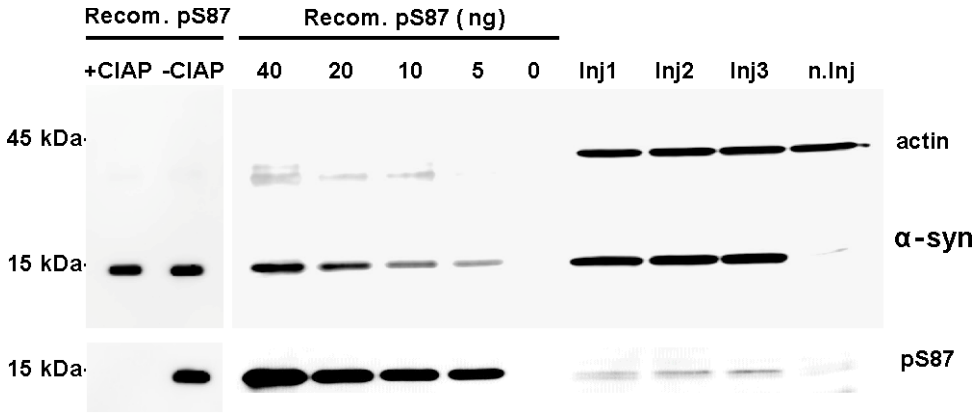
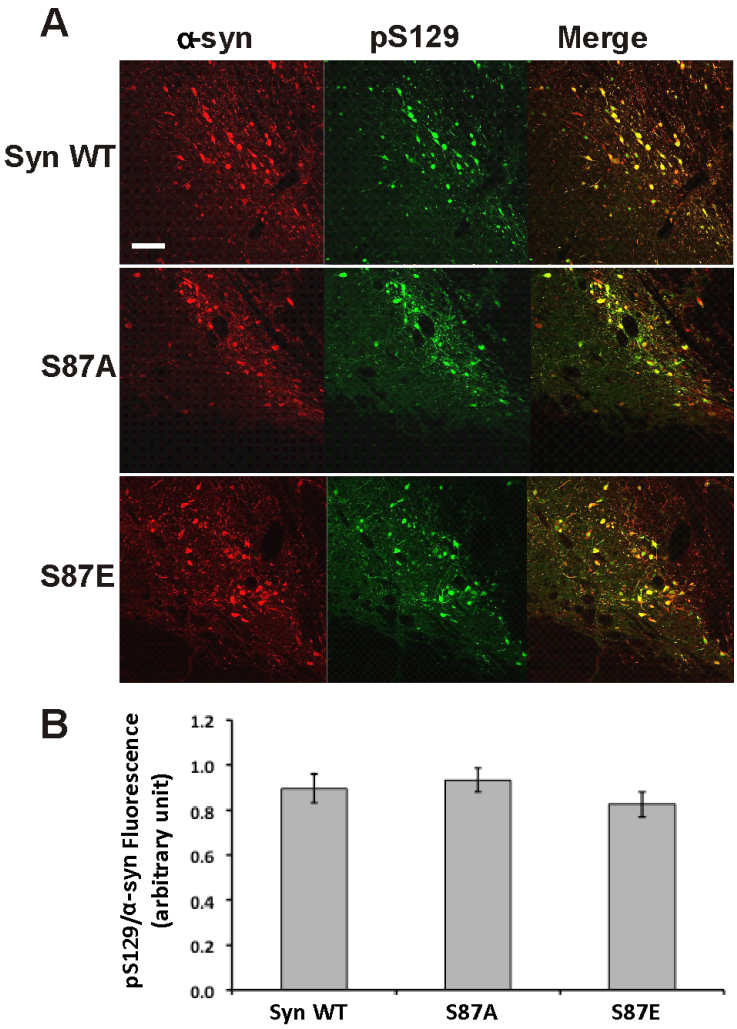
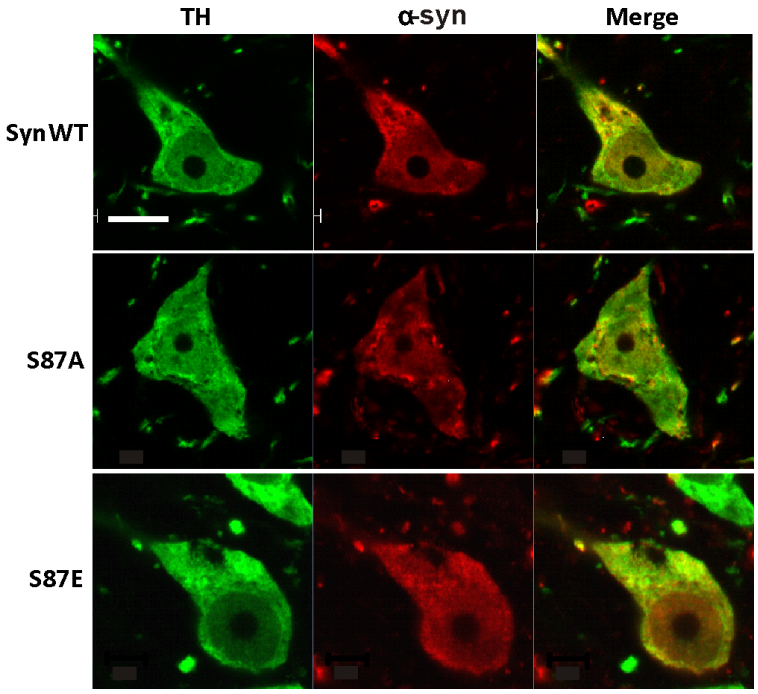
Supplemental materials



