In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model

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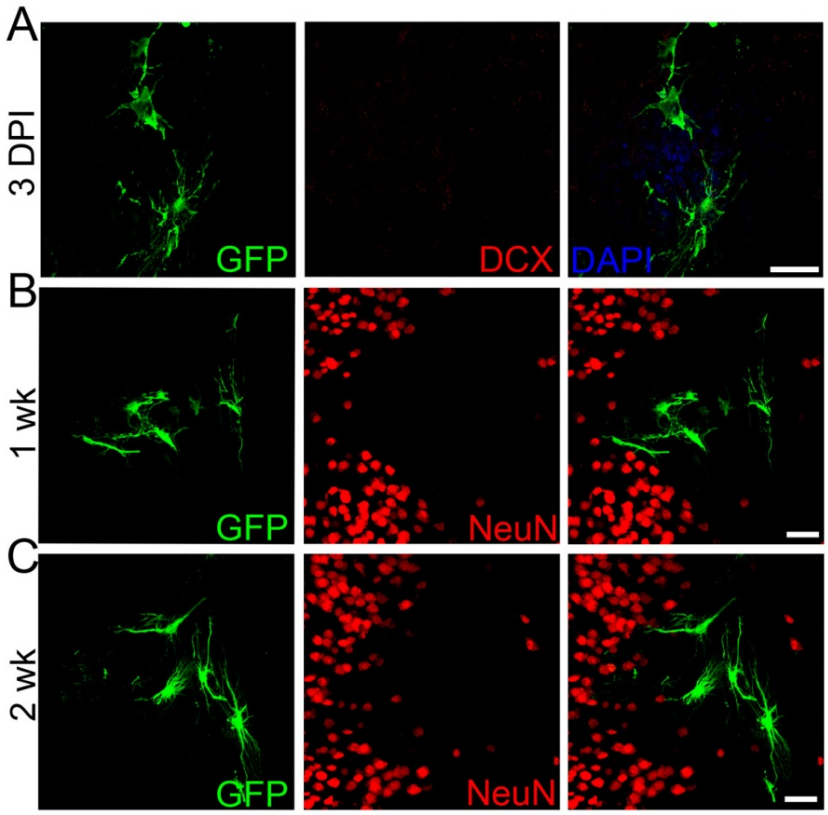
Supplementary figures:

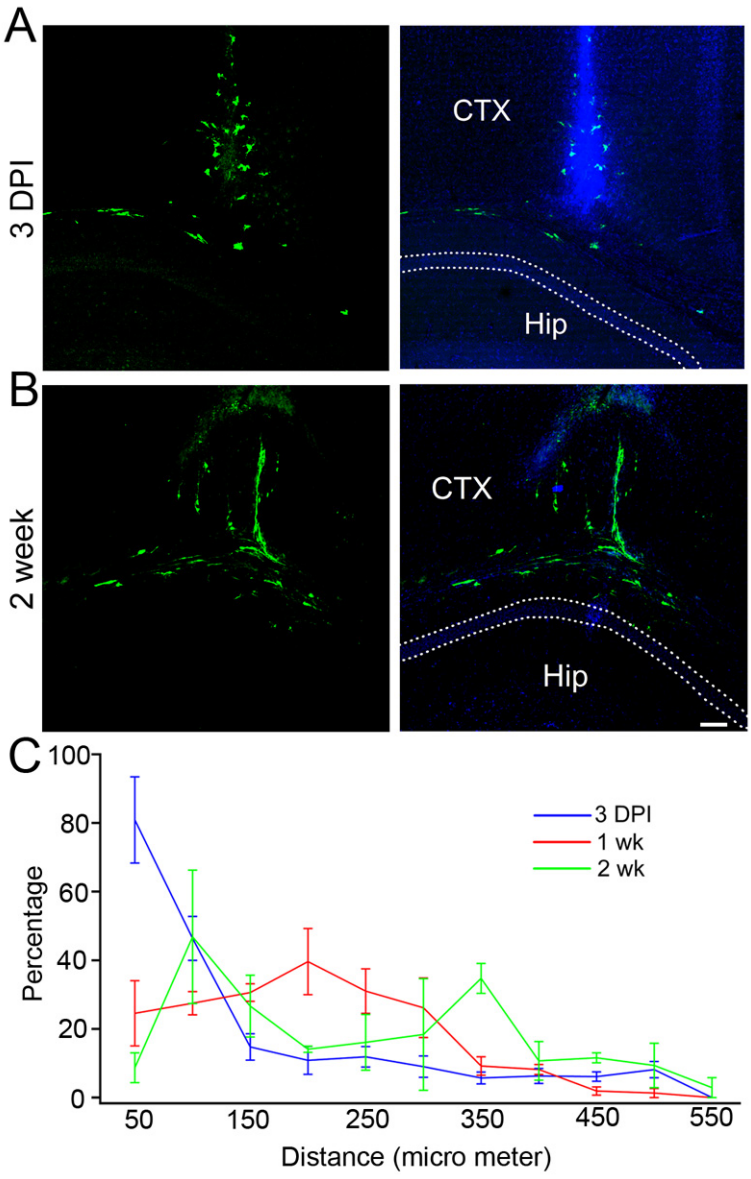
Suppl. Fig. 1. Control retrovirus expressing GFP alone did not infect any existing neurons, nor induced any new neurons, related to Figure 1.



