SUPPLEMENTAL MATERIAL

Correa et al., http://www.jem.org/cgi/content/full/jem.20101880/DC1

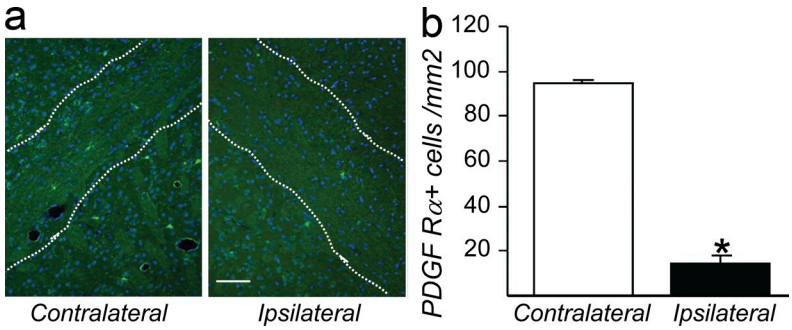

Figure S1. Loss of OL progenitor cells in the white matter after MCAO. (a) Representative image of PDGFR-α immunostaining (green) and DAPI staining (blue) in the contralateral and ipsilateral corpus callosum 24 h after permanent MCAO in 9-mo-old mice. *n* = 3 animals. Demarcations show the limits of corpus callosum. Bar, 100 µm. (b) Quantification of OLs (PDGFR-α^+) in the contralateral and ipsilateral corpus callosum after permanent MCAO in 9-mo-old mice (mean + SEM, *n* = 3). *, significantly ($P < 0.05$) different from contralateral, Mann-Whitney *U* test. *n* = 3 animals.

