

Supplementary material for:

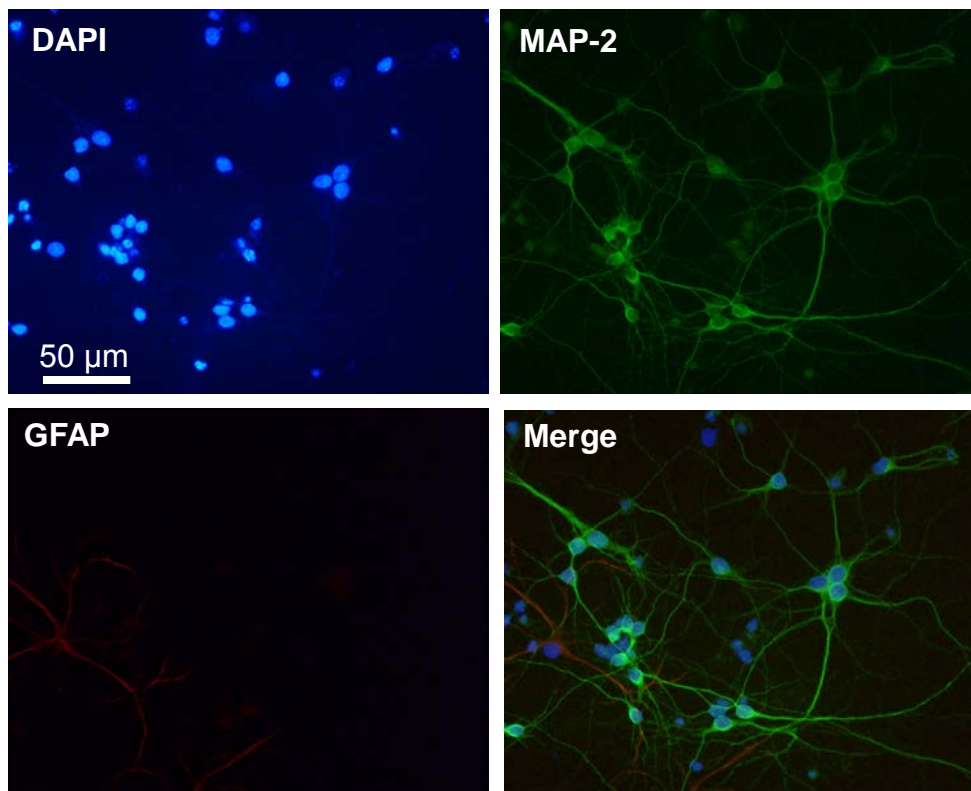
**Novel positive allosteric modulators of glutamate transport have neuroprotective properties in an *in vitro* excitotoxic model**

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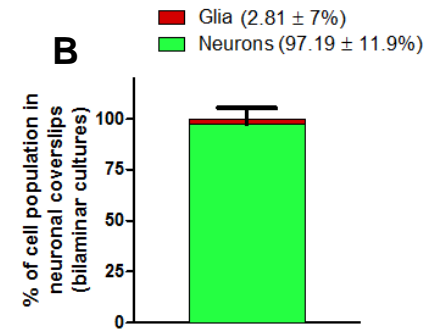
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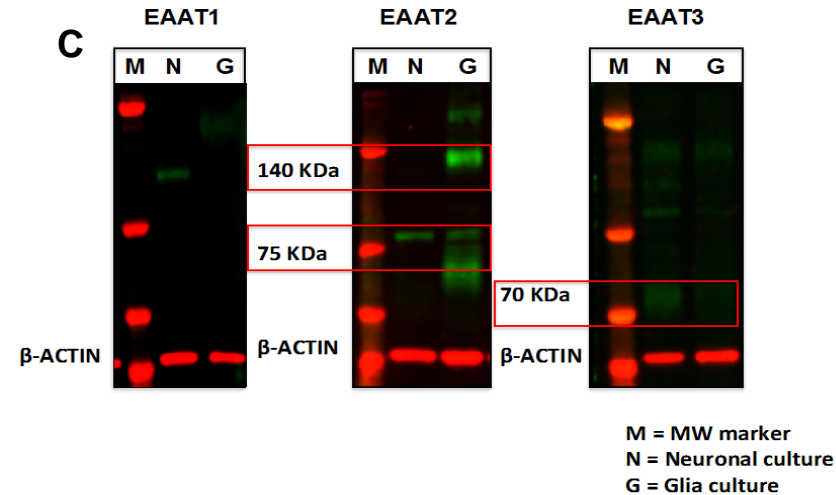
A



