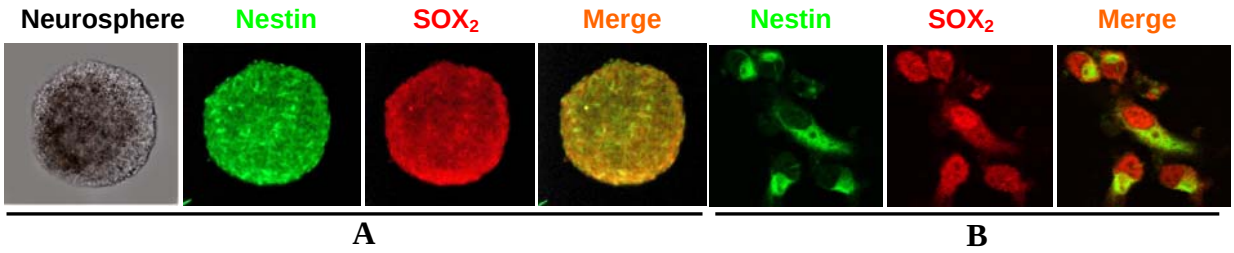

Fig. S1. Generation and characterization of aNSCs in vitro. aNSCs were isolated and expanded from the SVZ region of adult naïve C57BL/6 mice and cultured in DMEM/F-12 supplied with 20 ng/ml EGF, 20 ng/ml FGF-β and 2% B27 supplements. **(A)** Neurospheres at the 4th passage were immunostained for NSC markers nestin and SOX_2. Double-positive immunostaining for nestin (green) and SOX_2 (red) was observed by fluorescent microscopy. **(B)** Individual aNSCs dissociated from the 4th passage of neurospheres were positive for both nestin and SOX_2 as visualized by confocal microscopy. Magnification ×4 for A, ×65 for B.

