Supporting Information

Zhou *et al.* 10.1073/pnas.0802814105

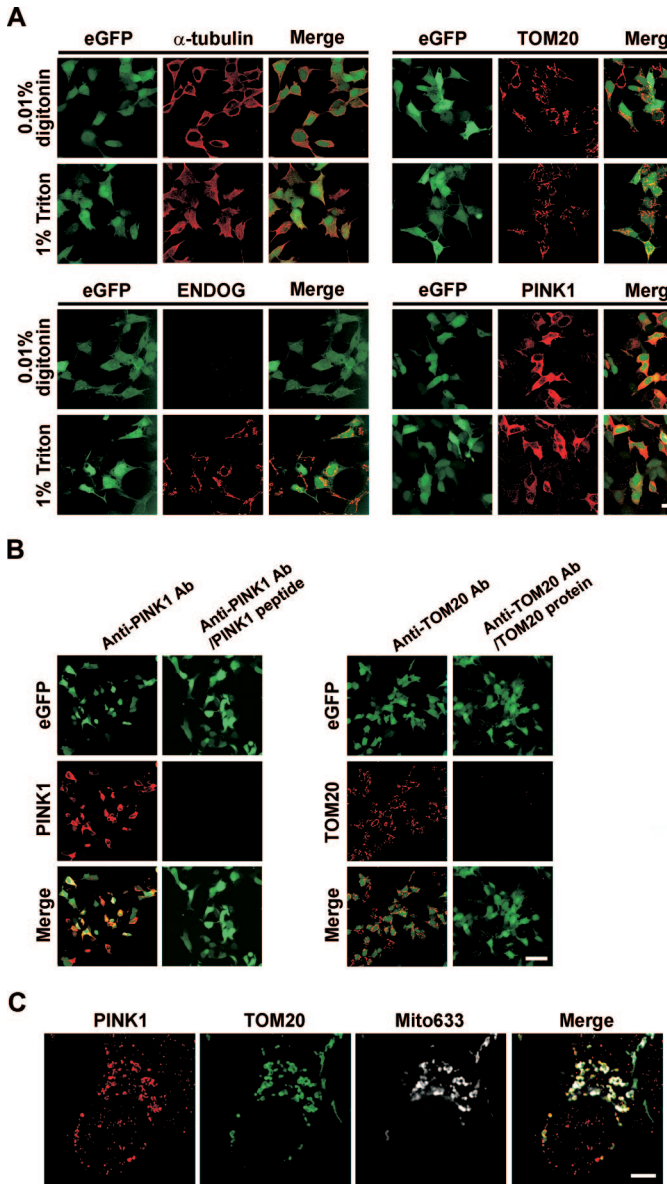

Fig. S1. (*A*) SH-SY5Y cells overexpressing PINK1 are treated and immunostained as in Fig. 1 by using the anti-PINK1$_{175–250}$ antibody. Scale bar, 50 μm. (*B*) SH-SY5Y cells overexpressing PINK1 are immunostained with the anti-PINK1$_{175–250}$ antibody and the anti-TOM20 antibody (red), respectively. For these two antibodies, PINK1 blocking peptide and TOM20 recombinant are used to confirm that immunostaining is abolished in the presence of an excess of antigen. eGFP is used as a general cell marker (green). Scale bar, 50 μm. (*C*) In SH-SY5Y cells expressing PINK1 stably, PINK1 immunofluorescent signal (red) colocalizes with TOM20 (green) and MitoTracker-Deep Red 633 (Mito633) (white). Scale bar, 5 μm.

