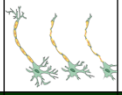
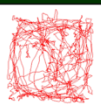
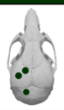
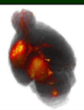
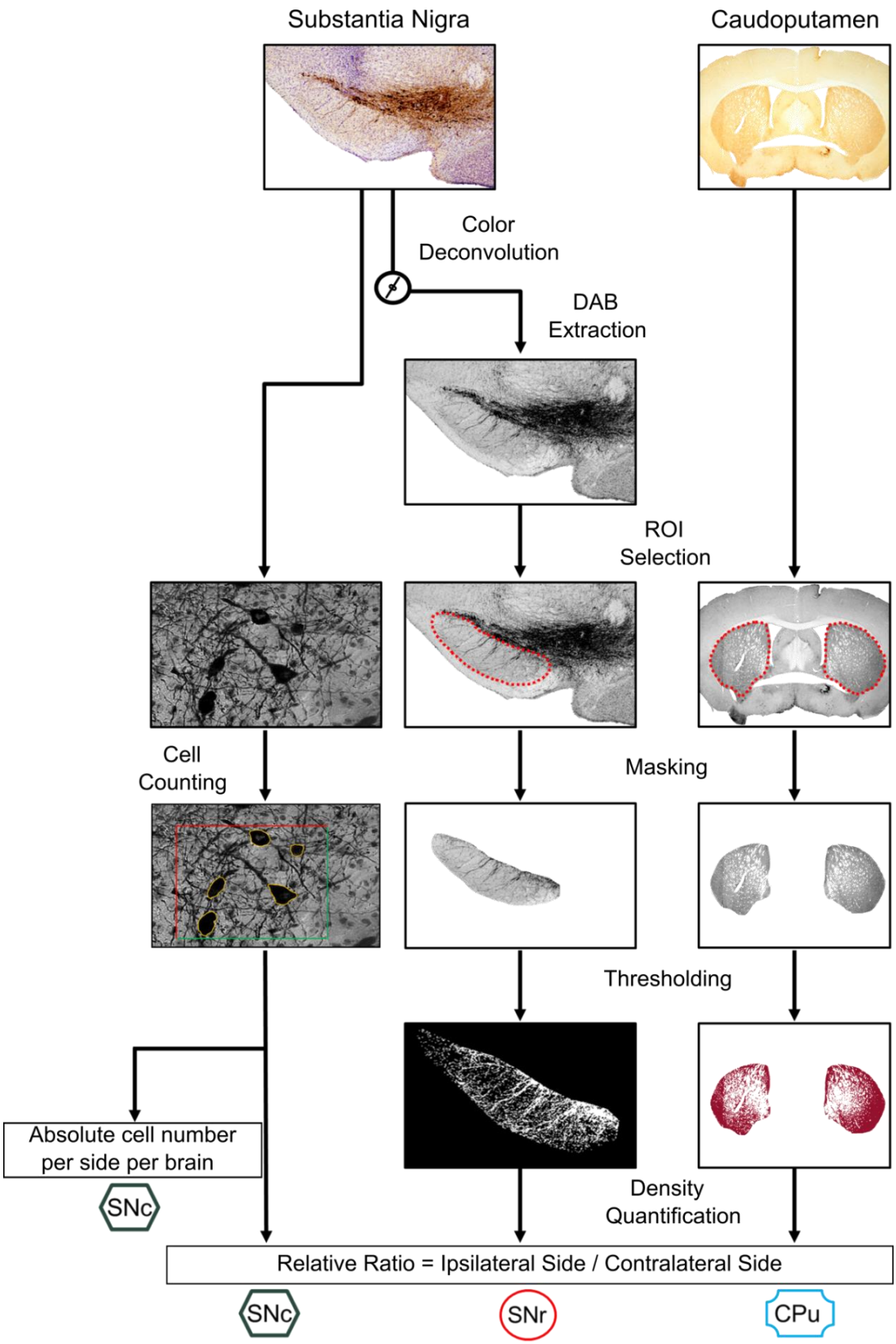
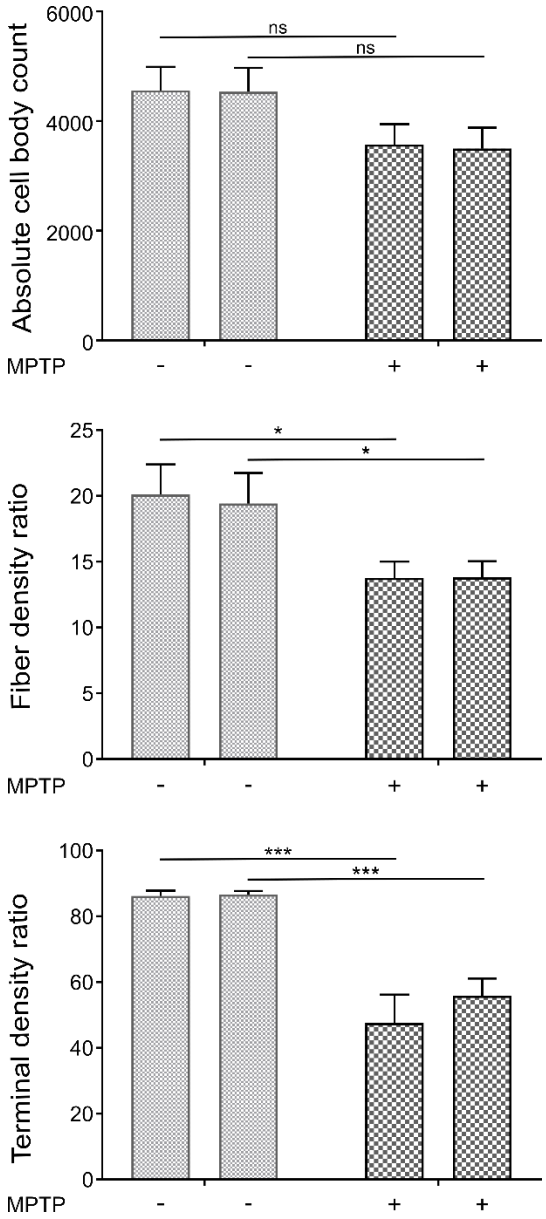
Supplementary Figures

