Supplementary Data for LOSS OF PINK1 FUNCTION PROMOTES MITOPHAGY THROUGH EFFECTS ON OXIDATIVE STRESS AND MITOCHONDRIAL FISSION
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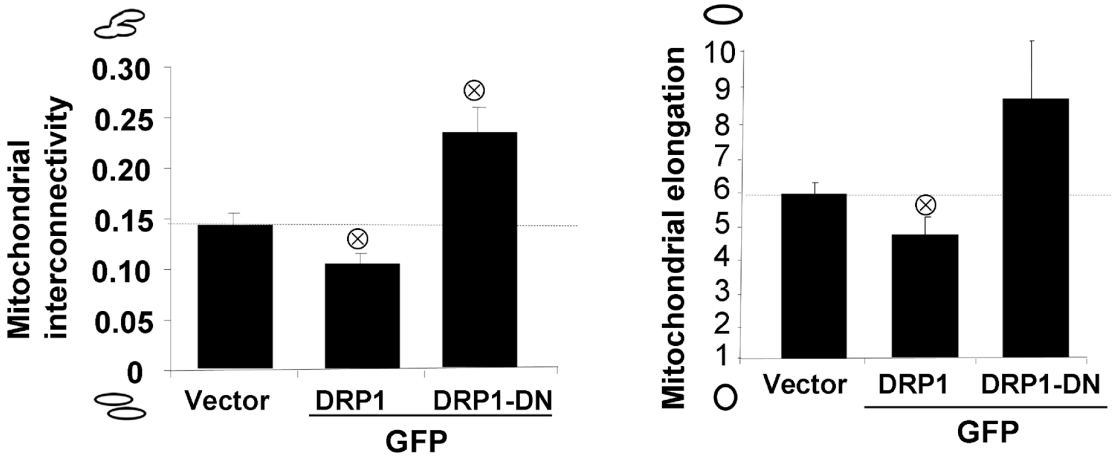


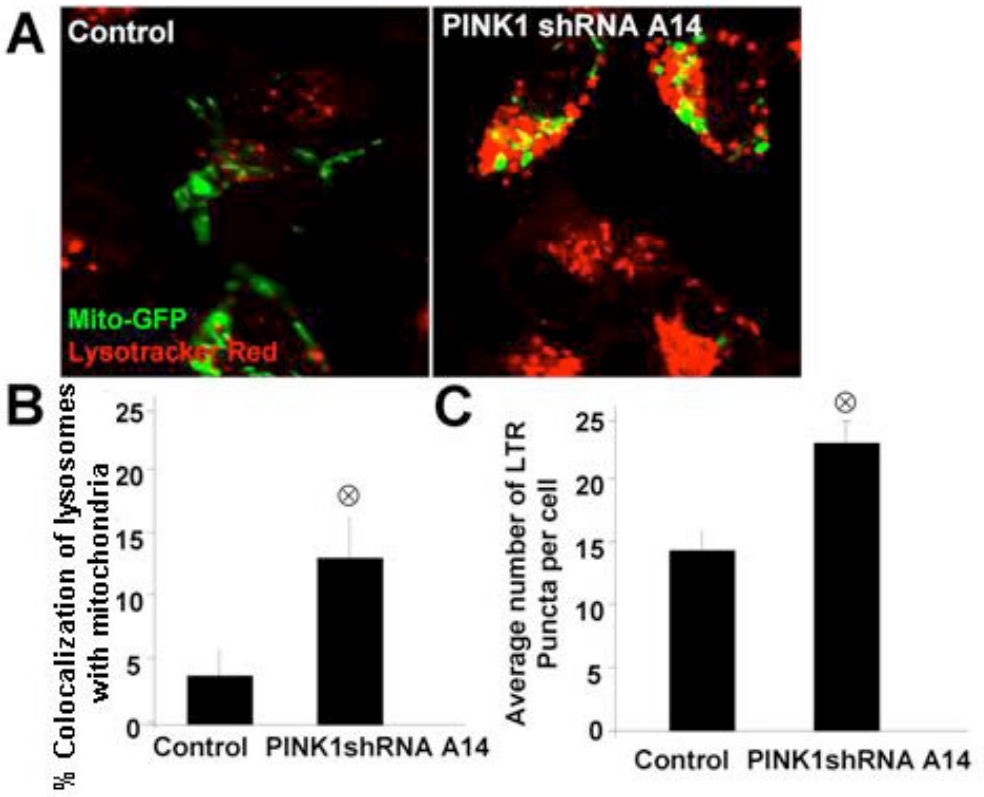
Supplement Figure S1. Algorithmic quantification of mitochondrial morphology in SH-SY5Y cells treated with known fission/fusion mediators.
Parental SH-SY5Y cells were transiently transfected with an empty vector control, with GFP tagged wild-type Drp1 (DRP1) or dominant negative Drp1 (DRP1-DN) constructs for three days prior to Image J mitochondrial morphometry analyses of MitoTracker red labeled mitochondria. (**Left graph**) Morphometry data demonstrating the effects of fission/fusion modulators on mitochondrial interconnectivity in SH-SY5Y cells. (⊗:$p<0.01$ vs. vector control cell line; n=25-30 cells analyzed per condition in a representative experiment of three).
(**Right graph**) Morphometry quantification demonstrating the effects of fission/fusion modulators on the mitochondrial elongation index (inverse circularity) in SH-SY5Y cells. (⊗:$p<0.05$ vs. vector control cell line; n=25-30 cells analyzed per condition in a representative experiment of three).

