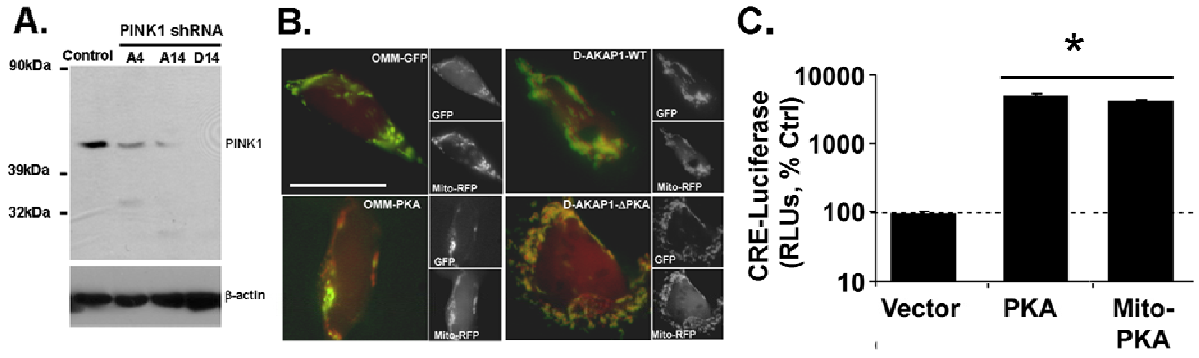
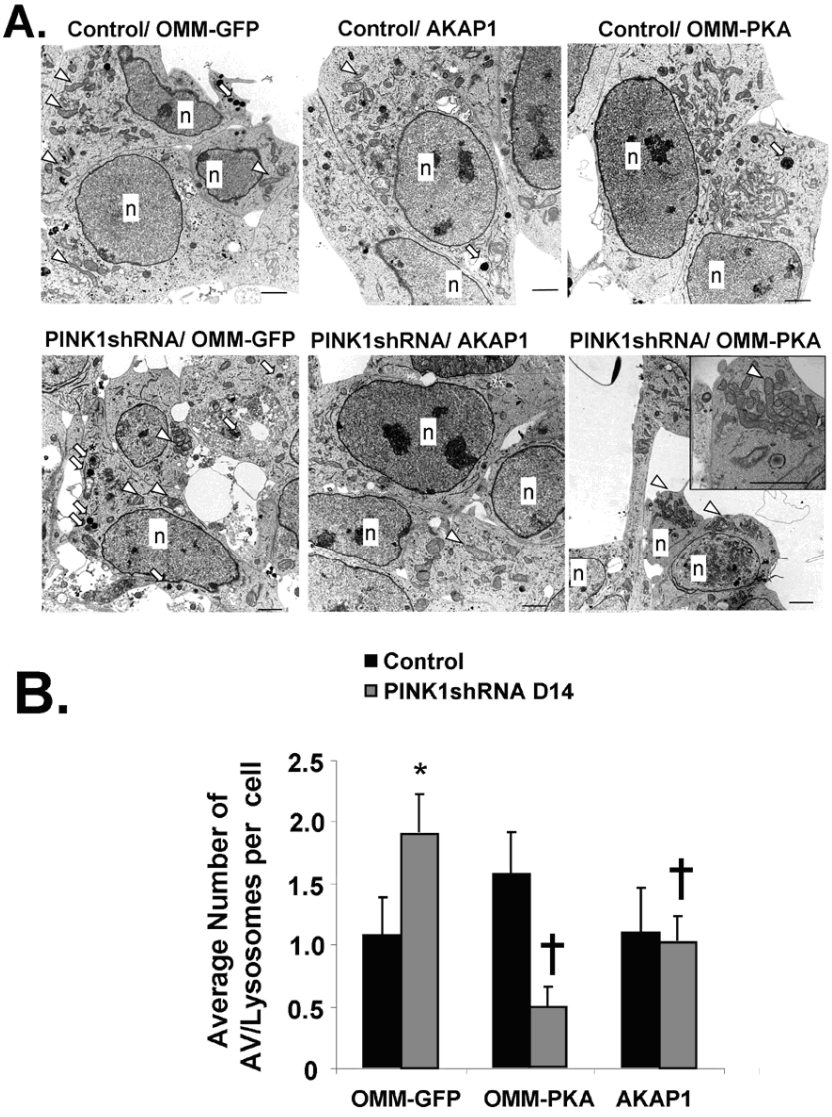


