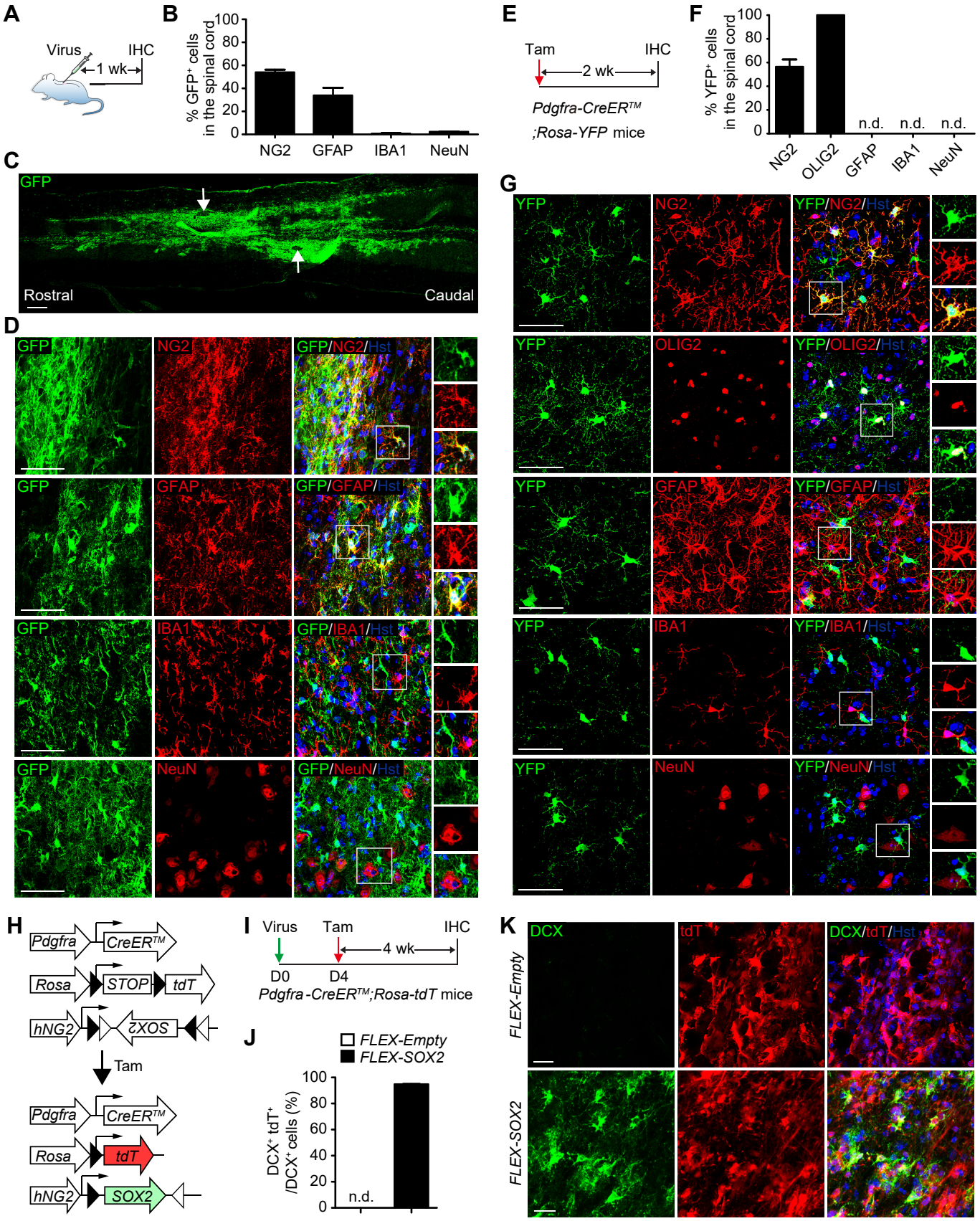
A
Virus
IHC
1 wk
B
% GFP⁺ cells in the spinal cord
100
80
60
40
20
0
NG2
GFAP
IBA1
NeuN
C
GFP
Rostral
Caudal
D
GFP
NG2
GFP/NG2/Hst
GFAP
GFP/GFAP/Hst
IBA1
GFP/IBA1/Hst
NeuN
GFP/NeuN/Hst
E
Tam
IHC
2 wk
Pdgfra-CreER™
;Rosa-YFP mice
F
% YFP⁺ cells in the spinal cord
n.d.
NG2
OLIG2
GFAP
IBA1
NeuN
G
YFP
NG2
YFP/NG2/Hst
OLIG2
YFP/OLIG2/Hst
GFAP
YFP/GFAP/Hst
IBA1
YFP/IBA1/Hst
NeuN
YFP/NeuN/Hst
H
Pdgfra
CreER™
Rosa
STOP
tdT
hNG2
ζXOS
Tam
SOX2
I
Virus
Tam
IHC
4 wk
D0
D4
Pdgfra-CreER™;Rosa-tdT mice
J
FLEX-Empty
FLEX-SOX2
DCX⁺ tdT⁺ /DCX⁺ cells (%)
n.d.
K
DCX
tdT
DCX/tdT/Hst
FLEX-Empty
FLEX-SOX2