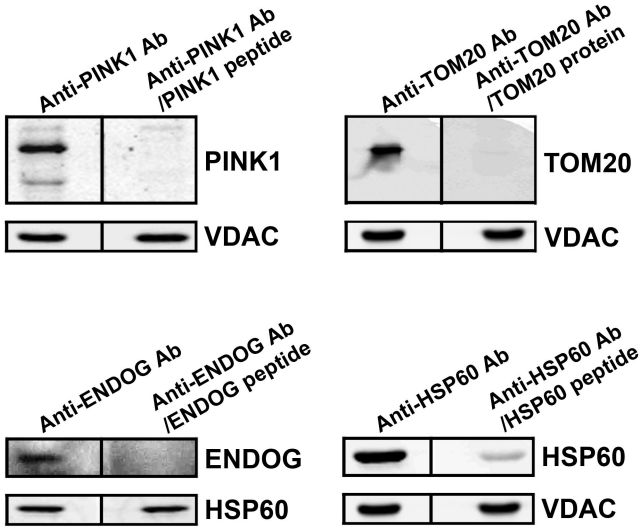

Fig. S2. Confirmation of antibody specificities. Mitochondrial fractions from SH-SY5Y cells overexpressing PINK1 are used for Western blot analyses using the anti-PINK1$_{175–250}$, anti-TOM20, anti-ENDOG, or anti-HSP60 antibodies. For each of these antibodies, their respective blocking peptides or recombinant proteins are used to confirm that the specific immunoreactive bands are abolished in the presence of an excess of antigen. VDAC and HSP60 are used as protein loading controls.