Groups	MPTP Lesions	Degeneration Interval	Behavioral	BBB Openings	MRI Validation	Injection	Application Interval	Restoration Interval	Behavioral	Staining and Imaging
										
	5 days	21 days	2 days	60 sec/ea	30 min	10 min	14 days	days	2 days	
Phase I										
A n=7	+	+	-	-	+	-	+	14	-	+
B n=7	+	+	-	+	+	-	+	14	-	+
C n=7	+	+	-	+	+	NTN	+	14	-	+
D n=5	+	+	-	-	+++	-	+++	14	-	+
E n=5	+	+	-	+++	+++	-	+++	14	-	+
F n=5	+	+	-	+++	+++	NTN	+++	14	-	+
Phase II										
G n=8	+	+	+	-	+		+	70	+	+
H n=8	+	+	+	-	+	AAV_1-GDNF	+	70	+	+
I n=8	+	+	+	+	+		+	70	+	+
J n=10	+	+	+	+	+	AAV_1-GDNF	+	70	+	+

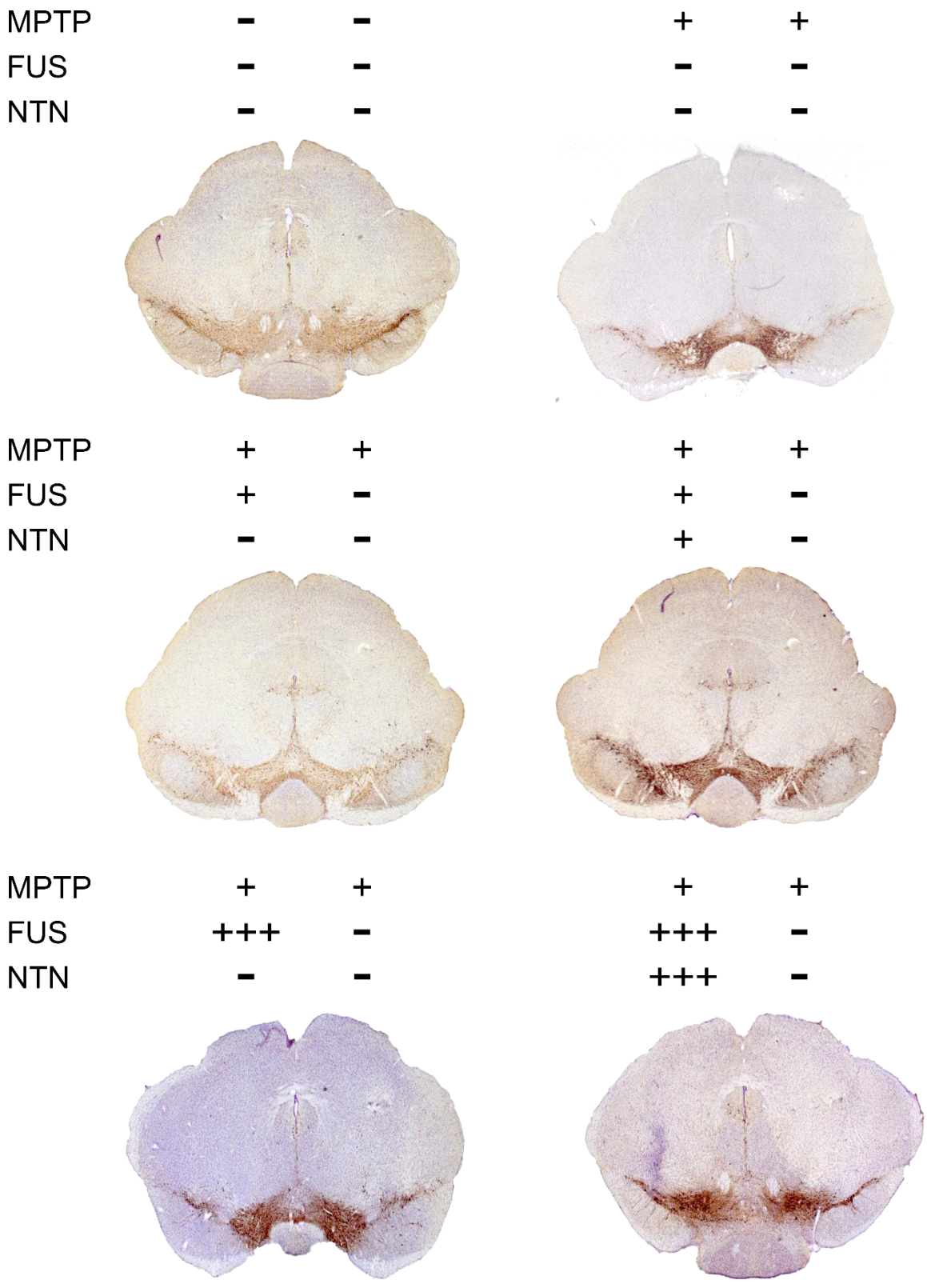
Supplementary Figure 1: Summary of the experimental groups and the procedures undergone.


Substantia Nigra
Caudoputamen
Color
Deconvolution
DAB
Extraction
ROI
Selection
Cell
Counting
Masking
Thresholding
Absolute cell number
per side per brain
SNc
Density
Quantification
Relative Ratio = Ipsilateral Side / Contralateral Side
SNc
SNr
CPu