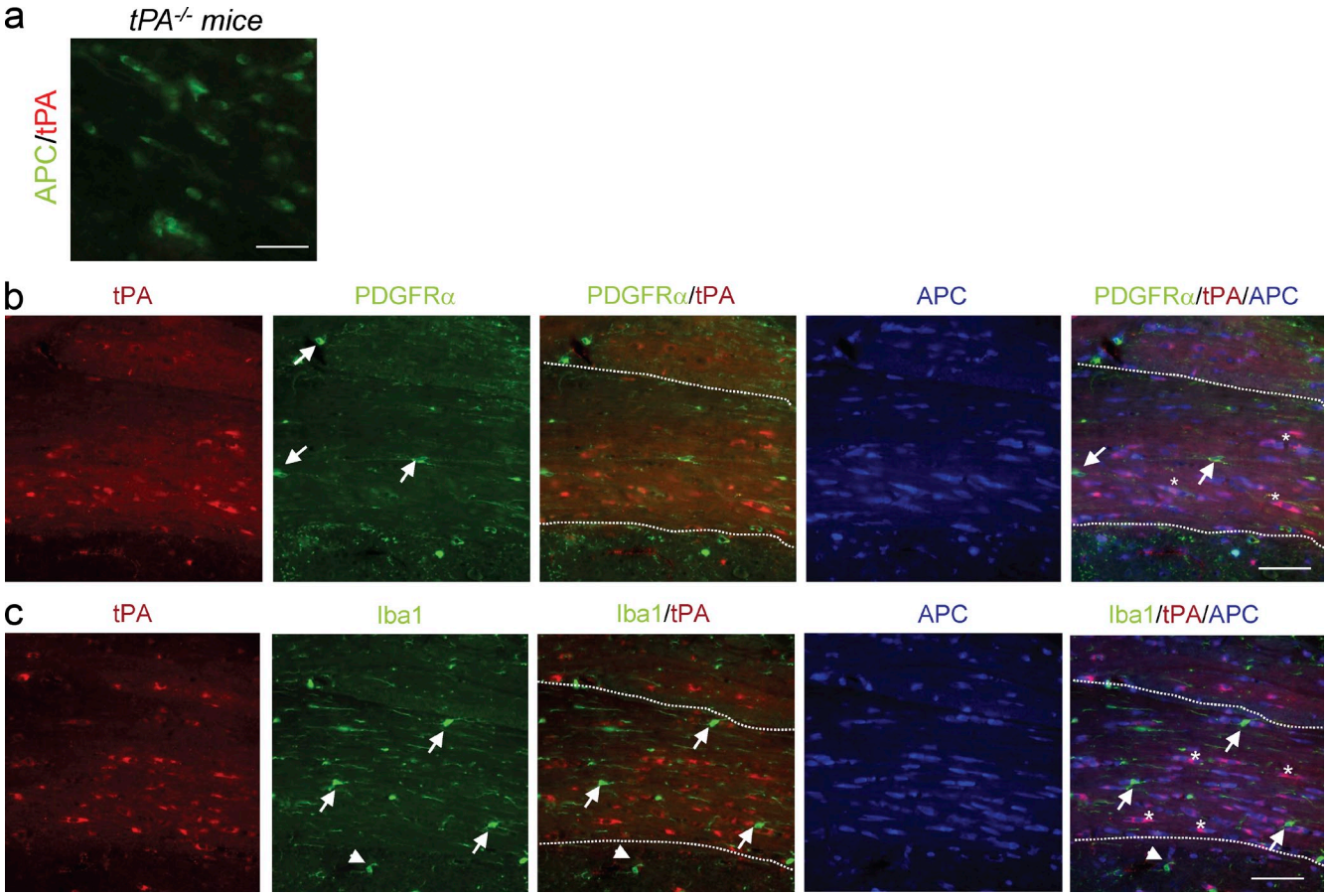

Figure S2. tPA immunostaining is absent in microglia and OL progenitor cells of white matter. (a) Representative image of tPA immunostaining (red) and APC immunostaining (green) in the corpus callosum of naive $tPA^{-/-}$ 4-mo-old mice. Note the absence of tPA immunostaining in these conditions. (b and c) Triple immunostaining for tPA (red), APC (blue), and cellular markers for OL progenitor cells (PDGFR-α; b) or microglial cells (Iba1; c). Arrows show PDGFR-α–positive cells (b) and Iba1-positive cells (c). Asterisks show cells double stained for tPA and APC. Bars, 100 µm.

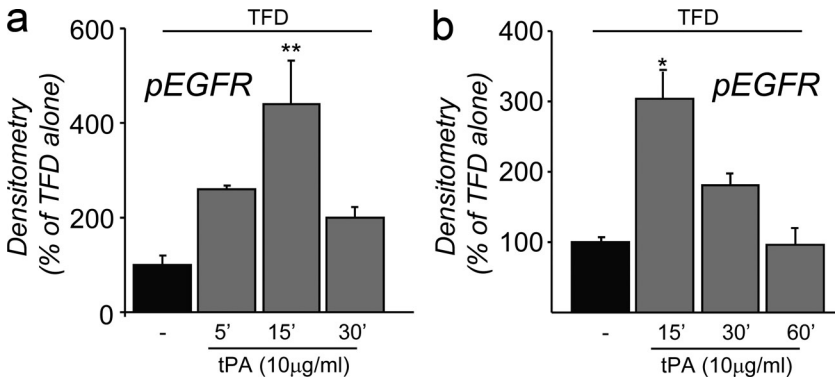

Figure S3. Quantification of Western blots showing the phosphorylation of EGFR after tPA treatment. Histograms show mean + SEM ($n = 3$) of densitometry of bands obtained in Western blots for the phosphorylated form of EGFR in OLs treated with tPA for 5–30 min (a) or for 15–60 min (b). See Fig. 3 c for representative Western blot image. * and **, significantly (respectively, $P < 0.05$ and $P < 0.01$) different from control (no tPA treatment). Mann-Whitney *U* test.

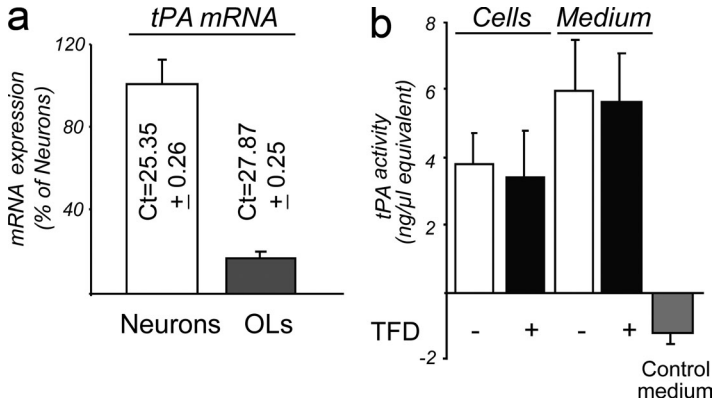

Figure S4. tPA expression in cultured OLs. (a) mRNA extracted from cultured OLs and neurons were subjected to quantitative RT-PCR for tPA. Histograms show mean + SEM of relative values of mRNA quantification, referred to the value in neurons (n = 3). Ct values are indicated. (b) Histograms show mean + SEM of tPA activity quantified by a spectrozyme assay from OL cell lysate or bathing medium (n = 3).

