Supplementary Data

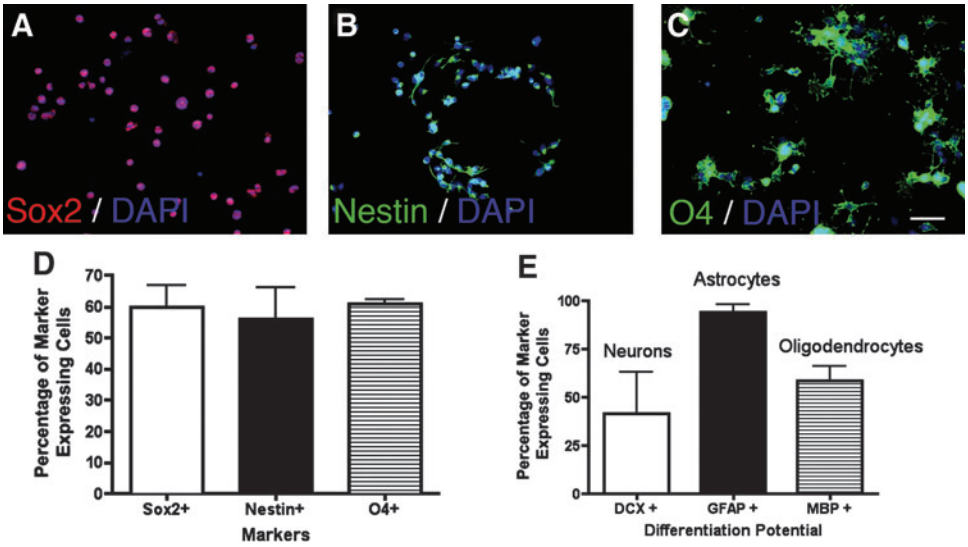
Supplementary Methods

Characterization of neural progenitor cells

To characterize the neural progenitor cells (NPCs) population that was used throughout this study, the expression of stem cell and progenitor markers and the differentiation potential of the NPCs were analyzed. To investigate the expression of stem/progenitor cell markers, NPCs were mechanically dissociated or with Accutase (Innovative Cell Technologies Inc., distributed by PAA) and plated on 100 μg/mL poly-L-ornithine (Sigma-Aldrich) and 5 μg/mL laminin-coated (Sigma-Aldrich) glass coverslips (2.5×104 cells/cm^2) in Dulbecco's modified Eagle medium knockout-20% Serum Replacement supplement (Gibco Invitrogen). After the cells were adherent (2–3 h) they were directly fixed for 30 min with 4% paraformaldehyde and processed for immunofluorescence stainings for Nestin, Sox2, and O4. To analyze the tripotent differentiation capacity of the NPCs, the cultures were dissociated with Accutase, diluted and single cells were seeded into 96-well plates. The presence of single cells-containing wells was confirmed by microscopy. Media was changed once a week. After primary spheres had formed the cells were dissociated and seeded for further culture expansion and secondary spheres formation. The tripotent differentiation potential was explored for dissociated secondary spheres that were stimulated with the following differentiation media: NB + B27 media supplemented with 1% fetal bovine serum (FBS) + 20 mM retinoic acid for neuronal differentiation, minimal essential medium alpha medium (αMEM)-10% FBS supplemented with 10 ng/mL bone morphogenetic protein (BMP)-2, and BMP-4 (R&D Systems GmbH) for astrocyte differentiation and conditioned medium derived from mesenchymal stem cell (MSC-CM) for oligodendrocyte differentiation. After 7 days the cells were fixed for 30 min with 4% paraformaldehyde and processed for immunofluorescence stainings for doublecortin (DCX), glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP).

Supplementary Results

To validate the stem/progenitor cell identity of the NPC population used throughout this study and because of the high amount of O4-expressing cells additional experiments were performed regarding the expression of neural stem cell markers and the differentiation capacity. Thus, neurospheres were mechanically dissociated to exclude possible effects of enzymatic treatment and stained for Sox2, Nestin, and O4. Approximately 55% to 60% of the cells were positive for the neural stem cell marker Sox2 and for Nestin (Supplementary Fig. S1A, B, D), while 60% of the cells were positive for the oligodendroglial marker O4 (Supplementary Fig. S1C, D). Similar numbers were obtained after



SUPPLEMENTARY FIG. S1. Neural stem/progenitor cell marker expression and tripotent differentiation potential of NPCs. **(A–D)** NPCs were mechanically dissociated, cells were seeded and fixed 2 h later. The marker expression profile was analyzed by immunocytochemistry. Illustrative fluorescence images of the immunostainings are shown for: Sox2 (*red*) and DAPI (*blue*) **(A)**; Nestin (*green*) and DAPI (*blue*) **(B)**; O4 (*green*) and DAPI (*blue*) **(C)**. Scale bar = 50 μm. Quantitative analysis show the percentage of positive cells for each marker **(D)**. The NPCs not only expressed the stem cell markers Sox2 and Nestin, but also the oligodendroglial marker O4. **(E)** Single cells derived from NPCs cultures were seeded into 96-well plates and incubated in proliferation media to produce growing spheres. The resulting spheres were dissociated and stimulated with differentiation media for neuronal, astrocyte, and oligodendrocyte differentiation. The cells were able to give rise to DCX-positive neurons, GFAP-positive astrocytes and MBP-positive oligodendrocytes. All experiments were performed at least in triplicate in 3 independent experiments. Data are shown as mean ± SD. NPCs, neural progenitor cells; DAPI, 4′, 6′-diamidino-2-phenylindole dihydrochloride; DCX, doublecortin; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; SD, standard deviation.

dissociation using Accutase treatment. Aberrances in the numbers of Nestin- and O4-positive cells cited here and mentioned in the text (Fig. 3) can be explained by the fact that the cells here were fixed 2 h after seeding. To confirm that the cells used in the present study were indeed multipotent central nervous system stem/progenitors, we tested for the capacity of clonal growth, self-renewal, and multipotency. Neurospheres that were derived from single cells under clonal (single well) conditions and that generated secondary spheres were dissociated and stimulated with differentiation media. The NPCs were able to generate DCX-positive neurons (~40%), GFAP-positive astrocytes (~90%), and MBP-positive oligodendrocytes (~60% Supplementary Fig. S1E).