

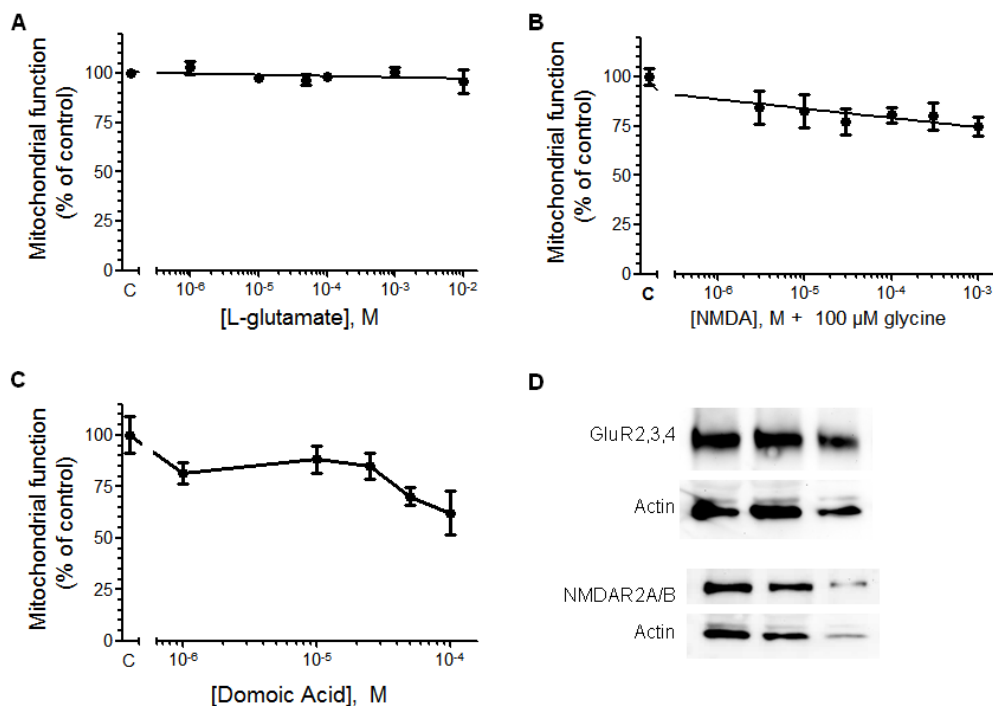
# In Vitro Effects of Chronic Spirolide Treatment on Human Neuronal Stem Cell Differentiation and Cholinergic System Development

Andrea Boente-Juncal<sup>†</sup>, Aida G. Méndez<sup>†</sup>, Carmen Vale<sup>†\*</sup>, Mercedes R. Vieytes<sup>‡</sup> and Luis M. Botana<sup>†\*</sup>

<sup>†</sup>Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain

<sup>‡</sup>Departamento de Fisiología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain

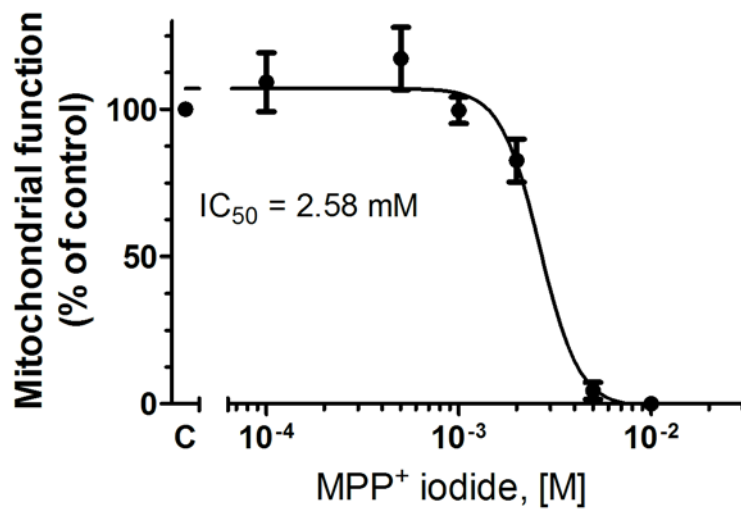
\* Email: Luis.botana@usc.es; mdelcarmen.vale@usc.es



Supplementary Figure 1

**Supplementary Figure 1. Ionotropic glutamate receptor agonists had little effect on CTX0E16 cell viability.** Effect of five days treatment of CTX0E16 cells with different glutamate **(A)**, NMDA **(B)** or domoic acid **(C)** concentrations on CTX0E16 cell viability. Cellular viability was determined by the MTT method in three different

experiments in the case of glutamate, five independent experiments in the case of NMDA and four experiments in the case of domoic acid. Data are mean  $\pm$  sem. **D.** Western blot band showing expression for the AMPAR subunits GluR2,3,4 and for the NMDA receptor subunits NMDA2A/B in differentiated CTX0E16 cells of 30 days of differentiation.



**Supplementary Figure 2.** Treatment of CTX0E16 cells during 24 hours with the neurotoxin MPP<sup>+</sup> at concentrations ranging from 0.1 to 10 mM caused a concentration dependent decrease in cellular viability. Cellular viability was determined by the MTT method. Data are mean  $\pm$  sem from three independent experiments.