Supporting Information

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SI Materials and Methods

FACS. Cells were transfected with plasmid DNA using Effectene (Invitrogen). Two days following transfection, the cells were split and cultured for 3 d in culture medium containing 400 μg/mL G418 (Sigma). After G418 selection, cells were trypsinized and resuspended in sorting medium (1× HBSS and 10% FBS). After sorting, cells were cultured in medium containing 1× triple antibiotic (GIBCO) until the next split. For membrane potential measurements, 4×10^5 cells were stained with 100 nM tetramethylrhodamine ethyl ester (TMRE) (Molecular Probe) in PBS (7.4) for 1 h at 37 °C, trypsinized, and resuspended in sorting medium for FACS analysis.

Fluorescence Microscopy. Cells for fluorescence microscopy were transfected using Fugene 6 (Roche). Cells were fixed with 4% paraformaldehyde (EMS) in PBS, treated with 0.15% TritonX-100 and blocked with 10% BSA. Cells were incubated with rabbit anti-Tom20 (Santa Cruz), mouse anti-FLAG (Invitrogen) or mouse anti-COXI (Invitrogen) for 2 h, washed with 10% BSA three times, incubated with AlexaFluor Goat anti-rabbit or anti-mouse IgG (Invitrogen) for 1 h, and washed with PBS for 10 min two times. The samples were stored in PBS and imaged on a Zeiss LSM510 microscope (63×/1.4 Oil DIC Plan Apo objective, 40×/1.3 Oil Plan Neo-Fluar objective). For assessment of membrane potential, cells were incubated with 2.5 nM of TMRE (Invitrogen) in culture medium for 1 h and imaged.

Western Blot. A 3.5-cm-diameter culture well of SH-SY5Y, 143B, cytochrome *c* oxidase subunit I gene (COXICA65), Cytb3.0, HeLa, Parkin M, and Parkin H cybrid cells were used to extract the whole-cell lysate with 1× Tris-Glycine sample buffer (Invitrogen) plus 1% β-mercaptoethanol and boiling for 30 min. Mouse tissues were collected and homogenized with RIPA buffer (Pierce) plus protease inhibitor (Roche). Protein concentration was measured by NanoDrop (ND-1000 spectrophotometer, Thermo Scientific). Protein samples were separated on 4 to 12% Tris-Glycine gel (Invitrogen) and blotted onto nitrocellulose membrane (Invitrogen). Rabbit anti-Parkin (PRK8, Santa Cruz), rabbit anti-GAPDH (Sigma), mouse anti-actin (Sigma), and rabbit anti-PINK1 (175-250, Novus Biologicals) were used for Western blot. ECL Plus Western Blotting Detection Reagents (GE Healthcare) were used for detection.

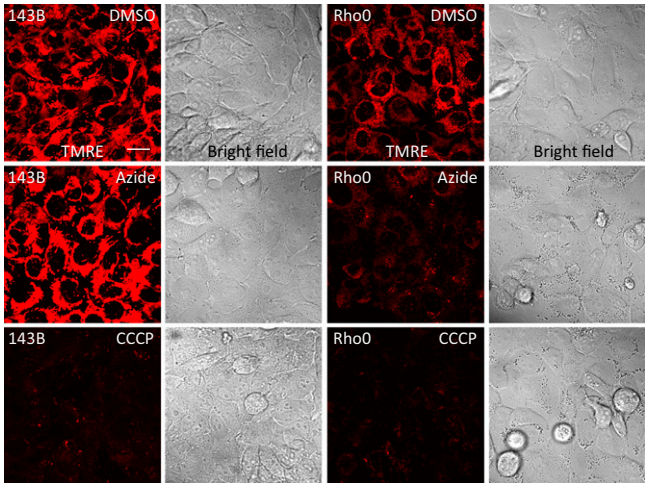

Fig. S1. TMRE intensity of 143B and Rho0 cells. The 143B and Rho0 cells were stained using 2.5 nM of TMRE (red) for 1 h and treated with DMSO, 100 μM sodium azide, or 10 μM carbonyl cyanide m-chorophenylhydrazone (CCCP) in medium containing 2.5 nM of TMRE, respectively, for 4 h. All TMRE images were taken using the same confocal microscope setting. (Scale bar, 20 μm.)