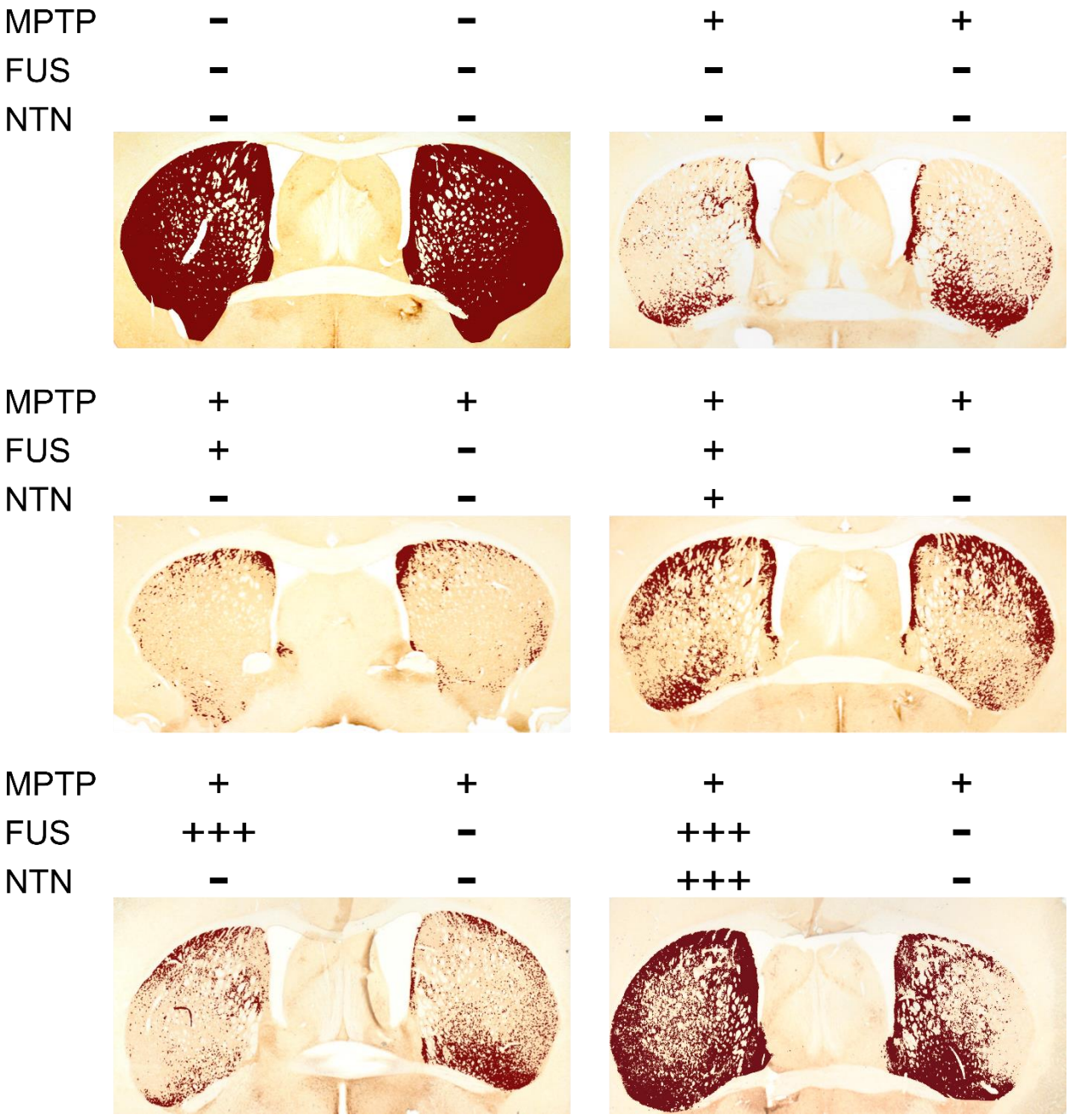
Supplementary Figure 2: Workflow of the quantification process. Brain sections at the level of the Substantia Nigra (SN) region were used for cell counting resulting in the absolute cell number per side of the brain. The double-stained images were color-deconvoluted after being white-balenced before further analysis. The Substantia Nigra reticulata (SNr) region was selected from the extraction of the DAB channel, masked and thresholded. The percent of the pixels surpassing the threshold was quantified. Respectively, the Caudate-Putamen region was selected and masked from the rest of the image. The pixels that surpassed the threshold were marked as dark red and are overlaid onto the raw image. The relative ratio of the ipsilateral over the contralateral side was quantified and is presented here.



