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Supplementary Figures.

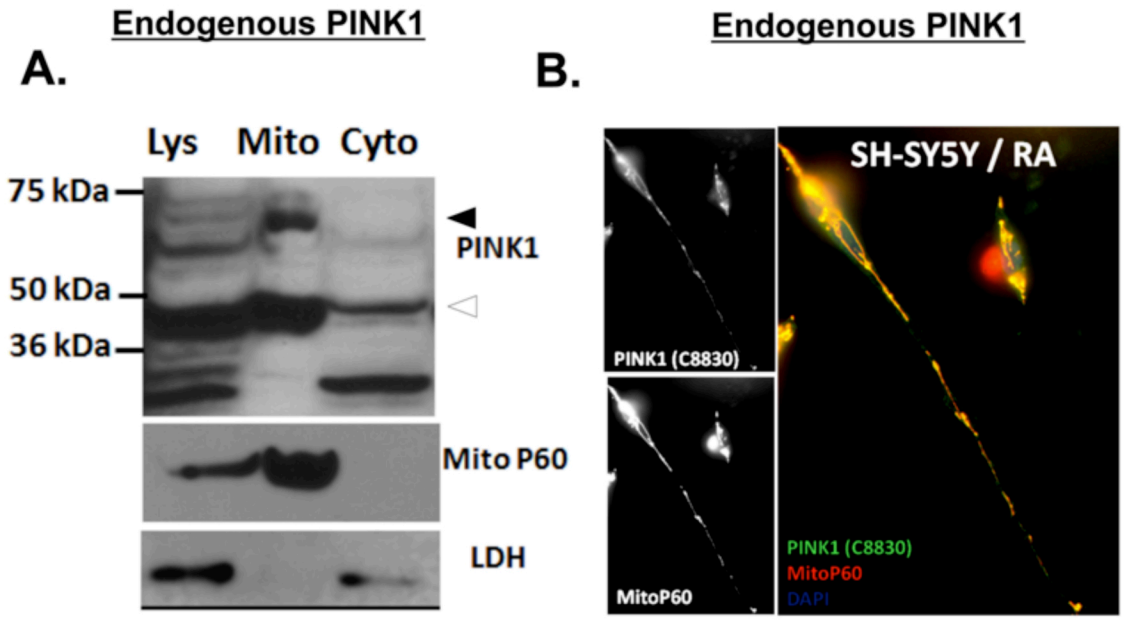

Figure S1. Subcellular localization of endogenous PINK1 in naïve and retinoic acid-differentiated cells

(A) Representative immunoblot demonstrating that full-length endogenous PINK1 (black arrowhead) is localized in mitochondrial fractions while cytosolic fractions of SH-SY5Y cells exhibit predominantly processed bands. Note that 25 μg of total cell lysate protein and 10 μg each of mitochondrial and cytosolic proteins were loaded into the gel, which accounts for 9.375%, 25% and 3.33% of the total lysate, mitochondrial or cytosolic proteins, respectively. Densitometry, with correction for the relative fraction of total mitochondrial and cytosolic proteins loaded on the gel, reveals that ~70% of the major processed endogenous PINK1 band (white arrowhead) is cytosolic. **(B)** Immunofluorescence of endogenous PINK1 (green) shows co-localization with the human mitochondrial antigen of 60kDa (red; MitoP60) upon a background of diffuse cytosolic labeling that includes the neurites of retinoic acid-differentiated SH-SY5Y cells.