**Figure S3**

**Figure S3. Related to Figure 3. The specificity of *hNG2* promoter and genetic tracing of NG2 glia and their derivatives.**

(A) Experimental design for examining the specificity of *hNG2* promoter.

(B) Quantification of marker expression in $GFP^+$ cells (mean ± SEM; n = 3 mice). At least 500 $GFP^+$ cells were analyzed for each marker.

(C) A stitched image of lower magnification view of a longitudinal section from the adult spinal cord injected with the *hNG2-GFP* lentivirus at one week post virus-injection (wpv). Arrows show the viral injection sites. Scale bar, 250 μm.

(D) Confocal images of $GFP^+$ cells in the adult spinal cord. Enlarged views of the boxed regions are shown on the right panels. Scale bars, 50 μm.

(E) Experimental design for genetic lineage tracing of NG2 glia.

(F) Quantification of marker expression in $YFP^+$ cells (mean ± SEM; n = 3 mice). At least 100 $YFP^+$ cells were analyzed for each marker. n.d., not detected.

(G) Confocal images of the indicated markers in the spinal cord of adult *Pdgfra-CreER$^{TM}$;Rosa-YFP* mice 2 weeks post tamoxifen administration. Enlarged views of the boxed regions are shown on the right panels. Scale bars, 50 μm.

(H) A schematic for restricted SOX2 expression in NG2 glia of *Pdgfra-CreER$^{TM}$;Rosa-tdT* mice.

(I) Experimental design.

(J) Quantification of SOX2-induced $DCX^+$ cells from NG2 glia (mean ± SEM; n = 3 mice). n.d., not detected.

(K) Confocal images of the indicated markers in the spinal cord of adult *Pdgfra-CreER$^{TM}$;Rosa-tdT* mice. Scale bars, 20 μm.

