Figure S1. Semi-quantitative PCR for MTP or MTP3 in brain tissues and in cells. A. RT-PCR was performed on RNA isolated from the adult mouse brain tissues (A) or from cell cultures (B). PCR product after 28, 29, and 30 cycles of amplification were shown. SVZ: subventrivular zone of the lateral ventricle; ST: striatum; HP: hippocampus; CX: neural cortex; NPC: Sox1-GFP+ neural progenitor cells; bEND: mouse brain endothelial
cells.

Fig. S1A

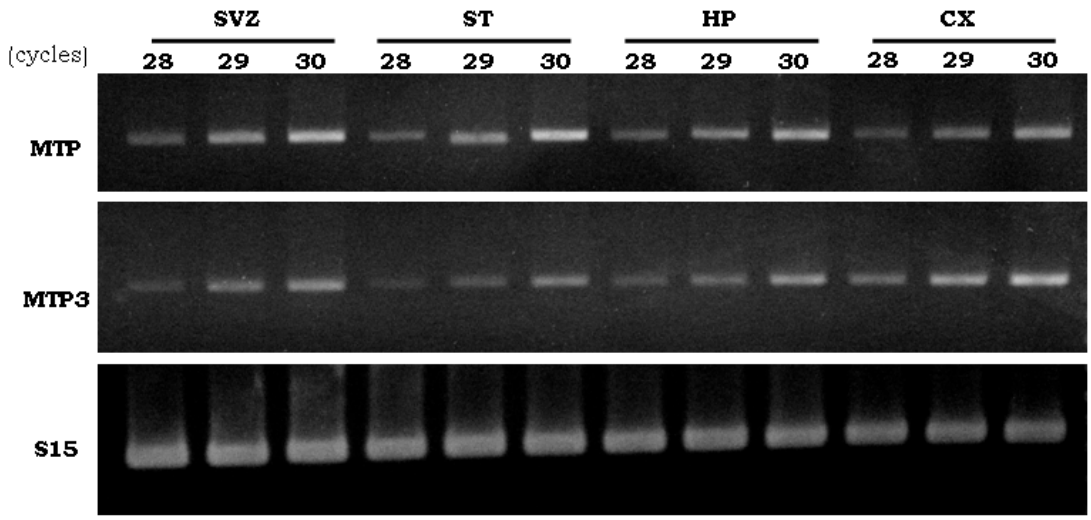

Fig. S1B

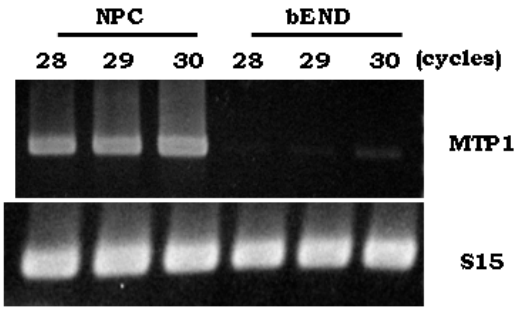

Figure S2. Immunohisochemical staining of MTP in adult mouse brain. Coronal sections of adult mouse brain were immuno-stained with antibody to MTP (A), NeuN (B) and TuJ1 (C). Image in D shows negative control staining with IgG of the same subtype as that of MTP antibody. Images E-K are high magnification images of the areas marked E-K in image A. Image in L shows cerebellum stained with MTP antibody. Some Purkinje neurons are indicated by arrows. E'-K' are negative control, respectively for E–K, stained with control immunoglobins of the same subtype. Bars: 500 μm in A-D, 50 μm in E-L and E'-L'.

