SUPPLEMENTARY FIGURE LEGEND

Supplementary figure 1. Caspase-8 activation is involved in TNF-α-induced apoptosis.

(A) Caspase-8 expression in MEFs was evaluated by western blot. The molecular weight markers are labeled on the left (kD). **(B)** Wild-type (WT) and caspase-8-KO MEFs were cultured with the indicated combination of recombinant human TNF-α and the Smac mimetic TL-32711. Cell viability was determined following 24 hour-exposure by measuring propidium iodide DNA dye exclusion. Data are presented as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate $P < 0.05$ (*), Student's unpaired t test.

Supplementary figure 2. Caspase-8 is not involved in C12-induced human tumor cell death.

(A) Caspase-8 expression in HCTT116 cells was stably reduced by shRNA. The expression levels of caspase-8 were determined by western blot. The molecular weight markers are labeled on the left (kD). **(B)** HCT116 cells were cultured with the indicated combination of recombinant human TNF-α protein and cycloheximide (CHX). Cell viability was determined following 48 hour-exposure. **(C)** The cytotoxicity of C12 on HCT116 cells was assessed 48 hours after C12 treatment. **(D)** Stable reduction of caspase-8 expression in PANC-1 cells was evaluated by western blot. The molecular weight markers are labeled on the left (kD). **(E)** TNF-α protein and cycloheximide (CHX) triggered more cell death in vector control PANC-1 cells than caspase-8 shRNA

cells following 24 hour exposure. **(F)** C12 induced equivalent cell death in vector-control and caspase-8-deficient PANC-1 cells after 24 hour treatment. Cell death data are presented as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate $P < 0.05$ (*), Student's unpaired t test. ns, no significance.

Supplementary figure 3. Caspase-9 activation is involved in chemotherapeutic drug-induced cell death.

(A) Caspase-9 expression in MEFs was determined by western blot. The molecular weight markers are labeled on the left (kD). **(B)** Wild-type (WT) and caspase-9-KO MEFs were treated for 48 hours with various concentrations of etoposide (etop), and cell viability was measured. **(C)** Wild-type and caspase-9-KO MEFs were cultured with or without 0.5 μg/ml actinomycin D (actD) for 24 hours, and cell viability was evaluated. All the data are shown as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate $P < 0.05$ (*), Student's unpaired t test.

Supplementary figure 4. Reexpression of caspase-9 sensitizes MEFs deficient in caspase-9 expression to C12.

(A) Murine caspase-9 was reexpressed in caspase-9-KO MEFs by lentiviral infection. Caspase-9 expression was determined by western blot. The molecular weight markers are labeled on the left (kD). **(B)** Caspase-9-KO MEFs reexpressing caspase-9 or its empty vector were treated with the indicated concentrations of C12 for 48 hours, and

cell viability was examined. The data are shown as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate P < 0.05 (*), Student's unpaired t test.

Supplementary figure 5. C12-induced cell death is dependent of caspase-9.

(A) Expression of Caspase-9 in MIA PaCa-2 cells was stably decreased by shRNA. Caspase-9 expression was evaluated by western blot. The molecular weight markers are labeled on the left (kD). **(B)** MIA PaCa-2 cells were cultured with the indicated concentrations of C12. Cell viability was determined following 24 hour-exposure. The data are shown as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate P < 0.05 (*), Student's unpaired t test.

Supplementary figure 6. C12-induced cell death is dependent of APAF1.

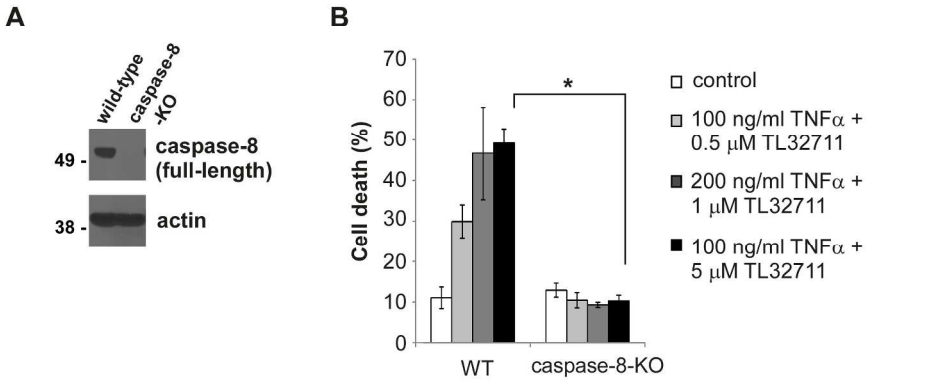
(A) APAF1 expression in APAF1-deficient primary MEFs and their wild-type counterparts was determined by western blot. The molecular weight markers are labeled on the left (kD). **(B)** Wild-type (WT) and APAF1-KO MEFs were treated with various concentrations of etoposide (etop) for 48 hours, and cell viability was determined. The data are shown as means ± standard deviations of three independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate P < 0.05 (*), Student's unpaired t test. **(C)** Cell viabilities of wild-type (WT) and APAF1-KO MEFs were measured 48 hours after treatment with different doses of C12. The data are presented as means ± standard deviations of three

independent experiments with duplicate samples measured in each independent experiment. Asterisks indicate P < 0.05 (*), Student's unpaired t test.

