Supplementary Figure Legends:

Supplementary Figure S1. Purification of parkin, PINK1, and DJ-1 fusion proteins. C-terminal fusion proteins of Parkin (lane 2, flag-myc) and PINK1 (lane 3, flag-VSVG) were generated using baculoviruses, affinity purified using anti-flag(M2)-conjugated agarose, and eluted with flag peptide. DJ-1 protein (lane 3) was made in bacteria as a GST fusion protein and purified using glutathione sepharose. 0.2 μg of each purified protein is separated on 4-20% Tris-Glycine SDS PAGE stained with Coomassie Blue. MW: molecular weight marker.

Supplementary Figure S2. Gel-filtration profile of reconstituted PPD-complex. Purified recombinant parkin, PINK1, and DJ-1 shown in **Supplementary Figure S1** were mixed to form the PPD complex in 0.4% NP-40 buffer and subjected to gel filtration on a Superdex S200 column. Immunoblotting analysis of the peak fractions corresponding to an apparent molecular weight of 200 kDa shows the presence of all three proteins.

Supplementary Figure S3. Mapping sequence motifs mediating the parkin, PINK1, and DJ-1 interaction.

A, Schematic illustration of parkin deletion mutants. Numbers at right indicate amino acid residues. Wild type parkin (WT) has 465 amino acids. Round-ended bar: ubiquitin-like domain (UBL). Thick black bars: ring finger domains. Oval bar: domains between ring fingers (IBR).

B. PINK1 deletion mutants. Numbers at right indicate amino acid residues. Wild type PINK1 (WT) has 581 amino acids. Round-ended bar: mitochondrial targeting sequences (MTS). Thick black bars: serine/threonine protein kinase domain.

C. Interaction between PINK1 and parkin deletion mutants. Cell lysates from SH-SY5Y cells transfected with plasmids encoding parkin fragments (indicated at top) and wild type PINK1 were immunoprecipitated with an anti-flag antibody (to precipitate PINK1), followed by immunoblotting with an anti-myc antibody (to detect parkin fragments; upper panel) or an anti-flag antibody (to detect PINK1; middle panel). Lysates were also immunoblotted with an anti-myc antibody to evaluate parkin fragment expression levels (lower panel). Co-immunoprecipitation of PINK1 with parkin 1-212 or parkin 1-245 but not parkin 1-93 indicates that PINK interaction requires parkin residues between 93 and 212 .

D. Interaction between DJ-1 and parkin deletion mutants. Lysates from SH-SY5Y cells transfected with plasmids encoding parkin fragments (indicated at top of the figure) and wild type DJ-1 were immunoprecipitated with an anti-flag antibody (to precipitate DJ-1) followed by immunoblotting an anti-myc antibody (to detect parkin fragments; upper panel) or an anti-flag antibody (to detect DJ-1; middle panel). Cell lysates were also analyzed by direct immunoblotting with an anti-myc antibody to show the expression of parkin fragments (lower panel). Note: coprecipitation of DJ-1 with parkin C-terminal fragments 77-465 and 217-465 was detected, indicating that DJ-1 binds to the parkin C-terminal domain.

E. Interaction between parkin and PINK1 deletion mutants. Cell lysates from SH-SY5Y cells transfected with plasmids encoding PINK1 fragments (indicated on the top of

the figure) and wild type parkin were immunoprecipitated with an anti-myc antibody (to precipitate parkin) followed by immunoblotting an anti-flag antibody (to detect PINK1 fragments; upper panel) or an anti-myc antibody (to detect parkin; middle panel). Lysates were also immunoblotted with an anti-flag antibody to show PINK1 fragment expression levels (lower panel). Parkin does not coimmunoprecipitate the PINK1 N-terminal amino acids 1-334, suggesting that parkin binds to the PINK1 C-terminal.

F. Co-immunoprecipitation of DJ-1 and PINK1 deletion mutants. Cell lysates from SH-SY5Y cells transfected with plasmids encoding PINK1 fragments (indicated on top of the figure) and wild type DJ-1 were immunoprecipitated with an anti-myc tag antibody (to precipitate DJ-1) followed by immunoblotting with an anti-flag antibody (to detect PINK1 fragments, upper panel) or an anti-myc antibody (to detect DJ-1, middle panel). Immunoblotting of lysates with an anti-flag antibody (to detect PINK1 fragments) serves as the input control (lower panel). PINK1 1-256 and 1-334 are equivalently expressed but only1-334 is efficiently coimmunoprecipitated with DJ-1, suggesting that DJ-1 interacts with the PINK1 253-334 motif (indicated by red arrow)(1).

