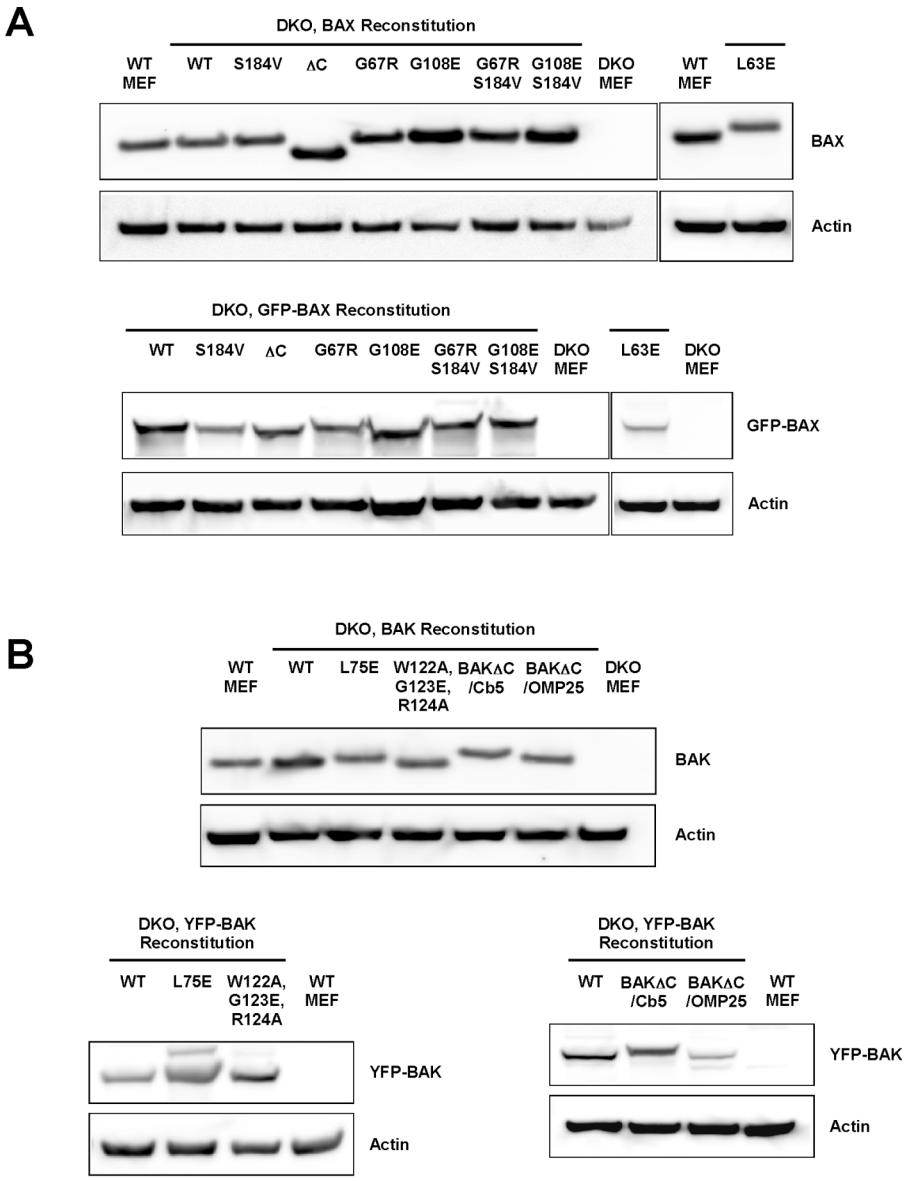

Figure S1. (A) Anti-BAX immunoblots detect comparable expression of wild-type or mutant BAX reconstituted into DKO cells by retroviral transduction. (B) Anti-BAK immunoblots detect comparable expression of wild-type or mutant BAK reconstituted into DKO cells by retroviral transduction. Of note, BAKΔC/OMP25 was less stable, and expressed at a lower level.

