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Neural and oligodendrocyte progenitor cells: transferrin effects on cell proliferation

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SUPPLEMENTARY DATA

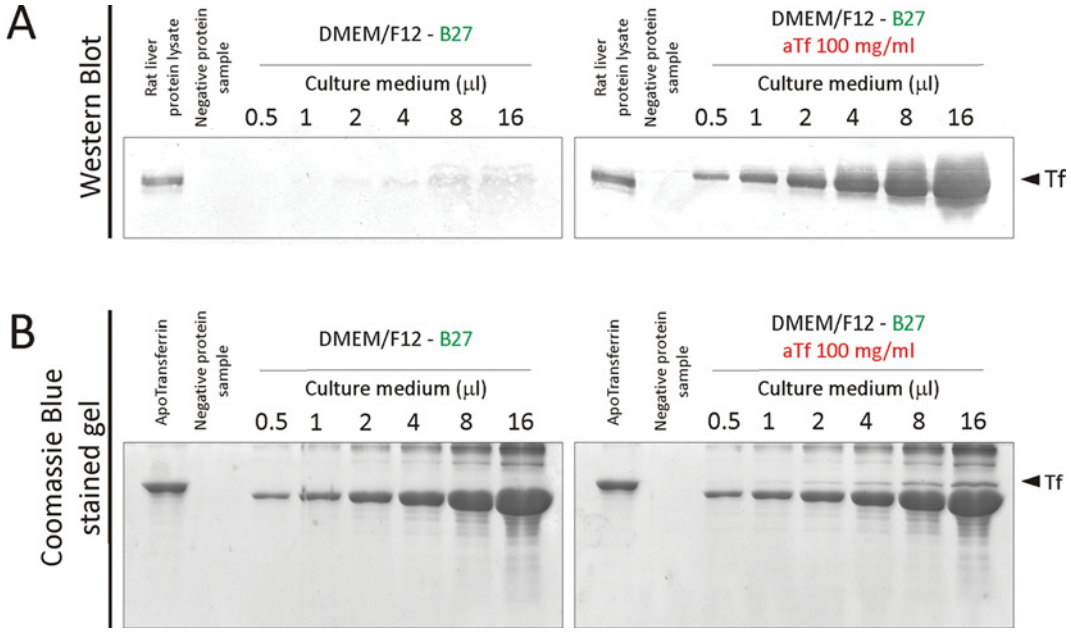

Figure S1 **Tf analysis in the culture medium**

(**A**) Western blot analysis of Tf in the DMEM/F12-B27 culture medium in the absence (left) and in the presence (right) of aTf. Increasing volumes of the culture medium (μl) were seeded onto each gel lane. (**B**) Coomassie Blue stained gels of total proteins in the DMEM/F12-B27 culture medium in the absence (left) or the presence (right) of aTf. Rat liver homogenates were used as positive controls, and the Laemmli loading buffer was used as a protein negative control. The expected molecular mass of Tf is indicated on the left of each image.

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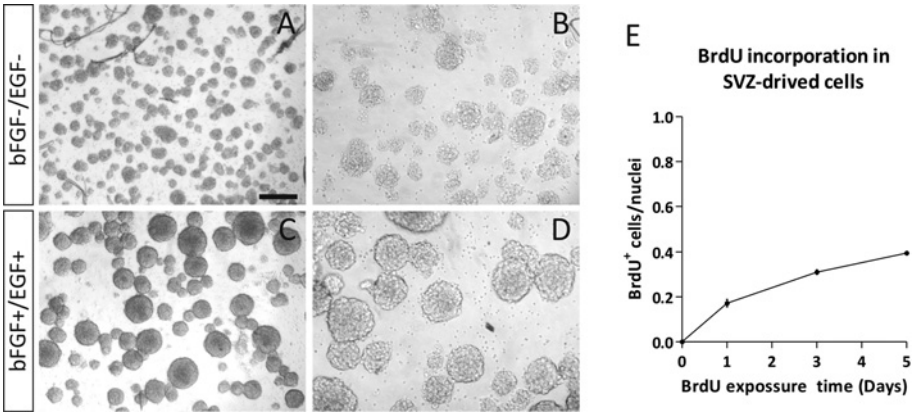

