# Supporting Information

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## SI Materials and Methods

**Electron Microscopy Studies.** After euthanasia by $CO_2$ inhalation, three *PINK1*$^{-/-}$ mice and three wild-type mice at the age of 3 months were transcardially perfused with 20 ml of PBS (PBS) and 200 ml 2.5% glutaraldehyde/ 2% paraformaldehyde in PBS (pH 7.4). Dorsal striata were removed and postfixed in 2.5% formaldehyde / glutaraldehyde in 0.1M Sodium cacodylate buffer (Electron Microscopy Sciences), pH 7.4 for 18 h at 4°C. Tissue pieces in immersion fixative were cut into 1–2 mm cubes and embedded in epon by routine protocol for embedding in resin with 1% osmium tetroxide fixation and 1% uranyl acetate counterstaining. Sections were cut at ultrathickness (~60–80 nm) and photographed at 4,600× on Tecnai G2 Spirit BioTWIN (Philips) to count the number of mitochondria. The number of mitochondria was determined by counting in images of five different fields for each mouse section, which corresponds to 0.057 $mm^2$. Mitochondrial morphology was determined at 18,500× on the same electron microscopy. Each area (size) of mitochondria in images of five different fields for each mouse is measured by ImageJ version 1.37 (NIH). Mitochondrial sizes are classified in seven size groups and the numbers in *PINK1*$^{-/-}$ mice and wild-type mice in the same size group are compared statistically. Both total numbers and the number of each size group of mitochondria in *PINK1*$^{-/-}$ mice and wild-type mice are finally set in 100 $\mu m^2$.

**Western Blot Analysis.** Mice were euthanized by $CO_2$ inhalation; cortices and striata were dissected following regular methods. Lysis was performed by three 15-second sonication shots at a setting of 60 (Fisher sonic dismembrator model 300) with a minimum 60 second cooling on ice in between shots in buffer A (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 0.1% Sodium Deoxycholate, 1% SDS, 0.5 mM PMSF, Phosphatase Inhibitors Mixture 1 and 2 (Sigma), and complete protease inhibitor Mixture (Roche, Indianapolis, IN)). After homogenization, samples were rotated at 4°C for 30 min for complete lysis. Protein concentrations were assayed using the Coomassie plus protein assay reagent (Pierce) using the manufacturer's directions. Proteins were separated on Nupage 4–12% Bis-Tris gel and electroblotted onto a nitrocellulose membrane (Protran). Primary antibodies used were anti-porin (Calbiochem), anti-AIF (Chemicon), anti-ICDH (Biogenesis) and anti Human total OXPHOS Complexes Detection Kit (Mitoscience), anti-GFAP (Sigma Aldrich), anti-Mn-SOD (Stressgen), anti-Cu-Zn-SOD (biosensis), anti-catalase (Calbiochem), and anti-G-6-PDH (Sigma Aldrich). Secondary antibodies were fluorescent labeled anti-mouse and anti-rabbit secondary antibodies (Licor System). Fluorescence was visualized on a Licor System laser scanner, and images were analyzed and quantified using the software provided by the manufacturer.

**Mitochondria Respiration Assay.** The metabolic capacity of mitochondria in *PINK1*$^{-/-}$ mice and wild-type controls was measured similarly to what we previously described (1). Briefly, mitochondria were isolated from cortical or striatal homogenates and resuspended at a final concentration of 0.4 mg/ml protein in 0.5 ml of special buffer (120 mM KCl, 3 mM Hepes, 1 mM EGTA, 5 mM $KH_2PO_4$, 1% wt/vol of BSA, pH 7.2) and assayed for respiration using each substrate for complex I (glutamate/malate), II (succinate), III/IV (TMPD ascorbate). ADP was added in limiting amounts, and state 3 respiration was measured. Following depletion of ADP, state 4 respiration was measured. Mitochondrial respiration was determined using a platinum electrode in a 0.35 ml chamber. For striatal samples, striata of two animals per genotype were pooled together and measured each day. Experimenter was blind to genotype.

