



Characterization of a Second-Hit Parkinson's Disease Mouse Model Utilizing α -synuclein Pre-Formed Fibrils in M83 Transgenic Mice

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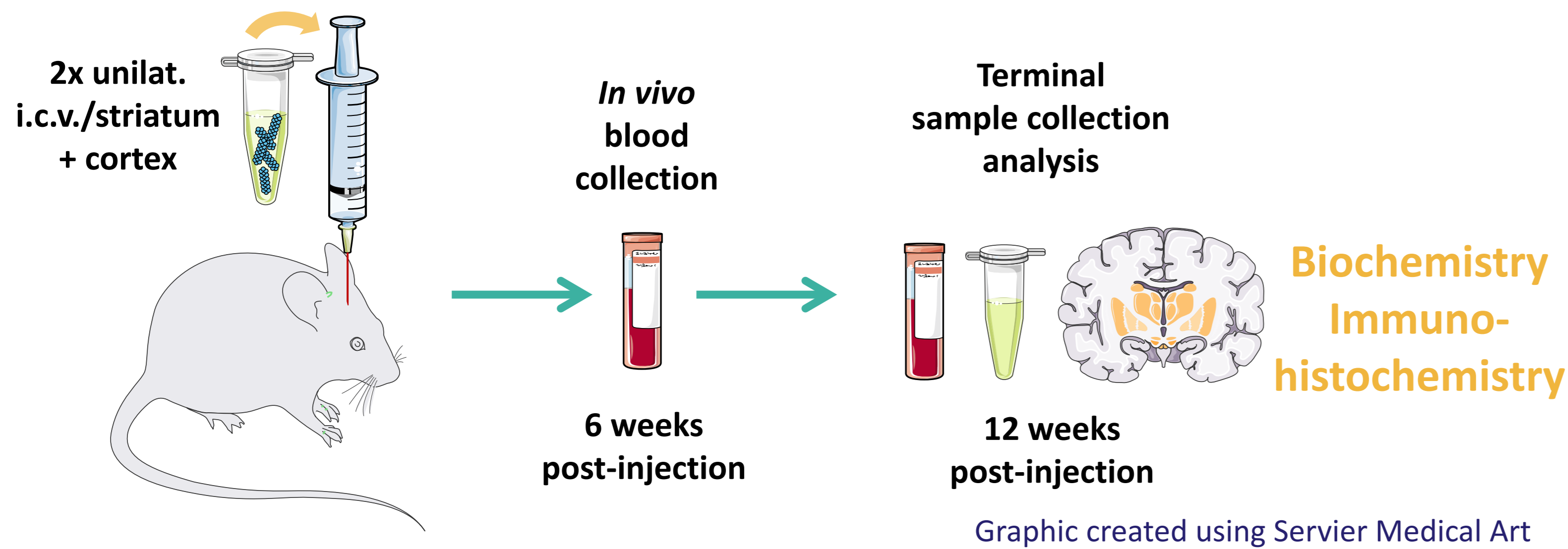
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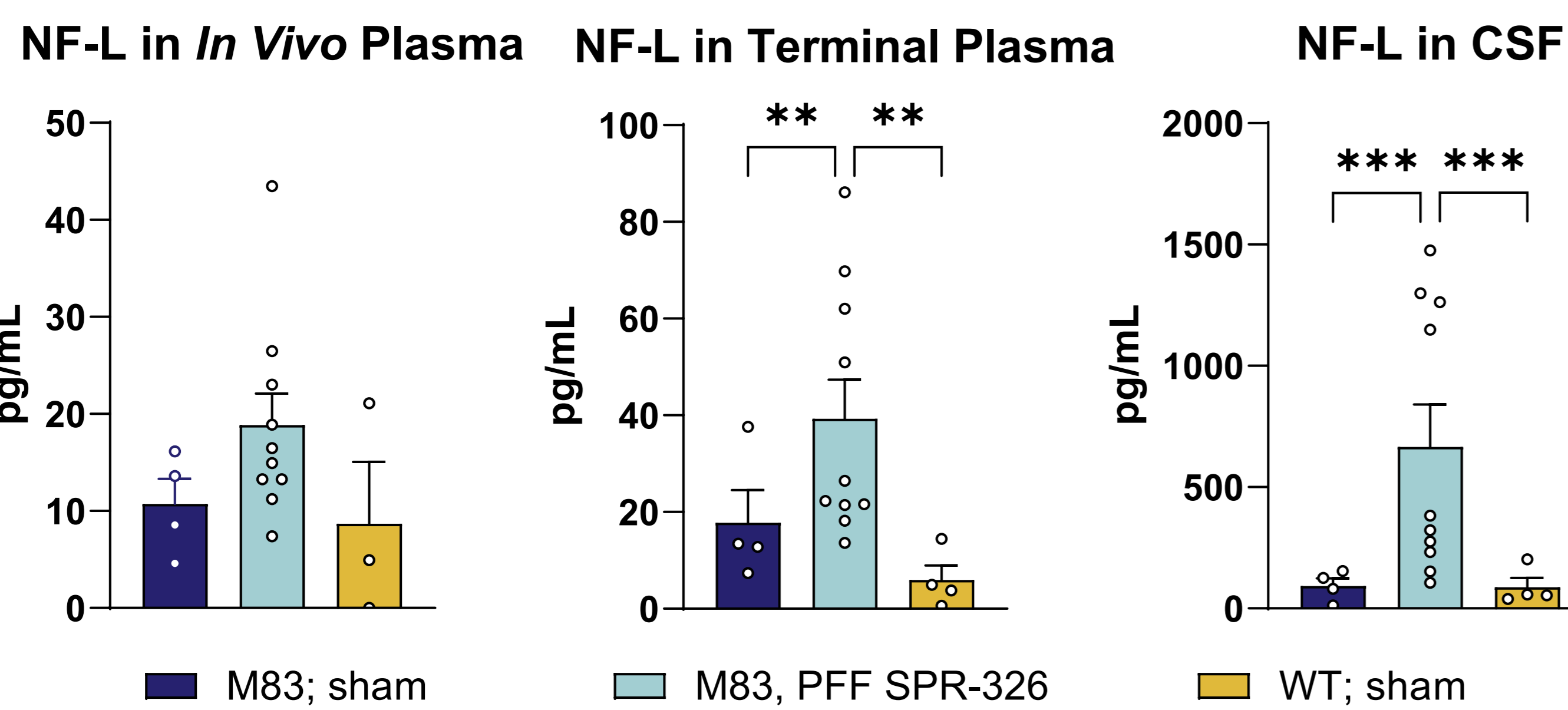
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Objectives

