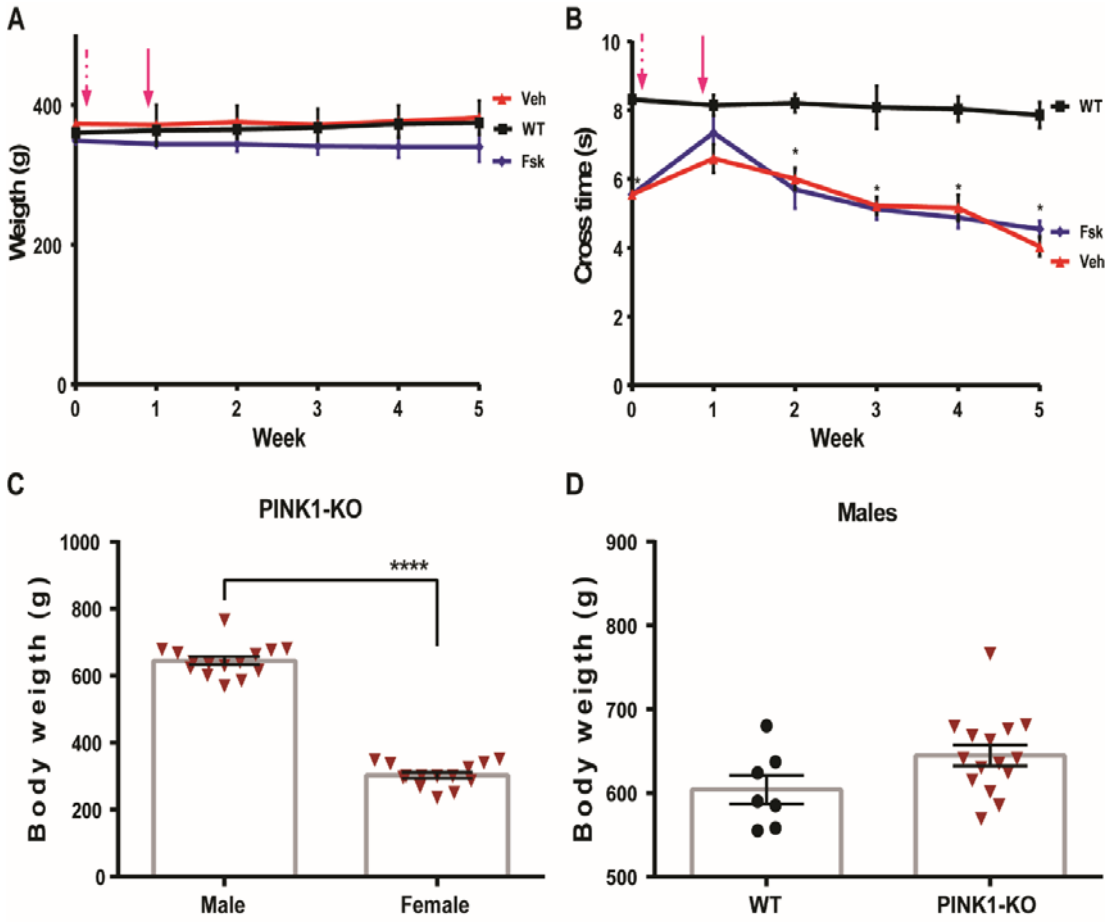
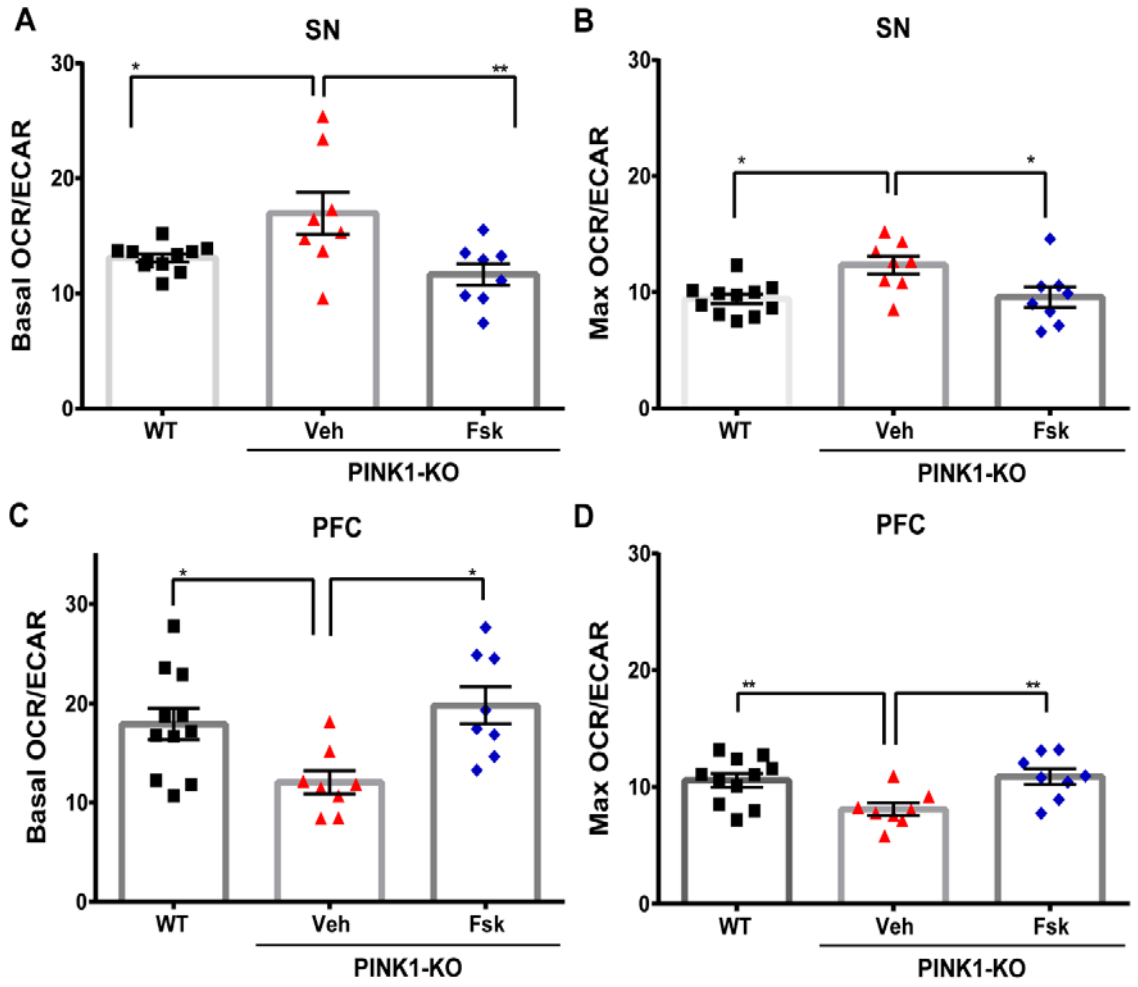
Supplementary Materials

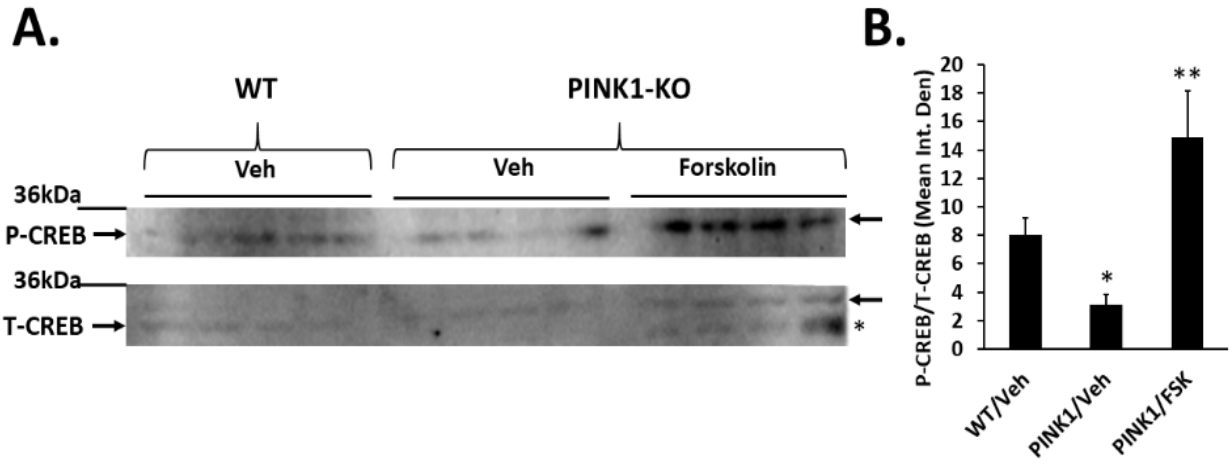
Intraperitoneal Administration of Forskolin Reverses Motor Symptoms and Loss of Midbrain Dopamine Neurons in PINK1 Knockout Rats



Supplementary Figure 1. Forskolin treatment has no effect on the mean body weight and crossing time of PINK1-KO rats. A) Line graph depicting mean body weight of rats throughout the 5 weeks in the same cohort of animals that were used for the washout studies that were conducted to compare the efficacy of Levodopa vs. Forskolin I.P. injections in reversing motor symptoms and loss of grip strength. Up to three animals per group were analyzed for mean body weight for six weeks. B) Line graph depicting mean crossing time on a beam balance per rat in the same cohort of animals that were used in the washout studies. The graph shows the mean quantification of the beam balance crossing times from three adult rats per group for six weeks. C) Mean body weight comparison in 10-month-old PINK1-KO males and females used for the motor and behavioral studies reported in Figs. 1-4. D) Mean body weight comparison in 10-month-old PINK1-KO and WT males used for the motor and physiological studies reported in Figs. 1-4. For both A and B: The dashed arrow