Supplementary Data

NitroSynapsin for the treatment of neurological manifestations of Tuberous Sclerosis Complex in a rodent model

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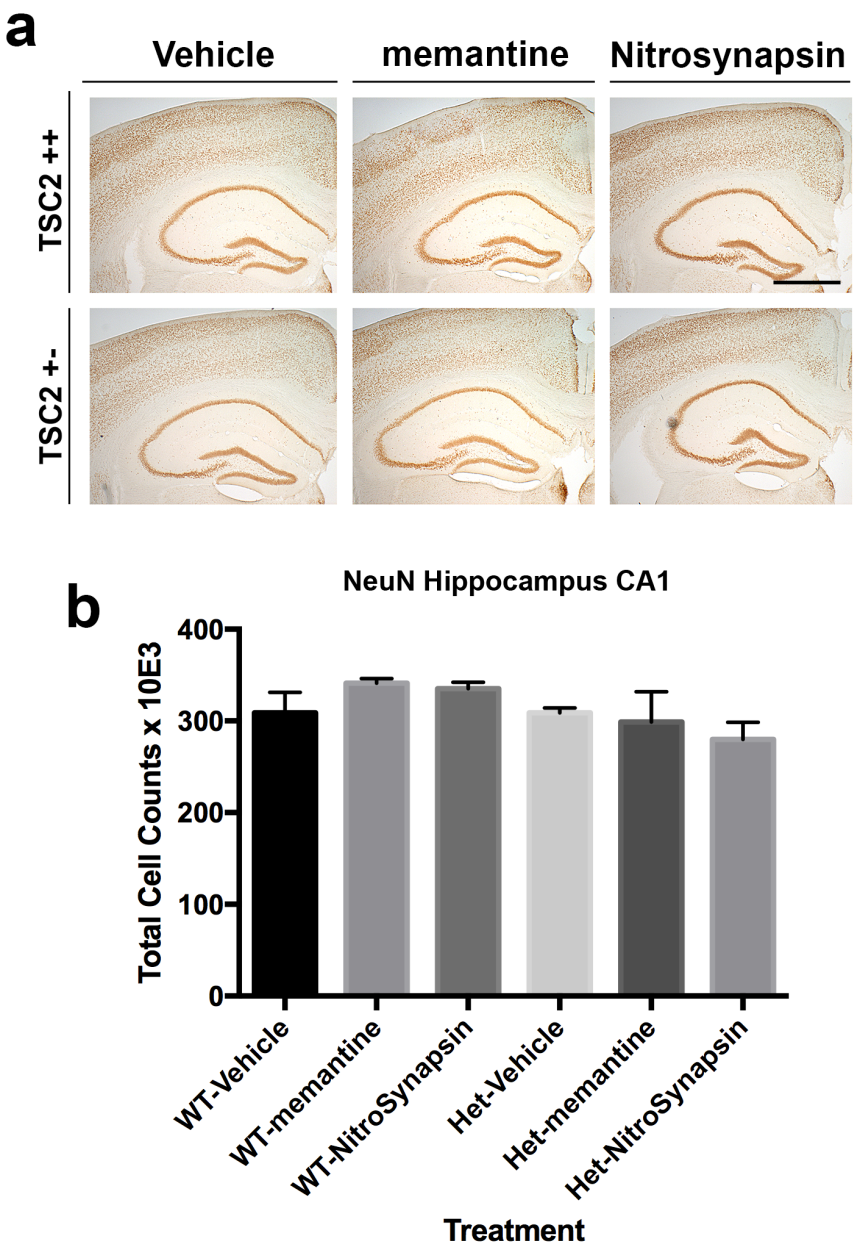
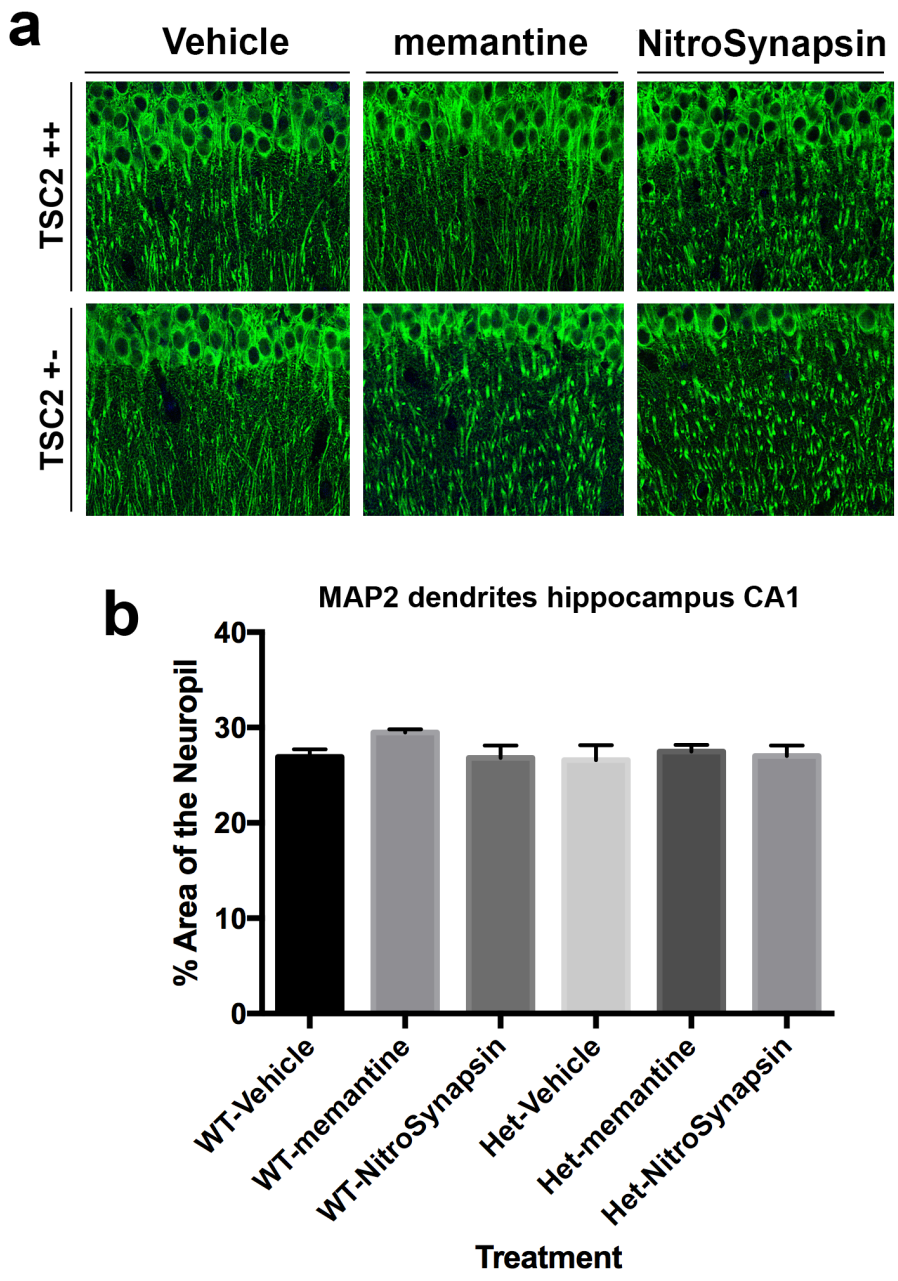
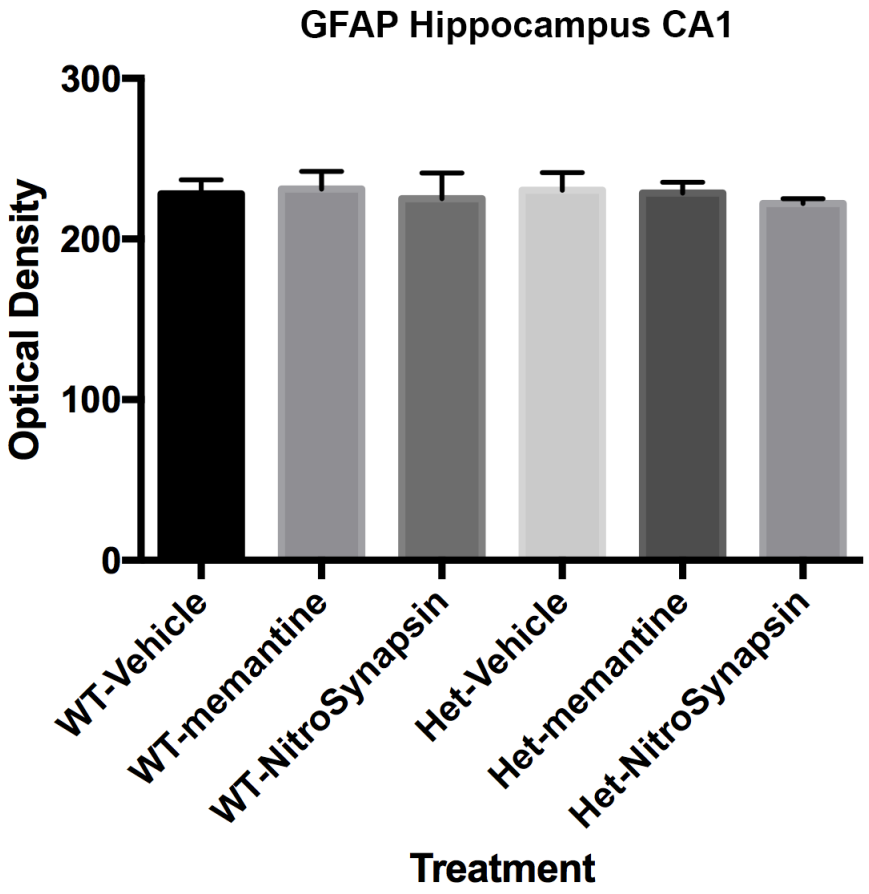


