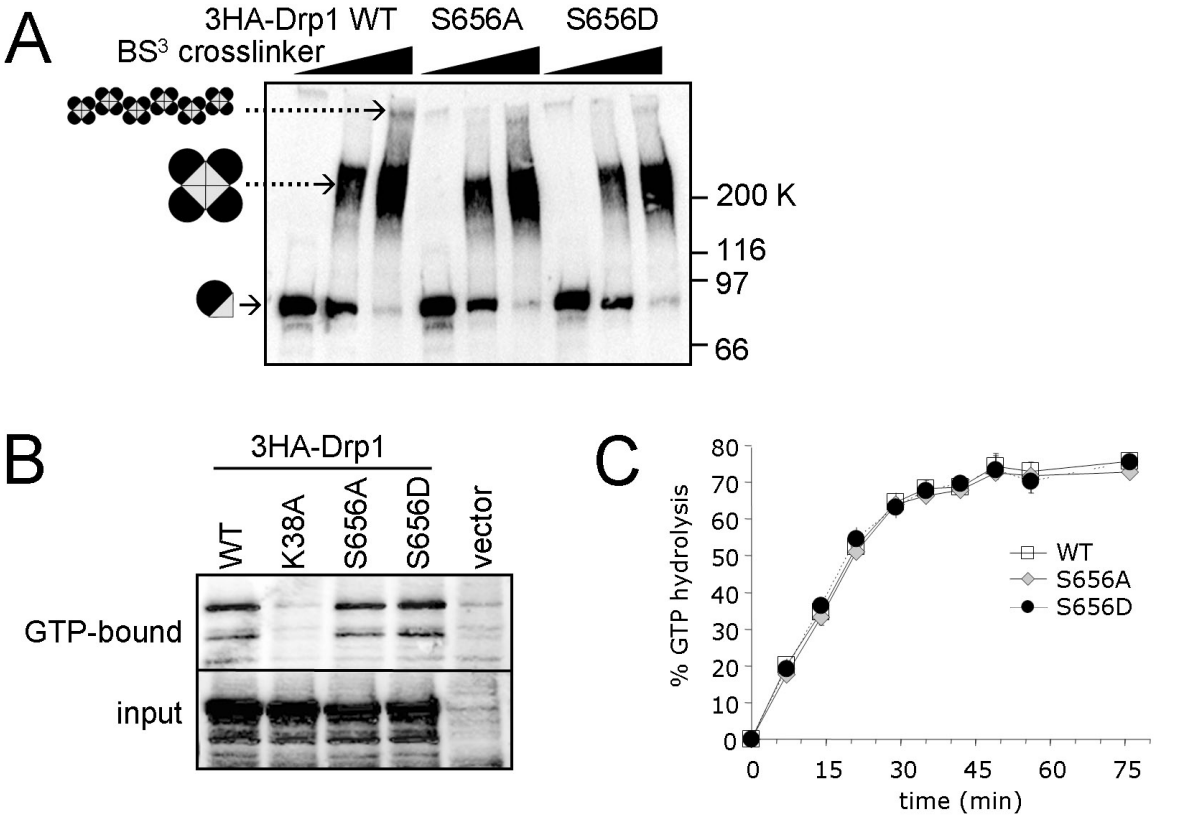
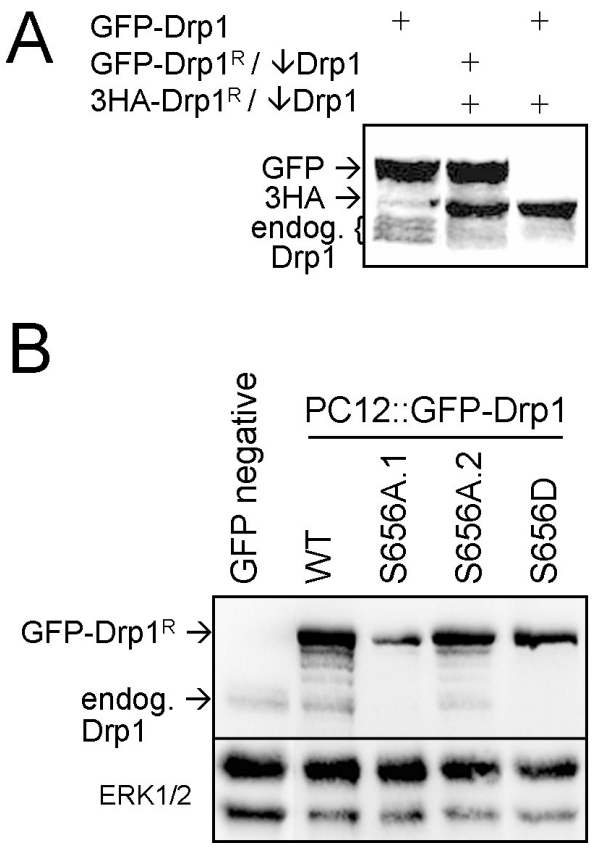
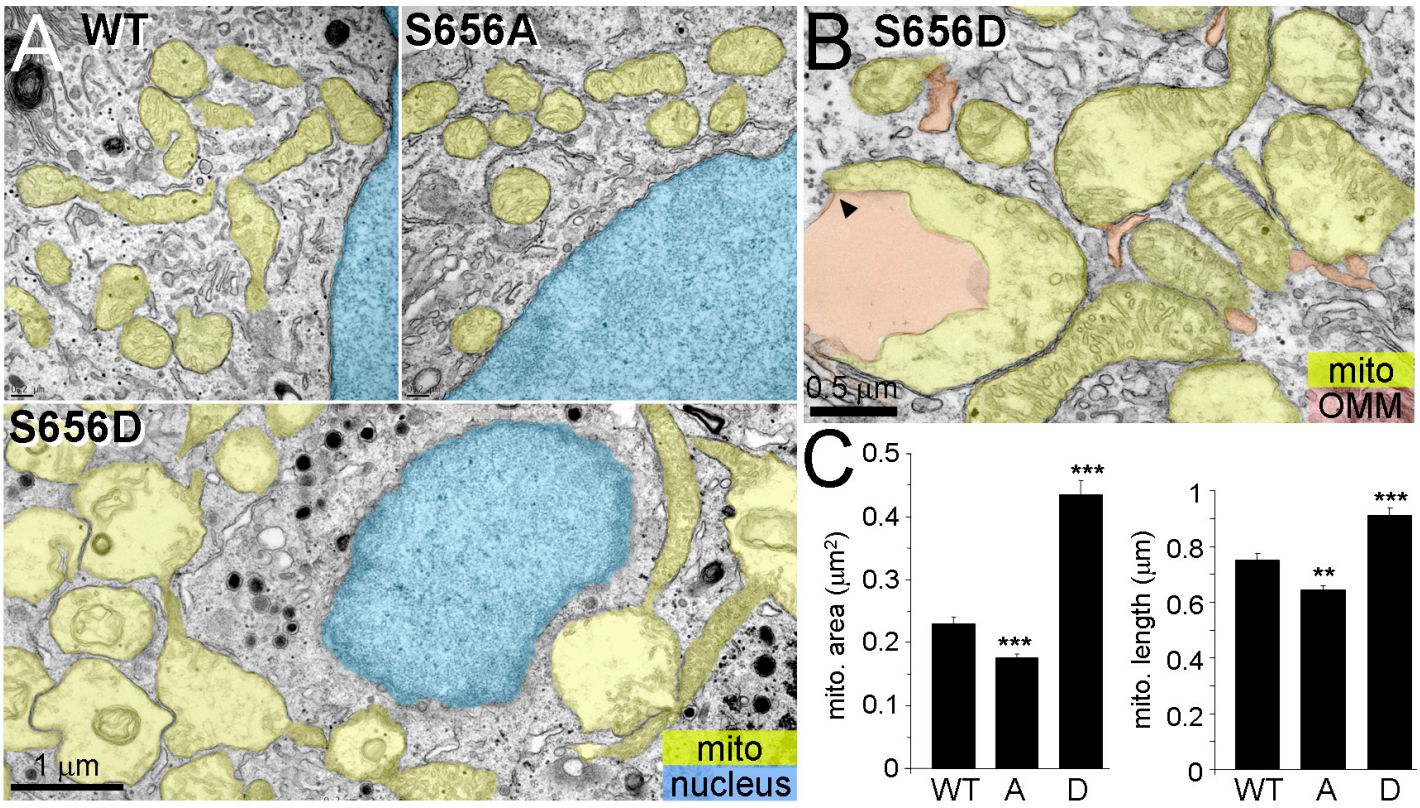
SUPPLEMENTARY FIGURES



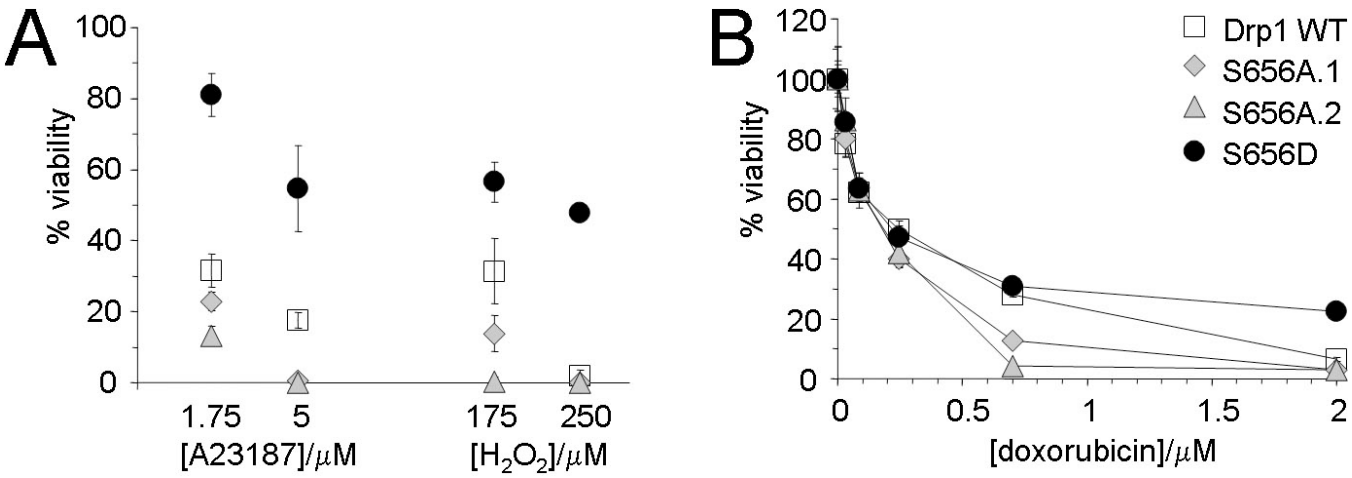
Supplementary Fig 1 | Biochemical characterization of Drp1 mutants. (**A**) Lysates of COS cells expressing 3 x HA-tagged Drp1 were chemically cross-linked (30 min, 4^{o}C) with increasing concentrations (0, 0.38, and 1.6 mM) of bis[sulfosuccinimidyl]-suberate (BS^3) and immunoblotted for the HA tag. Drp1 monomers, tetramers, and higher-order oligomers are indicated. (**B**) COS cell lysates expressing the indicated 3 x HA tagged Drp1 mutants (or empty vector) were affinity-purified on GTP-agarose and immunoblotted for HA. (**C**) Drp1 proteins purified from bacteria (adjusted to equal concentration by silver stain and immunoblotting, ~10 µg/ml) were assayed for [γ-^{32}P]GTP hydrolysis, separating bound from free ^{32}P by charcoal absorption.