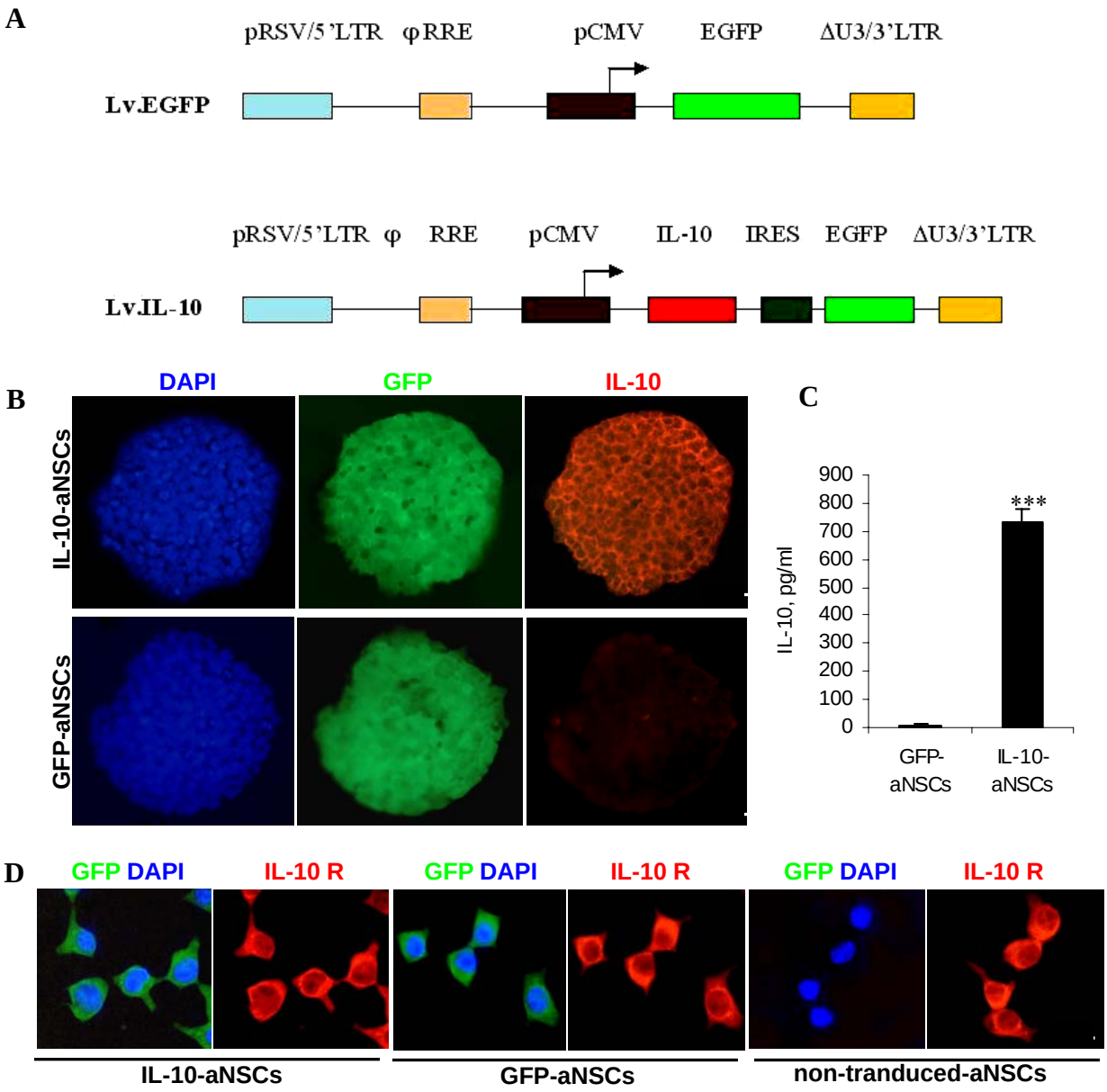

Fig. S2. Generation of lentiviral vectors and transduction into aNSCs. (A) Diagram of lentiviral vectors encoding GFP alone (Lv.GFP) and bicistronic vector encoding both IL-10 and GFP (Lv.IL-10) driven by a CMV promoter. Viral backbone plasmids were co-transfected with vector helper plasmids pLP1, pLP2, and pLP/VSV-G into 293T packaging cells. Conditioned supernatants were harvested for 3 days and concentrated by ultracentrifugation, achieving a viral titer of 5-10 × 10^8 infectious units/ml, then used to infect dissociated single aNSCs and small neurospheres at a multiplicity of infection (MOI) of 5-10. (B) GFP (green) expression in neurospheres transduced with

both vectors was visible by fluorescence microscopy 3 days after infection. Strong IL-10 staining (red) was visible in neurospheres transduced with Lv.IL-10, but not Lv.GFP. (C) IL-10 production in the supernatant of aNSCs after 3-day culture was measured by ELISA. Symbols represent mean values and SEM of 4 independent experiments. *** $p<0.001$. (D) IL-10 receptor (red) was expressed on IL-10-aNSCs, GFP-aNSCs and non-tranduced-aNSCs. Magnification ×10 for B, ×60 for D.

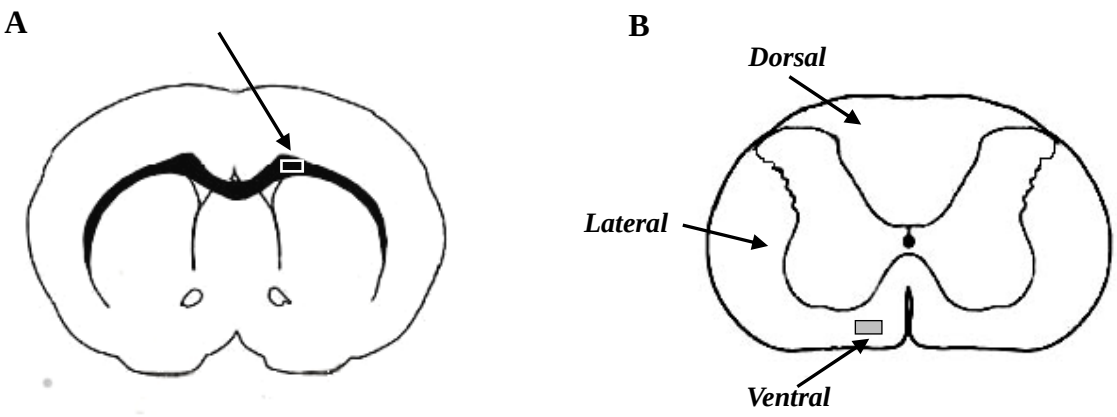

Fig. S3. Diagram showing 500 μm^2 fields ■ within the corpus callosum (A) and mid-lumbar spinal cord (B) cross-sections, where inflammation and demyelination were consistently observed in control mice. These specific areas were chosen for pathologic and immunohistochemical analysis in all animals in this study.