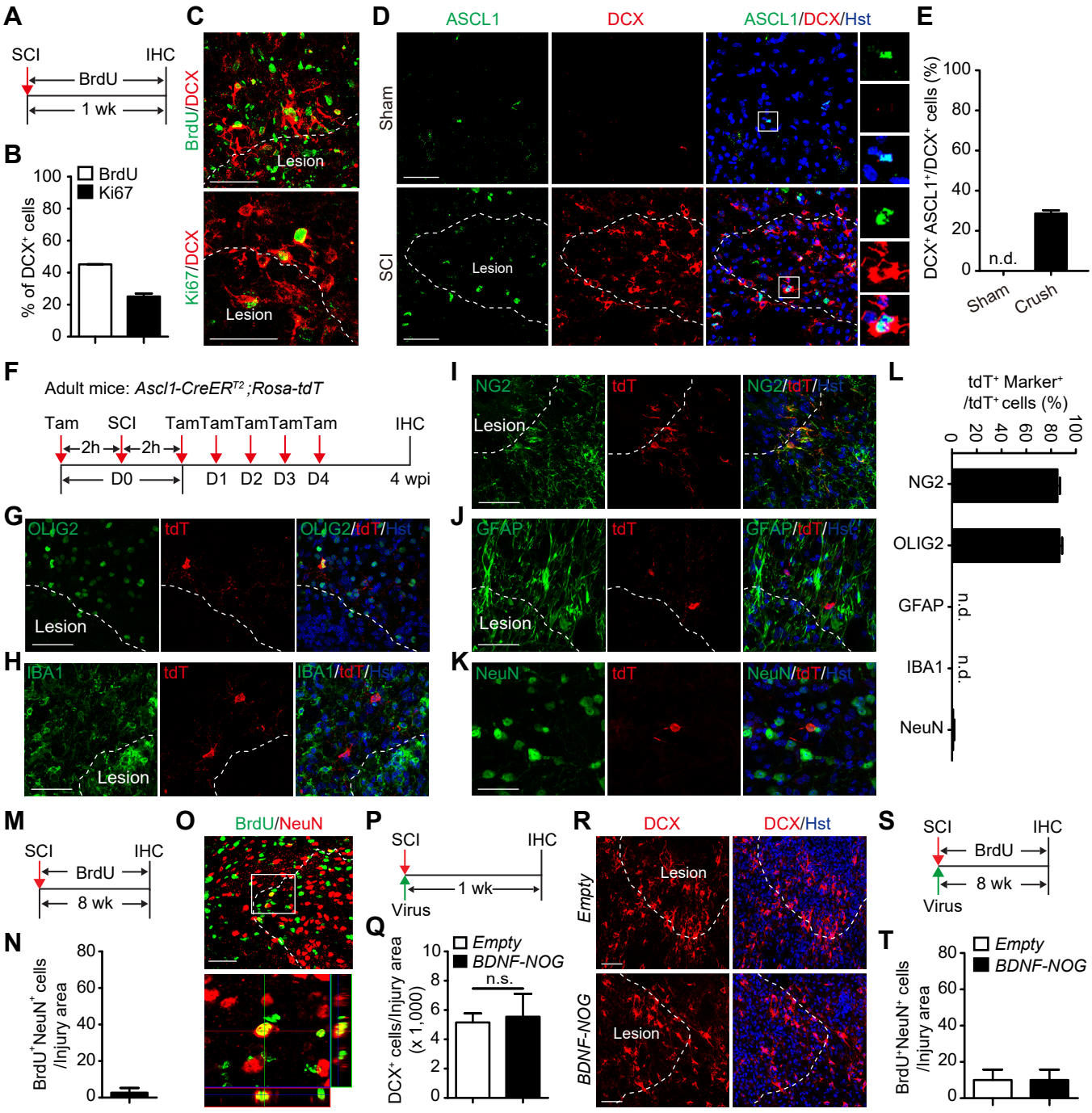
A
SCI
IHC
BrdU
1 wk
B
BrdU
Ki67
% of DCX+ cells
C
BrdU/DCX
Ki67/DCX
Lesion
D
ASCL1
DCX
ASCL1/DCX/Hst
Sham
SCI
Lesion
E
DCX+ ASCL1+/DCX+ cells (%)
n.d.
Sham
Crush
F
Adult mice: Ascl1-CreER^T2;Rosa-tdT
Tam
SCI
TamTamTamTamTam
IHC
2h
2h
D0
D1
D2
D3
D4
4 wpi
G
OLIG2
tdT
OLIG2/tdT/Hst
H
IBA1
IBA1/tdT/Hst
I
NG2
NG2/tdT/Hst
J
GFAP
GFAP/tdT/Hst
K
NeuN
NeuN/tdT/Hst
L
tdT+ Marker+ /tdT+ cells (%)
NG2
OLIG2
GFAP
IBA1
NeuN
n.d.
M
8 wk
N
BrdU+NeuN+ cells /Injury area
O
BrdU/NeuN
P
Virus
Q
Empty
BDNF-NOG
n.s.
DCX+ cells/Injury area (x 1,000)
R
DCX
DCX/Hst
Empty
BDNF-NOG
S
T
BrdU+NeuN+ cells /Injury area

