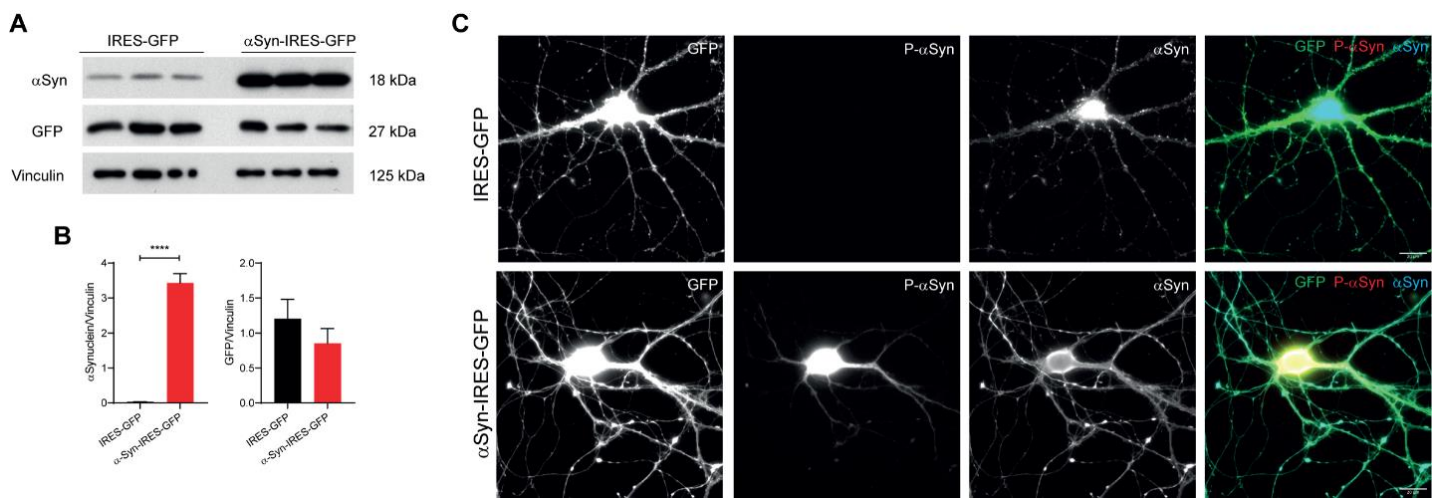


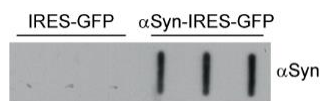
# Cofilin pathology is a new player on $\alpha$ -synuclein-induced spine impairment in models of hippocampal synucleinopathy

Oliveira da Silva MI<sup>1,2</sup>, Santejo M<sup>1</sup>, Babcock IW<sup>3</sup>, Magalhães A<sup>4</sup>, Minamide LS<sup>3</sup>, Castillo E<sup>5</sup> Gerhardt E<sup>6</sup>, Fahlbusch C<sup>6</sup>, Swanson RA<sup>5</sup>, Outeiro TF<sup>6,7,8,9,10</sup>, Bamberg JR<sup>3</sup>, Liz MA<sup>1</sup>

