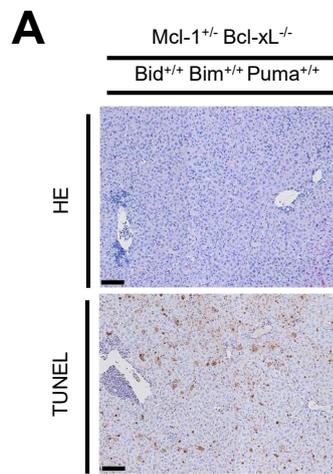
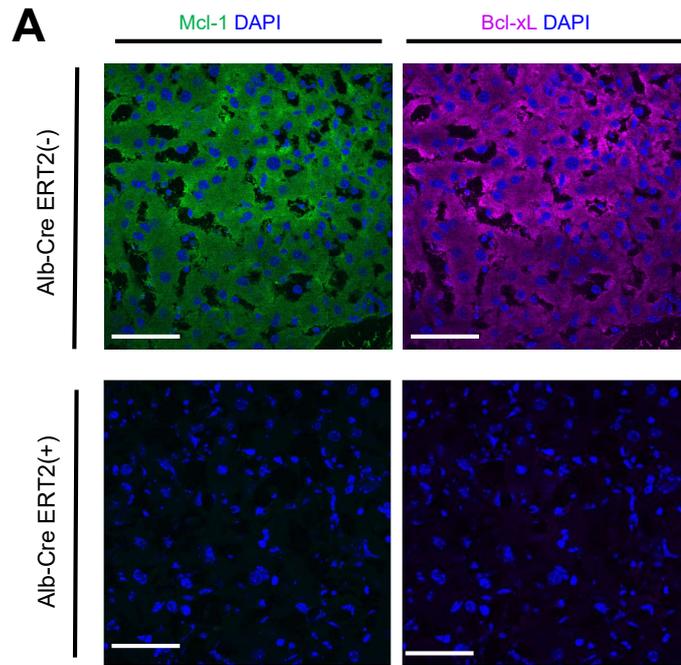


Supplementary Figure 1. Bcl-2 and Bcl-2A1 are nearly undetectable in *Mcl-1^{flox/flox} Alb-Cre Puma^{-/-}* mice, *Bcl-xL^{flox/flox} Alb-Cre Puma^{-/-}* mice and control mice.

(A) Western blots of Bcl-2, Bcl-2A1 and β-actin. The cell lysate of Hep-55.1 C cell line was used as a positive control. Lysate from wild-type C57BL/6J murine primary hepatocytes was used as “Primary Hepatocyte”.

