Supplementary Figures

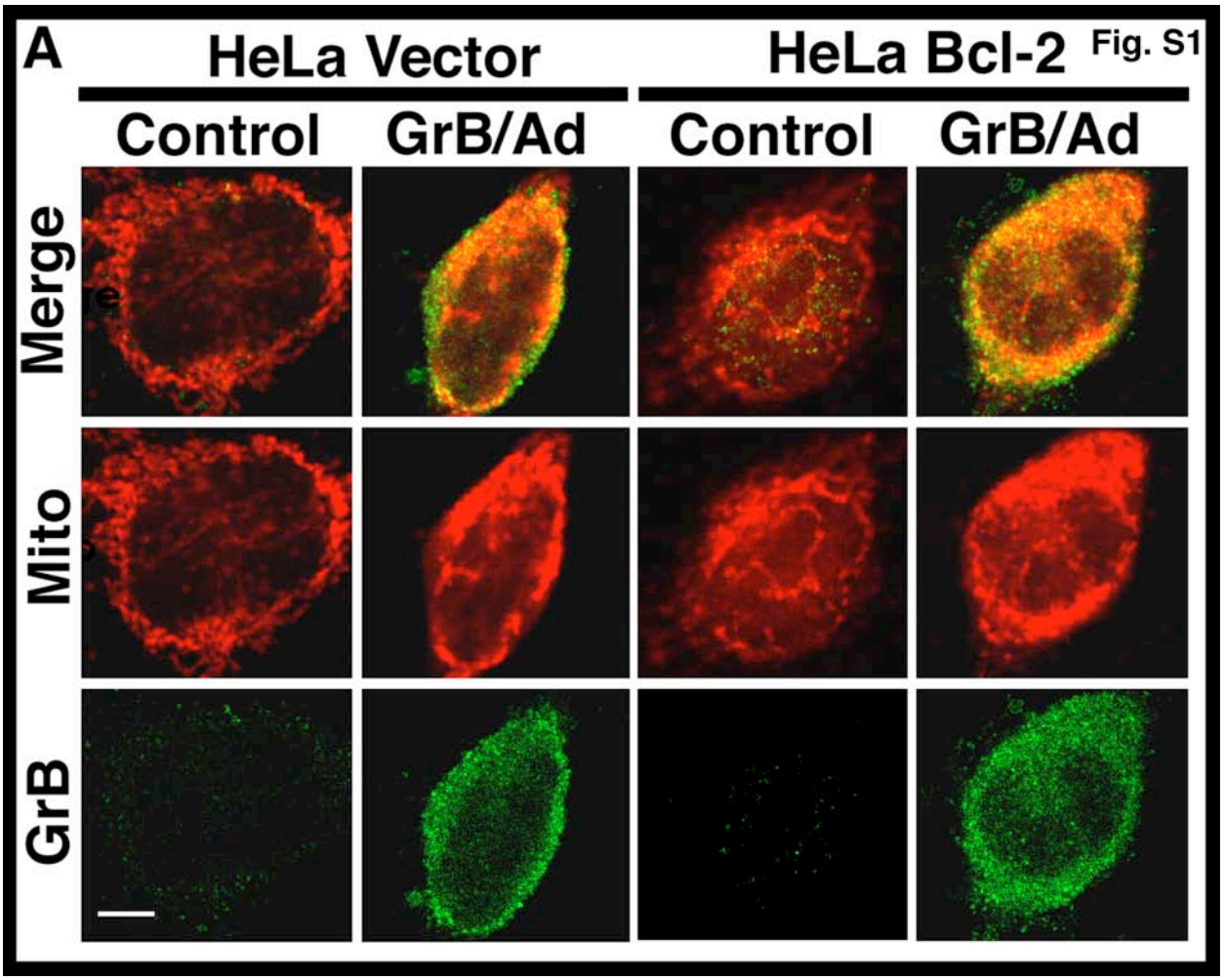

Fig. S1**. Higher magnification of Fig. 1A.** Mitochondrial localization of GrB. HeLa cells stably transfected with vector control or Bcl-2 were treated with GrB/Ad (33 nM/10 pfu/ml, respectively) for 10 min. The cells were then stained with the MitoTracker Deep Red and with anti-GrB Ab. Scale Bar = 10 μm