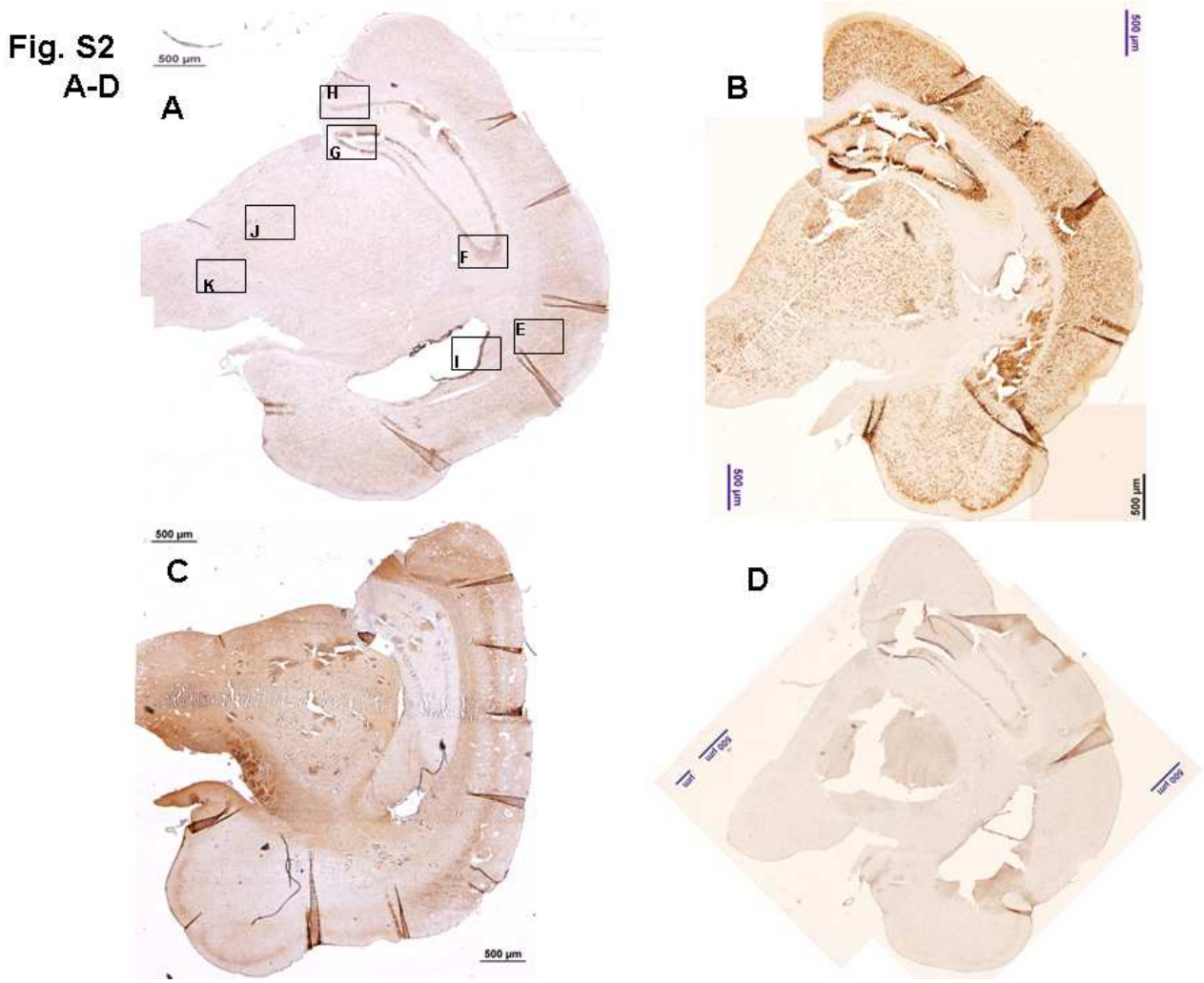

Fig. S2 E-L

