Supporting Information

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SI Materials and Methods

Plasmids, Antibodies, and Reagents. *Plasmids.* The full-length and truncated mutants for human parkin (ΔUBL, R1-IBR, and R2) were cloned into pRK5-myc vector (Stratagene). Ubiquitin was cloned into pRK5-HA vector (Stratagene). Full-length human FBP1 was kindly provided by David Levens (National Cancer Institute, National Institutes of Health, Bethesda, MD). FLAG-tagged aminoacyl-tRNA synthetase-interacting multifunctional protein type 2 (AIMP2) has been described previously (1). GFP-c-Abl-KA (kinase-active) and GFP-c-Abl-KD (kinase-dead) were kindly provided by Dr. Z.M. Yuan (Harvard School of Public Health, Boston, MA). Plasmids for c-Abl wild-type and c-Abl deletion mutants (F1–F3) were kindly provided by Dr. Baojie Li (Institute of Molecular and Cell Biology, Singapore). c-Abl deletion mutants (F4-F5) were cloned into pCMV-Tag2A-FLAG vector (Stratagene). GST-parkin was cloned into pGEX-4T-1 (Amersham Biosciences). V5-tagged parkin and V5-tagged Y143F parkin constructs were provided by Dr. S.Z. Imam (University of Texas Health Science Center, San Antonio, TX). A plasmid containing β-galactosidase, empty vector, or GFP cDNA was used as a control in the cell-culture experiments.

Antibodies. Primary antibodies used include the following: phospho-c-Abl (Tyr245) (2861; Cell Signaling), mouse anti-c-Abl (554148; BD Transduction Laboratories), mouse anti-c-Abl (A5844; Sigma), rabbit anti-c-Abl (Santa Cruz Biotechnology), mouse anti-parkin (4211; Cell Signaling), rabbit anti-parkin (2131; Cell Signaling), rabbit anti-phosphotyrosine antibody (610009; BD Transduction Laboratories), rabbit anti-phospho-CDK5 antibody (SC-12918-R; Santa Cruz Biotechnology), rabbit anti-CDK5 antibody (2506; Cell Signaling), rabbit anti-tyrosine hydroxylase (TH) (Novus Biologicals), rabbit anti-ubiquitin (DakoCytomation), rabbit anti-AIMP2 (p38/JTV1; ProteinTech Group), mouse anti-FBP1 (BD Transduction Laboratories), rabbit anti-GFP (ab290; Abcam), mouse anti-c-myc and rabbit anti-HA (Roche Diagnostics), monoclonal anti-FLAG M2 (Sigma), monoclonal anti-V5 (Invitrogen), and monoclonal anti-His antibody (Roche). Secondary antibodies used include the following: phosphotyrosine, 4G10 platinum-peroxidase (Millipore), actin-peroxidase and Flag-peroxidase (Sigma), V5-peroxidase (Invitrogen), HA-peroxidase and myc-peroxidase (Roche Diagnostics), anti-rabbit and -mouse IgG, peroxidase-linked species-specific whole antibody, and ECL (BD Biosciences).

Reagents. The following chemicals were used: Geneticin (G418), hygromycin B, and doxycycline (Dox; Invitrogen), c-Abl recombinant protein (SE 290; BIOMOL International), c-Abl kinase inhibitor STI-571 (Novartis Pharma AG), 1-methyl-4-phenylpyridinium (MPP^+; Sigma-Aldrich), dopamine (DA; Sigma-Aldrich), His_6-parkin recombinant protein (E3-150; Boston Biochem), protein-G Sepharose and glutathione-Sepharose 4B (GE Healthcare Sciences), Phosphatase mixture I and II [P2850 (I), P5726 (II) (Sigma-Aldrich)], and Lipofectamine Plus reagent and Lipofectamine 2000 (Invitrogen).

GST pull-down assay. GST fusion proteins were prepared following a standard protocol. For in vitro binding assays, parkin GST fusion proteins bound to the glutathione sepharose beads were incubated with c-Abl recombinant proteins. After washing, the bound proteins were separated by SDS/PAGE and immunoblotted with indicated antibodies.

Coimmunoprecipitation and immunoblot analysis. For coimmunoprecipitation from cell cultures, SH-SY5Y cells were transiently transfected with indicated plasmids. After 48 h, cells were washed with cold PBS and harvested in immunoprecipitation buffer (1% Triton X-100, 2 μg/mL aprotinin, and 100 μg/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 40 mL of protein-G Sepharose (Amersham Biosciences) preincubated with indicated antibodies, followed by rotating for 2 h or overnight at 4 °C. The protein-G Sepharose was pelleted and washed three times using immunoprecipitation buffer or buffer with additional 500 mM NaCl, followed by three washes with PBS. The precipitates were resolved on SDS/PAGE and subjected to immunoblot analysis. Immunoblot signals were visualized with chemiluminescence (Pierce). The tissues including human brain regions from striatum, substantia nigra, and cortex and mouse brain were homogenized in lysis buffer [10 mM Tris·HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 10 mM Na-β-glycerophosphate, Phosphate Inhibitor Mixture I and II (Sigma), and Complete Protease Inhibitor Mixture (Roche), using a Diax 900 homogenizer (Sigma). After homogenization, samples were rotated at 4 °C for 30 min for complete lysis, 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. The supernatant was used for immunoprecipitation with either of the following antibodies: mouse IgG (mIgG) or anti-parkin. The immunocomplexes were then washed with immunoprecipitation buffer six times and separated by SDS/PAGE and subjected to immunoblot analysis with indicated antibodies. Immunoblot signals were visualized with chemiluminescence.

