

Microglial CD300f immune receptor contributes to the maintenance of neuron viability *in vitro*
and after a penetrating brain injury

Daniela Alí-Ruiz^{1, 2}, Nathalia Vitureira³ and Hugo Peluffo^{*1, 2, 4, 5}

¹Neuroinflammation and Gene Therapy Lab., Institut Pasteur de Montevideo, Uruguay

²Dep. of Histology and Embryology, Faculty of Medicine, UdelaR, Montevideo, Uruguay

³Dep. of Physiology, Faculty of Medicine, UdelaR, Montevideo, Uruguay

⁴Unitat de Bioquímica i Biologia Molecular, Departamento de Biomedicina, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain

⁵Institut de Neurociències, Universitat de Barcelona (UB), Barcelona, Spain

* Corresponding author: Hugo Peluffo

email: hugo.peluffo@pasteur.edu.uy

Supplementary figure 1

% of different cell types in hippocampal neuron-mixed glia co-cultures

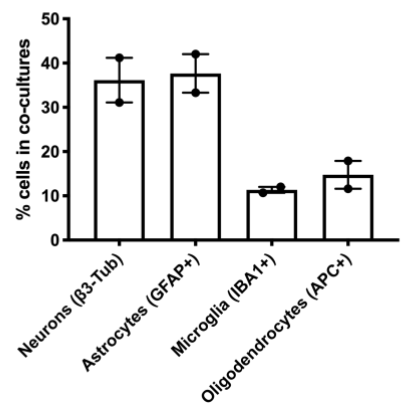


Figure 1S. The cellular composition of hippocampal neuron-glia cocultures. Immunolabeling against several cell-type specific markers. We detected 36% βIII-Tubulin positive neurons, 38% GFAP positive astrocytes, 11% IBA1 positive microglia and 15% APC positive oligodendrocytes. Total cells were stained with DAPI. Data show mean ± SEM.

Supplementary figure 2

Cell viability of cocultures

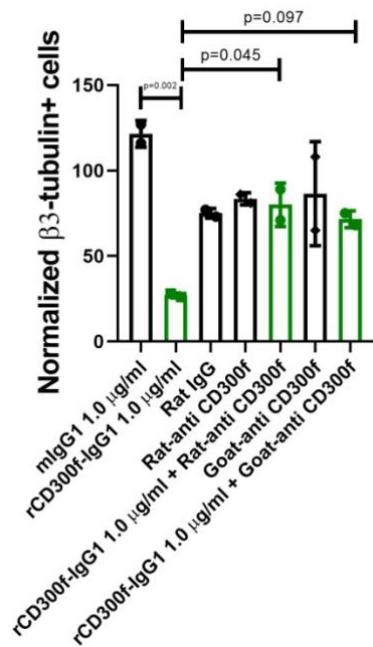


Figure 2S. The toxic effect for rCD300f-Fc was prevented by two different anti-CD300f antibodies. Cocultures were incubated with rCD300f-IgG1 (1μg/ml) or control IgG1 (1μg/ml) plus the following treatment: control IgGs or two polyclonal anti-CD300f antibodies (rat monoclonal IgG1 or goat polyclonal as indicated in the Methods section). Cultures were also incubated only with the anti-CD300f

antibodies alone. β 3-Tubulin positive cell numbers were counted 72 hours after and are represented as normalized to control β 3-Tubulin+cells. Data correspond to on experiment and show mean $\pm$ SEM; p corresponds to one way ANOVA followed by Tukey's test.

Supplementary figure 3

Cell viability of hippocampal neuron incubated with conditioned media from mixed-glia cultures

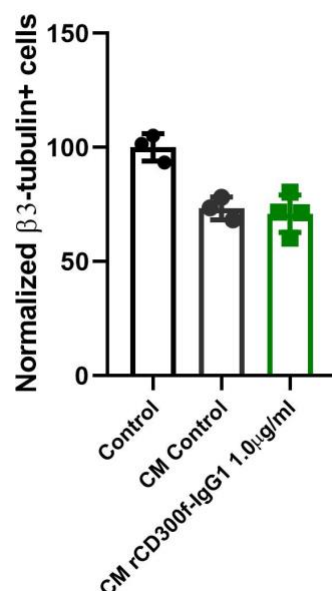


Figure 35. Conditioned media from CD300f-IgG1 treated mixed glial culture is not neurotoxic. In this experiment, mixed glial cultures were incubated with BME-based culture media (GibCo) containing 10% fetal calf serum (GibCo), Glutamax (GibCo), 20 mM D-glucose (GibCo), HEPES (1%), pyruvate (1%) and penicillin/streptomycin (GibCo) and rCD300f-IgG1 (1.0 μ g/ml) or control IgG1 (1.0 μ g/ml). Then, hippocampal neuron enriched cultures were incubated with the different mixed-glia conditioned media and β 3-Tubulin positive cell numbers were counted 72 hours after. Data are represented as normalized to control β 3-Tubulin+cells, correspond to on experiment and show mean $\pm$ SEM; p corresponds to one way ANOVA followed by Tukey's test.