To download image analysis macros developed by the Chu-Lab for ImageJ:
1) Type in the URL for the ImageJ Wiki site: http://imagejdocu.tudor.lu/doku.php

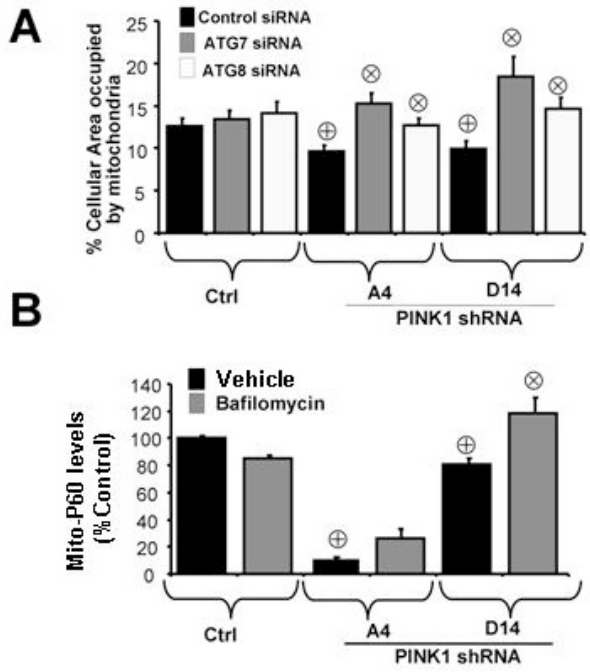
2) On the search field of the Image J website, type in "mitochondrial morphology" or search for "Chu Lab" or "Dagda" to find other Macros for autophagy (GFP-LC3) or neurite morphology.

3) Click on the green underlined title "Mitochondrial Morphology" in search results, which will lead you to the macro web-page. Copy the entire macro code (inside the box delineated by dotted lines) into a word processing program such as Word or NotePad and save it as a text file for insertion into the "macros" folder of the Image J program. Please refer to the macro web-page for the most up to date information on downloading and using the macro.

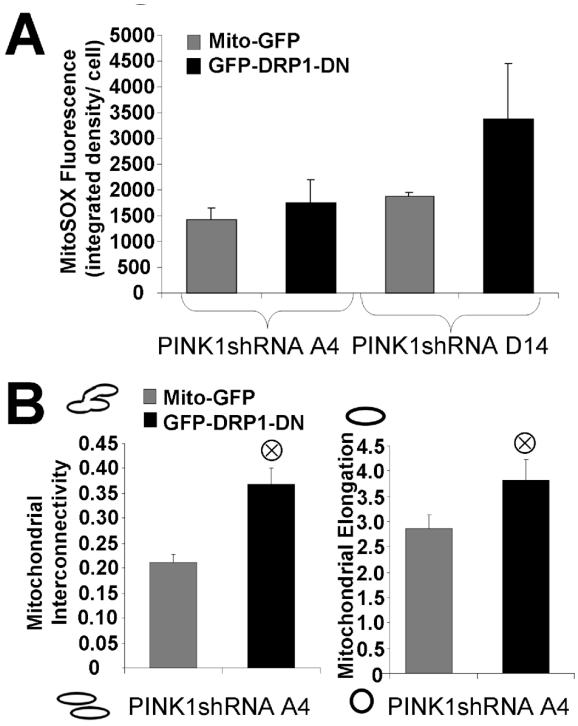
4) Please cite Dagda et al. 2009 *J Biol Chem* **284**: in press, for any publications or presentations resulting from use or modification of the macro.



