Perillyl alcohol attenuates NLRP3 inflammasome activation and rescues dopaminergic neurons in experimental in vitro and in vivo models of Parkinson's disease

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14. Supporting Information

Figure S1: (A): Immunofluorescence analysis of MitoTracker and MFN-1 in HMC-3 microglial cells, (B) CM-H2DCFDA and DHE staining in SH-SY5Y neuroblastoma cells.

Mitochondria is the main source of ROS production in the cell. While stress and any membrane damage can cause robust amount of ROS production. To investigate the plausible role of MFN-1 in the damaged mitochondria and the mitochondrial stabilizing effects of PA and CP (NLRP3 inhibitor) were measured through immunofluorescence **(Fig. S1-A).** PA and CP were found to restore the mitochondrial structure and regulate the levels of MFN-1 in human HMC-3 microglial cells. Similarly, the protective effects of PA were measured in SH-SY5Y human neuroblastoma cells by CM-H2DCFDA and DHE staining. Superoxide levels were markedly reduced in PA treated group compared to LPS+H_2O_2 and MPP^+ treated SH-SY5Y cells suggesting the neuroprotective role of PA and CP directly in the neuronal cells **(Fig. S1-D)**.

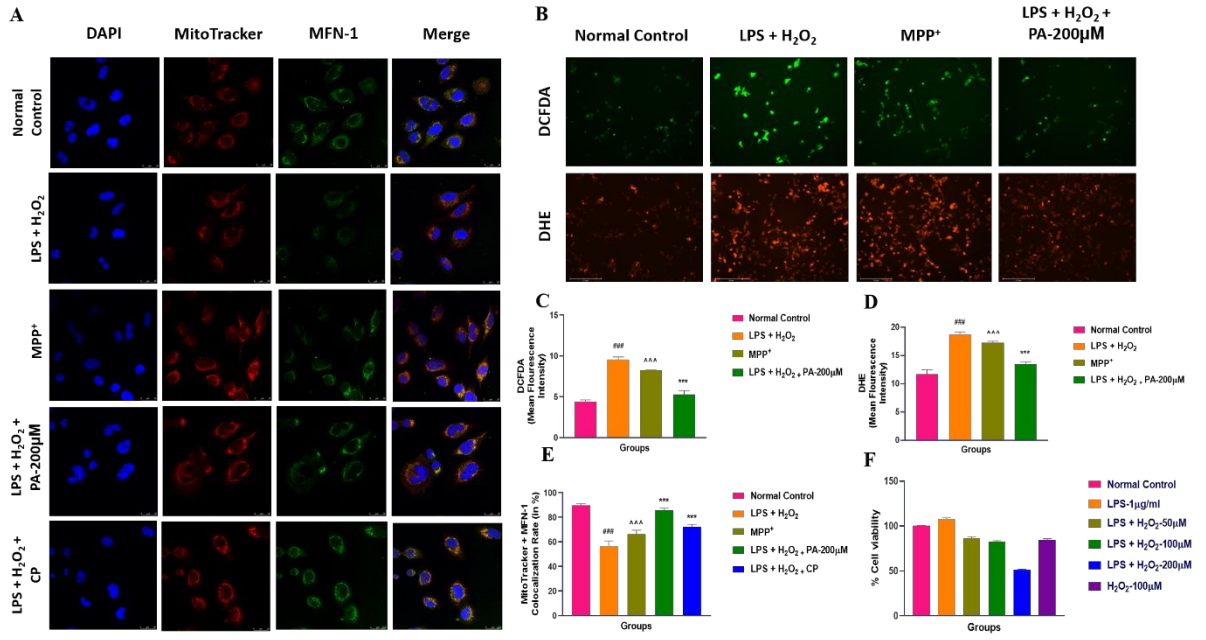

Figure S-1: A) Confocal fluorescent images of cells stained with MitoTracker Red and immunostained with MFN-1 antibody (Green) in HMC-3 microglial cells. **E)** PA and CP attenuate mitochondrial damage caused due to LPS primed H_2O_2 treated cells and stabilizes the

translocation of MFN-1 in the mitochondria. **B, C, D)** PA and CP reduces the intracellular ROS as evident from CM-H2DCFDA staining and DHE staining in SH-SY5Y cells treated with LPS + H_2O_2 and MPP^+. **F)** In vitro % cell viability by Alamar Blue assay in HMC-3 microglial cells, Data reported as % cell viability normalized to control cells. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc analysis where $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ represents control vs. LPS+H_2O_2 group, $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ represents control vs MPP^+ and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ represents LPS+H_2O_2 vs. different treatment group.