Supl. Figure 1: *Evaluation of pS87 levels in the injected ventral midbrains*. Western blot analysis shows the expression levels of total human α-syn (Sc-211) and pS87 in rat midbrains overexpressing human α-syn (Inj1, Inj2 and Inj3). No signal was observed in the non-injected hemisphere (n.inj). The evaluation of total α-syn and pS87 levels was performed using a standard curve based on the detection of known concentrations of recombinant human α-syn and pS87 α-syn (α-syn-S129A phosphorylated by CK1), repectively. The sepecificity of the pS87 antibody was demonstrated by the loss of signal when phosphorylated recombinant α-syn-S129A was incubated with Calf Intestinal Alkaline Phosphatse (CIAP) (Paleologou et al., 2010).



Supl. Fig 2: *S87 mutations do not affect α-syn phosphorylation at S129.* To investigate if S87 mutations affect α-syn phosphorylation at S129, we evaluated the level of pS129 in the dopaminergic neurons of animals injected with WT, S87A and S87E constructs. (A) Confocal imaging illustrates the expression levels of human α-syn (red) and pS129 (green) in the injected SN. Scale bar=100 µm (B) Histogram illustrating the relative levels of pS129/total α-syn ratio. pS129 and total α-syn levels were quantified in individual neurons using ImageJ software as previously described by Ramonet and collaborators (Ramonet et al., 2011). The quantification of the ratio of fluorescence intensity pS129/total human α-syn did not reveal any significant difference between cells overexpressed each of the S87-mutated α-syn constructs.



Supl. Fig 3: *S87 mutations do not affect human α-syn sub-cellular localization.* Confocal imaging of Tyrosine Hydroxylase (TH) (green) and human α-syn (red) immunofluorescence in the injected SN, revealing a similar sub-cellular localization of Syn WT and S87 mutants in the cytosolic and nuclear compartments of infected neurons. Scale bar=10µm

References:

Paleologou KE, Oueslati A, Shakked G, Rospigliosi CC, Kim HY, Lamberto GR, Fernandez CO, Schmid A, Chegini F, Gai WP, Chiappe D, Moniatte M, Schneider BL, Aebischer P, Eliezer D, Zweckstetter M, Masliah E, Lashuel HA (Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. J Neurosci 30:3184-3198.2010).

Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, Dawson TM, Dawson VL, Moore DJ (Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. PLoS One 6:e18568.2011).