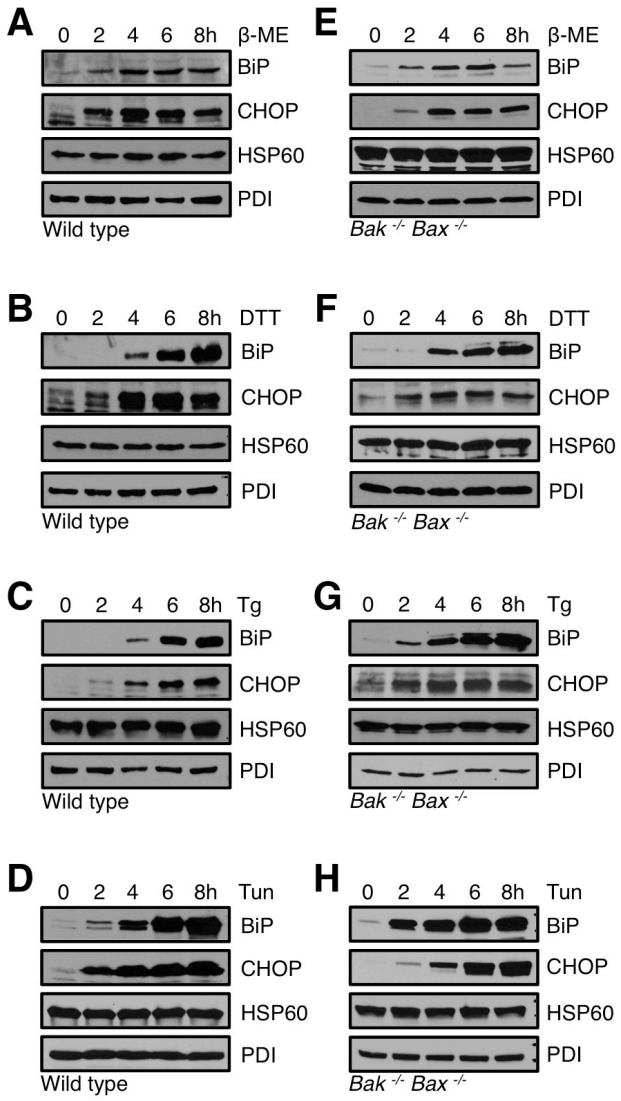

Figure S1. Wt and *Bak*$^{-/-}$*Bax*$^{-/-}$ MEFs induce similar levels of ER stress markers. ***Related to figure 1.*** **(A-H)** Whole cell lysates from ER stress treated Wt *(A-D)* and *Bak*$^{-/-}$*Bax*$^{-/-}$ *(E-H)* MEFs (0, 2, 4, 6, & 8 hours) were analyzed by western blot for BiP, CHOP, HSP60, and PDI expression. β-ME (15 mM), DTT (5 mM), Tg (1.5 μM), or Tun (2.5 μg/ml).

