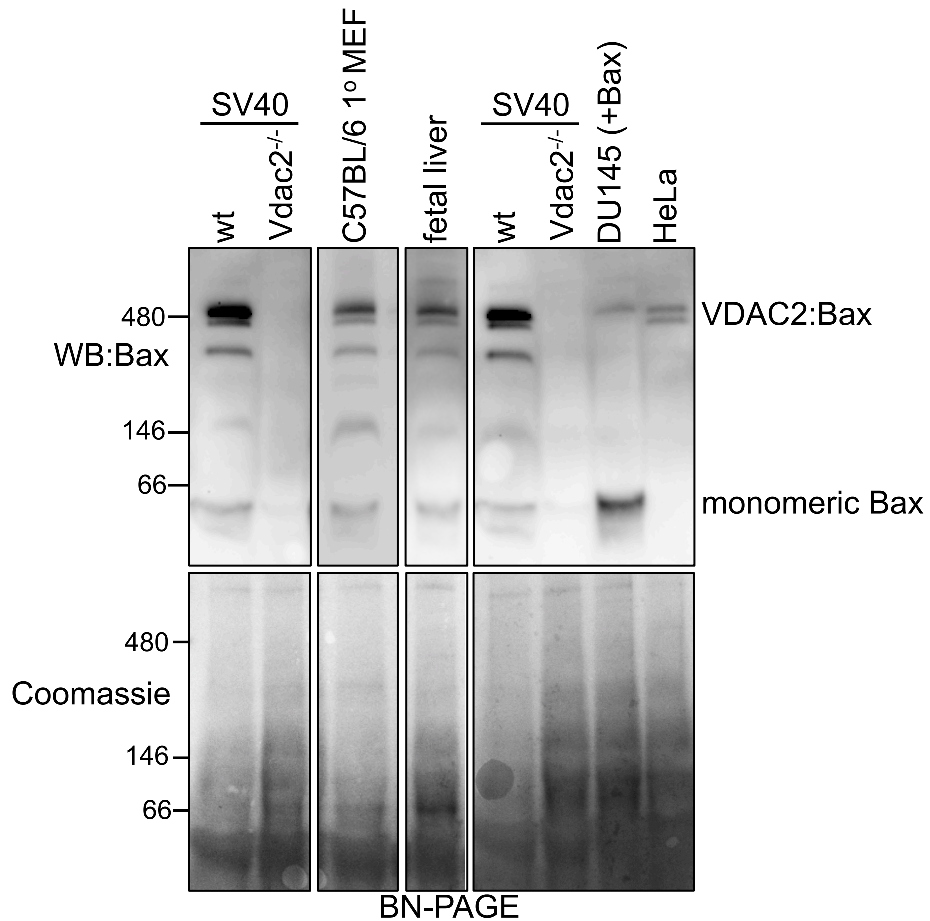

Figure S1. Endogenous and ectopically expressed Bax associates with a large molecular weight mitochondrial complex. Heavy membrane fractions from SV40 transformed wt or *Vdac2*$^{-/-}$ MEFs (mixed genetic background), untransformed early passage primary MEFs (C57BL/6), E14.5 fetal liver (C57BL/6), DU145 stably expressing hBax, or HeLa cells were solubilized in 1% digitonin prior to BN-PAGE and immunoblotting for Bax (*upper panels*) or Coomassie stained as a control for loading (*lower panels*).

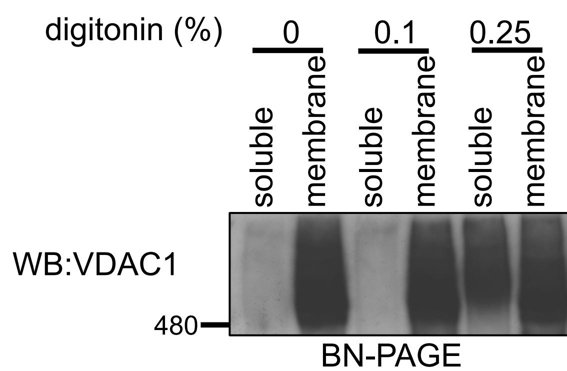

Figure S2. Membrane-integrated VDAC1 is solubilized from the mitochondrial outer membrane with digitonin. wt MEF membrane fraction was solubilized in the indicated concentrations (w/v) of digitonin prior to separation of soluble and membrane fractions by centrifugation and BN-PAGE. Note that VDAC1 remains in the membrane fraction unless solubilized with >0.25% digitonin.