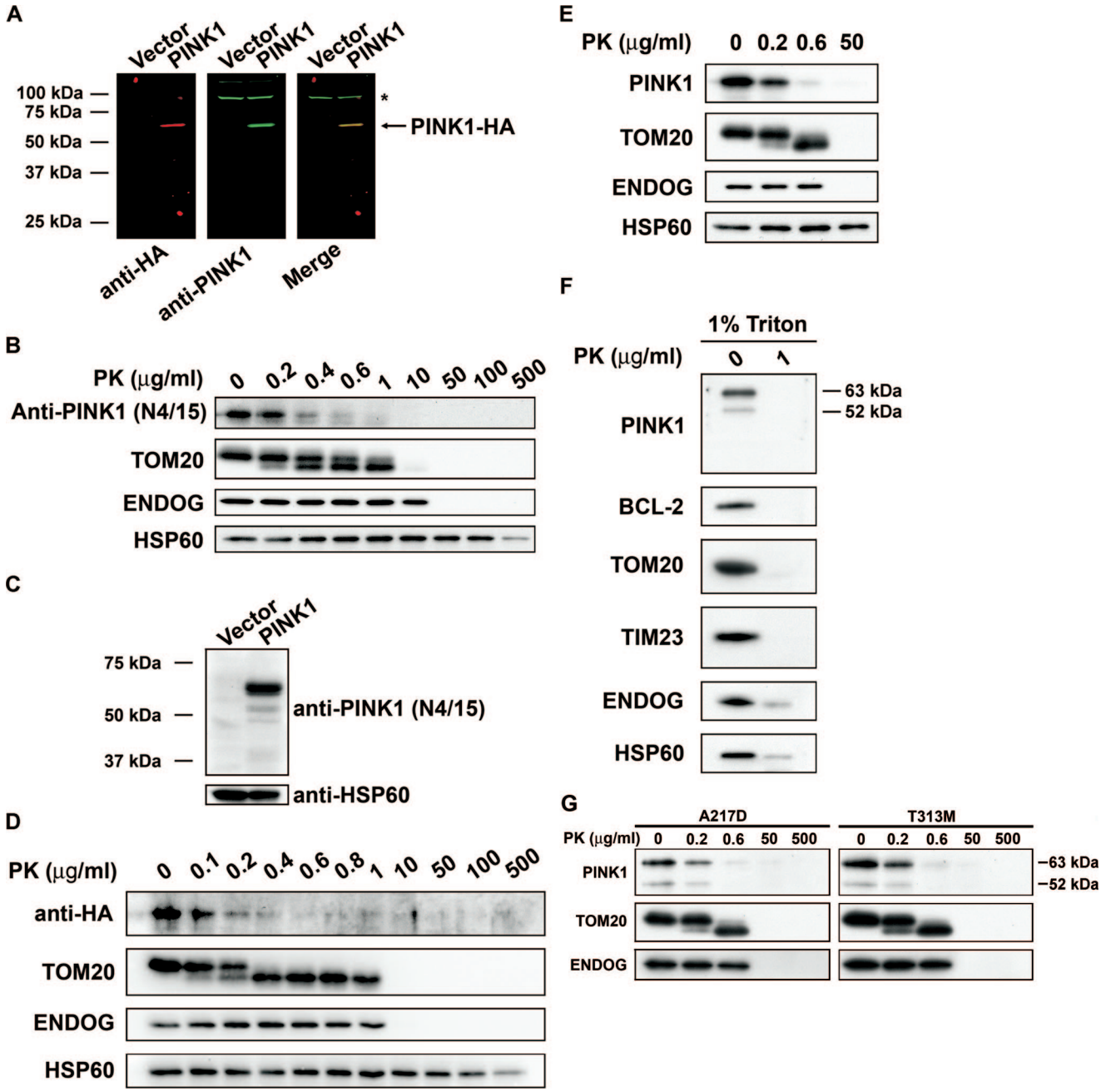

Fig. S3. (*A*) SH-SY5Y cells transfected with empty vector (Vector) or PINK1-HA (PINK1) stably are harvested and the whole-cell lysates are analyzed by Western blot analysis. Both the anti-PINK1$_{175–250}$ and the anti-HA antibodies detect full-length PINK1 (arrow) with a size of ≈63 kDa and the cleaved ≈52-kDa species in PINK1-overexpressing cells but not in vector control cells. By Odyssey Infrared Imaging system, anti-HA and anti-PINK1 antibodies reveal overlapping signals. *, nonspecific bands. (*B*) Mitochondria isolated from MES23.5 cells expressing PINK1 stably are treated with PK. PINK1 is detected by the anti-PINK1 N4/15 antibody. (*C*) Anti-PINK1 N4/15 antibody specifically detects overexpressed PINK1. (*D*) PK protection assay on purified mitochondria from SH-SY5Y expressing PINK1-HA using anti-HA antibodies. (*E*) Mitochondria isolated from M17 cells expressing PINK1 stably are used for PK protection assay. (*F*) Mitochondria isolated from SH-SY5Y cells expressing PINK1 stably are lysed with Triton X-100 and then used for PK digestion, followed by Western blot analysis. All proteins are completely digested. (*G*) Mitochondria isolated from SH-SY5Y cells expressing PINK1 mutants (A217D and T313M) stably are processed as in Fig. 2*C*. PINK1$_{175–250}$ antibody is used in *E*–*G*).

