



# Characterizing $\alpha$ -synuclein Pre-Formed Fibrils-Induced Parkinson's Disease Pathology in M83 Transgenic Mice

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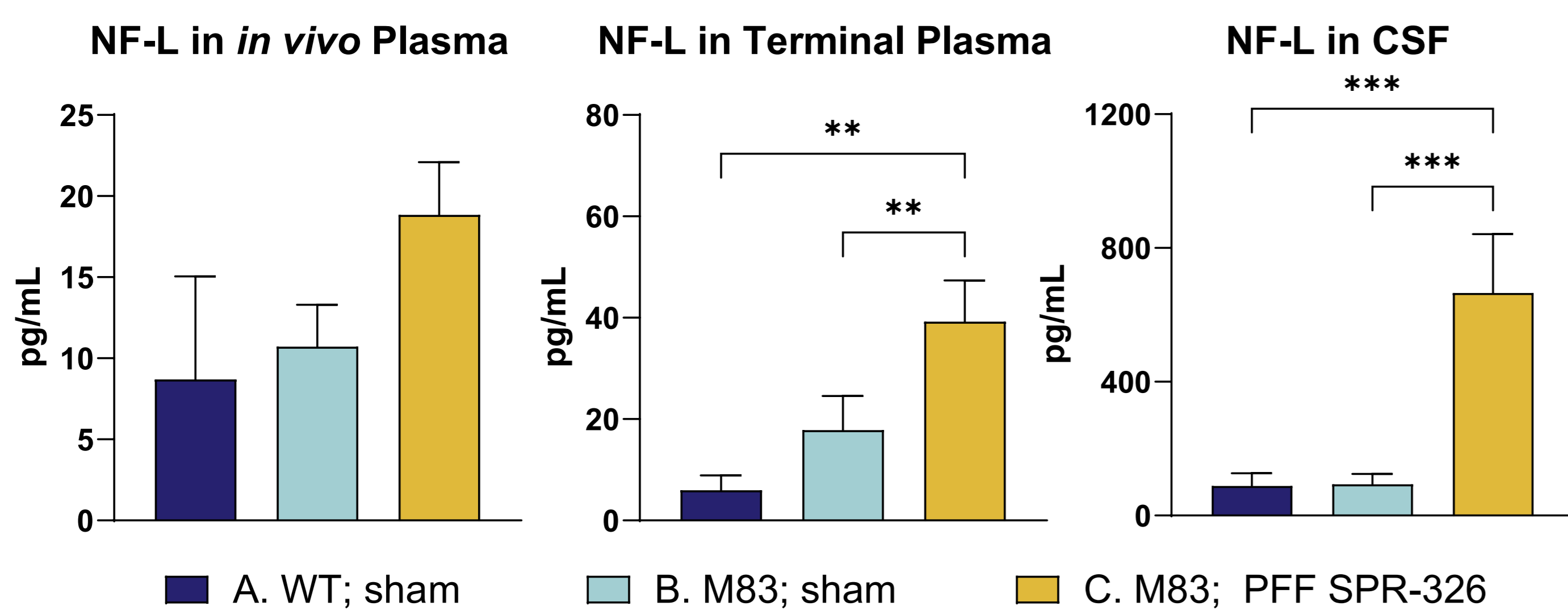


