Supplemental Figure 1. Gene targeting of ABIN-1 (tnip1) gene.

(A) Gene targeting construct and endogenous ABIN-1 (tnip1) locus, showing LoxP flanked neomycin expression cassette as well as third LoxP site. (B) Southern blot analyses of BamH1 digested ES cell genomic DNA confirming properly targeted ("T") ES cells (left blot) and Bgl2 digested tail genomic DNA confirming in vivo deletion ("-") of exons 12-15 (right blot). Probes used for Southern blotting analyses are shown by thick black bars. Predicted sizes of bands are indicated in table at left. (C) PCR genotyping of ABIN-1+/- allele using three PCR primers, indicated by half arrows, and genomic DNA from ABIN-1+/+, ABIN-1+/- and ABIN-1-/- MEFs. (D) ABIN-1 protein expression in ABIN-1+/+ and ABIN-1-/- MEFs.

Supplemental Figure 2. ABIN-1 is required for protecting cells from TNF and TNF plus CHX-induced PCD.

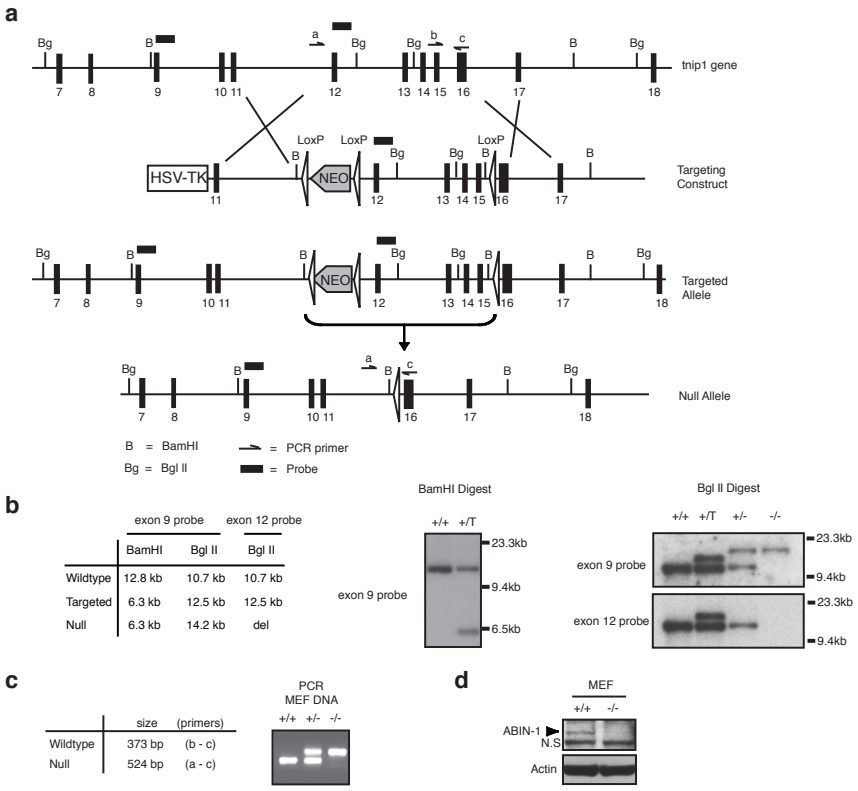
(A) TNF plus CHX-induced PCD of Jurkat T cells infected with ABIN-1 specific shRNA ("81" or "85" sequences) versus control Jurkats (* indicates $p < 0.01$ between either "81" or "85" ABIN-1 knockdown Jurkat and control cells; means and standard deviations indicated, n=3). Immunoblot analysis demonstrating efficacy of ABIN-1 protein knockdown (along with actin control) shown at left. (B) TNF-induced PCD of Jurkat T cells treated with both "81" and "85" ABIN-1 specific shRNAs versus control Jurkats (* indicates $p < 0.01$ between ABIN-1 knockdown and control Jurkats; means and standard deviations indicated, n=3). Immunoblot analysis demonstrating efficacy of ABIN-1 protein knockdown (along with actin control) shown at left. (C) TNF plus CHX-induced PCD of Hep G2 cells treated with both "81" and "85" ABIN-1 specific

