Supplemental Information

Supplemental Figures S1-S7

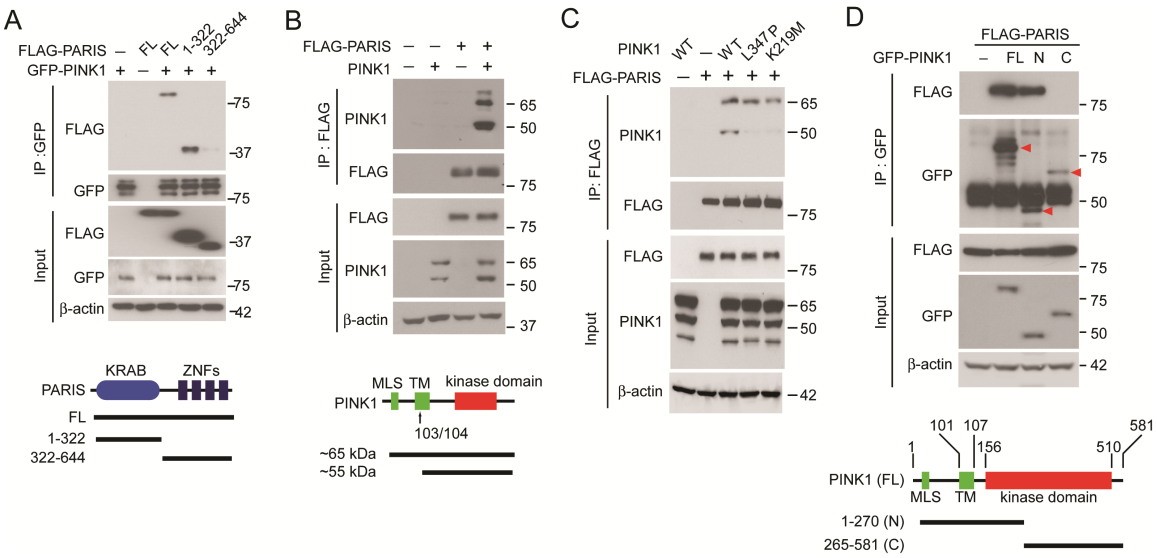

Figure S1. PARIS interacts with PINK1, Related to Figure 1
(A) Co-immunoprecipitation of full length (FL), N- (1-322) and C- (322-644) terminal fragments of FLAG-PARIS with GFP-PINK1 from SH-SY5Y cells transfected with the indicated constructs using a GFP antibody. Experiments were repeated two times (KRAB, Krüppel associated box; ZNF, zinc finger domain). (B) Co-immunoprecipitation of PINK1 and FLAG-PARIS from SH-SY5Y cells transfected with the indicated constructs using an anti-FLAG antibody. PINK1's modular structure is shown as a cartoon in the bottom panel. PARL mediated cleavage site (between aa 103/104) is indicated as an arrow. PARL mediated PINK1 cleavage generates ~55 kDa processed PINK1. Experiments were repeated three times (MLS, mitochondrial localization sequence; TM, transmembrane domain). (C) Interaction of wild type (WT), L347P, and K219M PINK1 with FLAG-PARIS in anti-FLAG immunoprecipitates from SH-SY5Y cells transfected with the indicated DNA constructs determined by western blots. (D) Mapping of PINK1 domains required for interaction with FLAG-PARIS in anti-GFP immunoprecipitates from SH-SY5Y cells transfected with GFP-tagged full-length (FL), 1-270 (N), and 265-581 (C) PINK1.

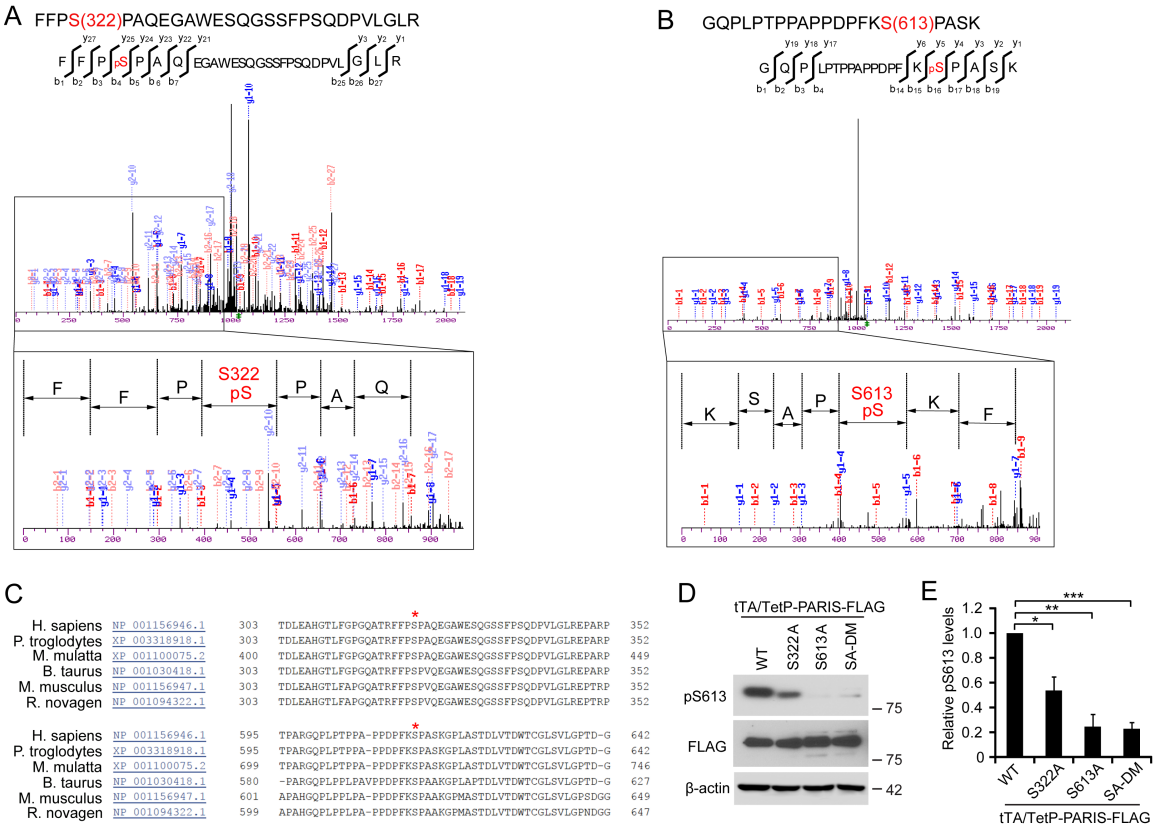

Figure S2. PINK1 phosphorylates PARIS at Serine 322 and 613, Related to Figure 2

(A) Mass spectrometry peaks showing PARIS phospho-peptides containing serine 322 or (B) serine 613 phosphorylation after PINK1 phosphorylation of FLAG-PARIS. (C) Alignment of amino acid sequence at human PARIS serine 322 (top panel) or serine 613 (bottom panel) among mammalian species. Phosphorylation sites are indicated with *. (D) Phosphorylation at S613 of PARIS for wild type and phospho-mutant PARIS with serine to alanine substitutions (S322A, and S613A) determined by western blots using pS613-PARIS antibodies for anti-FLAG immunoprecipitates from SH-SY5Y cells transfected with the indicated constructs. (E) Relative S613 phosphorylation normalized to total PARIS levels were quantified using densitometry and presented in the bar graph (n = 3 per group). Quantified data are expressed as mean ± s.e.m., $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, analysis of variance (ANOVA) test followed by Tukey HSD post-hoc analysis. β-actin was used as a loading control.