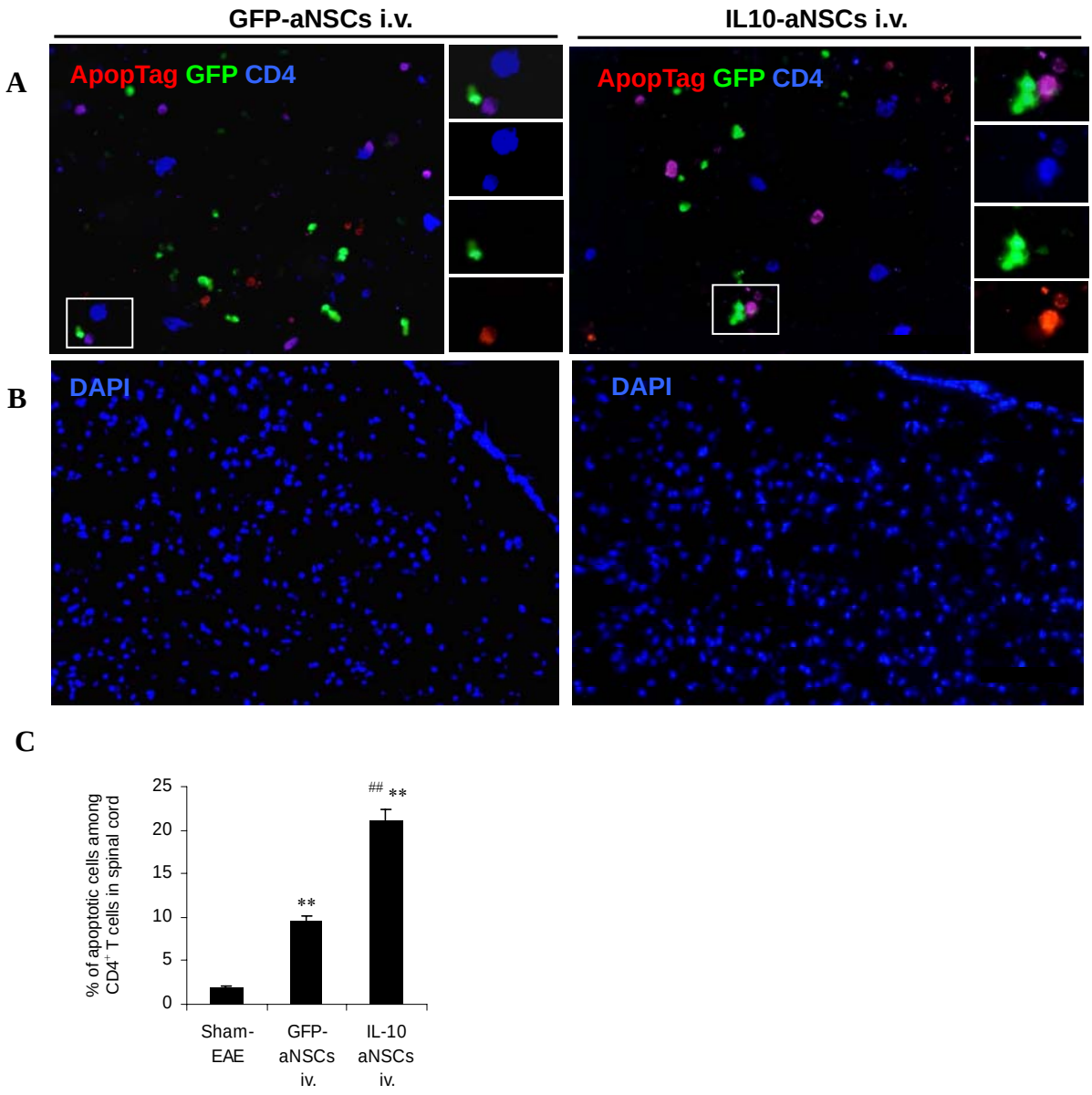

Fig. S4. Enhanced pro-apoptotic effect of IL-10-aNSCs on T cells. Mice treated with aNSCs i.v. at day 22 p.i. were sacrificed 6 weeks p.t. All groups were examined in the same regions of lymph nodes and the ventral column of lumbar spinal cords (L3) as shown in Fig. S3. **(A)** Spinal cord sections were stained for CD4 cells (blue) and apoptotic cells (red). Co-localization of red and blue (pink cells) indicated that some $CD4^+$ cells underwent apoptosis, while no aNSCs (green) underwent apoptosis. Magnification ×20, insets ×80. **(B)** No GFP^+ aNSCs and almost no apoptotic cells were found in lymph node sections at 6 weeks p.t. Nuclei in B were stained with DAPI (blue). Magnification ×10. **(C)** Quantitative analysis of apoptotic $CD4^+$ T cells in spinal cords. Symbols represent mean values and SD of 6-8 mice each group. ** $p<0.01$, comparisons between sham-EAE group and other groups; $^{\#\#}$ $p<0.01$, comparison between GFP-aNSC-i.v. and IL-10-aNSC-i.v.