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## Introduction

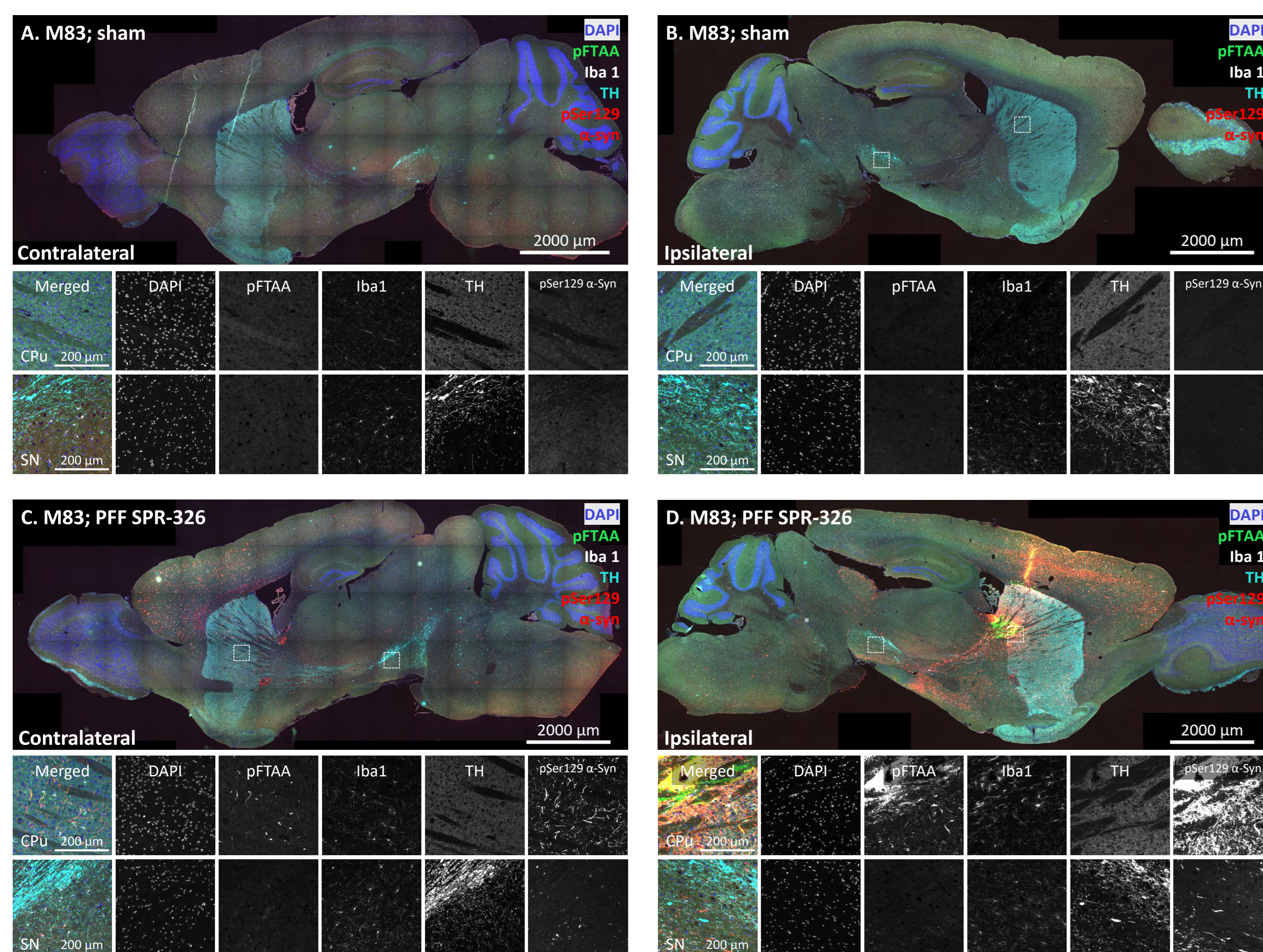
Transgenic mice are essential for studying the pathological mechanisms of Parkinson's disease (PD). However, they often fail to replicate the full disease spectrum, lack relevant molecular players, and show limited disease onset and progression. By challenging transgenic PD mice with induced lesions, these caveats can be alleviated. Here, we injected  $\alpha$ -synuclein ( $\alpha$ -syn) pre-formed fibrils (PFFs) into SNCA-A53T transgenic M83 mice to develop a "second hit" model.

## SNCA-A53T PFF Injections Exacerbate Neuronal Damage in M83 PD Mice



▲ Figure 2: Neurofilament light chain (NF-L) levels in plasma and cerebrospinal fluid (CSF). NF-L 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<sup>®</sup> ELISA 10-7001 CE from UmanDiagnostics was used. WT: n = 3-4; M83, sham: n = 4; M83, PFF SPR-326: n = 10. Two-way ANOVA followed by Bonferroni's multiple comparison test. Mean + SEM. \*\*p < 0.01; \*\*\*p < 0.001.