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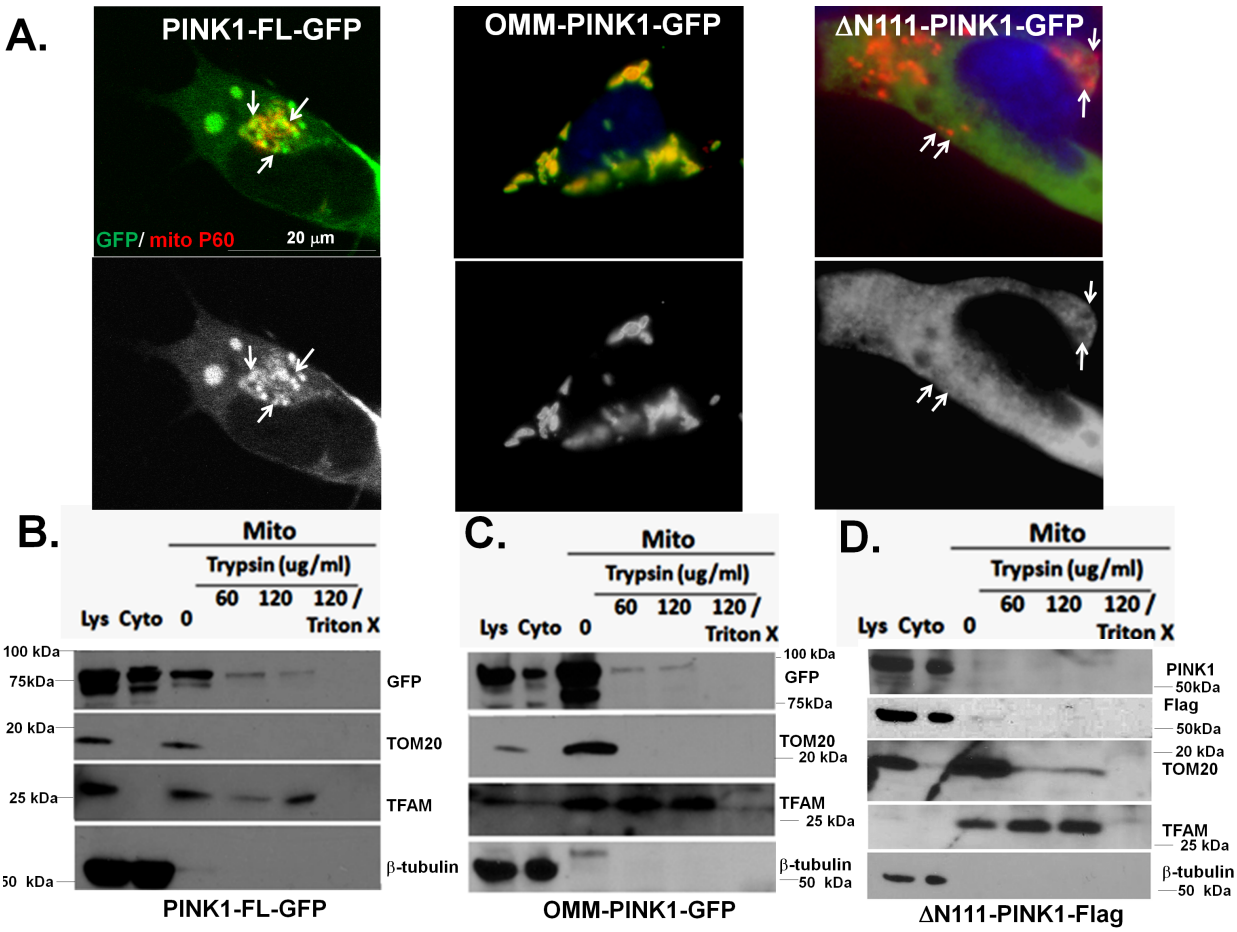

Figure S2. **Characterization of the subcellular distribution and expression levels of PINK1 fusion constructs.**

(A) Representative epifluorescence micrographs of SH-SY5Y cells transiently expressing the indicated chimeric constructs of PINK1 (green) were immunostained for GFP and for the mitochondrial marker mito-P60 (red). Note that full-length PINK1 has a dual mitochondrial (yellow colocalizing puncta, arrows) and diffuse cytosolic localization, while OMM-PINK1 localizes to mitochondria without the diffuse component, and Δ111N-PINK1-GFP shows diffuse cytosolic staining exclusive of mitochondria (red, arrows). Scale bar 20 μm. **(B-D)** Mitochondrial fractions (Mito) isolated from SH-SY5Y cells transiently transfected with PINK1-GFP (**B**), OMM-PINK1-GFP **(C)**, or ΔN111-PINK1-3X FLAG (**D**) were treated with increasing concentrations of trypsin (0-120 μg/ml) in the presence or absence of Triton X-100, then lysed and immmunoblotted for the indicated markers. Gels show total lysates (Lys) and cytosolic (Cyto) fractions for comparison.

