Supplemental Materials.

Supplemental Figure 1. **Cross-linkers caused BAX oligomerization in the solution without mitochondria. Substitution of non-fat milk for BSA as a blocking solution in western blotting improved detection of BAX oligomers.** In all experiments, the standard incubation medium was supplemented with 50 nM BAX and with 20 nM tBID as indicated. In ***a***, the medium was supplemented with a vehicle (1μl DMSO in 300μl of the incubation medium) or with 0.2 mM bismaleimidohexane (BMH, Pierce). The quenching was performed with 50 mM dithiothreitol (DTT) for 30 minutes with rocking at room temperature. In ***b***, the medium was supplemented with a vehicle (1μl DMSO in 300μl of the incubation medium) or with 0.5 mM ethylene glycol bis(succinimidyl succinate) (EGS, Pierce) or 0.5 mM disuccinimidyl suberate (DSS, Pierce), respectively. In both cases, the quenching was performed with 20 mM Tris-HCl, pH 7.5, for 30 minutes with rocking at room temperature. In ***a***, 5% non-fat milk was used as a blocking solution in western blotting. In ***b***, 5% BSA in PBS was used as a blocking solution.

Supplemental Figure 2. **Distribution of exogenous, recombinant BAX between the OMM and the incubation medium: a fraction of non-oligomerized BAX remained in the solution.** The standard incubation medium was supplemented with 50 nM BAX in a combination with 20 nM tBID or 1.4μmol Ca^{2+}/mg protein as indicated. In ***a***, alkali-resistant BAX insertion and oligomerization with tBID or Ca^{2+}. Mitochondria were treated for 30 minutes at 37ºC with 50 nM BAX and 20 nM tBID or 1.4μmol Ca^{2+}/mg protein as indicated. Following alkali treatment (30 minutes on ice in 0.1 M Na_2CO_3 at pH 11.5), mitochondrial membranes were solubilized with 1% Nonidet P-40 and BAX

was detected with western blotting. In ***d***, BAX detected in the supernatants (Super) obtained prior to alkali treatment of mitochondria. In ***b*** and ***e***, densitometry data obtained with individual BAX bands shown in *panels* ***a*** and ***d***, respectively. In ***c*** and ***f***, the averaged sum of densities of all BAX bands (1×BAX - 5×BAX) shown in *panels* ***a*** and ***d***, respectively.

Supplemental Figure 3. **Dithiothreitol disassembled BAX dimers.** 50 nM BAX was incubated for 30 minutes at 37°C in the standard incubation medium supplemented with 250μM Ca^{2+} or 20 nM tBID with or without 20 mM dithiothreitol (DTT) as indicated. Then, non-reducing SDS-PAGE and western blotting were performed.

Supplemental Figure 4. **Dithiothreitol decreased insertion and oligomerization of monomeric BAX.** Mitochondria were treated for 30 minutes at 37ºC with 50 nM BAX, as indicated. Where indicated, the incubation medium was supplemented with 20 mM dithiothreitol (DTT). Mitochondria were then pelleted by centrifugation and subjected to alkali treatment (30 minutes on ice in 0.1 M Na_2CO_3 at pH 11.5). Alkali-resistant BAX insertion was assessed by non-reducing SDS-PAGE followed by western blotting with mitochondrial membranes solubilized in 1% Nonidet P-40.