## Bilateral Pathology in PFF-Injected M83 Mice



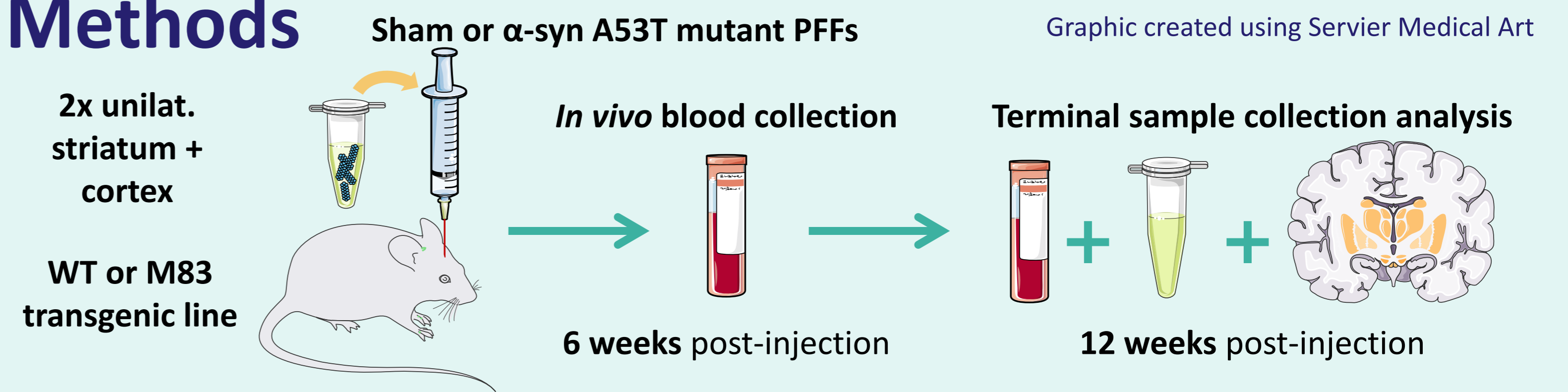
▲ Figure 3: Immunofluorescent labeling of terminal brain tissue. Representative images of pentameric formyl thiophene acetic acid (pFTAA), ionized calcium-binding adapter molecule 1 (Iba1), tyrosine hydroxylase (TH) and  $\alpha$ -syn phosphorylated at serine 129 (pSer129  $\alpha$ -Syn) in sagittal brain sections from M83 sham and M83 PFF injected animals. Panels show contralateral and ipsilateral hemispheres from both groups: contralateral M83 sham (A), ipsilateral M83 sham (B), contralateral M83 PFF SPR-326 (C) and ipsilateral M83 PFF SPR-326 (D). Nuclei were counterstained with DAPI. Single-channel magnifications show labeling in the caudate putamen (CPU) and substantia nigra (SN), corresponding to the regions indicated by the rectangles.

## Conclusions

Here we demonstrate that unilateral injection of  $\alpha$ -syn A53T-PFFs into M83 transgenic mice exacerbates PD pathology. Specifically, PFF injection increased neuronal damage (elevated NF-L levels in CSF and plasma), enhanced  $\alpha$ -syn phosphorylation and aggregation in CPU and SN of both hemispheres and activated Iba1-positive microglia in the targeted CPU. This establishes a robust bilateral seeding model for studying PD progression and evaluating therapeutic candidates, extending M83 utility in drug discovery.