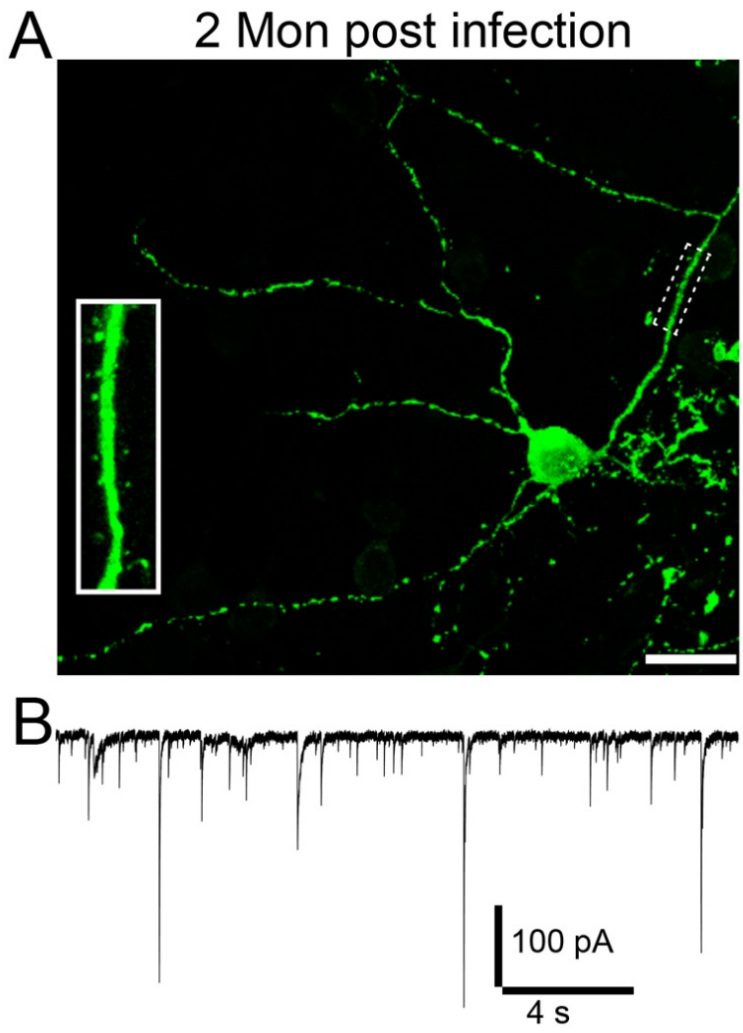
(A) GFP retrovirus-infected cells showing glial morphology at 3 DPI and not labeled by immature neuronal marker DCX. (B) At 7 DPI, GFP retrovirus-infected cells were negative for neuronal marker NeuN. (C) 2 weeks after viral injection, GFP-infected cells were still maintaining glial morphology and not labeled by NeuN. Scale bars, 40 μm.

Suppl. Figure 2. Migration of NeuroD1-converted neurons after retroviral injection into mouse cortex, related to Figure 1.