Figure S2 **The NS culture system**

Bright field images of the SVZ tissue cultured in the absence (**A**, **B**) or the presence (**C**, **D**) of the growth factors bFGF and EGF in the DMEM/F12.B27 culture medium. (**A** and **C**) Correspond to low magnification images, while (**B** and **D**) correspond to higher magnification images. (**E**) BrdU incorporation in dissociated NS cultures by immunocytochemical analysis. The BrdU incorporation ratio is represented as a function of the BrdU exposure time in SVZ-derived cultures. The values of each curve point represent the means ± S.E.M. Scale bar in (**A**) represents 500 μm for (**A**) and (**C**), 200 μm in (**C** and **D**).

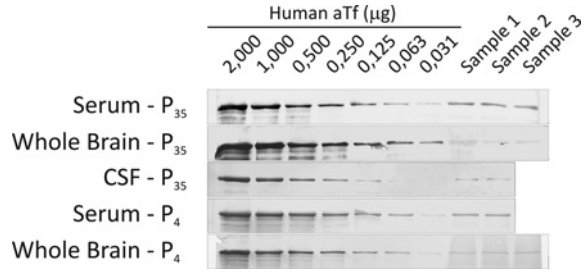

Figure S3 **Semiquantitative analysis of Tf by WB**

Scanned images of WB membranes used to semiquantify the Tf content in different tissue samples belonging to P_{35} and P_4 rats (serum, CSF and whole brain samples). The first seven lanes (left to right) correspond to decreasing amounts of the human aTf that were used to build a calibration curve. The last lanes belong to the samples of two or three different animals. All 'sample' lanes were loaded with 1 μl of each rat tissue sample. The serum samples of all animals were diluted 1/10 for the analysis due to their high albumin content that deformed the shape of the immunopositive bands, while the rest of the samples were loaded undiluted.

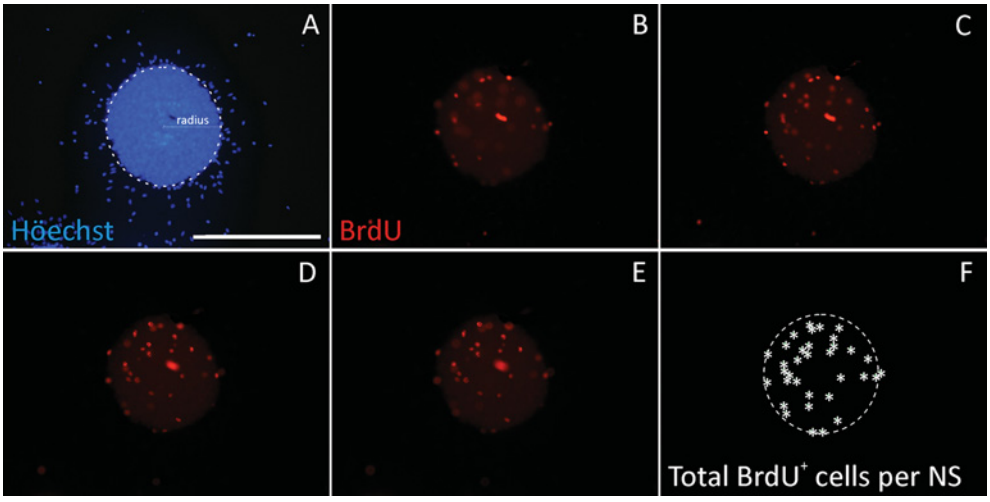

Figure S4 **BrdU analysis in whole NS**

(**A–F**) Epi-fluorescence microscopy images of Höechst-labelled nuclei (blue) and BrdU$^+$ nuclei (red) in a representative non-dissociated NS. (**B–E**) The BrdU$^+$ nuclei of a single NS are shown at different focal planes. (**F**) The sum of BrdU$^+$ nuclei at each focal plane is indicated with white asterisks. The total number of BrdU$^+$ nuclei per NS is normalized to the NS volume, where the volume is calculated from the radius length measurement. The scale bar in (**A**) represents 250 μm in all images.