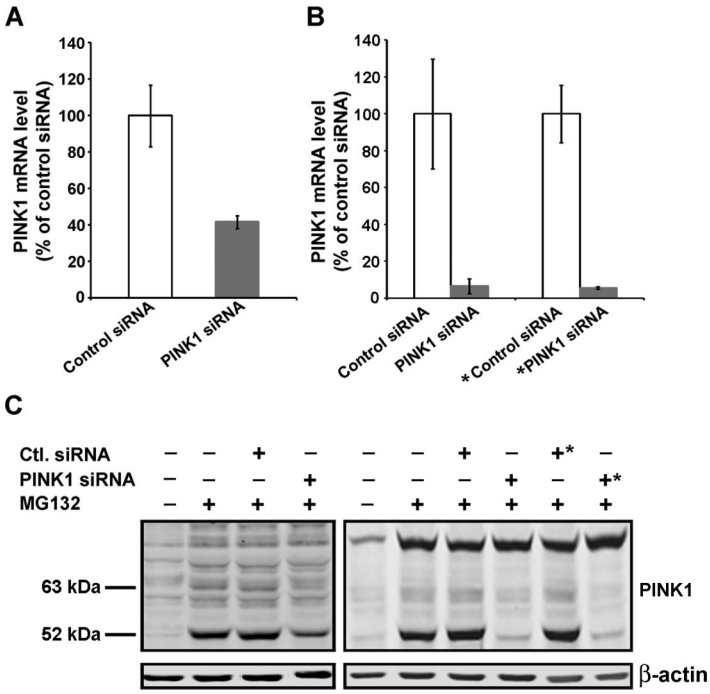

Fig. S4. Validation of knockdown efficiency of two different PINK1 siRNAs. (*A*) GFP^{u} SH-SY5Y cells are transfected with either 100 nM scramble control siRNA or 100 nM PINK1 siRNA (Ambion). After 24 h, PINK1 mRNA in PINK1-siRNA-treated cells is decreased by ≈60%, as evidenced by real-time PCR. (*B*) HeLa cells are transfected with 100 nM control siRNA or PINK1 siRNA (Ambion), or control siRNA or PINK1 siRNA (Qiagen). Asterisk indicates reagents from Qiagen. After 24 h, PINK1 siRNAs from both vendors resulted in a ≈95% decrease of PINK1 mRNAs, as evidenced by control siRNAs. Total RNA extracted from each sample is quantified by real-time PCR ($n = 3$). Values represent means ± SD. (*C*) GFP^{u} SH-SY5Y cells (*Left*) and HeLa cells (*Right*) are transfected with 100 nM control siRNA, PINK1 siRNA, *control siRNA, or *PINK1 siRNA respectively. Twenty-four hours later, media are replaced with fresh media containing either 5.0 μM MG132 for GFP^{u} SH-SY5Y cells or 2.0 μM MG132 for HeLa cells. Another 24 h later, cells are harvested and whole cell lysates are analyzed by Western blotting using PINK1$_{175–250}$ antibody.

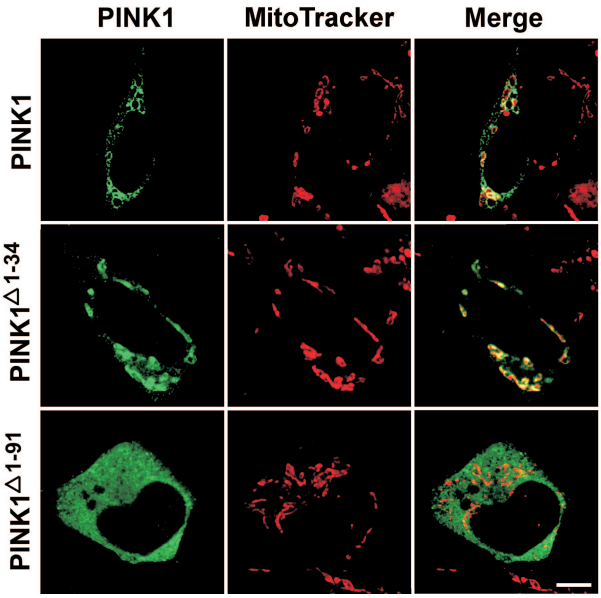

Fig. S5. Mitochondrial localization of representative PINK1 constructs. In SH-SY5Y cells transient transfected with PINK1 (*Top*) and PINK1$^{\Delta 1-34}$ (*Middle*), PINK1 immunofluorescent signals (green) localize with Mitotracker Red CMXRos signals (red), indicating that these PINK1 constructs colocalize with mitochondria. On the contrary, in PINK1$^{\Delta 1-91}$ transfected cells (*Bottom*), the PINK1 signal is more homogenously distributed throughout the cytoplasm. PINK1$_{175-250}$ antibody is used for detecting PINK1. Scale bar, 5 μm.

