Supplementary Materials for

TRPV1 on astrocytes rescues nigral dopamine neurons in the Parkinson's disease via CNTF

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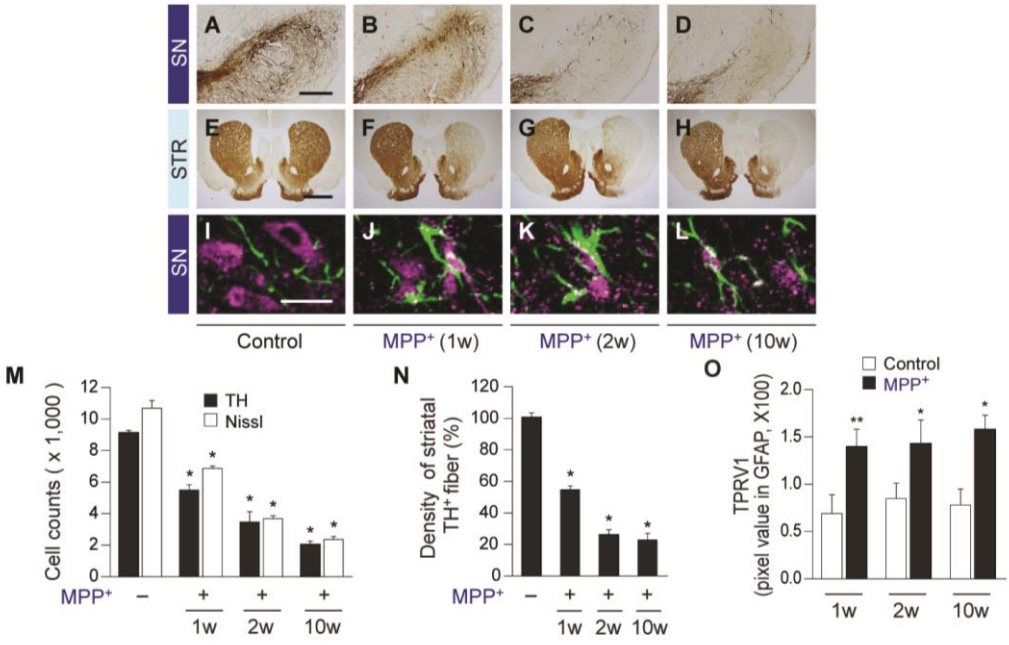
This PDF file includes:

Supplementary Figure and legends

Supplementary Figs 1-4

Supplementary Tables 1-3

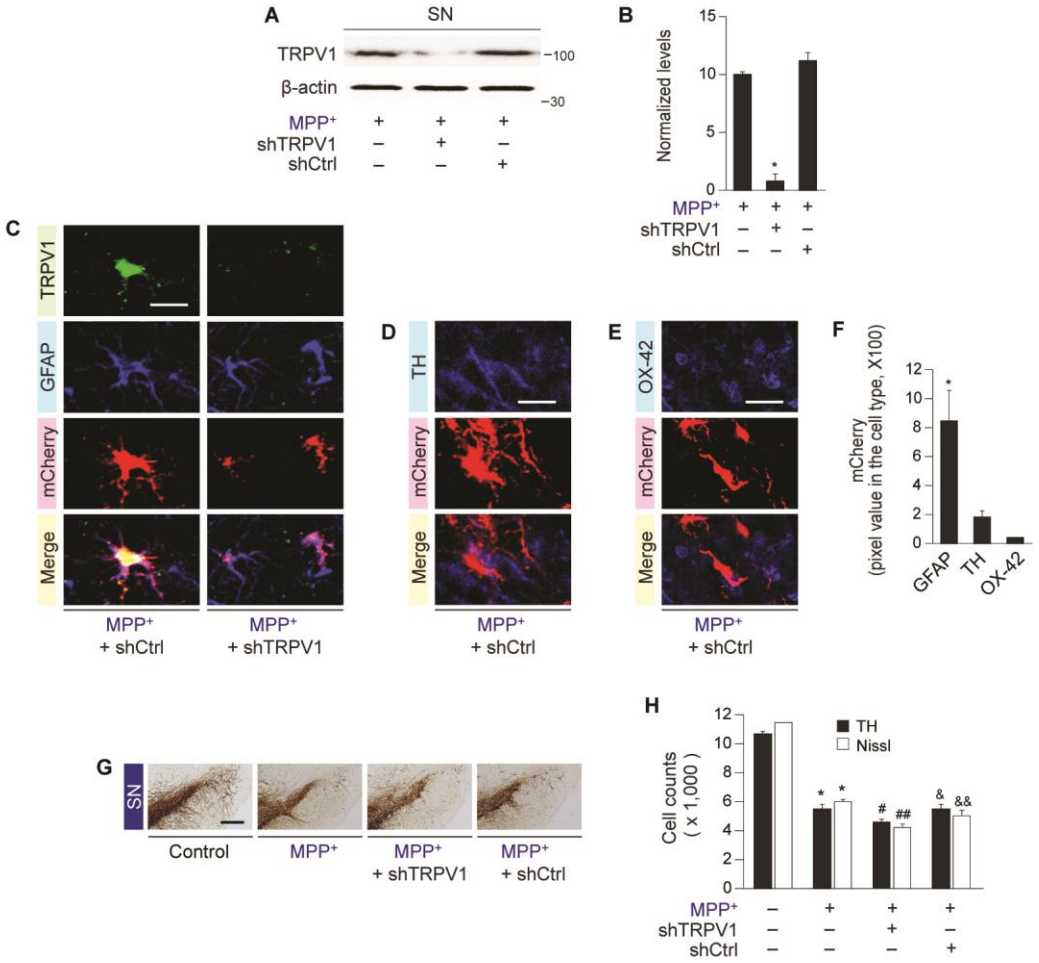
Supplementary Figure and legends



Supplementary Figure 1. **Degeneration of Dopamine neurons and expression of astrocytic TRPV1 in MPP$^+$-lesioned rat SNpc *in vivo*.**

(**A-L**) Photomicrographs of TH$^+$ cells in the substantia nigra (SN) (**A-D**), TH$^+$ fibers in the striatum (STR) (**E-H**) or colocalized cells of GFAP (green) and TRPV1 (red) (**I-L**) in the rat substantia nigra pars compacta (SNpc) at 1 (**B**, **F**,**J**) , 2 (**C**, **G**, **K**) and 10 (**D**, **H**, **L**) weeks after a unilateral MFB injection of MPP$^+$ or at 2 weeks after injection of PBS as a control (**A**, **E**, **I)**. (**M**) Number of TH$^+$ and Nissl$^+$ cells in the SNpc, $*P < 0.01$, significantly different from control. (**N**) Optical density of TH$^+$ fibers in the STR $*P < 0.01$, significantly different from control. Note no differences of remaining TH$^+$ cells in the SNpc and TH$^+$ fibers in the STR between 2 and 10 weeks post MPP$^+$, indicating that degeneration of nigrostriatal dopamine neurons was complete at 2 weeks post MPP$^+$ and sustained up to 10 weeks post MPP$^+$. (**O**) Quantification of TRPV1 expression colocalized with GFAP$^+$ astrocytes in the SNpc of MPP$^+$-lesioned rat brain at