Supplementary figure 7. C12 induces mitochondrial outer membrane permeabilzation *in vitro* in a time-dependent fashion.

(A) Mitochondria isolated from wild-type MEFs were incubated with C12 for the indicated time. One percent of DMSO was present in each sample. Cytochrome c released from mitochondria was determined by western blot. The molecular weight markers are marked on the left (kD). **(B)** Summary of the data shown in (A). As described in Materials and Methods, cytochrome c release is represented as a percentage of the sum of the protein intensity in mitochondrial fractions (P) and released fractions (S). Data represent the mean ± S.D. of three independent experiments. Asterisks indicate P < 0.05 (*) by Student's unpaired t test.

Supplementary figure 1



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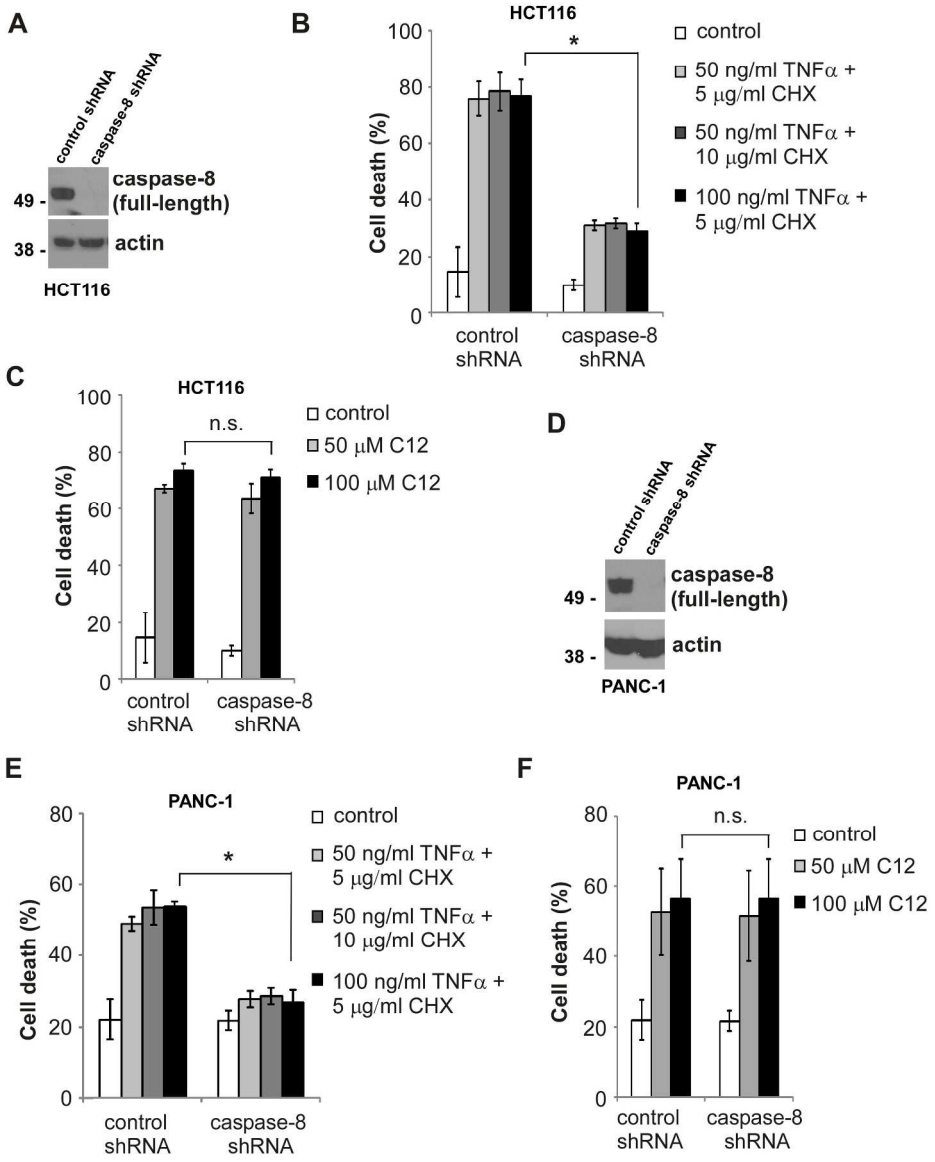
Supplementary figure 2