Supplementary Figure S4. Coimmunostaining of endogenous parkin, PINK1, and DJ-1 in human cortical neurons.

Human cortical neuronal cultures were fixed followed by immunostaining with a mouse monoclonal anti-parkin IgG (Green), a rabbit polyclonal anti-PINK1 IgG (Red), and a mouse monoclonal anti-DJ-1 IgM (Blue). Colocalization of the three proteins is shown mostly in cytoplasm (Merge).

Supplementary Figure S5. Subcellular fractination of PINK1.

Cell expressing PINK1 variants, including wildtype PINK1 (WT) and pathogenic mutants G309D, T313M and L399P, were fractionated to mitochondria (M) and cytosolic (C) fractions (shown on the top). Cytosolic marker tubulin (Tub) and mitochondria marker complex I (Cplx I) and were detected to demonstrate the fractionation effeciency. Note that the 64 KD full length PINK1 is largely detected in mitochondria. Control: untransfected cells.

Supplementary Figure S6. Inhibition of PINK1-mediated degradation of parkin and synphilin-1 by parkin or PINK1 siRNAs.

A. Knockdown of parkin by a mixture of parkin siRNAs. cDNAs from SH-SY5Y cells transfected with either unrelated control siRNAs (C+) or a mixture of parkin siRNAs (siRNA) were amplified with either parkin (upper panel) or GAPDH (lower panel) specific primers. C- is an amplification control without cDNA. Parkin expression is robustly reduced in cells transfected with the parkin siRNA mixture but not in cells transfected with control siRNA.

B. PINK1 mediated syphilin-1 degradation requires parkin. Cell lysates made from SH-SY5Y cells transfected with synphilin-1, PINK1, parkin siRNAs, and control siRNAs (siRNA-C) in various combinations shown at the top of the panel were immunoblotted with an anti-GFP antibody to detect synphilin-1 (upper panel). The membrane was reacted with an anti-actin antibody as a loading control (lower panel). Parkin siRNA transfection not only inhibited synphilin-1 degradation promoted by

PINK1 but resulted in synphilin-1 accumulation, consistent with observed parkin accumulation in transfected PINK1 knockout cells (Figure 4).

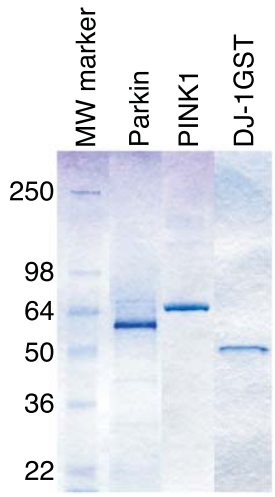
C. Knockdown of PINK1 by PINK1 RNAi. cDNAs from SH-SY5Y cells transfected with either unrelated control RNAi (C+) or two species of RNAi encoded by different siRNA constructs (siRNA1 and siRNA2) were amplified with either PINK1 (upper panel) or GAPDH (lower panel) specific primers. C- is an amplification control without cDNA. PINK1 expression is significantly reduced in cells transfected with PINK1 siRNA plasmids but not in cells transfected with control plasmid.

D. Knockdown PINK1 inhibits parkin ubiquitination. Cell lysates made from SH-SY5Y cells transfected with parkin, PINK1, two plasmids encoding PINK1 RNAi (siRNA1 and siRNA2), and HA-tagged ubiquitin (Ub) in various combinations shown at the top of panel were immunoprecipitated with an anti-vsvg antibody to precipitate parkin, followed by immunoblotting with either an anti-HA antibody to detect ubiquitin (poly-Ub, upper panel) or an anti-parkin antibody to detect parkin (parkin, lower panel). Note that PINK1 RNAi inhibits ubiquitination of parkin.

References:

1. Tang, B., Xiong, H., Sun, P., Zhang, Y., Wang, D., Hu, Z., Zhu, Z., Ma, H., Pan, Q., Xia, J.H. *et al.* (2006) Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. *Hum. Mol. Genet.,* **15,** 1816-1825.

