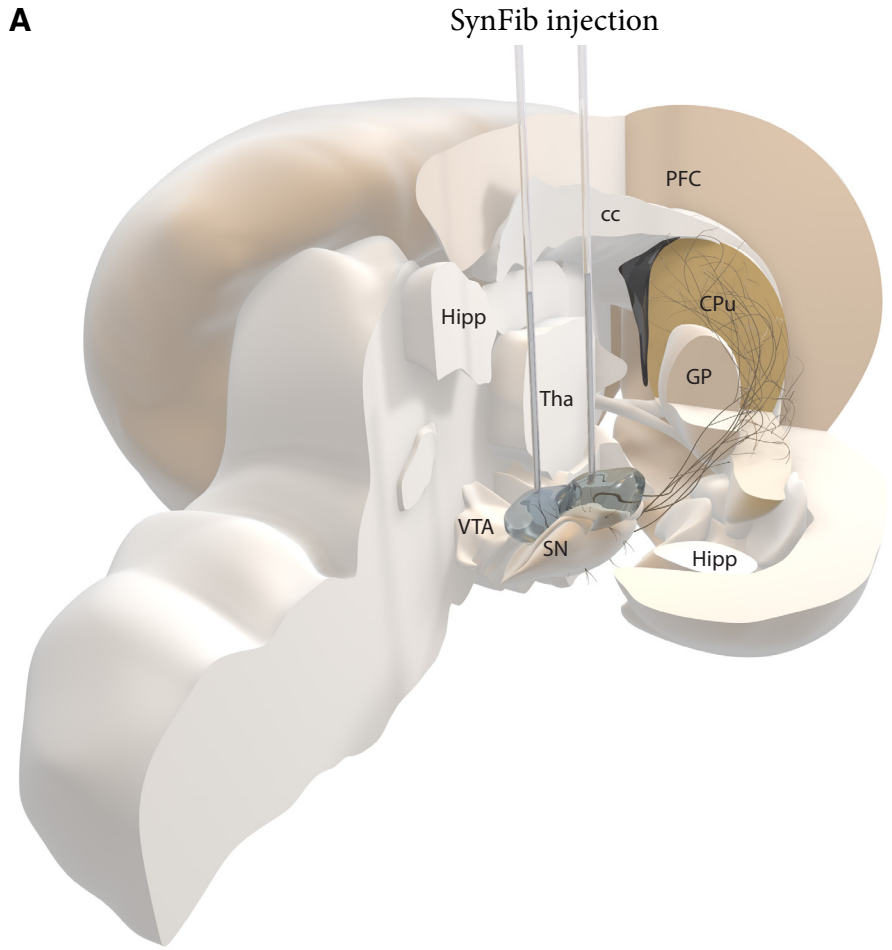

Figure S1. Experimental design of SynFib model induction.
Schematic of the brain shows the target sites in the substantia nigra used to simultaneously inject the AAV-α-syn and α-syn PFFs.

