

## Supplementary Data

### **Roflupram exerts neuroprotection via activation of CREB/PGC-1 $\alpha$ signaling in experimental models of Parkinson's disease**

Running Title: Roflupram protects against Parkinson's disease

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# Equal contribution

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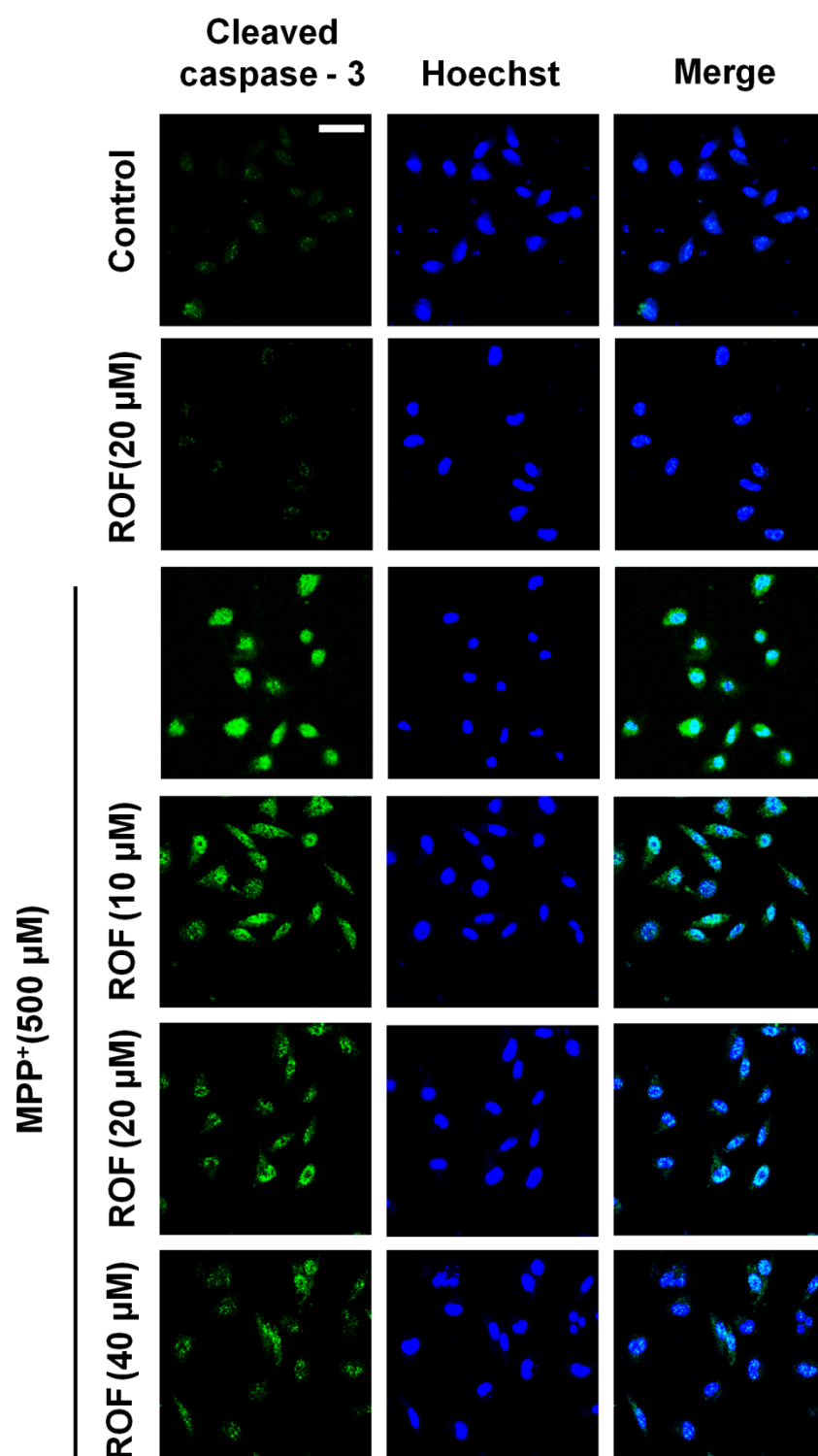
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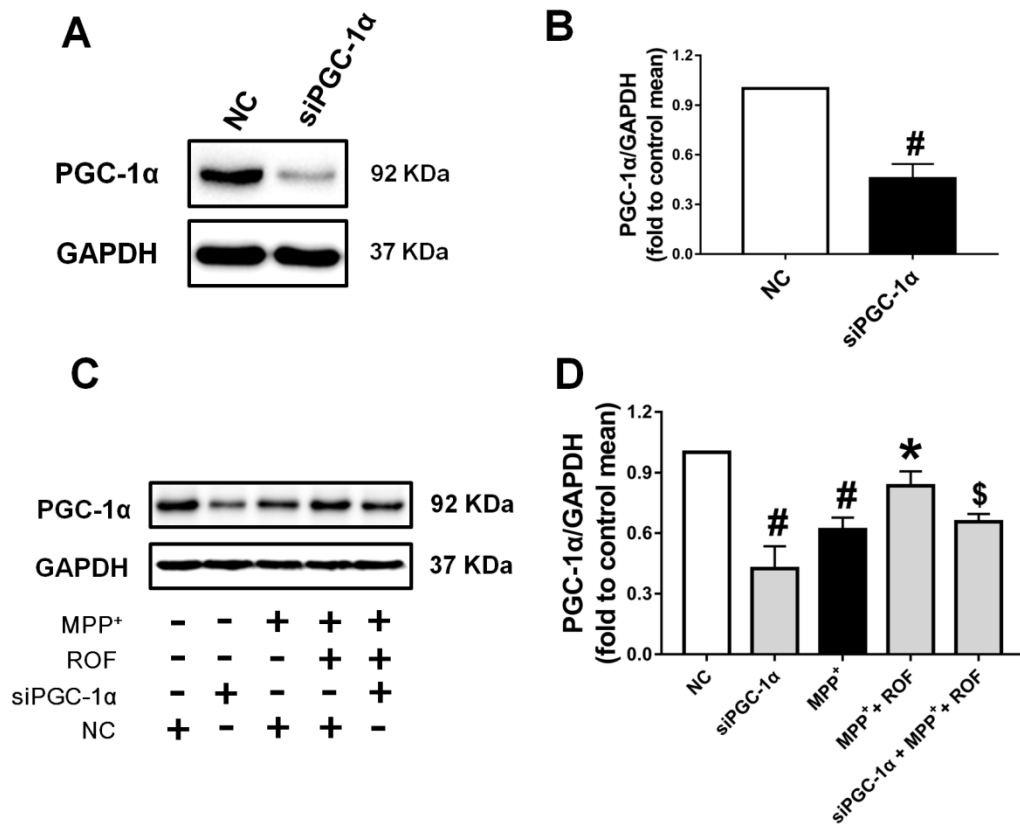
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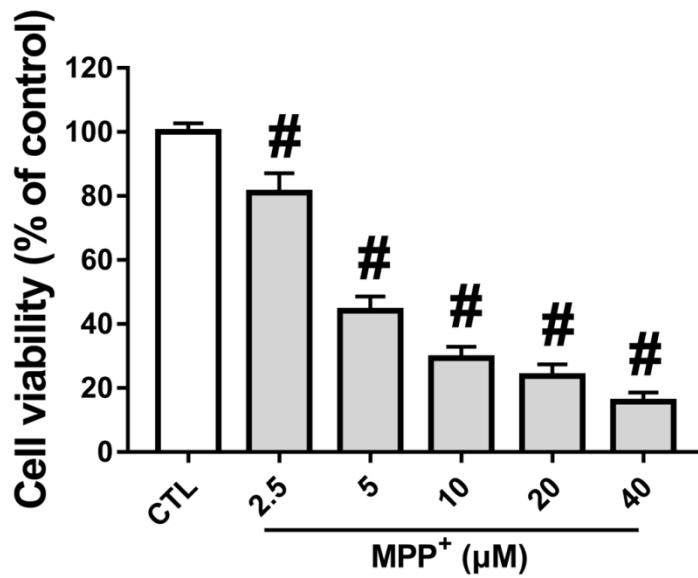