**Figure S4**

**Figure S4. Related to Figure 4. SCI-reprogrammed NG2 glia fail to generate mature neurons**.
(A) Experimental design for SCI-induced cell proliferation.
(B-C) Quantification and confocal images of SCI-induced $DCX^+$ cells going through proliferation (mean ± SEM; n=3 mice per group). Scale bars, 50 μm.
(D) Confocal images of the indicated marker expression at 1 wpi. Enlarged views of the boxed regions are shown on the right panels. Scale bars, 50 μm.
(E) Percentage of $DCX^+$ cells expressing ASCL1 (mean ± SEM; n = 3 mice per group).
(F) Experimental design for assessing the fate of SCI-induced $ASCL1^+$ cells.
(G-K) Confocal images of the indicated markers in the spinal cord of adult *Ascl1-CreER$^{T2}$;Rosa-tdT* mice at 4 wpi. Scale bars, 50 μm.
(L) Quantification of marker expression in $tdT^+$ cells (mean ± SEM; n = 3 mice).
(M) Experimental design for analyzing SCI-induced new neurons.
(N) Quantification of SCI-induced new neurons (mean ± SEM; n=3 mice per group).
(O) Confocal images of SCI-induced new neurons at 8 wpi. An orthogonal view of the boxed region is also shown in the bottom panel. Scale bars, 50 μm.
(P) Experimental design for investigating the effect of lentivirus injection on SCI-induced $DCX^+$ cells.
(Q) Quantification of $DCX^+$ cells (mean ± SEM; n = 3-4 mice per group).
(R) Confocal images of $DCX^+$ cells in the spinal cord one-week post SCI and virus injection. Scale bars, 50 μm.
(S) Experimental design for analyzing the effect of neurotrophic factors on SCI-induced neurons.
(T) Quantification of the effect of neurotrophic factors on SCI-induced new neurons (mean ± SEM; n=3 mice per group).