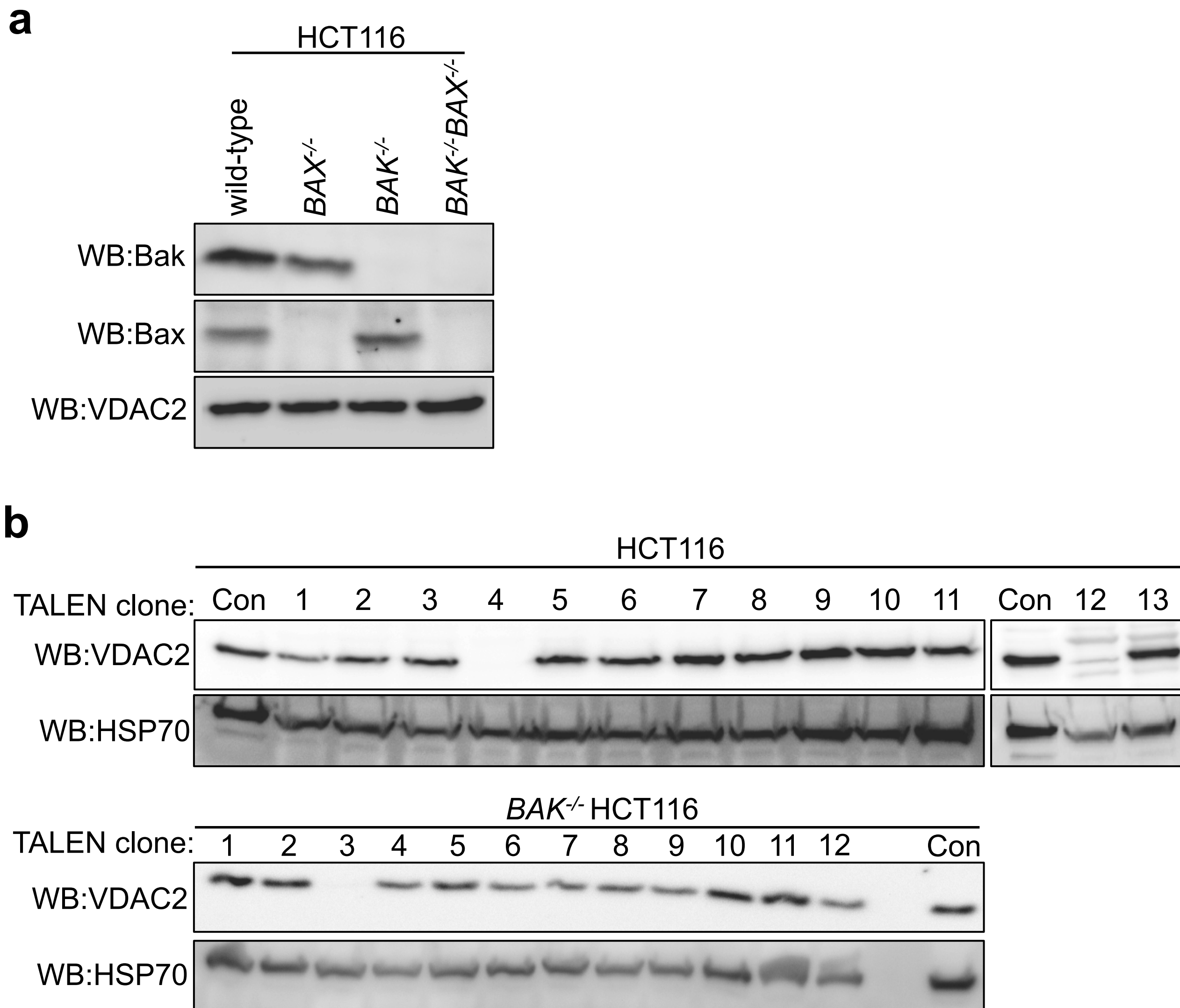

Figure S3. Characterization of Δ*VDAC2* HCT116 cell lines. (a) HCT116 cells of the indicated genotypes were immunoblotted for Bak, Bax or hVDAC2. **(b)** Screening of Δ*VDAC2* TALEN clones. Human *VDAC2* was disrupted using TALEN binding pairs targeting the common exon of human *VDAC2* isoforms in either wild-type (*upper panel*) or *BAK*$^{-/-}$ HCT116 cells (*lower panel*) and lysates immunoblotted for human VDAC2 or HSP70 as a loading control.