Figure S2: Uncropped western blot images of NLRP3, cleavage of pro-caspase-1, pro-IL-1β and pro-IL-18 to its active form caspase-1 p20, active IL-1β, and IL-18 form in the SNpc region of mouse brain (n=4).

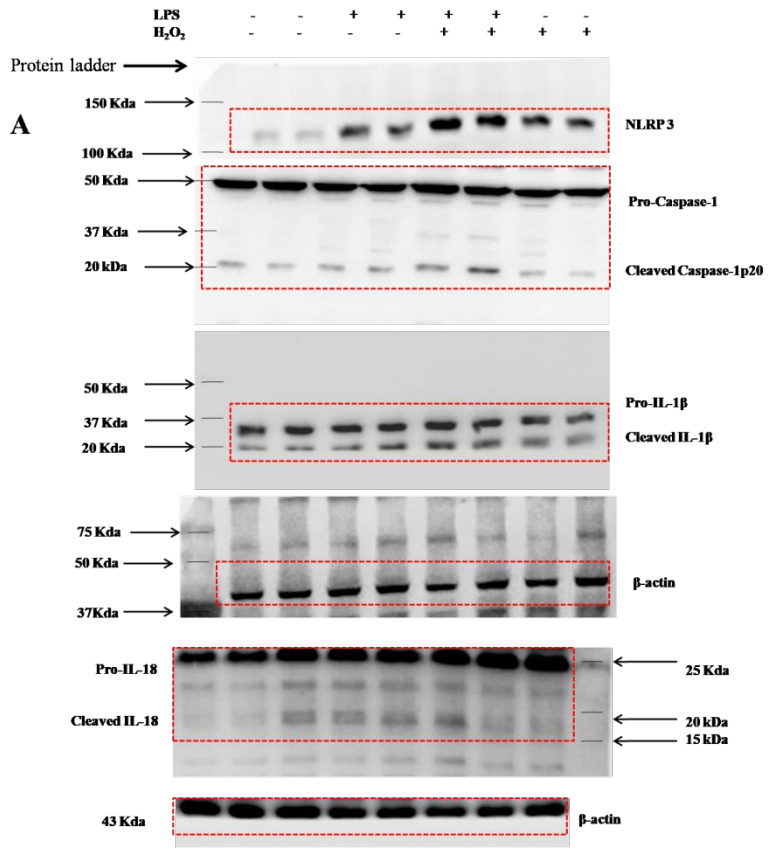

Figure S2 (A): Western blot showing protein expression of NLRP3, cleavage of pro-caspase-1, pro-IL-1β and pro-IL-18 to its active form caspase-1 p20, active IL-1β, and IL-18 form in the SNpc region of mouse brain (n=4).

