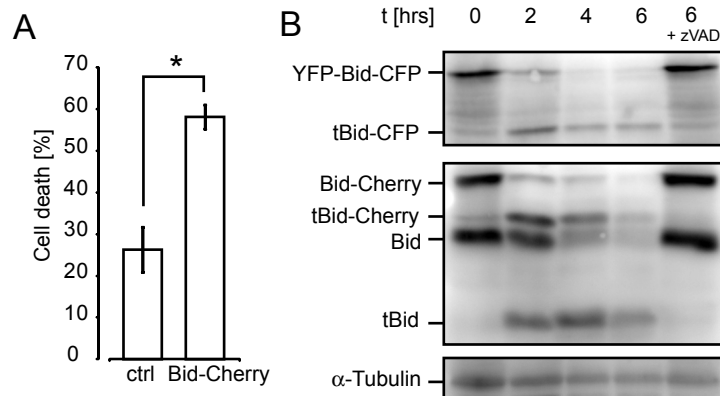


## Supplemental Figure 1



Supplemental Figure 1

(A) Bid-Cherry expression sensitizes to TRAIL-induced apoptosis. HeLa cells stably depleted of Bid expression (Kohler et al, 2008) were transfected with empty vector (pcDNA3) or pBid-Cherry. 24 hrs later, cells were exposed to 100 ng/ml TRAIL + 1  $\mu$ g/ml CHX. Cell death was determined after 6 hrs by Sytox Blue uptake using flow cytometry. Flow cytometry was performed using a BD LSR II station. Sytox Blue was purchased from Molecular Probes, Invitrogen. Data represent mean + S.D. from independent triplicates. The experiment was reproduced with similar results. (B) Cleavage of Bid-Cherry and Bid-FRET probe resembles the cleavage of native Bid. HeLa cells were transfected to express the YFP-Bid-CFP FRET probe and Bid-Cherry. 24 hrs later, cells were exposed to 100 ng/ml TRAIL + 1  $\mu$ g/ml CHX for the times indicated. In a control treatment, zVAD was added at 100  $\mu$ M to ensure that the observed Bid cleavage was caspase-dependent. Whole cell extracts were then used for immunoblotting. Full length and cleaved Bid variants were detected using a goat polyclonal Bid antibody (R&D Systems, Abingdon, UK). Tubulin served as a loading control and was detected using a mouse monoclonal  $\alpha$ -Tubulin antibody (Sigma).

### Reference:

Kohler, B., Anguissola, S., Concannon, C. G., Rehm, M., Kogel, D. and Prehn, J. H. (2008). Bid participates in genotoxic drug-induced apoptosis of HeLa cells and is essential for death receptor ligands' apoptotic and synergistic effects. PLoS ONE 3, e2844.