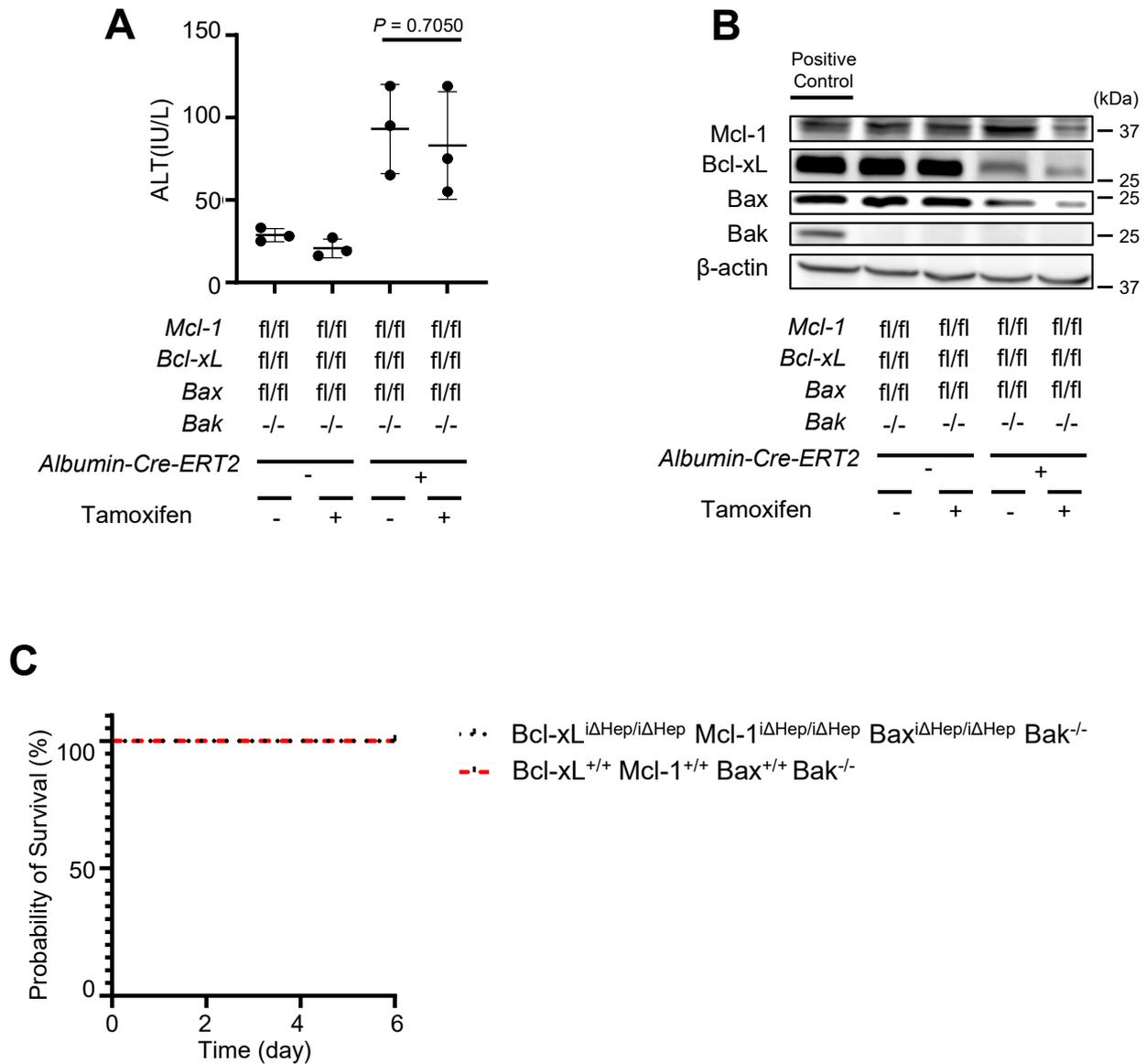


Supplementary Figure 2. *Bcl-xL*^{flox/flox} *Mcl-1*^{flox/+} *Alb-Cre* mice exhibit severe hepatocyte apoptosis.
(A) Representative images of H&E staining and TUNEL staining of *Bcl-xL*^{flox/flox} *Mcl-1*^{flox/+} *Alb-Cre* mice.
Scale bar (A): 100 μ m