Transgenic mice are invaluable in studying the disease mechanisms of Parkinson's disease (PD) and countless human diseases. However, they often do not constitute the full disease spectrum, involve all relevant molecular players, and are limited in disease onset and progression. By challenging transgenic PD mice with induced lesions, these caveats can be alleviated. Here, we thus injected α -synuclein (α -syn) pre-formed fibrils (PFFs) into SNCA-A53T transgenic mice to develop such a "second hit" model.

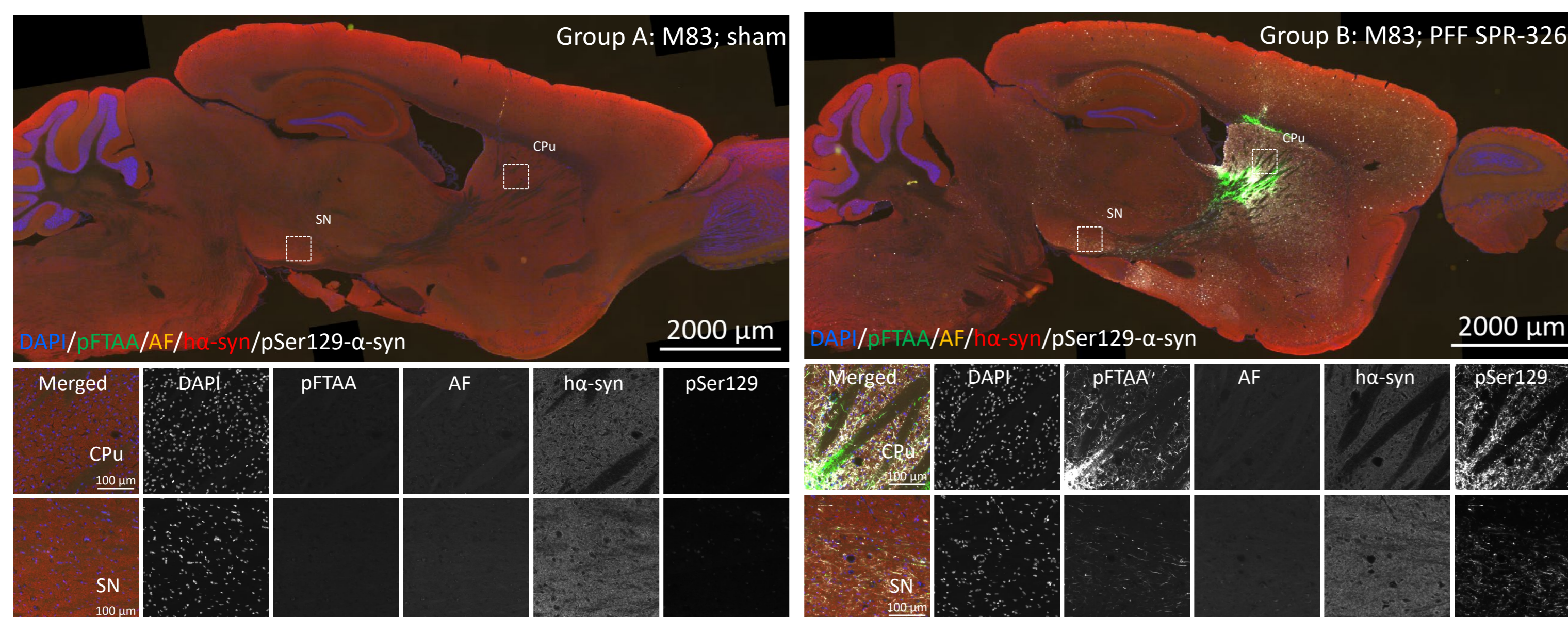


SNCA-A53T-PFF Injection Exacerbates Neuronal Damage in M83 PD Mice



▲ Figure 1: Neurofilament light chain (NF-L) levels in plasma and cerebrospinal fluid (CSF). NF-L baseline levels were measured from *in vivo* plasma collected 6 weeks post-PFF injection and terminal plasma as well as CSF collected 12 weeks post-PFF injection. NF-light® ELISA 10-7001 CE from UmanDiagnostics was used. M83, sham: n = 4; M83, PFF SPR-326: n = 10; WT: n = 3-4. Two-way ANOVA followed by Bonferroni's multiple comparison test. Mean + SEM. **p < 0.01; ***p < 0.001.

Immunohistochemistry Reveals Parenchymal Effects of SNCA-A53-PFF Injection



▲ Figure 2: Immunohistochemical analysis of terminal brain tissue. Examples of immunofluorescent labeling of α -syn, pSer129- α -syn and pFTAA on one animal of group A (M83; sham) and group B (M83; PFF SPR-326). Images show representative labeling on sagittal sections; nuclei are labeled with DAPI. Single channel magnifications show labeling in the caudate putamen (CPU) and substantia nigra (SN); images were taken at the position indicated by the rectangle.