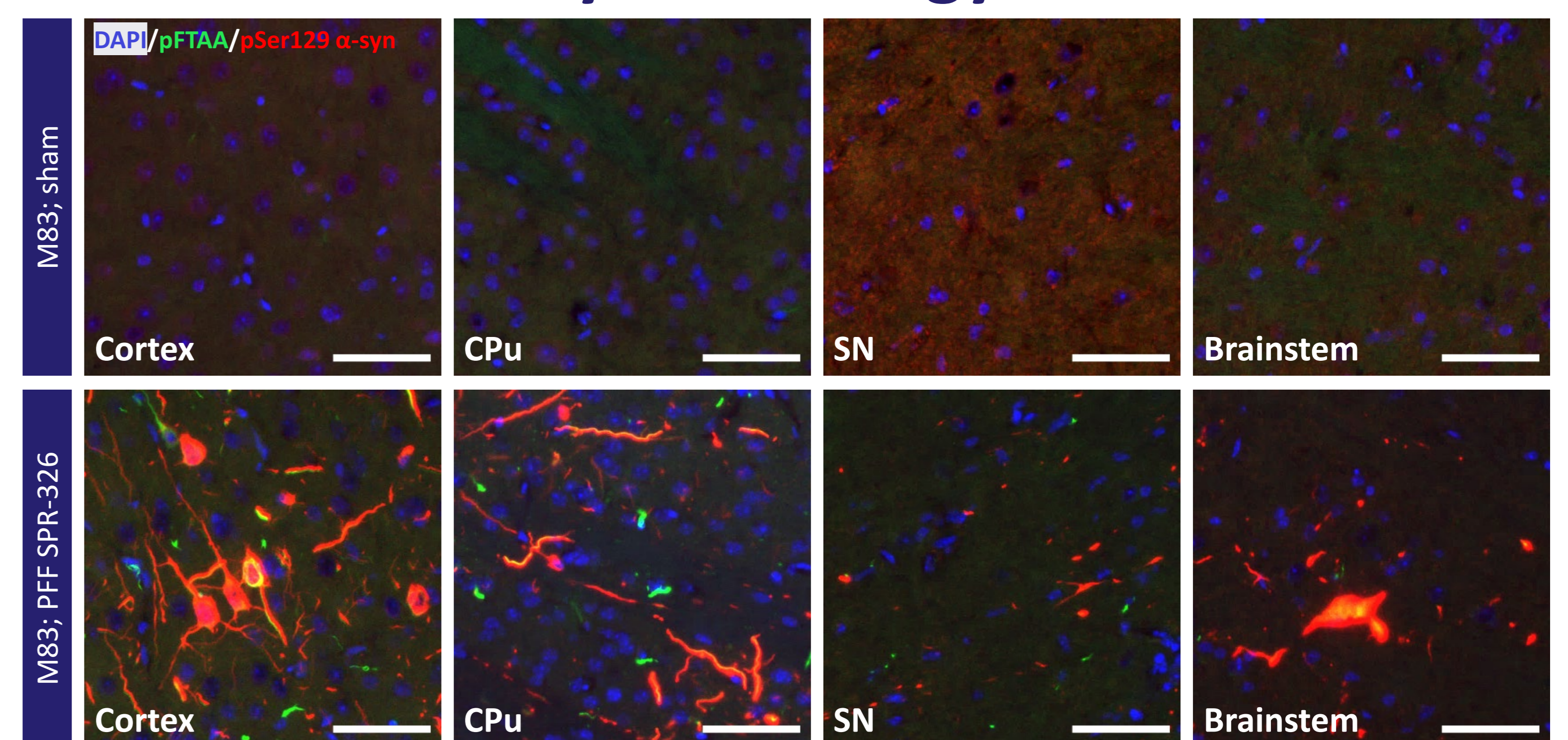
Parts of this project were performed in collaboration with Amyl Therapeutics [www.amyltx.com](http://www.amyltx.com)