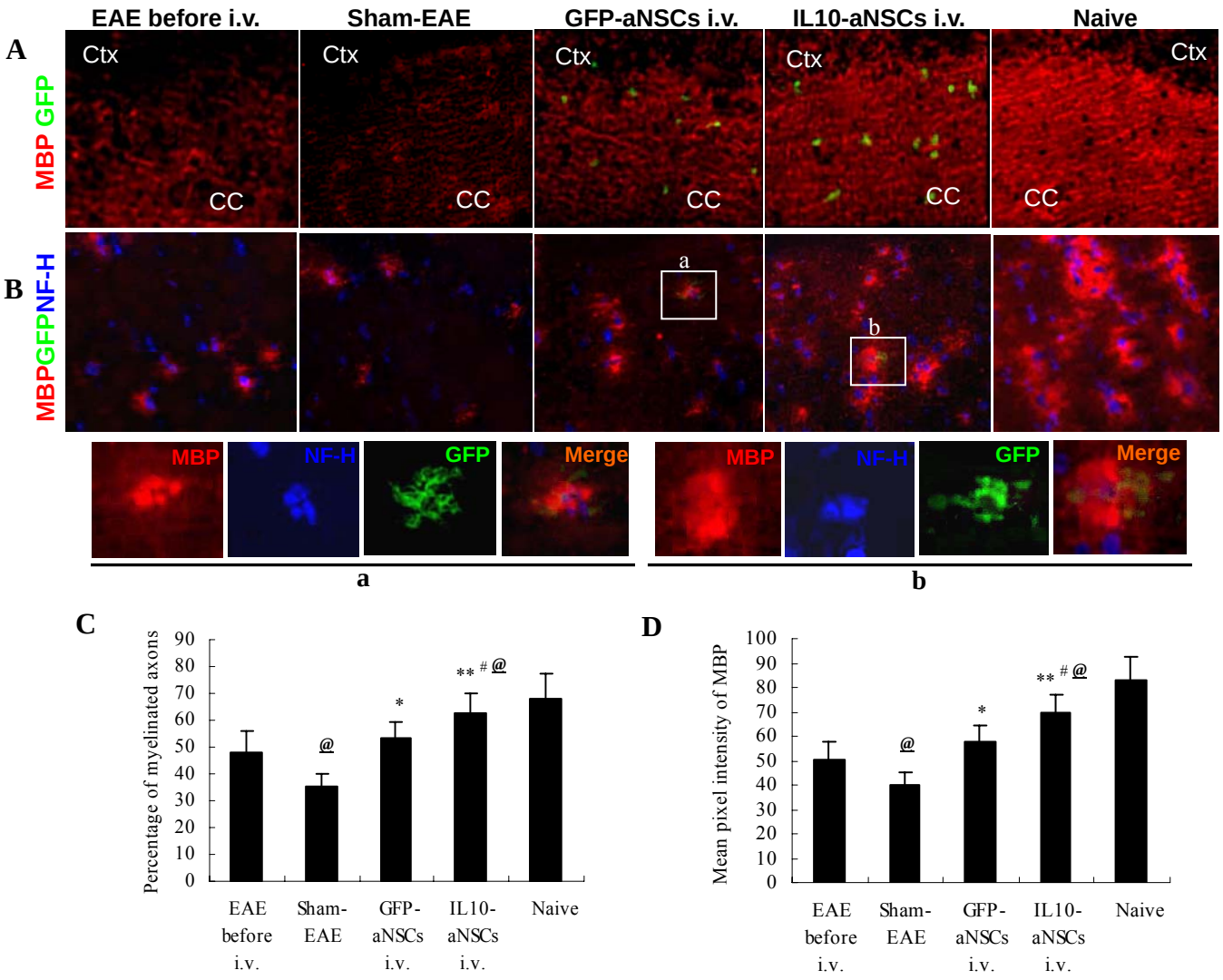

Fig. S5. IL-10-aNSCs reduce further demyelination and promote remyelination. At day 78 p.t., brains and spinal cords were harvested for immunohistology. All groups were examined in the same region of the corpus callosum of the brain and the ventral column of the lumbar spinal cord (L3) as shown in Fig. S3. **(A)** Markedly decreased MBP immunoreactivity (red; for myelin) was seen in the brain of EAE mice, while aNSC treatment increased MBP expression together with a high density of transplanted GFP^+ cells (green) present in the demyelinated lesions. Magnification ×20. **(B)** Double immunostaining of MBP (red) and NF-H (blue; for axons) showed that IL-10-aNSC-i.v. significantly increased MBP expression and the percentage of myelinated axons ($MBP^+NF\text{-}H^+$). Some of the GFP^+ aNSCs were MBP^+ and in close contact with axons, indicating the contribution of transplanted aNSCs to remyelination. Magnification ×40, insets ×100. **(C)** Quantification of percentage of myelinated axons ($MBP^+NF\text{-}H^+$) among total axons ($NF\text{-}H^+$). **(D)** Quantitative analysis of MBP expression. MBP

pixel intensity was measured at random areas of disease lesions in the spinal cord using ImageJ software. At least 25 images per mouse, 6-8 mice per group were evaluated. $^{@}$ $p<0.05$, comparisons between EAE before NSCs i.v. (day 22 p.i.) and other groups; * $p<0.05$, ** $p<0.01$, comparisons between sham-EAE and other groups; $^{\#}$ $p<0.05$, comparison between GFP-aNSC-i.v. and IL-10-aNSC-i.v.