Supplementary Figure S1 (*refers to Figure 3*). Effect of treatment with memantine or NitroSynapsin on neuronal counts in the hippocampus of *Tsc2*$^{+/-}$ mice. (**a**) Representative histological images of NeuN-stained brain slices from three-month-old WT and *Tsc2*$^{+/-}$ mice treated with vehicle, memantine or NitroSynapsin. (**b**) Quantification of NeuN-labeled neuronal cell bodies (total cell counts x 10E3) in the CA1 area of hippocampus (n = 17 mice evaluated).



Supplementary Figure S2 (*refers to Figure 3***). Effect of treatment with memantine or NitroSynapsin on MAP2-labeled neuropil of *Tsc2*$^{+/-}$ mice.** Representative histological images and quantification of brain slices from three-month-old WT and *Tsc2*$^{+/-}$ mice treated with vehicle, memantine or NitroSynapsin. (**a**) Staining for MAP2 positive neuronal dendrites (green) in the hippocampus. (**b**) Quantification of area occupied by MAP2-positive neuropil. No significant difference was observed between all treatment groups. Values presented as per cent area of neuropil + s.e.m. (n = 17 mice evaluated).



Supplementary Figure S3 (*refers to Figure 3***). Effect of treatment with memantine or NitroSynapsin on GFAP-labeled astrocytes *Tsc2*$^{+/-}$ mice.** Quantification of area occupied by GFAP-positive immunoreactivity in hippocampus. No significant difference was observed between all treatment groups. Values presented asper cent area occupied + s.e.m. (n = 17 mice evaluated).

Supplementary Table S1. Phosphoproteomic analysis after eNMDAR stimulation of rat cortical neurons compared to control cultures

Protein	Phosphopeptide spectral counts* (% eNMDAR-stimulated vs. control)
TSC1	100
TSC2	60
TSC2-pS1254	75
Rheb	-100
mTOR	74
Rps6kc1	72

Phosphoproteins detected in eNMDAR-activated cells over control. Spectral counts represent upregulation of phosphorylation by eNMDAR signaling (negative values, as for Rheb, indicate downregulation of phosphorylation after eNMDAR stimulation). Biological duplicate samples were analyzed by mass spectrometry in duplicate to yield an n = 4 for each sample.
*Each value was statistically significant from the control ($P < 0.05$).