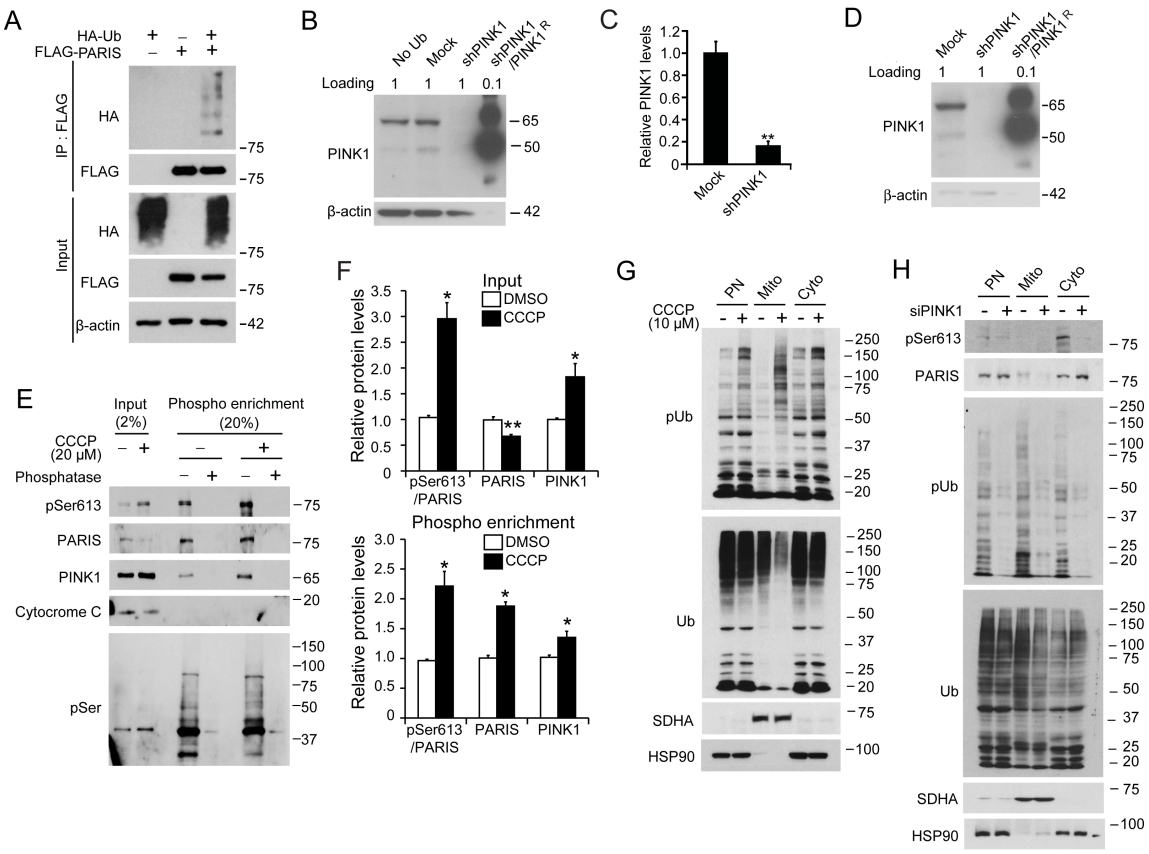

Figure S3. Efficient knockdown of endogenous PINK1 by shRNA and basal phosphorylation of PARIS and PINK1, Related to Figure 3

(A) PARIS ubiquitination was assessed in SH-SY5Y cells 2 days after transient transfection of HA-Ub ± FLAG-PARIS. PARIS ubiquitination was monitored by western blot using anti-HA antibodies in anti-FLAG PARIS immunoprecipitates from SH-SY5Y cells transfected with the indicated constructs. Similar results were obtained in three independent experiments. (B) Overexposed immunoblots from Figure 3B in order to demonstrate the degree of PINK1 knockdown (cotransfection of shPINK1 and shRNA resistant PINK1 was loaded with only one tenth of the protein of other groups in order to demonstrate the degree of PINK1 knockdown using extended western blot exposure). (C) Quantification of relative PINK1 protein levels in SH-SY5Y cells transfected with shRNA to PINK1 or control DNA (*n* = 3 per group). (D) Overexposed immunoblots from Figure 3E in order to demonstrate the degree of PINK1 knockdown (cotransfection of shPINK1 and shRNA resistant PINK1 was loaded with only one tenth of the protein of other groups in order to demonstrate the degree of PINK1 knockdown using extended western blot exposure). (E) Phosphorylation of PARIS and PINK1 in SH-SY5Y cells under basal condition or CCCP (20 μM) treatment for 3 h. Protein lysates from each treatment group were subjected to phospho-column purification to capture phosphorylated proteins. PARIS and PINK1 phosphorylation were assessed in the phosphoprotein enrichment fraction using indicated antibodies (anti-pSer613-PARIS and anti-PARIS antibodies for PARIS phosphorylation; anti-PINK1 antibody for PINK1 phosphorylation). Phosphatase treatment groups were used as a control to evaluate if the blots indicate phosphorylated form. Cytochrome C serves as non-phosphorylated protein control. Anti-phospho

serine (pSer) blot serves as loading control for phosphoprotein enrichment samples. Similar results were obtained in two independent experiments. (F) Quantification of PARIS, pSer613-PARIS, and PINK1 in input fraction (top panel) and phospho-protein enrichment fraction (bottom panel) prepared from SH-SY5Y cells treated with or without CCCP (n = 3 per group). (G) Immunoblot of phospho-ubiquitin (pUb), and ubiquitin in the subcellular fractions (PN, postnuclear; Mito, mitochondria; Cyto, cytoplasm) from SH-SY5Y cells treated by CCCP (10 uM) for 3 h. Heat shock protein 90 (HSP90), and succinate dehydrogenase (SDHA) were used as subcellular fraction markers. (H) Phosphorylation of PARIS and ubiquitin in the subcellular fractions (PN, postnuclear; Mito, mitochondria; Cyto, cytoplasm) determined by anti-pS613 and anti-pUb antibodies, respectively. Localization and protein levels of PARIS and total ubiquitin in each subcellular fraction were also assessed by western blots using indicated antibodies. Heat shock protein 90 (HSP90), and succinate dehydrogenase (SDHA) were used as subcellular fraction markers. Quantified data are expressed as mean ± s.e.m., $^{*}P < 0.05$, $^{**}P < 0.01$, unpaired Student's t test. β-actin was used as a loading control.