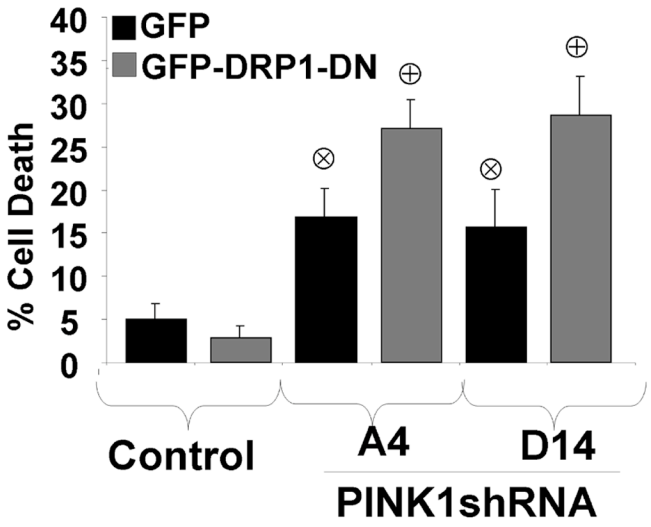
Supplement Figure S2. Loss of PINK1 promotes lysosomal expansion.
(A) Confocal images of control or a PINK1 knockdown stable cell line transiently transfected with mitochondrially targeted GFP (Mito-GFP) and stained with Lysotracker red-DND to visualize colocalization of lysosomes with mitochondria.
(B) Quantification of the percent of lysosomes that colocalize with mitochondria in control and PINK1 shRNA A14 clonal cell lines. (⊗:$p<0.03$ vs. control cell line; n=40-50 cells analyzed per condition in a representative experiment of two).
(**C**) Quantification of the average number of lysotracker (LTR) positive structures per cell in control and PINK1shRNA A14 cell lines. (⊗:$p<0.0005$ vs. control cell line; n=40-50 cells analyzed per condition in a representative experiment of two).