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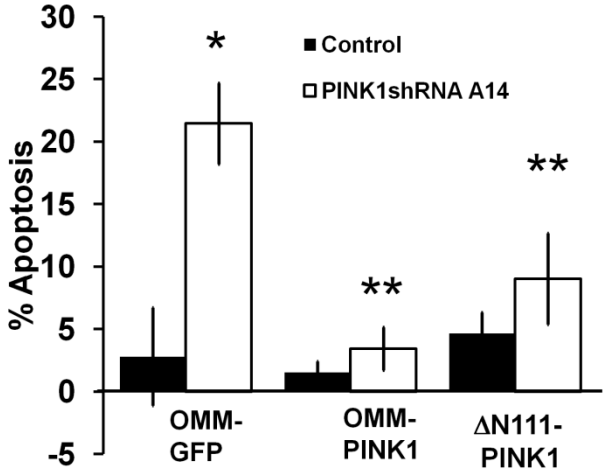

Figure S3. Mitochondrial and cytosolic pools of PINK1 are protective against cell death induced by loss of endogenous PINK1 levels

Control and PINK1 knockdown cells (clone A14) were transiently transfected with the indicated GFP tagged PINK1 constructs for two days and cells were fixed with paraformaldehyde and immunostained for GFP. The percentage of GFP positive cells that contained pyknotic or fragmented nuclei were scored as apoptotic. (Means ± SEM, n= 4-5 independent experiments, *:$p<0.05$ vs. Control /OMM-GFP, **:$p<0.05$ vs. PINK1shRNA A14/OMM-GFP).

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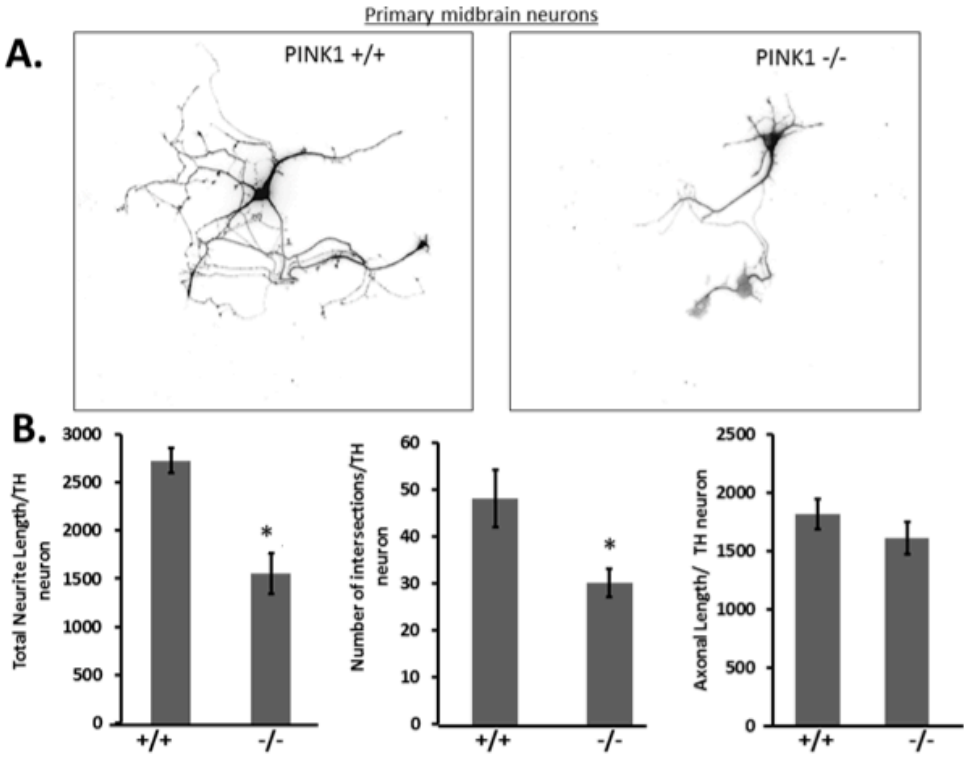