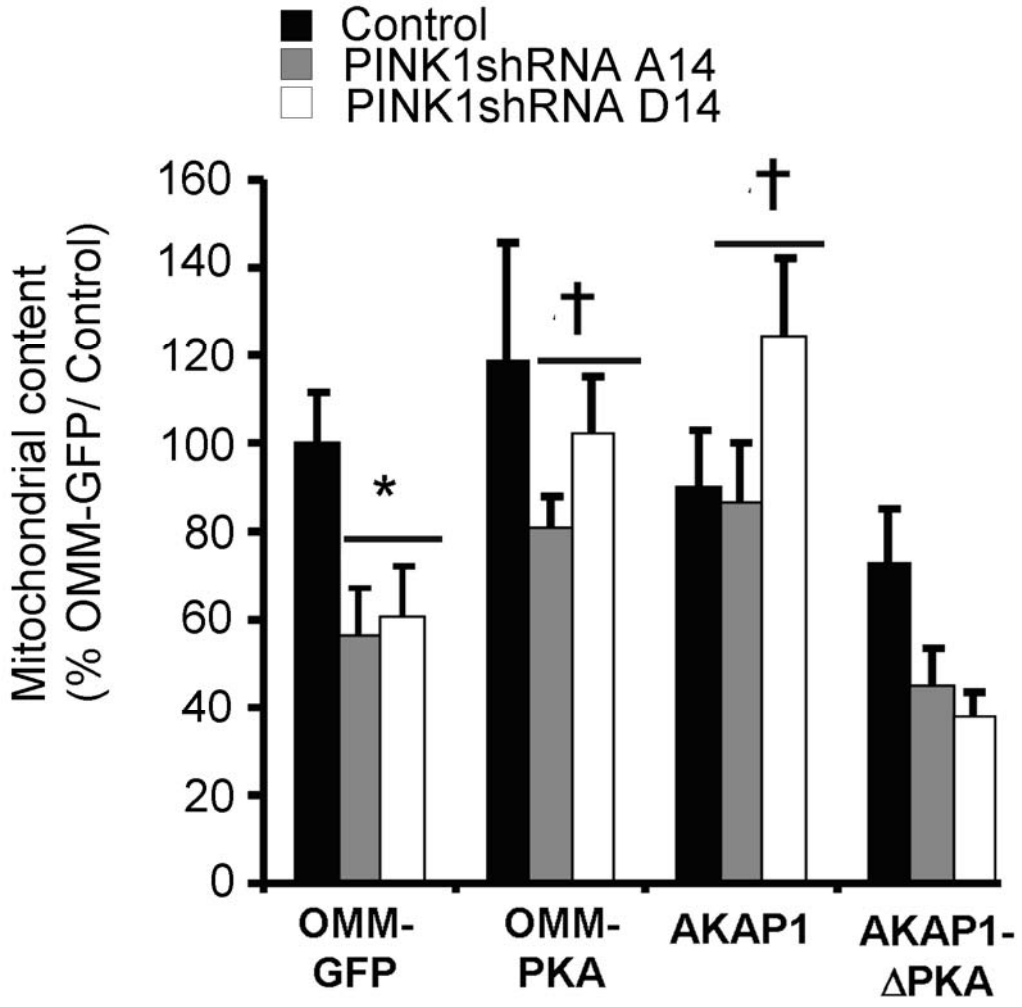
Supplementary Figure 1**. Characterization of PINK1shRNA cell lines and PKA constructs**