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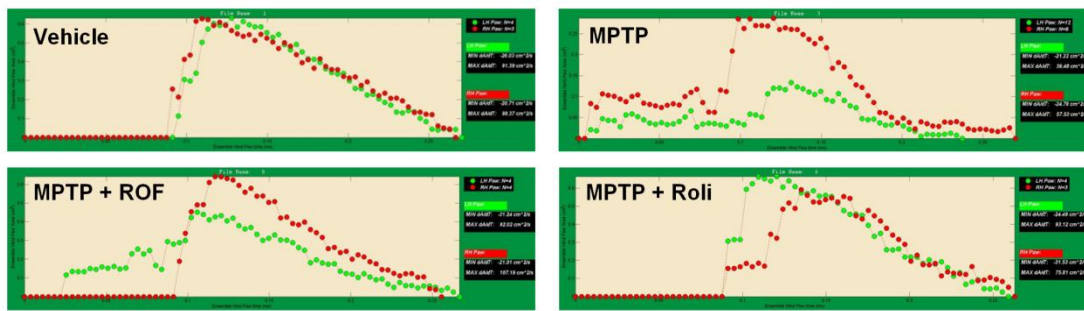
**Fig. S1. ROF decreases the level of cleaved caspase-3 in SH-SY5Y cells treated with MPP<sup>+</sup>.** The SH-SY5Y cells were pretreated with various concentrations of ROF for 1 h, and then stimulated with 500  $\mu$ M of MPP<sup>+</sup> for 48 h. Immunofluorescence was performed with anti-cleaved caspase-3 (green). Hoechst was used to stain the nuclei (blue). The stained cells were photographed using an inverted confocal microscope. Scale bar = 50  $\mu$ m.



