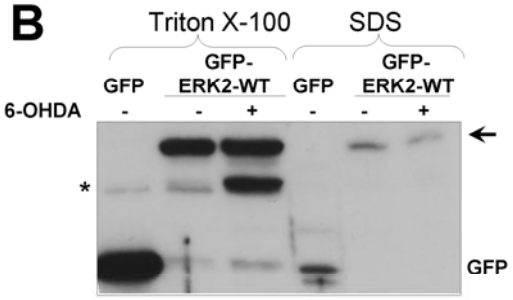
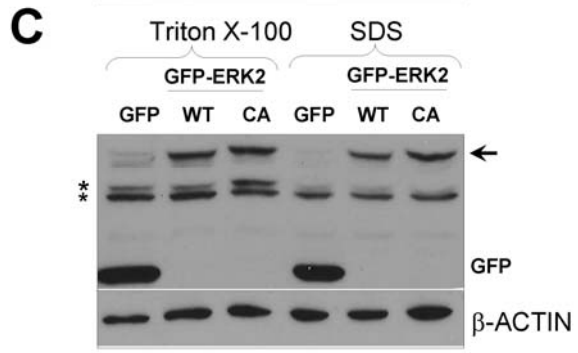
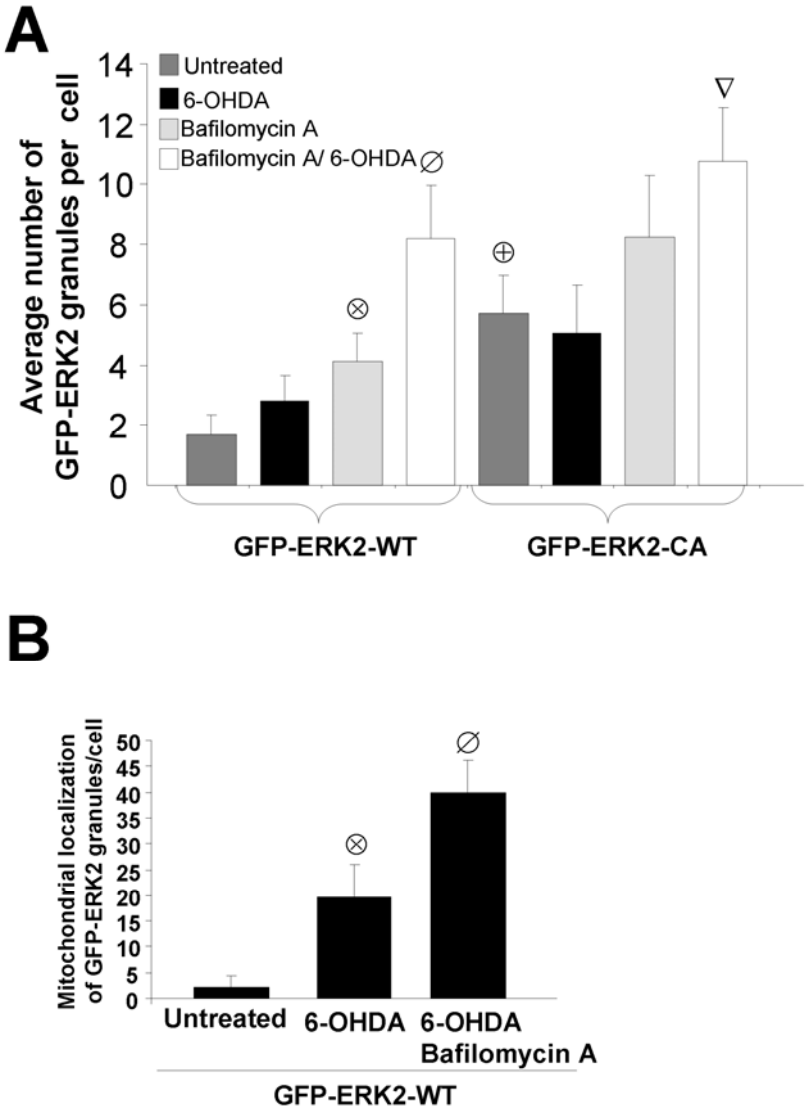
**A**

| | % transfection | std. error |
|---|---|---|
| GFP | 24.3 | 6.43 |
| GFP-ERK2-WT | 24.0 | 6.85 |
| GFP-ERK2-CA | 24 | 0 |
| GFP-ERK2-DN | 29.33 | 6.96 |