A. Cell lysates derived from shRNA-vector control SH-SY5Y cell line (control) and three PINK1shRNA clonal cell lines (A4, A14 and D14) were immunoblotted for endogenous PINK1 using anti-human PINK1 C8830 antibody, and re-blotted for β-actin as a loading control. The representative Western blot demonstrates that all three PINK1shRNA clonal cell lines show decreased levels of PINK1. **B**. Representative fluorescent micrographs showing mitochondrial localization of GFP tagged OMM-PKA, AKAP1-WT, and AKAP1-ΔPKA. Mitochondria were labeled with matrix-targeted RFP (mito-RFP) in SH-SY5Y cells. Scale: 20μm. **C**. CRE dual luciferase assay as a measure of intracellular PKA related activity in SH-SY5Y cells. Cells were co-transfected with the indicated panel of GFP tagged PKA constructs and a dual luciferase systems consisting of the CRE-luciferase reporter and a constitutively active *Renilla* luciferase as an internal transfection control. Luciferase activity was measured from cell lysates collected 48 h following transfection. Representative bar graph shows means ± S.E. (4-8 wells per transfection condition) and is representative of 4 independent experiments with similar results (*: $p<0.05$ vs. Vector).



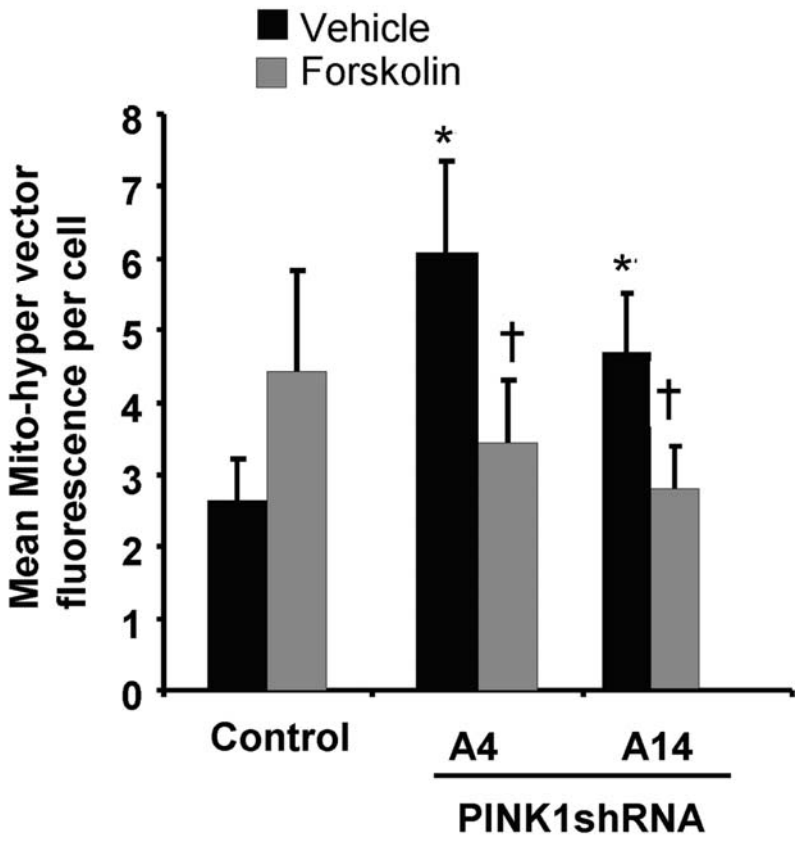
Supplementary Figure 2**. Ultrastructural analysis of control and PINK1 knockdown SH-SY5Y cell lines overexpressing AKAP1/PKA.**

A. Electron micrographs of shRNA-vector control cells co-expressing OMM-GFP, AKAP1, or OMM-PKA and of PINK1 D14 shRNA cells co-expressing the same plasmids. Arrowheads: mitochondria, Arrows: lysosomes, n: nuclei. Inset for bottom right panel: OMM-PKA expressing cell contained densely packed elongated mitochondria. Scale: 2μm. **B.** The average number of AVs (early and late) quantified per cell by EM analysis (mean ± S.E, 20 cells/condition, two independent experiments, *: $p<0.05$ vs. OMM-GFP/ control; †: $p<0.05$ vs. OMM-GFP/PINK1shRNA, ANOVA).