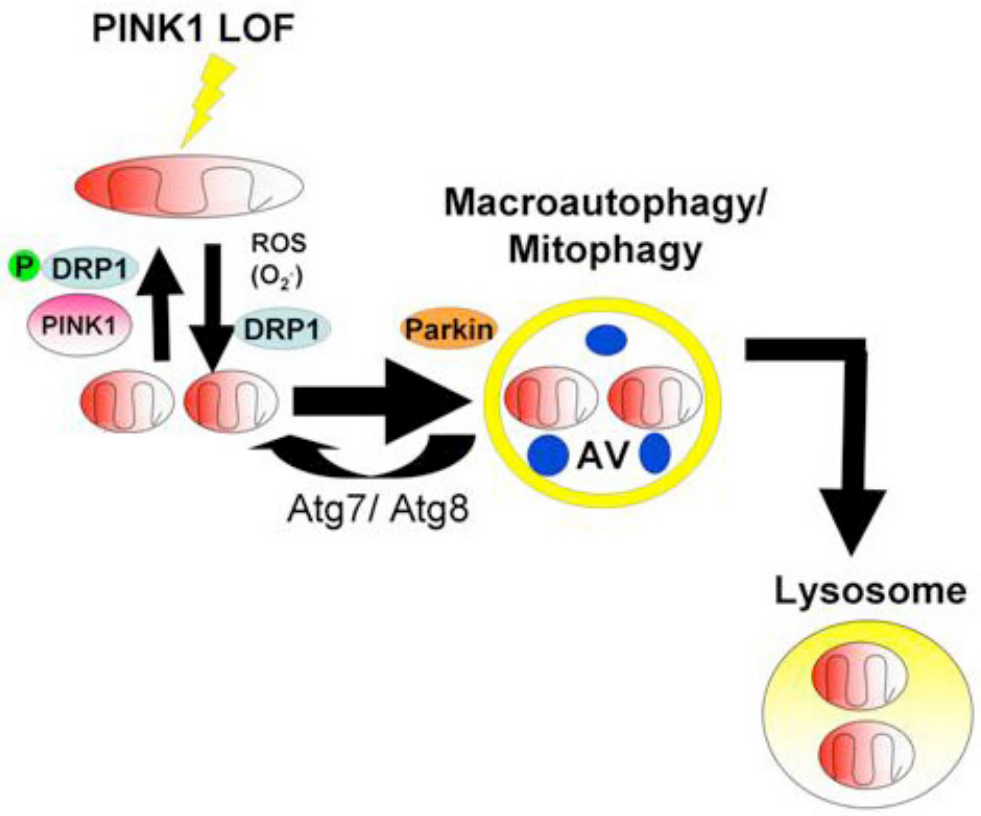
Supplement Figure S3. Loss of PINK1 promotes mitochondrial degradation through autophagy.
(A) Computer aided quantification of the percent cellular area occupied by mitochondria in cells transiently expressing mitochondrially targeted GFP (mito-GFP) and untargeted red fluorescence protein (RFP) to label the cellular perimeters. Representative bar graph shows means ±S.E. (⊕:$p<0.05$ vs. control cell line; ⊗:$p<0.004$ vs.respective shRNA line treated with control siRNA; n=25-35 cells analyzed per condition)
(B) Mitochondrial P60 protein content analyzed by quantifying the integrated densities of immunoreactive bands for human mitochondrial antigen of 60kDa in cell lysates of control or PINK1 knockdown stable cell lines treated for 4 h with either vehicle control or with bafilomycin-A. Means ±S.E. (⊕:$p<0.05$ vs. control clonal cell line; ⊗:$p<0.05$ vs.vehicle treated; n=3 immunoreactive lanes analyzed per condition).



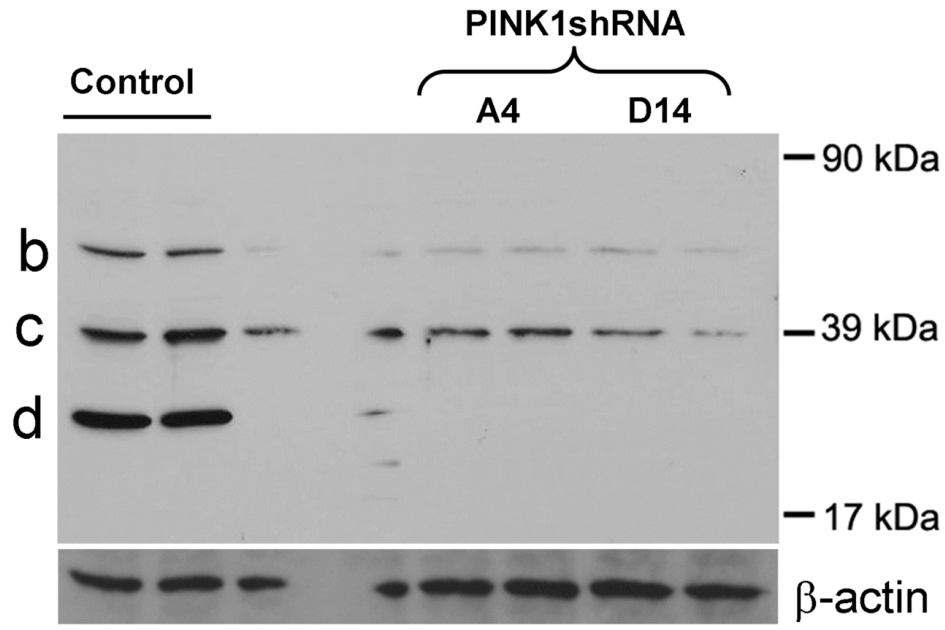
Supplement Figure S4. Mitochondrial ROS is upstream of mitochondrial fission induced by loss of PINK1 function.
(A) Quantification of the average integrated intensity of MitoSOX fluorescence per cell, as a measure of mitochondrial superoxide, in PINK1 knockdown clonal cell lines transiently expressing Mito-GFP as a control or GFP-tagged dominant negative Drp1 to induce mitochondrial fusion. No significant effects of transiently expressing GFP-DRP1-DN were seen compared to mito-GFP transfected cells in two independent experiments (n=25-40 cells analyzed per condition).
(**B**) Mitochondrial morphometry quantification in PINK1 shRNA A4 cell line demonstrates successful reversal of mitochondrial fragmentation by transiently expressing GFP-Drp1-DN. (⊗:$p < 0.05$ vs. mito-GFP Control; n=25-30 cells analyzed per condition in a representative experiment of two).