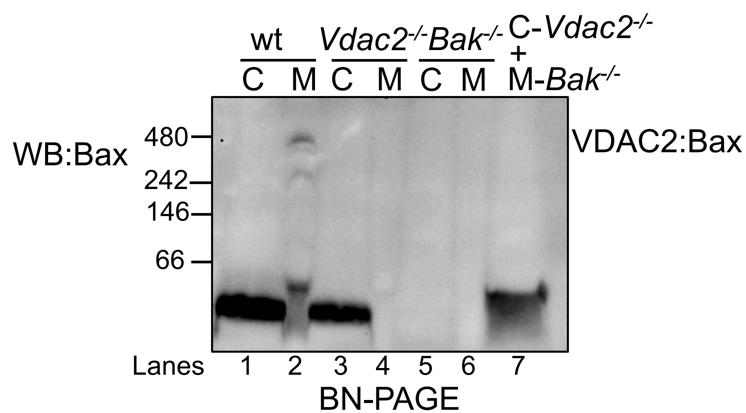

Figure S4. VDAC2:Bax complex is not driven by digitonin. Cytosol (C) and membrane (M) Fractions were isolated from the indicated MEFs. Membrane fractions were solubilized in 1% digitonin. Where indicated the membrane fraction from *Bak*$^{-/-}$ MEFs was resuspended in the cytosolic fraction from *Vdac2*$^{-/-}$ and 1% digitonin was added. Samples were then analyzed on BN-PAGE. Note the lack of the VDAC2:Bax complex when mitochondria lacking Bax were solubilized in presence of cytosolic Bax (*lane 7*). Note also the size shift of monomeric Bax in the absence (*lanes 1 and 3*) or presence (*lanes 2 and 7*) of 1% digitonin.

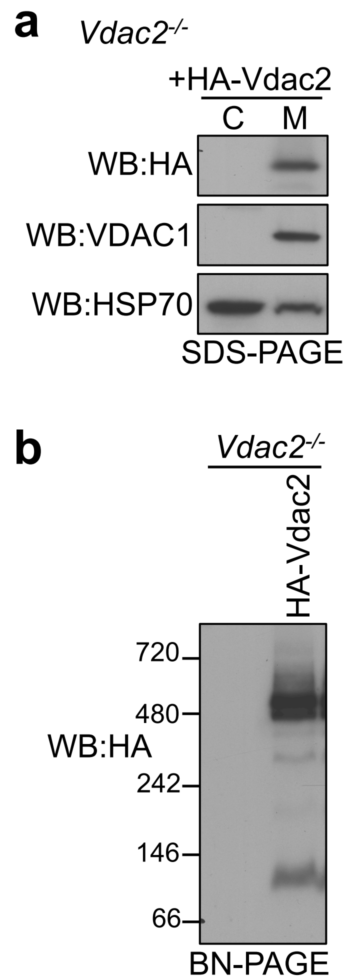

Figure S5. Reconstituted HA-tagged VDAC2 localizes to a large molecular weight complex at mitochondria. (a) *Vdac2*$^{-/-}$ MEFs stably expressing HA-VDAC2 were permeabilized and cytosol (C) and mitochondria-enriched heavy membrane fractions (M) were analyzed by SDS-PAGE. Data is representative of two independent experiments. **(b)** Reconstituted HA-tagged VDAC2 predominantly localizes to the 400 kDa complex. *Vdac2*$^{-/-}$ MEFs or *Vdac2*$^{-/-}$ MEFs stably expressing HA-VDAC2 were permeabilized and membrane fractions (M) were analyzed by BN-PAGE. Data is representative of two independent experiments.

