



Supplementary Figure S3. Over-expressed BOK induces apoptosis in various cell lines. (a) FLAG-BOK was induced in WT FDM/5xUAS-FLAG-BOK cells by addition of 0.1 μ M 4-OHT, in the presence or absence of 25 μ M Q-VD-OPh, and cell viability was determined by flow cytometry (GFP-Annexin V/PI exclusion). Anti-FLAG immunoblot of untreated and 4-OHT treated cells shows induction of FLAG-BOK at 24 h. Data are presented as means \pm SD from three independent experiments. (b) FLAG-BOK was induced as described in (a) in BAF/3-5xUAS-FLAG-BOK cells. Cell viability was measured at 24 and 48 h and caspase-3/-7 activity was quantified by using the fluorogenic caspase activity assay at 24 h using DEVD-AMC as a substrate. BAF/3 cells deprived of IL-3 for 15 h served as a positive control for caspase-3 activation. Anti-FLAG and anti-caspase-3 immunoblots of BAF/3 cell lysates after 0, 16, 24, 36 and 48 h of FLAG-BOK induction. Viability data are presented as means \pm SD from three independent experiments. DEVDase data are presented as means from 2 independent experiments. (c) Viability assay of Jurkat/5xUAS-FLAG-BOK cells after 0, 24, 48 and 72 h of FLAG-BOK induction. Induction of FLAG-BOK was verified by Western blot analysis using anti-FLAG specific antibody. Data are presented as means \pm SD from three independent experiments. (d) Confocal scanning micrographs of HeLa cells transiently transfected with a pEGFP-BOK expression construct for 12 h and stained with anti-cytochrome c antibody and Hoechst 33342 (nuclei). (e) Confocal scanning micrographs of HeLa cells transiently transfected with a FLAG-BOK/pcDNA3 expression construct for 18 h in the presence or absence of 25 μ M Q-VD-OPh. Cells were stained with antibodies to FLAG and active caspase-3 specific and Hoechst 33342 dye. (f) FLAG-BOK was induced for 24 or 48 h in WT and *Bok*^{-/-} SV40 immortalized MEF/5xUAS-FLAG-BOK cells and viability measured by flow cytometry (GFP-Annexin V/PI exclusion). Quantitative data are presented as means \pm SD of at least three independent experiments.