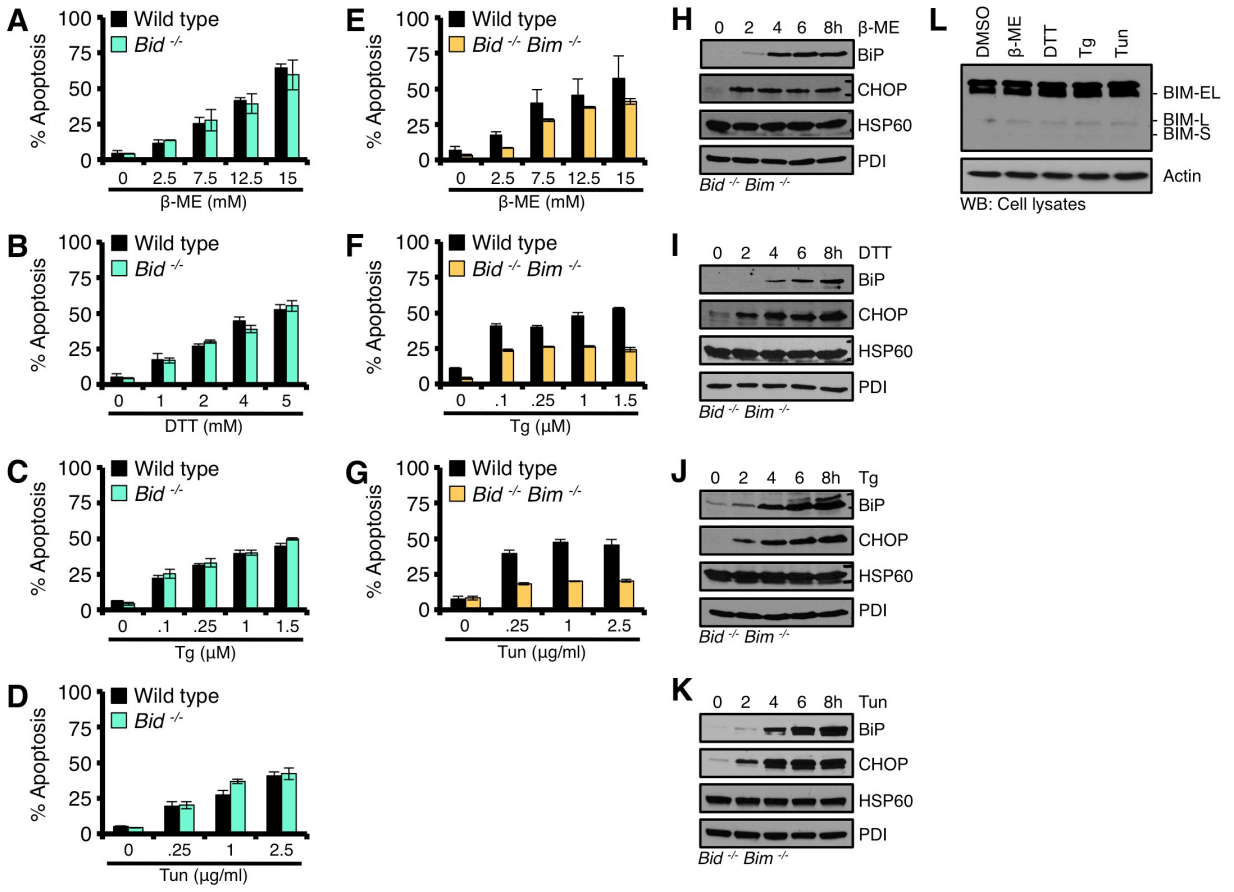

Figure S2. Terminal UPR responses are BIM-dependent. ***Related to figure 1.***

(A-D) *Bid*$^{-/-}$ MEFs were treated with indicated concentrations of β-ME, DTT, Tg, or Tun for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(E-G) Wt and *Bid*$^{-/-}$*Bim*$^{-/-}$ MEFs were treated with indicated concentrations of β-ME, DTT, or Tun for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(H-K) Whole cell lysates from ER stress treated *Bid*$^{-/-}$*Bim*$^{-/-}$ MEFs (0, 2, 4, 6, & 8 hours) were analyzed by western blot for BiP, CHOP, HSP60, and PDI expression. β-ME (15 mM), DTT (5 mM), Tg (1.5 μM), or Tun (2.5 μg/ml).

(L) Whole cell lysates from ER stress treated Wt MEFs (highest doses at 18 hours) were analyzed by western blot for BIM isoforms. Actin is a loading control.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

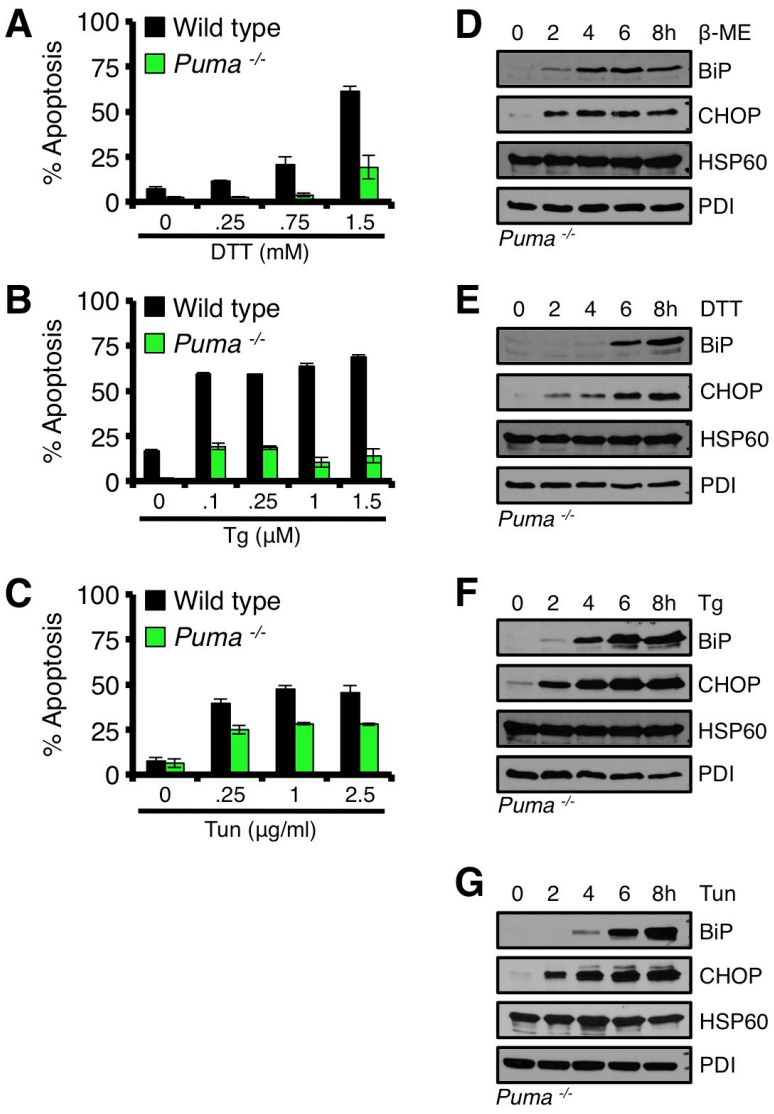

Figure S3. Terminal UPR responses require PUMA-mediated sensitization/de-repression. ***Related to figure 1.***

(A-C) Wt and *Puma*$^{-/-}$ MEFs were treated with indicated concentrations of DTT, Tg, or Tun for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining.

(D-G) Whole cell lysates from ER stress treated *Puma*$^{-/-}$ MEFs (highest doses; 0, 2, 4, 6, & 8 hours) were analyzed by western blot for BiP, CHOP, HSP60, and PDI expression. β-ME (15 mM), DTT (5 mM), Tg (1.5 μM), or Tun (2.5 μg/ml).