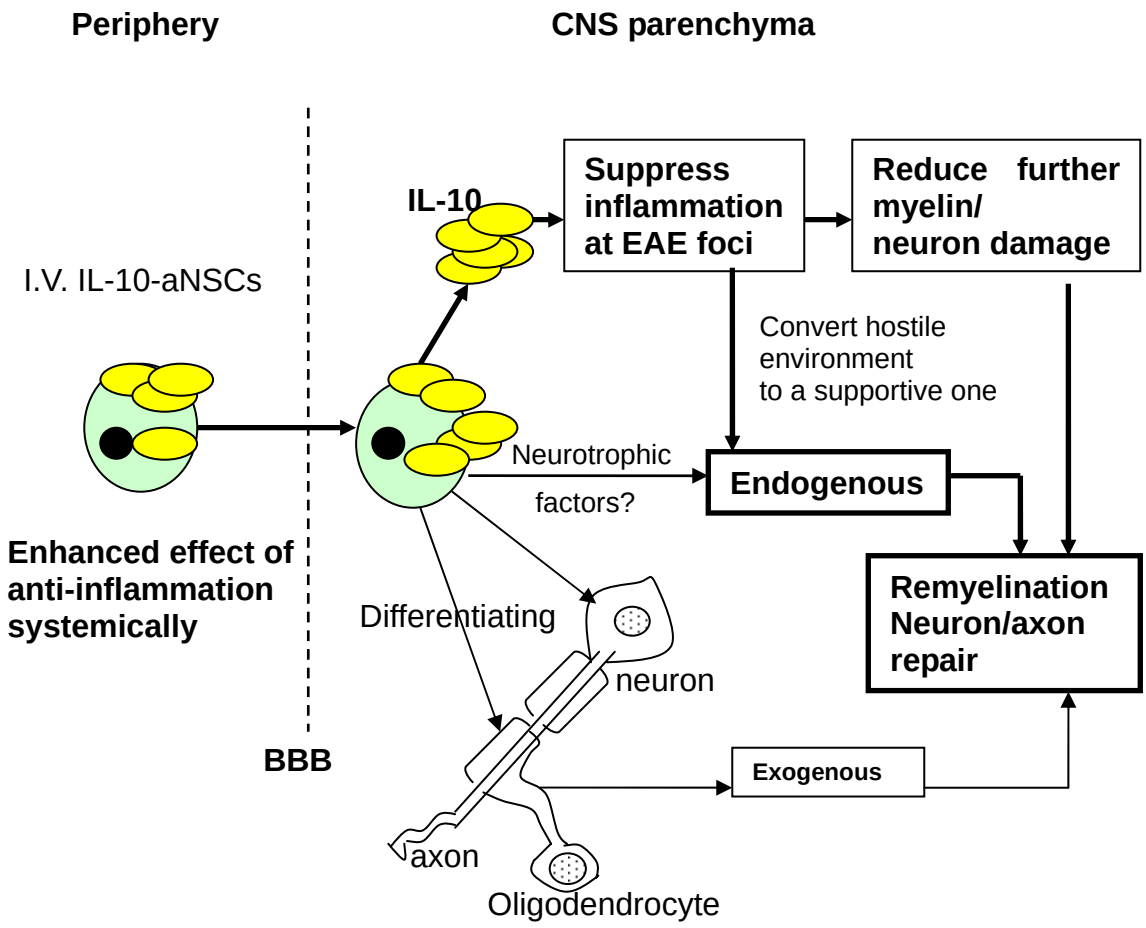

Fig. S6. Scheme: Proposed mechanisms of action of IL-10-aNSCs in EAE therapy. BBB: blood-brain barrier; NSCs: neural stem cells. Thick line/arrows: major pathways; thin line/arrows: secondary pathways.

Supplemental Methods

Adult neurosphere culture

Adult neurospheres were generated from the SVZ of adult C57BL/6 mice. Briefly, C57BL/6 mice at 8-12 wks of age were euthanized in a CO_2 chamber; the SVZ region of fresh brain was harvested under sterile conditions and placed in DMEM media. After a brief washing with DMEM media, tissues were cut into 1 mm^3 pieces and suspended in 2 ml 0.25% Trypsin with EDTA (Invitrogen), mechanically dissociated for 2 min and incubated at 37°C for 30 min. After filtration through a 70 μm cell strainer (BD Falcon), the cell suspension was washed twice with 10 ml DMEM medium. The cells were resuspended in serum-free DMEM/F-12 (Invitrogen) supplied with 2% B27 supplements (Invitrogen), 20 ng/ml epidermal growth factor (EGF, Peprotech) and 20 ng/ml basic fibroblast growth factor (b-FGF, Peprotech), along with 100 IU/ml penicillin and 100 μg/ml Streptomycin (Sigma). Cells were then transferred to poly-L-lysine coated 6-well plates (BD Bioscience, San Jose, CA) at a density of 0.25×10^6 cells/ml and maintained in culture at 37°C. Culture media were changed every 3-4 days. Neurospheres were formed after 3-5 days of culture. For passaging, free-floating neurospheres were collected and mechanically dissociated into small neurospheres or single cells and re-seeded at a density of 0.5×10^5 cells/ml in the same medium. aNSCs at passage 4-15 were used in all *in vivo* and *in vitro* experiments. To induce aNSC differentiation, dissociated single cells or small neurospheres were incubated in stem cell differentiation medium (NSC basal medium plus 10% NSC differentiation supplements, Stemcell Technologies) for 7 to 14 days and processed for immunocytochemistry staining.