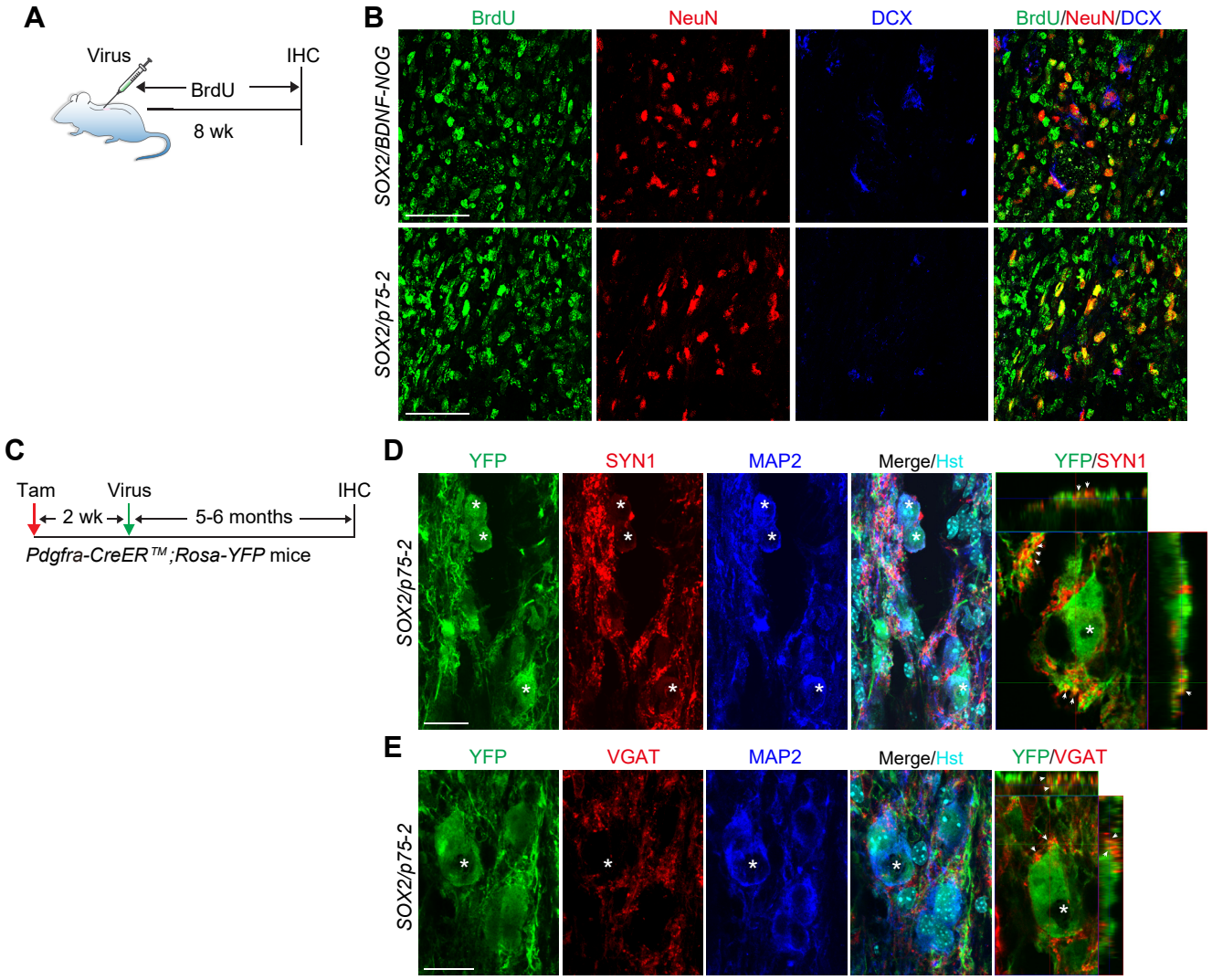


**Figure S5. Related to Figure 4. Additional analysis of SOX2-mediated reprogramming of NG2 glia.**
(A) Experimental design for BrdU-tracing.
(B) Confocal images of the indicated markers at 8 wpv, a time point with many $BrdU^+NeuN^+$ neurons but few $DCX^+$ cells. Scale bars, 50 µm.
(C) Experimental design for analyzing SOX2-induced neurons from NG2 glia.
(D) Expression of the presynaptic marker SYN1 in induced neurons. Asterisks indicate examples of NG2 glia-derived neurons, whereas arrowheads show signal co-localization in the orthogonal view. Scale bar, 20 µm.
(E) Expression of the inhibitory neuronal marker VGAT in induced neurons. Asterisk indicates an example of NG2 glia-derived neurons, whereas arrowheads show signal co-localization in the orthogonal view. Scale bar, 20 µm.