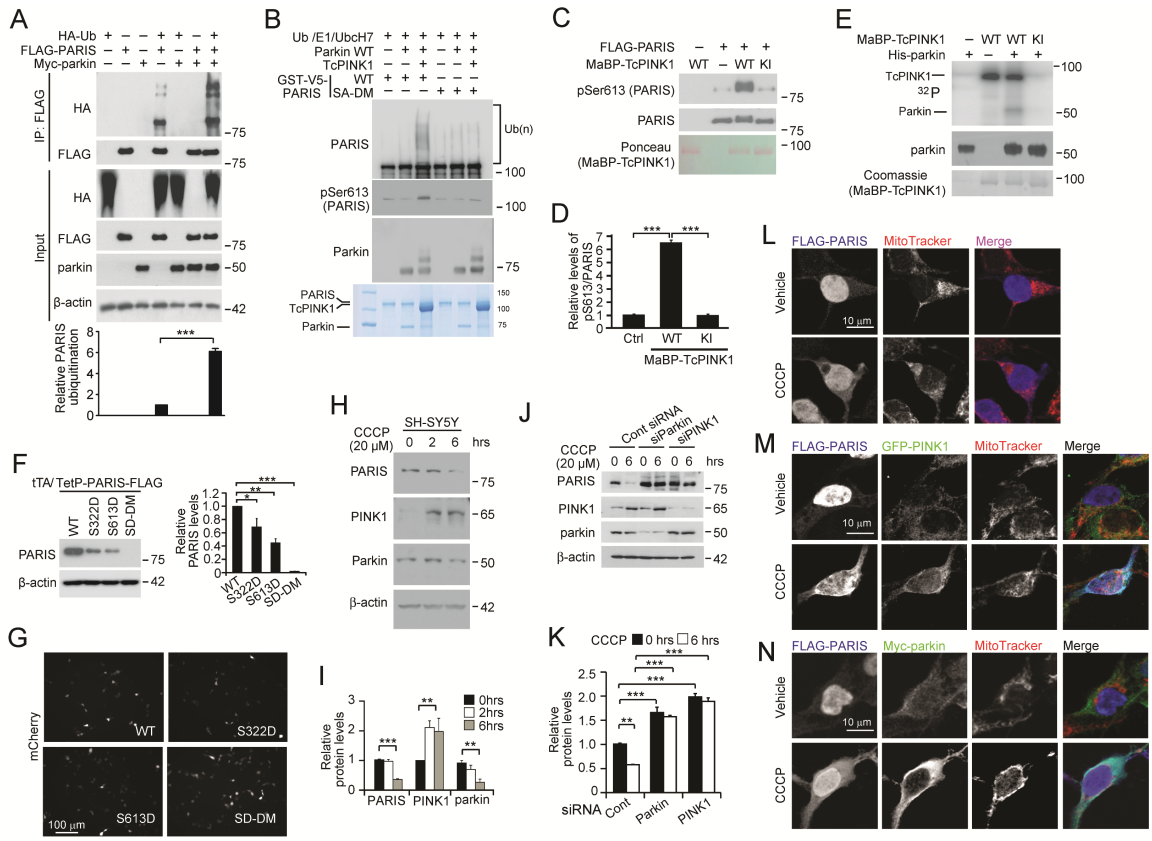

Figure S4. Phosphorylation of PARIS at S322 and S613 are required for parkin mediated ubiquitination of PARIS, Related to Figure 4

(A) PARIS ubiquitination was assessed in SH-SY5Y cells two days after transfection with the indicated combinations of HA-Ub, FLAG-PARIS, and Myc-parkin constructs. PARIS ubiquitination was monitored by western blot using anti-HA antibodies in anti-FLAG PARIS immunoprecipitates. Quantification of the levels of ubiquitination normalized to the levels of PARIS is shown in the bar graph. (B) *In vitro* ubiquitination assay demonstrating PARIS ubiquitination by rat parkin in the presence of TcPINK1 in a recombinant protein-reconstituted ubiquitination reaction. S322A/S613A double phosphorylation mutant PARIS fails to be ubiquitinated by parkin as determined by western blots using PARIS antibodies. The experiments were repeated three times. Polyubiquitinated PARIS species are indicated with (Ub)n. (C) *In vitro* kinase assay demonstrating PARIS S613 phosphorylation by wild type (WT) but not by kinase inactive mutant (KI) of TcPINK1 determined by pS613 PARIS specific antibodies. (D) Quantification of relative PARIS phosphorylation at S613 by TcPINK1 wild type or kinase inactive mutant in an *in vitro* kinase assay (n = 3 per group). (E) *In vitro* kinase assay demonstrating parkin phosphorylation by TcPINK1 WT but not by KI mutant determined by P^{32} autoradiogram. (F) Representative western blots showing the steady state protein levels of wild type (WT), and phospho-mimetic PARIS mutants (S322D, S613D, and S322/613D (SD-DM)) in SH-SY5Y cells transiently transfected with tTA and pTRE dual constructs expressing PARIS and reporter mCherry (TetP-PARIS-FLAG). Quantification of relative PARIS

levels normalized to β-actin analyzed by ImageJ densitometry is shown in the bar graph (the right panel, *n* = 3 per group). (G) Representative immunofluorescence images showing mCherry expression in the indicated groups of PARIS wild type and phospho-mimetic mutants. Scale bar = 100 μm. (H) The relative protein levels of PARIS, PINK1, and parkin in SH-SY5Y cells treated by CCCP (20 μM). (I) Quantification of Figure S4H, *n* = 3 per group. (J) PARIS, PINK1, parkin protein levels in SH-SY5Y cells treated with CCCP (20 uM, 6 h) two days after siParkin or siPINK1 transfection or scrambled control siRNA as determined by immunoblot. (K) Quantification of relative PARIS levels normalized to β-actin by ImageJ densitometry analysis for Figure S4J (*n* = 3 per group). (L) Representative confocal immunofluorescence images of FLAG-PARIS distribution in response to CCCP treatment (20 uM, 2 h) for SH-SY5Y cells. Mitochondria are labeled with Mitotracker Red. Scale bar = 10 μm. (M) Representative confocal immunofluorescence images of FLAG-PARIS and GFP-PINK1 in SH-SY5Y cells treated with CCCP (20 uM, 2 h). Mitochondria are labeled with Mitotracker Red. Scale bar = 10 μm. (N) Representative confocal immunofluorescence images of FLAG-PARIS and Myc-parkin in SH-SY5Y cells treated with CCCP (20 uM, 2 h). Mitochondria are labeled with Mitotracker Red. Scale bar = 10 μm. Quantified data are expressed as mean ± s.e.m., $^{**}P < 0.01$, $^{***}P < 0.001$, analysis of variance (ANOVA) test followed by Tukey HSD post-hoc analysis. β-actin was used as a loading control.

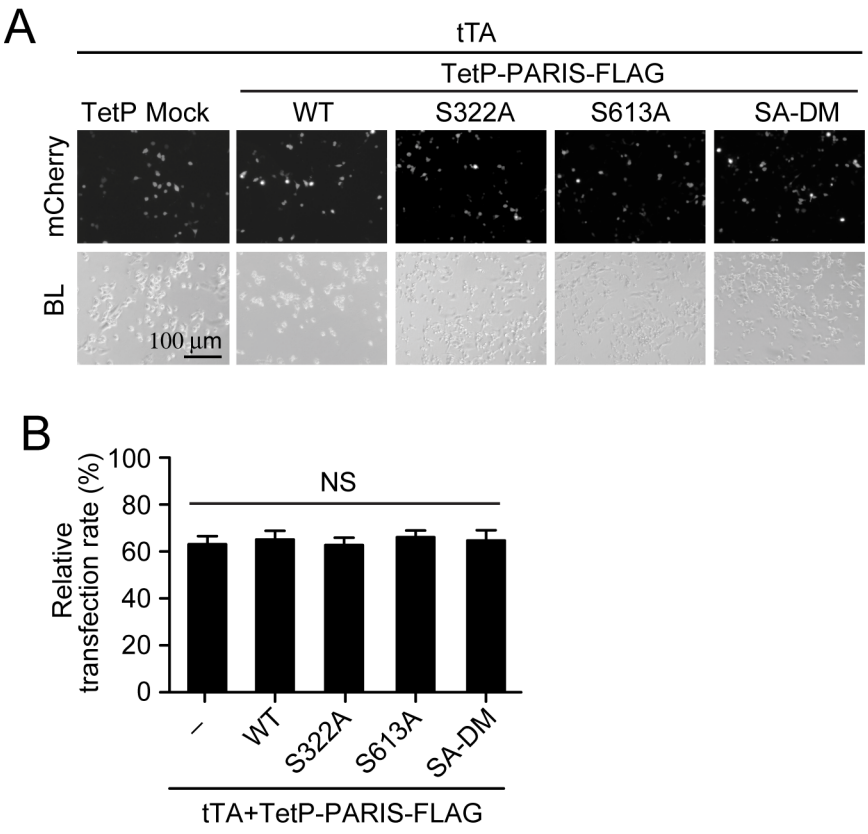

Figure S5. Tet-Off expression of the reporter protein mCherry in SH-SY5Y cells, Related to Figure 5.

(A) Representative fluorescence images of mCherry signal in SH-SY5Y cells transiently transfected with TetP-PARIS-FLAG wild type or phosphorylation deficient mutants together with pCMV-tTA construct. Bright field images are also shown in the bottom. Scale bar = 100 μm. (B) Quantification of percent cells expressing mCherry in SH-SY5Y cells transfected with respective DNA constructs (*n* = 3 experiments per group). Quantified data are expressed as mean ± s.e.m., analysis of variance (ANOVA) test followed by Tukey HSD post-hoc analysis.