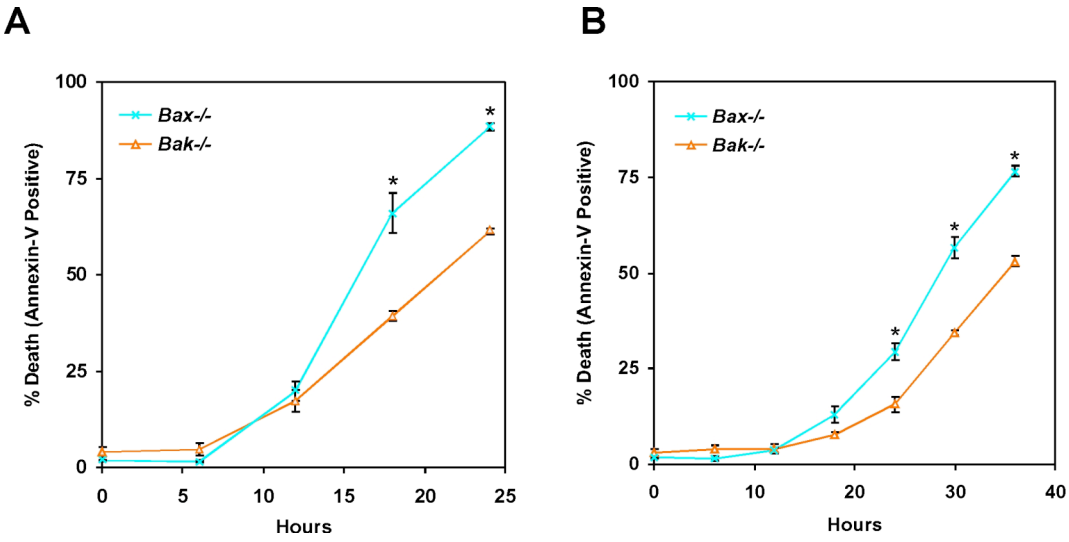

Figure S2. ***Bax*-deficient MEFs displays faster death kinetics in comparison with *Bak*-deficient MEFs.** *Bax*- or *Bak*-deficient MEFs were treated with etoposide (A) or tunicamycin (B) for the indicated time. Cell death was quantified by Annexin-V staining. Data shown are mean ± s.d. from three independent experiments. Asterisk, P<0.05.

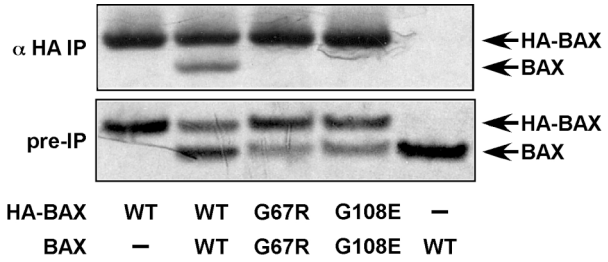

Figure S3. **BH1 and BH3 domain mutants of BAX fail to form homo-dimers.** In vitro transcribed/translated, radiolabeled N-terminal HA-tagged wild-type or mutant BAX plus non-tagged counterparts were immunoprecipitated with anti-HA antibody. Immunoprecipitates and pre-IP input were analyzed by Nu-PAGE and autoradiography.

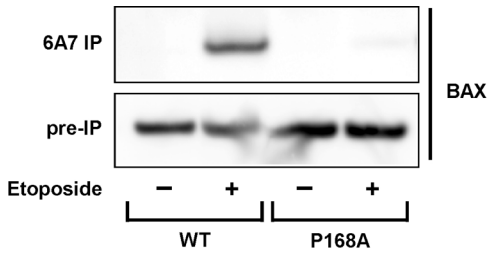

Figure S4. **BAX P168A fails to expose its N-terminus upon DNA damage.** DKO MEFs reconstituted with wild-type BAX or BAX P168A before or after treatment with etoposide for 15 hr were lysed in 1% CHAPS and then immunoprecipitated with the 6A7 anti-BAX antibody. Immunoprecipitates were analyzed by anti-BAX (N20) immunoblots.

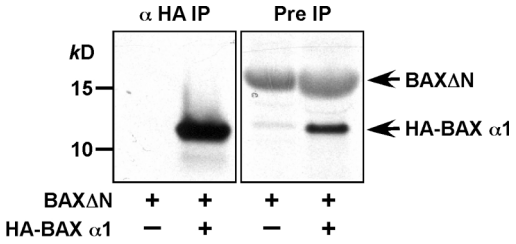

Figure S5. **NP-40 disrupts the binding of the α1 helix of BAX to BAXΔN.** Radiolabeled IVTT HA_3-tagged BAX α1 helix in combination with BAXΔN were subjected to anti-HA immunoprecipitation in 0.2 % NP-40. Immunoprecipitates and pre-IP input were analyzed by Nu-PAGE and autoradiography.