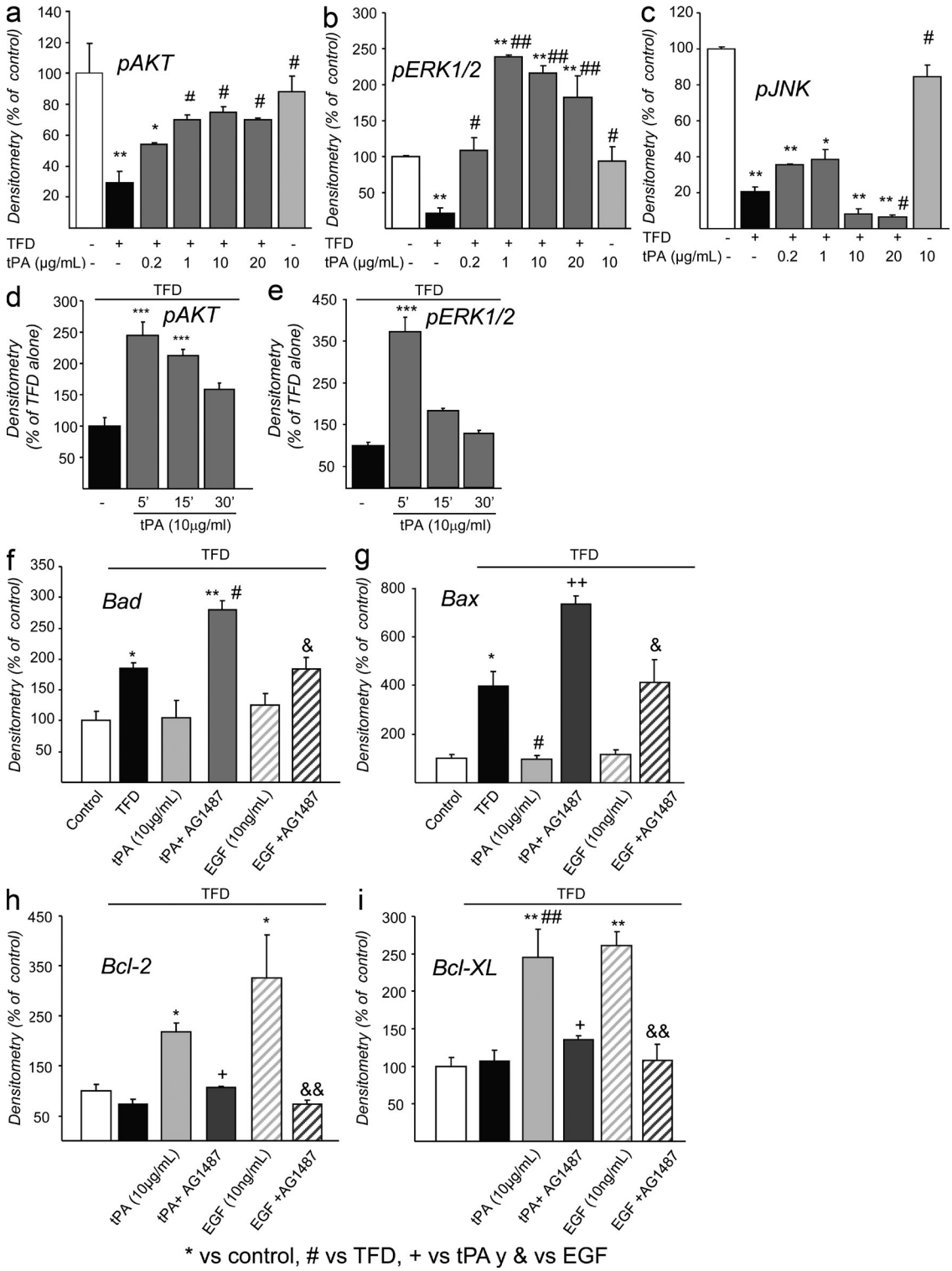

Figure S5. Quantification of Western blots for intracellular pathways and pro/antiapoptotic factors. Bars shows mean + SEM (n = 3) of densitometry of bands obtained in Western blots for the following: phosphorylated forms of Akt (a), Erk1/2 (b), and Jnk (c) in OLs treated with indicated doses of tPA for 24 h; the phosphorylated forms of Akt (d) and Erk1/2 (e) after tPA treatment (20 µg/ml; 5–30 min); and Bad (f), Bax (g), Bcl-2 (h), and Bcl-XL (i) in OLs in the indicated conditions after 24 h of treatment. See Fig. 6 (a, b, and i) for representative Western blot images. *, **, and ***, significantly (respectively, $P < 0.05$, $P < 0.01$, and $P < 0.005$) different from control; # and ##, significantly (respectively, $P < 0.05$ and $P < 0.01$) different from TFD; + and 2+, significantly (respectively, $P < 0.05$ and $P < 0.01$) different from TFD + tPA; & and &&, significantly (respectively, $P < 0.05$ and $P < 0.01$) different from TFD. Mann-Whitney U test.