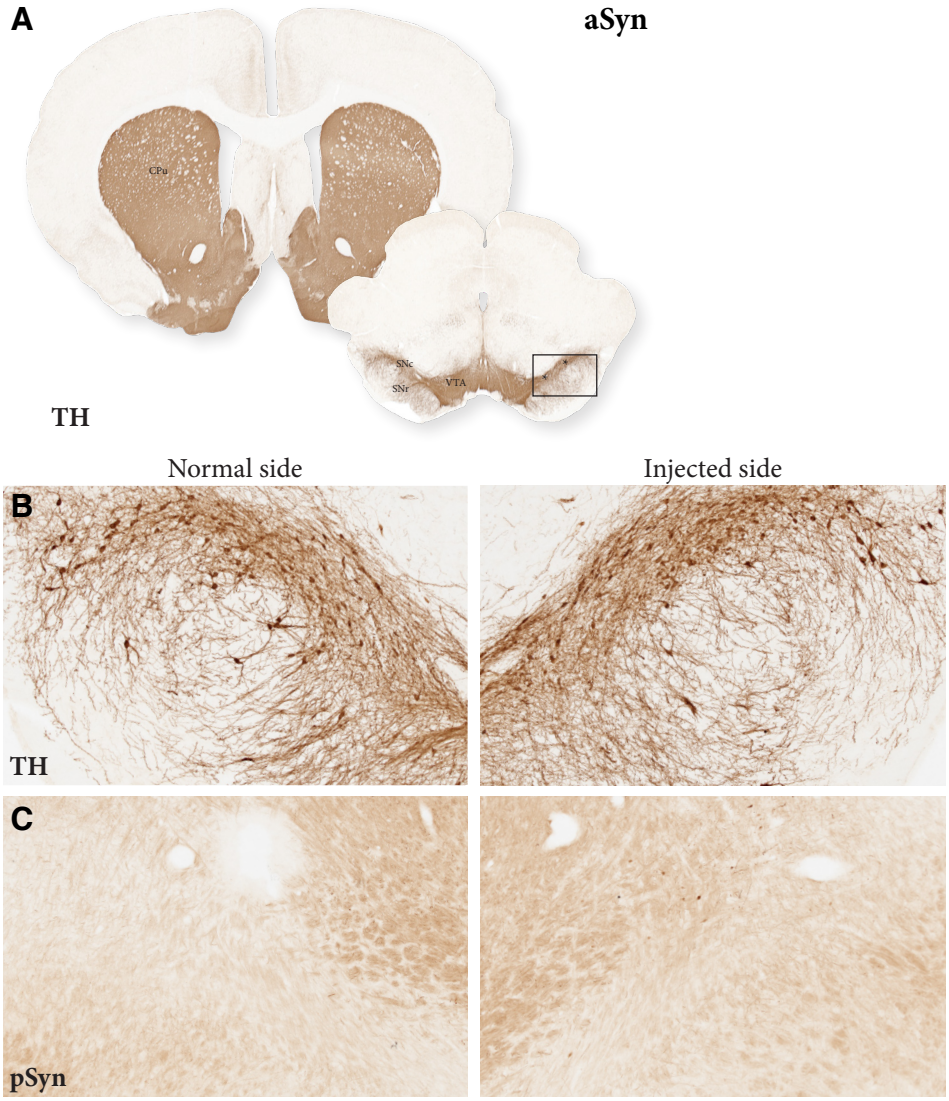

Figure S2. (Supplementary to Figure 3) Minimal TH loss and pathology in the aSyn animals.

In contrast to the extensive TH downregulation and pSyn expression observed in the STR and SN of SynFib animals (Fig 3), aSyn animals (that received AAV-α-syn alone) have minimal TH loss in the STR and SN (A,B) and pSyn expression in the SN (C), consistent with previous reports (1).