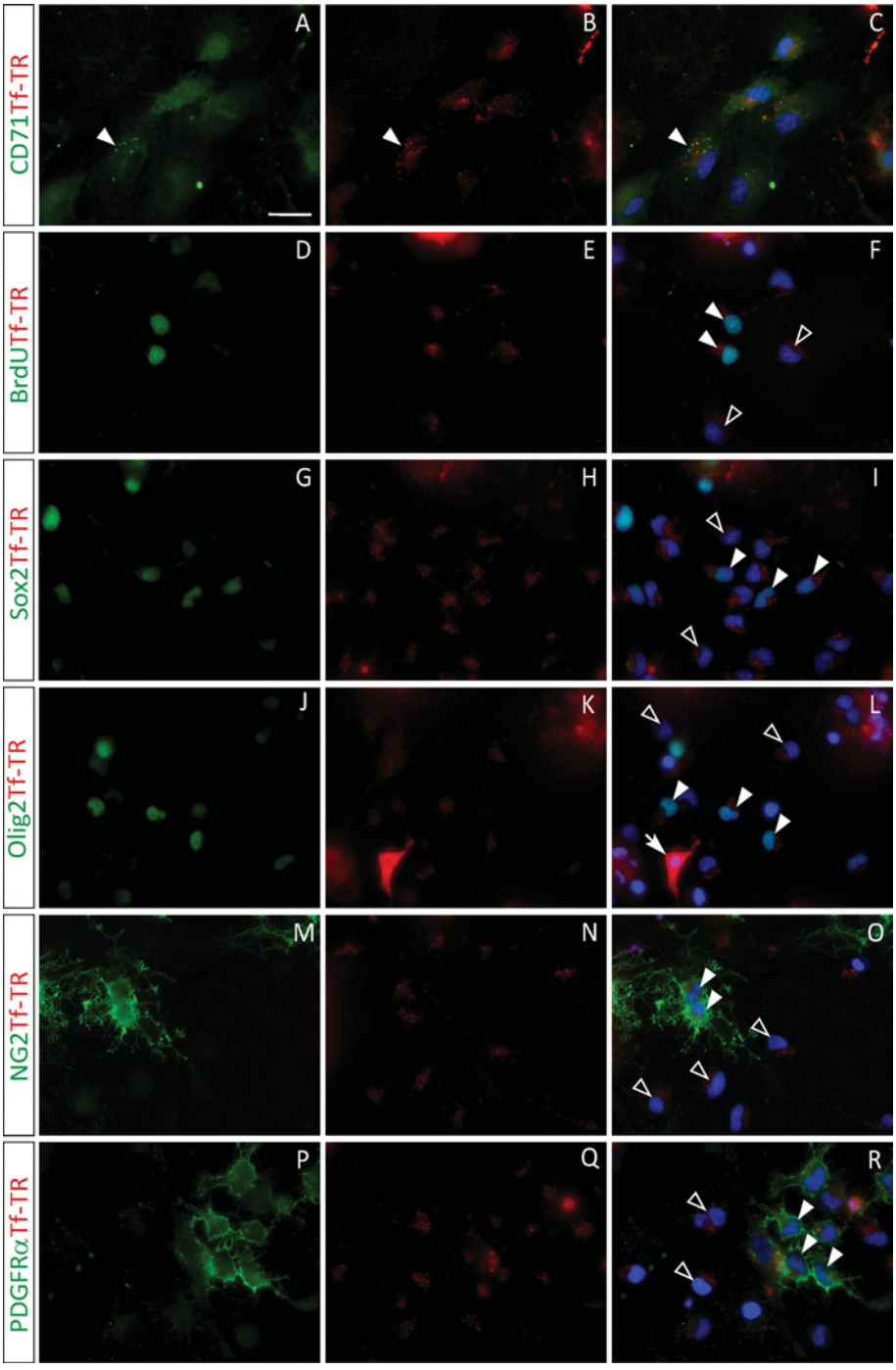

Figure S5 **Texas Red-labelled Tf incorporation in SVZ-derived cells *in vitro***