Construction of bicistronic lentiviral vectors encoding IL-10 and GFP and transduction into aNSCs

Lentiviral vector backbone plasmid pLenti6.2-GW/EmGFP (Invitrogen) was digested with EcoRV and EspI to remove GFP, PGK promoter and blasticitin, then replaced with a fragment containing viral IL-10 cDNA, poliovirus-derived internal ribosomal entry site (IRES) and enhanced GFP (EGFP), resulting in a bicistronic vector plasmid pLv.IL-10. The new generated pLv.IL-10 and three other helper vector plasmids pLP1, pLP2, pLP/VSV-G (Invitrogen) were amplified and their concentration was adjusted to 1 μg/μl. Ninety percentage confluent 293T cells in 100 mm dishes were transfected with plasmid DNA containing 15 μg pLv.IL-10/GFP or 15 μg pLenti6.2-GW/EmGFP (negative control vector), 6.5 μg pLP1, 2.5 μg pLP2 and 3.5 μg pLP/VSV-G in 60 μl Lipofectamine 2000 (Invitrogen) and 3 ml serum-free DMEM medium for 4-6 hrs, followed by 3 ml DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-Glutamine, 100 I.U. /ml penicillin and 100 μg/ml Streptomycin (Mediatech, Inc., Herndon VA) overnight. Supernatants were changed and harvested every day for a total of 3-4 days, centrifuged at 15000 rpm/min for 6 min to remove debris and transferred into a 36 ml ultra-centrifuge tube for ultra-centrifugation at 25,000 rpm/min for 3 hrs. The pellet containing lentiviral vectors was resuspended in NSC culture medium and aliquots were stored at -80°C. Viral vector titers were assayed by infection of 293T cells at different dilutions; the titers were 5~10 $\times$ 10^8 infectious units/ml after concentration by ultracentrifugation.