shRNA-parkin constructs and siRNA-c-Abl. MISSION short-hairpin RNA (shRNA) plasmids (Sigma) encoding small interfering RNAs (siRNAs) targeting parkin were purchased from Sigma. Two plasmids (#1: TRCN0000000281 and #3: TRCN0000000285) were effective in knocking down parkin expression. siRNA targeting c-Abl (S864 and S866) and scrambled control siRNA were purchased from Applied Biosystems.

2D gel electrophoresis and immunoblot. Recombinant His_6-tagged parkin or phosphorylated His_6-parkin by c-Abl was followed by 2D gel electrophoresis (2-DE) immunoblotting. Each sample was suspended in 1.5 mL of sample buffer consisting of 40 mM Tris, 7 M urea (Sigma), 2 M thiourea (Sigma), 4% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate) (Sigma), 65 mM DTT (Bio-Rad Laboratories), 1 mM EDTA, protease inhibitors mixture (Roche), and 1 mM phenylmethylsulfonyl chloride. The suspension was concentrated and desalted by a centrifugal filter (Millipore) at 3,500 rpm for 3 h. Samples were applied on immobilized pH 4–7 nonlinear gradient strips (13 cm). Focusing started at 200 V and the voltage was gradually increased to 8,000 V at 3 V/min (≈151,000 Vh). After the first dimension, strips were equilibrated for 15 min in the equilibration buffer containing 6 M urea, 20% glycerol, 2% SDS, and 2% DTT and then for 15 min in the same equilibration buffer containing 2.5% iodoacetamide instead of DTT. After equilibration, strips were loaded on 9–16% gradient SDS gels for second-dimensional separation. The gels (180 × 200 × 1.5 mm) were run at 40 mA per gel. Immediately after the second-dimension run, gels were transferred to PVDF membrane and anti-His or anti-phosphotyrosine antibody was applied for 2-DE immunoblotting. The corresponding phosphospot in Coomassie-stained 2-DE gel was used for identification of the phosphorylation site via MS/MS.

Phosphorylation analysis by LC-MS/MS. Excised 2-DE spots were subjected to a modified in-gel trypsin digestion procedure (2). Gel pieces were washed and dehydrated with acetonitrile for 10 min

followed by removal of acetonitrile. Pieces were then completely dried in a speed vac. Rehydration of the gel pieces was accomplished with 50 mM ammonium bicarbonate solution containing 12.5 ng/μL modified sequencing-grade trypsin (Promega) at 4 °C. Samples were then placed in a 37 °C room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed vac (~1 h). The samples were then stored at 4 °C until analysis. The samples were reconstituted in 5 mL of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nanoscale reverse-phase HPLC capillary column was created by packing 5-mm C18 spherical silica beads into a fused silica capillary (100-mm inner diameter × ~12-cm length) with a flame-drawn tip (3). After equilibrating the column, each sample was pressure-loaded off-line onto the column. The column was then reattached to the HPLC system. A gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid). As each peptide was eluted, it was subjected to electrospray ionization and then entered into an LTQ-Orbitrap mass spectrometer (Thermo Finnigan). Eluting peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein or translated nucleotide databases with the acquired fragmentation pattern by the software program Sequest (Thermo Finnigan) (4). The modification of 79.9663 mass units to serine, threonine, and tyrosine was included in the database searches to determine phosphopeptides. Each phosphopeptide that was determined by the Sequest program was also manually inspected to ensure confidence.

In vitro phosphorylation assays. For in vitro kinase assays, 2.5 μg His_6-parkin recombinant protein was incubated with 250 ng c-Abl recombinant protein, unless otherwise indicated, in a kinase buffer (20 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 5 mM EGTA, 150 mM NaCl, 20 mM β-glycerol phosphate) for 45 min at 30 °C. STI-571 was used at concentrations as indicated in the figure legends. Phosphorylated proteins were separated by SDS/PAGE or 2D gel electrophoresis (2-DE) and analyzed by immunoblot or by LC-MS/MS.