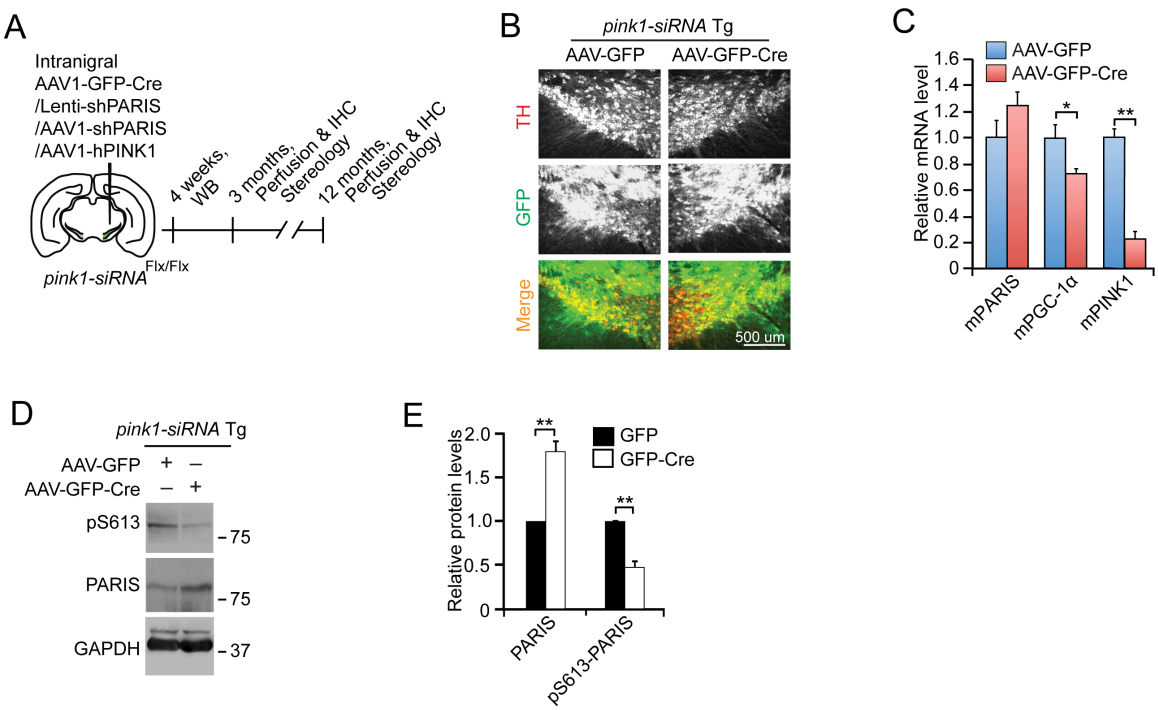

Figure S6. Conditional knockdown of PINK1 in mice leads to PARIS accumulation and PGC-1α repression, Related to Figure 6 and Table S1

(A) Schematic diagram showing the timetable of experimental procedures. *pink1-siRNA* conditional transgenic mice were injected with adenoassociated virus serotype 1 (AAV1) expressing GFP-Cre or GFP as a control into the substantia nigra (SN) for adult knockdown of PINK1. Lenti-shPARIS was coinjected for rescue experiments. WB, western blots; qRT-PCR, quantitative real-time PCR; IHC, immunohistochemistry. (B) Representative immunofluorescence images of ventral midbrain sections from conditional *pink1-siRNA* transgenic mice (*pink1-siRNA* Tg) SN injected with recombinant adeno-associated virus (AAV) expressing GFP or GFP-Cre stained with anti-tyrosine hydroxylase (TH, red)), and GFP (green) antibodies. Scale bar = 500 μm. (C) Quantification of relative messenger levels of PARIS, PINK1, and PGC-1α in the ventral midbrain of *pink1-siRNA* transgenic mice injected with either AAV-GFP or AAV-GFP-Cre determined by real-time quantitative PCR, and normalized to GAPDH levels (n = 4 per group). (D) Representative western blots of PARIS and pS613-PARIS in cytosolic subcellular fraction from the ventral midbrains of *pink1-siRNA* transgenic mice injected with AAV-GFP or AAV-GFP-Cre viruses. GAPDH was used as a cytosolic loading control. (E) Quantification of relative protein levels of PARIS, and pS613-PARIS normalized to GAPDH (n = 4 per group). Quantified data are expressed as mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, unpaired Student's t test.

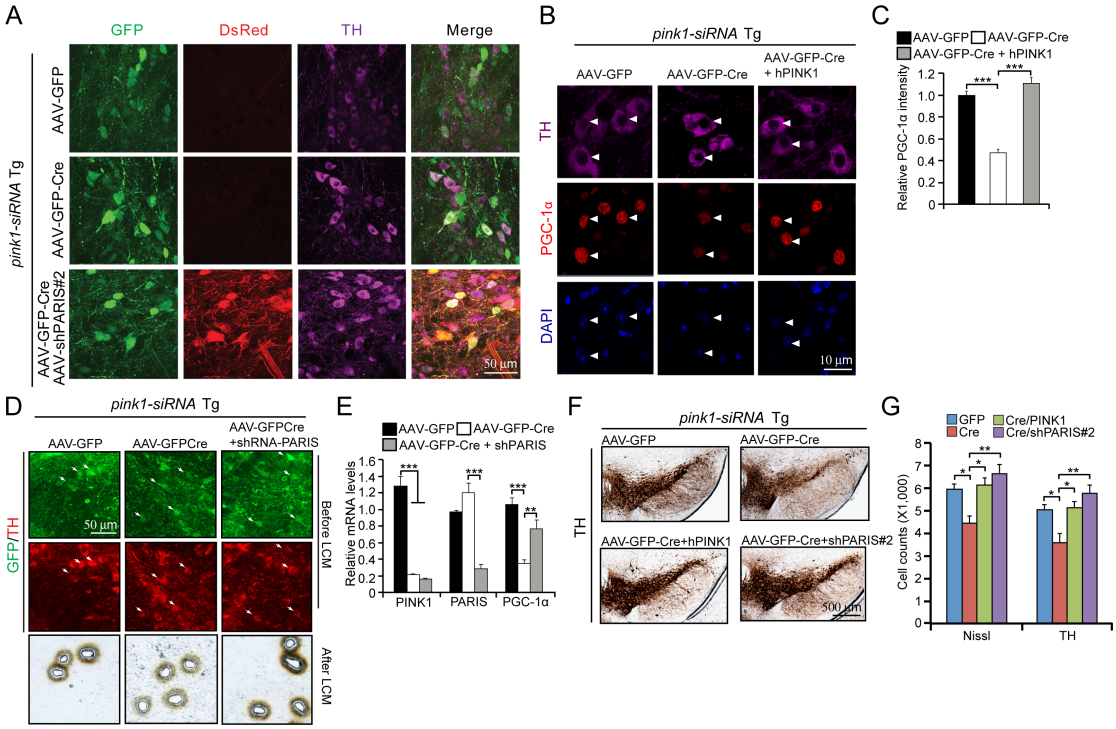

Figure S7. PGC-1α repression and cell toxicity in PINK1-depleted dopamine neurons are dependent on PARIS accumulation, Related to Figure 7

