Expanded View Figures

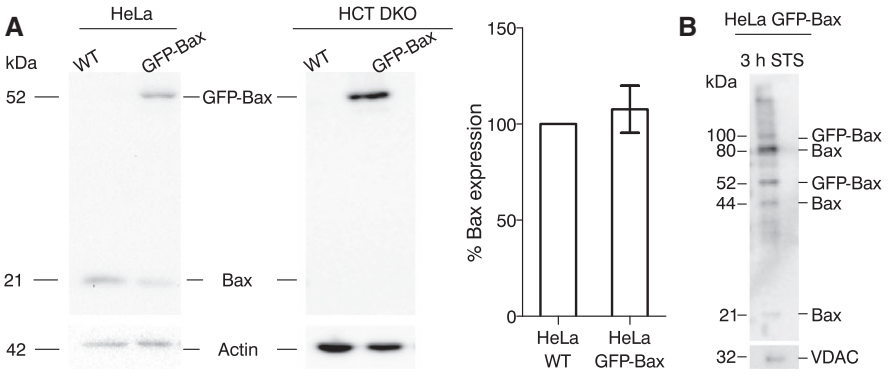

Figure EV1. Bax is active and oligomerized in the cells after apoptosis induction.

A Analysis of Bax protein levels in HeLa and HCT116 Bax/Bak$^{-/-}$ cells (wild-type and overexpressing GFP-Bax) by Western blot. The bar graph corresponds to the quantification of Bax expression in HeLa WT and HeLa GFP-Bax cells. Protein expression was first corrected with respect to the loading control (actin). Then, the expression of endogenous Bax from WT cells was normalized to 100% and compared with the total amount Bax (arising from endogenous protein and GFP-Bax) in transfected cells. Mean ± SEM of three independent experiments.

B Analysis of Bax translocation and oligomerization in mitochondria by Western blot. VDAC, loading control.

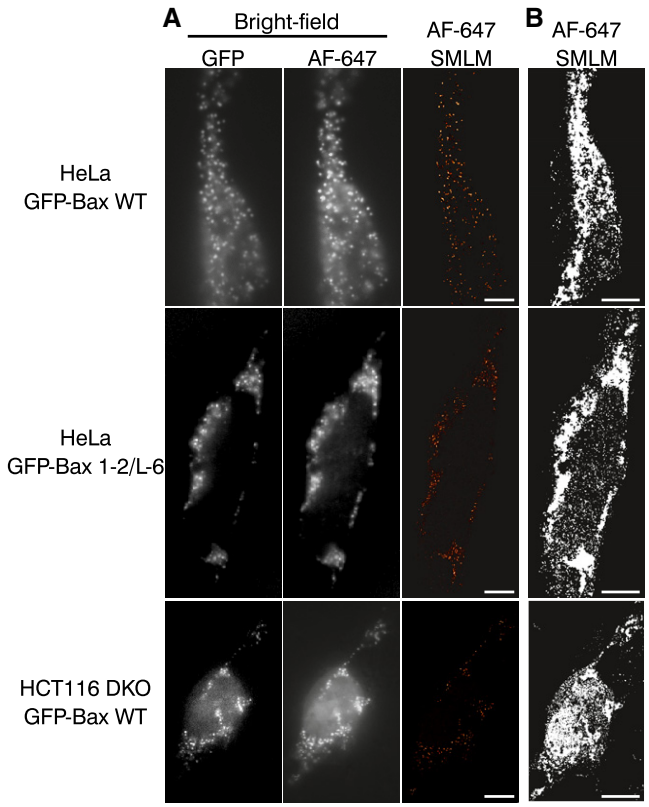

Figure EV2. Cell shape identification in SMLM images.

A Bright field images and SMLM overviews of HeLa and HCT116 DKO cells overexpressing GFP-Bax or GFP-Bax 1-2/L-6, respectively. Bright field images were taken in the GFP and AF647 channels before acquisition with single-molecule localization. Scale bars, 500 nm.

B SMLM images shown in (A) reconstructed with all the localizations and no cutoff applied. Pictures were converted to binary images and a black-white mask was applied to define the cell shape. Scale bars, 500 nm.

