Supporting Information for

Original article

Corynoxine B targets at HMGB1/2 to enhance autophagy for alpha-synuclein clearance in fly and rodent models of Parkinson's disease

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Received 14 October 2022; received in revised form 12 February 2023; 28 February 2023

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Running title: Corynoxine B targets at HMGB1/2 to enhance autophagy in PD

1. Supporting tables

Table S1 Reagents and antibodies

Reagents	Company name (Catalog number)	City/State, Country
Recombinant hHMGB1 protein	Abcam (#ab167718)	Cambridge, UK
Recombinant hHMGB2 protein	Abcam (#ab109962)	Cambridge, UK
Torin 1	LC Laboratories (#T-7887)	Massachusetts, US
SAR405	APExBIO (#A8883)	Houston, USA
EBSS	Gibco (#24010043)	Massachusetts, US
Chloroquine	Sigma-Aldrich (#C6628)	Louis Missouri, US
Kolliphor HS 15	Sigma-Aldrich (#42966)	Louis Missouri, US
DMEM for SILAC	Thermo Scientific (#88364)	Massachusetts, US
$^{13}C_6$ $^{15}N_2$ L-Lysine-2HCl	Thermo Scientific (#88209)	Massachusetts, US
L-Lysine-2HCl	Thermo Scientific (#88429)	Massachusetts, US
$^{13}C_6$ $^{15}N_4$ L-Arginine-HCl	Thermo Scientific (#89990)	Massachusetts, US
L-Arginine-HCl	Thermo Scientific (#89989)	Massachusetts, US
Dialyzed Fetal Bovine Serum	Thermo Scientific (#26400044)	Massachusetts, US
RIPA lysis (Medium)	Beyotime (#P0013C)	Shanghai, CN
RIPA lysis (Weak)	Beyotime (#P0013D)	Shanghai, CN
Poly-D-lysine	Beyotime (#ST508)	Shanghai, CN
Lipo8000	Beyotime (#C0533)	Shanghai, CN
Lipofectamine 3000	Invitrogen (#L30000015)	California, US
Anti-FLAG M2 Magnetic Beads	Sigma-Aldrich (#M8823)	Louis Missouri, US
Clean-Blot IP Detection Kit	Thermo Scientific (#21232)	Massachusetts, US
DAB Substrate Kit	Vectorlabs (#SK-4100)	California, US
Antibodies	Company name (Catalog number)	City/State, Country
Anti-phospho-AMPKα (Thr172)	Cell Signaling Technology (#5256)	Massachusetts, US
Anti-AMPK	Cell Signaling Technology (#5831)	Massachusetts, US
Anti-Atg14	MBL(#PD026)	Hokkaido Prefecture, JP
Anti-PI 3-Kinase p100 (VPS34)	Santa Cruz (#sc365404)	Texas, USA
Anti-BECN1 (Beclin 1)	Santa Cruz (#sc11427)	Texas, USA
Anti-HMGB1	Abcam (#ab18256)	Cambridge, UK
Anti-HMGB2	Abcam (#ab124670)	Cambridge, UK
Anti-SQSTM1	Abcam (#ab109012)	Cambridge, UK
Anti-LC3	Novus Biologicals (#NB100-2220)	Colorado, US
Anti-Tyrosine hydroxylase	Pel-Freez (#P40101-0)	Arkansas, US
Anti-β-actin	Cell Signaling Technology (#4970)	Massachusetts, US
Anti-α-synuclein	BD Transduction Laboratories (#610786)	California, US
Purified anti-α-synuclein phospho-(Ser129) antibody	BioLegend (#MMS-5091)	California, US
Goat anti-mouse secondary antibody	Cell Signaling Technology (#7076)	Massachusetts, US
Goat anti-rabbit secondary antibody	Cell Signaling Technology (#7074)	Massachusetts, US
Goat anti-Rabbit Secondary Antibody, Alexa Fluor 555	Invitrogen (#A-21428)	California, US
Goat anti-Mouse IgG Secondary Antibody, Alexa Fluor 488	Invitrogen (#A-11001)	California, US
Goat anti-Rabbit Secondary Antibody, Alexa Fluor 488	Invitrogen (#A-11008)	California, US