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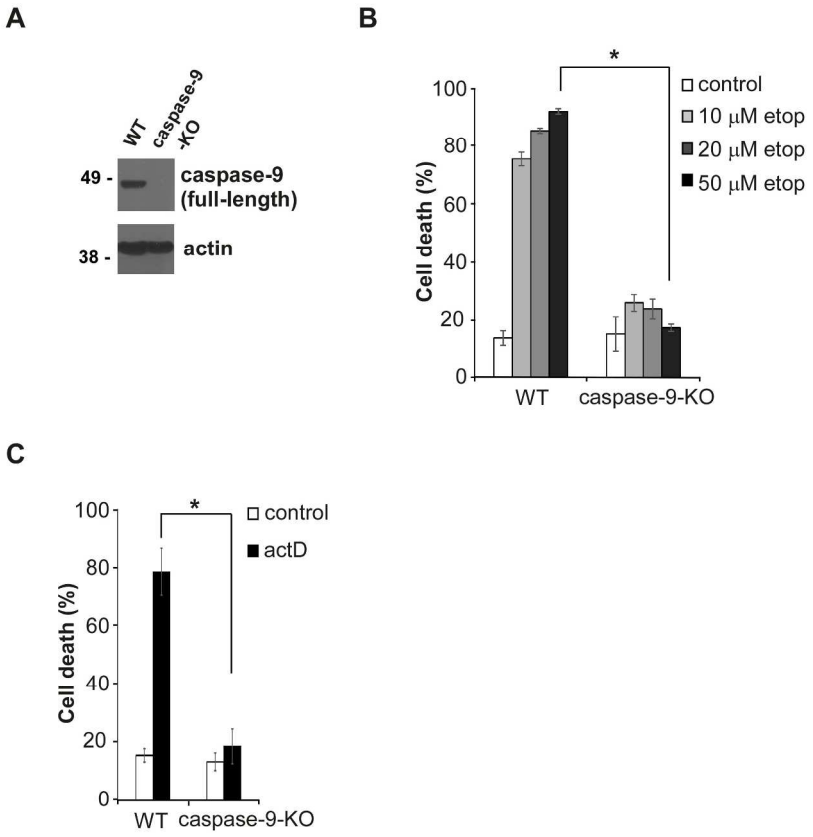

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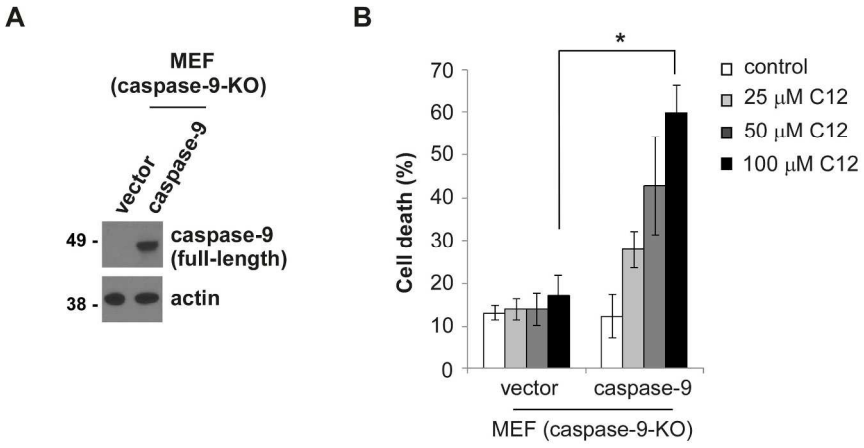

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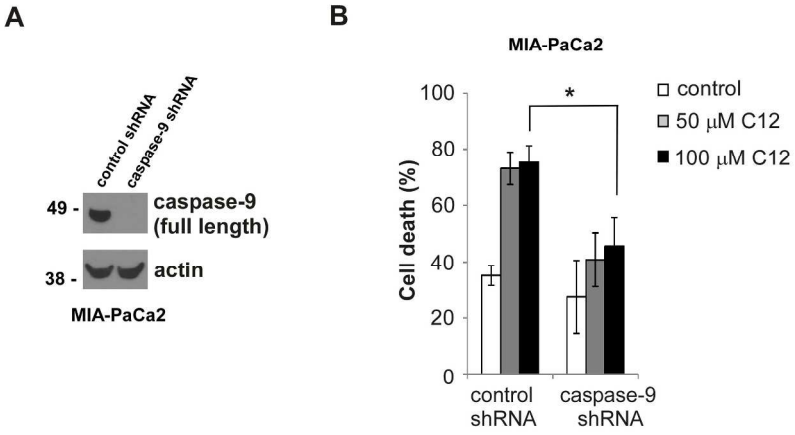

