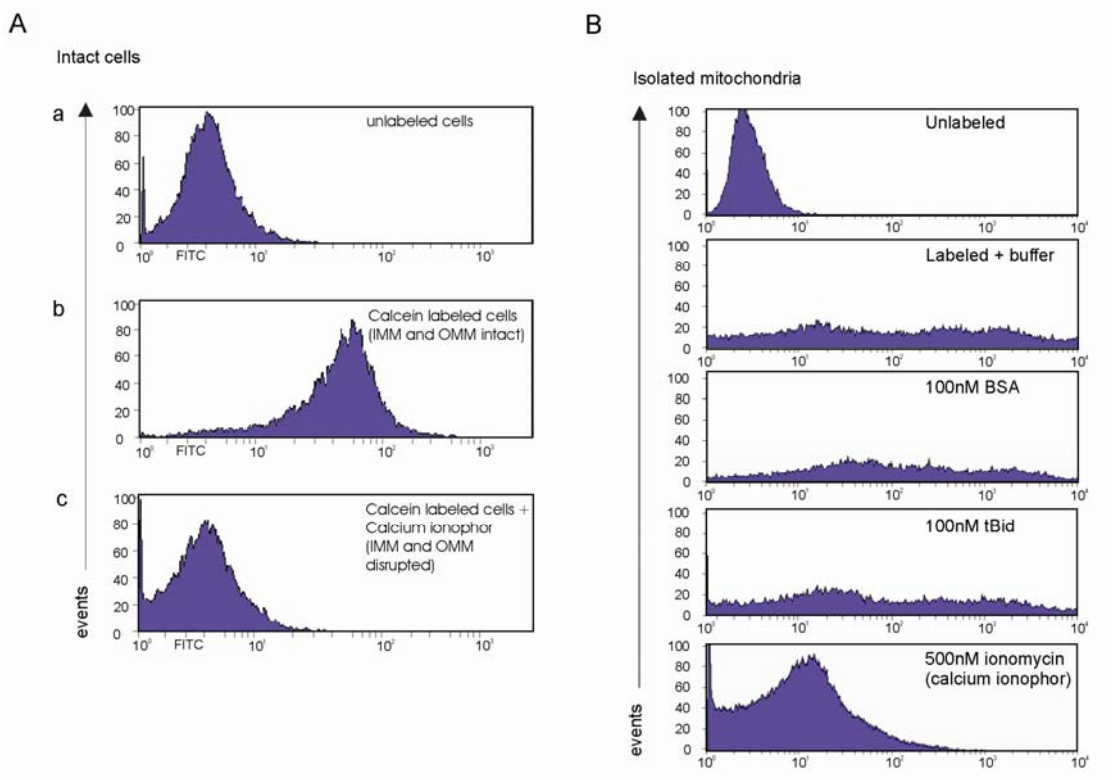

Supplementary information, Figure S2 A. HCT116 WT cells were loaded *in situ* with the fluorescent dye calcein that gets trapped in the mitochondrial matrix. Cytoplasmic fluorescence was quenched with $CoCl_2$. Labeled cells (**b**) show increased fluorescence by one order of magnitude compared to unlabeled cells (**a**), as measured by FACS. Retained fluorescent signal in mitochondria on x-axis. (**c)** The supraphysiological calcium ionophore ionomycin (500 nM) severely disrupts both inner and outer membrane, as indicated by the abolished fluorescence signal. **B.** tBid opens the Bax/Bak lipid pore at the outer mitochondrial membrane but does not disrupt the integrity of the inner membrane. Purified mitochondria from HCT116 WT cells were labeled *in vitro* with calcein prior to adding purified tBid or BSA protein (100 nM each) or ionomycin (500 nM). Calcein release was measured by FACS as loss of retained mitochondrial fluorescence.