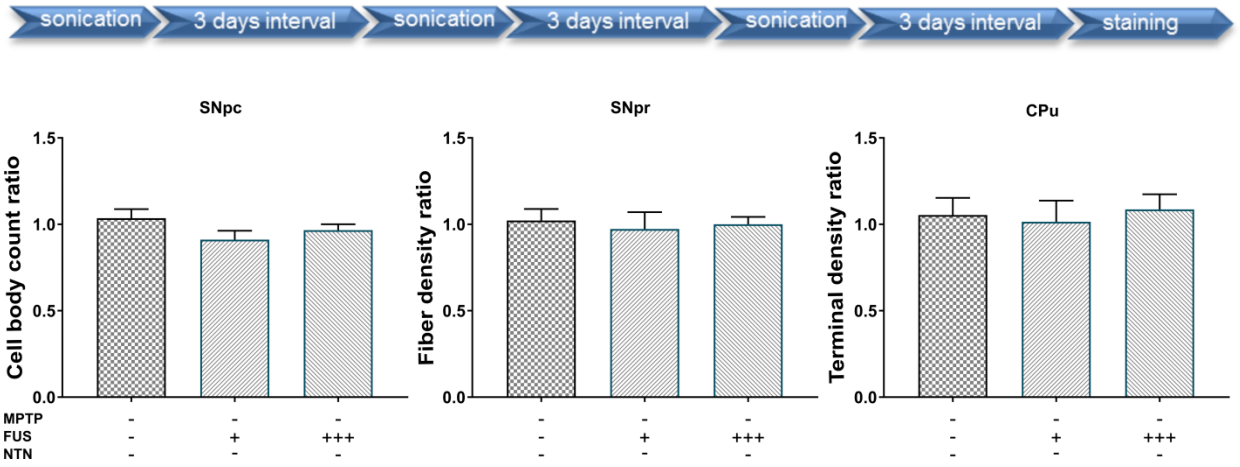
Supplementary Figure 3: Comparison of the MPTP-treated brains with the control group. There was a ~22% reduction in TH-positive neurons compared to saline-injected controls (two way ANOVA, $F_{[1, 12]} = 3.191$; P=0.0993), a ~33% reduction of TH-immunoreactivity in the SNr region (two way ANOVA, $F_{[1, 8]} = 5.335$; P=0.0497) and a ~40% in the CPu region (two way ANOVA, $F_{[1, 8]} = 25.64$; P=0.0010).



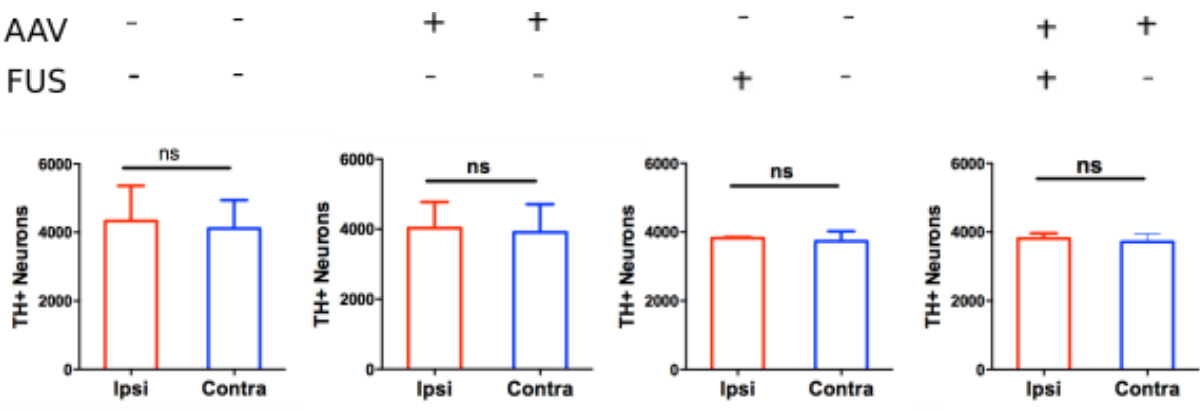
Supplementary Figure 4: Large version of the Substantia Nigra images presented in Figures 1 and 3.