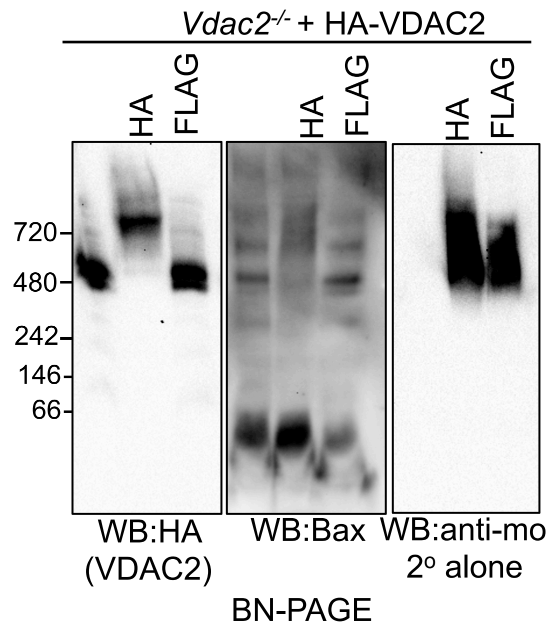

Figure S6. Bax and VDAC2 reside in the same mitochondrial complex. Membrane fraction from *Vdac2*$^{-/-}$ MEFs expressing HA-VDAC2 were incubated without antibody or with mouse monoclonal antibodies recognising either HA or FLAG as a control. Membranes were solubilized in digitonin prior to BN-PAGE and immunoblotted for Bax, HA or with an anti-mouse secondary antibody only.

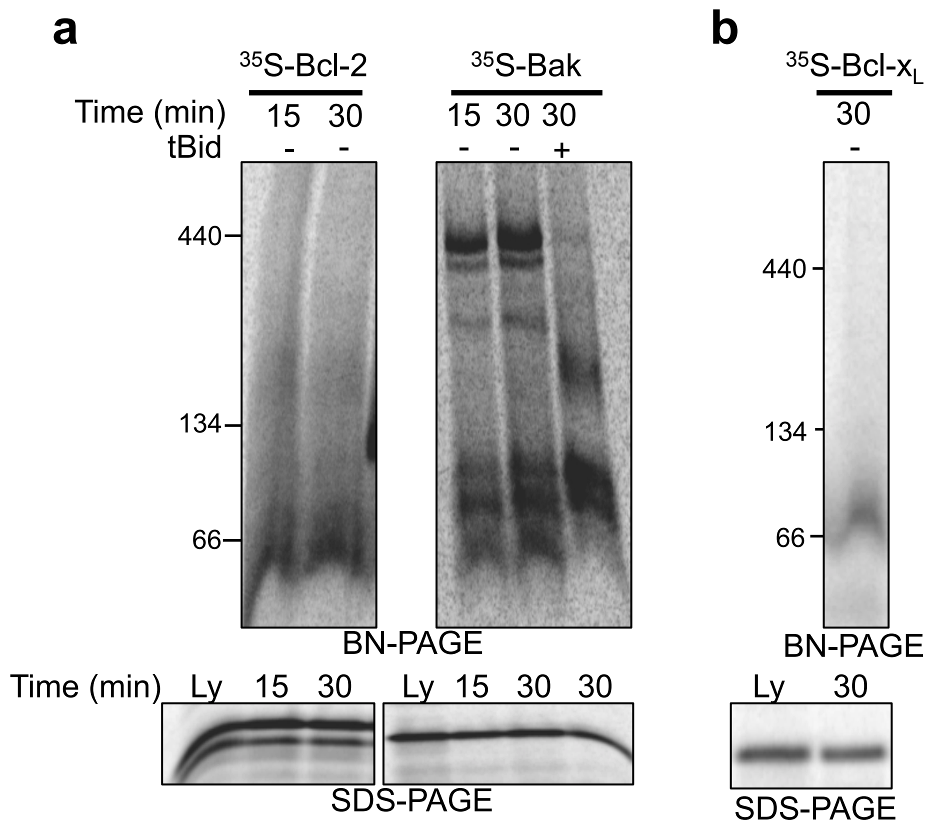

Figure S7. Neither Bcl-x$_L$ or Bcl-2 associate with the VDAC2 complex. Mitochondria isolated from wild-type MEFs were incubated with radiolabelled Bcl-2, Bak **(a)** or Bcl-x$_L$ **(b)** for the indicated times. Following import mitochondria were incubated with or without tBid where indicated prior to BN-PAGE or SDS-PAGE and autoradiography. Lysates (Ly) are translated ^{35}S-labelled protein, 20% input. In (a) the images are from the same gel but intervening lanes have been removed.