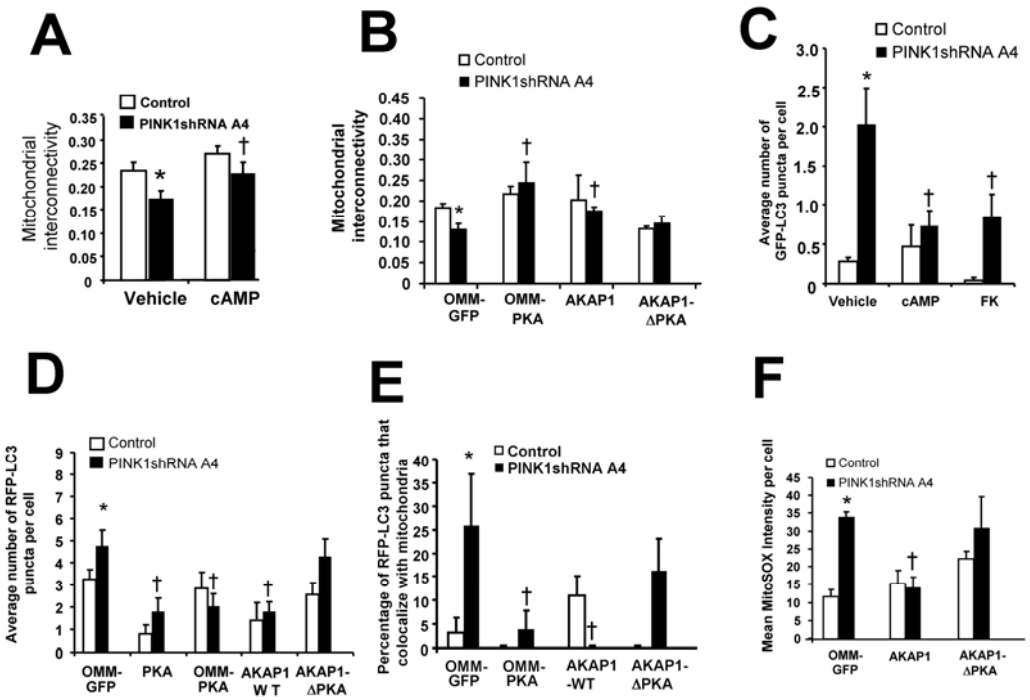
Supplementary Figure 3. **AKAP/PKA partially restores mitochondrial content in PINK1shRNA cells.**

Quantification of the percent cytosolic area occupied by mitochondria as a measure of cellular mitochondrial content. (mean ± S.E, 30 cells/condition, two independent experiments *:p <0.05 vs. OMM-GFP/ control cell line; †:p <0.05 vs. OMM-GFP/PINK1shRNA cell line, ANOVA).