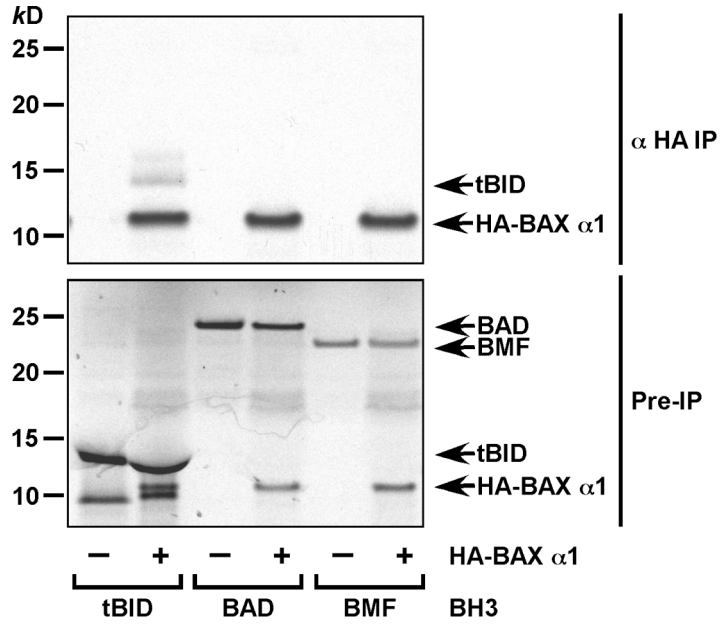

Figure S6. **tBID, but not BAD or BMF, binds to the α1 helix of BAX.** Radiolabeled IVTT HA_3-tagged BAX α1 helix in combination with tBID, BAD, or BMF were subjected to anti-HA immunoprecipitation in 1 % CHAPS. Immunoprecipitates and pre-IP input were analyzed by Nu-PAGE and autoradiography.

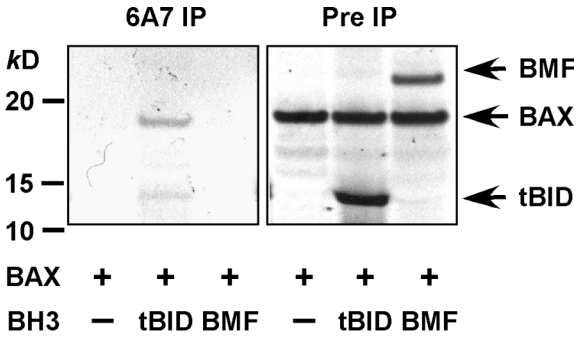

Figure S7. **tBID, but not BMF, induces the N-terminal exposure of BAX and remains associated with the N-terminally exposed BAX.** Radiolabeled IVTT BAX incubated with tBID, or BMF were immunoprecipitated with the 6A7 antibody. Immunoprecipitates and pre-IP input were analyzed by Nu-PAGE and autoradiography.

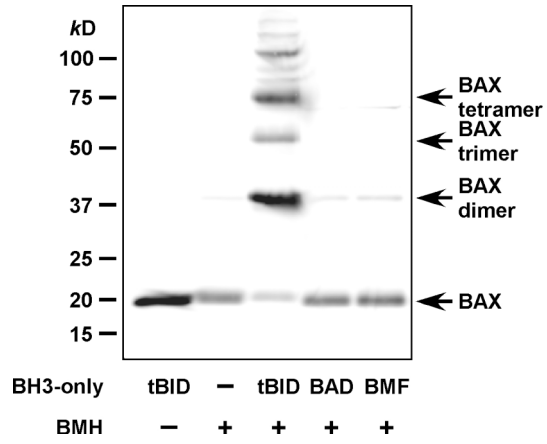

Figure S8. **tBID, but not BAD or BMF, induces the homo-oligomerization of BAX S184V.** Mitochondria isolated from DKO MEFs reconstituted with BAX S184V were incubated with IVTT tBID, BAD, or BMF for 30 min and then treated with BMH crosslinker. The BAX oligomers were detected by an anti-BAX immunoblot.