Figure S4. PINK1 regulates neurite lengths in primary dopamine midbrain neurons

(A) DIV9 primary midbrain neurons from *Pink1*+/+ and *Pink1*-/- embryonic mouse brains were immunolabeled for tyrosine hydroxylase (TH) to identify dopamine neurons. Representative images demonstrate that Pink1-deficient dopaminergic neurons (top right panel) show significantly reduced neurite complexity and lengths compared to wild-type neurons (top left panel). **(B)** Quantification of total (summated) neurite lengths per neuron (left graph), complexity (middle) as assessed by the number of intersections per neuron, and MAP2-negative axon length (right graph) in primary dopaminergic midbrain neurons from wild type and homozygous *Pink1* knockout littermates (Means ± S.EM., n=22-43 primary midbrain neurons pooled from 3-5 prenatal mouse pups per genotype, *:$p<0.05$ vs. wild type control neurons).

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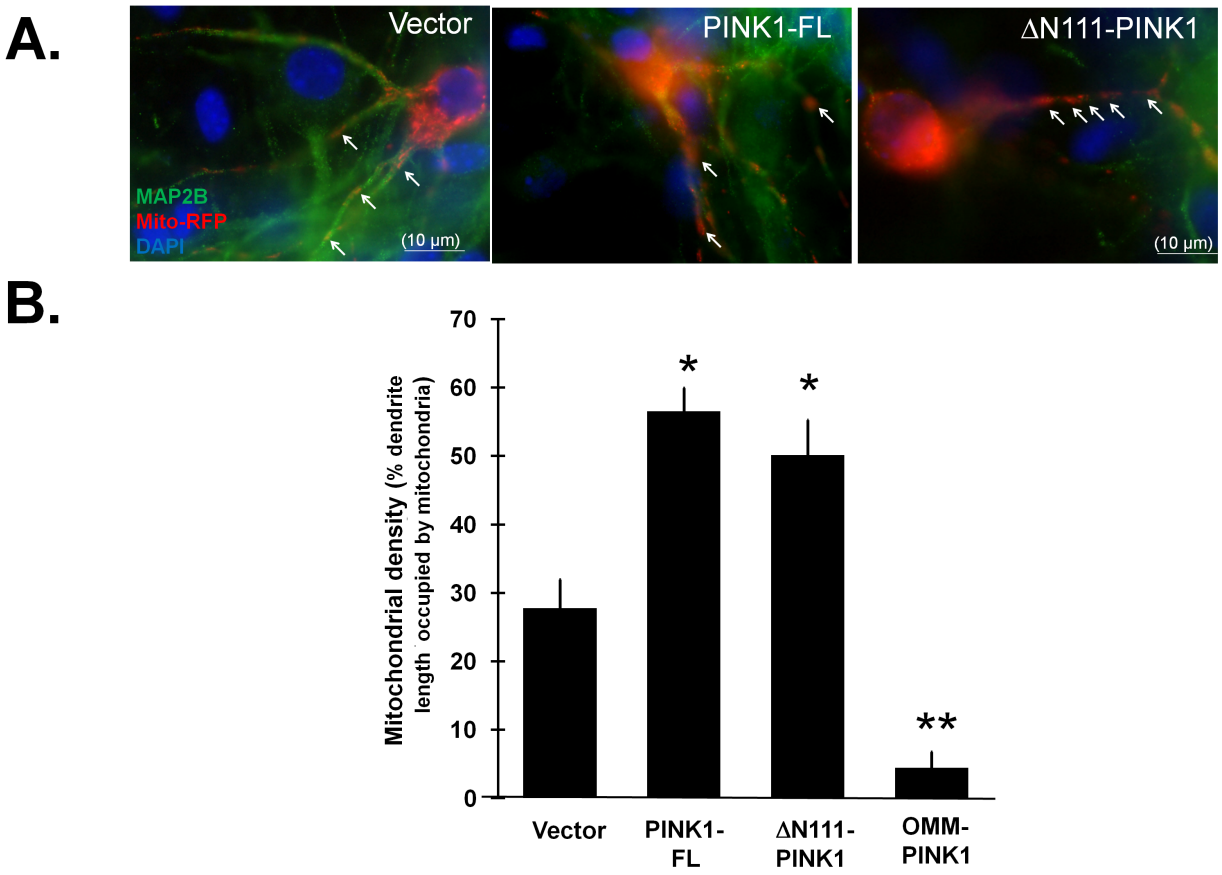

