



Supplemental Figure 1. Astrocyte viability after 24 h treatment with MnCl₂ and TNF α . Assessment of astrocyte after 24 h with 30 μ M with Mn and 10 pg/ml TNF α (Mn+TNF α) verified viable cells for co-culture studies. (a-b) Representative images of astrocytes treated with saline or Mn+TNF α and examined by immunofluorescence for expression of GFAP (1:500, Cell Signaling, Green) and the high affinity glutamate transporter, GLAST (2 μ g/mL, Miltenyi, red). Treated astrocyte display an activated phenotype, with formation of stress fibers and hypertrophy of cytoplasmic processes, without loss of GLAST expression. (c) Percent of total cells treated with Mn+TNF α were positive for nuclear staining with the propidium iodide (5 μ g/mL; Sigma). Triton-X treatment (1%, 15 min) was used as a positive control. (d) Dose-response viability data using the live/dead assay with calcein and propidium iodide in astrocytes treated with increasing concentrations of Mn in the presence of 10 pg/ml TNF α . *** $p < 0.001$.