points to the start of the treatment whereas the solid arrow indicates the end of I.P. administration with Forskolin at 1.6 mg/kg for ten days with I.P. injections that occurred on days 1, 3, 5, 7, and 9. n=3 per group, addition (Mean ± SEM. *p≤0.05, One-way ANOVA followed by Tukey's Multiple Comparisons test). C, D) WT and PINK1-KO untreated male rats, (Mean ± SEM. *p≤0.05, unpaired t-test). n=7-15 per group. Treatment groups: WT, Long Evans wt rats treated with PBS-DMSO 10%; Fsk, Long Evans PINK1-KO rats treated with Forskolin; Veh, Long Evans PINK1-KO rats treated with PBS-DMSO 10%.



Supplementary Figure 2. Oxidative phosphorylation rate relative to glycolysis in the basal state (basal OCR/ECAR) and following FCCP was added (max OCR/ECAR). By employing an XF24^{e} Seahorse BioAnalyzer, different bioenergetic parameters (oxygen consumption rates/OCR or Extracellular Acidification Rates, ECARs) were analyzed in brain slices from the SN and PFC from 10-month-old female (F) and male (M) WT rats treated with vehicle (WT) or from PINK1-KO rats treated with vehicle (Veh) or with 1.6mg/kg of Forskolin for ten days (Fsk). Bar graphs showing the mean ratio of the basal OCR/ECAR shown in A) or maximal OCRs/ECARs shown in B) in the SN from 10-month-old WT or PINK1-KO rats treated with the indicated pharmacological