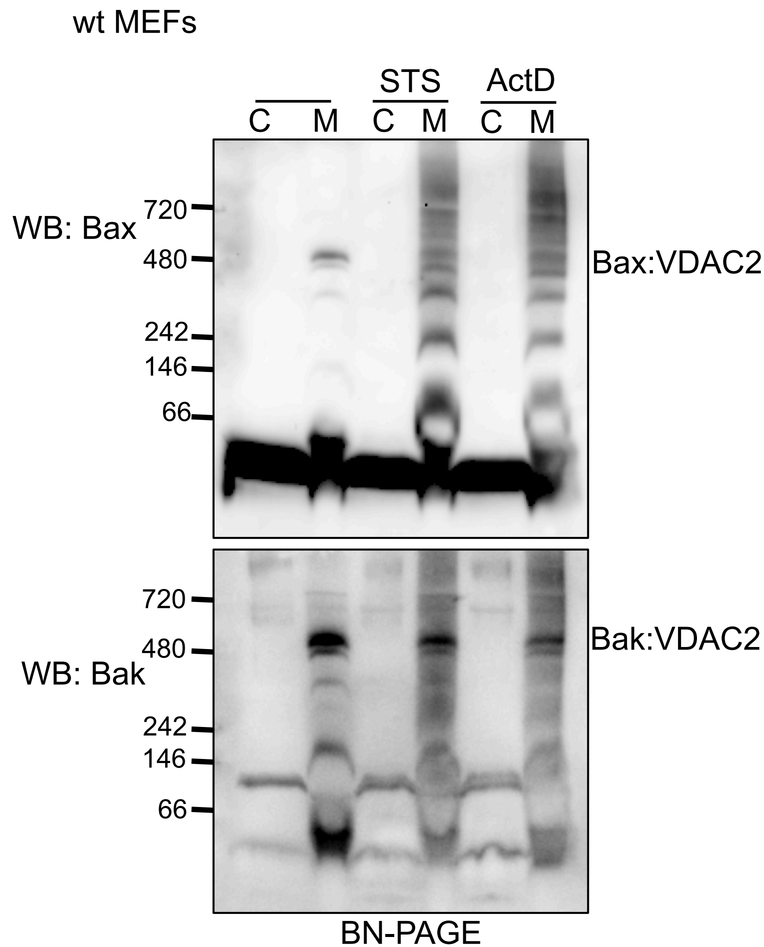

Figure S8. Bax and Bak dissociate from the VDAC2 complex to self-associate in response to apoptotic stimuli. wt MEFs were untreated or treated with staurosporine (0.1 μM) or actinomycin D (1 μM) for 24 h in the presence of Q-VD.oph (50 μM). Cells were harvested and fractionated into cytosol (C) and membrane (M) and analyzed on BN-PAGE.