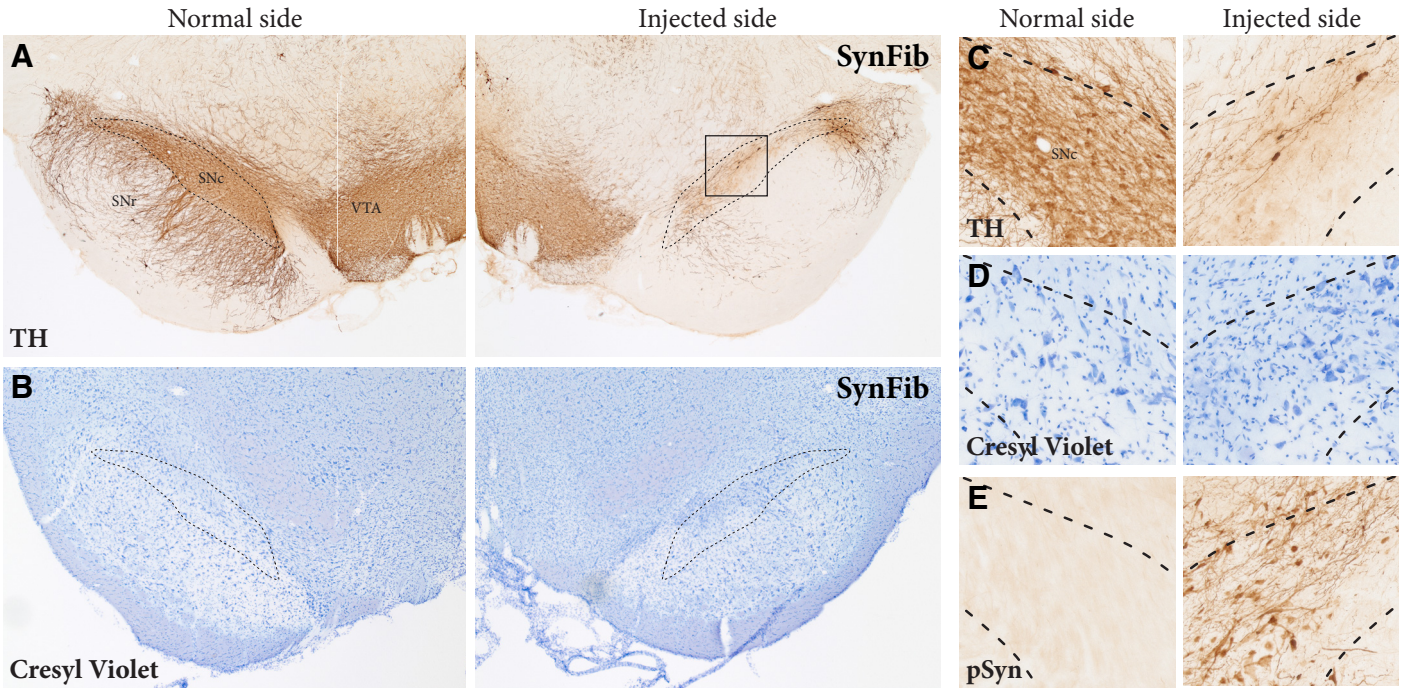

Figure S3. (Supplementary to Figure 3) Downregulation of TH in the SynFib model.
Representative images show reduced TH immunostaining (A,C) coupled with maintained cresyl violet (B,D) and pSyn (E) immunostaining in the SN indicating TH downregulation in the SynFib animals. In addition, a large number of nuclei are present in the SN (right in D) likely from the infiltration of microglia seen in Fig 2.

Antigen	Company	Catalogue No.	Host	Dilution
α-synuclein	Millipore	ab5038	Rabbit	1:1000
Calbindin	Swant	CB38	Rabbit	1:1000
GFP	Abcam	ab13970	Chicken	1:1000
Girk2	Abcam	ab65096	Goat	1:500
hNCAM	Santa Cruz	sc106	Mouse	1:1000
Iba1	Wako	019-19741	Rabbit	1:1000
mCherry	SICGEN	ab0040-200	Goat	1:1000
Nurr1	Abcam	ab41917	Mouse	1:500
Pitx3	Thomas Perlmann	Gift	Guinea Pig	1:500
pSyn 81A	Kelvin Luk	Gift	Mouse	1:10000
pSyn129	Abcam	ab51253	Rabbit	1:1000
Stem121	Takara	40410	Mouse	1:300
TH	Millipore	ab152	Rabbit	1:2000
TH	Millipore	ab1542	Sheep	1:1000
VMAT2	Sigma	V6637	Guinea Pig	1:2000

Figure S4. Antibodies used in the study.
The table shows the antigen, host and dilution used for each immunohistochemical staining in the study. The company and catalogue numbers are also included.

Movie S1. (in .mp4 file, Supplementary to Fig. 2). pSyn pathology in the SynFib model.

Extensive pSyn pathology was found in SynFib injected animals in the SN after 12 weeks. This was visualised used iDISCO clearing and staining as previously described (2) and light sheet microscopy, as described in the Materials and Methods. In this movie, we can see the large number of pSyn+ cell bodies at the site of injection in the SN. This pathology extends throughout the brain to form a dense network of pSyn+ fibers in the striatum.

Supplementary References:

1) Thakur, P., et al. Modeling Parkinson's disease pathology by combination of fibril seeds and alpha-synuclein overexpression in the rat brain. Proc Natl Acad Sci U S A 114, E8284-E8293 (2017).

2) Etessami, R., et al. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an *in vitro* and *in vivo* study. J Gen Virol 81, 2147-2153 (2000).