(A) Representative immunofluorescent images showing effective transduction of TH-positive DA neurons by AAV-GFP or AAV-GFP-Cre or AAV-GFP-Cre with AAV expressing RFP and shRNA to mouse PARIS (AAV-shPARIS#2). Scale bar = 50 μm. (B) Representative immunofluorescent images of ventral midbrains of *pink1-siRNA* transgenic mice injected with AAV-GFP or AAV-GFP-Cre or AAV-GFP-Cre/hPINK1 viruses immunostained for tyrosine hydroxylase (TH, purple), PGC-1α (red), and DAPI (blue). Scale bar = 10 μm. (C) Quantification of PGC-1α intensity in the TH-positive DA neurons of the substantia nigra pars compacta of AAV-GFP or GFP-Cre injected *pink1-siRNA* transgenic mice (*n* = 23 for AAV-GFP and 21 for AAV-GFP-Cre positive DA neurons with clear morphologies of TH staining from three mice injected with the indicated viruses). (D) Representative immunofluorescent images taken before and after laser capture microdissection of DA neurons from ventral midbrains of *pink1-siRNA* transgenic mice injected with AAV-GFP or AAV-GFP-Cre viruses or AAV-GFP-Cre with shRNA-PARIS immunostained for tyrosine hydroxylase (TH, red), and GFP (green). Scale bar = 50 μm. (E) Quantification of relative mRNA levels of PINK1, PARIS, and PGC-1α in laser captured DA neurons transduced with AAV-GFP, AAV-GFP-Cre, or AAV-GFP-Cre/shPARIS determined by real-time PCR (*n* = 4 mice per group, in each group approximately 500-600 DA neurons were collected for RNA extraction). (F) Representative tyrosine hydroxylase immunohistochemistry images of ventral midbrains of *pink1-siRNA* transgenic mice SN injected with the indicated AAV viruses. Scale bar = 500 μm. (G) Quantification of TH-positive cells and Nissl stained DA neurons in the substantia nigra of the groups of mice in (*n* = 6 injections for AAV-GFP, 5 injections for AAV-GFP-Cre, 8 injections for AAV-GFP-Cre+hPINK1 and AAV-

GFP-Cre+shPARIS#2). Quantified data are expressed as mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001 analysis of variance (ANOVA) test followed by Tukey HSD post-hoc analysis.

Supplemental Table

Table S1. Primers used for real-time qPCR, related to Figure 5.

Target genes		Primers (5'-3') for human genes	Primers (5'-3') for mouse genes
GAPDH	F	AAACCCATCACCATCTTCCAG	AAACCCATCACCATCTTCCAG
	R	AGGGGCCATCCACAGTCTTCT	AGGGGCCATCCACAGTCTTCT
PINK1	F	GCCTCATCGAGGAAAAACAGG	GCTTGCCAATCCCTTCTATG
	R	GTCTCGTGTCCAACGGGTC	CTCTCGCTGGAGCAGTGAC
PARIS	F	GCTGGAATTTCCGGTGTAAACC	AGTTGGACTCTGGAGCAGGA
	R	GGGGTCCAAGATGGCCTCT	GCTGCTGTGTTGAGCTTCAG
PGC-1α	F	TCCTCACAGAGACACTAGACA	AGCCGTGACCACTGACAACGAG
	R	CTGGTGCCAGTAAGAGCTTCT	GCTGCATGGTTCTGAGTGCTAAG

F, forward; R, reverse

Supplemental Experimental Procedures

Generation of stable cell lines.

A stable SH-SY5Y cell line was established, which expresses the PGC-1α promoter firefly luciferase reporter. pGreenFire1 lenti-reporter vector backbone (System Biosciences, Inc., Mountain View, CA) was used for expression of human PGC-1α promoter region (AF108193; GenBank, -992 to +90 bp) so that the dscGFP reporter gene followed by the self-cleaving T2A peptide and the firefly luciferase gene was under the control of the PGC-1α promoter. The WPRE element enhances the expression level of the reporter genes. Puromycin (2 μg per ml) was used for selection of stably transduced SH-SY5Y cells.

Luciferase Assay.

Cells were harvested 48 h after transfection with corresponding DNA constructs and lysates were assayed for firefly luciferases, using the Luciferase Reporter Assay System (Promega, Madison, WI) with a Monolight 3010 luminometer (Analytical luminescence Lab, Los Angeles, CA), according to the manufacturer's instructions.

Antibodies.

The following antibodies were used. For primary antibodies, mouse antibody to PARIS (cat# 75-195, 1:1,000, NeuroMab), rabbit antibody to PARIS (Shin et al., 2011), mouse antibody to phosphoserine (cat# P3430, 1:2,000, Sigma), rabbit antibody to PINK1 (cat# B100-494, 1:1,000, Novus Biologicals), mouse antibody to FLAG (M2, 1:5,000, Sigma-Aldrich), mouse antibody to Myc (cat# 11 667 149 001, 1:1,000, Roche Diagnostics), rabbit antibody to Myc tag (cat# 2272, 1:1,000, Cell Signaling) mouse antibody to PGC-1α (4C1.3, 1:1,000, Calbiochem), mouse antibody to parkin (Park8, 1:2,000, Cell Signaling), rabbit antibody to tyrosine hydroxylase (NB300-109, 1:2,000, Novus Biologicals), mouse antibody to tyrosine hydroxylase (TH-16, 1:1,000, Sigma-Aldrich), rabbit antibody to GFP (ab290, 1:1,000, Abcam), mouse antibody to GFP (GSN149, 1:1,000, Sigma Aldrich), goat antibody to GFP (ab6673, 1:1,000, Abcam), rabbit antibody to RFP (ab62341, 1:1,000, Abcam), mouse antibody to GFAP (GA5, 1:5,000, Cell Signaling Technology), rabbit antibody to mCherry (cat# ab167453, 1:3,000, Abcam), mouse antibody to GAPDH (GT239, 1:5,000, GeneTex), mouse antibody to Succinate Dehydrogenase (SDHA) (ab 14715, 1:2,000, Abcam), mouse antibody to HSP90 (ab13492, 1:3,000, Abcam). Rabbit antibody to phosphorylated ubiquitin was used as described (Fiesel et al., 2015). Horseradish peroxidase (HRP)-conjugated antibody to HA (Roche), HRP-conjugated mouse antibody to GST (GE Healthcare), HRP-conjugated mouse antibody to β-actin (AC15, Sigma-Aldrich). For secondary antibodies. we used HRP-conjugated sheep antibody to mouse IgG (cat# RPN4301, 1:5,000, GE Healthcare), HRP-conjugated donkey antibody to rabbit IgG (cat# RPN4101, 1:5,000, GE Healthcare), biotin-conjugated goat antibody to mouse IgG (cat# BA-2000, 1:1,000, Vector Laboratories), biotin-conjugated goat antibody to rabbit IgG (cat# BA-1000, 1:1,000, Vector Laboratories), Alexa Fluor 488–conjugated donkey antibody to mouse or rabbit or goat IgG (H+L) (cat# A21202, A21206, A11055, 1:1,000, Invitrogen), Alexa Fluor 568–conjugated donkey antibody to mouse or rabbit IgG (cat# A10037, A10042, 1:1,000, Invitrogen). Alexa Fluor 647-conjugated donkey antibody to

mouse IgG (cat# A10038, 1:1,000, Invitrogen).

Plasmids.
Phospho-mimetic PARIS mutants were generated by site-directed mutagenesis of S322 and/or S613 of PARIS into aspartate (TetP-PARIS-FLAG-S322D, S613D, and S322D/S613D). Recombinant GST-V5-PARIS and recombinant GST-FLAG-parkin constructs were generated by the gateway cloning technologies following the manufacturer's instruction (Invitrogen). Briefly, attL-containing PARIS and parkin entry clones and attR-containing destination vector were recombined in the presence of LR Clonase (Cat #: 11791-019, Invitrogen) and the resulting expression clones were transformed into BL21 E.coli for expression of recombinant proteins. N-terminal GFP-tagged PINK1 deletion mutants were generated by PCR amplifying N-terminal (aa 1-270) or C-terminal (aa 268-581) regions of human PINK1 and cloning into EcoRI/XbaI restriction sites of pEGFP-C3 plasmid. Construct integrity was verified by sequencing. pLKO-shPINK1 constructs were screened to select and verify efficient knockdown of human PINK1. pLKO-shPINK1 #4 (TRCN0000007100, target sequence: GTTCCTCGTTATGAAGAACTA, Sigma) was selected. As a control, shRNA-DsRed coexpressing GFP and short hairpin sequence (AGTTCCAGTACGGCTCCAA) under the control of the EF1a and human U6 promoter was used (shDsRed). shRNA resistant PINK1 was constructed by introducing silent mutation at the targeted sequence (QuikChange® II XL Site-Directed Mutagenesis Kit, Stratagene). The siRNA to human PINK1 was purchased (#1116919, Bioneer, Daejeon, South Korea). Control siRNA (#sc-37007), and parkin siRNA (#sc-42158) were purchased from Santa Cruz biotechnology (Dallas, USA). pCMV-tTA was purchased from Clontech. pEGFP-C1-PINK1 and rat parkin $^{219-465}$ RBR were purchased from Addgene. The following constructs used here were described previously: N-terminal FLAG tagged full length PARIS and N-terminus and C-terminus deletion mutants, HA-ubiquitin, Myc-parkin, shDsRed, pLKO-shparkin, pLKO-shPARIS (Shin et al., 2011), PINK1 wild type, A217D, G309D, L347P, and K219M (Vives-Bauza et al., 2010).