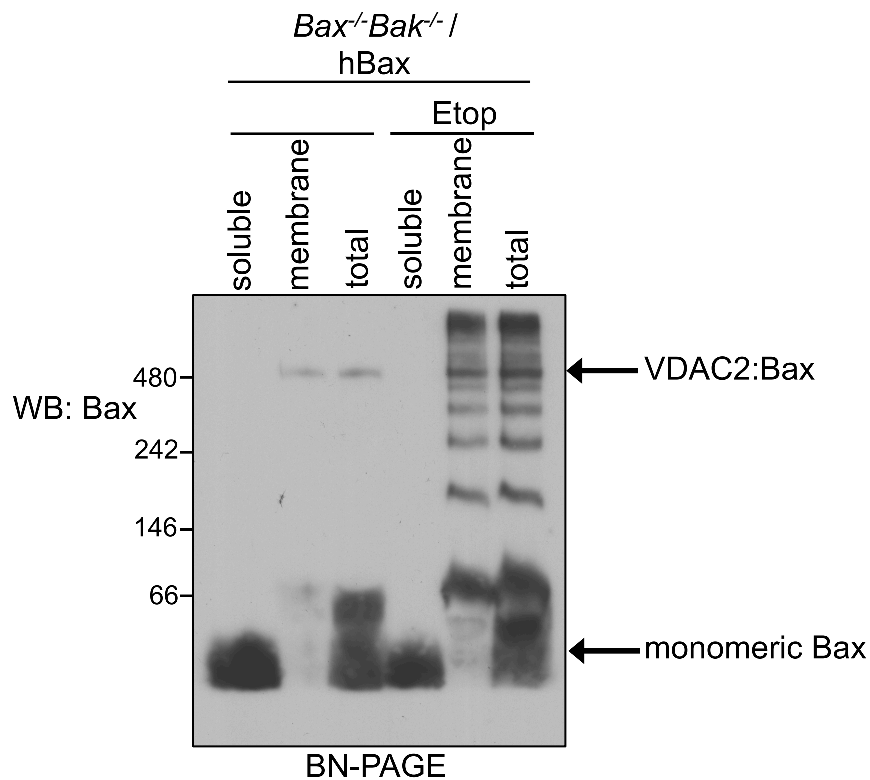

Figure S9. Oligomerized Bax in healthy or apoptotic cells is membrane bound. *Bak*$^{-/-}$ *Bax*$^{-/-}$ MEFs expressing hBax were treated or not with etoposide in the presence of Q.VD-oph. Cells were permeabilized in digitonin and the membrane fractions were resuspended in buffer without detergent, prior to separation into soluble and membrane-bound fractions. Membranes were solubilized in digitonin prior to BN-PAGE. Note that monomeric Bax was released from the membrane fraction upon resuspension whereas the VDAC2 complex in healthy cells and Bax homo-oligomers in etoposide-treated cells remained membrane bound.