## Methods



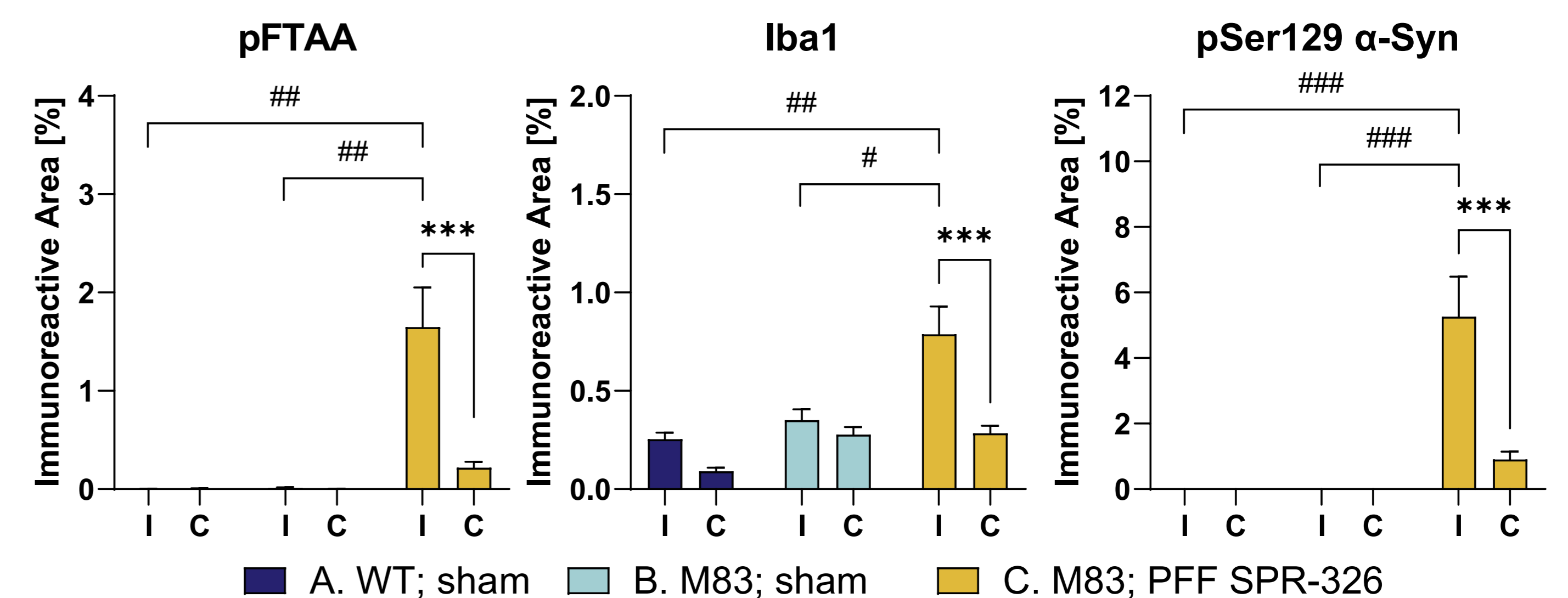
▲ Figure 1: Experimental timeline for unilateral  $\alpha$ -syn A53T or sham PFF injection in M83 and WT mice.