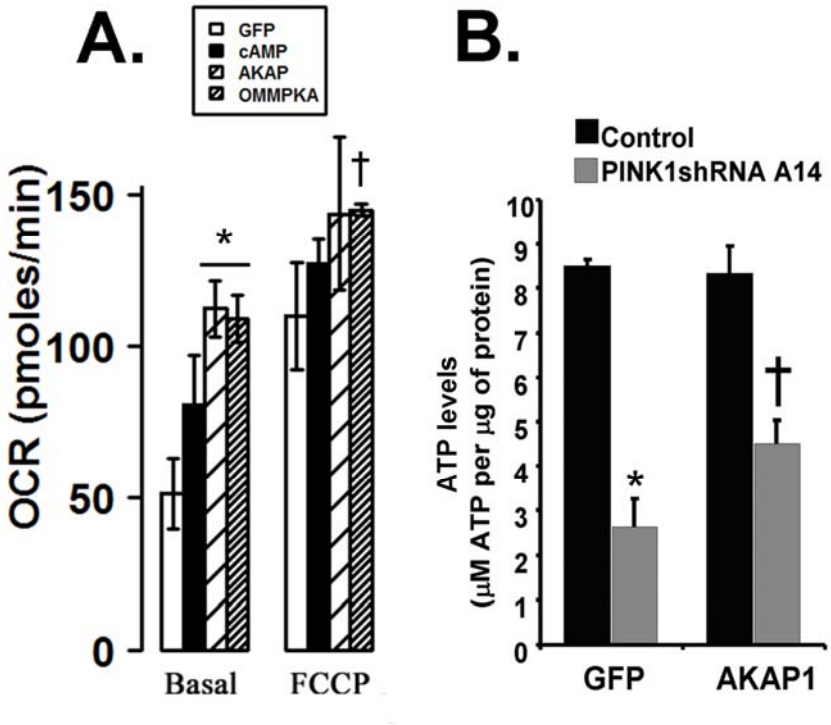


Supplementary Figure 4**. Activating PKA related signaling reverses the increased mitochondrial hydrogen peroxide signal in PINK1 deficient cell lines**

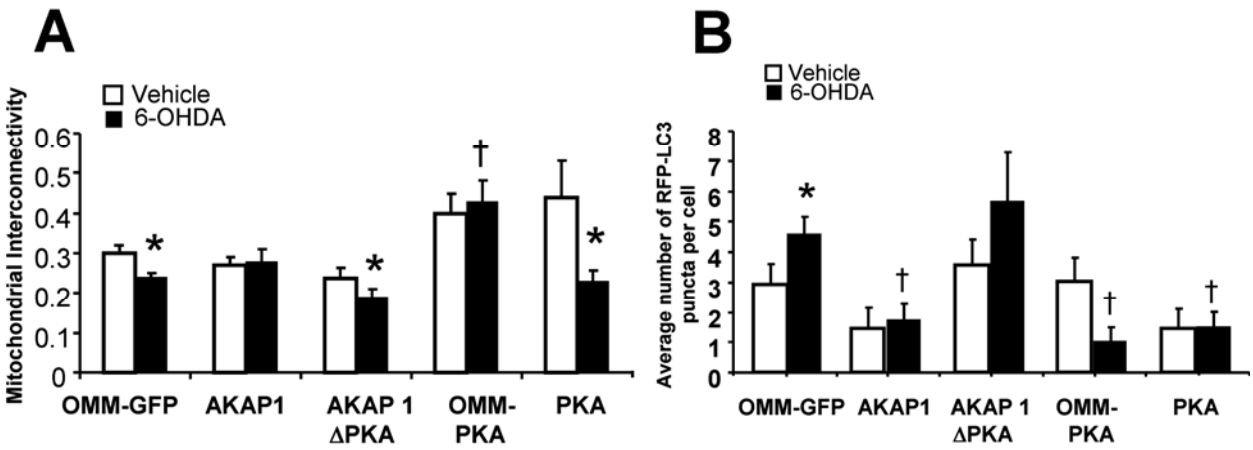
To measure intracellular levels of hydrogen peroxide near mitochondria, PINK1 deficient cell lines were transiently transfected with a mitochondrially targeted hydrogen peroxide sensor based on the OxyR regulatory domain (Mito-HyPer) and co-transfected with cytosolic RFP as a transfection marker. Two days post-transfection, cells were treated with forskolin x 4 h. The bar graph shows the mean fluorescence intensity per cell. *:p <0.05 vs. Control cell line/ vehicle; †:p <0.05 vs. PINK1shRNA cell line/ vehicle, ANOVA).