Conclusions

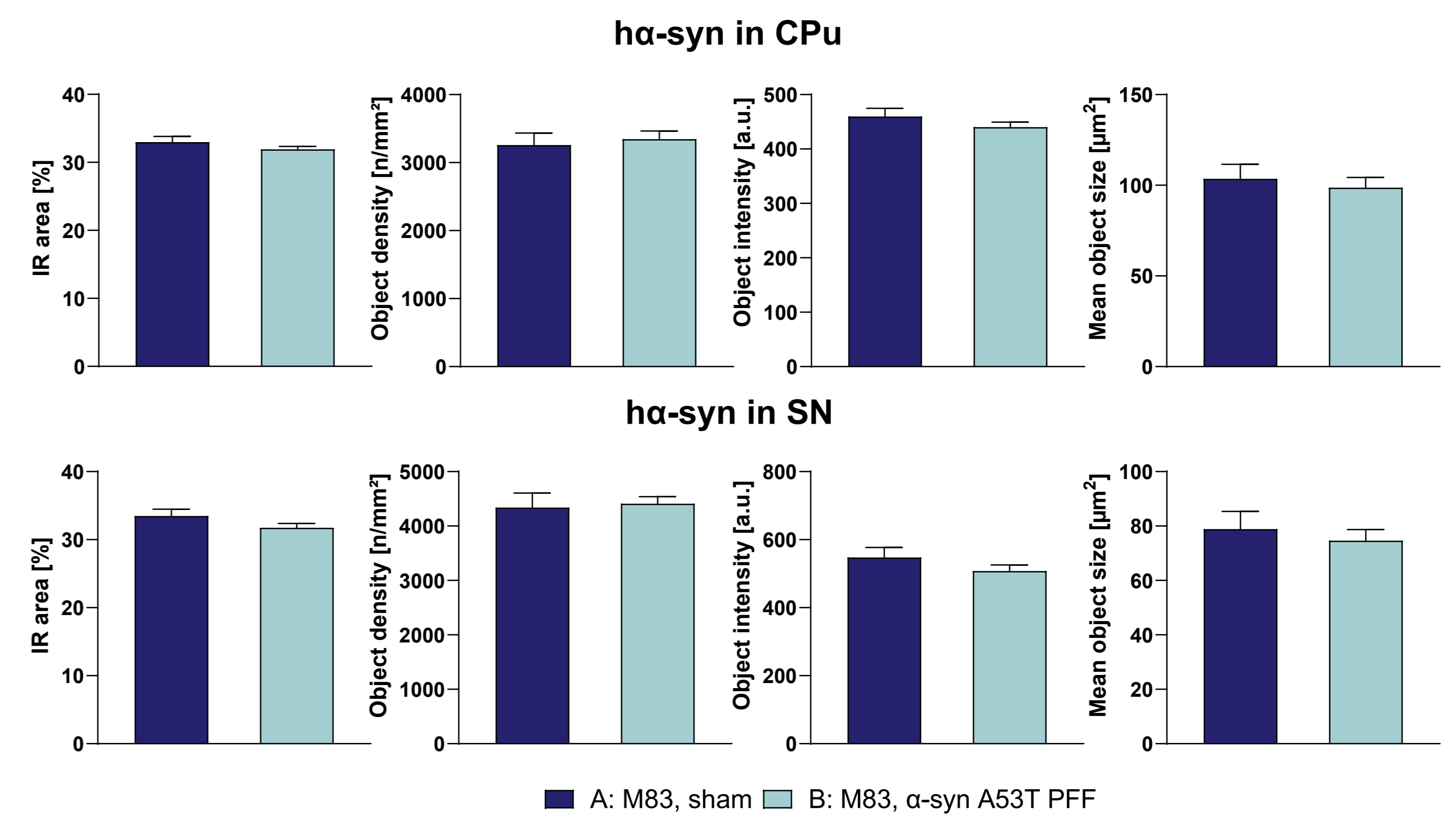
Here we demonstrate that challenging M83 transgenic mice with α -syn A53T-PFFs exacerbates PD pathology in a synergistic manner. In particular, PFF injection increased neuronal damage (indicated by NF-L levels in CSF and plasma), increased α -syn phosphorylation on disease relevant Ser129, and increased α -syn aggregation in CPU and SN. Overall α -syn levels were not affected. This suggests that this is a viable model to study additional disease aspects and potential drug candidates and thus extending the usability of M83 mice for drug discovery.

Parts of this project were performed in collaboration with Amyl Therapeutics www.amyltx.com