**Enzymatic Assays.** Citrate Synthase activity in the striatum was measured as previously described (2). Briefly striata were dissected using regular methods and then sonicated on ice by two 15-second shots in non-denaturing buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Triton, protease inhibitors mixture (Roche Diagnostic)). Samples were spun at 21,000 × g for 2 min, protein content assayed using Coomassie plus protein assay reagent (Pierce), and concentrations were equalized to 1 mg/ml. The reaction was started with the addition of 995 $\mu$l of incubation buffer (0.1M Tris·HCl pH 8.0, 10 mM Triethanolamine, 0.25% Triton X-100, 120 $\mu$M AcCoA, 500 $\mu$M Oxaloacetate, 100 $\mu$M DTNB) to 5 $\mu$l of lysates and monitored spectrophotometrically at 412 nm for 200 s on a Beckman Coulter DU-800 spectrophotometer (Carlsbad, CA). Samples were run in duplicate. Aconitase activity was measured by monitoring absorbance at 340 nm in a freshly prepared reaction mix containing 0.2 mM $NADP^+$, 5 mM sodium citrate, and a 1.8 unit/ml concentration of isocitrate dehydrogenase in Tris·HCl pH 7.4 plus MnCl2 buffer. To start the assay, 150 $\mu$l of freshly prepared assay buffer was added to 50 $\mu$g of striatal preparation. Measurements at 340 nm were recorded in a 96-well plate wells at intervals of 12 seconds. Aconitase activity was calculated from the linear increase in absorbance at 340 nm. Activity was calculated by using an extinction coefficient for NADPH of 6.22 $mM^{-1}{\cdot}cm^{-1}$ assuming the conversion of one molecule of citrate to one molecule of NADPH via isocitrate dehydrogenase and then normalized to the citrate synthase activity of the same sample. Glucokinase activity was measured as the formation of NADPH at 340 nm as described earlier after the addition of 100 $\mu$l of a freshly prepared reaction mix (75 mM Tris, pH 9.0, 20 mM $MgCl_2$, 4 mM ATP, 12 mM $\beta$-D-glucose, 1 mM $\beta$-$NADP^+$, 20 U/ml G6PDH) to 10 $\mu$g of striatal preparation. Malate dehydrogenase activity was followed as the formation of NADH at 340 nm as described earlier after the addition of 100 $\mu$l of freshly prepared assay buffer (200 $\mu$M Oxaloacetate, 100 $\mu$M NADH in PBS) to 10 $\mu$g of striatal preparation. Complex IV activity was measured as previously described (3), and the Cytochrome c oxidase activity determined by following the oxidation of cytochrome *c* at 550 nm ($\varepsilon$ M = 18.500) in a buffer composed of 30 mM $KH_2PO_4$, pH 7.4, 1 mM EDTA, 56 $\mu$M cytochrome *c*, and 5 $\mu$g mitochondrial proteins.

**JC-1 Assay.** Mitochondria (25 $\mu$g/ml) were incubated at 25°C for 15 min in the presence of 200 nM JC-1 in an assay buffer supplied by manufacturer. Fluorescence emission spectra (500–650 nm) were then recorded on AlexaFluor fluorometer using 495 nm excitation wavelength. To check the effect of oxidative stress mitochondria were previously incubated 15 min in the presence of 100 $\mu$M $H_20_2$, and emission spectra recorded and normalized to the basal conditions. Total depolarization by the protonophore FCCP (0.5 $\mu$M) was used as positive control.

**Mitochondria Swelling Assay.** Measures were completed on mitochondria purified following the previous method except that in the last step mitochondria were resuspended in swelling buffer (0.2 M sucrose, 10 mM Tris-Mops, pH 7.4, 5 mM succinate, 1 mM Pi). Swelling was followed as a function of light scattering

at 545 nm on a plate reader (Benchmark plus, Bio-Rad) by using the kinetic mode of the software provided by the manufacturer. Measures were taken every 20 seconds with 10 seconds strong shaking in the interval to avoid mitochondria deposition at the bottom of the well. A volume equivalent to 100 $\mu$g of mitochondrial protein was deposited at the bottom of the well and the swelling reaction was started by the addition of 150 $\mu$l of swelling assay buffer containing 100 $\mu$M $Ca^{2+}$.


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3. Yoshino H, Nakagawa-Hattori Y, Kondo T, Mizuno Y (1992) Mitochondrial complex I and II activities of lymphocytes and platelets in Parkinson's disease. *J Neural Transm Park Dis Dement Sect* 4:27–34.


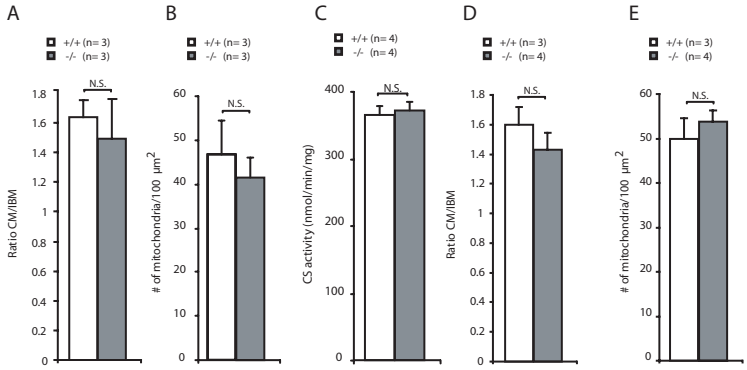


**Fig. S1.** (*A*) Equivalent outer membrane/ inner boundary membrane ratio at 3–4 months in *PINK1*$^{-/-}$ mice and WT controls account for unchanged equilibrium between these two suborganellar compartments. (*B*) Similar numbers of mitochondria in an area of 100 $\mu$m$^2$ in the striatum at 3–4 months in *PINK1*$^{-/-}$ mice and WT controls ($n = 3$, $P > 0.05$). (*C*) Normal citrate synthase activity in total striatal lysates in *PINK1*$^{-/-}$ mice and WT controls at 3–4 months (372 $\pm$ 11 versus 366 $\pm$ 12 nmol/min/mg, $n = 4$, $P > 0.05$). (*D*) Equivalent outer membrane/ inner boundary membrane ratio account for unchanged equilibrium between these two suborganellar compartment at 22–24 months in *PINK1*$^{-/-}$ mice and WT controls ($n = 3$–4, $P > 0.05$). (*E*) Similar numbers of mitochondria in an area of 100 $\mu$m$^2$ in the striatum at 22–24 months in *PINK1*$^{-/-}$ mice and WT controls ($n = 3$–4, $P > 0.05$).