Supplementary Figure 5. **Mitochondrial PKA reverses mitochondrial fragmentation, macroautophagy, mitophagy and ROS in a third PINK1 deficient clone.**
A. Control and PINK1shRNA cell line A4 transiently expressing mito-GFP in the presence or absence of db-cAMP x 4 h were measured for mitochondrial interconnectivity (means ± S.E., 25-30 cells/condition, two independent experiments, *: $p<0.05$ vs. Control/Vehicle; †:$p<0.05$ vs. PINK1shRNA A4/Vehicle, ANOVA) **B.** Control and PINK1shRNA cell line A4 transiently co-expressing mito-GFP and the depicted PKA constructs were measured for mitochondrial interconnectivity (means ± S.E., 25-30 cells/condition, two independent experiments; *: $p<0.05$ vs. Control/OMM-GFP; †:$p<0.05$ vs. PINK1shRNA A4/ OMM-GFP, ANOVA). **C**. The average number of GFP-LC3 puncta per cell in control and PINK1 shRNA clonal cell line A4 treated with pharmacological activators of PKA or DMSO as a vehicle control (means ± S.E., 25-30 cells/condition, two independent experiments; *: $p<0.05$ vs. Control/Vehicle; †:$p<0.05$ vs. PINK1shRNA A4/ Vehicle, ANOVA). **D.** The average number of RFP-LC3 puncta per cell in control and PINK1shRNA cell line A4 transiently co-expressing the indicated GFP-tagged PKA constructs. (Mean ± S.E, 20-30 cells/condition, three independent experiments.*:$p<0.05$ vs. OMM-GFP/control cell line; †:$p<0.05$ vs. OMM-GFP/PINK1shRNA A4, ANOVA). **E.** RFP-LC3 colocalization with mitochondria as an index of mitophagy (means ± S.E., 25-35 cells/condition, three independent experiments, *:$p<0.05$ vs. OMM-GFP/control cell line; †:$p<0.05$ vs. OMM-GFP/PINK1shRNA A4, One-way ANOVA). **F**. MitoSOX fluorescence/cell in the indicated cell lines transiently expressing the indicated forms of AKAP1 or OMM-GFP (means ± S.E, 40-50 cells/condition, 2 representative experiments; *:$p<0.05$ vs. OMM-GFP/control cell line; †:$p<0.05$ vs. OMM-GFP/PINK1shRNA cell A4, ANOVA).