shRNAs versus control HepG2 cells (* indicates $p < 0.01$ between ABIN-1 knockdown and control HepG2 cells; means and standard deviations indicated, n=3). Immunoblot analysis demonstrating efficacy of ABIN-1 protein knockdown (along with actin control) shown at left. (D) TNF plus CHX-induced caspase-8 and BID expression in ABIN-1 deficient and control Jurkat cells. Actin expression shown below as protein loading control.

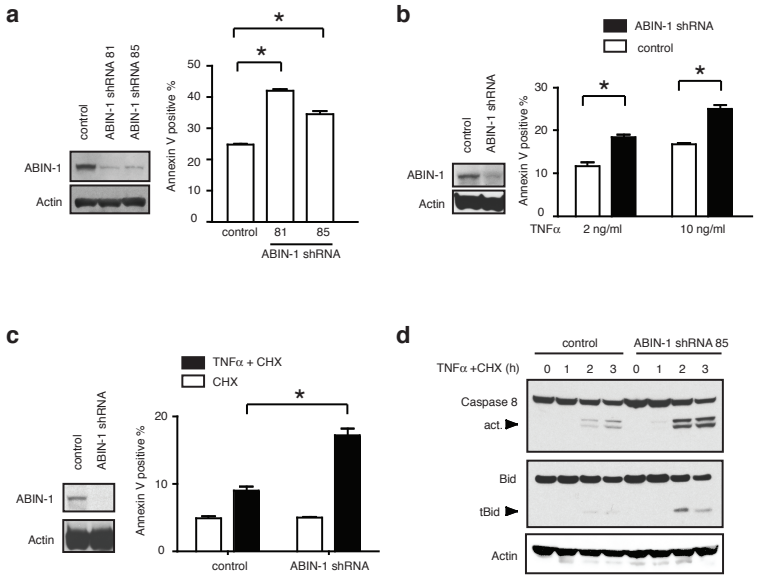
Supplemental Figure 3. ABIN-1-/- cells exhibit largely normal NFkB signaling and expression of NFkB dependent genes. (A) TNF-induced expression of pIκBα and IκBα expression in ABIN-1-/-, A20-/- and control MEFs. Numbers indicate ratios of signal intensities of phospho-IkBa to IkBa, normalized to actin for each sample, with all values then normalized to the ratio of the 60 minute time point in wild type cells (arbitrarily set to 1.0). (B) TNF-induced IKK kinase activity of wild type, ABIN-1-/- and A20-/- MEFs. Immunoblot for phospho-IkBa shown in upper panel and IKKg immunoblot of immunoprecipitated sample shown below as control. Numbers indicate ratios of signal intensities of phospho-IkBa to IKKg calculated for each sample, with all values normalized to the 10 minute time point in wild type cells (arbitrarily set to 1.0). (C) EMSA analyses of TNF-induced NFkB DNA binding of wild type, ABIN-1-/- and A20-/- MEFs. (D) Real time (RT) PCR analyses of TNF-induced expression of NFkB dependent genes. Fold induction indicates the ratio of measured RT-PCR value to wild type cells at time=0 (except iNOS values which are normalized to time = 45 mins). (E) ELISA analysis of TNF-induced production of IL-6 protein by ABIN-1-/-, A20-/- and control MEFs. * over columns indicates $p < 0.05$ statistical

difference between indicated sample and corresponding value in wild type cells; ** over columns indicates $p < 0.05$ difference between indicated sample and corresponding value in ABIN-1-/- cells; n.d. = none detected; means and standard deviations indicated, n=3 for both (D) and (E) above.

Supplementary
Fig.1



Supplementary
Fig.2



Supplementary
Fig. 3