Supplemental Fig. S1



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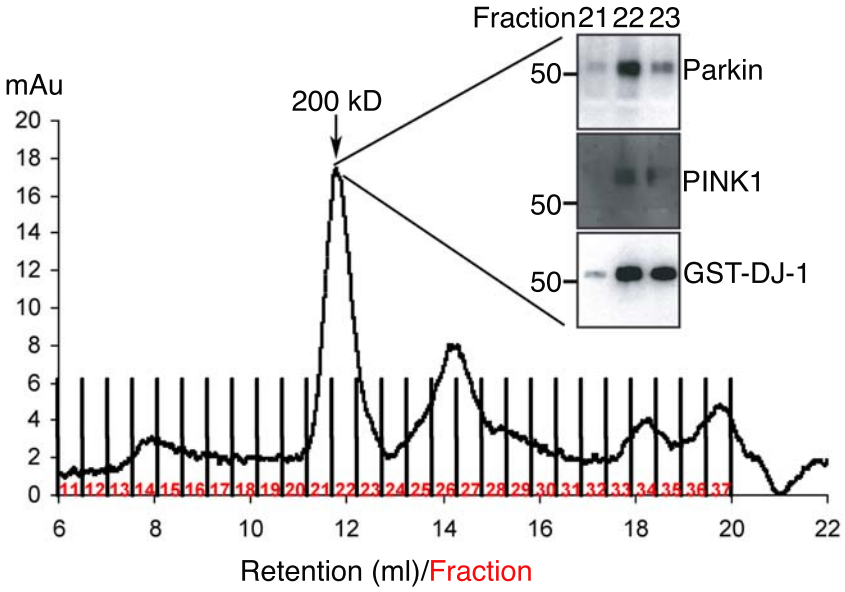

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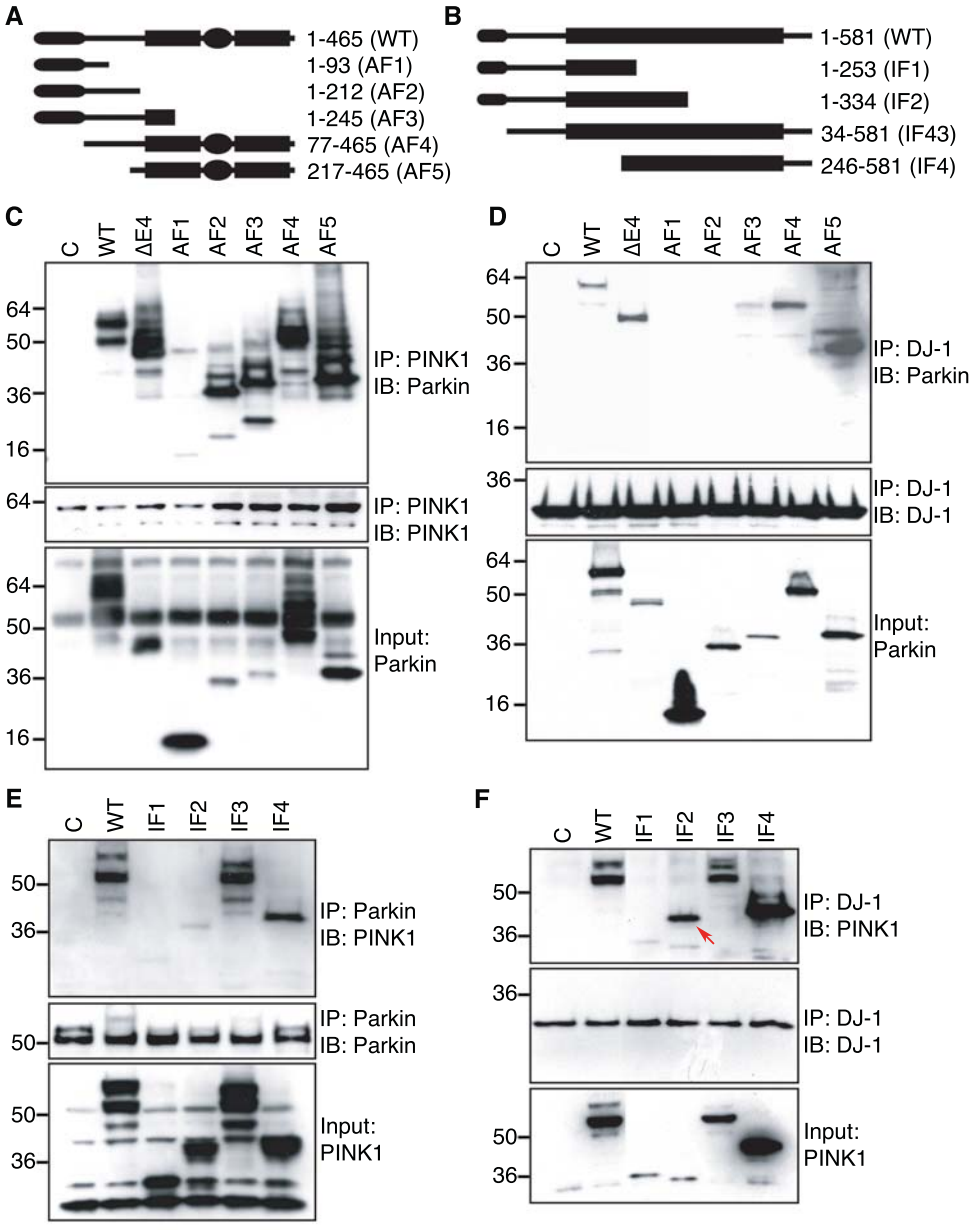