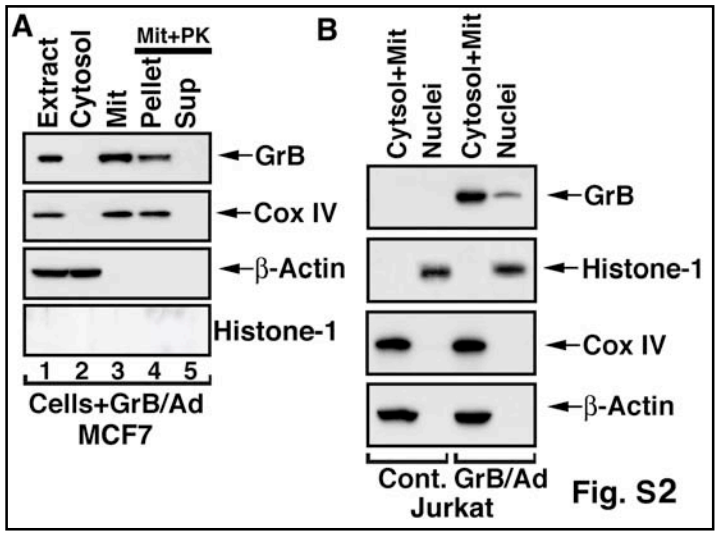

Figure S2. **Mitochondrial translocation of GrB is independent of its nuclear translocation.** (A) Detection of GrB in a proteinase K-resistant mitochondrial compartment in cells treated with GrB/Ad. MCF7 cells were treated with GrB/Ad (33 nM/10 pfu/ml) for 4 hr at 37°C. Following addition of GrB inhibitor (Z-IETD-CHO, 500 μM) the cells were Dounce homogenized and subjected to subcellular fractionation to obtain the mitochondrial and cytosolic fractions. The mitochondria were then treated with proteinase K and spun to obtain the mitochondrial pellet and wash supernatant. The cell extract and the fractionated proteins were resolved by SDS-PAGE and immunoblotted with Abs to the indicated proteins. Exclusion of nuclei from all fractions is indicated by the lack of histone-1 expression. (B) Exogenous GrB localizes to purified nuclei. Jurkat cells treated with GrB/Ad as described in A were subjected to nuclei purification. The SDS-PAGE resolved proteins were assessed by immunoblotting.