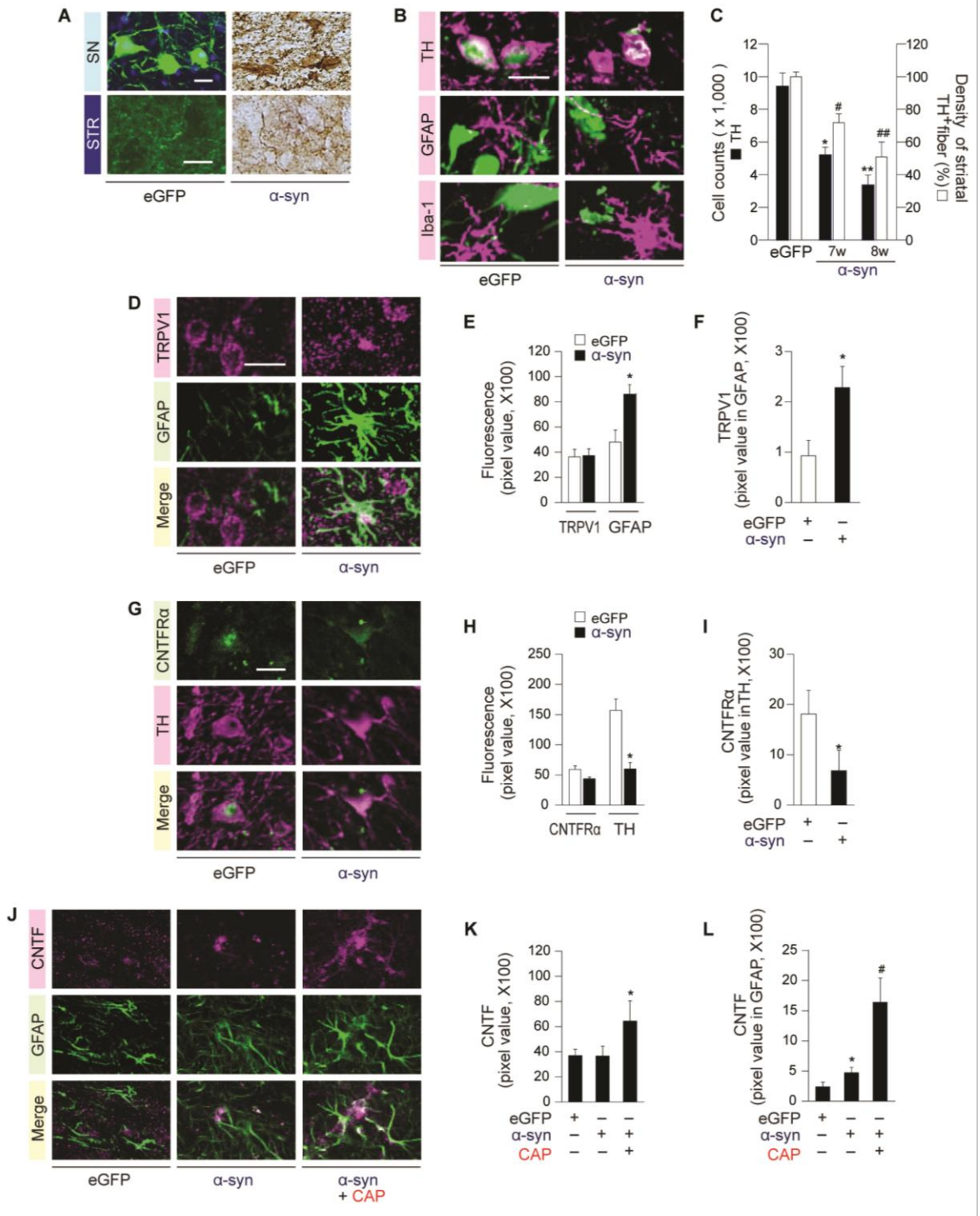
indicated time point *P < 0.05, **P < 0.01, significantly different from control. Scale bars: 400 μm (A-D, SN), 2 mm (E-H, STR), 20 μm (I-L). Mean ± s.e.m..; M-O, n = 5 to 10 in each group; ANOVA and Student-Newman-Keuls analysis.



Supplementary Figure 2. **Efficiency and specificity of TRPV1 knockdown.**

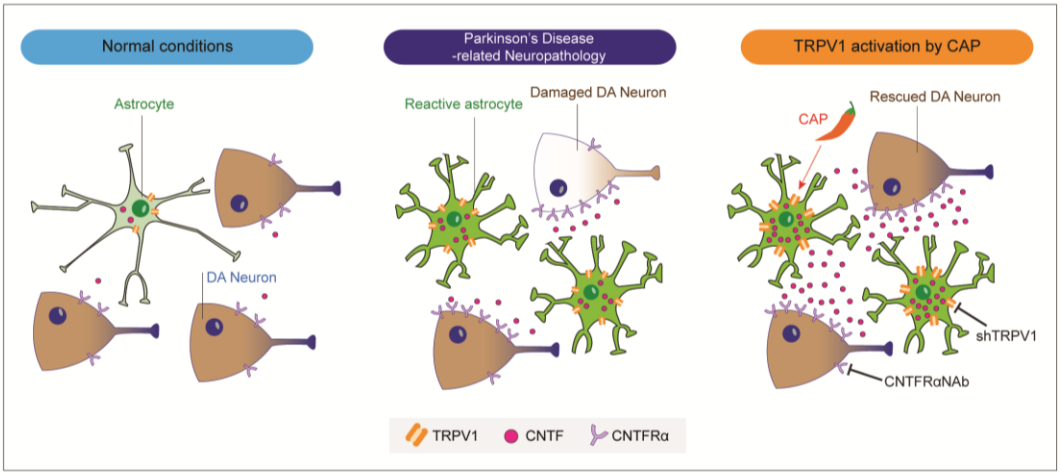
Rats were given a unilateral medial forebrain bundle (MFB) injection of MPP^+ followed by injection of shTRPV1 or shCtrl (control) into the substantia nigra (SN) (See **Fig. 1A**). At 1 week post MPP^+, brain tissues were prepared for Western blot (**A** and **B;***P < 0.001, significantly different from MPP^+) or immunohistochemical analysis (**C**-**H**). shTRPV1 reduced TRPV1 protein levels (**A** and **B**) or TRPV1 immunoreactivity (green, **C**) within $GFAP^+$ astrocytes (blue, **C**) in the substantia nigra pars compacta (SNpc), compared to shCtrl (**A**-**C**). The viruses