B



C

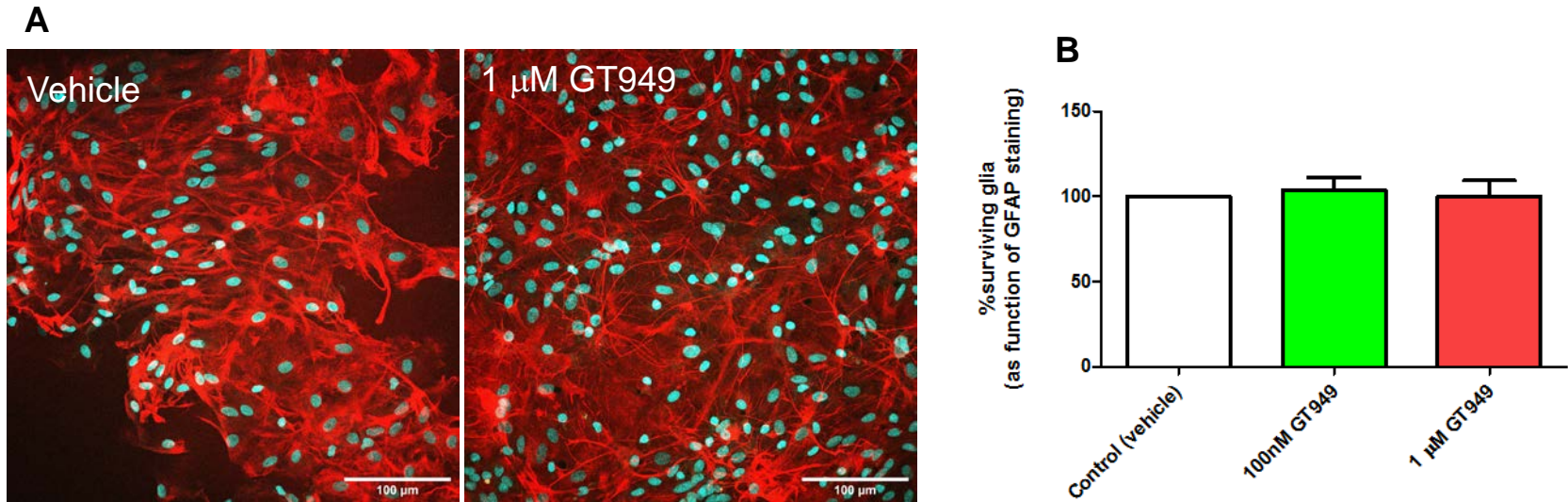


**Supplemental Figure 1. EAAT expression and purity of the neuronal layer in bilaminar cultures.** Immunocytochemical staining of MAP-2 and GFAP was performed as described to evaluate the purity of neuronal coverslips in bilaminar cultures.

A. Representative images show that the majority of cells are MAP-2 positive neurons (green), but some GFAP positive glia (red) is also present.

B. Quantification of cell types. Ten random fields from four different coverslips were examined to quantify the neuronal purity in the bilaminar culture. We found that ~97% of counted cells were MAP-2 positive, indicating that the vast majority of cells on neuronal coverslips are neurons. Additionally, ~2-3% of cells were GFAP positive, indicating minimal astroglia contamination (< 3%), in agreement with other studies using the same system.

C. Expression of glutamate transporters subtypes EAAT1, EAAT2 and EAAT3 in 14 DIV bilaminar co-culture system. Representative immunoblots are shown for all three EAAT subtypes in separate lysates from neuronal (N) and glial (G) layers. A qualitative assessment of the expression of the transporter subtypes suggest that EAAT1, as shown in the 140 Kda band, is present in the neuronal cultures band, but not in glia. For EAAT2, both monomer (75 Kda) and dimer (140 Kda) are highly expressed in glia, but not in neurons. EAAT3 is mostly expressed in neurons, as shown in the 70 Kda band. 40μg of protein per sample was loaded/lane. MW= Molecular weight. N= neuronal culture and G= glia cultures.