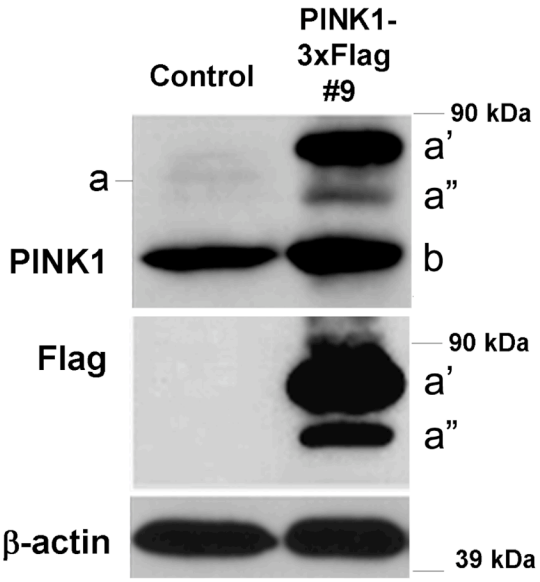
Supplement Figure S5. Mitochondrial fission plays a protective role in stable PINK1 deficient cell lines.
Cell death quantification as determined by the percent of GFP immunolabeled cells exhibiting pyknotic or fragmented nuclei in control and PINK1shRNA clonal cell lines expressing either GFP alone as a control or GFP-DRP1-DN. (⊗:$p < 0.05$ vs. control cell line; ⊕: $p < 0.05$ vs. respective GFP transfected shRNA line; n=250-300 cells analyzed per condition in a representative experiment of three).



Supplement Figure S6. Working model for a role of PINK1 in mitophagy and mitochondrial morphology regulation.
PINK1 loss of function (LOF) elicits increases in mitochondrial superoxide, Drp1-dependent fragmentation of mitochondria, and compensatory Drp1-dependent macroautophagy/mitophagy that is further amplified by increasing parkin levels within the cell. Overexpression of PINK1 promotes mitochondrial fusion and inhibits toxin-induced autophagy/mitophagy. Interestingly, mitochondrial fragmentation is a coordinated process mediated by both the mitochondrial fission-fusion machinery and the autophagic machinery, suggesting a feed forward loop of mitochondrial fragmentation and turnover. Fission induced mitophagy appears to represent a compensatory process in the setting of PINK1 deficiency.



Supplement Figure S7. Uncropped version of the blot shown in Figure 1F.
PINK1 immunoblot showing reduction of endogenous PINK1 in two stable PINK1shRNA clonal cell lines compared to vector control cell line, with β actin serving as loading control. Lanes 3 and 4 which were cropped from Figure 1E, showed incomplete protein transfer and were thus not analyzed.



Supplement Figure S8. PINK1-3X-Flag expression and processing in clone #9.
Cell lysates from stable vector control SH-SY5Y clonal cell line and a PINK1 overexpressing clonal cell line (#9) were immunoblotted for PINK1 with C8830 and re-probed for Flag and for β-actin as a loading control. Overexpressed PINK1-3X Flag (band a') and a second processed overexpressed PINK1-3X Flag (band a'') were recognized by antibodies for both FLAG and for PINK1 (C8830). The position of a faint band corresponding to full length endogenous PINK1 is indicated in the control lane (band a). The ~50 kDa major processed endogenous band (band b) is not recognized by anti-Flag antibody, suggesting that it could be C-terminally truncated. Bands b and c (see also Fig. 1E and S7) are also recognized by the Novus antibody for PINK1, although the commercial antibody preferentially labels the higher molecular weight overexpressed bands (S. Cherra and CT Chu, unpublished observations).