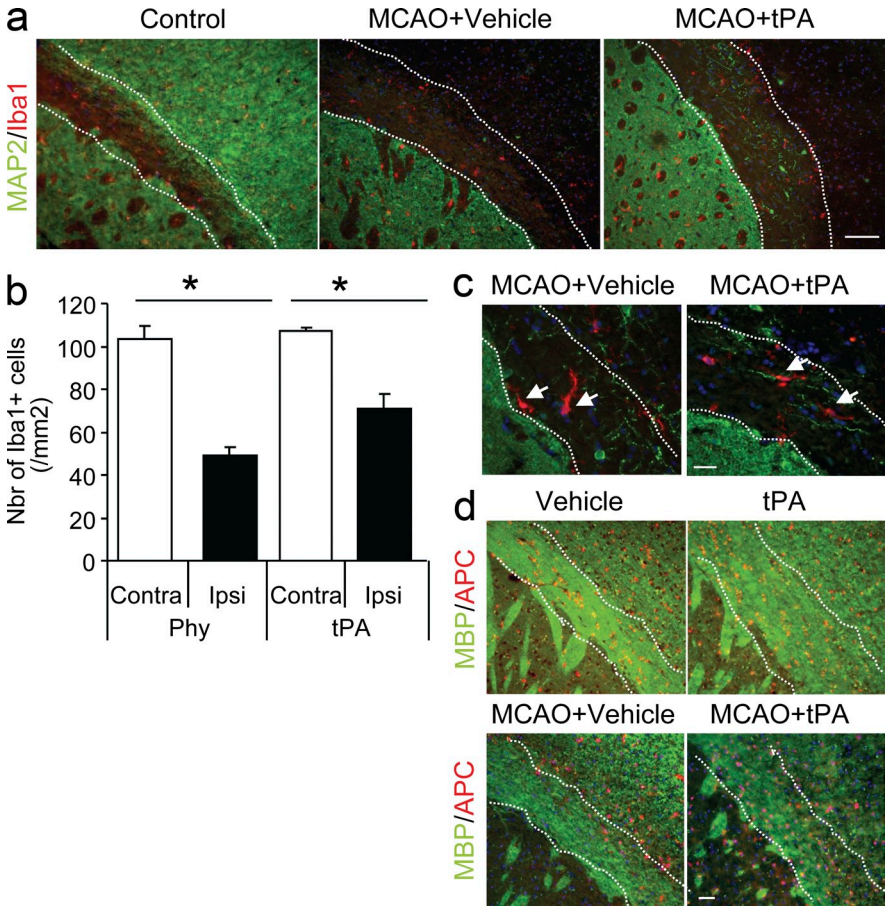

Figure S6. tPA does not influence microglial response or myelin integrity in the white matter after MCAO. (a) Photomicrographs of 9-mo-old mice tissue sections show MAP-2 (green) and Iba1 (red) immunoreactivities in the corpus callosum in the indicated conditions. Control animals, MCAO (ipsilateral hemisphere) with or without i.v. injection of recombinant tPA (Actilyse). Bar, 100 µm. (b) Quantification of microglia ($Iba1^+$) in the contralateral and ipsilateral corpus callosum after permanent MCAO in 9-mo-old, vehicle, or tPA-injected animals (mean + SEM, $n = 3$). *, significantly ($P < 0.05$) different from contralateral, Mann-Whitney *U* test. (c) Photomicrographs of 9-mo-old mice tissue sections show MAP-2 (green) and Iba1 (red) immunoreactivities in the corpus callosum in the indicated conditions. Note the typical morphology of activated microglia both in vehicle and tPA-injected animals (arrows). Bar, 100 µm. (d) Photomicrographs of 9-mo-old mice tissue sections show MBP (green) and APC (red) immunoreactivities in the corpus callosum in the indicated conditions. Note the conservation of MBP staining in all conditions, the loss of APC^+ cells in the MCAO + Vehicle condition, and the rescue of these cells the MCAO + tPA condition. Bar, 100 µm.