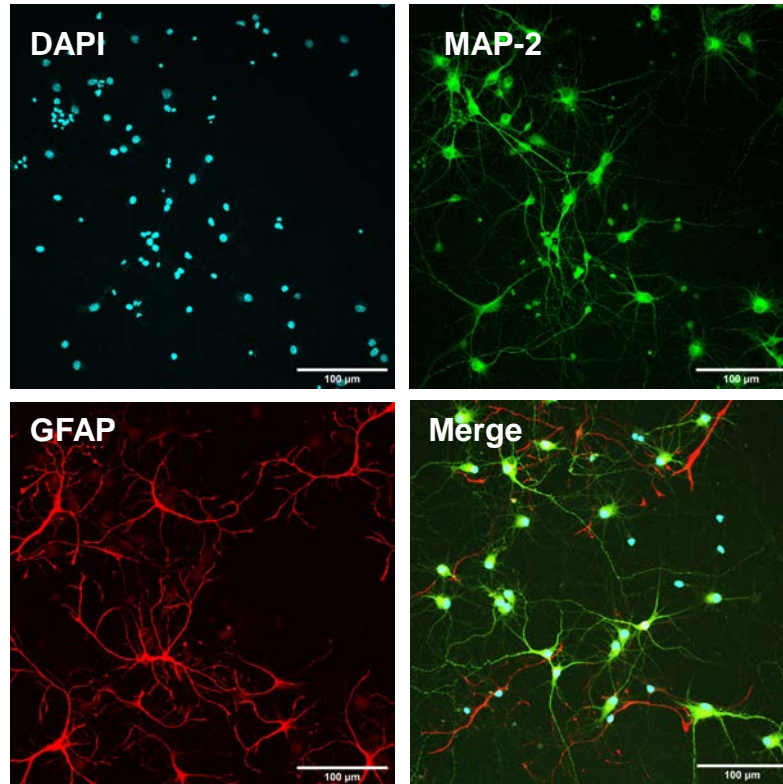


### Supplemental Figure 2. Lack of toxicity of GT949 in glia.

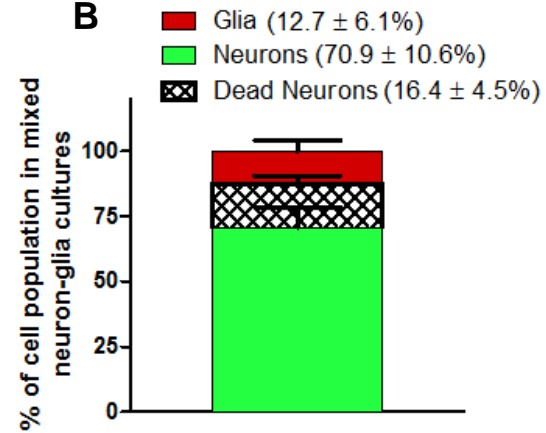
A. 14 DIV pure glia cultures were incubated with vehicle (control) and 100 nM (not shown) and 1  $\mu$ M GT949 for 24 hours, then stained for glial marker GFAP and nuclei marker DAPI. Images indicate preservation of glial morphology in both treatment conditions.

B. Quantification of GFAP+ cells in cultures incubated 24 hours with vehicle (control), and 100 nM and 1  $\mu$ M GT949 for 24 hours indicates no toxicity of the compound, as there is no significant changes between the number of GFAP+ cells among groups. Data is representative of 3 independent experiments.

A



B



**Supplemental Figure 3. Optimization of mixed neuron-glia cultures.**

A. Representative images of mixed neuron-glia cultures, grown to 14 DIV, fixed, and immunostained against MAP-2 (green) and GFAP (red), with counter-staining of DAPI (blue) to quantify the abundance of each cell type.

B. Quantification of neurons and glia in mixed cultures. Neurons and glia were quantified in 5 random fields from 3 separate coverslips from optimized mixed cultures from 3 individual experiments. Roughly, 70% of cells in these cultures were neurons, while roughly 15% were glia.