Methods

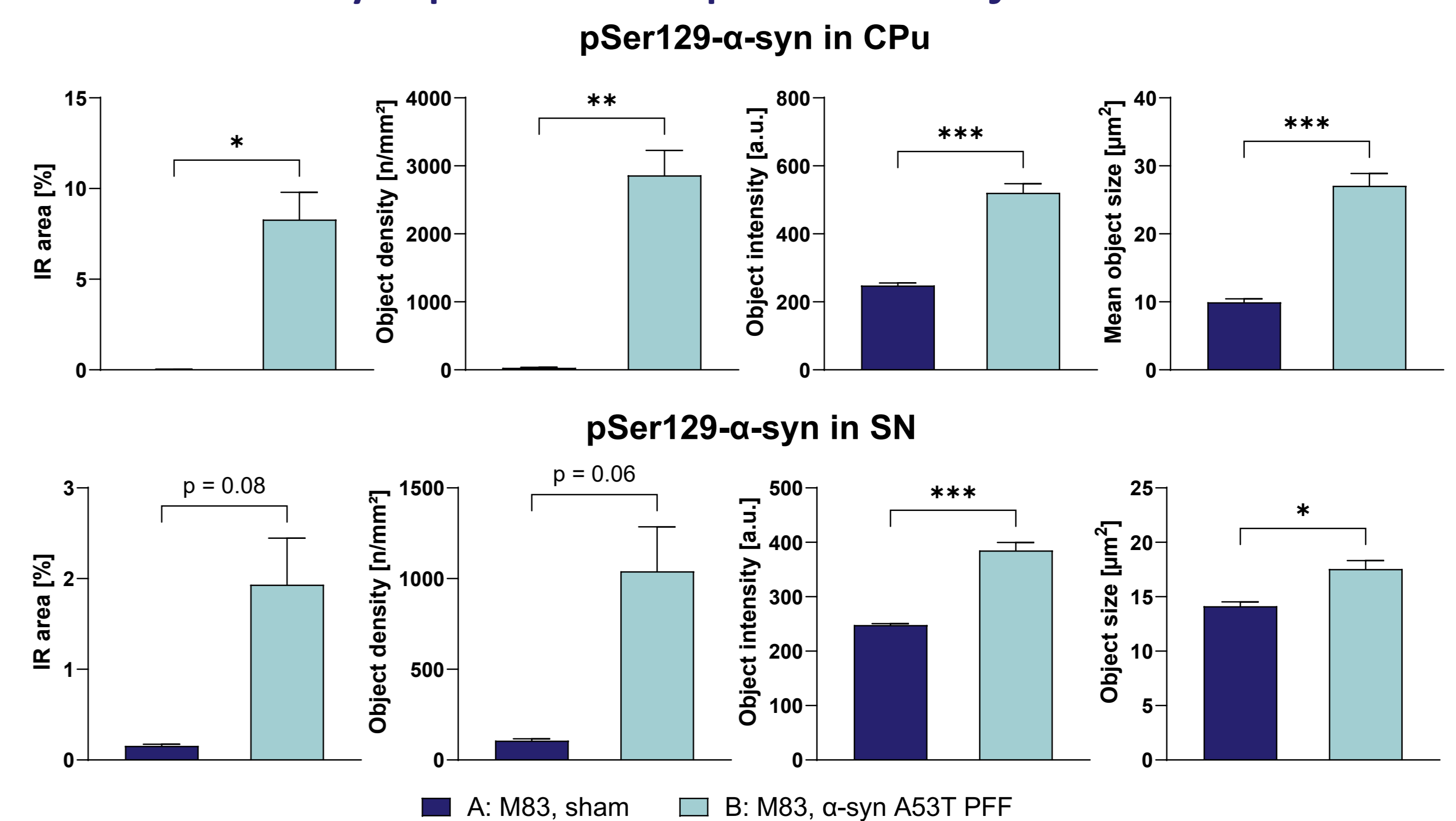
The A53T α -syn transgenic mouse model M83, expressing mutant human A53T α -syn from a murine prion promoter, was used. M83 mice and wild type (WT) littermates were unilaterally injected with recombinant α -syn A53T mutant PFFs in two injection sites of the somatosensory cortex and the dorsal striatum. Animals' motor performance as well as clinical presentations were assessed longitudinally for up to 13 weeks. Furthermore, a wide array of biochemical and histological readouts was measured to assess PD pathology.