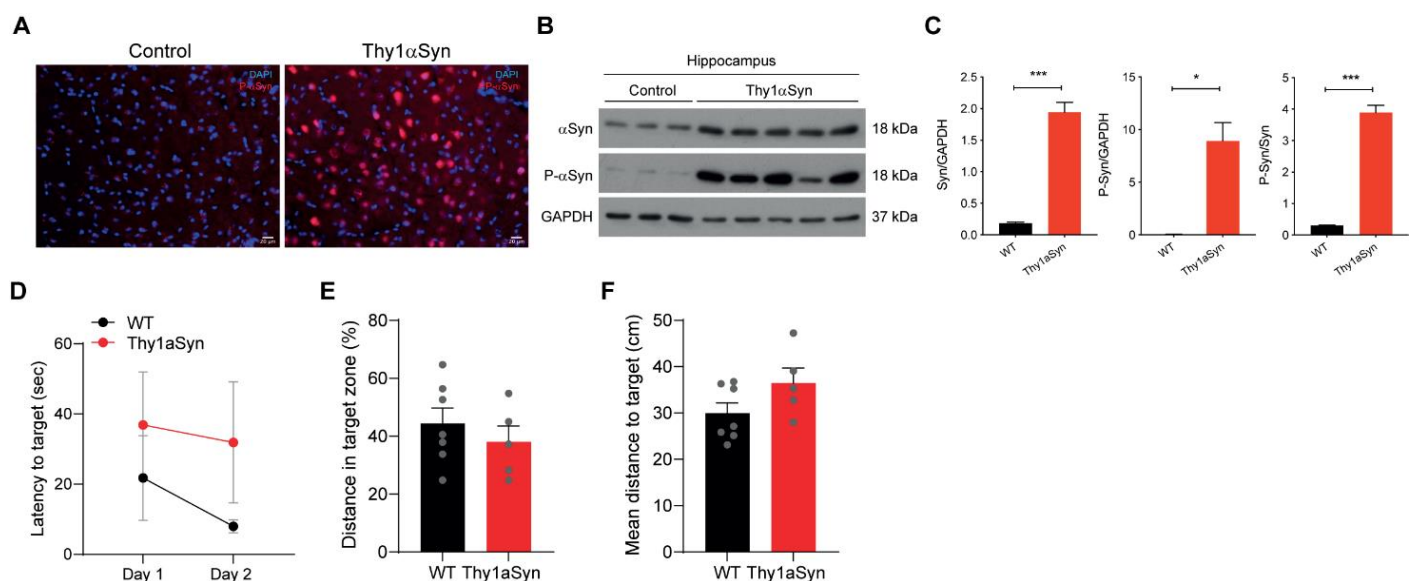
## Supplementary Material



**Supplementary Figure S1 - Overexpression of  $\alpha$ Syn in mature hippocampal neurons.** (A, B) Western blot analysis (A) and respective quantification by densitometry (B) of  $\alpha$ -Syn and GFP levels in DIV14 transduced hippocampal neurons. Vinculin was used as loading control. Data represent mean  $\pm$  SEM (n=3 replicates/treatment). \*\*\*\*p<0.0001 by Student's t test. (C) Representative images of primary hippocampal neurons infected at DIV4 with IRES-GFP or WT- $\alpha$ Syn-IRES-GFP lentivirus and immunostained at DIV14 with  $\alpha$ -Syn pS129 (red) and  $\alpha$ -Syn (blue). Scale bar: 20  $\mu$ m.



**Supplementary Figure S2 -  $\alpha$ Syn is released from hippocampal neurons in culture.** Dot blot analysis of  $\alpha$ Syn levels in the supernatants from IRES-GFP or WT- $\alpha$ Syn-IRES-GFP transduced neurons at DIV7.



**Supplementary Figure S3 - Thy1-aSyn mice show increased  $\alpha$ Synuclein phosphoS129 in hippocampus and cognitive defects.** (A) Representative images of brain sections from 6-month Control and Thy1-aSyn mice stained for DAPI (blue) and  $\alpha$ -Syn pS129 (red). Scale bar: 20 $\mu$ m. (B, C) Western blot analysis (B) and respective quantification by densitometry (C) of  $\alpha$ -Syn and  $\alpha$ -Syn pS129 levels in hippocampus from Control and Thy1-aSyn mice. GAPDH was used as loading control. Data represent mean $\pm$ SEM (n=3-5 animals/condition). \*p<0.05, \*\*\*p<0.001 by Student's *t* test. (D) Latency to target during cued learning in MWM. (E) Distance in target zone in MWM. (F) Mean distance to target in MWM. Data represent mean $\pm$ SEM (n=5-7 animals/condition).