Tandem affinity purification (TAP) of PARIS interacting proteins
SH-SY5Y cells were transiently transfected with N-terminal tandem affinity tag (SBP-CBP)-conjugated human PARIS (pNTAP-PARIS) or mock vector as a control followed by sequential purification steps according to the manufacturer's instructions (Interplay mammalian TAP system, Agilent Technologies). Briefly, total protein lysates were sequentially incubated in streptavidin and calmodulin resins to purify interacting proteins highly specific to TAP tagged PARIS. pNTAP-PARIS interacting proteins were subjected to western blots using anti-parkin or PINK1 antibodies to examine physical association of these proteins with PARIS. SBP, streptavidin binding peptide. CBP, calmodulin binding peptide.

Purification of GST-V5-PARIS WT or SA-DM (S322A/S613A) mutant, GST-PINK1, GST-FLAG-parkin, rat Parkin and MaBP-TcPINK1 recombinant proteins.
For purification of recombinant GST fusion proteins, the plasmids (pDEST-GST-V5-PARIS WT or SA-DM (S322A/S613A), pDEST-GST-FLAG-parkin, pGEX-6P-1-rat Parkin (Addgene #45969), pGEX-6P-1-rat Parkin$^{219-465}$ (Addgene #45972), pGEX-6P-1-

PINK1 wild type and kinase dead mutant) were transformed to BL21 pLys, which were then grown in the presence of 0.1 mM IPTG for 4 h at 30° C. Cells were lysed by sonication in a TNE buffer (10 mM Tris–HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA) containing 0.1% Triton X-100 (vol/vol) and protease inhibitors and finally centrifuged at 14,000 rpm for 30 min at 4° C. After centrifugation, the supernatant was recovered, and the GST-V5-PARIS, GST-FLAG-parkin and GST-PINK1 were purified with glutathione Sepharose 4B (GE Healthcare). The GST tag was removed by PreScission protease (GE Healthcare) from GST-V5-PARIS and GST-FLAG-parkin following the manufacturer's instructions. For purification of maltose-binding protein (MaBP) fusion proteins, Tc PINK1 wild type and kinase inactive (D359A) (Woodroof et al., 2011) were transformed to BL21 pLys, which were then grown with 250 μM IPTG for 15–16 h at 16° C. Cells were lysed by sonication in the lysis buffer. Lysates were clarified by centrifugation at 30,000g for 30 min at 48° C. After centrifugation the supernatant was recovered and the MaBP TcPINK1 wild type and kinase dead were purified with amylose resin for 1.5 h at 48° C.

Cell culture and transfection.
Human neuroblastoma SH-SY5Y cells (ATCC, Manassas, VA) were grown in DMEM containing 10% FBS (vol/vol) and antibiotics in a humidified 5% CO2/95% air atmosphere at 37° C. For transient transfection, cells were transfected with indicated target vectors using Fugene reagent (Promega) according to manufacturer's instructions. The ratio of amounts of DNA constructs used in transfection is as follows, PARIS:PINK1=1:4 for cellular kinase assay; HA-Ub:PARIS:PINK1=4:1:4 for cellular ubiquitination assay. Unless otherwise indicated, lysates were prepared 48 h after transfection. For the luciferase assay SH-SY5Y cells were transiently transfected with pCMV-empty vector or pCMV-FLAG-PARIS or pCMV-tTA together with TetP-PARIS-FLAG wild type or phosphorylation mutants with either wild type or K219M PINK1, or pGL3-Basic, pGL3-PGC-1α promoter-Luciferase (a gift from Akyoshi Fukamizu, University of Tsukuba, Japan) for firefly Luciferase assay and 0.1 mg pRL-TK vector (Promega) for Renilla luciferase control.

Subcellular fractionation.
SH-SY5Y cells and mouse brain tissues were subcellularly fractionated into the cytosol, mitochondria, and the nucleus by using Qproteome Mitochondria Isolation Kit (Qiagen) following the instructions in the manual. Cytosolic fractions were further concentrated with acetone precipitation. The purity of each fraction was validated with western blots using antibodies to marker proteins for cytosolic (GAPDH), mitochondrial (SDHA), and nuclear (PARP1) subcellular fractions.

***In vitro* interaction assay.**
For *in vitro* protein-protein interaction assays, 0.2 mg of V5–PARIS and protein G Sepharose preincubated with anti-PARIS antibodies were mixed and incubated with rGST-PINK1 over night at 4° C in 10 ml binding buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% NP-40 (vol/vol), 0.1% SDS (wt/vol)) including the protease inhibitor cocktails (Roche). After extensive washing, retained proteins were eluted by boiling in SDS protein loading buffer and analyzed by immunoblotting using rabbit anti-

PINK1 (Novus Biologicals) and rabbit anti-PARIS antibodies. For *in vitro* complex formation with recombinant FLAG-parkin, V5-PARIS/rGST-PINK1/FLAG-parkin were incubated in combinations in binding buffer as described previously. The pull-down samples by a mouse anti-parkin antibody were subjected to western blots analysis with the indicated antibodies.

***In vitro* kinase assay.**
MaBP-TcPINK1 wild type and the kinase inactive recombinant proteins were prepared as described in the Method section of the Purification of recombinant proteins above. FLAG-PARIS was purified by anti-FLAG immunoprecipitation from SH-SY5Y cells transfected with the cFUGW-FLAG-PARIS construct. PARIS was eluted by incubating beads with FLAG peptides (N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C, Sigma-Aldrich, 100 μg per ml in PBS) for 3 h at 4° C. FLAG peptide-eluted PARIS-FLAG wild type proteins (200 ng) were used in *in vitro* kinase reaction with MaBP-TcPINK1 (Figure 2A, and Figure S4C). The *in vitro* PARIS phosphorylation assay protein preparations were incubated at 30° C for 45-60 min in a mixture of 10-20 mM $MgCl_2$, 2-3 mM $MnCl_2$, 1 mM dithiothreitol, 20% glycerol (vol/vol), protease inhibitor cocktail, 1-2 mM ATP (when [γ-P^{32}]-ATP was used in the reaction, the final concentration per reaction was set to be 4 μCi), and 20 mM Tris or 40 mM HEPES (pH 7.4). The reaction was stopped by adding sample buffer and boiling. The mixtures were separated on a SDS–polyacrylamide gel and PARIS phosphorylation was determined by western blot with phosphoserine specific antibodies or pS613-PARIS specific antibodies or ^{32}P autoradiography.