Figure S5. **Cytosolic PINK1 increases mitochondrial occupancy in proximal dendrites**

(A) Primary cortical neurons (DIV14) were co-transfected with the indicated Flag-tagged PINK1 constructs (FL: full-length) and mito-RFP to label mitochondria, fixed in paraformaldehyde 3 days later, immunolabeled for the dendrite marker MAP2B (green) and counterstained with DAPI to visualize nuclei (blue). Representative epifluorescence micrographs demonstrate that transient expression of either PINK1-FL or ΔN111-PINK1 increases the density of mitochondria in dendrites compared to vector. (**B)** Mitochondrial density was measured by calculating the percentage of dendrite length occupied by mitochondria. White arrows indicate mitochondria in proximal dendrites (Means ± S.EM, n= 3 experiments, *:$p<0.05$ vs. vector, **:$p<0.05$ vs. ΔN111-PINK1-GFP).

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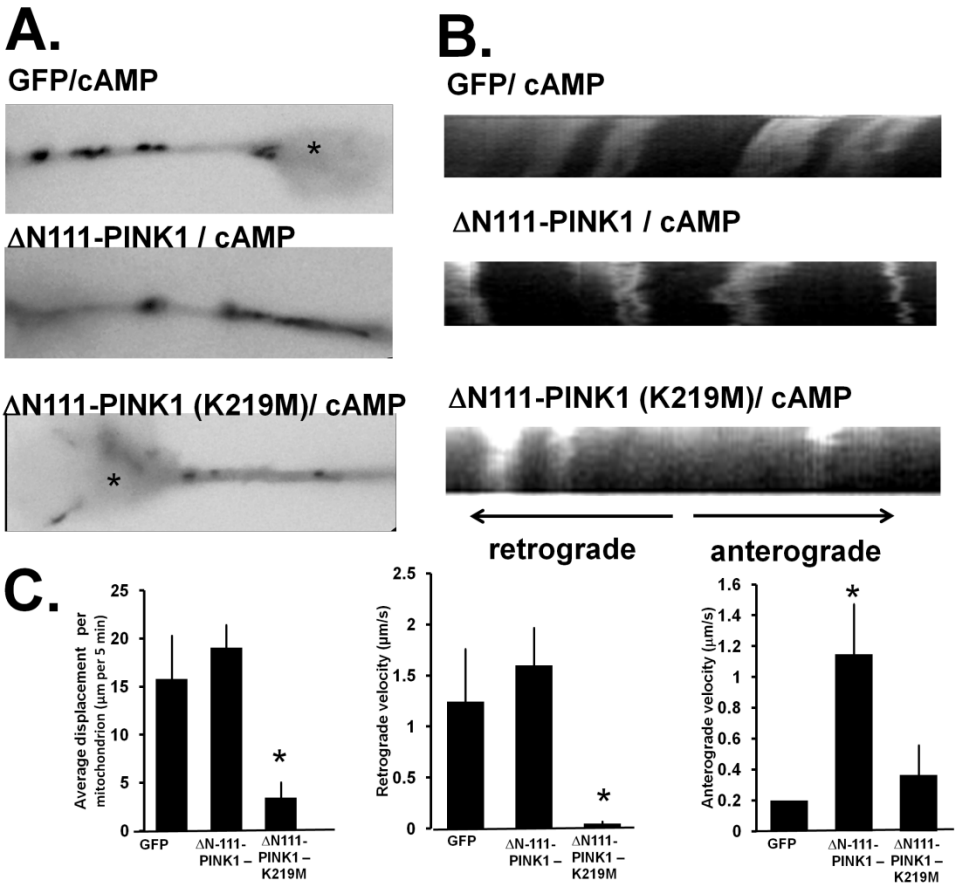