conditions. C) Bar graphs showing the mean basal ratios of OCR/ECAR or D) maximal OCR/ECAR in the PFC from 10-month old WT or PINK1-KO rats treated with the indicated pharmacological conditions. For both C and D, note that the loss of endogenous PINK1 induces an increase in glycolysis compared to oxidative phosphorylation following FCCP addition to collapse the mitochondrial transmembrane potential, whereas Forskolin treatment increases the OCR/ECAR ratio similar to WT rats (Mean ± SEM. *p≤0.05, One-way ANOVA followed by Tukey's multiple comparisons test, n = 8-11 animals per treatment group).



Supplementary Figure 3. Intraperitoneal administration of Forskolin reverses the loss in CREB phosphorylation in PINK1-KO rats. A)Western blot of lysates from *substantia nigra* derived from 10 month old wild-type (WT) or PINK1-KO rats intraperitoneally treated with Vehicle (Veh) or Forskolin (FSK) and immunoblotted for phosphorylated CREB (Ser 133) or for total CREB. Arrows indicate the bands specific for p-CREB (top western blot) or total CREB (Bottom western blot). Asterisk represents a non-specific immunoreactive band. B) Bar graph depicting the mean denstiometry analysis of p-CREB normalized to total CREB. (Mean ± SEM. *p≤0.05, One-way ANOVA followed by Tukey's multiple comparisons test, n = 4 animals per treatment group).