Supplementary Fig 2 | Combined endogenous Drp1 knockdown and mutant Drp1 expression. (**A**) COS cells were transfected with the indicated combinations of plasmids expressing GFP-Drp1 and plasmids expressing RNAi-resistant Drp1 and Drp1-directed shRNA in tandem (GFP/3HA-Drp1^{R} /↓Drp1). Immunoblotting for Drp1 3 d post-transfection shows RNAi-mediated knockdown of GFP-Drp1 and endogenous Drp1, but not Drp1^{R}. (**B**) Total lysates of the indicated clonal PC12 cell lines were immunoblotted for Drp1 and ERK1/2 (loading control). “GFP negative” cells were recovered in the same selection and express endogenous Drp1 at levels similar to naïve cells. While endogenous Drp1 is undetectable in two cell lines (S656A.1, S656D), a ladder of GFP-Drp1 breakdown products obscures the area of the blot where endogenous Drp1 would migrate in lysates of two other lines (WT, S656A.2).



Supplementary Fig 3 | Mitochondrial shape and ultrastructure in PC12 cells, in which endogenous Drp1 was stably replaced with Ser656-mutant GFP-Drp1. (**A,B**) Color overlays in representative transmission electron micrographs highlight mitochondria (yellow) and nuclei (blue). In Drp1 S656D-expressing PC12 cells (**B**), we sometimes observed inner/outer mitochondrial membrane separation (arrow head) and outer mitochondrial membrane (OMM)-enclosed vesicular structures (pink). (**C**) Area and length (major axis) of mitochondrial cross-sections (mean ± s.e.m. of 389-505 mitochondrial profiles from two cultures) in PC12 cells substituted with WT, S656A (A), and S656D (D) GFP-Drp1. $^{**}p<5\times10^{-4}$, $^{***}p<1\times10^{-5}$ by Student's t-test.



Supplementary Fig 4 | Drp1 Ser656 determines sensitivity to select apoptotic insults. PC12 cells stably substituting WT and Ser656-mutant GFP-Drp1 for endogenous Drp1 were challenged for 48 h with the indicated doses of various cytotoxic agents, followed by colorimetric and fluorometric viability assays in 96-well plate format. Data (mean ± s.d. of quadruplicate determinations) are representative of three or more independent experiments. For each Drp1 genotype, two to three clonal lines were analyzed (two Drp1 S656A lines are shown here for comparison).