



SUPPLEMENTARY FIG. S1. MPP+ treatment-induced ROS accumulation, ATP depletion, and neuronal apoptosis. Cell viability was measured by MTT in N-2a cells under different concentrations of MPP+ for 24 h (A) and with 500 μM MPP+ for different times (B), and in mouse primary midbrain neurons under different concentrations of MPP+ for 24 h (C) and with 50 μM MPP+ for different times (D). (E) Cellular ROS were measured by flow-cytometric analysis with DCFH-DA staining under MPP+ treatment. (F) Mitochondrial ROS were measured by flow-cytometric analysis with Mito-Sox staining under MPP+ treatment. (G) SOD2 activity was measured by an SOD Assay Kit under MPP+ treatment. (H) Protein carbonyl content was quantified spectrophotometrically by reacting with 2, 4-DNPH under MPP+ treatment. (I) MDA level was measured by a MDA Assay Kit under MPP+ treatment. (J) ATP level was measured by an ATP Assay Kit under MPP+ treatment. The expression of PARP-1 and caspase 3 was measured by Western blotting in N-2a cells (K) and mouse primary midbrain neurons (L). (M) The mRNA level of Sirt3 was measured in N-2a cells and mouse primary midbrain neurons transfected with Sirt3 siRNA. Quantitative data = mean ± SEM, $n=3$, $^{\#}p<0.001$; $^{**}p<0.01$ or $^{###}p<0.01$; $^{***}p<0.001$ or $^{####}p<0.001$, * represents significance between N-2a cells, while # represents significance between mouse primary midbrain neurons, compared with untreated, paired two-tailed Student's *t*-test. (N) Sirt3 expression was measured by Western blotting in Sirt3 knockout mice. ATP, adenosine triphosphate; MPP+, 1-methyl-4-phenylpyridinium iodide; N-2a, Neuro-2a; PARP-1, poly (ADP-ribose) polymerase-1; ROS, reactive oxygen species; Sirt3, sirtuin 3; SOD2, manganese superoxide dismutase.