Ubiquitination assay. SH-SY5Y cells were transiently transfected with various plasmid combinations as indicated. Forty-eight hours later the cells were lysed in 2% SDS buffer [2% SDS, 150 mM NaCl, 10 mM Tris·HCl, pH 8.0, 1% Phosphatase Inhibitor Mixtures I and II (Sigma), protease inhibitors] and boiled for 10 min followed by sonication. Lysates were diluted 1:10 in dilution buffer (10 mM Tris·HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100) and incubated at 4 °C for 1 h with rotation. The samples were split for use as input and for immunoprecipitation. Immunoprecipitated proteins were washed with washing buffer (10 mM Tris·HCl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1% Nonidet P-40), boiled in SDS sample buffer, and separated on SDS/PAGE. Immunoblot analysis experiments were performed using standard procedures. Detection was performed with Super Signal West Pico and Femto chemiluminescent substrates (Pierce Biotechnology).

Targeting the c-Abl gene in neuronal cells. Mice possessing the loxP-flanked *c-Abl* gene exon 5 (c-$Abl^{KO/flox}$/nestin Cre^{-}) were previously described and used in this study (5). c-$Abl^{KO/flox}$/nestin Cre^{-} (male) and c-$Abl^{KO/flox}$/nestin Cre^{-} (female) were crossed to generate c-$Abl^{flox/flox}$/nestin Cre^{-}. The c-$Abl^{flox/flox}$/nestin Cre^{-} mice were crossed to c-$Abl^{KO/flox}$/nestin Cre^{+}, and the offspring were bred to homozygosity at the loxP-flanked c-Abl locus. This enabled the Cre recombinase to inactivate the *c-Abl* gene specifically in cells in which the Nestin promoter is active (i.e., neuronal cells). The presence of the floxed *c-Abl* gene was determined by PCR around the 3′ loxP site using the primer (primer-2) 5′-TGT GCA TAG CAG GAA GTC CTC CAG AG-3′ and (primer-3) 5′-AGT TAA CAC ACC TCC AGA GTG AGT GCC CT-3′, yielding a PCR product of ~200 bp for WT and a product of ~239 bp for the floxed allele. Cre-mediated excision of c-Abl genomic sequences was detected by PCR on DNA isolated from tail snips of mice using the forward primer (primer-1) 5′-GAT GTC TCT ACA GGG TTA AG A TTA AGA GCA-3′ and the reverse primer (primer-3) 5′-AGT TAA CAC ACC TCC AGA GTG AGT GCC CT-3′, giving no band for nonrecombined allele and a product of ~300 for recombined floxed allele, resulting in the deletion of *c-Abl* gene as described previously (5). PCR to detect the presence of Nestin-Cre allele was performed using Cre primers, Cre forward primer 5′-GGA AGG TGT CCA ATT TAC TGA CCG TA-3′, and Cre reverse primer 5′-AAA TGT TGC TGG ATA GTT TTT ACT GC-3′ yielding a product of ~200 bp.

AIMP2-inducible cell lines. PC12 cells were grown in DMEM containing 10% horse serum, 5% Tet-approved FBS in a 5% CO_2 atmosphere. To create Tet-off PC12 cell lines inducibly expressing AIMP2, PC12 Tet-off cells containing pTet-off were purchased from Clontech. PC12 Tet-off cells (Clontech) were used to create PC12 cell lines expressing inducible AIMP2. A Tet-responsive AIMP2 expression construct was engineered by cloning the full-length cDNA of AIMP2 into pTRE (Clontech) vector. The AIMP2 construct was cotransfected into PC12 Tet-off cells with pTK-Hyg (Clontech) at a 10:1 molar ratio. Single colonies were obtained by limiting dilution of cells cultured in the presence of 100 μg/mL G418, 200 μg/mL hygromycin B (Clontech), and 200 ng/mL Dox for 2–3 wk. Clones were analyzed for the expression of AIMP2 by immunoblotting. Differentiation was initiated by the addition of 100 ng/mL NGF to the culture medium (DMEM with N2 supplement). NGF was replenished every day after differentiation.

Cell culture, transfections, and siRNA experiments. Human neuroblastoma SH-SY5Y cells were cultured as described previously (1) and AIMP2-inducible Tet-off PC12 cell lines were maintained in DMEM supplemented with 10% horse serum, 5% Tet-approved FBS, and the following antibiotics: 100 U/mL penicillin/streptomycin (Invitrogen), 100 μg/mL G418 (Invitrogen), and 200 μg/mL hygromycin (Invitrogen). Transient transfection with siRNA targeting c-Abl or scrambled control siRNA was carried out by using the Lipofectamine 2000 reagent (Invitrogen). All transient transfections were done with Lipofectamine PLUS or Lipofectamine 2000 reagent according to the manufacturer's instructions (Invitrogen).

