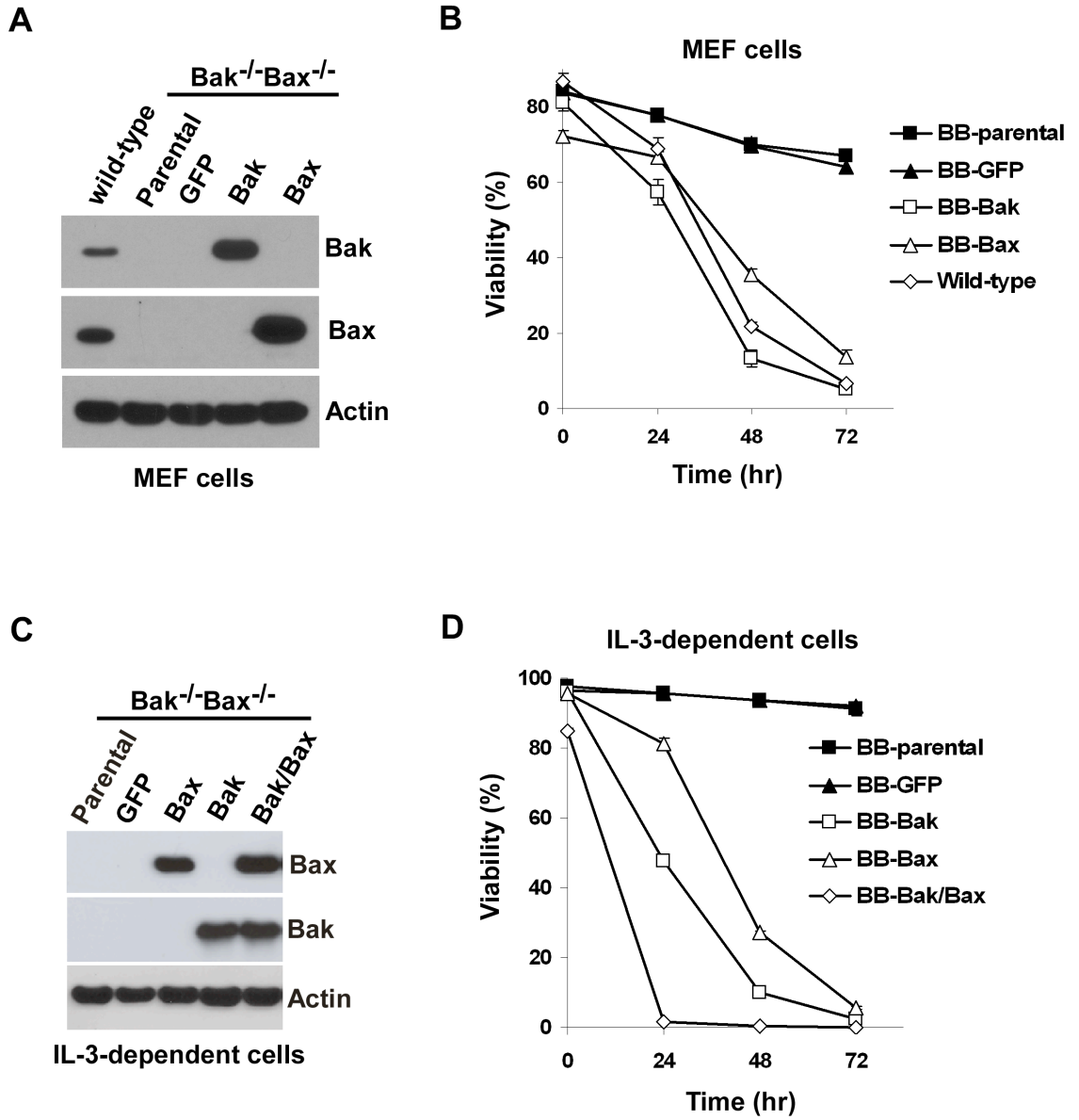
Supplementary Figure 1



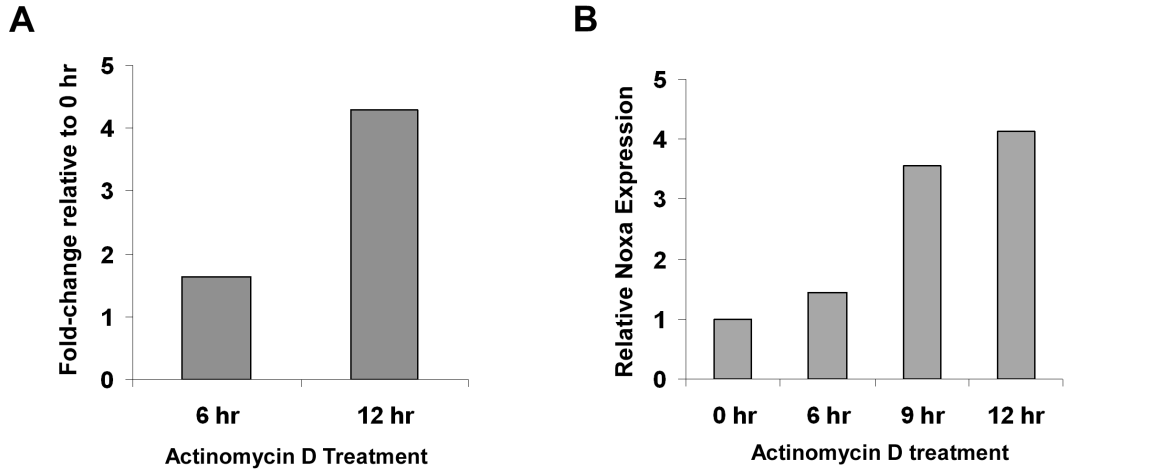
Supplementary figure 1. Actinomycin D-induced cell death is mediated by Bak or Bax.