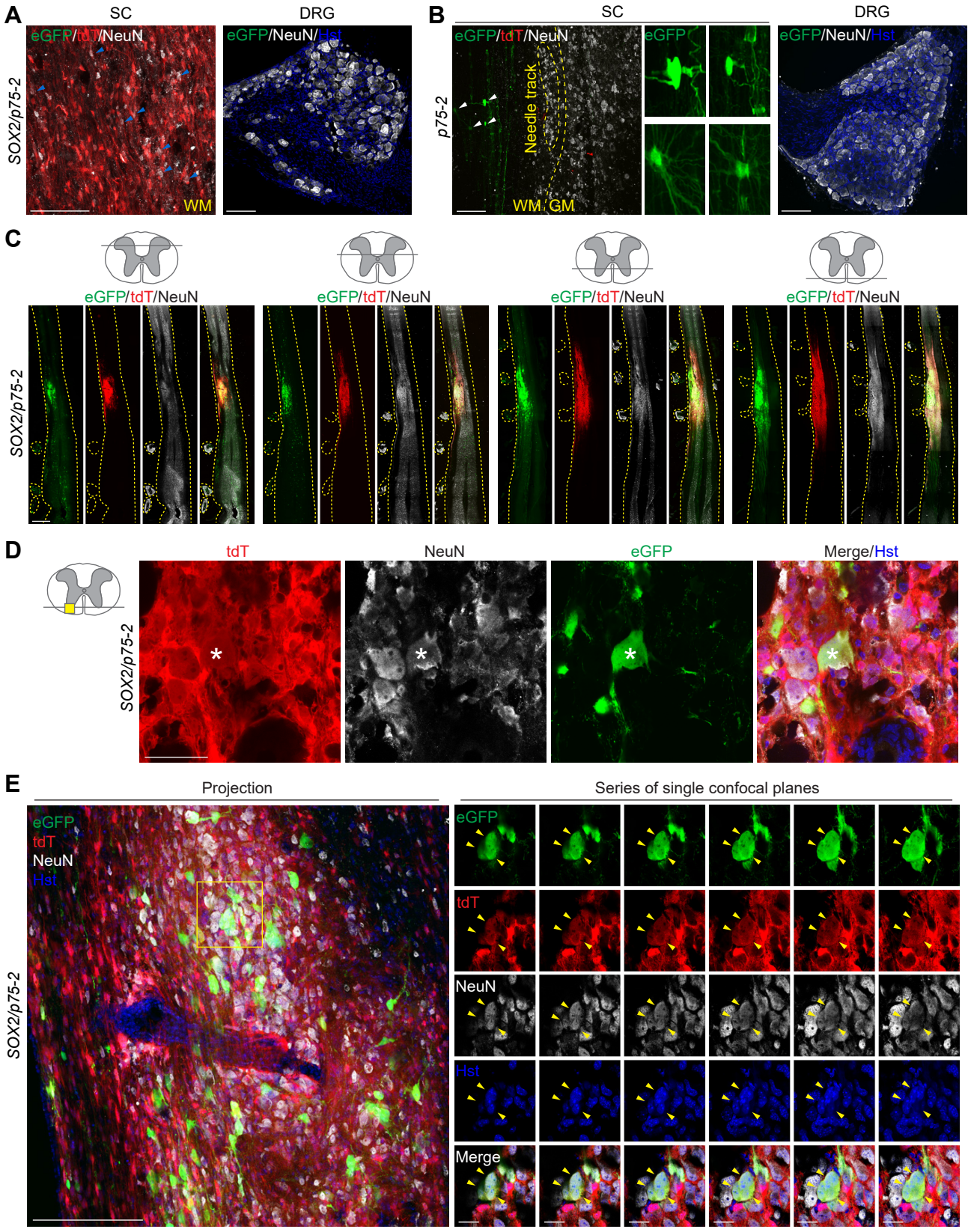
A
SC
DRG
eGFP/tdT/NeuN
eGFP/NeuN/Hst
SOX2/p75-2
WM
B
SC
DRG
eGFP/tdT/NeuN
eGFP
Needle track
p75-2
WM
GM
eGFP/NeuN/Hst
C
eGFP/tdT/NeuN
eGFP/tdT/NeuN
eGFP/tdT/NeuN
eGFP/tdT/NeuN
SOX2/p75-2
D
tdT
NeuN
eGFP
Merge/Hst
SOX2/p75-2
E
Projection
Series of single confocal planes
eGFP
tdT
NeuN
Hst
SOX2/p75-2
eGFP
tdT
NeuN
Hst
Merge

**Figure S6**

**Figure S6. Related to Figure 5. Rabies virus-mediated tracing of synaptic connections of NG2 glia-derived neurons.**

(A) Confocal images of cells in the spinal cord (SC) and dorsal root ganglia (DRG) of the control *Ascl1-CreER$^{T2}$;Rosa-tdT* mice that were injected with *SOX2/p75-2* virus and the engineered rabies virus. Induced tdT$^{+}$NeuN$^{+}$ neurons were only observed in the SC and were not infected with the engineered rabies virus due to the lack of TVA expression (blue arrowheads for examples; n=4). WM, white matter. Scale bars, 100 μm.