(A) NeuroD1-infected cells were mostly clustered around the injection sites at 3 DPI, except a few spreading in the cingulate cortex region. (B) Two weeks after viral injection, NeuroD1-infected cells migrated away from the center of injury sites and spread more broadly in the deep layer. (C) Plot of the percentage of NeuroD1-infected cells versus the distance measured from the center of injury sites. The number of NeuroD1-infected cells was 263 for 3 dpi, 214 for 1 week, and 124 for 2 weeks. n = 3 animals for each group. Scale bar, 100 μm.



Suppl. Fig. 3. Long-term survival of NeuroD1-converted neurons in mouse brain *in vivo*, related to Figure 1.



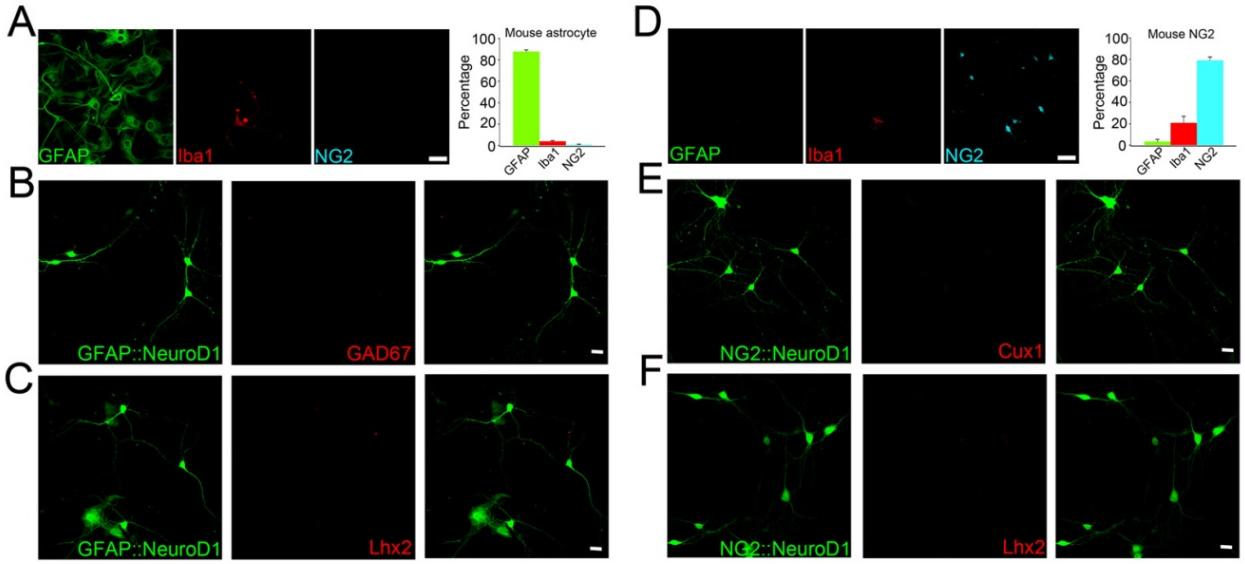
(A) A NeuroD1-converted neuron observed 2 months after viral injection. The converted neuron showed clear dendritic spines (arrows). Scale bar, 20 μm.

(B) Cortical slice recording revealed large spontaneous synaptic events from a 2-month old NeuroD1-converted neuron.

Suppl. Fig. 4. Characterization of NeuroD1-converted neurons in cultured mouse astrocytes or NG2 cells, related to Figure 2 and 3.