In all images, the Tf-TR incorporation is shown in red and the Höechst-labelled nuclei in blue. The different cell markers identified on cells are shown in green. **(A–C)** Tf-TR is shown together with CD71$^+$ cells. The white arrowhead indicates a cell with the Tf-TR characteristic puncta co-localizing with TfRc1. **(D–F)** Tf-TR is shown in BrdU$^+$ cells (white arrowheads) as well as in BrdU$^-$ cells (empty arrowhead). **(G–I)** Tf-TR in Sox2$^+$ cells (white arrowheads) and in Sox2$^-$ cells (empty arrowheads). **(J–L)** Tf-TR in Olig2$^+$ cells (white arrowheads) and in Olig2$^-$ cells (empty arrowheads). **(M–O)** Tf-TR in NG2$^+$ cells (white arrowheads) and in NG2$^-$ cells (empty arrowheads). **(P–R)** Tf-TR in PDGFRα$^+$ cells (white arrowheads) and in PDGFRα$^-$ cells (empty arrowheads). The blue colour on images belongs to the Höechst nuclear dye. The scale bar in **(A)** represents 50 μm for all images in this Figure.

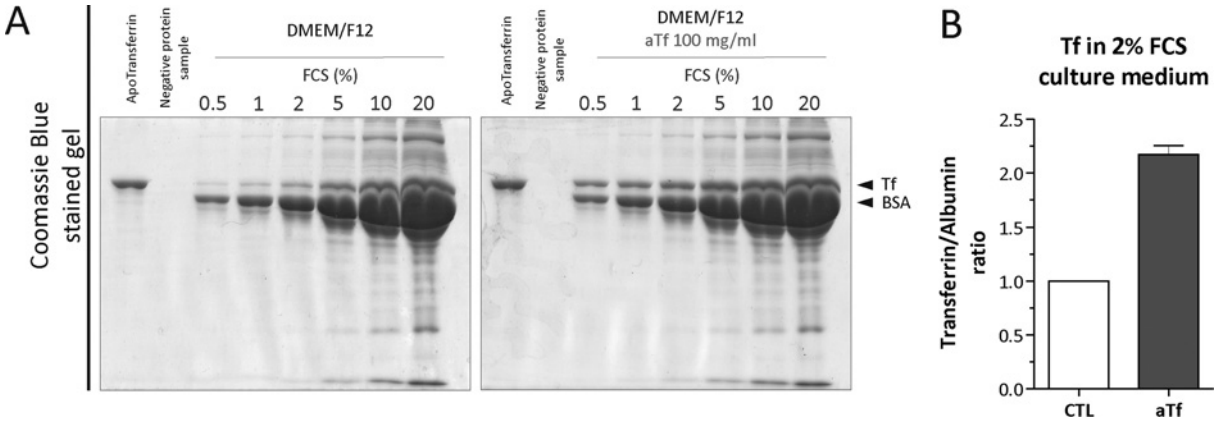

Figure S6 **Culture medium protein analysis by SDS/PAGE**

(**A**) Comparative analysis of the total proteins present in the DMEM-F12 culture medium supplemented with increasing percentages of FBS, and in the absence or presence of added aTf (100 μg/ml). All the wells containing culture medium were loaded with equal amounts (10 μl) of the medium sample. The commercial aTf of human origin that was used to supplement the culture medium was used as a molecular mass marker. (**B**) The Tf content in samples belonging to 2% FBS–DMEM/F12 culture medium (CTL) and the 2% FBS–DMEM/F12–aTf (100 μg/ml) is compared. A Tf to BSA ratio is used to normalize the Tf content in the samples. The major protein band in the Coomassie Blue-stained gels is considered to be BSA, the most abundant protein in the FBS. Bars in (**B**) represent means ± S.E.M.