transduced the fluorescent mCherry protein (red, **A**-**E**), indicating infected cells. $mCherry^+$ cells were mainly colocalized within $GFAP^+$ astrocytes (**C** and **F**) whereas there was minimal $mCherry^+$ cells colocalized in TH^+ neurons (**D** and **F**) or OX-42^+ microglia (**E** and **F**) in the SNpc of MPP^+-lesioned rats, **;***$P < 0.001$, significantly different from mCherry cells colocalized in TH^+ or OX-42^+ cells. Photomicrographs of TH^+ cells in the SNpc (**G**) and number of TH^+ or $Nissl^+$ cells in the SNpc (**H**) *$P < 0.01$, significantly different from control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, significantly different from MPP^+, $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, significantly different from MPP^+ + shTRPV1. Note that TRPV1 knockdown enhances MPP^+ neurotoxicity in the SNpc *in vivo*. Scale bars; C-E 20 μm. g, 400 μm. Mean ± s.e.m..; B, $n = 4$; F and H, $n = 5$. ANOVA and Student-Newman-Keuls analysis.


A
SN
STR
eGFP
α-syn
B
TH
GFAP
Iba-1
eGFP
α-syn
C
Cell counts (x 1,000)
TH
Density of striatal TH+fiber (%)
eGFP
7w
8w
α-syn
D
TRPV1
GFAP
Merge
eGFP
α-syn
E
eGFP
α-syn
Fluorescence (pixel value, X100)
TRPV1
GFAP
F
TRPV1 (pixel value in GFAP, X100)
eGFP
α-syn
G
CNTFRα
TH
Merge
eGFP
α-syn
H
eGFP
α-syn
Fluorescence (pixel value, X100)
CNTFRα
TH
I
CNTFRα (pixel value in TH, X100)
eGFP
α-syn
J
CNTF
GFAP
Merge
eGFP
α-syn
α-syn + CAP
K
CNTF (pixel value, X100)
eGFP
α-syn
CAP
L
CNTF (pixel value in GFAP, X100)
eGFP
α-syn
CAP

Supplementary Figure 3**. Expression of TRPV1, CNTFRα and CNTF in α-synuclein-lesioned rat brain.**

(**A**-**L**) Rats were given a unilateral injection of AAV2-α-synuclein (α-syn) or AAV2-eGFP (eGFP, control) into the substantia nigra (SN) and transcardially perfused for immunohistochemical analysis at 7 weeks after transduction (See **Fig. 6A**). Photomicrographs of $eGFP^+$ or $\alpha\text{-syn}^+$ cells in the SN and fibers in the striatum (STR) (**A**). Fluorescence images showing colocalization of $eGFP^+$ (green) or $\alpha\text{-syn}^+$ (green) cells in TH^+ (dopamine neurons; red) cells, but not in $GFAP^+$ (astrocytes; red) or $Iba\text{-}1^+$ (microglia; red) cells, respectively (**B**). (**C**) Number of TH^+ cells in the SNpc and optical density of TH^+ fibers in the STR, $^*P < 0.001$, $^{\#}P < 0.01$, significantly different from control, $^{**}P < 0.05$, $^{\#\#}P < 0.05$ significantly different from 7 weeks post α-syn. Fluorescence images of TRPV1 (red) and GFAP (green), and both images are merged in the substantia nigra pars compacta (SNpc) (**D**), and quantification of TRPV1 and GFAP expression (**E;** $^*P < 0.01$, significantly different from eGFP), and TRPV1 expression colocalized in $GFAP^+$ astrocytes (**F;** $^*P < 0.05$, significantly different from eGFP) in the SNpc. Fluorescence images of CNTFRα (green) and TH (red), and both images are merged in the SNpc (**G**), and quantification of CNTFRα or TH expression (**H;** $^*P < 0.001$, significantly different from eGFP) and CNTFRα expression colocalized in TH^+ dopamine neurons (**I;** $^*P < 0.01$, significantly different from eGFP) in the SNpc. Sections adjacent to those used in **Fig. 3** were immunostained. Fluorescence images of CNTF (red) and GFAP (green), and both images are merged in the SNpc at 8 weeks post α-syn (**J**), and quantification of CNTF expression (**K;** $^*P < 0.05$, significantly different from α-syn + CAP) or CNTF expression colocalized with $GFAP^+$ astrocytes (**L;** $^*P < 0.05$, significantly different from eGFP, $^{\#}P < 0.01$, significantly different from α-syn) in the SNpc at 8 weeks post α-syn. Fluorescence images of GFAP (eGFP, **D** and **J**) and CNTFRα (eGFP, **G**) were pseudo-colored from blue to green. Scale bars; 20 μm. Mean ± s.e.m.; C, $n = 4$ to 7 in each group; E, F, $n = 4$ to 14 in each group; H, I, $n = 6$ in each group; K, L, $n = 4$ to 6 in each group. ANOVA and Student-Newman-Keuls analysis.