Co-immunoprecipitation.
For co-immunoprecipitation from SH-SY5Y cells, 48 h after transfection cells were washed with cold PBS and harvested in immunoprecipitation buffer (1% NP-40 (vol/vol), 2 mg per ml aprotinin, and 100 mg per ml PMSF in PBS). The lysate was then rotated at 4 °C for 1 h, followed by centrifugation at 14,000 rpm for 20 min. The supernatants were then combined with protein G Sepharose beads (Amersham Biosciences) preincubated with antibodies against FLAG or GFP, followed by rotating at 4 °C overnight. The protein G Sepharose was pelleted and washed two times using immunoprecipitation buffer and additional two times with 500 mM NaCl buffer. The precipitates were resolved on SDS-PAGE gel and subjected to immunoblot analysis. Immunoblot signals were visualized with chemiluminescence (Pierce, Rockford, IL). For coimmunoprecipitation of endogenous parkin and associated protein complex from mouse brain, brains were homogenized in lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40 (vol/vol), 10 mM Na-beta-glycerophosphate, Phosphatase Inhibitor Cocktail I and II (Sigma), and Complete Protease Inhibitor Mixture (Roche)), using a Diax 900 homogenizer (Heidolph). The tissue homogenate was incubated on ice for 30 min and mixed twice for complete lysis. The samples were then centrifuged at 52,000 rpm at 4° C for 20 min. The supernatant was used for immunoprecipitation with an anti-parkin antibody. Immunoprecipitates were separated by SDS-PAGE and subjected to immunoblot analysis with an anti-PARIS or anti-PINK1 antibodies. Immunoblot signals were visualized with chemiluminescence. For mapping of the binding region between PINK1 and PARIS, FLAG-tagged PARIS full length or deletion constructs were transfected with GFP-tagged PINK1, or FLAG-PARIS was co-

transfected with PINK1. Transfections and co-immunoprecipitation was performed as described above.

Phosphoprotein purification.
Total protein was extracted from SH-SY5Y cells treated with CCCP or DMSO by using lysis buffer provided in the phosphoprotein column purification kit (#90003, Thermo scientific, Waltham, MA, USA). Total proteins were further processed by incubating with either phosphatase inhibitor or recombinant phosphatase (cat# P0753S, NEB, Ipswich, MA, UK) for 1 h followed by phosphoprotein column purification according to the manufacturer's instruction. Phosphoprotein enrichment samples were prepared by directly adding sample buffer and subjected to western blots.

Cellular ubiquitination assay.
For the ubiquitination assay, SH-SY5Y cells were transiently transfected with pMT123-HA-ubiquitin, FLAG-PARIS wild type or phosphorylation mutants in the presence of wild type or kinase deficient PINK1, or shPINK1, or shparkin. 48 h after transfection total cell lysates were prepared by harvesting the cells after washing with PBS, followed by solubilizing the pellets in 200 μl of 2% SDS (wt/vol), followed by sonication. The lysates were then rotated at 4° C for 1 h, diluted to 1 ml with PBS, and then boiled and sonicated. The samples were used as input and for immunoprecipitation. Immunoprecipitation was performed with an antibody against FLAG. The precipitates were subjected to immunoblotting with anti-HA for ubiquitin modification and anti-FLAG antibodies for normalization of PARIS.

Tet-off mediated pulse chase study.
SH-SY5Y cells were transfected with pCMV-tTA and TetP- PARIS-FLAG constructs with indicated other DNA constructs. On the following day, SH-SY5Y cells were treated with doxycycline (200 ng per ml, Invitrogen) to stop further transcription of PARIS-FLAG and the levels of PARIS-FLAG degradation were monitored at the indicated time points by collecting total protein lysates using western blots with anti-FLAG antibodies. To monitor proteasomal clearance of Tet-off expressed PARIS-FLAG, SH-SY5Y cells were treated with the proteasome inhibitor MG132 (10 uM, Sigma, C2211) for 4 h at 16 h following transient transfection with the indicated DNA constructs. The prepared total protein lysates were subjected to immunoblot.

***In vitro* ubiquitination/phosphorylation assay.**
Recombinant PARIS wild type or phosphorylation (S322A/S613A) mutant (SA-DM) proteins (200 ng, recombinant proteins were prepared as described in the Method section of the Purification of recombinant proteins above) were incubated in the ubiquitin conjugation reaction buffer (Boston Biochem) with supplementation of 2 mM ATP and with the addition of ubiquitin S65A (10 μg), UBE1 (250 ng), UbcH7 (500 ng), and rat Parkin (full length, aa 219-465, 2 μg) at 37° C for 40 min. The reaction was stopped by boiling the samples in SDS sample buffer and the boiled supernatants were separated in 8-16% gradient SDS-PAGE (wt/vol). Ubiquitinated proteins were detected by immunoblots with antibodies specific for each protein. All the recombinant proteins except for rat Parkin, PARIS WT and SA-DM mutant were purchased from Boston

Biochem.

Virally induced conditional PINK1 knockdown mouse model.
Conditional *pink1-siRNA* transgenic mice were generously given by Dr. Xugang Xia (Thomas Jefferson University). In this mouse strain, expression of shRNA to mouse PINK1 is blocked by a floxed STOP sequence in between the U6 promoter and shPINK1 sequence (Zhou et al., 2007). Conditional *pink1-siRNA* transgenic mice were genotyped by PCR using primers annealing to a part of the U6 promoter region and the stuffer sequence (forward primer: 5'-AGACTTGTGGGAGAAGCTCG-3'; reverse primer: 5'-GCCTCTTCATCGGGAATGC-3'). To achieve PINK1 knockdown in adult mice, a recombinant adeno-associated virus expressing GFP-fused Cre recombinase (AAV-GFP-Cre) was stereotaxically introduced into two month-old conditional *pink1-siRNA* transgenic mice using the substantia nigra coordinates (1.3 mm lateral, 3.2 mm caudal, 4.3 mm ventral relative to Bregma). Lentiviral shRNA-PARIS was co-administered along with AAV-GFP-Cre to determine whether the changes in PGC-1α and toxicity were PARIS dependent. Additional AAV viruses were used to address potential off-target effects by shRNA to mouse PINK1 or mouse PARIS (AAV-human PINK1; AAV-scrambled shRNA-GFP control with targeting sequence: CCGG-CAACAAGATGAAGAGCACCAA-CTCGAG-TTGGTGCTCTTCATCTTGTTG-TTTTTT; AAV-shPARIS#2-RFP with targeting sequence: TGCTGTTGACAGTGAGCGACTGGACTATGCCATCTCCAAATAGTGAAGCCACAGATGTATTTGGAGATGGCATAGTCCAGGTGCCTACTGCCTCGGA).

Preparation of tissues for immunoblot.
Mouse brain tissues harvested 1 month after virus injection were homogenized in lysis buffer [10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40 (vol/vol), 10 mM Na-β-glycerophosphate, Phosphate Inhibitor Cocktail I and II (Sigma), and Complete Protease Inhibitor Mixture (Roche)], using a Diax 900 homogenizer. After homogenization, samples were rotated at 4° C for 30 min for complete lysis, then the homogenate was centrifuged at 52,000 rpm for 20 min, and the resulting fractions were collected. Protein levels were quantified using the BCA kit (Pierce) with BSA standards and analyzed by immunoblot. Immunoblotting was performed with an antibody of interest with chemiluminescence visualization (Pierce). The densitometric analyses of the bands were performed using ImageJ (NIH, http://rsb.info.nih.gov/ij/).

Chromatin immunoprecipitation.
Chromatin immunoprecipitation was carried out per the manufacturer's instructions (Cell signaling). Briefly, SH-SY5Y cells were fixed with 1% formaldehyde (wt/vol) for 10 min at 37° C. Glycine quenched samples were lysed in 500 μl of SDS buffer containing protease inhibitors. Chromatin was digested with micrococcal nuclease and sonicated to 100-250bp DNA/Protein complex using a Diagenode UCD-300. The samples were centrifuged at 10,000 x g at 4° C for 10 min and the supernatant was taken. Pre-cleared samples were incubated with mouse anti-PARIS antibodies (Neuromab) overnight and protein G Sepharose beads followed by several washes. Elutes were subjected to reverse cross-linking and DNA was recovered by phenol-chloroform-ethanol purification. Real-time PCR was performed using template DNA and the following primers (cycle

number: 25-40): hPGC-1α promoter (forward primer: 5'-ACCTGCATTAGCCCTCATTGTCTC-3'; reverse primer: 5'-TAGCGTTTCCTCCCTAACTGCC-3'), hGAPDH promoter (forward primer: 5'-GGAAAGGCAATCCCAGAAAGGC-3'; reverse primer: 5'-ATGGGAGGGTGCTGAACACTTG-3').