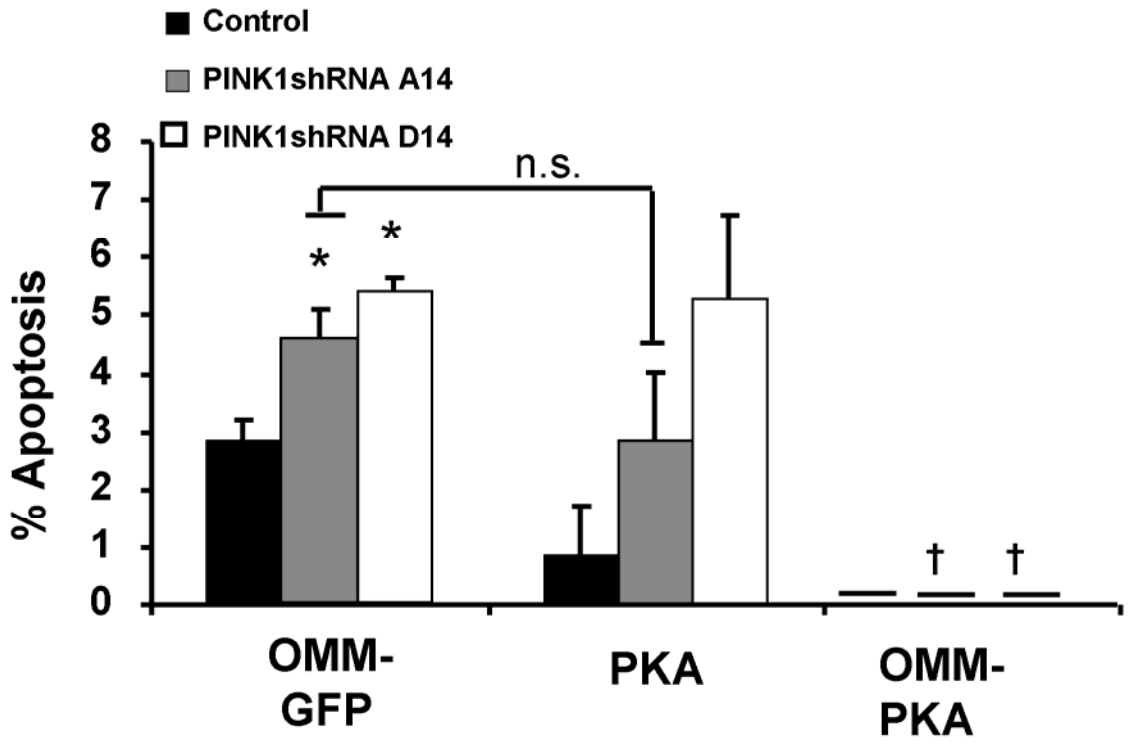


Supplementary Figure 6. **Decrease in oxygen consumption and mitochondrial-dependent ATP levels are partially reversed by transient expression of AKAP1 in the PINK1shRNA A14 line. A.** The effects of mitochondrial PKA as elicited by treating cells with a 24 hr. dose of db-cAMP and by transient expression of OMM-PKA or AKAP1 on basal oxygen consumption levels and maximal respiratory capacity (FCCP- rotenone) in a PINK1shRNA clonal line (A14) (*: $p < 0.05$ vs. GFP/basal; †:<0.05 vs. GFP/FCCP). **B.** Mitochondrial-dependent ATP levels were quantified in the stable control or PINK1 shRNA line A14, expressing GFP or AKAP1-GFP, and grown in galactose media to suppress glycolytic potential. Mitochondrial complex inhibitors reveal that >70% of ATP levels are derived from mitochondria under these conditions (12 wells per condition, mean ± S.E, *:p <0.05 vs. Control cell line/GFP; †:p <0.05 vs. PINK1shRNA/GFP, ANOVA).



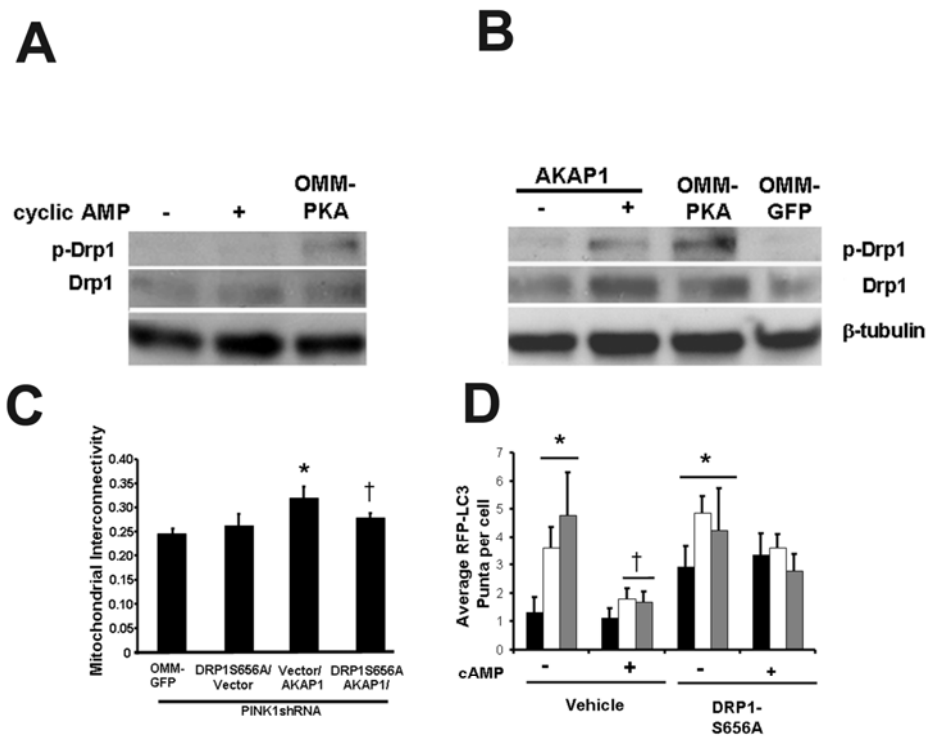
Supplementary Figure 7**. Mitochondrial PKA also prevents 6-hydroxydopamine induced mitochondrial fragmentation, and either form of PKA blocks toxin-induced autophagy.**