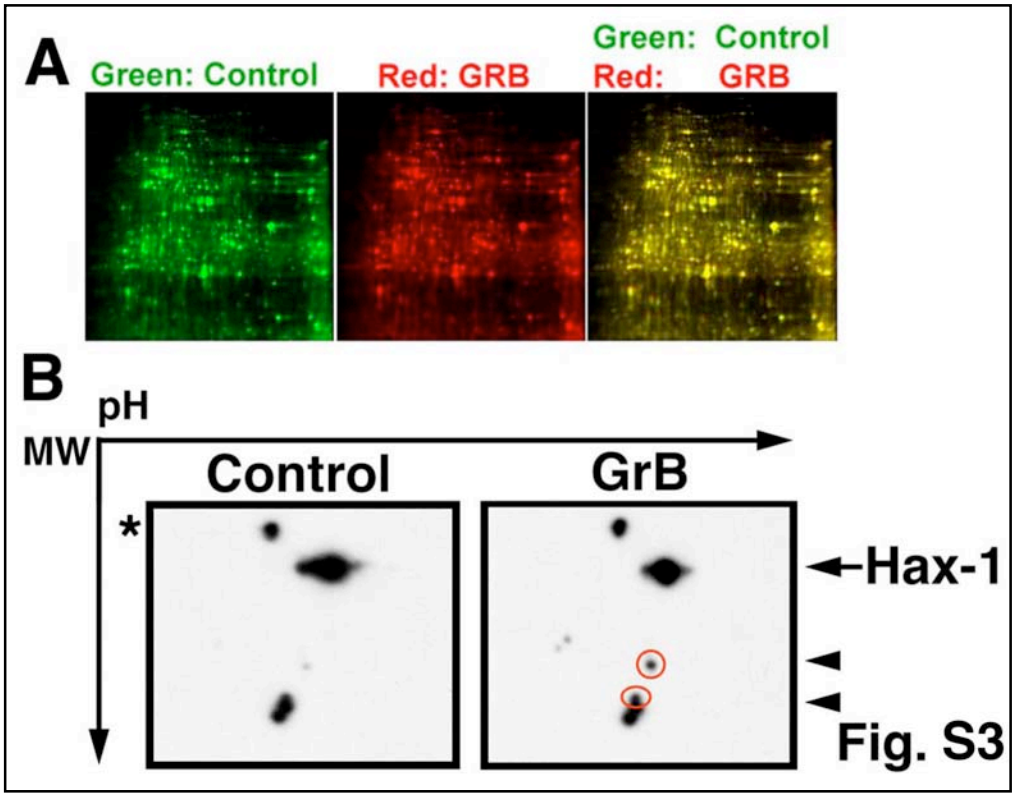

Figure S3. **2D-DIGE analysis of GrB-treated mitochondria.** (A) 2D DIGE analysis of GrB-treated mitochondria. Mitochondria purified from Jurkat cells were treated with GrB (66 nM for 30 min) and then with a GrB inhibitor (Z-IETD-CHO, 500 mM). The mitochondrial supernatant was removed, and the pellet washed and lysed. Control samples were labeled with Cy2 and GrB-treated samples - with Cy3. The proteins were resolved by 2D DIGE (IEF and SDS-PAGE) and analyzed by the Typhoon imager, and DeCyder software. (B) Immunoblott analysis of 2D-PAGE for Hax-1 and its GrB-generated fragments. Mitochondria purified from WT Jurkat cells were treated with purified GrB for 30 min. The mitochondrial pellet was then lysed and assessed by 2D PAGE with a pH range of 3-7 in the 1st dimension. Following membrane electroblotting, the membrane was probed with an anti-Hax-1 Ab. Arrowheads indicate Hax-1 cleavage fragments. Similar running patterns of the Hax-1 fragment were detected in three independent experiments.

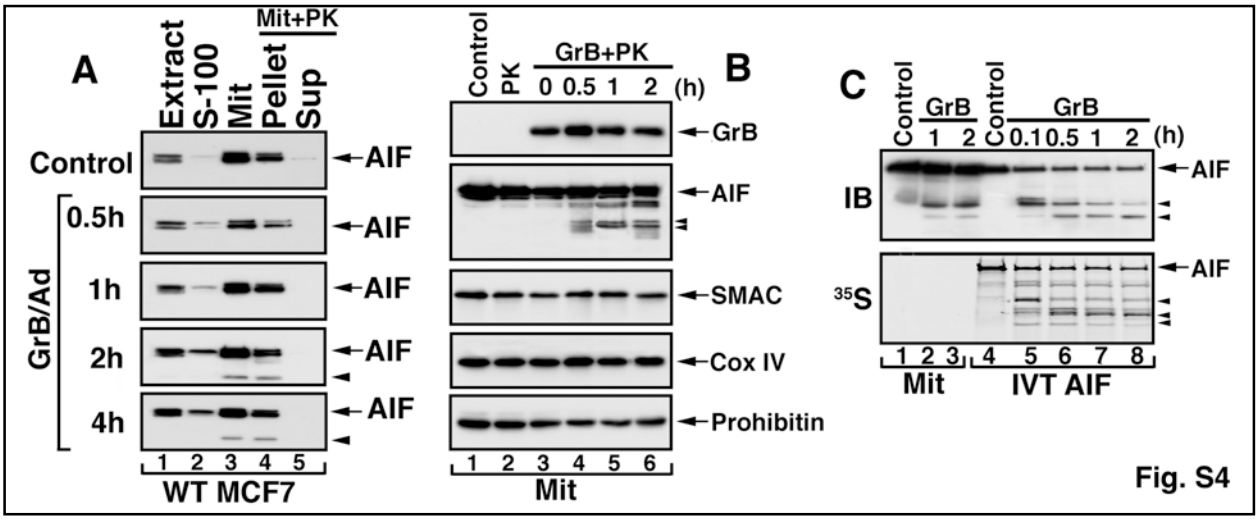

Figure S4. **AIF cleavage by GrB. (A) Kinetics of AIF release from mitochondria in GrB-treated MCF7 cells.** MCF7 cells were treated with GrB/Ad as described (33nM/10 pfu/ml for 0.5-4 hr). AIF released from mitochondria was detected in the cytoplasm at the earliest time-point tested (30 min). A cleavage product of AIF was detected in the mitochondrial pellet after 2-4 h of treatment with GrB/Ad (arrow heads). (B) GrB-mediated cleavage of AIF within a proteinase K-resistant compartment of mitochondria. Purified mitochondria from Jurkat cells were treated with GrB for the indicated time periods. The mitochondria were then treated with proteinase K. After addition of PMSF to block proteinase K activity, the mitochondrial pellets were lysed and assessed by immunoblotting for the indicated mitochondrial proteins. Cleavage products of AIF are indicated by arrow heads. (C) ^{35}S-labeled in vitro translated AIF was incubated with GrB for the indicated time periods. The reaction products were then run on SDS-PAGE (lanes 4-8) side-by-side with lysates of GrB-treated mitochondria (lanes 2,3), as described in B. The AIF cleavage products were detected by immunoblotting (top panel) or autoradiography (bottom panel). At least, four AIF cleavage products were detected by autoradiography, and two of them were co-detected by immunoblotting. The cleavage products of the in vitro translated AIF co-migrated with the mitochondrial AIF fragments.