Figure S6. **The cytosolic pool of PINK1 enhances anterograde movement of mitochondria to neurites**

SH-SY5Y cells grown in complete DMEM/FBS media and transiently expressing the indicated PINK1 fusion constructs together with mito-RFP were treated with a single dose of cAMP to promote neurite outgrowth 24 h prior to analysis. (**A)** Representative epifluorescence micrographs show that transient expression of kinase deficient ΔN111-PINK1(K219M) depletes neurites of mitochondria in cAMP treated SH-SY5Y cells. (**B)** Mitochondrial movement was analyzed in neurites by time lapse epifluorescence microscopy in live cells for 5 minutes with 5 second intervals per capture and images were compiled into kymographs. (**C)** The average distance traveled (left graph), average retrograde velocity (middle graph), or average anterograde velocity (right graph) per mitochondrion were quantified using the "Line Analyzer" function of Image J. Note that transient expression of ΔN111-PINK1(K219M) stalls mitochondria movement in both directions, while ΔN111-PINK1-GFP promotes anterograde movement towards the distal ends of the neurites. (Means ± S.EM, n=200 mitochondria quantified from 8 neurons per condition, *:$p<0.05$ vs. GFP).

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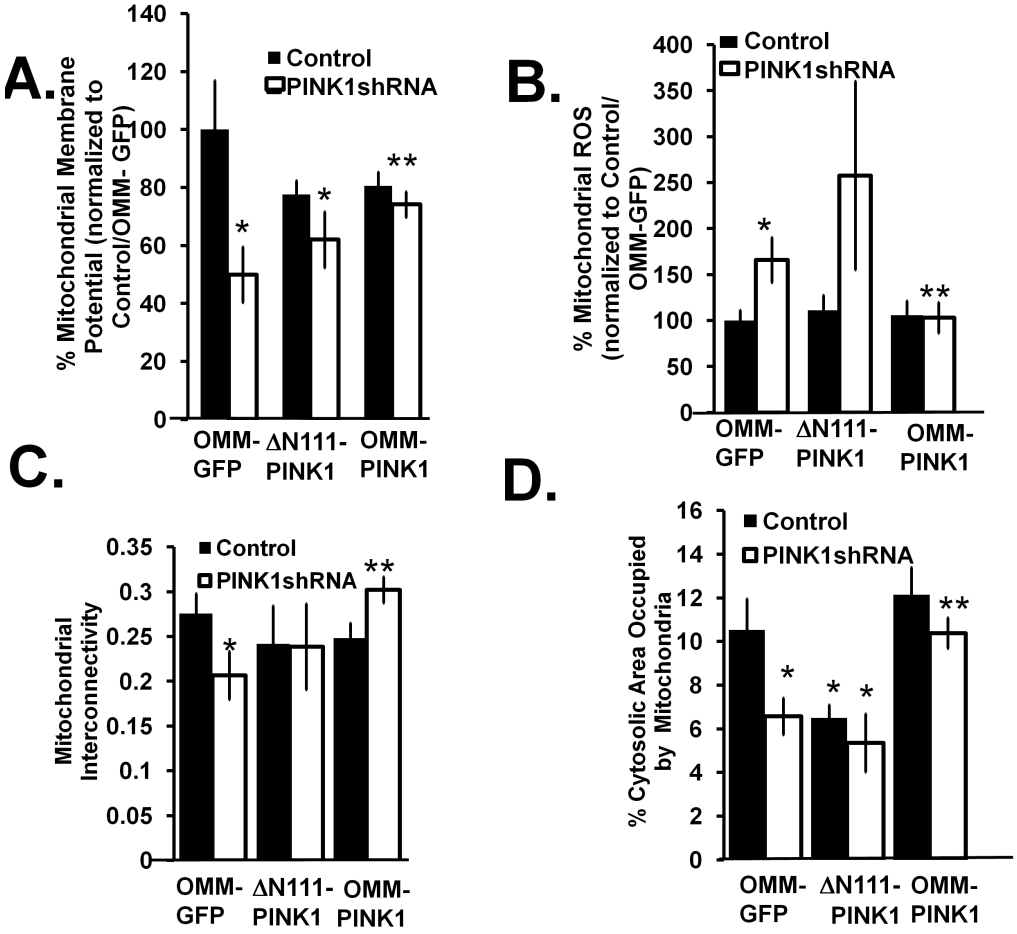