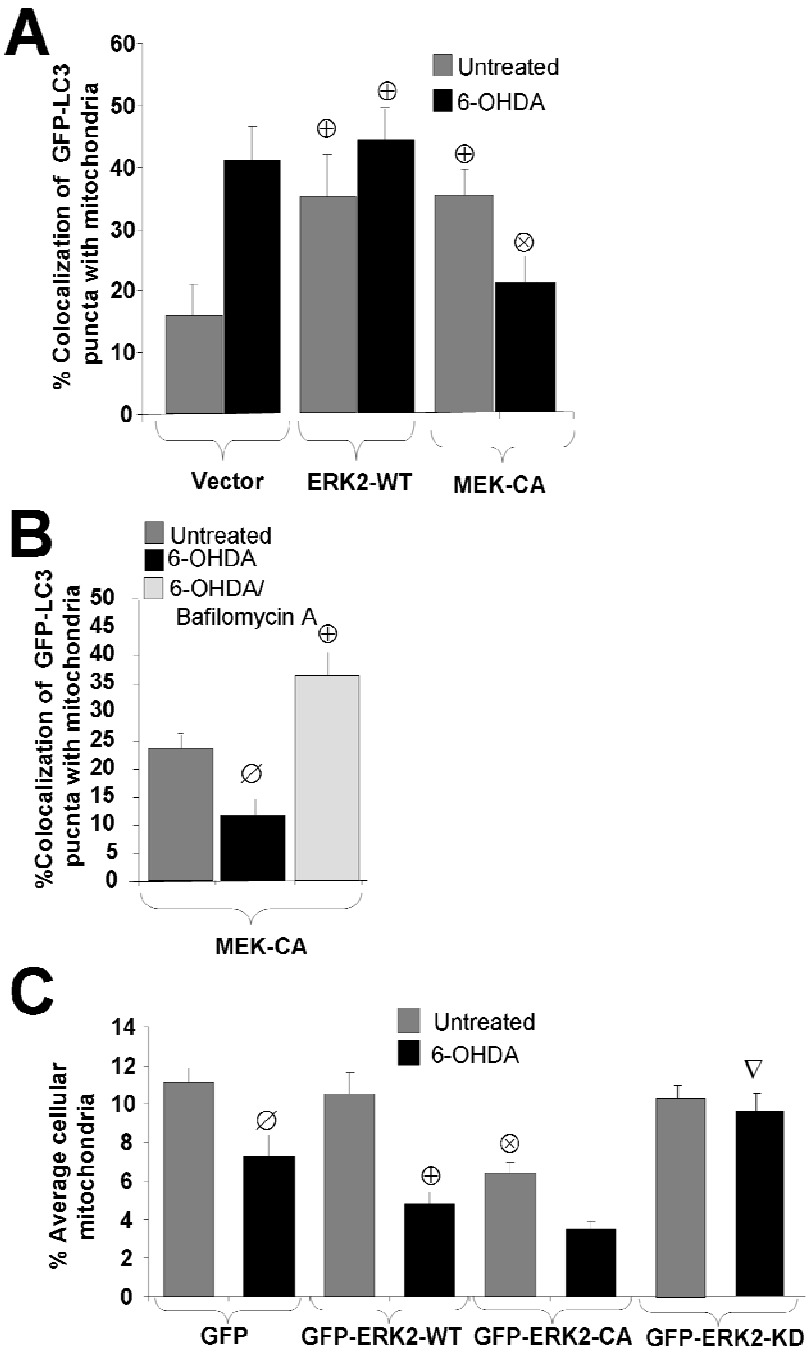



**Supplementary Figure 1. Transfection efficiency and lack of aggregation in GFP-ERK2 transfected cells.** (**a**) Summary table of the percent transfection efficiency achieved in SH-SY5Y cells transiently transfected with GFP or the indicated GFP tagged constructs of ERK2 for 48 h. (**b**) Representative GFP Western blots of cell lysates from SH-SY5Y cells transfected with GFP control or GFP-ERK2-WT treated with or without 6-OHDA for 4 h. Protein lysates were prepared by sequential detergent extractions of cells in lysis buffer containing Triton X-100 followed by protein extraction in SDS sample buffer. Note that the vast majority of GFP-ERK-WT from untreated or 6-OHDA treated cells is extracted by Triton X-100. Only a small amount of GFP-ERK2 is left behind for subsequent SDS extraction, and there is no increase in Triton-resistant forms induced by 6-OHDA. Black arrows point to immunoreactive bands of recombinant GFP-tagged ERK2-WT. The reason for the more intense GFP-immunoreactive band that migrates near non-specific bands (*) which occurs only in Triton-extracts of 6-OHDA treated cells is unclear. (**c**) Representative GFP Western blot