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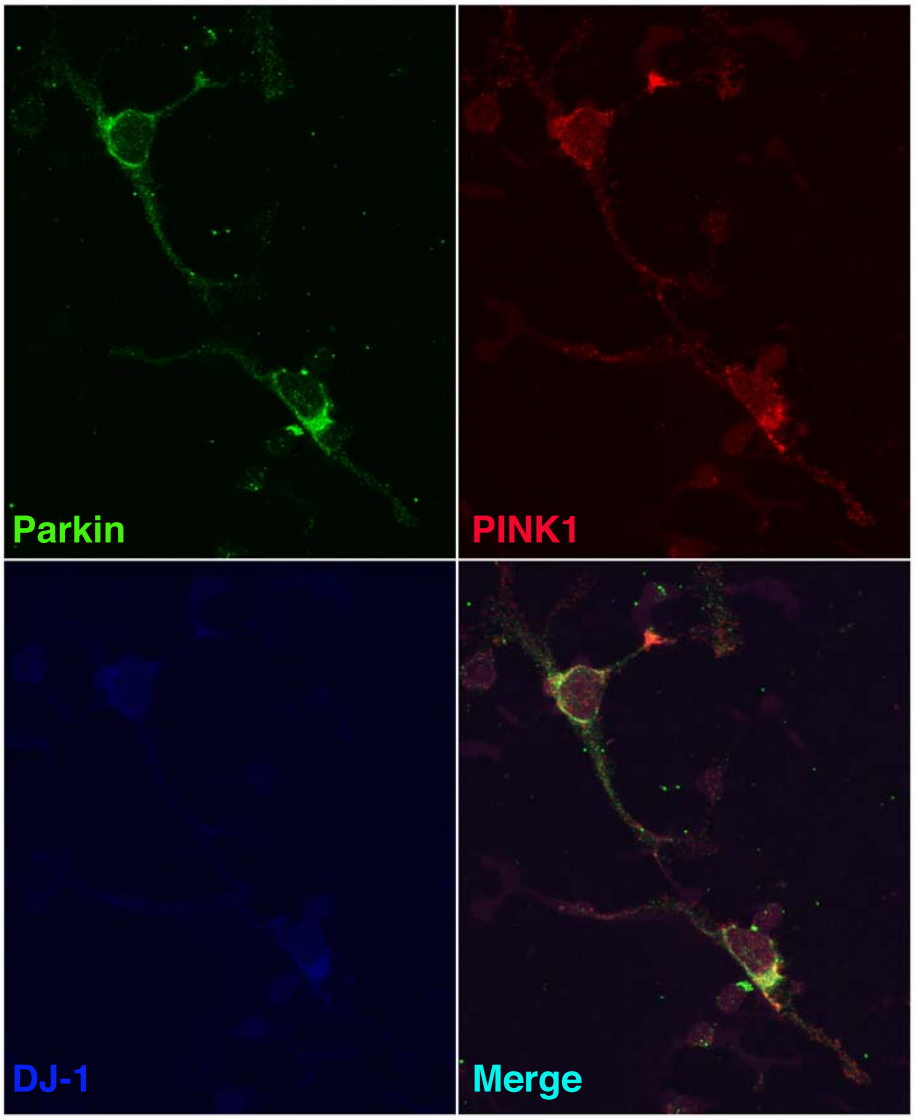