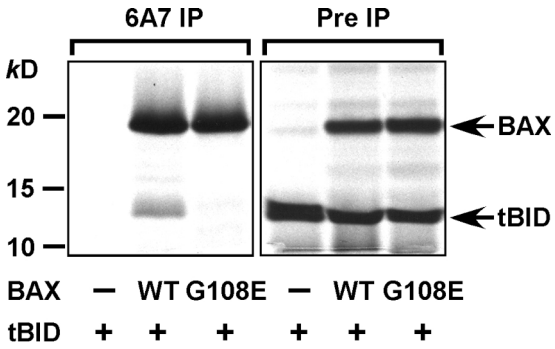

Figure S9. **BH1 domain is required for N-terminally exposed BAX to interact with tBID.** Radiolabeled IVTT BAX incubated with tBID were immunoprecipitated with the 6A7 antibody in 0.2% NP-40. Immunoprecipitates and pre-IP input were analyzed by Nu-PAGE and autoradiography.

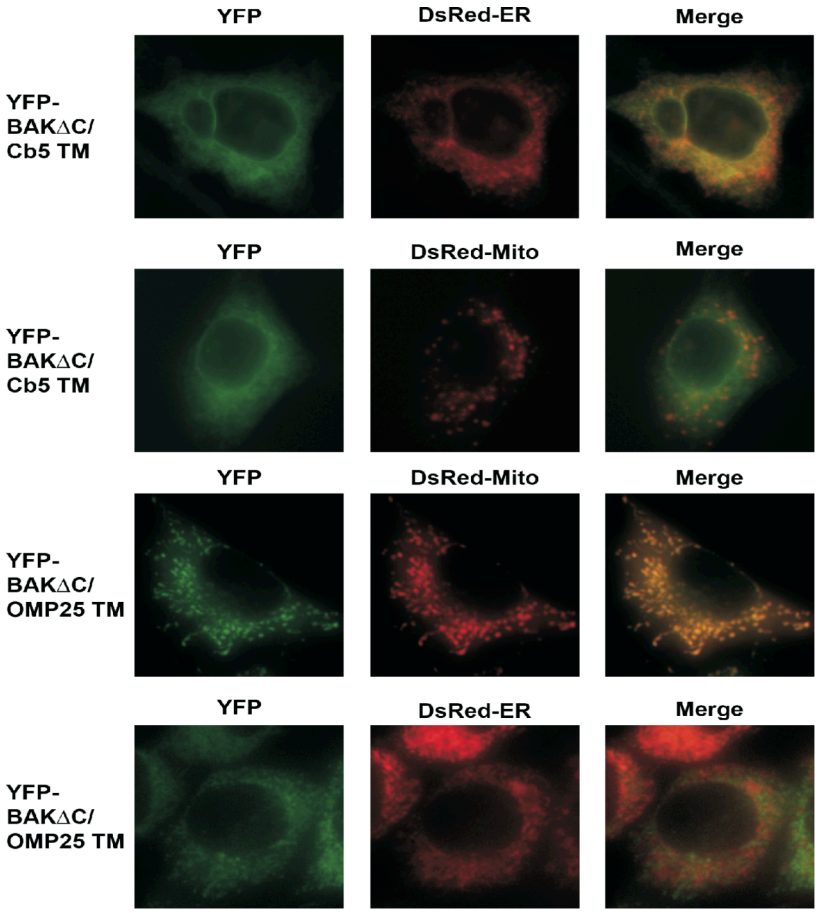

Figure S10. **YFP-BAKΔC/Cb5 TM colocalizes with DsRed-ER whereas YFP-BAKΔC/OMP25 TM colocalizes with DsRed-Mito.** Fluorescence microscopy of DKO MEFs stably reconstituted with indicated N-terminal YFP-tagged BAK chimera followed by retroviral transduction of DsRed-ER or DsRed-Mito.