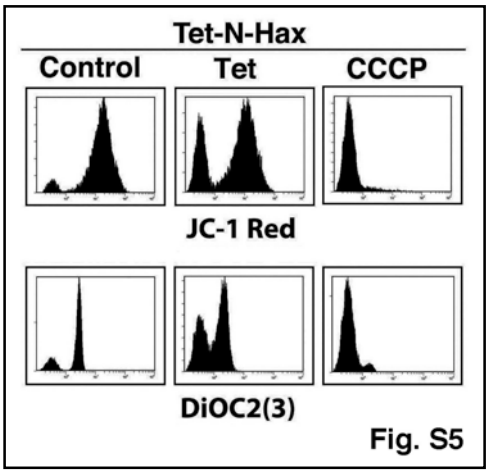

Fig. S5. **Mitochondrial depolarization following Tet-induction of the N-terminal Hax-1 fragment.** T-REx-293 cells stably transfected with Tet-inducible N-terminal Hax-1 fragment were treated with tetracycline (1 μg/ml, 16 hr) and assessed by flow cytometry for staining by the two $\Delta\Psi_m$-dependent dyes, JC-1 (red) and DiOC2(3) (green).

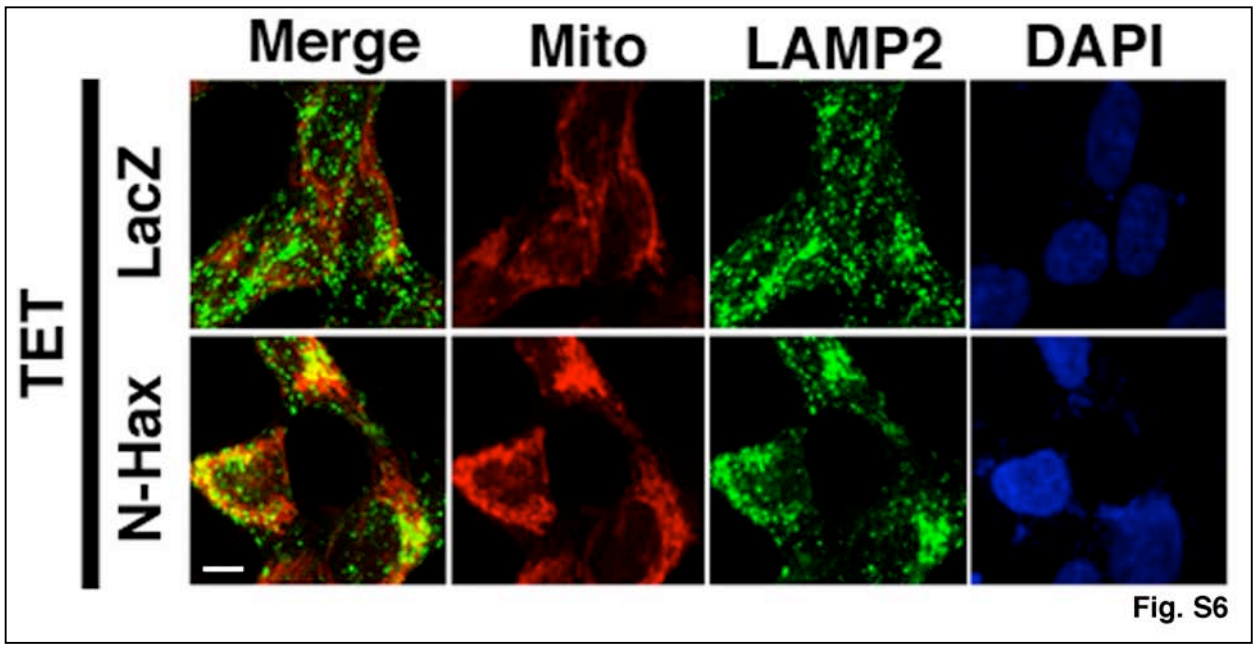

Fig. S6. **Higher magnification of Fig. 6D.** Enhanced co-localization of mitochondria and lysosomes following Tet-induction of the N-terminal Hax-1 fragment. T-REx-293 cells stably transfected with Tet-inducible lacZ or N-terminal Hax-1 fragment were treated with tetracycline (1 μg/ml, 16 hr) and assessed by confocal microscopy for a merge between MitoTracker Deep Red and anti-LAMP2 Ab detected by a secondary green fluorescent Ab. Scale bar = 40 μm.