## Unilateral SNCA-A53T PFF Injections Induce Contralateral $\alpha$ -syn Pathology



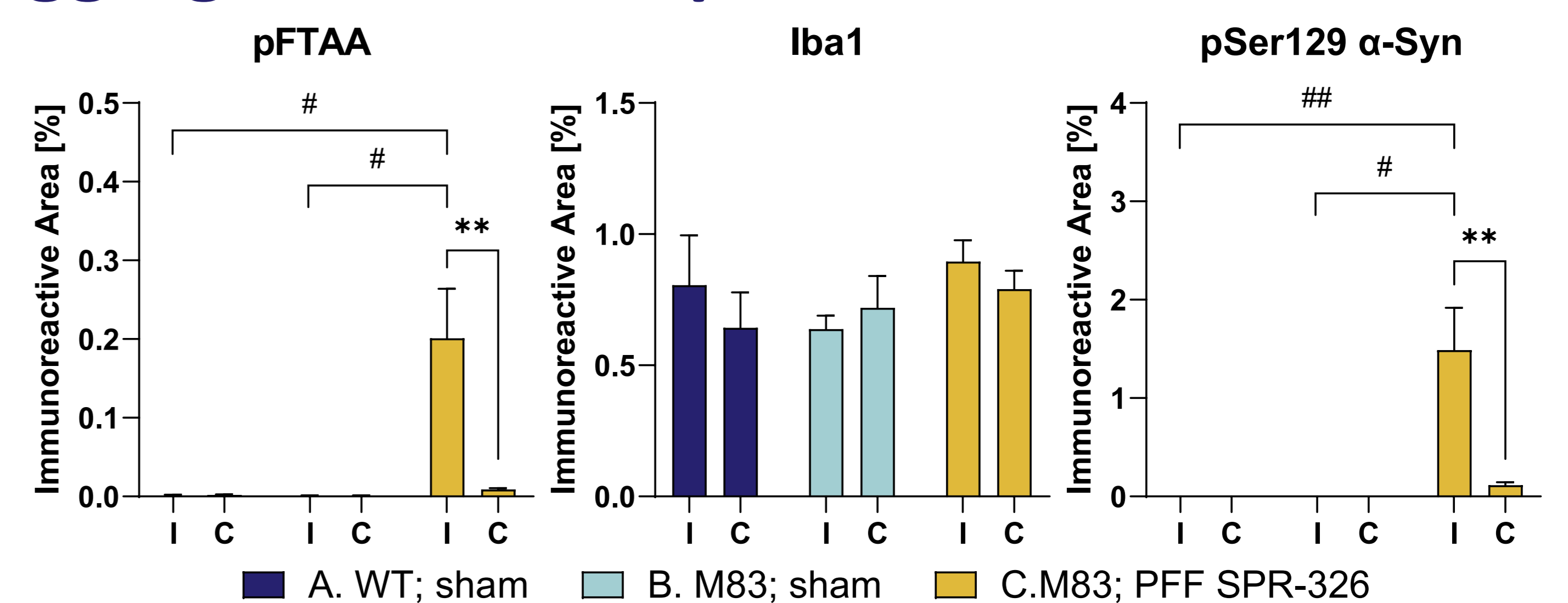
▲ Figure 4: Contralateral  $\alpha$ -synuclein pathology after PFF injection. Representative immunofluorescent images of brain compartments—cortex, caudate putamen (CPU), substantia nigra (SN) and brainstem—of the contralateral hemisphere of M83 mice after sham or SNCA-A53T PFF injection. Images show a merge of DAPI counterstaining in blue, pFTAA labelling in green and pSer129  $\alpha$ -syn immunoreactivity in red. Partial colocalization between pFTAA and pSer129  $\alpha$ -syn suggests a mixed population of  $\alpha$ -syn aggregates, with some showing both fibrillar (pFTAA-positive) and phosphorylated pathology (pSer129  $\alpha$ -syn-positive) and others only being positive for one of the two markers. Scale bar 50  $\mu$ m.

## $\alpha$ -syn Pathology and Microglial Activation in the Targeted CPU Following PFF Injections



▲ Figure 5: Quantification of pFTAA, Iba1 and pSer129  $\alpha$ -syn in the ipsilateral and contralateral CPU. Graphs present the means of immunofluorescent signal on five brain sections per mouse. WT: n = 4; M83, sham: n = 3; M83, PFF SPR-326: n = 10. Two-way ANOVA with Bonferroni's multiple comparisons test. Mean + SEM. \*\*\*p < 0.001; #p < 0.05; ###p < 0.01; ####p < 0.001. \* for C-I comparisons and # for comparisons between groups. C: contralateral; I: ipsilateral.

## Increased $\alpha$ -syn Phosphorylation and Aggregation in the Ipsilateral SN



▲ Figure 6: Quantification of pFTAA, Iba1 and pSer129  $\alpha$ -syn in the ipsilateral and contralateral SN. Graphs present the means of immunofluorescent signal on five brain sections per mouse. WT: n = 4; M83, sham: n = 3; M83, PFF SPR-326: n = 10. Two-way ANOVA with Bonferroni's multiple comparisons test. Mean + SEM. \*\*p < 0.01; #p < 0.05; ###p < 0.01. \* for C-I comparisons and # for comparisons between groups. C: contralateral; I: ipsilateral.

For more information about the models please visit: [www.scantox.com](http://www.scantox.com)  
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