2. Supporting figures

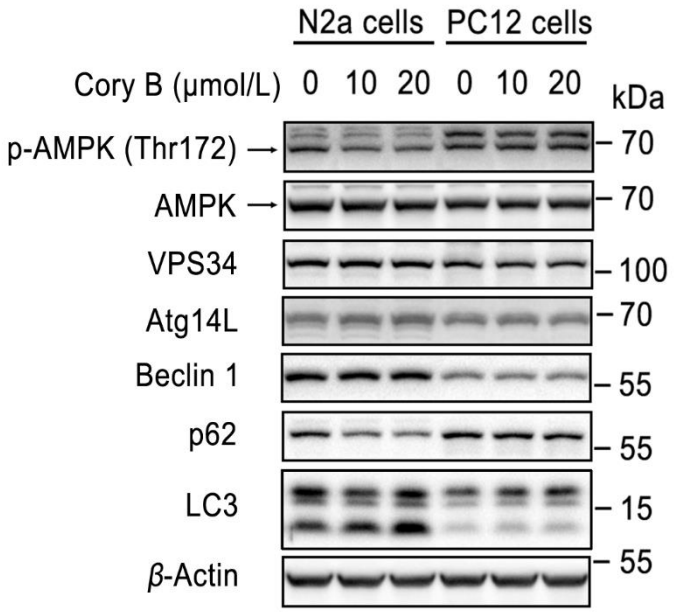

Figure S1 Effects of corynoxine B (Cory B) on the expression levels of autophagy related proteins in N2a and PC12 cells. N2a cells and PC12 cells were treated with different concentrations of Cory B for 6 h. Expression levels of phospho-AMPK*α* (Thr172), total AMPK, VPS34, Atg14L, Beclin1, p62 and LC3 were analyzed by Western blot, and band intensities of phospho-AMPK*α* (Thr172) /total AMPK were used to compare AMPK activation.

We examined the effects of Cory B on autophagy after knocking down *Hmgb1*, *Hmgb2*, or both in N2a cells. Short interfering RNA (siRNA) for *Hmgb1* (siHMGB1) was synthesized based on previous study, and four different pairs of siRNAs for *Hmgb2* (siHMGB2) were synthesized and verified as Fig. S2A. siHMGB2-4 was chosen for further experiment. Results showed that transfection of siRNA for *Hmgb1* and *Hmgb2* individually decreased protein expression levels of HMGB1 and HMGB2, respectively. In addition, the dual knockdown of both *Hmgb1* and *Hmgb*2 decreased their expression levels simultaneously (Fig. S2B). The loss of function resulting from individual knockdown of *Hmgb1* and *Hmgb*2 markedly inhibited autophagy level in N2a cells, and dual knockdown synergistically enhanced the inhibitory effect (Fig. S2B–S2D).

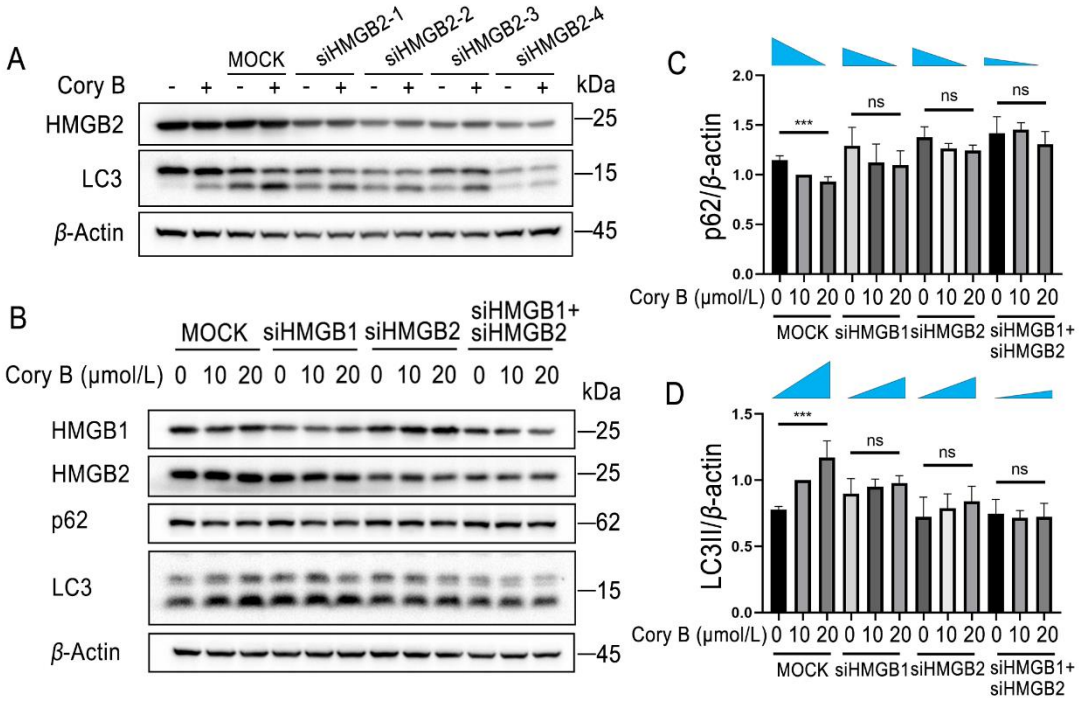

Figure S2 Effects of corynoxine B (Cory B) on autophagy after knocking down *Hmgb1*, *Hmgb2*, or both in N2a cells by siRNA. *Hmgb1* knockout N2a cells were untreated or transfected with non-target and four different pairs of siRNAs for *Hmgb2*. After transfection for 48 h, 50 μmol/L Cory B were added for another 12 h. Cell lysates were subjected to Western blot analysis. Expression levels of HMGB2 and LC3 were analyzed by Western blot. (B) N2a cells were transfected with non-target siRNA, *Hmgb1*/*2* specific siRNA or *Hmgb1*&*2* siRNAs for 48 h followed by indicated concentration of Cory B treatment for another 6 h. Cell lysates were subjected to Western blot analysis. (C, D) Normalized band intensities of p62/*β*-actin and LC3II/*β*-actin from three independent experiments were presented as mean ± SD (***P<0.001; ns, no significance).