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

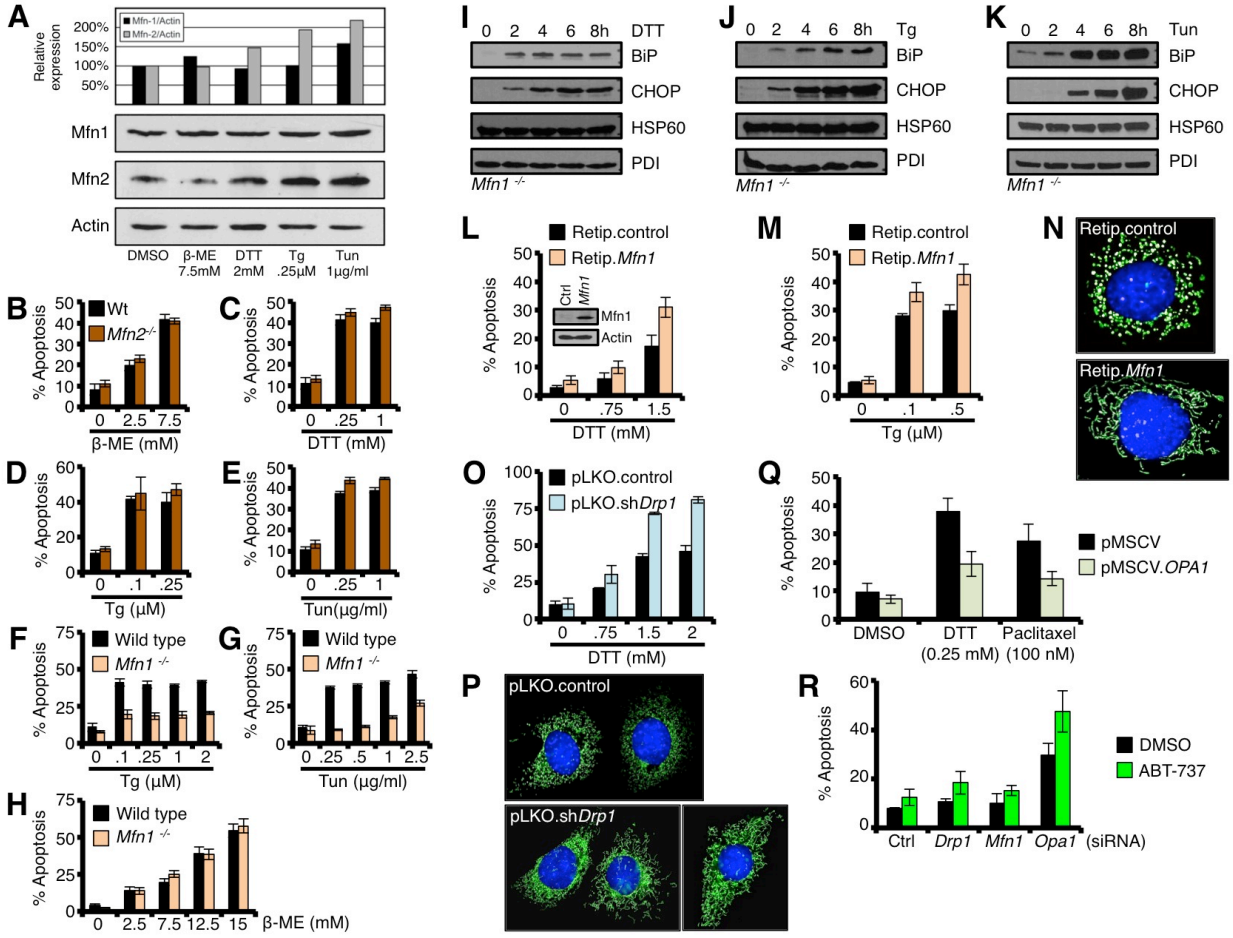

Figure S4. ***Mfn2*** **is induced following UPR; and** ***mfn1*** **increases terminal UPR efficiency, except for β-ME.** ***Related to figure 3.***

(A) Whole cell lysates from ER stress treated Wt MEFs (12 hours, concentration indicated) were analyzed by western blot for Mfn1, Mfn2, and Actin. Mfn1 and Mfn2 band intensities were quantified and normalized to Actin.

(B-E) Wt and *Mfn2*$^{-/-}$ MEFs were treated with indicated concentrations of β-ME, DTT, Tg, or Tun for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(F-H) Wt and *Mfn1*$^{-/-}$ MEFs were treated with indicated concentrations of β-ME, Tg, or Tun for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(I-K) Whole cell lysates from ER stress treated *Mfn1*$^{-/-}$ MEFs (0, 2, 4, 6, & 8 hours) were analyzed by western blot for BiP, CHOP, HSP60, and PDI expression. β-ME (15 mM), Tg (1.5 μM), or Tun (2.5 μg/ml).

(L-M) *Mfn1*$^{-/-}$ MEFs expressing Retip.control or Retip.*Mfn1* were treated with DTT or Tg (indicated concentrations) for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow

cytometry. In panel *L*, whole cell lysates from *Mfn1*$^{-/-}$ MEFs expressing Retip.control or Retip.*Mfn1* were analyzed by western blot for Mfn1 and Actin.

(N) *Mfn1*$^{-/-}$ MEFs expressing Retip.control or Retip.*Mfn1* were loaded with MitoTracker Green® (50 nM) and Hoechst 33342 (20 μM) before live cell imaging (400×).

(O) *Mfn1*$^{-/-}$ MEFs expressing pLKO.control or pLKO.sh*Drp1* were treated with DTT (indicated concentrations) for 18 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(P) *Mfn1*$^{-/-}$ MEFs expressing pLKO.control or pLKO.sh*Drp1* were loaded with MitoTracker Green® (50 nM) and Hoechst 33342 (20 μM) before live cell imaging (400×).

(Q) Wt MEFs expressing pMSCV or pMSCV.*OPA1* were treated with DTT (0.25 mM) or Paclitaxel (100 nM) for 24 hours, and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry.

(R) Wt MEFs were transfected with indicated siRNAs (20 nM) for 48 hours in the presence of ABT-737 (1 μM), and analyzed for % apoptosis by AnnexinV-FITC staining and flow cytometry. These data suggest that *Opa1* silencing leads to cell stress and apoptosis, while silencing *Drp1* or *Mfn1* is tolerated. All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