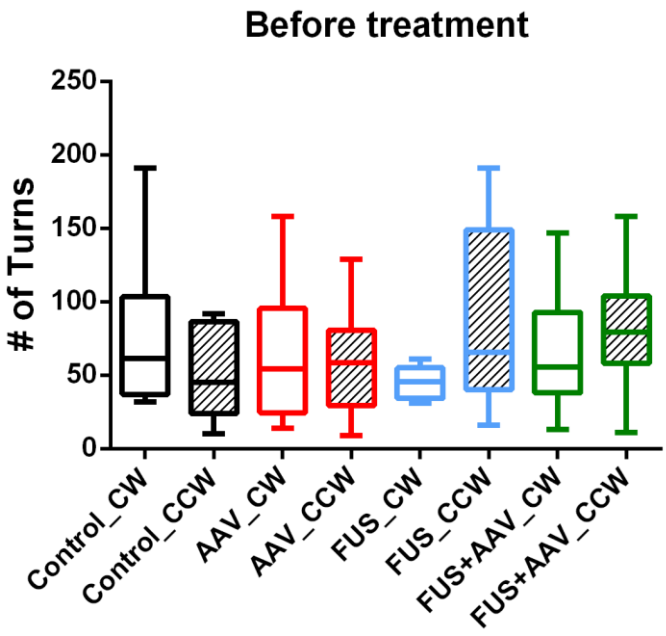
Supplementary Figure 5: Large version of the Caudoputamen images presented in Figures 1 and 4.