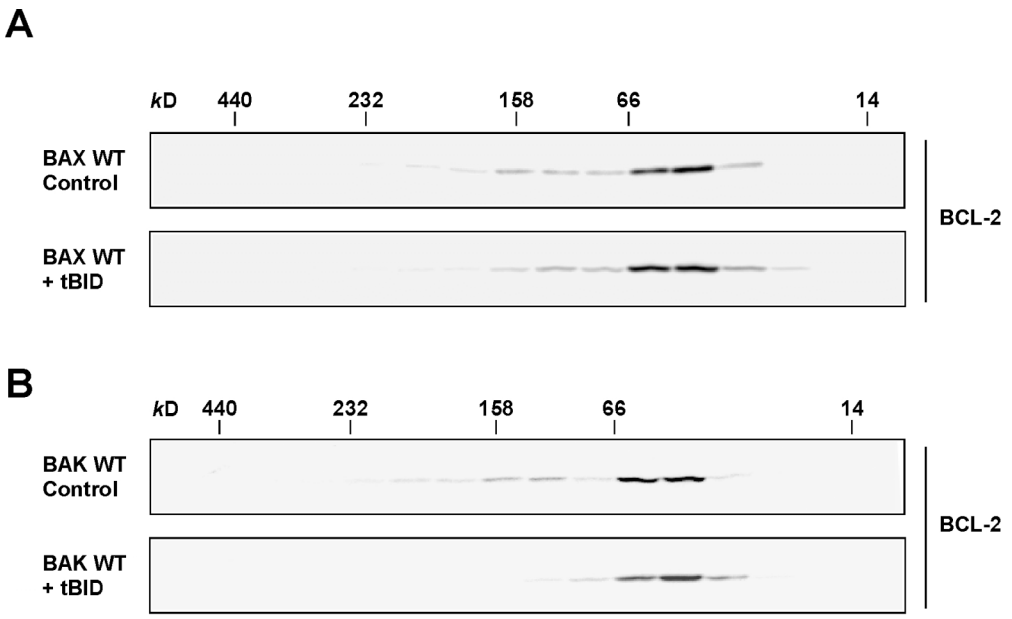

Figure S11. **BCL-2 does not homo-oligomerize in response to tBID.** (A) Mitochondria isolated from DKO MEFs reconstituted with wild-type BAX were treated with recombinant tBID (1 ng/μl) and solubilized in 2 % CHAPS buffer. The protein lysates (200 μg) were subjected to Superdex 200 (HR 10/30) gel filtration chromatography. The membranes utilized in Fig. 2A were stripped and probed with anti-BCL-2. (B) Mitochondria isolated from DKO MEFs reconstituted with wild-type BAK were treated with recombinant tBID (1 ng/μl) and solubilized in 2 % CHAPS buffer. The protein lysates (200 μg) were subjected to Superdex 200 (HR 10/30) gel filtration chromatography. The membranes utilized in Fig. 5A were stripped and probed with anti-BCL-2.

Mutation	Domain	Localization	Bind BH3s	Defect
BAX L63E	BH3	Mitochondria	yes	oligomerization
BAX S184V	TM	Mitochondria	ND	no
BAX G67R	BH3	Cytosol	ND	Mitochondrial insertion & oligomerization
BAX G108E	BH1	Cytosol	no	Mitochondrial insertion & oligomerization
BAX G67R/S184V	BH3 & TM	Mitochondria/Cytosol	ND	oligomerization
BAX G108E/S184V	BH1 & TM	Mitochondria	ND	oligomerization
BAK L75E	BH3	Mitochondria	yes	oligomerization
BAK W122A/G123E/R124A	BH1	Mitochondria	no	oligomerization

Table S1. Summary of BAX and BAK mutants. ND, not determined.

Antibody and reagents

Antibodies used for immunoblots are listed as followed: anti-BAK (NT, Upstate), anti-BAK (G23, Santa Cruz), anti-BAX (N-20, Santa Cruz), anti-BAX (6A7, Trevigen), anti-BIM (Calbiochem), anti-PUMA (Sigma), anti-HA (12CA5), and anti-BCL-2 (3F11, BD Bioscience). The chemicals with concentration used are as followed: etoposide 10 μg/ml; staurosporine 1.2 μM; tunicamycin 1 μg/ml; thapsigargin 2 μM; arachidonic acid 500 μM (Sigma); and bismaleimidohexane (BMH) 5 mM (Pierce).