(B) Confocal images of cells in the SC and DRG of *Ascl1-CreER$^{T2}$;Rosa-tdT;Rosa-TVAg* mice that were injected with the control *p75-2* virus and the engineered rabies virus. tdT$^{+}$NeuN$^{+}$ neurons were not detected. Only rare glial cells were infected with the engineered rabies virus (white arrowheads and enlarged views). GM, gray matter. Scale bars, 100 μm.

(C) Stitched images of lower magnification views of the spinal cord and the attached DRGs (outlined) of *Ascl1-CreER$^{T2}$;Rosa-tdT;Rosa-TVAg* mice that were injected with *SOX2/p75-2* virus and the engineered rabies virus. The relative positions of the longitudinal sections are diagramed on the top panels. Scale bar, 1 mm.

(D) Confocal images of cells from the ventral white matter surrounding the virus-injected spinal cord. Induced neurons are traced with tdT, whereas cells harboring the engineered rabies virus are indicated by eGFP. Asterisks show an example of an eGFP$^{+}$tdT$^{+}$NeuN$^{+}$ "starter" cell. Scale bar, 50 μm.

(E) Confocal images of clustered "starter" cells in the virus-injected spinal cord. Both maximum projections (left, scale bar, 100 μm) and a series of single confocal planes (right, scale bars, 20 μm) are shown.

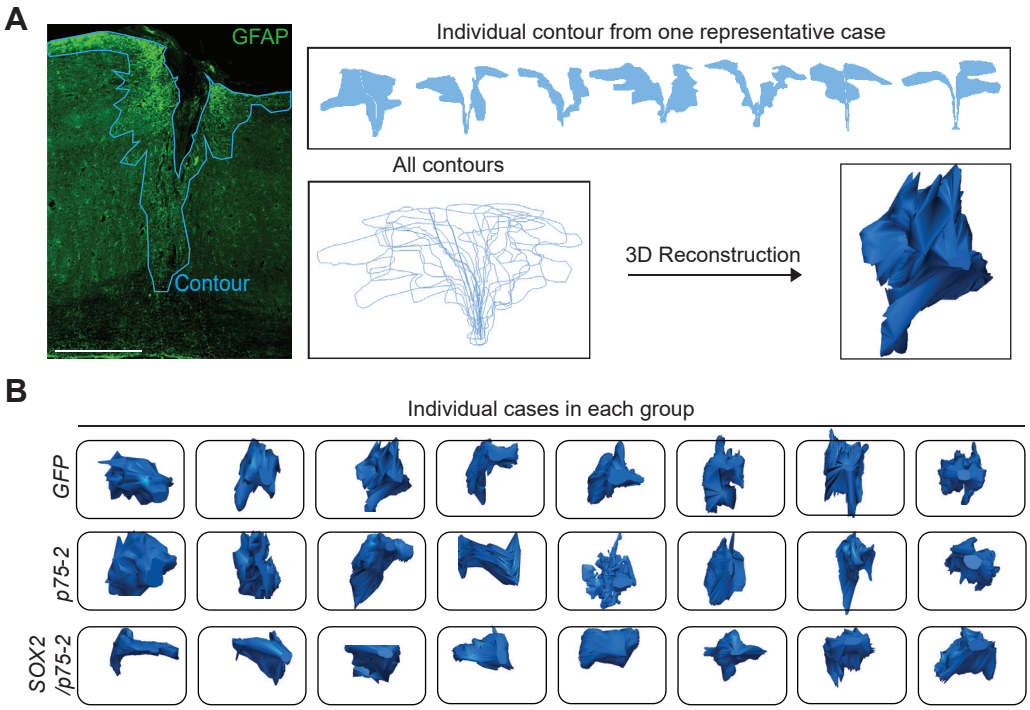


**Figure S7. Related to Figure 6. 3D reconstructed astroglial scar.**

(A) Contouring of the astroglial scar. Left, a contour line in a representative GFAP-labeled astroglial scar in a representative stitched image (scale bar, 500 μm); Right, Neurolucida 3D reconstruction of contours of the astroglial scar.

(B) 3D reconstructed astroglial scar from each case in all groups.