Cell-viability analysis. SH-SY5Y cells were plated in a six-well plate at 0.5×10^6 cells for a viability assay. The cells were transfected with indicated siRNAs and shRNAs. After 24 or 48 h, cells were treated with MPP^+ (200 μM) for 24 h. In some cases, STI-571 (10 μM) was added to the cells 6 h before MPP^+ treatment. Trypan blue exclusion was used to measure cell death by counting the number of dead (blue) and live cells in the cultures. AIMP2-inducible PC12 cells were plated in a six-well plate at 0.5×10^6 cells for viability assays. Cells were transfected with indicated plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were incubated with MPP^+ (200 μM) or DA (200 μM) for 24 h after 2 d of induction and differentiation. In some cases, STI-571 (10 μM) was added to the cells 6 h before MPP^+ and DA treatment. To assess cell viability, we used the trypan blue exclusion assay. We counted the percentage of blue-stained cells among total cells (2,400–3,600 cells in total) in the trypan blue exclusion assay.

MPTP Injections and Analysis of Striatal MPP^+ Levels. All procedures involving animals were approved by and conformed to the guidelines of the Institutional Animal Care Committee of Johns Hopkins University. c-Abl WT and KO animals received four injections i.p. of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP·HCl; 20 mg/kg free base; Sigma-Aldrich) or saline control at 2-h intervals. Animals were decapitated 7 d after the last MPTP injection, and brains were dissected and

processed for immunoblot analysis. Animals injected with vehicle and MPTP were killed for the measurement of MPP^+. MPP^+ was estimated following the method described previously (6) using HPLC-UV detection at 295 nm. c-Abl WT and KO mice were injected i.p. with four doses of MPTP (20 mg/kg, i.p. every 2 h) and killed at 2 h after the final vehicle or MPTP injection. Striatums were dissected out and stored at −80 °C until analysis. Striatal tissues were sonicated in 10 volumes of 5% trichloroacetic acid containing 5 μg/mL of 4-phenylpyridine (Sigma) as an internal standard. The samples were centrifuged at 14,000 × *g* for 10 min and the supernatant was injected onto a cation-exchange Ultracyl-CS column (Beckman). The mobile phase consisted of 90% of a solution of 0.1 M acetic acid and 75 mM triethylamine·HCl (pH 2.35 adjusted with formic acid), and 10% acetonitrile. The flow rate was 1.5 mL. Results are presented as ng/mg tissue.

Immunohistochemistry and Stereological Cell Counts. Mice were perfused with ice-cold PBS and 4% paraformaldehyde/PBS (pH 7.4). Brains were removed and postfixed 4 h in the same fixative. After cryoprotection in 20% and 30% sucrose/PBS (pH 7.4), brains were frozen and serial coronal sections (30-μm sections) were cut with a microtome. Free-floating 30-μm sections were blocked with 4% goat serum/PBS plus 0.2% Triton X-100 and incubated with antibody against tyrosine hydroxylase (rabbit polyclonal; Novus Biologicals) followed by incubation with biotin-conjugated anti-rabbit antibody (goat polyclonal; Jackson ImmunoResearch), ABC reagents (Vector Laboratories), and SigmaFast DAB Peroxidase Substrate (Sigma-Aldrich). Sections were counterstained with Nissl (0.09% thionin). TH-positive or Nissl-positive cells from the substantia nigra pars compacta region of the left hemisphere were counted by using an optical fractionator, an unbiased method for cell counting. This method was carried out by using a computer-assisted image analysis system consisting of an Axiophot photomicroscope (Carl Zeiss Vision) equipped with a computer-controlled motorized stage (Ludl Electronics), a Hitachi HV C20 video camera, and Stereo Investigator software (MicroBrightField). The total number of TH- and Nissl-stained neurons was calculated by using the formula previously described for this method (7). For densitometry analysis of the fiber density in the striatum stained with an anti-TH antibody, the data were analyzed by using optical density measurement tools in ImageJ software (National Institutes of Health). The mean density of striatal regions from each group of mice was normalized by the intensity of the region outside of the striatum; the normalized intensities were compared for each group.

Statistical Analysis. Pooled results were expressed as means ± SEM. Statistical significance was determined by one-way ANOVA and Tukey's post hoc test or a two-tailed nonpaired Student's *t* test unless otherwise noted in the figure legend. Significance was set at $P \leq 0.05$.

1. Ko HS, et al. (2005) Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. *J Neurosci* 25: 7968–7978.
2. Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Anal Chem* 68:850–858.
3. Peng J, Gygi SP (2001) Proteomics: The move to mixtures. *J Mass Spectrom* 36: 1083–1091.
4. Griffin PR, et al. (1995) Direct database searching with MALDI-PSD spectra of peptides. *Rapid Commun Mass Spectrom* 9:1546–1551.
5. Moresco EM, Donaldson S, Williamson A, Koleske AJ (2005) Integrin-mediated dendrite branch maintenance requires Abelson (Abl) family kinases. *J Neurosci* 25:6105–6118.
6. Mandir AS, et al. (1999) Poly(ADP-ribose) polymerase activation mediates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. *Proc Natl Acad Sci USA* 96:5774–5779.
7. Andres-Mateos E, et al. (2007) DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. *Proc Natl Acad Sci USA* 104:14807–14812.