To modify aNSCs with GFP and IL-10 genes, dissociated neurospheres were infected with lentiviral vectors Lv.IL-10 encoding both IL-10 and GFP, or Lv.GFP encoding GFP alone as a control. After 24 hrs of infection, cells were harvested and washed with PBS, re-suspended in growth medium and then transferred to poly-L-lysine coated 6-well plates. After 3-4 days in culture, cells were dissociated and washed again, and GFP positive cells were sorted by FACS to a purity of >99%. Then these cells were re-seeded at 0.5 $\times$ 10^5 cells/ml in growth medium for further expansion. IL-10

production was measured by ELISA (BD Bioscience) from the supernatant of cultured aNSCs.

Antibodies for immunohistochemistry

To identify cell infiltration, sections were labeled with anti-mouse CD45 (hematopoietic cells, AbD seroTec), CD4 and CD8 (T cells), and CD68 (macrophages/microglia) all from Santa Cruz Biotechnology. To determine potential debris clearance, sections were stained with monoclonal antibody directed against LAMP2 (Abcam) as described (1). CNS blood vessels were stained with anti-laminin antibody (Sigma). To identify specific markers of undifferentiated and differentiated aNSCs, immunohistochemistry was performed on 7 μm cryosections of the brain and spinal cord at day 78 p.t. Antibodies to nestin and SOX_2 (undifferentiated NSCs, BD Biosciences), NG2 (oligodendrocyte precursors, upstate cell signaling solutions), GalC (oligodendrocytes, Chemicon), NF-M and NeuN (neurons, Chemicon), NF-H (axons, Chemicon), GFAP (astrocytes, StemCell Tech.) and MBP (myelin, Santa Cruz Biotechnology) were used. These antibodies, as well as antibodies to IL-10 and IL-10 receptor (Santa Cruz Biotechnology), were also used in the cells in *in vitro* studies.

Quantification of transplanted GFP^+ cells within the CNS of EAE mice

Immunohistochemistry was performed with anti-neural specific antibodies and DAPI on six non-adjacent sections from each mouse under identical conditions. Five digital photographs were taken using the same exposure parameters from each section, and 5 mice per group were evaluated. Cells triple-labeled with GFP, neural specific markers and DAPI were identified as transplanted aNSCs, and other cells double-labeled with DAPI and neural specific markers were identified as endogenous cells in the CNS. Cell counter of ImageJ software (NIH ImageJ; National Institutes of Health, Bethesda, MD), developed by the NIH and available as a free download from http://rsb.info.nih.gov/ij/, was used

to count cells, and mean numbers were used for analysis.

Measurement of pixel of MBP immunofluorescence

To quantify the amount of MBP expression, pixel intensity of MBP immunofluorescence was measured according to previous reports (2, 3). All slides were immunostained with anti-MBP and NF-H antibodies under identical conditions, and all digital images were acquired using the same exposure parameters. MBP pixel intensity was measured at random areas of disease lesions in same image size using ImageJ software. Total axon numbers between each measured area did not differ significantly. The mean pixel intensity was calculated from at least 25 images per mouse, 4 mice per group were evaluated.

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2. Aguirre, A., Dupree, J.L., Mangin, J.M., and Gallo, V. 2007. A functional role for EGFR signaling in myelination and remyelination. *Nat Neurosci* 10:990-1002.
3. Paunescu, T.G., Russo, L.M., Da Silva, N., Kovacikova, J., Mohebbi, N., Van Hoek, A.N., McKee, M., Wagner, C.A., Breton, S., and Brown, D. 2007. Compensatory membrane expression of the V-ATPase B2 subunit isoform in renal medullary intercalated cells of B1-deficient mice. *Am J Physiol Renal Physiol* 293:F1915-1926.