Real-time quantitative PCR.
Total RNA was extracted with RNeasy Micro Kit (Qiagen) followed by in-column DNase I treatment to eliminate trace DNA contamination. cDNA was synthesized from total RNA (1.5 μg) using a First-strand cDNA synthesis kit (Invitrogen). Aliquots of cDNA were used as templates for real-time qPCR procedure. Relative quantities of mRNA expression were analyzed using real-time PCR (Applied Biosystems ABI Prism 7700 Sequence Detection System, Applied Biosystems). The SYBR greenER reagent (Invitrogen) was used per the manufacturer's instruction. The primer sequences for real-time amplification of genes are listed in Table S1.

Cell viability analysis.
SH-SY5Y cells were plated in a 6-well plate at a seeding density of 0.5 x10^6 cells per well. Following transient transfection with indicated constructs, the cells were grown in DMEM containing low serum (2.5 % FBS (vol/vol)) for two more days. After the indicated days of induction, cultures were trypsinized yielding single cell suspensions that were washed twice with PBS before resuspension in serum free DMEM. Resuspended cells were mixed with an equal volume of 0.4% trypan blue (wt/vol) and incubated for 2 min at room temperature. Live and dead cells were counted under a light microscope using a hemacytometer.

Immuofluorescence.
4% paraformaldehyde/PBS (pH 7.4)-fixed coronal brain sections were blocked with 4% donkey serum (Sigma-Aldrich)/PBS plus 0.2% Triton X-100, followed by incubation with mouse antibodies to TH, and rabbit antibodies to GFP (Figure 6B, and S6B) or rabbit antibodies to TH, goat antibodies to GFP (Figure S7A) and mouse antibodies to PGC-1α (Figure S7B). After brief washes with PBS containing 0.2% Triton X-100, floating brain sections were incubated with corresponding secondary antibodies conjugated with fluorescent dyes (Alexa Fluor 488–conjugated donkey antibody to rabbit IgG for GFP, and Alexa Fluor 568–conjugated donkey antibody to mouse IgG for tyrosine hydroxylase or Alexa Fluor 647-conjugated donkey antibody to rabbit IgG for TH, Alexa Fluor 488–conjugated donkey antibody to goat IgG for GFP, and Alexa Fluor 568–conjugated donkey antibody to mouse IgG for PGC-1α). Images were obtained using fluorescent microscope (Zeiss Axiovert 200M) or confocal microscope (Zeiss Confocal LASM 710). For SH-SY5Y cells, 24 h after transient transfection with the indicated constructs and treatment of 20 μM CCCP for 2 h, cells were incubated with mitotracker Red CMXRos (200 nM, 10 min, Molecular Probes, Invitrogen) followed by fixation in 4% paraformaldehyde/PBS (pH 7.4) for 15 min. Fixed cells were blocked with 10% donkey serum (Sigma-Aldrich)/PBS plus 0.3% Triton X-100 for 1 h at room temperature and incubated overnight at 4° C with antibodies against mouse antibodies to FLAG and rabbit antibodies to Myc or mouse antibodies to FLAG and rabbit antibodies to GFP.

After brief washes with PBS, cells on glass plates were incubated with corresponding secondary antibodies conjugated with fluorescent dyes and mounted with mounting solution (Aqua-Mount, Thermo scientific) onto microscope slides (Superfrost Plus Microscope Slides, Fisher Scientific). Images were obtained using confocal microscope (Zeiss Confocal LASM 710).

Laser capture microdissection (LCM).
Approximately three month old *pink1-siRNA* transgenic mice injected with either AAV-GFP (*n* = 4) or AAV-GFP-Cre (*n* = 3) or AAV-GFP-Cre/shPARIS at two month of age were sacrificed. The brains were rapidly removed and frozen on dry ice with OCT tissue freezing medium for cryostat sectioning (Tissue-Tek). To preserve fluorescence and RNA integrity, a RNase inhibitor and autoclaved PBS were used during all staining procedures. Fifteen micron-thick coronal sections of the midbrain on superfrost glass slide were incubated with blocking solution for 30 min and rinsed with 1 x PBS followed by incubation with mouse anti-TH (1:50) and rabbit anti-GFP (1:50) for 3-4 h. Rinsed sections were incubated with Alexa Fluor 568-conjugated anti-mouse (1:25) and Alexa Fluor 488-conjugated anti-rabbit (1:25) for 1 h. Sections were rinsed with 1 x PBS three times and were washed once again with DEPC-treated water. Double, TH and GFP, positive neurons were obtained by LCM (Leica P.A.L.M. laser microdissection). Microdissected cells were directly used for RNA extraction.

Stereotaxic injections and stereological assessments.
For stereotaxic injection of adeno-associated virus serotype 1 (AAV1) overexpressing GFP, or GFP-Cre, or PARIS, or PARIS SA-DM, or hPINK1, or shRNA-PARIS into mice, experimental procedures were followed according to the guidelines of Laboratory Animal Manual of the National Institute of Health Guide to the Care and Use of Animals, which were approved by the Johns Hopkins Medical Institute Animal Care Committee. Two month old wild type C57BL/6J or PINK1 siRNA conditional transgenic mice (pU6-STOP fl/fl-shPINK1) were anesthetized with pentobarbital (60 mg per kg). An injection cannula (26.5 gauge) was applied stereotaxically into the substantia nigra (anteroposterior, 3.2 mm from bregma; mediolateral, 1.3 mm; dorsoventral, 4.3 mm). The infusion was performed at a rate of 0.2 ml per min and wound healing and recovery were monitored after the injection was done. Four weeks, 3 months, and 12 months after injection, animals were anesthetized and perfused with PBS followed by 4% paraformaldehyde (wt/vol). Brains were post-fixed with 4% paraformaldehyde (wt/vol), cryoprotected in 30% sucrose (wt/vol), and processed for immunohistochemistry. Forty μm coronal sections were cut throughout the brain including substantia nigra and every 4th section was utilized for analysis. For tyrosine hydroxylase (TH), sections were incubated with a 1:1000 dilution of rabbit polyclonal anti-TH (Novus) and visualized with biotinylated goat anti-rabbit IgG, followed by application of streptavidin-conjugated horseradish peroxidase (HRP) (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Positive immunostaining was visualized with 3,3-diaminobenzidine (DAB, cat# D4293, Sigma). Stained sections were mounted onto slides and counterstained with thionin for Nissl substance. Total numbers of TH-positive and Nissl-stained neurons in substantia nigra pars compacta were counted using the Optical Fractionator probe of Stereo Investigator software (MicroBrightfield, Williston, VT). For Nissl counting, a cell

was defined as a bright blue-stained neuronal perikarya with a nucleolus. Nissl positive counts were calculated by combining $Nissl^+/TH^+$ neurons along with large $Nissl^+$ neurons with DA-like morphology, that contain little or no TH immunostaining (Experimenters were blinded for treatments of mice during stereological counting).

Statistics.
Power analysis was performed by using G*Power 3.1 software to determine approximate sample size for tyrosine hydroxylase stereological analysis of virally induced PD mouse models. On the basis of mean difference from our preliminary experiments, total samples size of 12 was calculated for AAV-GFP or PARIS or PARIS/PINK1 injection models (Figure 6E and 6F. effect size f = 1.414 for 40% mean difference, 0.2 s.d. value in each group; α = 0.05). Total samples size of 12 was calculated for conditional PINK1 knockdown models (Figure 7C~7D, and Figure S7F~S7G. effect size f = 1.6499 for 30% mean difference, 0.2 s.d. value in each group; α = 0.05). Quantitative data are presented as the mean ± s.e.m. Normality of the data was tested with the Shapiro-Wilk test. The equality of variance was determined with Levene statistics. Statistical significance was assessed either via an unpaired two-tailed Student's *t*-test or nonparametric Mann-Whitney *U*-test for two-group comparison or an ANOVA test with Tukey's HSD post hoc analysis for comparison of more than three groups. Assessments were considered significant with a $P < 0.05$.

Supplemental References

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