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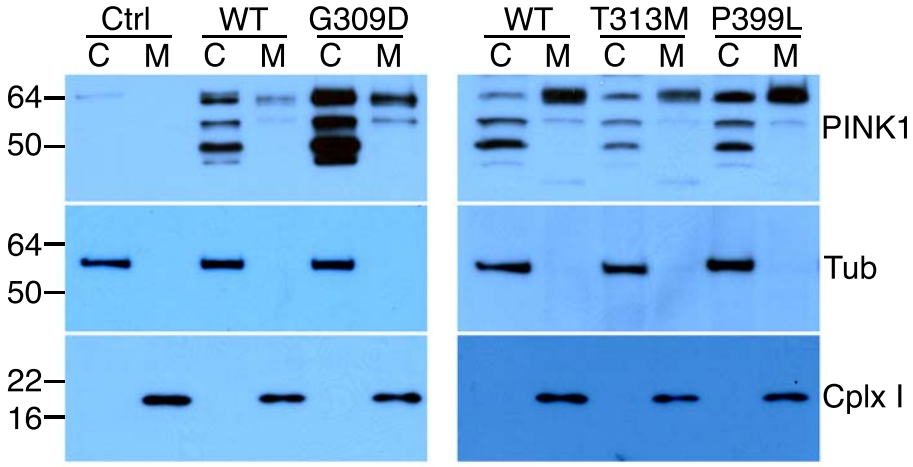

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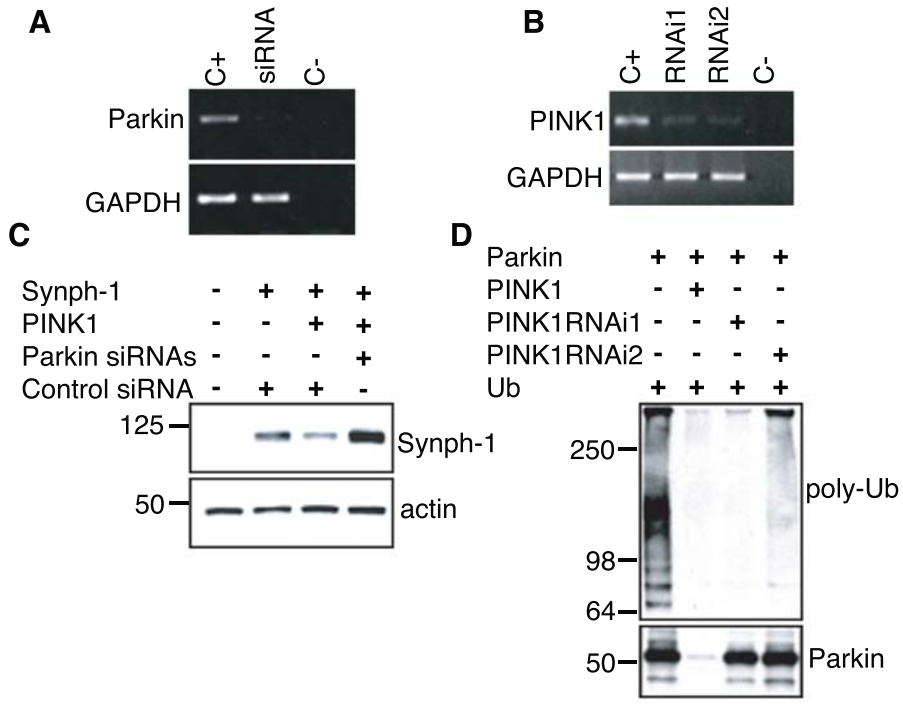

Supplemental Table 1. Association of PINK1 with endogenous parkin and DJ-1

RefSeq	Protein Name	Sequence Coverage (%)	Unique Spectra	Total Spectra
NP_115785	PINK1	29.3%	25	251
NP_004553	Parkin	20%	8	11
NP_009193	DJ-1	15%	3	7

Note: Indicated are Flag-tagged PINK1, endogenous parkin and DJ-1 in SH-SY5Y cells, their percentage sequence coverage, number of unique spectra, and number of total spectra as detected by mass spectrometry .