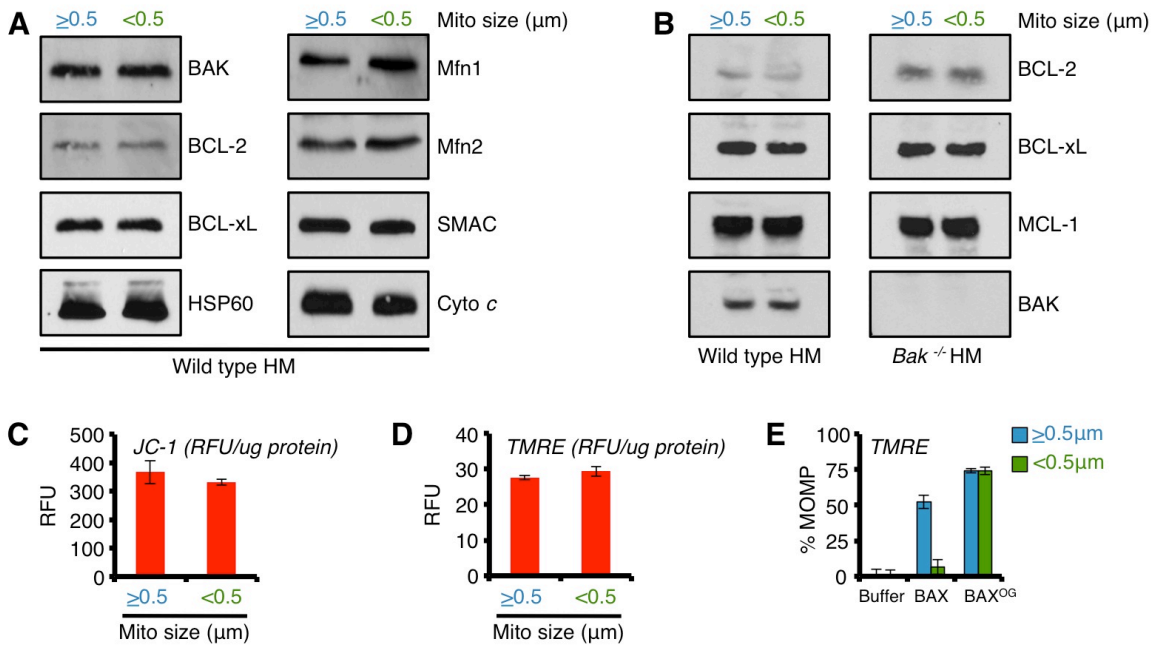

Figure S5. Characterization of large and small mitochondria. ***Related to figure 4.***

(A-B) Lysates from large (≥0.5 μm) and small (<0.5 μm) mitochondria (Wild type or *Bak*$^{-/-}$) were subjected to western blot analyses for indicated proteins.

(C-D) Liver mitochondria (250 μg) were loaded with JC-1 (100 nM; *panel C*) or TMRE (100 nM; *panel D*) for 10 minutes at 37°C, fractionated (≥0.5 μm and <0.5 μm), lysed in RIPA buffer, fluorescence was determined, and reported as RFU/μg mitochondrial protein.

(E) TMRE loaded Wt liver mitochondria were fractionated by size. Larger (>0.5 μm) and smaller (<0.5 μm) mitochondria were treated with BAX (100 nM) or OG-BAX (100 nM) for 60 minutes at 37°C. Only endpoint measurements are shown because TMRE rapidly fades; therefore, after treatments, mitochondria were centrifuged, lysed in RIPA, and the remaining fluorescence was determined. Untreated mitochondria and 0.5% CHAPS were considered 0% and 100% MOMP, respectively. These results parallel data presented in figure 4H, therefore JC-1 and TMRE loading does not alter BAX function.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