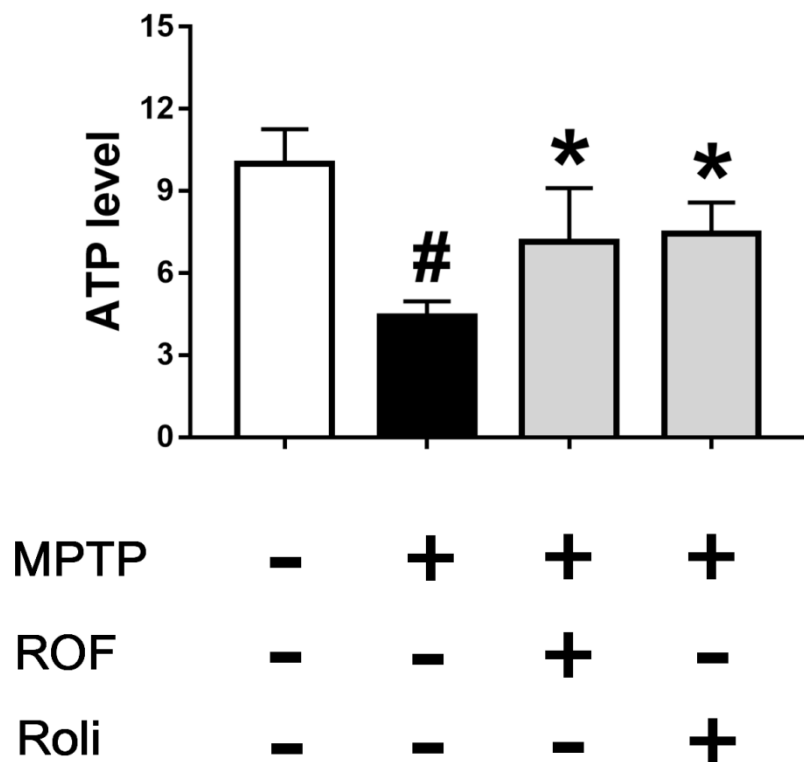
**Fig. S2. Verification of siRNA-mediated knock down of PGC-1 $\alpha$  in SH-SY5Y cells.** (A) Knocking-down efficiency of PGC-1 $\alpha$  siRNA (siPGC-1 $\alpha$ ) (50 nM) in SH-SY5Y cells was verified via detecting the protein expression of PGC-1 $\alpha$  by Western blotting. (B) Densitometric quantification of PGC-1 $\alpha$ /GAPDH in (A). (C) SH-SY5Y cells were transfected with PGC-1 $\alpha$  siRNA or random siRNA for 24 h. Transfected cells were then treated with MPP<sup>+</sup> (500  $\mu$ M) and ROF (20  $\mu$ M) for an additional 24 h. the expression level of PGC-1 $\alpha$  was detected by Western blotting. (D) Densitometric quantification of PGC-1 $\alpha$ /GAPDH in (C). (D) Densitometric quantification of PGC-1 $\alpha$ /GAPDH in (C). Data are presented as mean  $\pm$  SD (n = 5). <sup>#</sup>*P* < 0.05 versus negative control (NC) group. \**P* < 0.05 versus MPP<sup>+</sup>-treated group. <sup>\$</sup>*P* < 0.05 versus MPP<sup>+</sup> + ROF-treated group.