Figure S7. **Mitochondrial, but not cytosolic, PINK1 restores parameters of mitochondrial function in PINK1-deficient SH-SY5Y cells**

Control and PINK1 knockdown cells (clone A14) were transiently transfected with the respective GFP tagged PINK1 constructs for two days and analyzed for mitochondrial membrane potential by image analysis quantification of TMRM fluorescence (**A**), for mitochondrial ROS levels by measuring the mean fluorescence intensity at the mitochondria in MitoSOX stained cells (**B**), for mitochondrial morphology as measured by quantifying the area/perimeter ratios of transfected cells (**C**) and for mitochondrial content as quantified by calculating the percentage of cytosolic area occupied by mitochondria per cell (**D**) (Means ± SEM, n= 3-5 experiments per condition, *:$p<0.05$ vs. control/OMM-GFP, **:$p<0.05$ vs. PINK1shRNA A14/OMM-GFP).

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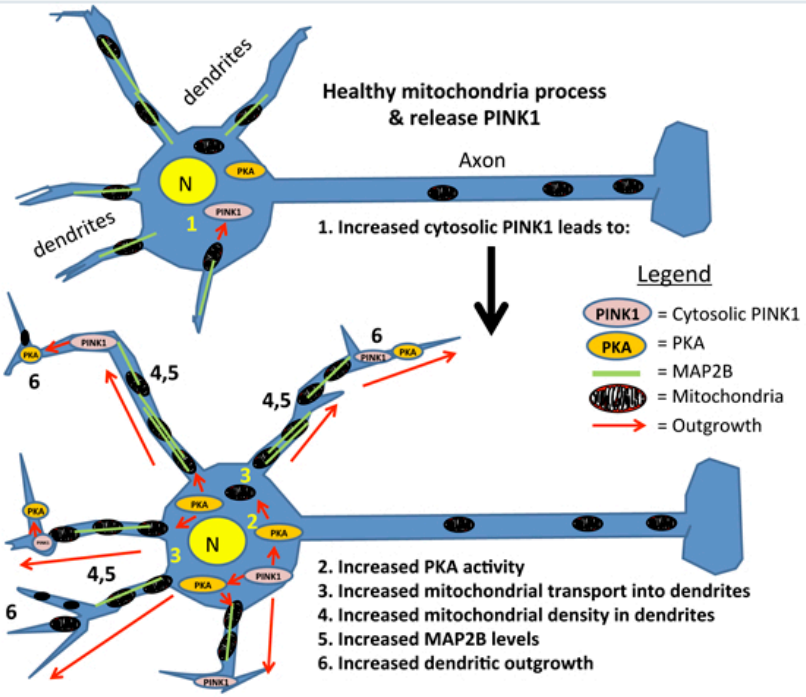

Mutations in *PINK1* cause recessive Parkinson's disease, but the neuronal function(s) of the PINK1 protein remain elusive. We found that cytosolic PINK1 promotes neuronal differentiation in naïve cells, reversing dendritic shortening and cell death in *Pink1*$^{-/-}$ neurons, by increasing PKA activity and mitochondrial transport to dendrites. Release of processed PINK1 from healthy mitochondria may serve as a pro-differentiation signal in cortical and dopaminergic neurons.