Overall α -syn Levels are Unaffected by PFF Injection



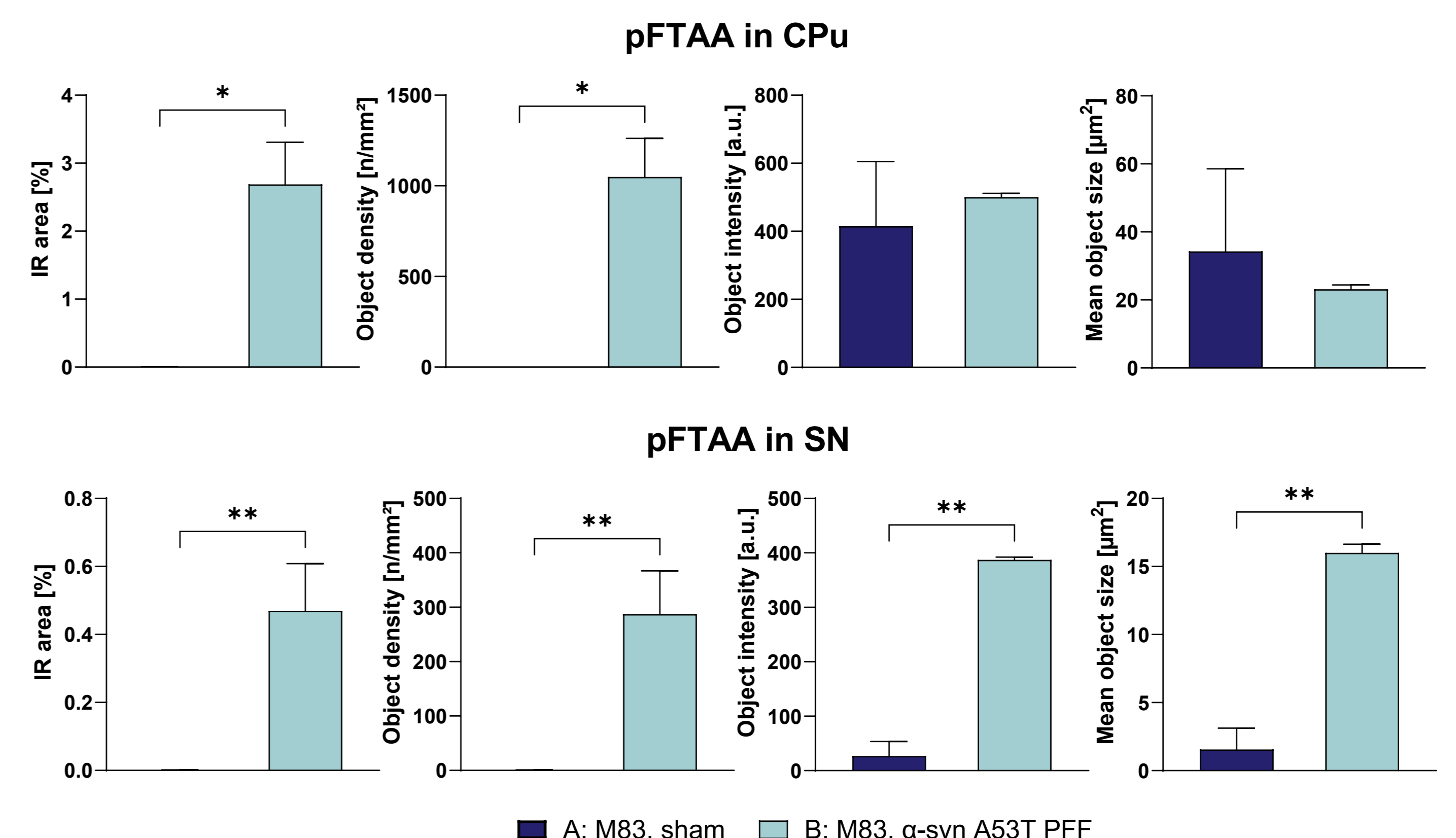
▲ Figure 3: Quantification of human α -syn in the ipsilateral caudate putamen (CPU) and substantia nigra (SN). Graphs present the means of immunofluorescent signal on five brain sections per mouse (A: n = 3; B: n = 9). Unpaired two-tailed T-test [n.s.]. Mean + SEM.

Increased α -syn pSer129 upon PFF Injection



▲ Figure 4: Quantification of human α -syn (pSer129) in the ipsilateral caudate putamen (CPU) and substantia nigra (SN). Graphs present the means of immunofluorescent signal on five brain sections per mouse (A: n = 3; B: n = 9). Unpaired two-tailed T-test. Mean + SEM. *p < 0.05; **p < 0.01; ***p < 0.001.

Increased α -syn Aggregation upon PFF Injection



▲ Figure 5: Quantification of pFTAA immunofluorescence in the ipsilateral caudate putamen (CPU) and substantia nigra (SN). Graphs present the means of immunofluorescent signal on five brain sections per mouse (A: n = 3; B: n = 9). Unpaired two-tailed T-test. Mean + SEM. *p < 0.05; **p < 0.01.

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