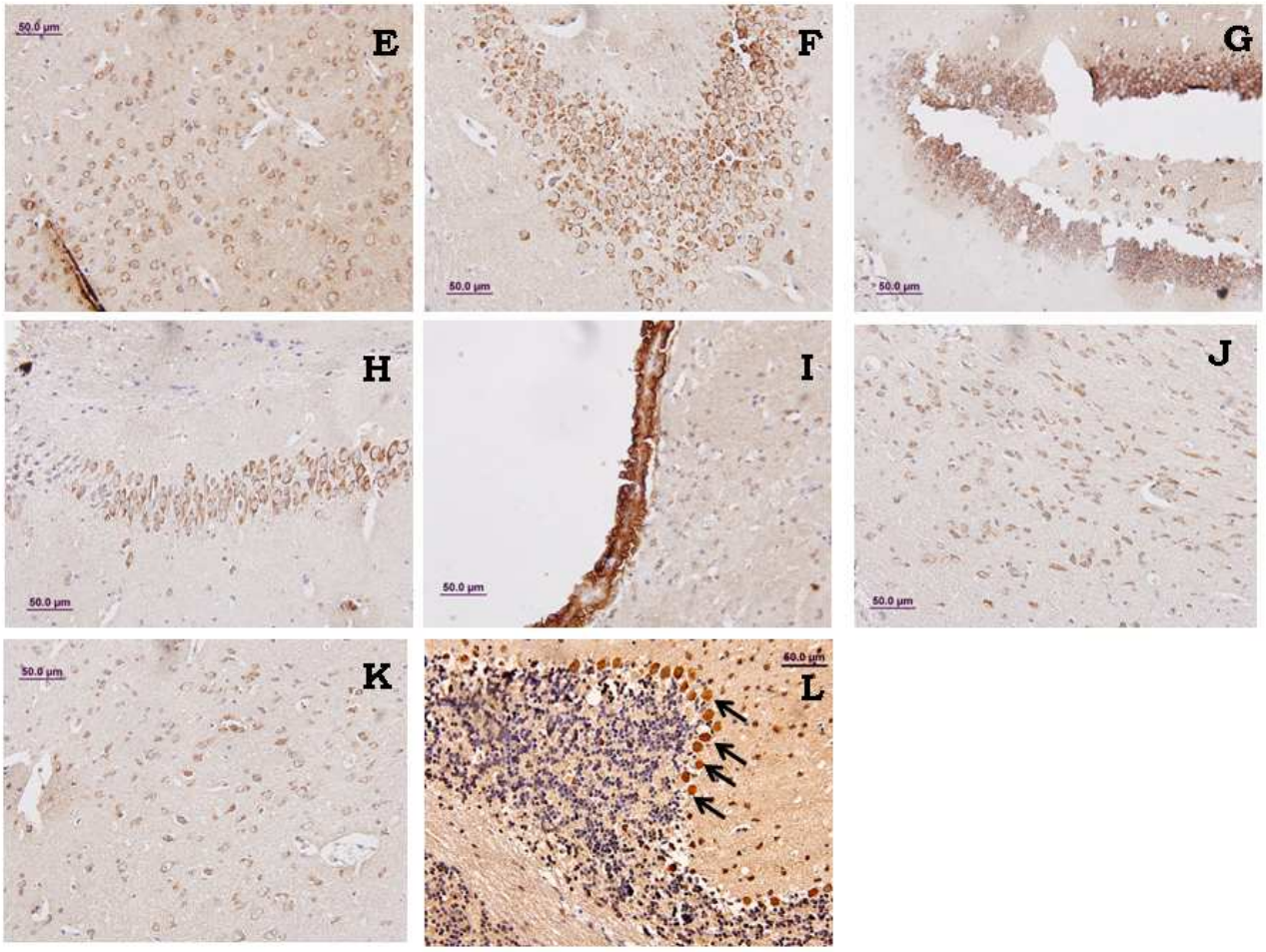

Fig. S2 E'-L'