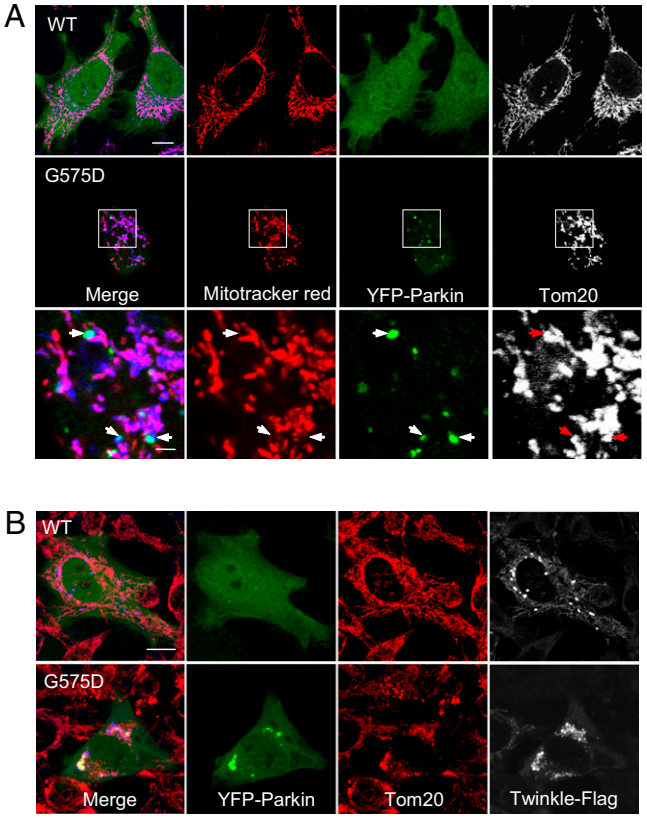

Fig. S2. YFP-Parkin accumulates on mitochondria following loss of mitochondrial DNA integrity. HeLa transiently expressed YFP-Parkin and Flag-tagged wild-type Twinkle or Flag-tagged G575D mutant Twinkle for 5 d. (*A*) Cells were stained with MitoTracker red, fixed, and immunostained for Tom20 (mitochondria, white; blue in merged images). The arrows indicated the mitochondria enclosed by YFP-Parkin lost MitoTracker red signal. (*B*) Cells were immunostained for Tom20 (mitochondria, red) and Flag (Twinkle, white; blue in merged image). (Scale bars, 10 μm; 5 μm in the magnified images.)

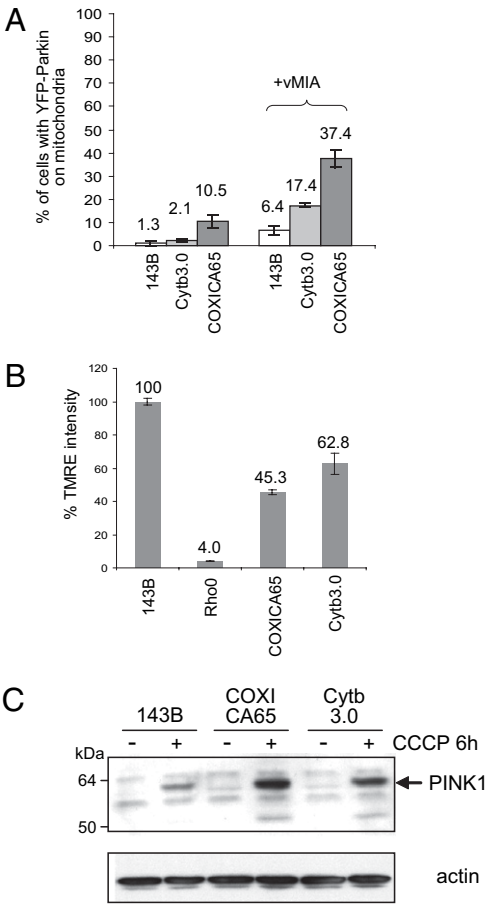

Fig. S3. Parkin translocation in 143B, COXICA65, and Cytb3.0. The 143B, COXICA65, and Cytb3.0 cybrid cells were transfected with YFP-Parkin, fixed, and immunostained for Tom20. (*A*) Cells were scored for YFP-Parkin on mitochondria in the presence or absence of vMIA-myc. Greater than 60 cells were counted in each sample. The mean and SD were calculated from three experiments. (*B*) Quantitative FACS analysis of TMRE intensity. 143B, Rho0, COXICA65, and Cytb3.0 cells were stained with TMRE. Twenty-thousand cells were analyzed for each sample. The percentages of TMRE intensity were normalized to that of 143B cells (=100%). The mean and SD were calculated from three replicates. (*C*) The whole-cell lysate was extracted from cells treated without or with 20 μM CCCP for 6 h. Forty-five micrograms of proteins from each sample was loaded.