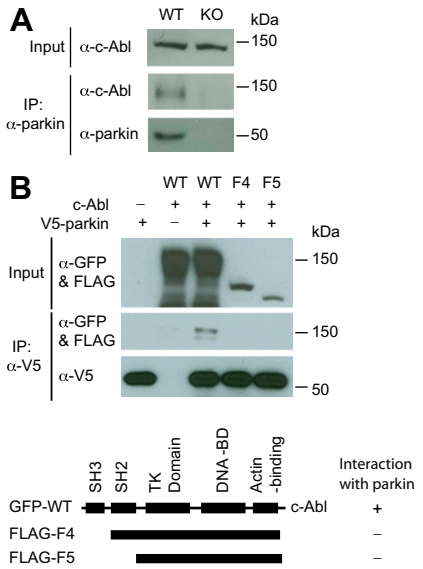

Fig. S1. (*A*) Interaction between parkin and c-Abl in mouse brain. Lysates prepared from parkin WT and KO mouse brain subjected to immunoprecipitation with anti-parkin and anti-IgG. Immunoprecipitates with antibodies to parkin and mouse IgG were probed with antibodies to c-Abl and parkin, respectively. (*B*) Protein interaction mapping between parkin and c-Abl. Immunoprecipitated V5-parkin interacts with WT but not the SH3-deficient domain mutants of c-Abl. The deletion domains of c-Abl used are shown at the bottom of the panel. All experiments were repeated at least three times and representative images of the immunoblots are shown.

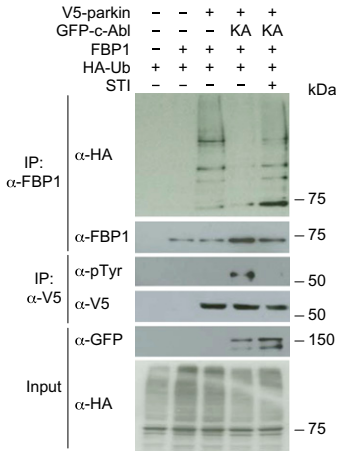

Fig. S2. Phosphorylated parkin is unable to ubiquitinate its substrate, FBP1. SH-SY5Y cells were transfected with FBP1, V5-WT-parkin, HA-ubiquitin, and GFP-c-Abl-KA. Immunoprecipitation with anti-FBP1 antibody and immunoblotting with anti-HA show the amount of ubiquitination of FBP1 and anti-FBP1 shows equivalent immunoprecipitated FBP1. Immunoprecipitation with anti-V5 and immunoblotting with anti-phosphotyrosine antibody show parkin phosphorylation and anti-V5 shows equivalent immunoprecipitated parkin. Inputs were immunoblotted with anti-HA and anti-GFP to show equivalent expression. All experiments were repeated at least three times and representative images of the immunoblots are shown.