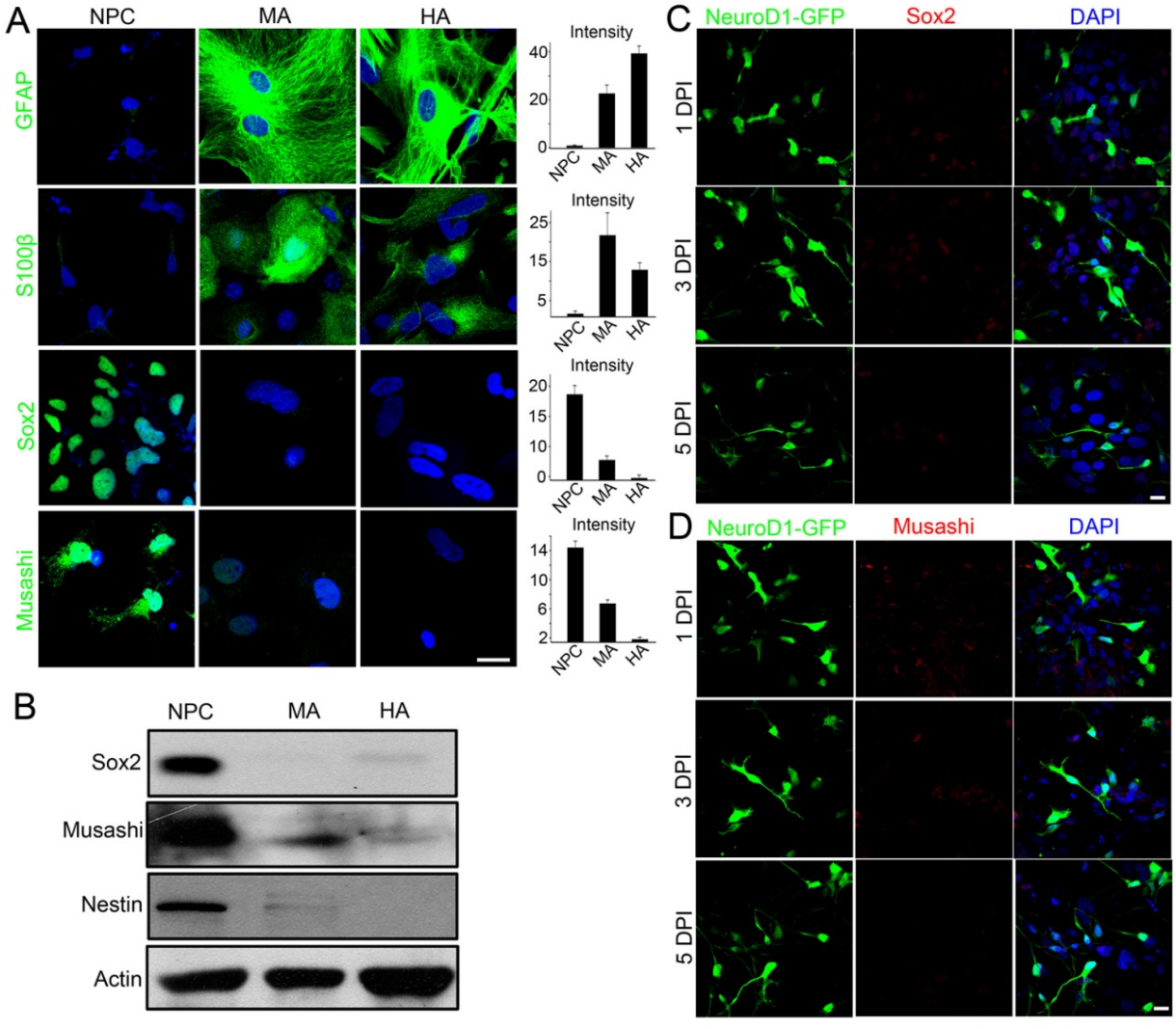
(A) Our cultured mouse astrocytes were mostly immunopositive for astrocytic marker GFAP (87.8 ± 1.4%), with a few positive for Iba1 but rarely NG2.

(B) GFAP::NeuroD1-GFP retrovirus-infected cells (green) were immunonegative for GAD67. (C) GFAP::NeuroD1-GFP retrovirus-infected cells were immunonegative for cortical superficial layer marker Lhx2. (D) The majority of cells in our NG2 culture were immunopositive for NG2 (~80%) with ~20% positive for microglia marker Iba1. (E-F) NG2::NeuroD1 retrovirus-infected cells were immunonegative for cortical superficial layer marker Cux1 (E) and Lhx2 (F). Scale bars, 40 μm for A and D; 20 μm for B, C, E and F.