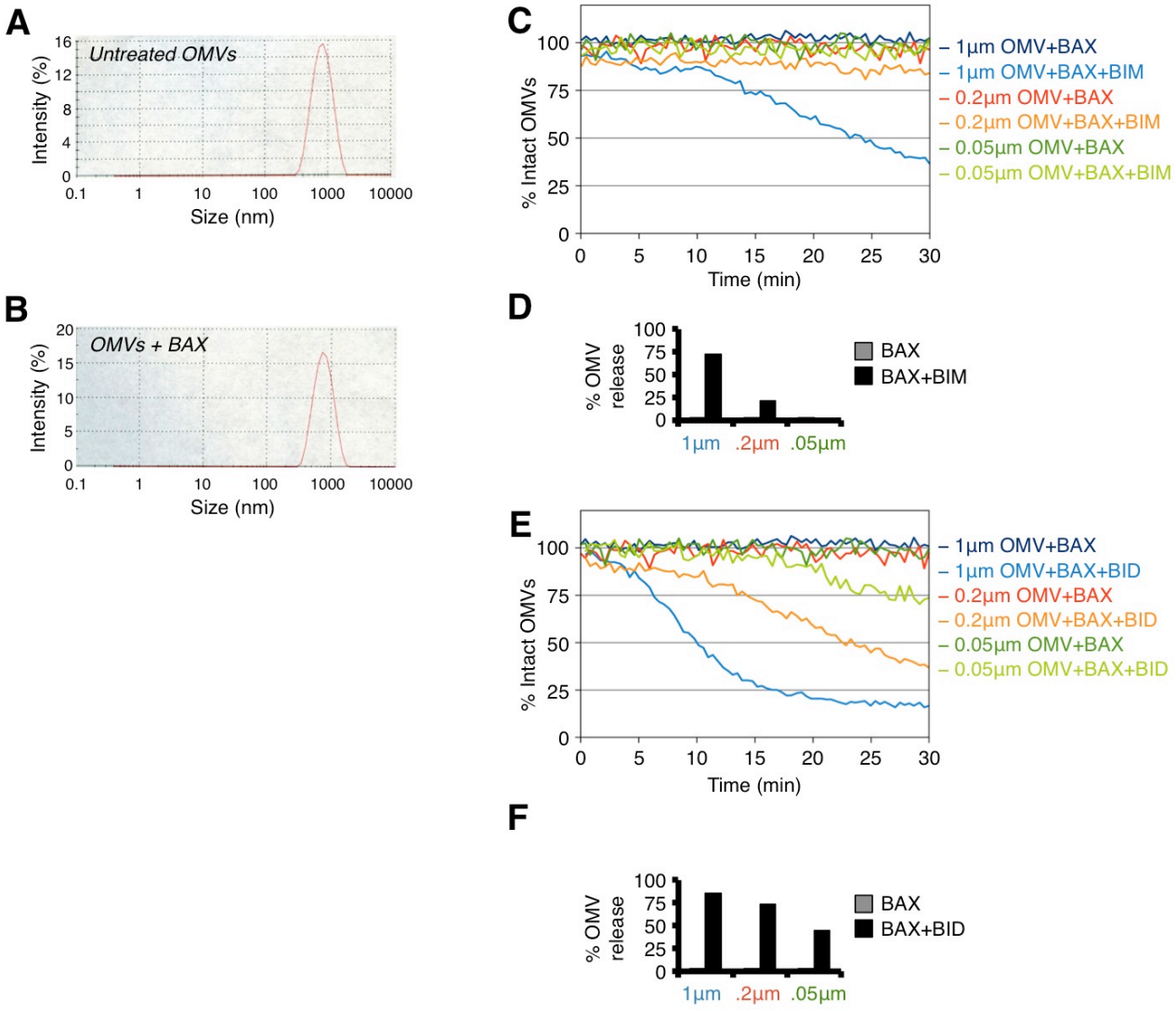

Figure S6. Characterization and kinetic analyses of OMV permeabilization. ***Related to figure 5.***

(A-B) Dynamic light scattering analyses of unextruded OMVs ± BAX (100 nM) for 30 minutes.

(C) Kinetic traces of indicated OMVs incubated with BAX (40 nM) and BIM BH3 (2.5 µM) for 30 minutes at 37°C.

(D) The 30 minutes endpoint data for *C* are shown.

(E) Kinetic traces of indicated OMVs incubated with BAX (40 nM) and N/C-BID (25 nM) for 30 minutes at 37°C.

(F) The 30 minutes endpoint data for *E* are shown.

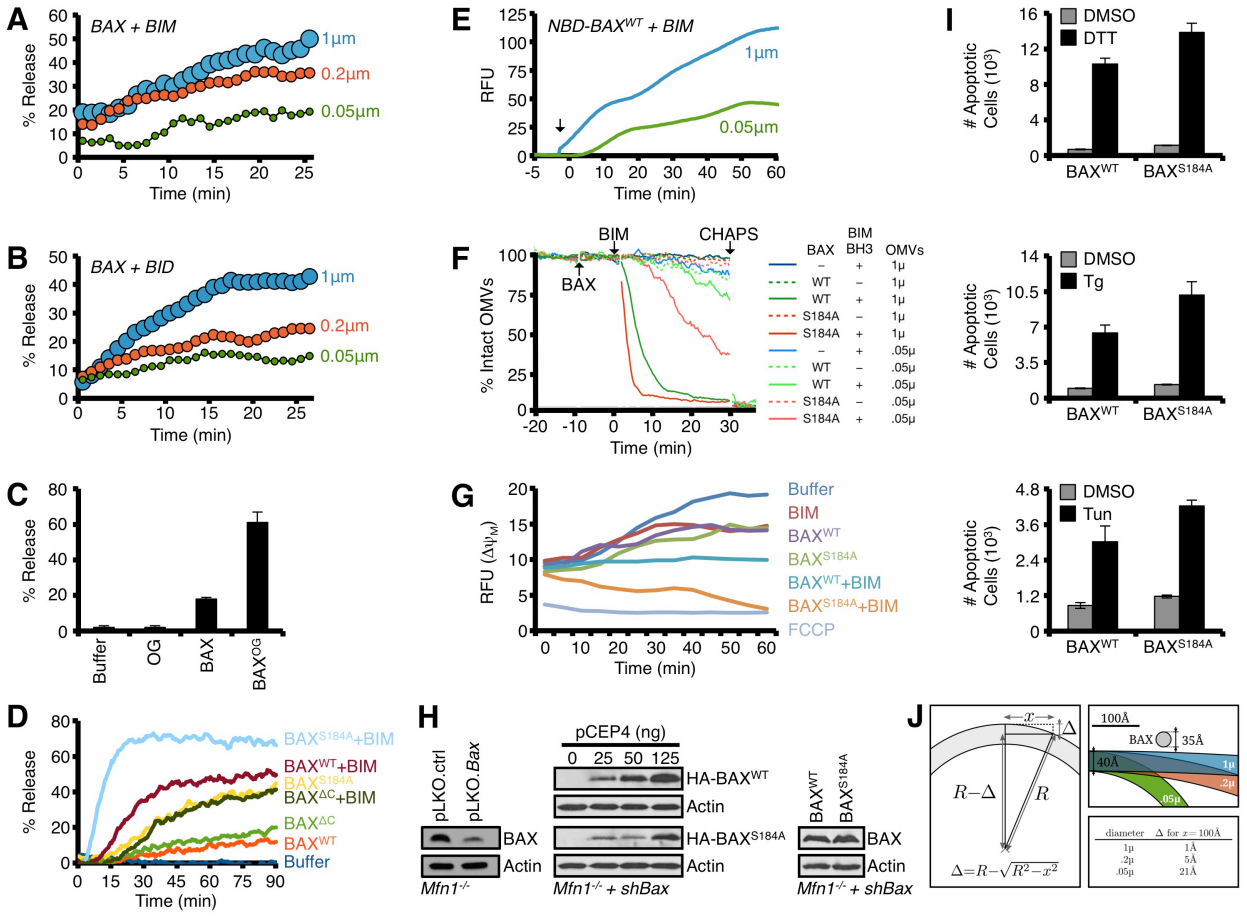

Figure S7. Characterization and kinetic analyses of LUV permeabilization, and functional comparisons between BAX variants. ***Related to figures 6 & 7.***

(A) Kinetic traces of LUV permeabilization with BAX and BIM BH3 peptide (2.5 µM) for 25 minutes at 37°C.

(B) Same as in *A*, but with N/C-BID protein (20 nM) instead of BIM.