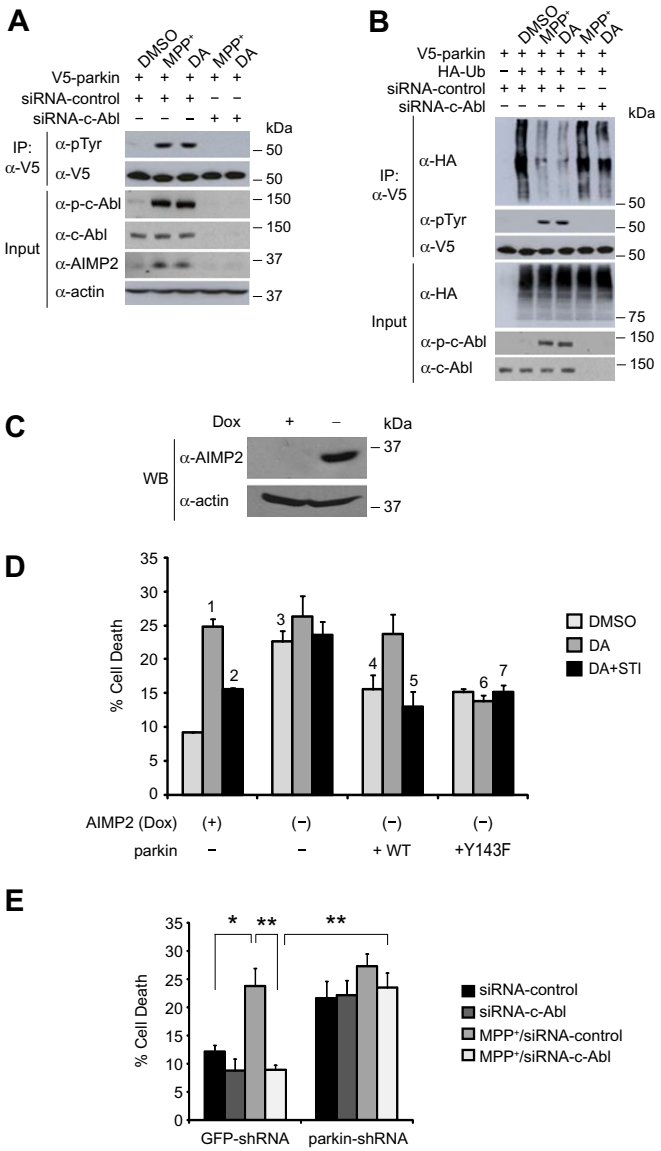

Fig. S3. Parkin is tyrosine-phosphorylated and its protective function is compromised under dopaminergic stress. (*A*) SH-SY5Y cells were transiently transfected with V5-parkin and treated with MPP+ (250 μM) or dopamine (DA) (250 μM) for 24 h. Some samples were transfected together with c-Abl-siRNA or scrambled control siRNA. Lysates were immunoprecipitated with an anti-V5 antibody and immunoblotted with an anti-phosphotyrosine to show phosphorylated parkin, and anti-parkin to show equivalent immunoprecipitation (IP). Inputs were immunoblotted with anti-phospho-c-Abl to show activated c-Abl, with anti-c-Abl to show knockdown of c-Abl, and with anti-AIMP2 or anti-actin antibodies. (*B*) SH-SY5Y cells were transiently transfected with V5-parkin and HA-ubiquitin and treated with MPP^+ (250 μM) or DA (250 μM) for 24 h. Some samples were transfected together with c-Abl-siRNA or scrambled control siRNA. Cell lysates were immunoprecipitated with an anti-V5 antibody and immunoblotted with anti-HA to monitor ubiquitination of parkin, anti-phosphotyrosine to show phosphorylated parkin, and anti-V5 to show equivalent levels of immunoprecipitated parkin. Inputs were immunoblotted with anti-phospho-c-Abl to show activated c-Abl, with anti-c-Abl to show knockdown of c-Abl, and anti-HA to show equivalent expression. (*C*) Inducible AIMP2-PC12 cell lines. Confirmation of expression levels of AIMP2 by Western blot (WB) analysis in induced doxycycline $(Dox)^-$ or noninduced Dox^+ conditions. Representative images from three separate experiments are shown. (*D*) PC12-AIMP2-inducible cells were transfected with V5-WT-parkin and V5-Y143F-parkin in the presence or absence of DA (200 μM) for 24 h. Some samples were incubated with 10 μM STI-571. Cell death was assessed by trypan blue exclusion. Both pretreatment with STI-571 and transfection with phosphorylation-resistant Y143F mutant of parkin protected against AIPM2-induced cell death after DA treatment. +, uninduced condition; –, induced condition. Data are mean ± SEM for three separate experiments performed in duplicate. Statistical significance was determined by one-way ANOVA and Tukey's post hoc test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; **1: DMSO versus DA–Dox (+); *2: DA versus STI+DA–Dox (+); *3: DMSO–Dox (+) versus AIMP2–Dox (–); ***4: AIMP2–Dox (–) versus AIMP2–Dox (–) + WT-parkin; **5: DA/AIMP2–Dox (–) + WT-parkin versus STI + DA/AIMP2–Dox (–) + WT-parkin; **6: DMSO/AIMP2–Dox (–) versus AIMP2–Dox (–) + Y143F-parkin; **7: DA/AIMP2–Dox (–) versus DA/AIMP2–Dox (–) + Y143F-parkin. (*E*) Trypan blue cell death assessments in SH-SY5Y neuroblastoma cells that were transiently transfected with parkin-shRNA or GFP-shRNA and treated with MPP^+ (200 μM). Some samples were transiently transfected together with c-Abl siRNA or scrambled control siRNA. Data are shown as mean ± SEM from four separate experiments. Statistical significance was determined by one-way ANOVA and Tukey's post hoc test. $*P < 0.05$, $**P < 0.01$.