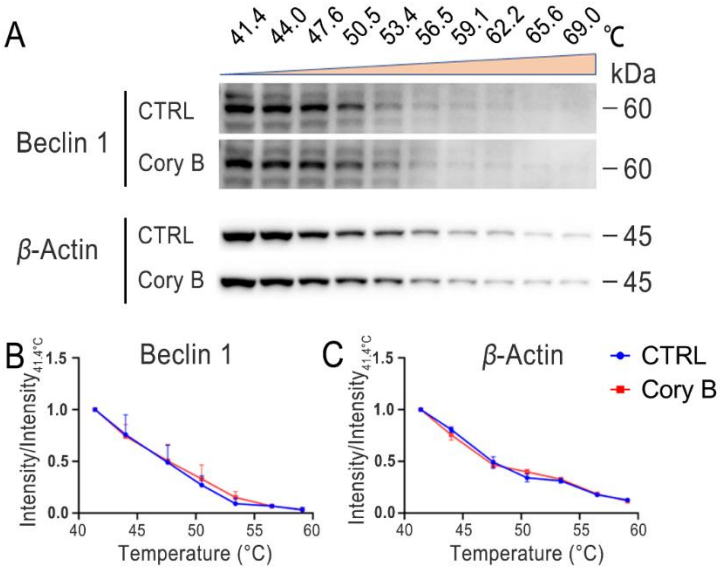

Figure S3 The thermal stability of Beclin 1 did not change after 1 h of corynoxine B (Cory B) treatment. (A) Cellular thermal shift assay (CETSA) was performed in N2a cells. Cell lysates in control group and Cory B treatment group were subjected to Western blot analysis. (B, C) CETSA curves of Beclin 1 or *β*-actin in N2a cells were determined in the absence and presence of Cory B. Normalized band intensities ratio from three independent experiments are presented as mean ± SD.

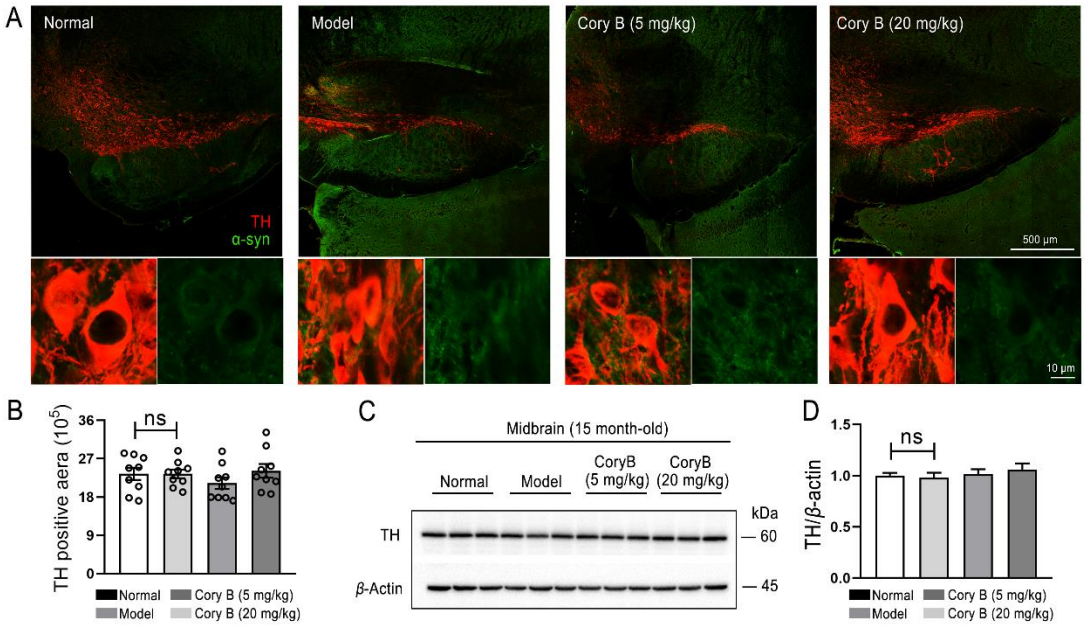

Figure S4 No obvious dopaminergic neuron loss was observed in aged heterozygous A53T a-syn transgenic mice. Corynoxine B (Cory B) reduced α-synuclein (α-syn) in tyrosine hydroxylase (TH) positive neurons. Representative images of TH expression in the midbrain assessed by immunofluorescence in 15-month-old PD transgenic mice. (B) Quantifications of TH in three ventral midbrain regions of 3 mice per group. (C, D) The midbrain of 15-month-old mice were sequentially extracted with Triton-X100 soluble and insoluble buffers, as described in Materials and Methods. The expression levels of TH in Triton-X100 soluble fraction were analyzed by Western blot.