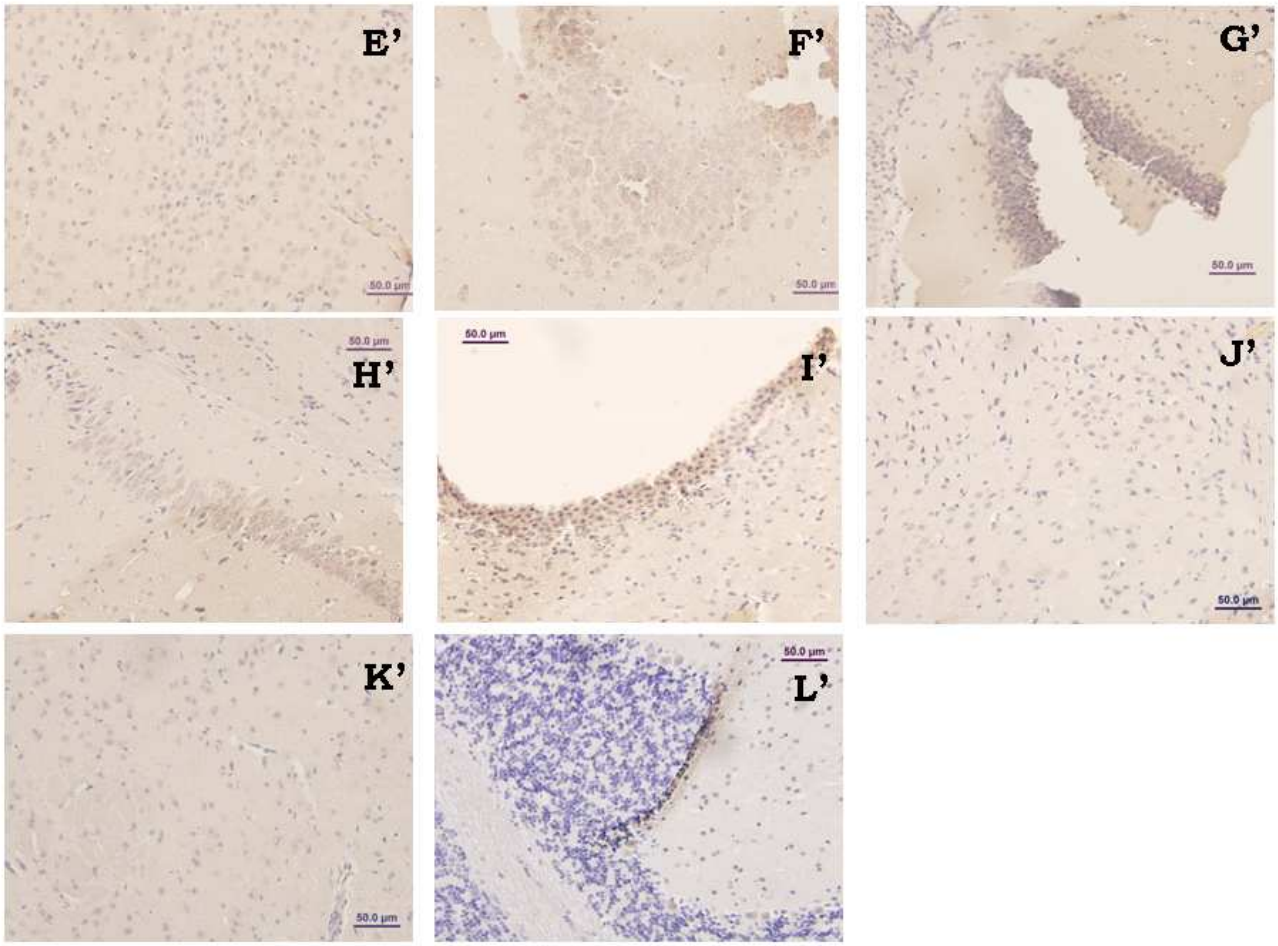

Figure S3. Neural differentiation of 46C ES cells in feeder-free monolayer culture. 46C ES cells were seeded on collagen-coated surface and cultured in neural differentiation culture condition as described in "Methods". Cells were fixed at 2 days (d2), 4 days (d4) and 7 days (d7) post-culture. Day-fourteen (d14) cells are cells from the d7 cultures seeded on a PDL/laminin-coated surface and cultured for an additional 14 days. After fixation, cells were stained with anti-GFP antibody and the antibodies to neuronal markers βIII–tubulin (TuJ1) or NeuN.