Supplementary Figure 4**. Model of TRPV1-mediated CNTF production in astrocytes and neuroprotection of nigral dopamine neurons of Parkinson's disease.**

There is a dramatic expression of TRPV1 and CNTF in reactive astrocytes in the substantia nigra pars compacta of human Parkinson's disease and Parkinson's disease-related neuropathology (MPP^+- or α-synuclein-lesioned rats), compared to the respective controls. Note that astrocytic TRPV1 activation by CAP significantly increases CNTF production and rescues dopamine neurons through over-expressed CNTF receptor alpha on DA neurons in the SNpc of Parkinson's disease *in vivo*. TRPV1, Transient Receptor Potential Vanilloid 1. shTRPV1, TRPV1 shRNA lentivirus. DA, Dopamine. CNTF, Ciliary Neurotrophic Factor. CNTFRα, CNTF receptor alpha. CNTFRαNAb, CNTF receptor alpha neutralizing antibody. CAP, capsaicin.

Supplementary Tables 1-3

Supplementary Table 1. Human postmortem tissues used for Western blots in **Fig. 7 A-D**.

Sample No	Final Diagnosis	Age	Sex	Race	PMD	Tissue
1	Control	73	M	W	9	SN
2		62	M	W	14	
3		68	M	W	14	
4		66	M	W	10	
1	PD W/D	83	M	W	16.5	SN
2	PD W/D	75	M	W	6	
3	PD	76	M	W	7.5	
4	PD W/D	74	M	W	19	
5	PD W/D	75	F	W	24	

W/D, with dementia; M, Male; F, Female; W, White;
PMD, Postmortem delays; SN, Substantia nigra

Supplementary Table 2. Human postmortem tissues used for Western blots in **Fig 7E** and **F**.

Sample No.	Final Diagnosis	Age	Sex	Race	PMD	Tissue
1	Control	73	M	W	9	Cortex
2		62	M	W	14	
3		80	M	B	21	
4		80	F	W	6	
5		79	M	W	16	
1	PD W/D	75	M	W	6	Cortex
2	PD	75	F	W	24	
3	PD W/D	83	M	W	7	
4	PD W/D	85	M	W	11	

W/D, with dementia; M, Male; F, Female; W, White;
PMD, Postmortem delays.

Supplementary Table 3. Human postmortem tissues used for immunostaining in **Fig. 7G** and **H** (GFAP+TRPV1), **I** and **J** (GFAP+CNTF) and **K-O** (CNTFRα).

Sample No	Final Diagnosis	Age	Sex	PMD	Staining	Tissue
10-303	Control	43.2	M	44	GFAP+TRPV1	SN
08-026		67.3	M	24		
07-787		66.5	M	19		
04-112		73.5	M	22	GFAP+CNTF	
V11-038		39.2	M	51		
07-239		78.8	M	19		
08-260		67.3	F	24	CNTFRα	
V11-038		39.2	M	51		
04-424		75.1	F	22.5		
08-319	PD	70	M	32.5	GFAP+TRPV1	SN
07-566		78.4	M	32.5		
09-260		66.8	F	20		
V11-042		72.1	M	25	GFAP+CNTF	
03-819		63.6	F	56		
09-260		66.8	F	20		
09-260		66.8	F	20	CNTFRα	
V11-730		80	M	65		
07-566		78.4	M	32.5		

M, Male; F, Female;
PMD, Postmortem delays; SN, Substantia nigra