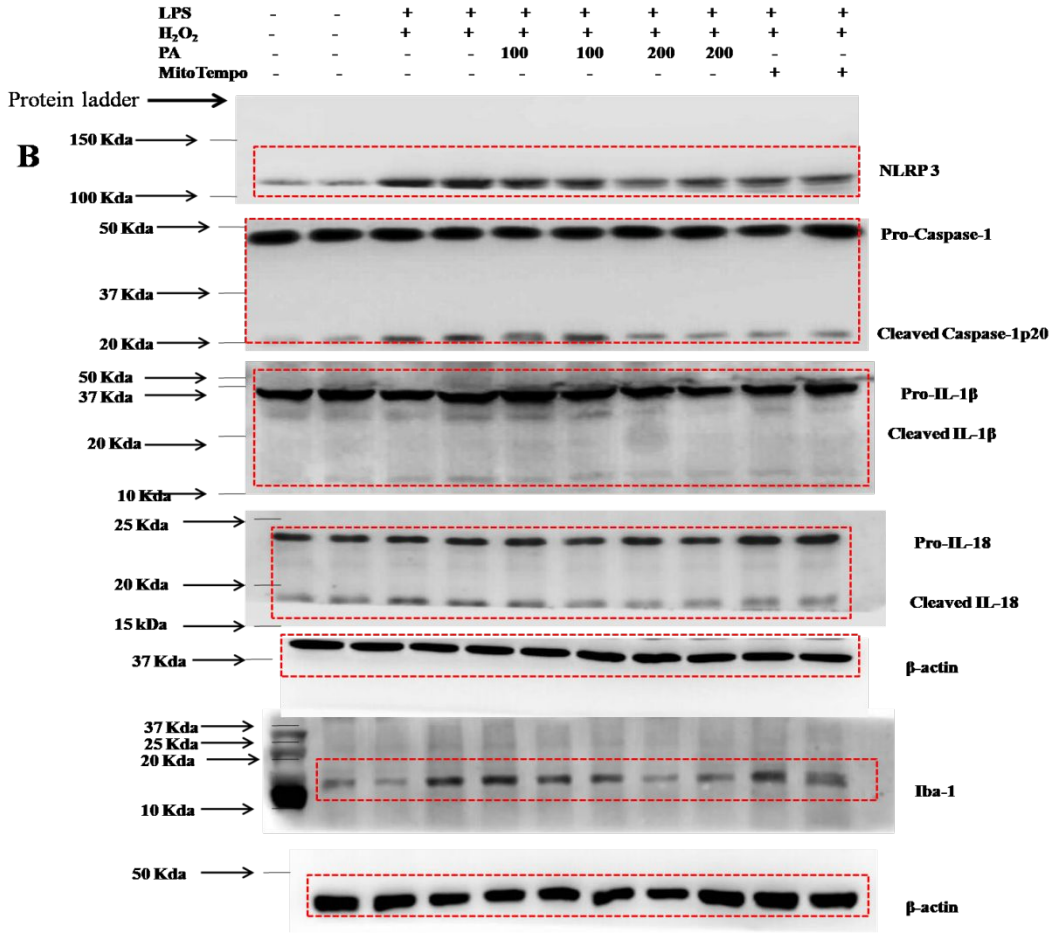

Figure S2 (B): Western blot showing protein expression of NLRP3, cleavage of pro-caspase-1, pro-IL-1β and pro-IL-18 to its active form caspase-1 p20, active IL-1β, and IL-18 form in the SNpc region of mouse brain (n=4).

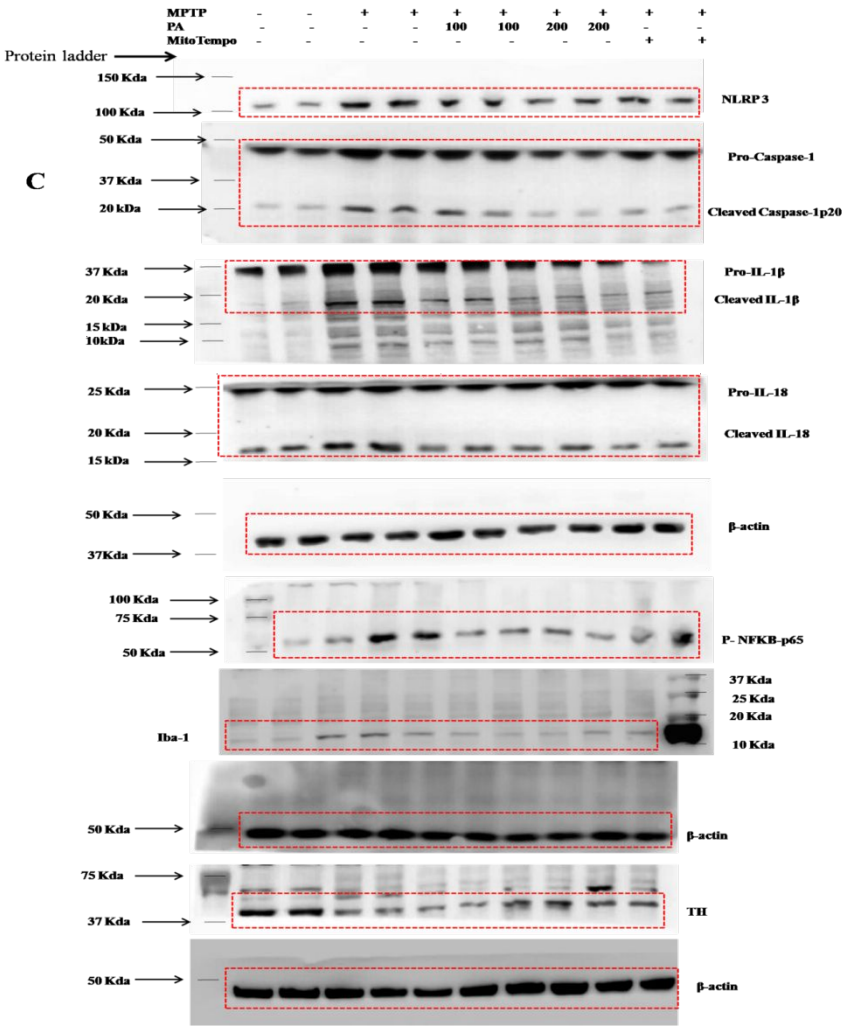

Figure S2 (C): Western blot showing protein expression of NLRP3, cleavage of pro-caspase-1, pro-IL-1β and pro-IL-18 to its active form caspase-1 p20, active IL-1β, and IL-18 form in the SNpc region of mouse brain (n=4).