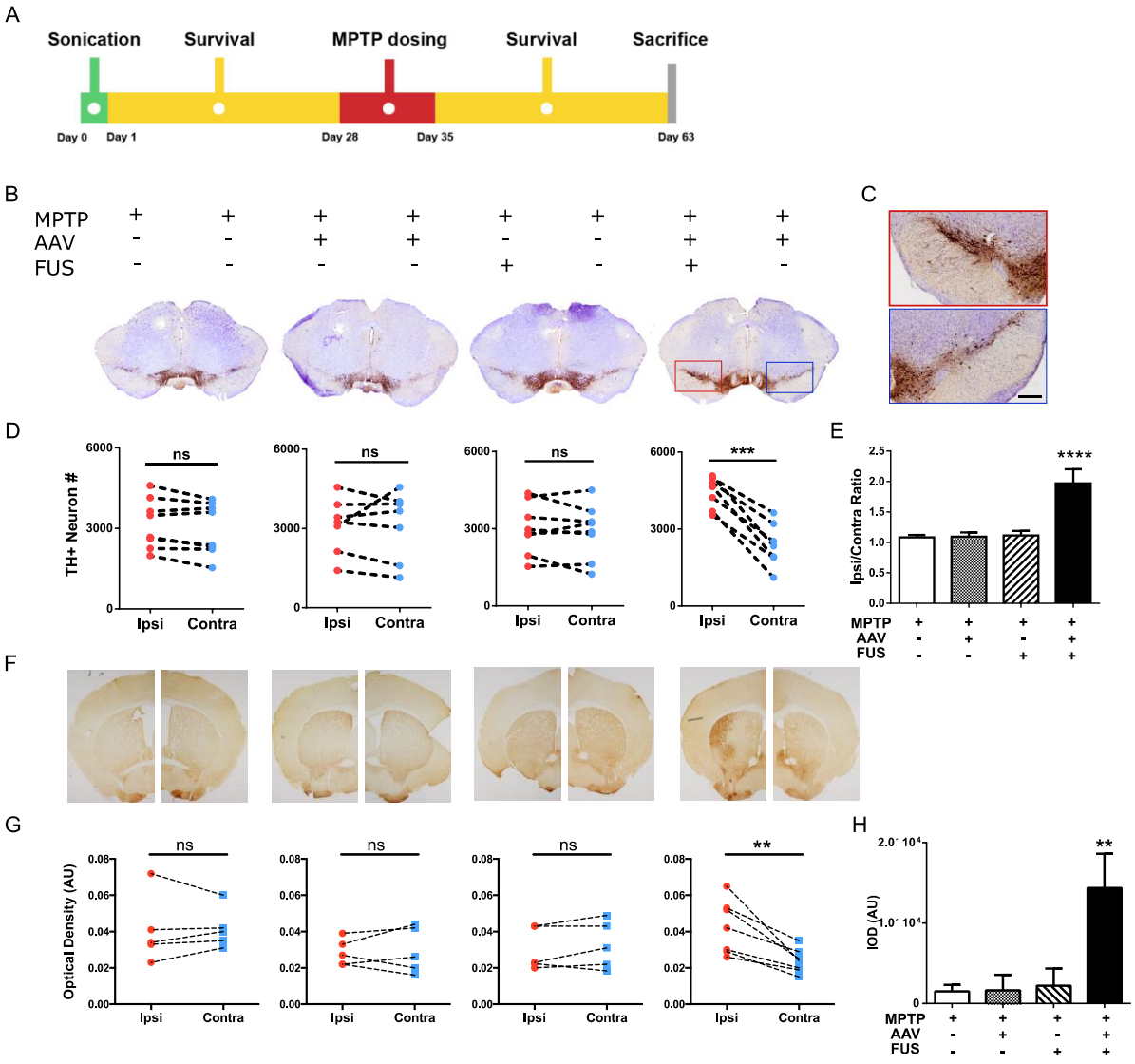
Supplementary Figure 6: Wild-type mice were sonicated none, one or three times in the SN and CPu regions to investigate the effect of ultrasound on the nigrostriatal pathway. One-way ANOVA (mean ± SEM) showed no significant differences in the cell body (one way ANOVA, $F_{[2, 12]} = 1.755$; P=0.2145), fiber (one way ANOVA, $F_{[2, 12]} = 0.1158$; P=0.8917) and terminal (one way ANOVA, $F_{[2, 12]} = 0.1193$; P=0.8886) density ratios of the ipsilateral over the contralateral side.



Supplementary Figure 7: AAV and FUS treatment does not alter TH+ neuron distribution in intraperitoneal saline-injected mice (n=3). No intra-group significant differences were found.



Supplementary Figure 8: Amphetamine-induced behavioral studies were performed before treatment where no intra-group significant rotational difference was observed (n=6-10).



Supplementary Figure 9: FUS-facilitated AAV-GDNF delivery induced neuroprotection in MPTP mice. A) Experimental timeline for neuroprotection. B) An example of TH-positive neurons and projections in the substantia nigra (counter-stained with Nissl, scale bar = 10 □m). C) TH staining in the substantia nigra in 4 experimental groups that received a combination of AAV and FUS treatment. TH+ neurons are stained in brown (n=7-8). D) High magnification of the SN region: top image AAV+/FUS+; bottom image AAV+/FUS-. E) Total number of TH+ neurons in each hemisphere of the four groups corresponding to the experimental conditions indicated in C. F) The ratio of the number of TH+ neurons on the ipsilateral to contralateral side. The AAV+/FUS+ mice showed a significantly higher ratio based on two-way ANOVA analysis. G) TH

staining of the Striatum revealed prominent dopaminergic projection preservation on the AAV+/FUS+ side. H) Quantitative analysis of optical density (OD) of the striatum. A significantly higher OD was observed on the AAV+/FUS+ side when compared to the contralateral side. I) Integrated optical density (IOD) was calculated and two-way ANOVA revealed significant difference was noted in the AAV+/FUS+ group. All error bars represent standard deviation. **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.