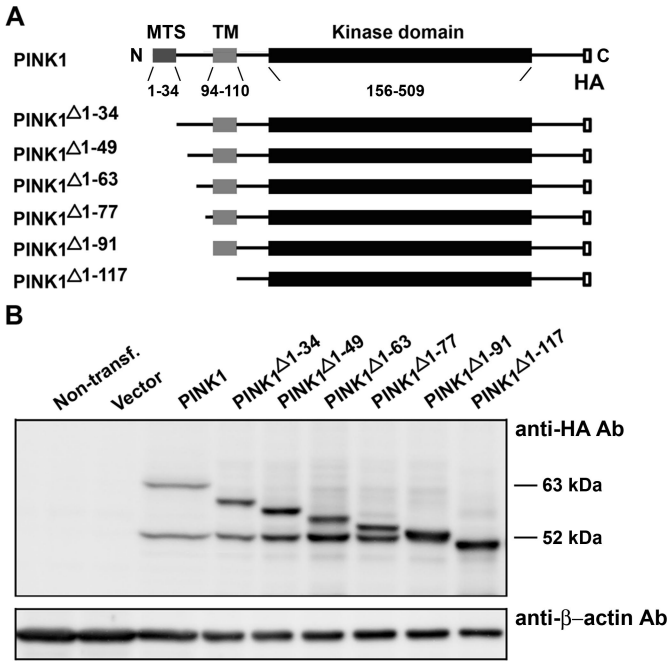

Fig. S6. The ≈52-kDa species of PINK1 is due to a cleavage around amino acids 91–101 at the N terminus of PINK1. (*A*) Schematic representation of various N-terminal deletions of PINK1. (*B*) COS7 cells transiently transfected with C-terminal HA-tagged PINK1 and various PINK1 deletions are harvested, and the whole cell lysates are prepared for Western blot analysis. The wild-type PINK1 sample is loaded with a double amount of protein compared with other constructs because of its low expression level. Using the anti-HA antibody, except for PINK1$^{\Delta 1–91}$ and PINK1$^{\Delta 1–117}$, all other PINK1 constructs showed a N-terminal-cleaved PINK1 species at ≈52 kDa. The size of this ≈52-kDa PINK1 species is between the sizes of PINK1$^{\Delta 1–91}$ and PINK1$^{\Delta 1–117}$.

Table S1. Western blotting using various anti-PINK1 antibodies

	Epitope[†]		Overexpression in cells		Endogenous in tissue			
Antibody	Human	Mouse, %	Human	Mouse	Cells	Mice^{WT}	Mice^{KO}	Human
BC100–494[‡] (Novus)	175–250 aa	72%	+	—	—	—	—	—
N4/15 (NeuroMab)	112–496 aa	82%	+	+	—	—	—	—
NB100–493 (Novus)	1–50 aa	74%	+	—	—	—	—	—
10006283 (Cayman)	484–504 aa	86%	+	?	—	—	—	—
N4/49 (NeuroMab)	112–496 aa	82%	—	?	—	—	—	?
PAB-10983 (Orbigen)	1–18 aa	100%	—	—	—	—	—	—
IMG-4942 (Imgenex)	258–274 aa	82%	—	?	—	?	?	?
BC100–644 (Novus)	258–274 aa	82%	—	+	—	—	—	—
BC100–505 (Novus)	84%	400–500 aa	—	+	—	—	—	—
BC100–506 (Novus)	84%	550–580 aa	—	?	—	—	—	—
sc-32584 (Santa Cruz Biotechnology)	—	?	—	?	—	?	?	—
Ab1[§]	—	?	—	?	—	—	—	?
Ab2[§]	—	?	—	?	—	—	—	?
Culvenor 04/8[¶]	—	?	—	?	—	—	—	—
Ab3[‖]	135–149 aa	60%	—	?	—	?	?	?

Various anti-PINK1 antibodies, either commercially available or acquired from other researchers, were tested for their ability to detect overexpressed PINK1 or endogenous PINK1 using both cell and mouse brain extracts; siRNA PINK1 knockdown is used as a control. Mammalian cell lines tested were COS7, HEK293, HEK293T, SH-SY5Y, M17, MES23.5, HeLa. +, detected specific PINK1 bands by Western blotting; —, no detected specific PINK1 bands by Western blotting; ?, not tested.

[†]Percentage values refer to the homology percentage.

[‡]BC100–494 from Novus can detect endogenous PINK1 in SH-SY5Y cells upon MG132 treatment.

[§]Provided by Anurag Tandon, University of Toronto, Toronto, ON, Canada.

[¶]Provided by Janetta Culvenor, University of Melbourne, Mebourne, Australia.

[‖]Provided by Sonia Gandhi, Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London, UK.