(C) 1.0 µm LUVs were combined with BAX (100 nM), OG (0.025%), or BAX^{OG} for 30 minutes at 37°C.

(D) 1.0 µm LUVs were combined with indicated recombinant BAX proteins (100 nM) ± BIM BH3 (2.5 µM) for 90 minutes to demonstrate different assay length requirements based on various BAX proteins.

(E) Kinetic traces of BIM BH3 peptide (2.5 µM) induced NBD-BAX^{WT} association with indicated OMVs for 60 minutes at 37°C. BIM BH3 addition is indicated by the arrow. NBD-BAX^{WT} alone fails to associate with OMVs (-5 minutes to 0 minutes, and data not shown).

(F) Indicated OMVs were treated with BAX^{WT} or BAX^{S184A} (50 nM), ± BIM BH3 (2.5 µM), for 30 minutes at 37°C.

(G) Digitonin-permeabilized, JC-1 loaded *Mfn1*$^{-/-}$ MEFs were incubated with indicated combinations of BIM BH3 (0.1 μM), BAX^{WT} (50 nM), and BAX^{S184A} (50 nM), and mitochondrial depolarization ($\Delta\psi_M$) was determined.

(H) Whole cell lysates from *Mfn1*$^{-/-}$ MEFs expressing pLKO.control or pLKO.*Bax* were analyzed by western blot for BAX and Actin (*left panels*). *Mfn1*$^{-/-}$ MEFs expressing pLKO.*Bax* reconstituted with pCEP4.human-HA-BAX^{WT} or pCEP4.human-HA-BAX^{S184A} were analyzed by western blot for BAX and Actin (*middle panels*). As BAX^{WT} and BAX^{S184A} expressed differently, we adjusted the transfections to ensure equal expression *(right panels)*.

(I) *Mfn1*$^{-/-}$ MEFs expressing sh*Bax* were reconstituted with human BAX^{WT} or BAX^{S184A}, treated with DTT (1.5 mM), Tg (0.25 μM), or Tun (1 μg/ml), and the kinetics of cell death was evaluated by IncuCyte. Data from the 20 hour time point are shown.

(J) Modeling and comparing membrane curvatures for 1, 0.2, and 0.05 μm vesicles within a 100Å increment. The size of BAX is ~ 35Å, based on PDB-1F16 and PyMol measurements. Membrane curvature is defined by Δ.

All data are representative of at least triplicate experiments, and reported as ± S.D., (or ± S.E. for IncuCyte) as required.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animal studies. Mouse protocols were approved by the Icahn School of Medicine at Mount Sinai IACUC. 8 week-old male C57BL/6 mice (Charles River Breeding Laboratories) were housed under controlled temperature, humidity and light cycles (12h:12h). Standard chow diet and water were available *ad libitum*. To induce ER stress mice were injected *i.p.* with tunicamycin (Calbiochem Lot # D00127619; 2 mg/kg) or PBS control. 24 h after the injection mice were sacrificed by cervical dislocation, tissue was snap frozen in liquid nitrogen and stored at -80°C until further analysis.

Heavy membrane isolations. At least 2 × 15 cm dishes at 90 - 95% confluency were used per treatment. Cells were harvested by trypsinization, and pelleted by centrifugation at 1000 × *g* for 10 minutes. The cell pellet was washed once with mitochondrial isolation buffer (MIB: 200 mM mannitol, 68 mM sucrose, 10 mM HEPES-KOH pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 0.1% BSA), and resuspended in MIB supplemented with protease inhibitors (HALT, Pierce Biotechnology). The cell suspension was incubated on ice for 20 minutes, and homogenized using a 2 ml Potter-Elvehjem dounce. The homogenate was centrifuged for 10 minutes at 800 × *g* at 4ºC, the supernatant collected, and centrifuged again using the same conditions to ensure that no unlysed cells or nuclei were present. The resulting supernatant was centrifuged for 10 minutes at 8000 × *g* at 4ºC. The supernatant was collected as the S8 cytosol; the pellet was collected as the heavy membrane fraction and lysed using RIPA buffer. For liver mitochondrial isolations, we employed a common protocol (Renault et al., 2013).

Western blot analysis. Whole cell protein lysates were made from trypsinized cells, pelleted, resuspended in RIPA buffer supplemented with protease inhibitors, incubated on ice for 10 minutes and centrifuged for 10 minutes at 21,000 × *g*. Protein concentrations were determined by using a standard BCA kit and the lysates were then adjusted with RIPA buffer to equal the protein concentrations. Proteins (25-100 μg/lane) were subjected to SDS-PAGE before transferring to nitrocellulose by standard western conditions, blocked in 5% milk/TBST and primary antibodies (1:1000 in blocking buffer; incubated overnight at 4°C). The secondary antibody (1:5000 in blocking buffer) was incubated at 25°C for 1 hour before standard enhanced chemiluminescence detection.

For analysis from fresh liver, the tissue was homogenized in 20 mM MOPS, 2 mM EGTA, 5 mM EDTA, 30 mM sodium fluoride, 40 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 0.5% NP-40 and complete protease inhibitor cocktail (Roche) and centrifuged at 13,000 × *g* for 20 minutes at 4°C. Protein concentration in the supernatant was measured with a BCA protein quantification kit.

Live cell imaging. Cells were seeded on rat-tail collagen I coated plates for 24 hours before indicated treatments. Mitochondria and nuclei were labeled with MitoTracker Green® (50 nM) and Hoechst 33342 (20 μM) for 30 minutes at 37ºC, respectively. Phenol red free media was used for all imaging performed on a Zeiss Imager.Z1 equipped with a N-Achroplan 40×/0.75 water immersion lens and an AxioCAM MRm digital camera; images were captured using AxioVision 4.8 software. At least 300 cells per condition were quantified, where required.

