

## SUPPLEMENTARY MATERIAL

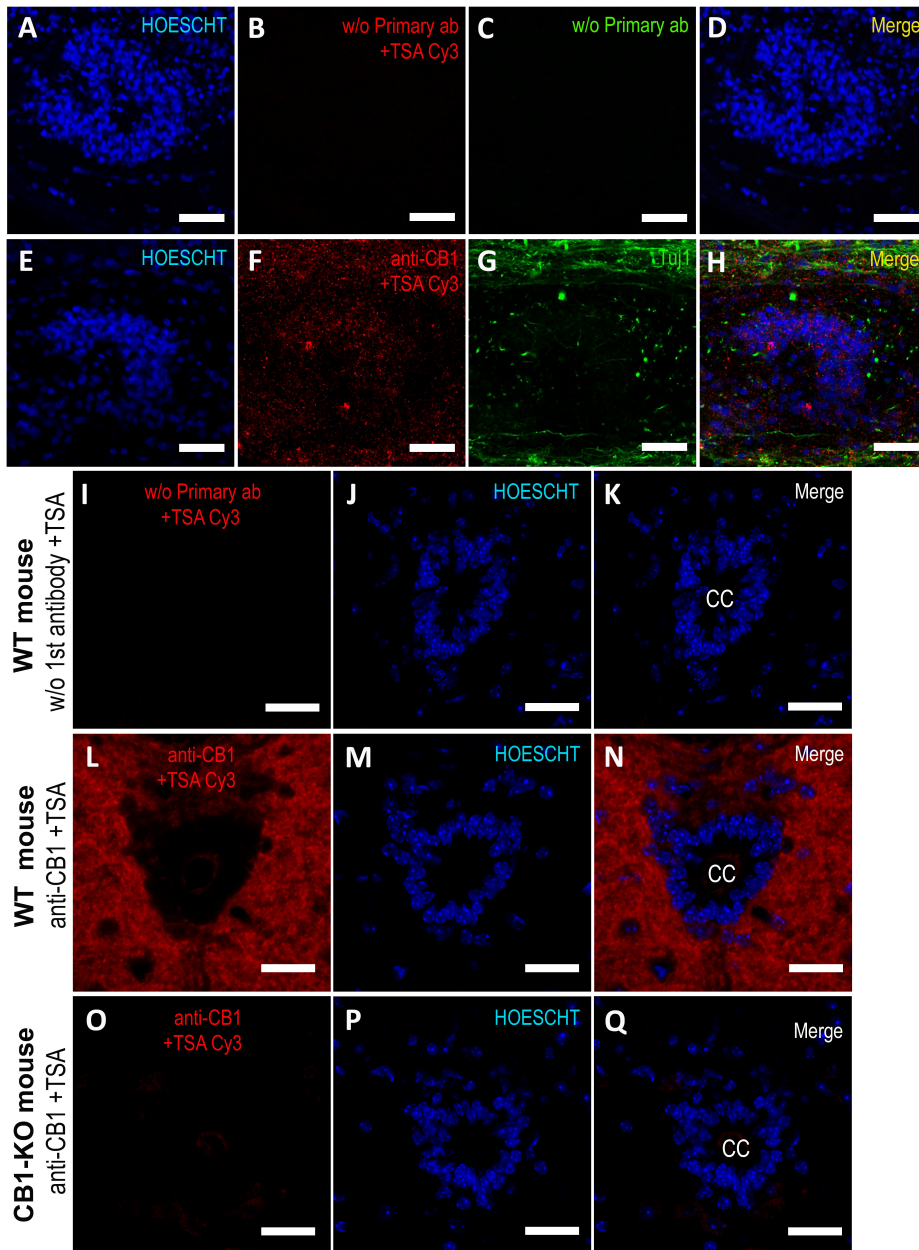
### **CB<sub>1</sub> cannabinoid receptor enrichment in the ependymal region of the adult human spinal cord**

Beatriz Paniagua-Torija<sup>1</sup>, Angel Arevalo-Martin<sup>1</sup>, Isidro Ferrer<sup>3</sup>, Eduardo Molina-Holgado<sup>1\*</sup>,  
Daniel Garcia-Ovejero<sup>1\*</sup>,

<sup>1</sup>Laboratory of Neuroinflammation, Hospital Nacional de Paraplejicos (SESCAM), Toledo, Spain

<sup>3</sup>Institut de Neuropatologia, Servei d'Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona, L'Hospitalet de Llobregat, Spain

**\*Corresponding authors:** Eduardo Molina-Holgado, Ph.D., Daniel Garcia-Ovejero, Ph.D., Laboratory of Neuroinflammation, Hospital Nacional de Paraplejicos, Finca La Peraleda s/n, 45071-Toledo, Spain. Ph: +34925247754, Fax: +34925247745; [eduardom@sescam.jccm.es](mailto:eduardom@sescam.jccm.es), [dgarciao@sescam.jccm.es](mailto:dgarciao@sescam.jccm.es);



**Supplementary Figure 1.** Representative images of controls for background signal and CB1 antibody specificity. **(A-H)** Absence of primary antibodies completely abolishes CB1 immunoreactivity in human tissue after amplification with Cy3-Tyramide amplification System (TSA-Cy3). BetaIII tubulin + axons are shown in green. **(I-K)** Background staining is also absent in C57/BL6 mice after omitting CB1 antibody using the same staining protocol and restrictive microscope settings. **(L-N)** When using TSA, a very intense CB1 immunoreactivity is observed in C57/BL6 mice that is in accordance to the pattern previously described (Garcia-Ovejero et al., 2013). Images show CB1 staining in ependymal region. **(O-Q)** When using the same microscope settings, only a dim staining can be found in CB1 knockout mice, supporting the specificity of this CB1 antibody under our conditions used here. CC, central canal. Magnification bars: A-H = 50  $\mu$ m; I-Q = 25  $\mu$ m.