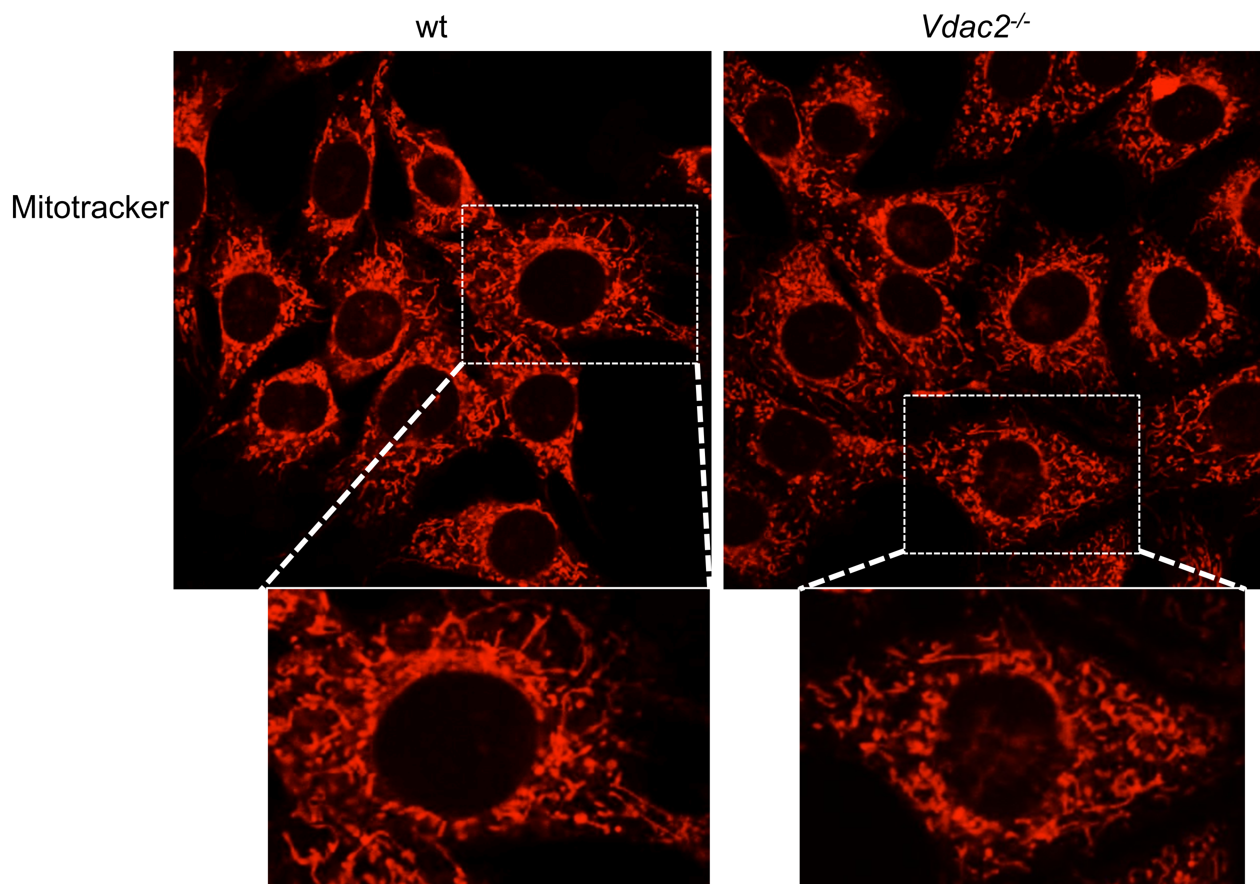

Figure S10. *Vdac2$^{-/-}$* MEFs have grossly normal mitochondria dynamics. wt and *Vdac2$^{-/-}$* MEFs were incubated with Mitotracker prior to confocal analysis.

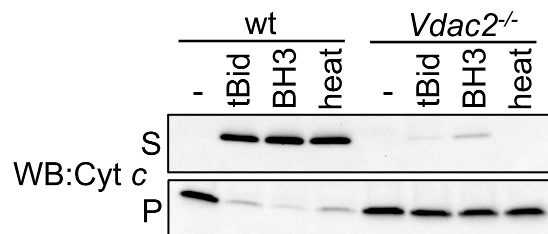

Figure S11. Mitochondrial Bak in *Vdac2*$^{-/-}$ MEFs cannot mediate cytochrome *c* release. Membrane fractions from wt or *Vdac2*$^{-/-}$ MEFs were treated with recombinant tBid or Bid BH3 peptide at 30°C for 30 mins, or alternatively incubated at 43°C for 30 mins in the absence of additional stimuli prior to separation of supernatant (S) and pellet (P) and immunoblotting for cytochrome *c*. Data is representative of three independent experiments.

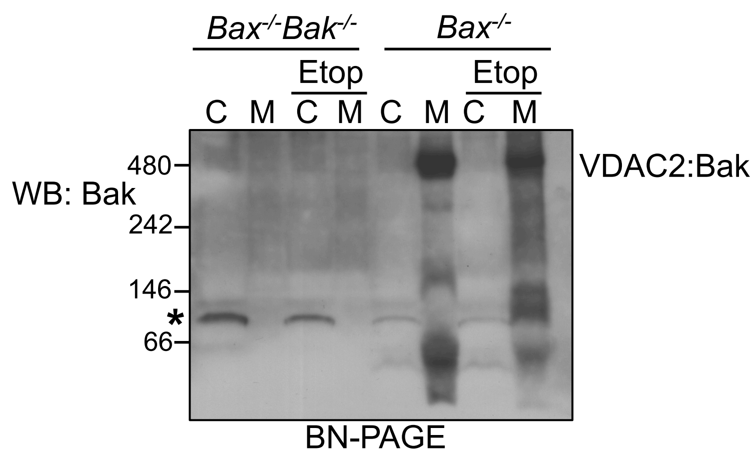

Figure S12. Cytosolic band detected by the anti-Bak antibody on BN-PAGE is not Bak. *Bak$^{-/-}$ Bax$^{-/-}$* or *Bax$^{-/-}$* MEFs were treated or not with etoposide/Q-VD.oph and then permeabilized into cytosol (C) and membrane (M) fractions. Membranes were solubilized in digitonin and fractions analyzed on BN-PAGE. * is a non-specific protein detected in *Bak$^{-/-}$ Bax$^{-/-}$* cytosol with a rabbit polyclonal antibody (aa23-38) raised against Bak.