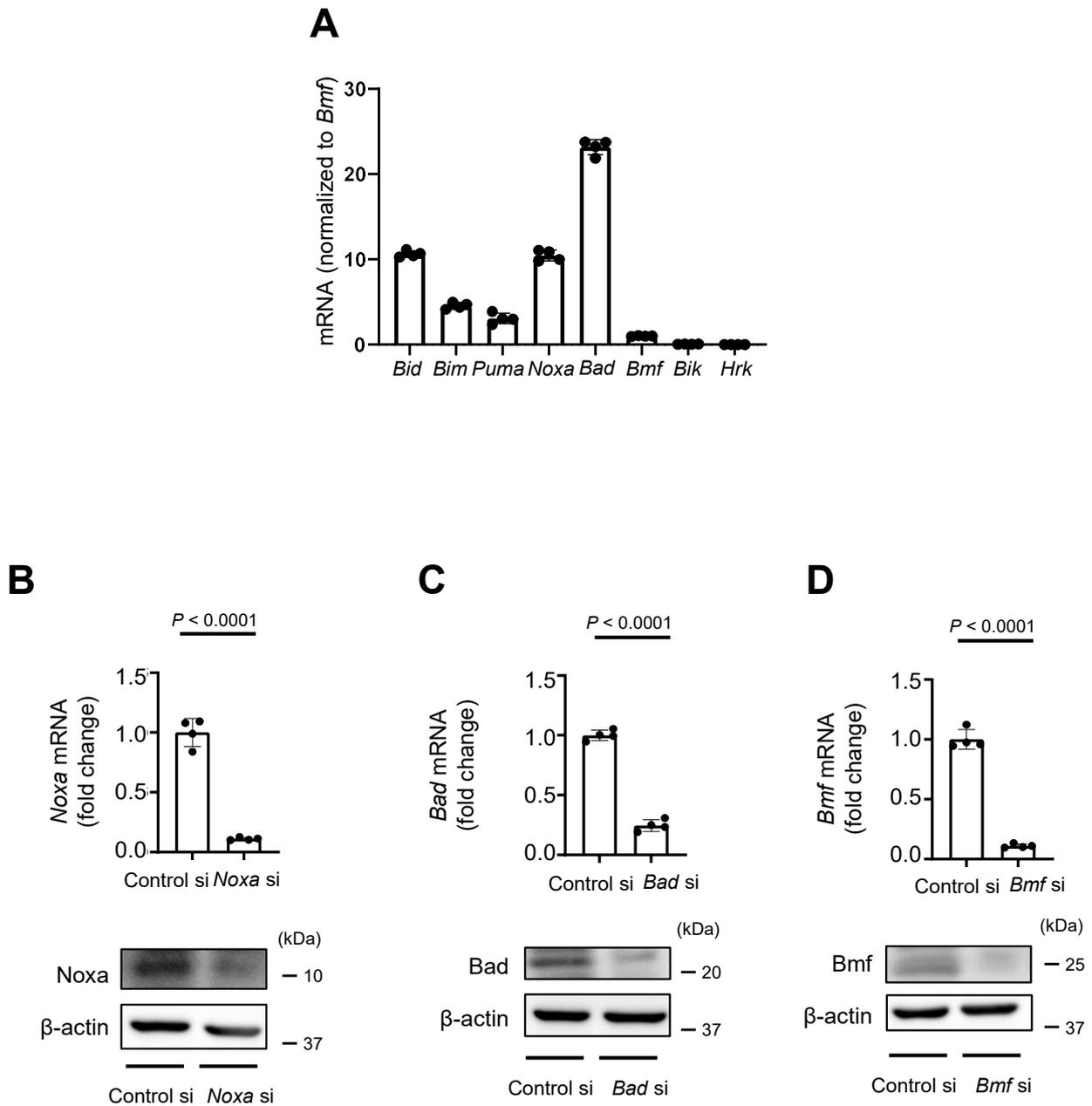
Supplementary Figure 3. Tamoxifen injection reduces the expression of Bcl-xL and Mcl-1.

Bcl-xL^{flox/flox} Mcl-1^{flox/flox} Alb-Cre^{ERT2} Bid^{-/-} Bim^{-/-} Puma^{-/-} mice and control (*Bcl-xL^{flox/flox} Mcl-1^{flox/flox} Bid^{-/-} Bim^{-/-} Puma^{-/-}*) mice were injected intraperitoneally with 1 mg of tamoxifen for 3 consecutive days and analyzed 24 hours after the last tamoxifen injection. (A) Immunofluorescence staining for Mcl-1 and Bcl-xL. Scale bar (A): 50 μ m



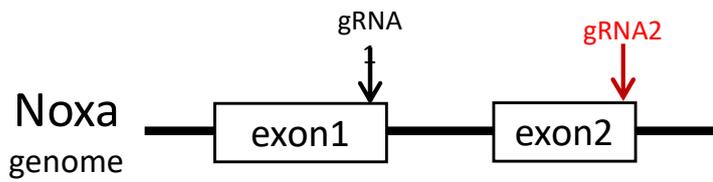
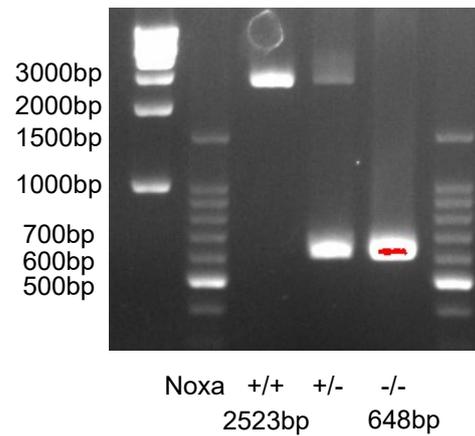
Supplementary Figure 4. Bax/Bak deficiency blocks the hepatocyte apoptosis induced by Mcl-1/Bcl-xL deficiency.