A. Quantification of mitochondrial interconnectivity in parental SH-SY5Y cells transiently co-expressing the indicated panel of GFP tagged fusion constructs of PKA-modulating plasmids and mito-RFP to visualize mitochondria following vehicle or 6-hydroxydopamine (120μM x 4 h) (Representative experiment of 2, mean ± S.E , 20-25 cells analyzed per condition, *: $p<0.05$ vs. vehicle/OMM-GFP; †: $p<0.05$ vs. 6-OHDA/OMM-GFP, ANOVA). **B.** The average RFP-LC3 puncta per cell in parental SH-SY5Y cells co-expressing the indicated GFP tagged constructs following treatment with vehicle or 6-hydroxydopamine (120μM x 4 h) (Representative experiment of 2, mean ± S.E, 15-25 cells analyzed for per condition,*: $p<0.05$ vs. vehicle/ OMM-GFP; †: $p<0.05$ vs. 6-OHDA/OMM-GFP, ANOVA)



Supplementary Figure 8. **Mitochondrial but not untargeted PKA reduces cell death elicited by loss of PINK1 in PINK1 knockdown cell lines**

Control and PINK1shRNA cell lines were transiently transfected with GFP or the GFP tagged PKA modulators for 48hrs. Cell death was determined by quantifying the percentage of GFP positive cells that exhibit apoptotic DAPI stained nuclei per epifluorescence micrograph field (means ± S.E., 150-200 cells/condition, two independent experiments, *: $p<0.05$ vs. OMM-GFP/Control; †:$p<0.05$ vs. respective OMM-GFP/PINK1shRNA cell line, n.s.= not significant, One-way ANOVA).



Supplementary Figure 9. **The effects of enhanced PKA signaling on mitochondrial fusion and suppression of autophagy in PINK1 knockdown cells involve Drp1**

A. Western blot analyses of phospho-Drp1 (PKA site) in cells transiently expressing OMM-PKA, or an empty vector control in the presence or absence of db-cAMP (500μM x 40 min), and re-probed for total levels of Drp1 and β-tubulin as a loading control. **B**. Western blot analyses of phospho-Drp1 in cells transiently expressing OMM-GFP, OMM-PKA, or AKAP1 +/- db-cAMP. **C.** Quantification of mitochondrial interconnectivity in a PINK1shRNA line A14 transiently co-expressing vector or AKAP1-GFP in the presence or absence of co-transfected non-phosphorylatable Drp1 S656A mutant, and stained with MitoTracker red to visualize mitochondria (means ± S.E, n=20-35 cells, two independent experiments, *: $p<0.05$ vs. OMM-GFP/PINK1shRNA A14, †: $p<0.05$ vs. AKAP1/PINK1shRNA A14, ANOVA). **D**. Quantification of RFP-LC3 puncta in PINK1 shRNA clonal cell lines co-transfected with GFP tagged Drp1 S656A (non-phosphorylatable fission-active) and with RFP-LC3, and treated with vehicle or db-cAMP (means ± S.E, n =20-40 cells/condition, three independent experiments, *: $p<0.05$ vs. Vehicle/control cell line, †: $p<0.05$ vs. respective vehicle-treated PINK1shRNA cell line clonal cell line, ANOVA)