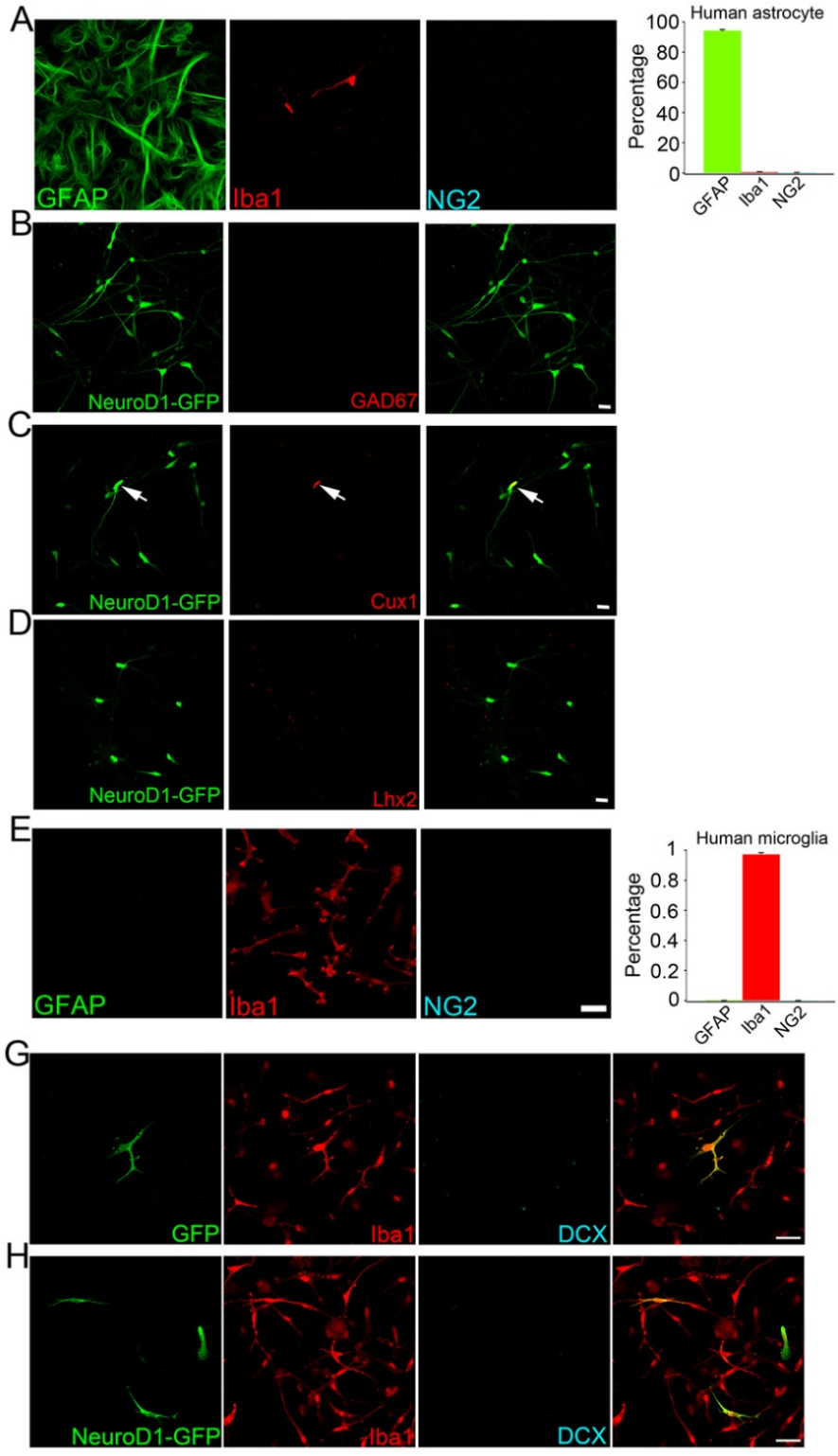


Suppl. Figure 5. No intermediate neuroprogenitor stage during human astrocyte-neuron conversion, related to Figure 5.

(A) Characterization of human astrocytes by comparing to human neuroprogenitor cells (NPC) or mouse astrocytes in primary culture. Human and mouse astrocytes were immunopositive for GFAP and S100β but negative for neural stem cell marker Sox2 or Musashi. (B) Western blot confirmed that our cultured human astrocytes were different from human NPCs. (C-D) NeuroD1-infected cells (green) did not show any increase in the expression of neural stem cell marker Sox2 (C) or Musashi (D) over 1, 3 and 5 days post infection. Scale bar, 20 μm; n = 3 cultures.



Suppl. Fig. 6. Human astrocytes can be converted into neurons but microglia cannot be converted, related to Figure 5.