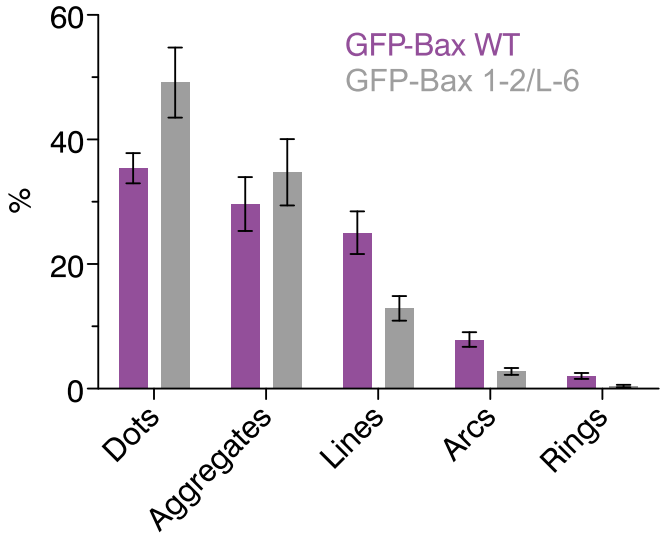

Figure EV3. Frequency of Bax assemblies in the cells.
Quantification of the frequency of the different types of Bax assemblies in HeLa cells with respect to the total number of assemblies found in each condition (GFP-Bax WT or GFP-Bax mutant 1-2/L-6). Mean ± SEM of 14 independent cells for each condition.

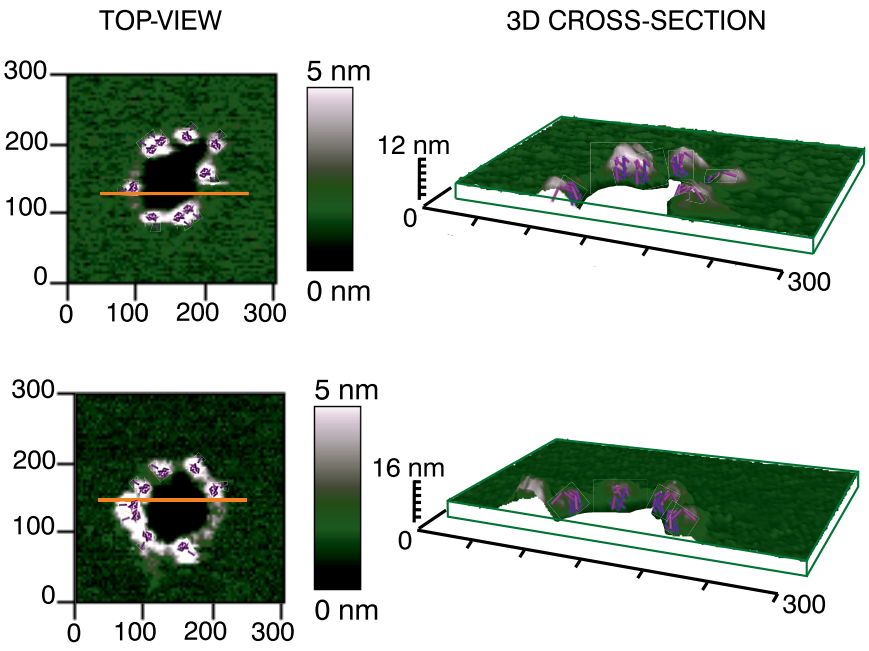

Figure EV4. Cartoon of Bax proteins localization around full rings assemblies associated with membrane pores imaged by AFM.
Top-views and 3D cross sections of two representative pores detected by AFM in a SLB prepared from LUVs with a mitochondrial-like lipid composition pre-incubated with heat-activated Bax, similar to the ones shown in Fig 6. The 3D cross section corresponds to the orange line in the top-view image. Images in the 3D cross section are shown in a 69*x*, 4*y*, 30*z* tilted representation. The green box allows for a better visualization of the 3D representation and thickness of the bilayer. Both top-view images reveal a circular dark hole that spans the lipid membrane (green). Dimers of Bax molecules (pink and purple sticks represent the two monomeric units, respectively), illustrated according to the clamp model (Bleicken *et al*, 2014), are displayed in scale around the rim of the pore in correspondence with the white-gray protrusions from the AFM images. The placement of the dimeric units takes into account the best accommodation between the length of the dimer (reproduced in scale according to the 3D model in (Bleicken *et al*, 2014)) and the height profile of the protrusion (plus membrane thickness), and allows for a very rough estimate of the number of Bax dimers lining the pore, with 9 and 8 dimer units in the upper and lower images, respectively. This illustration does not account for the presence of side chains and water in the Bax dimer, which could contribute to the overall size of the complex, as well as for the AFM resolution limit of our pictures. Importantly, there is no scientific ground other than reasonable overlap behind the inclusion of the 3D structures of the Bax dimers in the AFM images, and therefore, this cartoon representation should be considered with care and as indicative only.