RNA extractions and quantitative real-time PCR. Total RNA was obtained from frozen liver with the RNeasy Tissue Kit (Qiagen). Purified total RNA was used for first strand cDNA synthesis with Quanta QScript cDNA Supermix (Quanta Biosciences). We ran quantitative real-time PCR with Quanta PerfeCTa SybrGreen FastMix (Quanta Biosciences) on a 7900HT sequence detection system (Applied Biosystems). Data were analyzed with the comparative Ct method (Schmittgen and Livak, 2008). Primers: *CHOP* Forward: 5'-CATGAAGGAGAAGGAGCAG-3'; *CHOP* Reverse: 5'-CTTCCGGAGAGACAGACAGG-3'; *BIP* Forward: 5'-AGTGGTGGCCACTAATGGAG-3'; *BIP* Reverse: 5'-CAATCCTTGCTTGATGCTGA-3'; *Mfn1* Forward: 5'-TCTCCAAGCCCAACATCTTCA-3'; *Mfn1* Reverse 5'-ACTCCGGCTCCGAAGCA-3'; *Mfn2* Forward 5'-ACAGCCTCAGCCGACAGCAT-3'; *Mfn2* Reverse 5'-TGCCGAAGGAGCAGACCTT-3'.

Isolated mitochondria cytochrome c release assays. 5×10^6 cells were treated as indicated, trypsinized, and washed twice in cold PBS. The cell pellet was resuspended in permeability buffer (250 mM sucrose, 20 mM HEPES, pH 7.4, 100 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, supplemented with 0.015% digitonin and 1X HALT Protease and Phosphatase inhibitor cocktail) and incubated on ice for 10 minutes. The permeabilized pellet was divided into the required number of samples, drugs/proteins were added, and the reactions were incubated at 37ºC for 1 hour. The reactions were then centrifuged at 8000 × *g* for 10 minutes, and the supernatants were analyzed for cytochrome c by SDS-PAGE and western blot analysis. Total cytochrome c was determined by a sample treated with 1% CHAPS. For cell treatment induced cytochrome c release, 1 x 10^6 cells were treated as indicated, harvested, and subjected to the same permeability procedure listed above. The resulting supernatant and pellet fractions after the 8000 × *g* centrifugation were then analyzed for the presence of cytochrome c.

BAX activation assays. For 6A7 studies, cells were treated as indicated, trypsinized, pelleted, lysed in 6A7 amino terminal capture buffer (10 mM HEPES, 135 mM NaCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 1% glycerol + 1% CHAPS, added fresh; pH 7.4), incubated on ice for 10 minutes, and centrifuged at 20000 × *g* for 10 minutes at 4°C. The supernatants were combined with 1 μg of 6A7 antibody,

incubated for 2 hours at 4°C with end/end mixing, an appropriate volume of protein A/G-agarose conjugate was added, and incubated for an additional hour. The beads were washed with each of the following buffers twice, 1 ml per wash: (1) Wash A: 10 mM HEPES, 135 NaCl, 2% CHAPS; pH 7.4 (2) Wash B: 10 mM HEPES, 135 NaCl, 0.2% CHAPS; pH 7.4 and (3) Wash C: 100 mM Tris HCl, 100 mM NaCl, pH 8.0. Proteins are eluted by the addition of 1X SDS-PAGE loading buffer (50 μl), denatured for 10 minutes at 95°C, centrifuged for 1 minute at 15,000 × *g*, and the supernatant was subjected to SDS-PAGE and western blot analysis using clone N-20 for BAX detection. 0.25% TX100 treatment of samples is a positive control.

For oligomeric BAX trypsinization studies, mitochondria from untreated and treated cells were isolated as indicated above but in the absence of protease inhibitors. Mitochondria were pelleted at 5,500 × *g* (1 minute at 4°C), resuspended in KCl buffer (125 mM KCl, 2 mM $MgCl_2$, 5 mM KH_2PO_4, 10 mM HEPES; pH 7.4) at a concentration of 4 μg mitochondrial protein/μl, and proteolysis was initiated by the addition of proteomics grade trypsin (0.17 μg/μl final concentration). The samples were incubated at 25°C for 90 minutes, combined with 4X SDS-PAGE loading buffer, denatured by boiling, and analyzed with SDS-PAGE and western blot for BAX (clone Δ21). The proteolysis will lead to either the complete degradation of monomeric BAX, or to the retention of a BAX 15 kDa fragment, suggestive of activation and oligomerization.

Large unilamellar vesicle assays. Large unilamellar vesicles (LUVs) release assays were prepared as described (Asciolla et al., 2012; Kuwana et al., 2002). Briefly, phosphatidylcholine, phosphatidylethanoloamine, phosphatidylserine, phosphatidylinositol and cardiolipin at a ratio of 47:28:9:9:7 (4 mg total) were dried and resuspended in LUV buffer (0.2 mM EDTA, 10 mM HEPES [pH 7], 200 mM KCl, 5 mM $MgCl_2$) containing a polyanionic dye (12.5 mM ANTS: 8-aminonaphthalene-1,3,6-trisulfonic acid) and cationic quencher (45 mM DPX: *p*-xylene-bis-pyridinium bromide) using a water bath sonicator. Unilamellar vesicles were formed by extrusion of the suspension through indicated polycarbonate membranes (0.05, 0.2, and 1.0 μm; Avanti). The unincorporated DPX and ANTS were removed by using a 10 ml Sepharose S-500 gravity flow column. Using a 96 well format, 100 μl total volume per condition, LUVs, proteins, peptides, drugs, and buffers were combined as indicated, incubated for 45 minutes at 37°C, and analyzed for fluorescence using a Synergy H1 hybrid multi-mode microplate reader (BioTek). Excitation wavelength: 355 nm; Emission wavelength: 520 nm; Gain (voltage): 125; Optics position: Top; Read height: 5.5 mm. The percentage of release was calculated between the baseline provided by the buffer control and 100% release obtained by LUVs solubilized in 1% CHAPS. Recombinant BCL-2 family protein concentrations were determined by extensive titrations, and the lowest concentration for maximal activity for each protein was used, and this was determined for

each recombinant protein preparation; *e.g.,* the optimal BAX range is between usually between 75 and 150 nM. For kinetic studies, the first 15 minutes of permeabilization are not included in the analyses as the fluorescence stabilizes during this time. For BAX-LUV association studies, LUVs were combined with BAX (~100 nM) ± BID (20 nM) or BIM (2.5 μM), incubated for 30 minutes, then pelleted at 147,000 × *g* for 1 hour prior to solubilization in 1X Laemmli buffer, SDS-PAGE, and western blot.