(A) The majority of cells in our human astrocyte cultures were immunopositive for astrocytic marker GFAP (93.7 ± 1%). (B) NeuroD1-converted neurons from human astrocytes were immunonegative for GAD67. (C-D) Human astrocyte-converted neurons were largely negative for cortical superficial layer marker Cux1 (C) and Lhx2 (D). (E) The majority of cells in our human microglia culture were positive for Iba1 (97.1 ± 1.1%). (G-H) Human microglia not converted into neurons by NeuroD1 (20 DPI, DCX negative). n = 3-5 cultures. Scale bars, 40 μm for (A) and (E-H); 20 μm for (B-D).

Supplemental Experimental procedures:

Antibodies. The following primary antibodies were used: polyclonal anti-green fluorescent protein (GFP, chicken, 1:1000, Abcam, AB13970), polyclonal anti-glial fibrillary acidic protein (GFAP, rabbit, 1:500, Abcam, Z0334; and chicken, 1:500, Millipore, AB5541), monoclonal anti S100β (mouse, 1:500,Abcam, ab66028), polyclonal anti-vesicular glutamate transporter 1 (vGluT1, rabbit, 1:500, Synaptic Systems), polyclonal anti-SV2 (rabbit, 1:2000, Developmental Studies Hybridoma Bank, Iowa City), polyclonal anti-microtubule associated protein 2 (MAP2, Chicken, 1:1000, Abcam, AB5392; and rabbit, 1:500, Chemicon, AB5622), polyclonal anti- T-box brain 1 (Tbr1, 1:300, rabbit, Abcam, AB31940), polyclonal anti-Musashi-1 (rabbit, 1:300, Neuromics, RA14128), monoclonal anti-Sox-2 (mouse , 1:300, Abcam, AB79351), polyclonal anti-Sox-2 (rabbit, 1:500, Millipore, AB5603), monoclonal anti-Nestin (mouse, 1:500, Neuromics, MO15056), monoclonal anti-Actin (mouse, 1:1000, BD, 612656), monoclonal anti-βIII tubulin (Tuj1, mouse, 1:500, COVANCE, MMS-435P), polyclonal anti-Doublecortin (DCX, rabbit, 1:500, Abcam, AB18723), polyclonal anti-NeuN (rabbit, 1:500, Millipore, ABN78), monoclonal anti-NG2 (mouse, 1:200, Abcam, AB50009), polyclonal anti-Iba1 (goat, 1:200, Abcam, AB5076; and rabbit, 1:1000, Wako, 019-19741), monoclonal anti-NeuroD1 (mouse, 1:1000, Abcam, ab60704) , monoclonal anti-Ctip2 (Rat, 1:1000, Abcam, ab18465), polyclonal anti-LIM homeobox protein Lhx2 (Rabbit, 1:1000, Millipore, AB5756), polyclonal anti-Otx1 (mouse, 1:200, Developmental Studies Hybridoma Bank, Iowa City), polyclonal anti-Cux1 (Rabbit, 1:1000, Santa Cruz, sc-13024), monoclonal anti GAD67 (Mouse, 1:1000, Millipore, MAB5406), monoclonal anti GAD65 (GAD-6, mouse, 1:1000, Developmental Studies Hybridoma Bank, Iowa City).

Western blotting. Cells were washed in cold PBS and lysed with ice-cold Pierce IP lysis buffer. Lysates were run on 10% SDS-Tris glycine gels and transferred to 45 µm PVDF membranes, which were blocked in 5% w/v skim milk in TBST and stained in 5% w/v BSA in TBST. Detection was performed with film (GeneMate) by SRX-101A film processing device (Konica Minoltal). Antibodies and dilutions were used as following: mouse monoclonal anti-Sox2 (Abcam ab79351, 1:800); mouse monoclonal

anti-Musashi-1 (Abcam ab109994, 1:500); mouse anti-Nestin (Neuromics MO15056, 1:300); mouse anti-Actin (BD 612656, 1:1000).

Data analysis. Cell counts were performed by taking images of several randomly chosen views per coverslip and analyzed by Image J software. The intensity or pixel area was analyzed by Image J software. Data were represented as mean ± SEM. Student's *t*-test (paired or unpaired) was used for statistical analysis. $*$ $P < 0.05$, $**$ $P < 0.01$, $***$ $P < 0.001$.