SUPPLEMENTARY MATERIAL
Figure S1 and Figure S2

Cannabinoid regulation of angiotensin II-induced calcium signaling in striatal neurons

Rafael Rivas-Santisteban[1,2*], Ana Muñoz[2,3], Jaume Lillo[2,4], Iu Raïch[2,4], Ana I. Rodríguez-Pérez[2,3], Gemma Navarro[2,4,5#], José L. Labandeira-García[2,3#], Rafael Franco[2,6,7#].

[1] Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Autonomous University of Barcelona. Campus Bellaterra. 08193 Barcelona. Spain

[2] Network Center for Biomedical Research in Neurodegenerative Diseases. CiberNed., Spanish National Health Institute Carlos iii. Av. Monforte de Lemos, 3-5. 28029 Madrid. Spain.

[3] Cellular and Molecular Neurobiology of Parkinson's Disease, Research Center for Molecular Medicine and Chronic Diseases (CIMUS), IDIS, University of Santiago de Compostela, Santiago de Compostela; Spain.

[4] Department of Biochemistry and Physiology. School of Pharmacy and Food Sciences. Universitat de Barcelona. 08028 Barcelona. Spain.

[5] Institute of Neuroscience of the University of Barcelona. Universitat de Barcelona. 08028 Barcelona. Spain.

[6] Molecular Neurobiology laboratory. Dept. Biochemistry and Molecular Biomedicine. Facultat de Biologia. Universitat de Barcelona. 08028 Barcelona. Spain.

[7] School of Chemistry. Universitat de Barcelona. Barcelona. Spain.