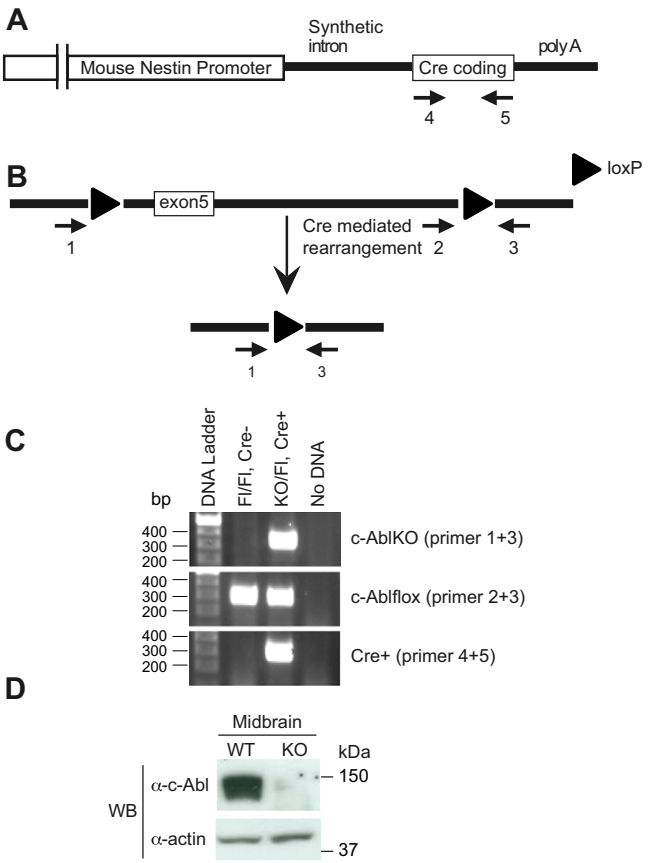

Fig. S4. Targeting the *c-Abl* gene in neuronal cells. (*A*) A Nestin-Cre construct possessing a 9.0-kb fragment of the mouse Nestin promoter, synthetic intron, Cre cDNA, and an SV40 polyadenylation sequence. Numbered arrows represent primers used to screen for the Cre-coding sequence. (*B*) Map of the c-Abl locus containing a floxed version of the gene and the predicted product of Cre-mediated recombination, including the elimination the 5′-untranslated sequence, exon 5, and intervening DNA. Numbered arrows represent PCR primers. (*C*) Ethidium bromide-stained 2.0% agarose gel of PCR products amplified from mouse tails of indicated mouse strains. PCR primers 1 and 3 demonstrate selective deletion of DNA between loxP sites in the presence of Cre recombinase in vivo. Each lane represents tail DNA samples isolated from an individual mouse of the indicated genotype. (*D*) Midbrain from WT and c-Abl KO mice were analyzed by immunoblot analysis to show knockout of the c-Abl protein. Representative images from two separate experiments are shown.

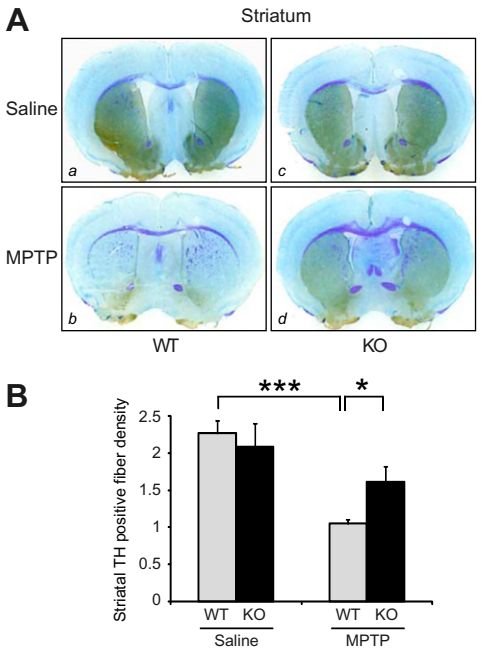

Fig. S5. (*A*) Photomicrographs of TH-immunostained sections in the striatum of the c-Abl WT (*a* and *b*) and KO (*c* and *d*) mice treated with saline vehicle (*a* and *c*) and MPTP (*b* and *d*). (*B*) Quantitative analysis of TH-immunoreactive fibers using optical density measurement tools in ImageJ software in the striatum. The data are the mean ± SEM (n = 6). Statistical significance was determined by one-way ANOVA and Tukey's post hoc test. *$P < 0.05$, ***$P < 0.001$.