of cell lysates prepared by lysing cells directly in either lysis buffer containing Triton X-100 or in SDS sample buffer. Note that no differences were found in extraction of GFP or GFP tagged ERK2. Non-specific GFP immunoreactive bands are denoted by an asterisk (*).

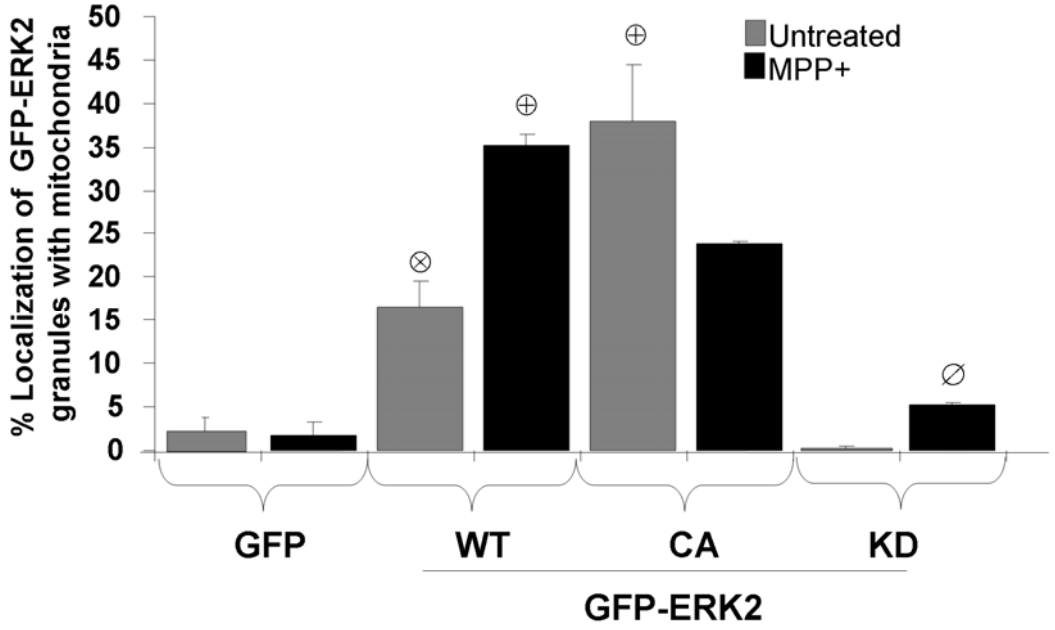



**Supplementary Figure 2. GFP-ERK2 granules are cleared by autophagolysosomal mechanisms.** (**a**) Quantification of the average number of GFP-ERK2 granules per cell following vehicle or 6-OHDA treatment of SH-SY5Y cells in the presence or absence of bafilomycin-A. Representative bar graph of at least three independent experiments showing means from 25-30 cells each ± s.e.m. (⊗: $p<0.05$ vs. GFP-ERK2-WT, ∅: $p<0.01$ vs. GFP-ER2-WT with 6-OHDA, ⊕: $p<0.01$ vs. GFP-ERK2-WT, ∇: $p<0.02$ vs. GFP-ERK2-CA with 6-OHDA). (**b**) Summary quantification of the percent of GFP-ERK2-WT granules colocalizing with mitochondria in the presence or absence of 6-OHDA with or without bafilomycin-A co-treatment (Representative bar graph of at least three independent experiments showing means from 25-30 cells each ± s.e.m; ⊗: $p=0.05$ vs. GFP-ERK2-WT, ∅: $p=0.05$ vs. GFP-ERK2-WT with 6-OHDA).