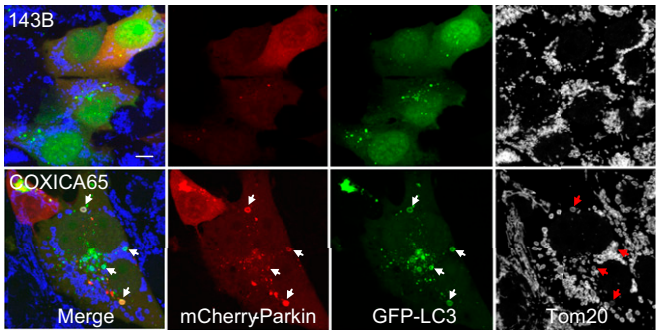

Fig. S4. LC3 colocalized with Parkin on mitochondria in COXICA65 cybrid cells. The 143B and COXICA65 cells were cotransfected with mCherry-Parkin (red) and GFP-LC3 (green), fixed, and immunostained with Tom20 (white, blue in merged images). The arrows indicate GFP-LC3 signal colocalized with mCherry–Parkin on mitochondria. (Scale bar, 10 μm.)

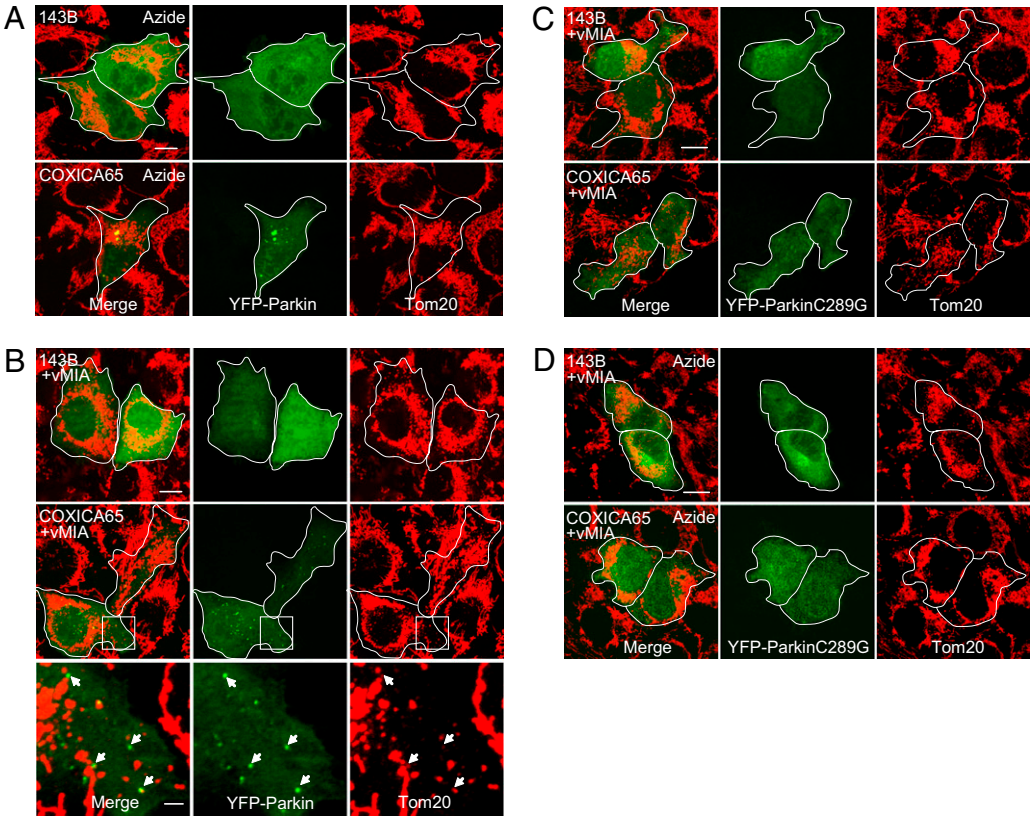

Fig. S5. Parkin patient mutant did not translocate to mitochondria in COXICA65. The parental 143B and COXICA65 cybrid cells transfected with YFP-tagged wild-type Parkin (green) (*A*), wild-type Parkin plus vMIA-myc (*B*), or C289G mutant Parkin plus vMIA-myc (*C* and *D*) were incubated without (*B* and *C*) or with 100 μM sodium azide (*A* and *D*) for 4 h. Cells were fixed and immunostained for Tom20 (mitochondria, red). Arrows in *B* represent YFP-Parkin localized to a subset of mitochondria in zoom image of white box of COXICA65 cells. (Scale bars, 10 μm.)