(A-C) *Bcl-xL^{flox/flox} Mcl-1^{flox/flox} Bax^{flox/flox} Alb-Cre ERT2 Bak^{-/-}* mice and control (*Bcl-xL^{flox/flox} Mcl-1^{flox/flox} Bax^{flox/flox} Bak^{-/-}*) mice were injected intraperitoneally with 1 mg of tamoxifen for 5 consecutive days and analyzed at day 6; n=3 per group. (A) Serum ALT levels before and after tamoxifen injection. (B) Western blot analysis before and after tamoxifen injection. (C) Probability of survival after tamoxifen injection. Data was analyzed by two-tailed unpaired *t*-test (A) or the log-rank test (C).



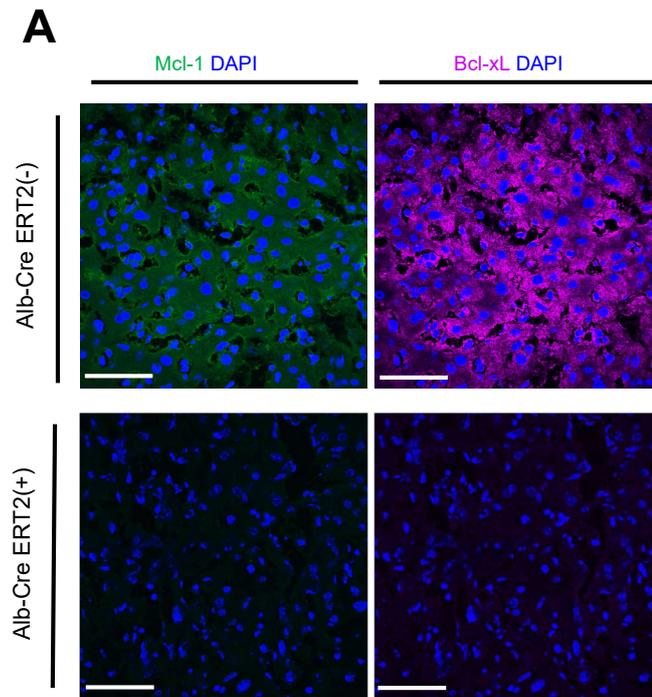
Supplementary Figure 5. Knockdown of Noxa suppresses apoptosis in Bcl-xL-, Mcl-1-, Bid-, Bim- and Puma-deficient hepatocytes.

(A) Relative mRNA expression levels of all BH3-only proteins in the wild-type C57BL/6J murine primary hepatocytes. Expression levels of each protein were normalized to *Bmf* mRNA level. (B-D) Relative mRNA expression levels and Western blots of (B) *Noxa*, (C) *Bad* and (D) *Bmf* after siRNA transfection. Data is represented as mean \pm SD. Data was analyzed by two-tailed unpaired *t*-tests (B-D).