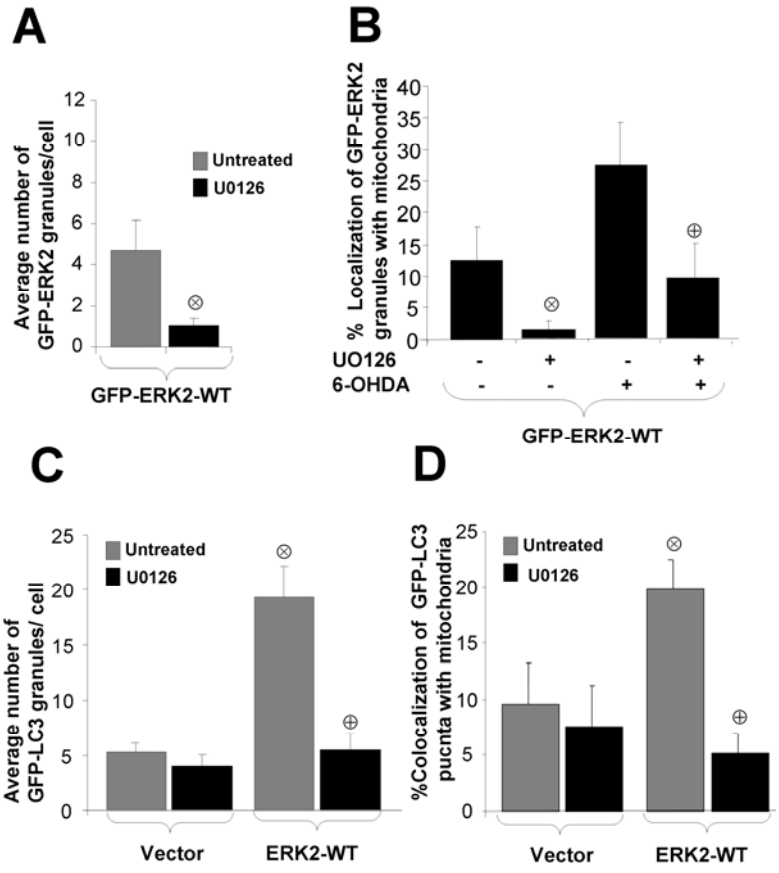



**Supplementary Figure 3. Transient expression of MEK or ERK2 elevates mitophagy to levels similar to that induced by 6-OHDA**. **(a)** Quantification of the percentage of GFP-LC3 puncta colocalizing with mitochondria per cell in SH-SY5Y cells transiently transfected with empty vector, wild-type ERK2 and constitutively active MEK2 (MEK-CA) plasmids in the presence or absence of 6-OHDA. The representative bar graph shows means ± s.e.m. (25-30 cells analyzed per condition) and is representative of at least 3 independent experiments (⊕: $p<0.03$ vs untreated vector, ⊗: $p<0.02$ vs. untreated MEK-CA). **(b)** Quantification of the percentage of GFP-LC3 puncta colocalizing with mitochondria per cell in SH-SY5Y cells transiently

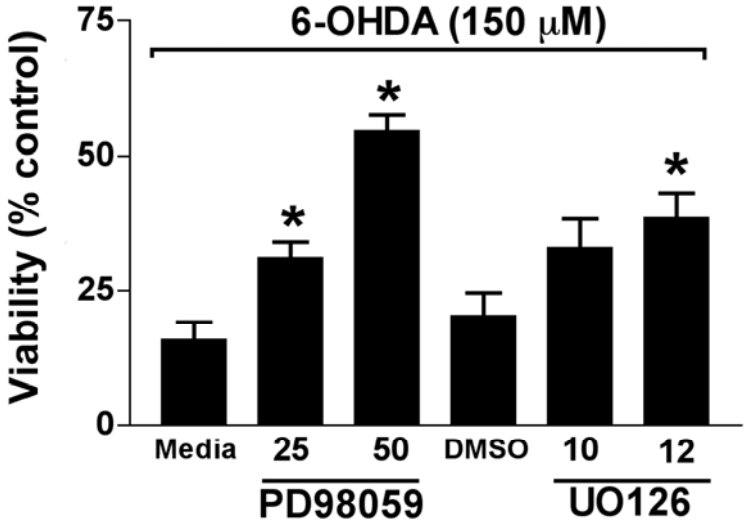
transfected with MEK-CA co-treated with either vehicle control or 6-OHDA in the presence or absence of bafilomycin-A. The representative bar graph shows means ± s.e.m. (25-30 cells analyzed per condition) and is representative of at least 3 independent experiments (∅:p<0.005 vs. untreated MEK-CA, ⊕: p<0.0001 vs. MEK-CA with 6-OHDA). (**c**) SH-SY5Y cells were transiently transfected with the indicated GFP tagged ERK2 constructs for 48 h. prior to treating cells with 6-OHDA for 5 h. Cells were then fixed and immunostained for human mitochondrial antigen of 60kDa. The mitochondrial content per cell was measured using a custom NIH ImageJ macro that calculates the percent mitochondrial area occupied in the cell and the results were graphed for at least 30 cells per condition. Note that transient expression of ERK2-CA but not ERK2-WT significantly decreases basal cellular mitochondrial content while ERK2-WT exacerbates the 6-OHDA-induced decrease in mitochondrial content, consistent with activity-level dependent effects on mitophagy. The representative bar graph shows means ± s.e.m. (25-30 cells analyzed per condition) and is representative of 2 independent experiments (∅:p<0.01 vs. untreated GFP, ⊕: p<0.05 vs. GFP with 6-OHDA, ⊗: p<0.01 vs. ERK2-WT, ∇: p<0.0005 vs. ERK2-WT with 6-OHDA).