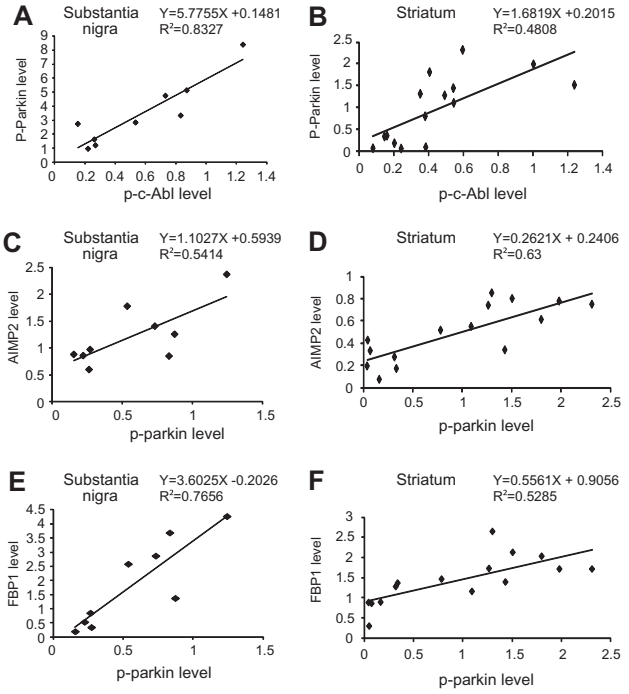

Fig. S6. Correlation between phosphorylated c-Abl and phosphorylated parkin and between phosphorylated parkin and levels of AIMP2 and FBP1 in substantia nigra and striatum of Parkinson disease (PD) brains. There is a strong positive correlation between phosphorylated c-Abl and phosphorylated parkin in substantia nigra (R^2 = 0.8327, $P < 0.0005$) (*A*) and striatum (R^2 = 0.4808, $P < 0.005$) (*B*) and between phosphorylated parkin and AIMP2 in substantia nigra (R^2 = 0.5414, $P < 0.05$) (*C*) and striatum (R^2 = 0.63, $P < 0.0005$) (*D*) and between phosphorylated parkin and FBP1 in substantia nigra (R^2 = 7656, $P < 0.001$) (*E*) and striatum (R^2 = 0.5285, $P < 0.005$) (*F*).

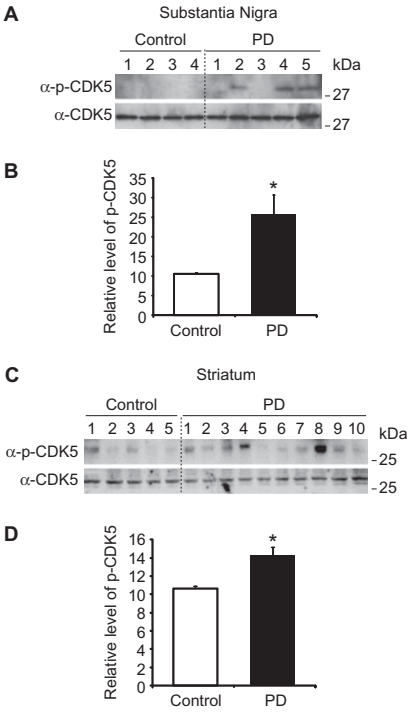

Fig. S7. CDK5 is tyrosine-phosphorylated in the striatum and substantia nigra of PD patients. Brain lysates from substantia nigra (*A*) and striatum (*C*) were immunoblotted with anti-phospho-CDK5 antibody to monitor tyrosine-phosphorylated CDK5 on tyrosine 15. Relative phospho-CDK5 levels normalized to CDK5 are indicated in *B* (substantia nigra) and *D* (striatum). The data are the mean ± SEM. Statistical significance was determined by applying the unpaired two-tailed Student's *t* test. $*P < 0.05$.

Table S1. Human postmortem tissues used for immunoblot in Fig. 6*A*.

	Final diagnosis	Age	Sex	Race	PMD	Tissue
Control	Control	89	M	W	8.5	SN
	Control	85	M	B	6	SN
	Control	73	F	W	9	SN
	Control	71	M	W	16	SN
PD	PD, neocortical	71	M	W	8	SN
	PD, W/D, ND, OI	83	M	W	5	SN
	PD, W/D	76	M	W	17	SN
	PD, W/D	73	M	W	6.5	SN
	PD, MIC	80	F	W	6	SN

B, black; F, female; M, male; MIC, multiple infarcts/contusions; ND, neurodegeneration; OI, occipital infarction; PMD, postmortem delay; SN, substantia nigra; W, white; W/D, with dementia.

Table S2. Human postmortem tissues used for immunoblots in Fig. 6 *B* and *C*.

	Final diagnosis	Age	Sex	Race	PMD	Tissue
Control	Control	66	M	W	12	Str, Ctx
	Control	49	F	W	15	Str, Ctx
	Control	74	M	W	4	Str, Ctx
	Control	91	F	W	8	Str, Ctx
	Control	79	M	W	16	Str, Ctx
PD	PD, LB	86	F	W	16	Str, Ctx
	PD, W/LB	70	M	W	26	Str, Ctx
	PD, LB	88	F	W	19.5	Str, Ctx
	PD, VD	95	F	W	12	Str, Ctx
	PD, W/D, CVD	83	M	W	16.5	Str, Ctx
	PD, W/D	75	M	W	6	Str, Ctx
	PD, W/D	76	M	W	17	Str, Ctx
	PD, CVD	85	F	W	9	Str, Ctx
	PD, W/D	60	M	W	15.5	Str, Ctx
	PD, W/D	71	M	W	24	Str, Ctx

B, black; Ctx, cortex; CVD, cerebrovascular disease; F, female; LB, Lewy body; M, male; PMD, postmortem delay; Str, striatum; VD, vascular disease; W, white; W/D, with dementia; W/LB, with Lewy body.