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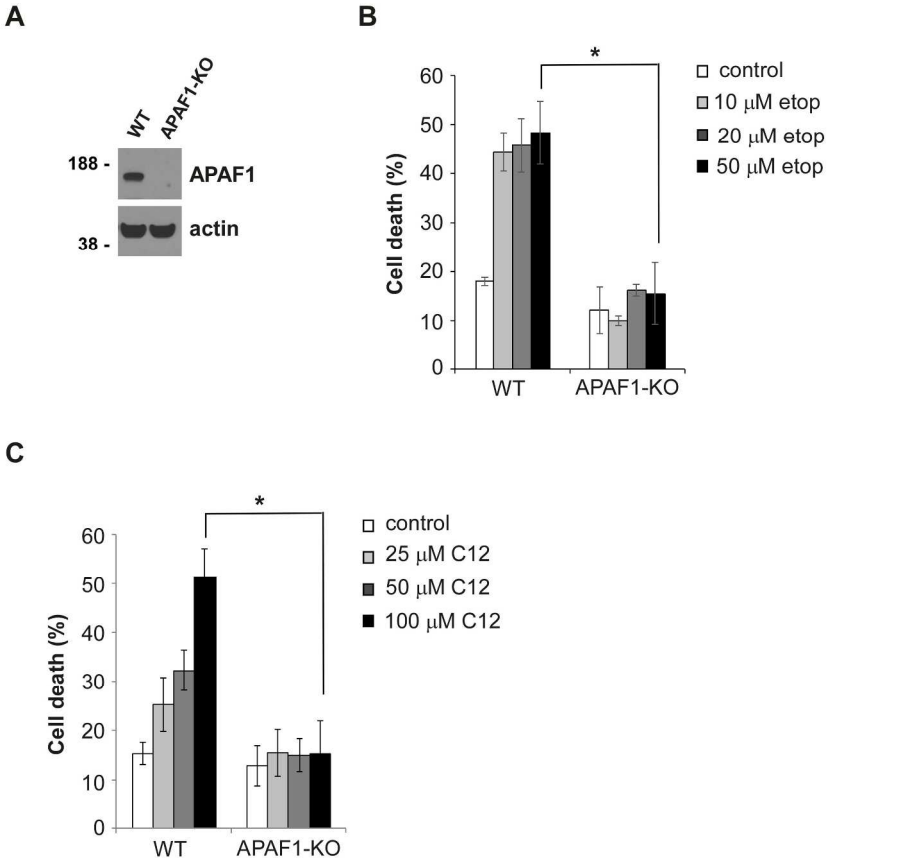

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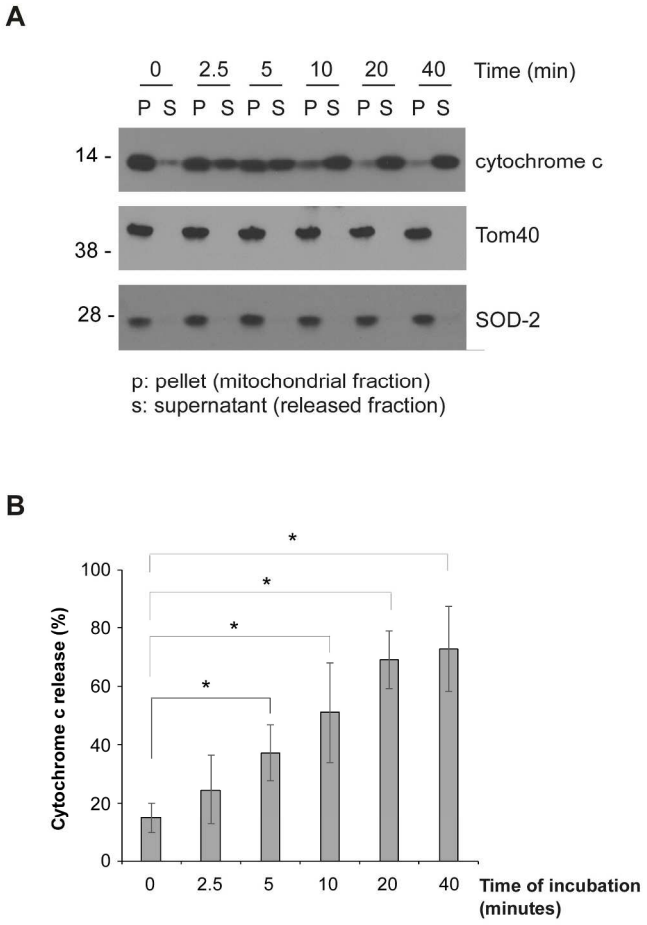

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