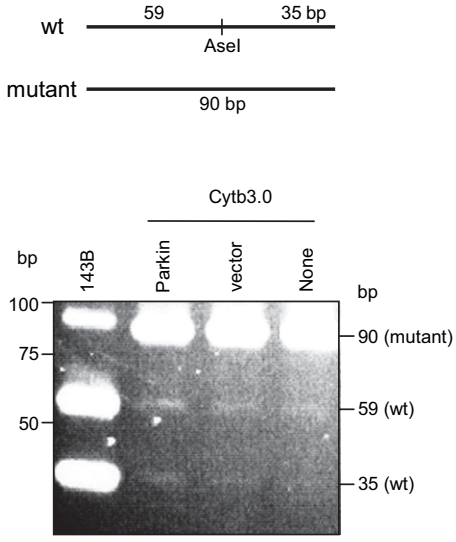

Fig. S6. Restriction fragment-length polymorphism analysis of PCR (PCR-RFLP) of Cytb3.0 cybrid cells. Cytb3.0 cybrid cells with ~90% mutant mtDNA (4-bp deletion in cytochrome *b* gene) were transfected with YFP-Parkin (Parkin), YFP vector (vector), or left untransfected (None). The transfected cells were enriched with YFP signal by FACS following transfection for 120 d. The 94-bp and 90-bp fragments were amplified from wild-type and mutant mitochondrial DNA, respectively. Following AseI digestion, the wild-type mtDNA (which possess one AseI site) showed 59- and 35-bp fragments and mutant mtDNA (which possess no AseI sites) showed 90-bp fragment. The 59- and 35-bp fragments of wild-type mtDNA in Cytb3.0 cybrid cells transfected without (None) or with YFP-Parkin or vector were observed under long exposure and displayed similar amounts of wild-type mtDNA.

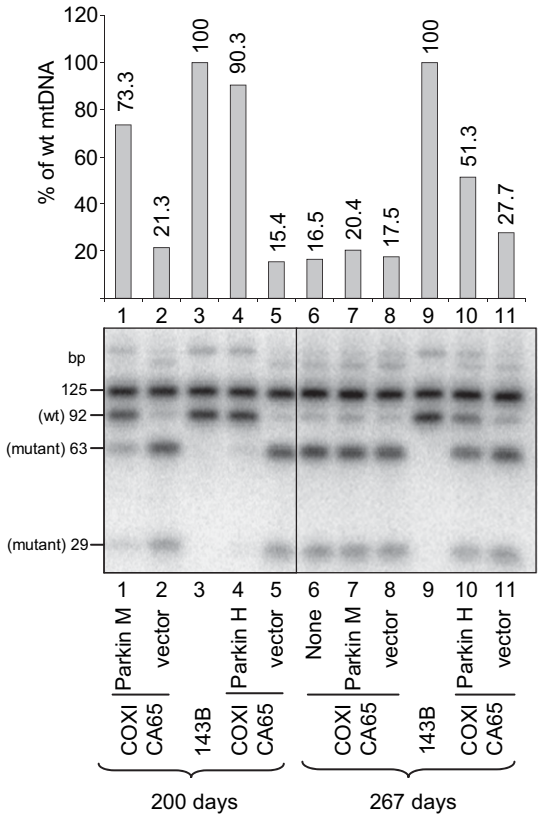

Fig. S7. Quantification of ^{32}P-labeled PCR-RFLP. The samples analyzed in Fig. 4 *C* and *E* were labeled with [α-^{32}P] dCTP at the last cycle of PCR. PCR products were run on 10% polyacrylamide gels (*Lower*) and ^{32}P radiation was detected using the phosphoImage system. The intensity of each band was quantified and normalized to intensity of 125-bp fragment for each sample. The percentage of wild-type mtDNA (*Upper*) was calculated by dividing the 92-bp fragment intensity by the sum of the 92-bp, 63-bp, and 29-bp fragment intensities.

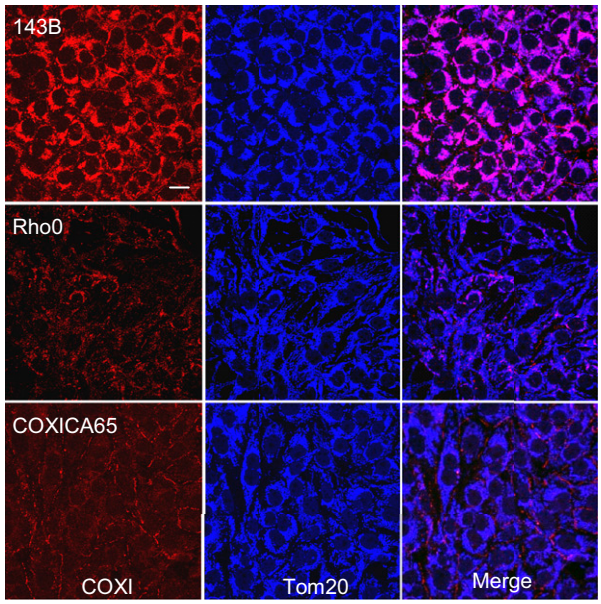

Fig. S8. COXI expression in 143B, Rho0 and COXICA65 cybrid. Cells were fixed and immunostained for Tom20 (blue) and COXI (red). All images were scanned using the same confocal settings as were used in Fig. 5*B*. (Scale bar, 20 μm.)