**Supplementary Figure 4. Mitochondrial colocalization of GFP-ERK2 granules is increased with MPP+ toxicity.** Summary quantification of the percent of GFP-ERK2 granules colocalizing with mitochondria in the presence or absence of MPP+ for 5 h in transiently transfected cells expressing the indicated forms of ERK2 (means ± s.e.m. of n= 3-7 experiments with 25-30 cells each). (⊗:p<0.02 vs. untreated GFP, ⊕: p<0.05 vs. GFP-ERK2-WT, ∅:P<0.0001 vs. GFP-ERK2-WT treated with MPP+).




**Supplementary Figure 5. Pharmacological inhibition of MEK blocks the formation and mitochondrial colocalization of GFP-ERK2 granules (a,b), as well as macroautophagy and mitophagy induced by ERK2-WT (c,d).** (**a**) Quantification of the average number of GFP-ERK2 granules per cell in transiently transfected cells treated with the MEK inhibitor U0126 for 4 h. Representative bar graph shows ± s.e.m. of 25-30 cells each condition for 3 independent experiments (⊗:$p<0.006$). (**b**) Representative quantification of the percent colocalization of GFP-ERK2 granules with mitochondria in cells transiently expressing GFP-ERK2 treated with 6-OHDA for 4 h in the presence or absence of the MEK inhibitor U0126. This representative bar graph of two independent experiments shows means ± s.e.m. of 25-30 cells each condition (⊗:$p<0.01$ vs. untreated GFP-ERK2-WT, ⊕:$p<0.05$ vs. GFP-ERK2-WT treated with 6-OHDA). (**c**) Quantification of the average number of GFP-LC3 puncta per cell in SH-SY5Y cells transiently transfected with empty vector or wild-type ERK2 in the presence or absence of MEK inhibitor U0126. The bar graph shows means ± s.e.m. (25-30 cells analyzed per condition) and is representative of at least 3 independent experiments (⊗: $p<0.01$ vs. Vector, ⊕: $p<0.01$ vs. ERK2-WT). (**d**) Quantification of the percentage of GFP-LC3 puncta colocalizing with mitochondria per cell in SH-SY5Y cells transiently transfected with empty vector or wild-type ERK2 in the presence or absence of MEK inhibitor U0126. The bar graph shows means ± s.e.m. (25-30 cells analyzed per condition) and is representative of 2 independent experiments. (⊗: $p<0.05$ vs. Vector, ⊕: $p<0.0001$ vs. ERK2-WT).




**Supplementary Figure 6. Pharmacologic inhibition of MEK/ERK signaling confers protection from 6-OHDA toxicity.** SH-SY5Y cells were treated with 6-OHDA, at a dose that causes 85% lethality, in the presence or absence of two pharmacologic inhibitors of MEK, PD98059 and UO126 at the indicated μM concentrations, or their respective vehicles. After 18 h, cell death was quantified using the MTS assay as previously described.[34] Note that both compounds confer significant protection. * $p < 0.05$ by ANOVA and multiple comparison testing using Tukey's HSD.