(A) Bak or Bax was stably re-expressed in $Bak^{-/-}Bax^{-/-}$ (BB) MEF cells by retroviral infection, and cell lysates of 1x10^5 MEF cells were examined by western blot. GFP represents the empty expression vector (pBabeIRESGFP). Actin was a loading control.

(B) Actinomycin D (0.2 μg/ml) induced cell death in Bak- or Bax-expressing MEF cells.

(C) Re-expression levels of Bak or Bax in IL-3-dependent $Bak^{-/-}Bax^{-/-}$ hematopoietic cells were determined. Lysates of 5x10^4 cells were analyzed by western blot. **(D)** The indicated IL-3-dependent cells were treated with 0.2 μg/ml actinomycin D and cell viability was examined. All cell viability data are representative of three independent experiments. Mean ± standard deviation of triplicate experiments are shown. BB, $Bak^{-/-}$ $Bax^{-/-}$.

Supplementary Figure 2

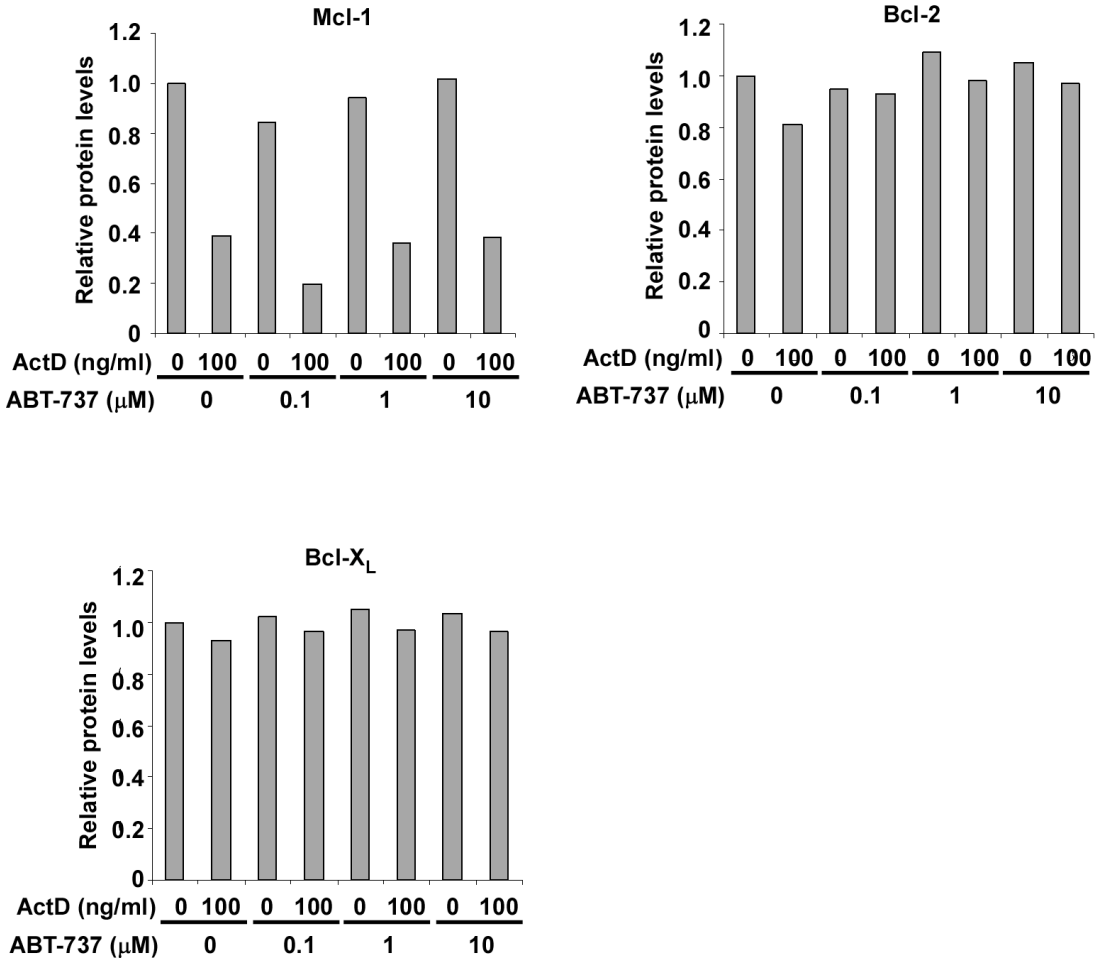


Supplementary figure 2. Actinomycin D treatment leads to an increase in Noxa expression.

(A) The relative increase in Noxa mRNA levels after 6 and 12 hours of 0.2 μg/ml actinomycin D treatment in wild-type MEF cells was determined by microarray analysis. Data are represented as fold-changes in mRNA compared to untreated cells. **(B)** Relative mRNA levels of Noxa were assessed by RT-qPCR. Data are normalized to actin

transcript expression and are depicted relative to mRNA levels at the zero hour time point.

Supplementary Figure 3



Supplementary figure 3. Mcl-1 protein levels were down-regulated in MEF cells treated with actinomycin D.

The intensities of proteins shown in the western blot data of Figure 3A were quantified using ImageJ software (NIH). The data are normalized to actin protein levels and represented relative to the protein levels at the zero hour time point.