**Fig. S3. MPP<sup>+</sup> could decrease cell viability of LUHMES cells in a concentration-dependent manner.** After 6 days of differentiation, LUHMES cells were treated with MPP<sup>+</sup> (2.5–40 μM) for 48 h, and the cell viability of LUHMES was reduced in a concentration-dependent manner. MPP<sup>+</sup>-induced a comparable level of toxicity at a concentration of 5 μM (56% cell viability reduction). Data are presented as mean ± SD (n = 5) and represent five independent experiments. #*P* < 0.05 versus control group.



**Fig. S4. ROF treatments improved limb coordination and motor coordination in MPTP-treated mice.** The mice were walked for about 5 sec on a transparent treadmill belt at a speed of 30 cm/s. The gait analysis system continuously imaged the underside of the mice. Digital paw prints and dynamic gait signals for each of the 4 limbs were generated by the gait dynamic system (DigiGait Analysis Software Version 14.5). Representative of the hind limbs of mice in each group is shown.



**Fig. S5. ROF increases the level of ATP in the SN of MPTP-induced mice.** The concentration of ATP in the SN of MPTP-induced mice was measured by an enzyme-linked immunoassay kit, and the value was measured by a luminometer. Data are presented as mean  $\pm$ SD (n = 5). #*P* < 0.05 versus vehicle group. \**P* < 0.05 versus MPTP-treated group.

**Movie S1. Representative pole test videos of MPTP-treated mice with and without ROF.** Mice were placed on top of the straight rod and we recorded the time that the mice climbed along the wood to the bottom of the rod.

**Movie S2. Representative rotarod test videos of MPTP-treated mice with and without ROF.** The mice were placed on a rotarod with a rolling speed of 12 rpm, and the duration that the mice stayed on the rotarod were observed and recorded.

**Movie S3. Representative pole test videos of MPTP-treated mice with and without ROF.** The mice were placed in a walking compartment with a width of 7 cm and a length of 30 cm. A high-speed camera at the lower end of the transparent treadmill belt continuously captured the walking images of the mice. The mice walked for about 5 s on the running belt at a speed of 30 cm/s. Digital paw prints and dynamic gait signals for each of the 4 limbs were generated by the gait dynamic system.