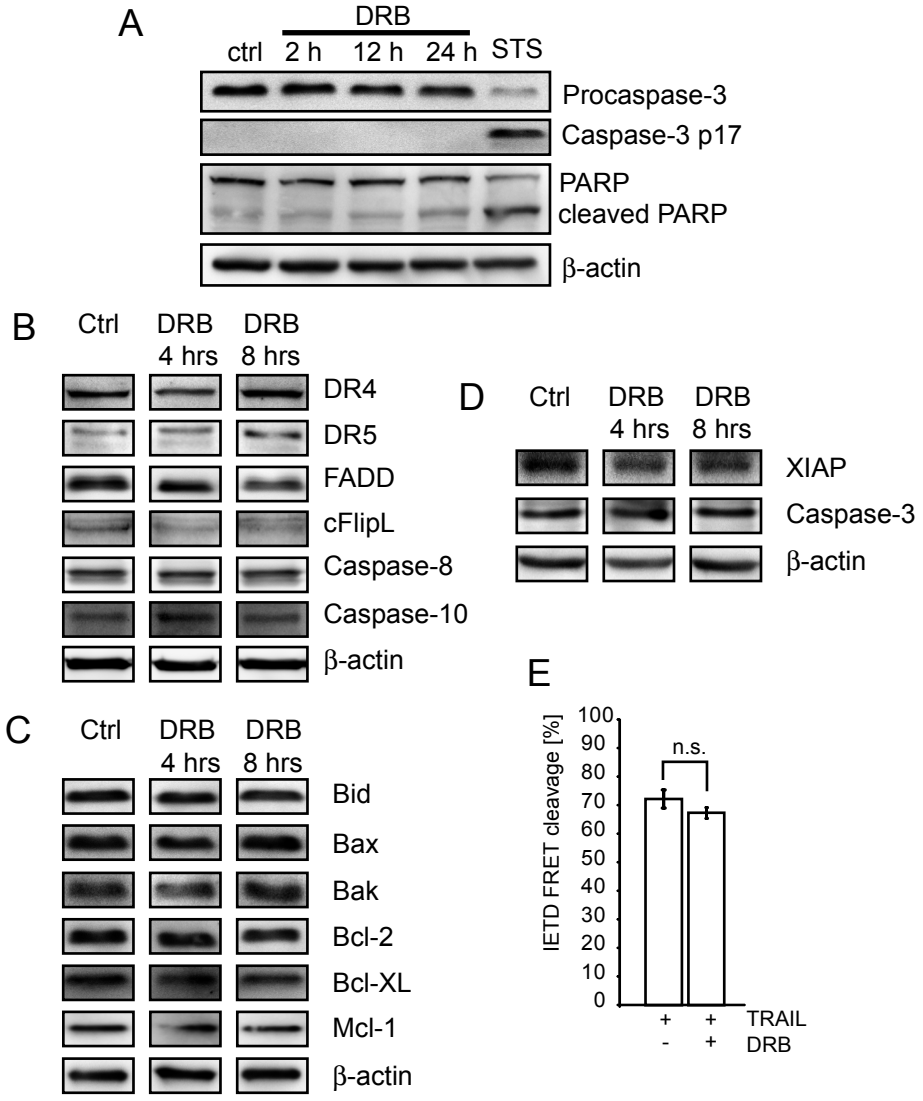
Supplemental Figure 2



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(A) Kinase inhibitor DRB does not induce effector caspase activation. Immunoblots of HeLa cells treated with 50 μM DRB for the indicated times or with 1 μM staurosporine (STS) for 6 hrs. Processing of procaspase-3 into the active p17 subunit and cleavage of caspase-3 substrate PARP can only be detected in response to the known apoptosis inducer STS. (B-D) Steady state protein levels in response to 50 μM DRB exposure. For DISC components, the levels of DR4, caspase-8 and caspase-10 remained unchanged in response to DRB, while DR5 levels mildly increased and cFlipL and, to a smaller extent, also FADD levels, decreased slightly upon prolonged DRB exposure. The balance of key proteins involved in mitochondrial pore formation did not noticeably change. Caspase-8 substrate Bid, as well as Bax and Bak as proapoptotic Bcl-2 family members, and Bcl-xL, Mcl-1, and Bcl-2 as antiapoptotic family members remained constant Even though HeLa cells are type II signalling cells (Engels et al., 2000), i.e. they require the mitochondrial pathway to execute apoptosis, we investigated whether changes could be detected in the direct caspase-3 activation pathway (type I signalling). The levels of neither caspase-3 nor its inhibitor x-linked inhibitor of apoptosis protein (XIAP) noticeably changed, suggesting that direct activation of caspase-3 by caspase-8 is still as efficiently blocked as in parental HeLa cells. The following antibodies were used: a rabbit polyclonal caspase-3 antibody (Cell Signaling Technology, Danvers, MA), a mouse monoclonal caspase-8 antibody (Alexis Biochemicals, San Diego, CA), a goat polyclonal Bid antibody (R&D Systems, Abingdon, UK), a rabbit polyclonal DR4 antibody (abcam, Cambridge, UK), a rabbit polyclonal DR5 antibody (abcam), a mouse monoclonal cFLIP antibody (Alexis Biochemicals), a mouse monoclonal FADD antibody (BD Bioscience, Oxford, UK), a mouse monoclonal Caspase-10 antibody (MBL International, Buckingham, UK), a rabbit polyclonal Bax antibody (Upstate Biotechnology, Dundee, Scotland), a mouse monoclonal Bak antibody (Santa Cruz, Heidelberg, Germany), a mouse monoclonal Bcl-2 antibody (Santa Cruz), a rabbit polyclonal Bcl-xL antibody (BD Bioscience), a mouse monoclonal Mcl-1 antibody (BD Bioscience), a mouse monoclonal XIAP antibody (BD Bioscience), a mouse monoclonal PARP antibody (Sigma), and a mouse monoclonal β-actin antibody (Sigma). (E) HeLa cells over-expressing Bcl-2 were transfected to express the IETD FRET probe and 24 h later were exposed to 100 ng/ml TRAIL + 1 μg/ml CHX either in presence or absence of 50 μM DRB. IETD FRET probe cleavage was measured by flow cytometry on a BD LSR II station. Cells with a cleaved probe presented with high CFP fluorescence and low emission in the FRET channel. Data represent mean + S.D. from independent triplicates. The experiment was reproduced with similar results.