Fig. S3

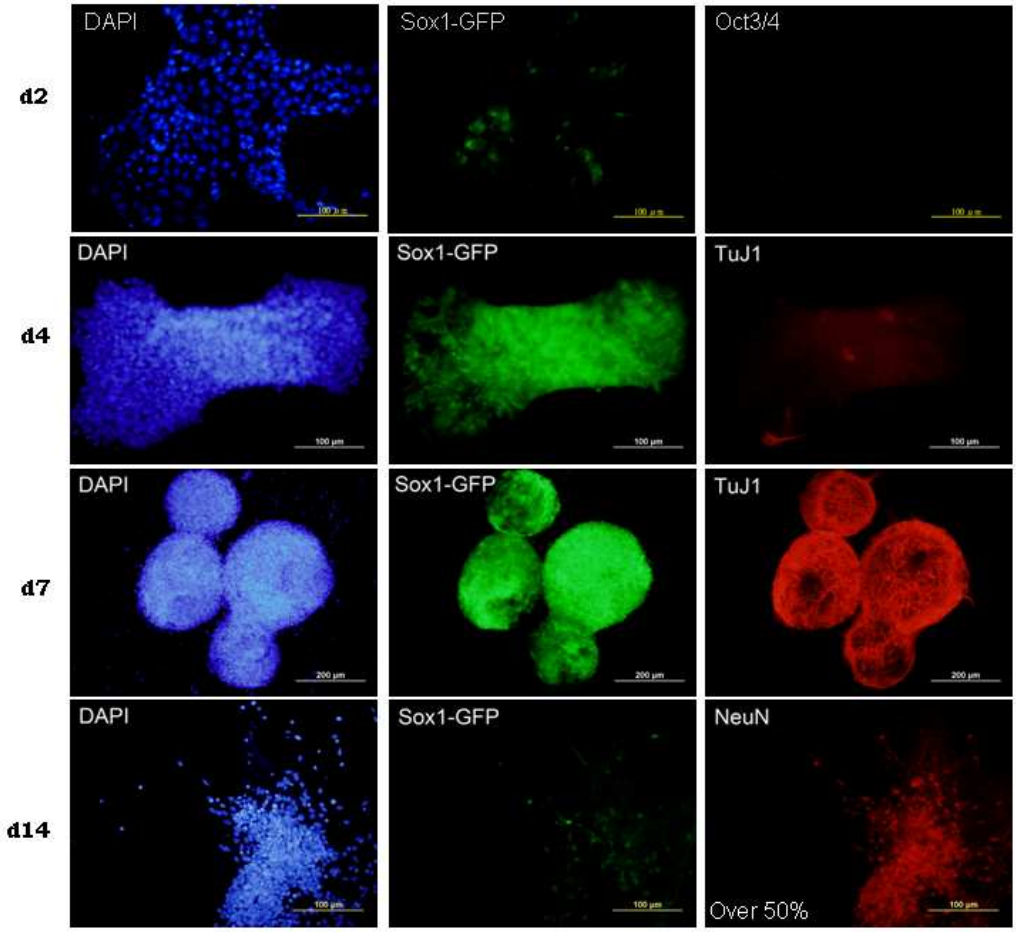

Figure S4. No effect of MTP on Sox-GFP+ NS/P cell proliferation and cell death. A. Total number of cells after 24, 48 or 72 hours culture with (MTPi) or without (CTRL) 2 μM of MTP inhibitor. B. The number and size of neurospheres generated in cells cultured in the absence (CTRL) or presence (MTPi) of MTP inhibitor (2 or 5 μM). C. The control (CTRL), non-silencing control siRNA transfected (siNeg), MTP siRNA transfected (siMTP), or MTP expression plasmid transfected (pMTP) day-4 NS/P cells were treated with BrdU as described in "Methods". Graph shows the percentage of BrdU labeled cells to the total cells. D. The percentage of trypan blue stained (for inhibitor treated cells) or 7-AAD labeled (for siRNA treated cells) cells was normalized, respectively, to that of cell without MTPi treatment or transfected with the non-silence control siRNA (shown in dark dotted line). Data were collected from three independent experiments.