A**B**

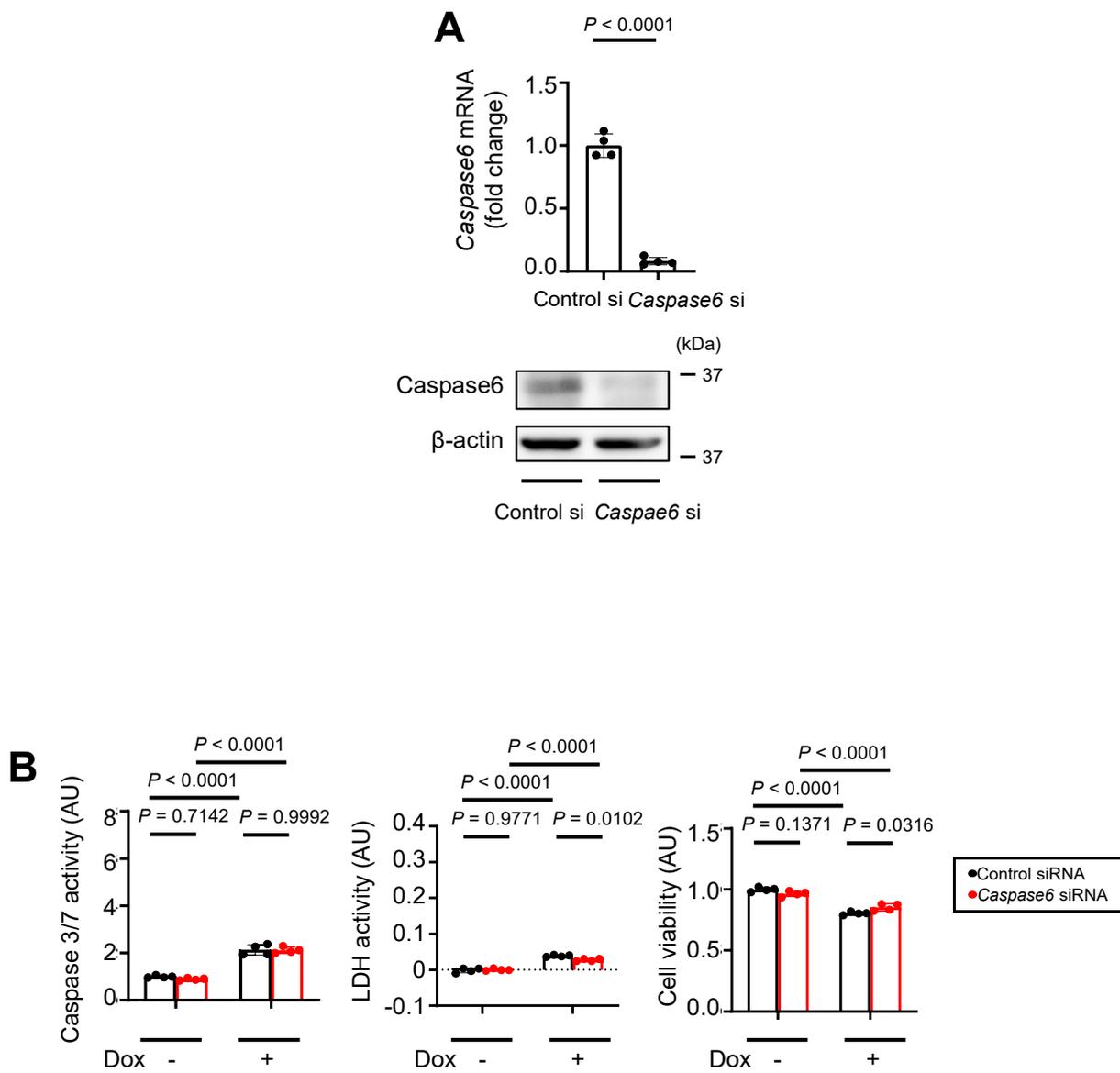
Supplementary Figure 6. Generation of Noxa knockout mice by CRISPR-Cas9 technology.

(A) Schematic of Noxa knockout in mice using CRISPR-Cas9 system. The gRNA sequences were designed using the online tools <http://crispor.tefor.net/> and <https://crispr.dbcls.jp/> to predict unique target sites throughout the mouse genome (gRNA1: 5'- ACGCGCCAGTGAACCCAACG -3' and gRNA2: 5'- CTGGGAAGTCGCAAAAGAGC -3'). Specific Alt-R CRISPR-Cas9 crRNA was procured from IDT and assembled with Alt-R CRISPR-Cas9 tracrRNA, following the manufacturer's instructions. (B) Genotyping band of Noxa knockout mice. *Noxa*^{+/+}: 2523 bp, *Noxa*^{+/-}: 2523 bp + 648 bp, *Noxa*^{-/-}: 648 bp.