# equal contribution

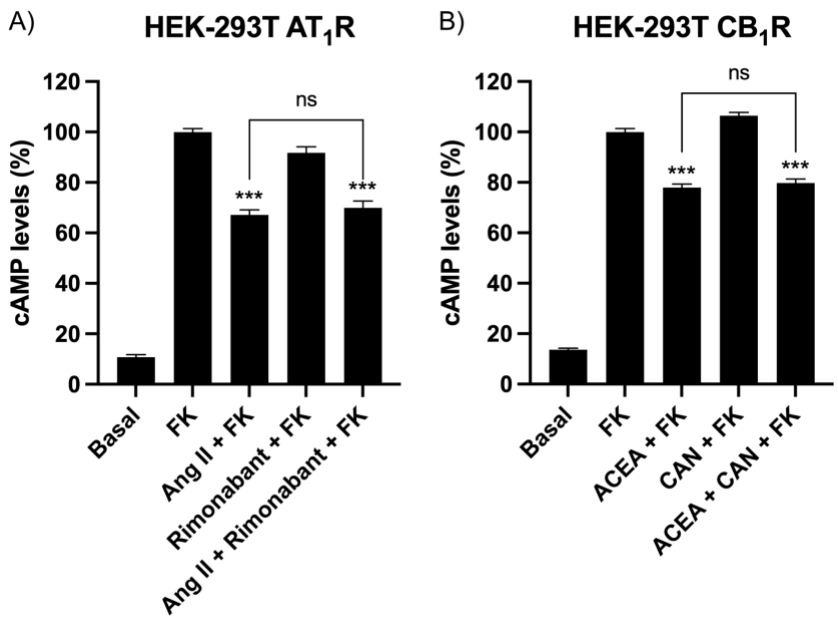
*** Corresponding authors**

Rafael Rivas-Santisteban
rrivasbioq@gmail.com

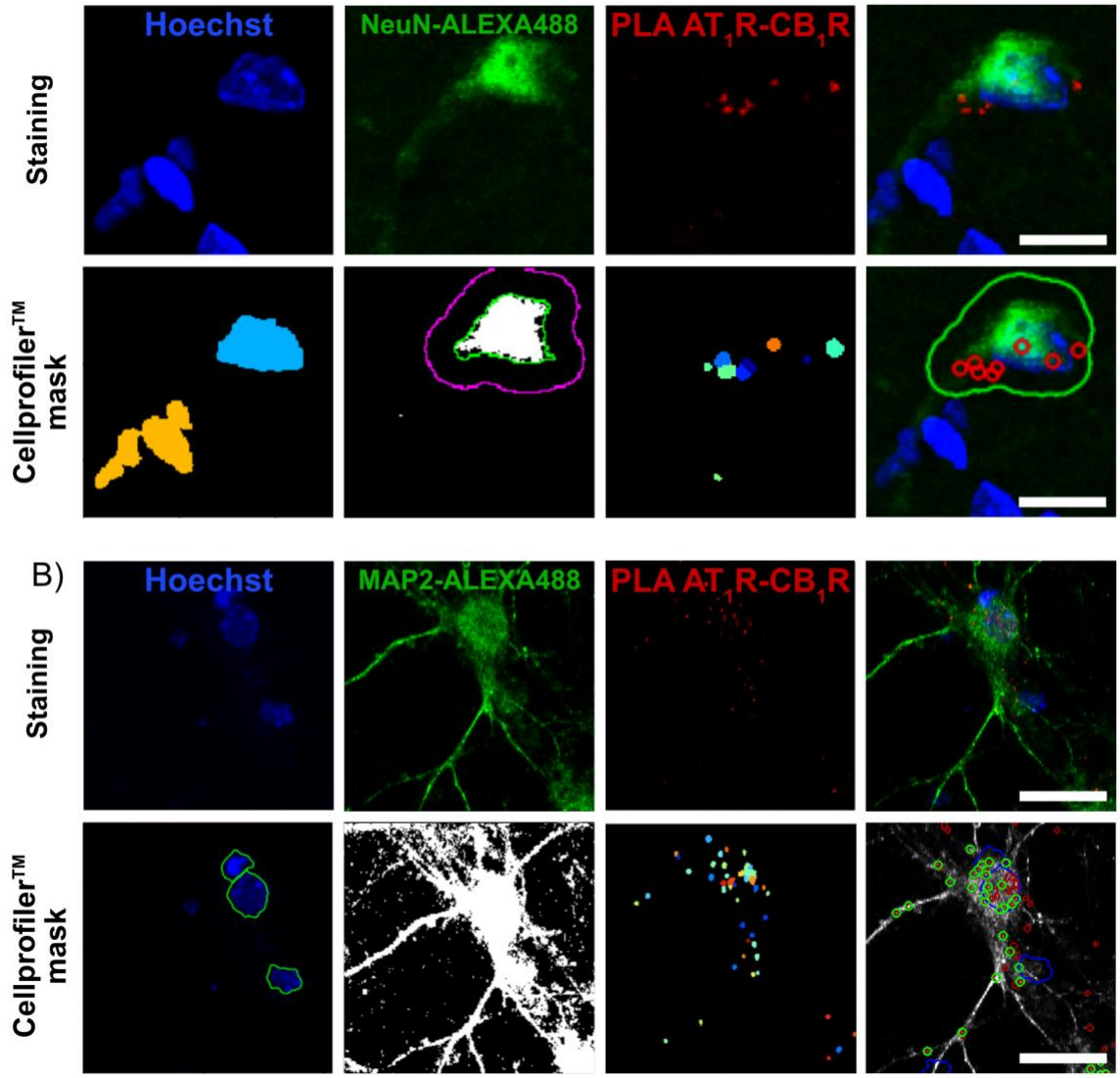
Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Autonomous University of Barcelona, Campus Bellaterra, 08193 Barcelona. Spain

Rafael Franco
rfranco@ub.edu; rfranco123@gmail.com

Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, Universitat de Barcelona, 08028 Barcelona, Spain.



Supplementary Figure S1. cAMP control assays testing the antagonists of the two receptors: candesartan and rimonabant. HEK-293T cells expressing AT_1 (A) or CB_1 (B) receptors were pretreated with the solvent of ligands (vehicle) or receptor antagonists (1 µM candesartan for CB_1R or 1 µM rimonabant for AT_1R) and subsequently treated with selective agonists (100 nM Ang II for AT_1R and/or 100 nM ACEA for CB_1R). Panels A-B. G_i protein-coupling was assessed by measuring the decreases in forskolin (FK)-induced cAMP levels; 0.5 µM FK was used (added 15 min after the treatment with agonists). Values are the mean ± S.E.M. of 3 independent experiments performed in triplicates. One-way ANOVA followed by Bonferroni's *post-hoc* test was used to compare cAMP levels (***p< 0.001, versus FK condition) and (ns. versus Ang II (A) or ACEA (B) condition).



Supplementary Figure S2. Cellprofiler™ segmentation of nuclei and red dots for PLA quantification in neurons. A) Neurons labelled for NeuN were were detected by a CellProfiler™ pipeline (blue shadow mask) and distinguished from those without the neuronal marker (orange shadow mask). Only red dots surrounding cells presenting NeuN are quantified (red circles). Scale bar: 10 µm. B) Cells labeled with MAP2 were detected by a CellProfiler™ pipeline. Only red dots in $MAP2^{+}$ cells were considered (green circles), excluding the other red dots (red circles). Scale bar: 20 µm.