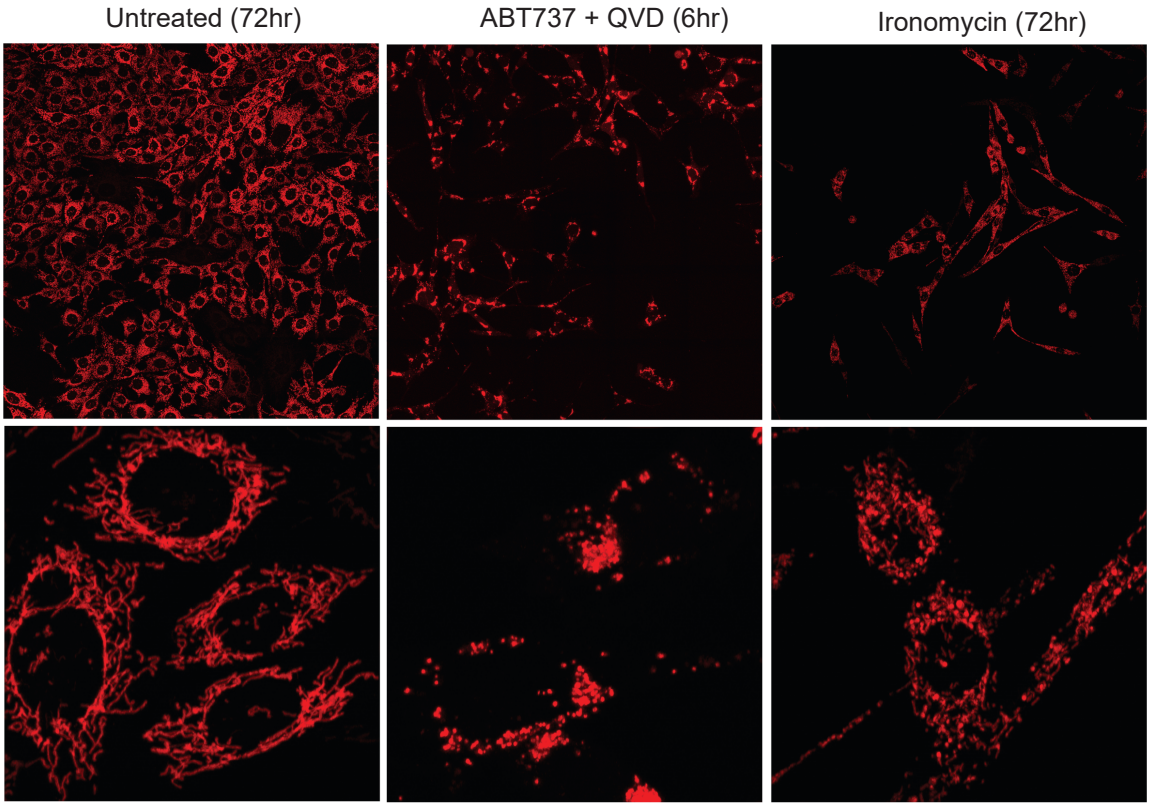
Figure S6

A



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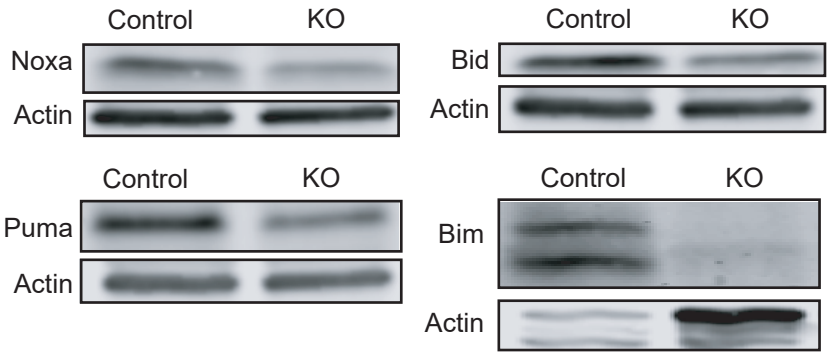
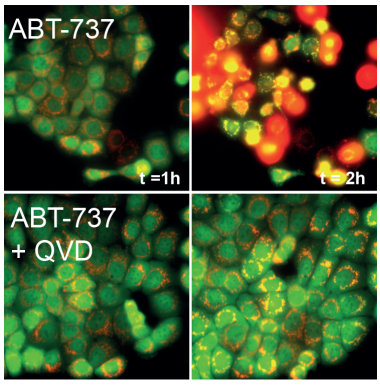


C



D

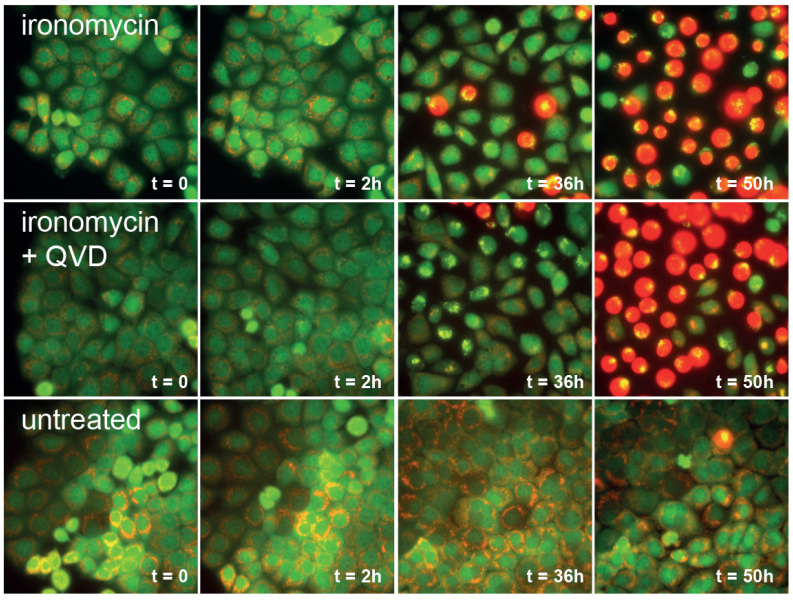


Figure S6| (related to Figure 6). Ironomycin induced cell death is distinct from canonical apoptosis. A, Mitochondrial network morphology in Mcl-1-/- MEFs during BH3-mimetic induced apoptosis and ironomycin-induced death. MEFs stably over-express TOMM20-Halo stained with JF-646 dye (red) in order to visualise the OMM. Cells were treated with 20 μM QVD-Oph, 1 μM ABT737 or 3 μM ironomycin before live imaging by confocal microscopy. **B** Immunoblot showing protein expression in MV4;11 expressing Cas9 transfected with sgRNAs targeting NOXA, PUMA, BIM and BID. **C-D,** *Mcl-1*$^{-/-}$ *Bax*$^{-/-}$ *Bak*$^{-/-}$ MEFs expressing mNeonGreen-tagged Bax (green) and TOMM20-Halo (red) were stained with JF646 (green), treated with 1 μM ABT-737 (**C**) or 500 nM ironomycin (**D**) with or without 20 μM QVD-OPH and imaged in the presence of propidium iodide [0.5 μg/mL] (red). Early events of cell death can be seen when Bax foci appear, localized to mitochondria, with the later event of plasma membrane rupture evident at the point at which cells become PI positive – obvious from the rapid acquisition of very bright (overexposed) red signal. Movies (included as a supplemental file) are representative of at least three independent imaging experiments.