Outer membrane vesicle assays. Rat OMVs were prepared as described (Kushnareva et al., 2012); thus far, murine OMVs have not been successfully isolated. In brief, mitochondria were isolated from the livers of male Sprague-Dawley rats by standard differential centrifugation techniques (Lapidus and Sokolove, 1993). The isolation buffer contained 210 mM mannitol, 70 mM sucrose, 10 mM HEPES-KOH (pH 7.4), 2 mM EGTA, and 0.1% bovine serum albumin (essentially fatty acid free, Sigma). Isolated mitochondria were further purified in a step gradient of iodixanol (OptiPrep, AxisShield-Sigma) as described previously with some modifications (Andreyev et al., 2010). The mitochondrial pellet was resuspended in 36% iodixanol diluted in SHE buffer (250 mM sucrose, 10 mM HEPES-KOH, pH7.4, 2 mM EGTA), to a final volume of 9–10 ml. The iodixanol gradients, consisting of 3 ml SHE, 5 ml 17.5% iodixanol, 5 ml 25% iodixanol, and ~ 1.5 ml of the mitochondrial suspension in 36% iodixanol, were prepared in six 16 ml tubes. The gradients were centrifuged at 50,000 × *g* for 2 hours. Purified mitochondria were recovered from the 17.5%/25% interface. For preparation of OMVs, purified mitochondria were diluted 7–8 times in a hypotonic buffer (10 mM KOH, pH 7.4, 0.5 mM EGTA, 4 mM KCl) and incubated for 10 minutes. After hypotonic treatment, mitochondria were centrifuged at 12,000 × *g* for 10 minutes and the pellet was resuspended in 2 ml of the hypotonic buffer supplemented with 5 mg of 70 kDa dextran-fluorescein (Sigma). The suspension was homogenized in a 7 ml glass Dounce homogenizer using a tight-fitting pestle (40–50 strokes). The homogenate volume was then adjusted to 6 ml and OMVs were purified in iodixanol step gradients prepared in three 16 ml tubes. Each tube contained 2 ml of the homogenate, 5.5 ml 8% iodixanol, 5.5 ml 17.5% iodixanol, and 1.5 ml 25% iodixanol. The gradients were centrifuged at 50,000 g for 2 hours and OMVs were collected from the 8%/17.5% interface. Unincorporated fluorescent dextran was separated from OMVs by the 8% iodixanol layer. Remaining mitochondria partially devoid of the outer membrane (mitoplasts) banded on the 17.5%/25% interface. This fraction was used in analyses of the purity of OMVs. Additional removal of external dextran was achieved by diluting OMVs 10-fold in the hypotonic buffer and concentrating them by centrifugation at 100,000 × *g* for 20 minutes. The pellet was resuspended in 200 μl of the hypotonic buffer. Typical protein concentration in OMV suspension was ~ 2 mg/ml as determined by BCA assay (Pierce). Dynamic light scattering measurements were performed with Malvern or a Brookhaven

Instruments ZetaPALS; LUVs and OMVs were diluted 1:25 in LUV buffer and hypotonic buffer, respectively.

RNA interference. The mouse pLKO.sh*Drp1* and pLKO.sh*Bax* plasmids were purchased from Sigma-Aldrich (Mission^{R} shRNA). The pLKO.control construct was kindly provided by the laboratory of Dr. E. Premkumar Reddy. The 293T cell line was used to produce retroviral particles for the generation of stable cell lines. Virus was harvested at 24 and 48 h, pooled, and 0.45 μm filtered. RNAi studies with siRNAs were performed with MISSION oligos (20 nM, Sigma-Aldrich) and indicated treatments were performed 48 hours after silencing.

Retroviral Mfn1 expression. The mouse Retip.Mfn1 plasmid was generated by cloning into the Retip retroviral construct (a gift from Dr. Adrian Ting at Mount Sinai). The 293T cell line was used to produce retroviral particles for the generation of stable cell lines. Virus was harvested at 24 and 48 h, pooled, 0.45 μm filtered, added to *Mfn1*$^{-/-}$ MEFs for 48 h, and selection using puromycin was performed.

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The abbreviations used are: β-ME, beta-mercaptoethanol; BAK, BCL-2 homologous antagonist killer; BAX, BCL-2 associated X protein; BCL-2, B cell lymphoma 2; BCL-xL, B cell lymphoma extra large; BH3, BCL-2 homology domain 3; BID, BH3 interacting domain death agonist; BIM, BCL-2 interacting mediator of cell death; BiP, 78-kDa glucose-regulated protein/binding immunoglobulin protein; CHOP, transcription factor C/EBP homologous protein; DLS, dynamic light scattering; DRP-1, dynamin related protein 1; DTT, dithiothreitol; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; HM, heavy membrane; JC-1, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; LUV, large unilamellar vesicles; MCL-1, myeloid cell leukemia sequence 1; MEF, mouse embryonic fibroblasts; Mfn1, mitofusin 1; Mfn2, mitofusin 2; MOMP, mitochondrial outer membrane permeabilization; NBD, N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4yl)ethylenediamine; OMM, outer mitochondrial membrane; OMV, outer membrane vesicle; PUMA, p53 upregulated modulator of apoptosis; Tg, thapsigargin; TIB, trehalose isolation buffer; TMRE, tetramethylrhodamine ethyl ester; Tun, tunicamycin; UPR, unfolded protein response; and Wt, wild type.