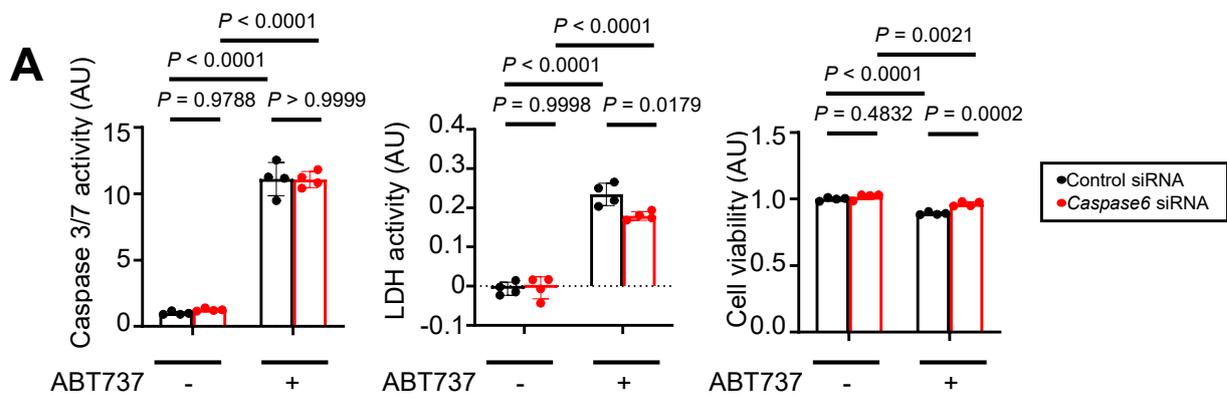
Supplementary Figure 7. Bcl-xL and Mcl-1 are decreased after tamoxifen injection.

Bcl-xL^{fllox/fllox} Mcl-1^{fllox/fllox} Alb-Cre ERT2 Bid^{-/-} Bim^{-/-} Puma^{-/-} Noxa^{-/-} mice and control (*Bcl-xL^{fllox/fllox} Mcl-1^{fllox/fllox} Bid^{-/-} Bim^{-/-} Puma^{-/-} Noxa^{-/-}*) mice were injected intraperitoneally with 1 mg of tamoxifen and sacrificed at 12 hours after tamoxifen injection. (A) Immunofluorescence staining for Mcl-1 and Bcl-xL. Scale bar (A): 50 μ m



Supplementary Figure 8. Knockdown of Caspase-6 increases relative cell viability in Bcl-xL-, Mcl-1-, Bid-, Bim-, Puma-, and Noxa-deficient hepatocytes.

Immortalized *Bcl-xL*^{flox/flox} *Mcl-1*^{flox/flox} *Bid*^{-/-} *Bim*^{-/-} *Puma*^{-/-} *Noxa*^{-/-} mouse primary hepatocytes with doxycycline-inducible Cre recombinase were treated with 0.3 μ M doxycycline for 48 hours after transfection of *caspase-6* siRNA or control siRNA. (A) Relative mRNA expression levels and Western blotting of caspase-6 after siRNA transfection. (B) Caspase 3/7 activity and LDH activity in the culture supernatant, cell viability assessed with a WST assay after *caspase-6* knockdown. Data is represented as mean \pm SD. Data was analyzed by two-tailed unpaired *t*-test (A) or One-way ANOVA with Sidak's multiple comparisons test (B).



Supplementary Figure 9. Knockdown of Caspase-6 improves the relative cell viability of BNL.CL2 cells treated with ABT-737.

BNL.CL2 cells were treated with 4 μ M ABT-737, which is the Bcl-xL inhibitor, for 24 hours after transfection of *caspase-6* siRNA or control siRNA. (A) Caspase 3/7 activity and LDH activity in the culture supernatant, cell viability assessed with a WST assay after *caspase-6* knockdown. Data is represented as mean \pm SD. Data was analyzed by One-way ANOVA with Sidak's multiple comparisons test (A).