

Figure S1: Effects of siRNA knock-down of selected BH3-only proteins on TRAIL-induced Bak activation and apoptosis in HeLa cells: (A) Left panel: western blot analysis of HeLa cell extracts after transfection with the indicated siRNA duplexes. Right panel: Following siRNA transfection, HeLa cells were treated with TRAIL (10 ng/ml) and cell death assay by Hoechst staining was carried out 4 hrs and 8 hrs after treatment. (B) Cells were transfected with the indicated siRNAs and Bak activation was assayed by immunoprecipitation using a conformation-specific antibody against Bak (Ab-1) 6 hrs after TRAIL (10 ng/ml) treatment.

Figure S2: Requirement of Bid and Noxa for TRAIL-induced apoptosis in H460 cells: (A) Following siRNA transfection, H460 cells were treated with TRAIL (10 ng/ml) and cell death was assayed 6 hrs after treatment. (B) Western Blot analysis after siRNA transfection.

Figure S3: Bcl-xL or Bcl-2 overexpressing cells are resistant to apoptosis: (A) Cell lysates were diluted in lysis buffer at the indicated dilution ratios and loaded onto SDS-PAGE. Western blot was probed with anti-Bcl-xL antibody. (B) Western Blot analysis showing expression levels of His-tagged Bcl-xL and Bcl-2 (C) Control HeLa cells, HeLa cells overexpressing His₉Bcl-2, and HeLa cells overexpressing His₉-Bcl-xL were treated with TRAIL (10 ng/ml), TG (5 μM), or MG-132 (10 μM) and cell death was assayed 6 hrs after TRAIL treatment, or 16 hrs after TG and MG-132 treatment.

Figure S4: Increase of “Noxa free” Mcl-1 after Noxa knock-down: (A) Western Blot analysis showing knock-down of Noxa. (B) Increased binding of GST-Noxa to Mcl-1 after knock-down of endogenous Noxa. GST pull-down after Noxa knock-down using recombinant GST, GST-Noxa, and GST-Noxa L29E mutant. (C) Western Blot analysis showing levels of His₉-Mcl-1 expression in 3 different cell lines. (D) Low levels of Mcl-1 over-expression is sufficient to block TRAIL-induced apoptosis. Control HeLa cells and HeLa cells over-expressing low levels of Mcl-1 were treated with TRAIL (10 ng/ml), and cell death was assayed 6 hrs after treatment.

Figure S5: Effects of Noxa knock-down on the association between Mcl-1 and active Bak: (A) Western Blot analysis showing knock-down of Noxa. (B) Noxa knock-down causes an increase in the association between Bak and Mcl-1. Following siRNA transfection, cells were harvested and lysed in EBC buffer containing 0.5% NP-40. Immunoprecipitation was performed using either Bak antibody (Ab-1) or IgG control antibody following siRNA transfection with control siRNA or siRNA targeting Noxa. Samples were blotted for Bak and Mcl-1.

Figure S6: Effects of siRNA knock-down of selected BH3-only proteins on TG-induced Bak activation and apoptosis in HeLa cells: (A) Left panel: Bim and Noxa knock-down block TG-induced apoptosis in HeLa cells. Cells were treated with TG (5 μM) and cell death was assayed 10hrs, 14hrs, and 18hrs after treatment. Right Panel: Western blot analysis showing knock-down of Bim, Bmf, Puma, and Noxa. (B) Forty–eight hours after siRNA transfection with the indicated siRNAs duplexes, cells were harvested and lysed in lysis buffer containing 2% CHAPS. Bak activation was assayed by immunoprecipitation using a conformation-specific antibody (Ab-1) against Bak 16hrs after TG (5 μM) treatment.

Figure S7: ER stress-induced interaction between Noxa and Bcl-xL in CHAPS buffer: HeLa cells over-expressing Bcl-xL were treated with TG (5 μM) for the indicated time before

lysed in a buffer containing 1.5% CHAPS. Nickel beads pull-down was performed and the proteins were detected by western blot using antibodies against Bcl-xL and Noxa.

Figure S8: Effects of siRNA knock-down of selected BH3-only proteins on MG-132-induced Bak activation and apoptosis in HeLa cells: (A) Left panel: Noxa knock-down blocks MG-132-induced apoptosis in HeLa cells. Cells were treated with MG-132 (10 μ M) and cell death was assayed 10hrs, 14hrs, and 18hrs after treatment. Right Panel: Western blot and RT-PCR analysis showing knock-down of Noxa, Bid, Bmf, and Bik. (B) HeLa cells were transfected with the indicated siRNAs and Bak activation was assayed by immunoprecipitation using conformation-specific antibodies against Bak 16hrs after MG-132 (10 μ M) treatment.

Figure S9: MG-132-induced interaction between Noxa and Bcl-xL in CHAPS buffer.

HeLa cells over-expressing Bcl-xL were treated with MG-132 (10 μ M) for the indicated time before lysed in a buffer containing 2% CHAPS. Nickel beads pull-down was performed and the proteins were detected by western blot using antibodies against Bcl-xL and Noxa.

Figure S10: Binding of BH3-only proteins to Bcl-xL following TG treatment: (A) His₉Bcl-xL HeLa cells were treated with TG (5 μ M) for the indicated time before lysis in EBC buffer containing 0.5% NP-40. Nickel beads pull-down was performed and the proteins were detected by western blot using antibodies against Bcl-xL and Noxa. (B) Western Blot analysis of HeLa cell extracts following siRNA transfection. (C) Following siRNA transfection with the indicated siRNA duplexes, HeLa cells were treated with TG (5 μ M) and cell death was assayed 16 hrs after treatment.

Figure S11: Binding of BH3-only proteins to Bcl-xL following MG-132 treatment: His₉Bcl-xL HeLa cells were treated with MG-132 (10 μ M) for the indicated time before lysis in EBC buffer containing 0.5% NP-40. Nickel beads pull-down was performed and the proteins were detected by western blot using antibodies against Bcl-xL and Noxa. (B) Western Blot analysis of HeLa cell extracts following siRNA transfection. (C) Following siRNA transfection with the indicated siRNA duplexes, HeLa cells were treated with MG-132 (10 μ M) and cell death was assayed 16 hrs after treatment.

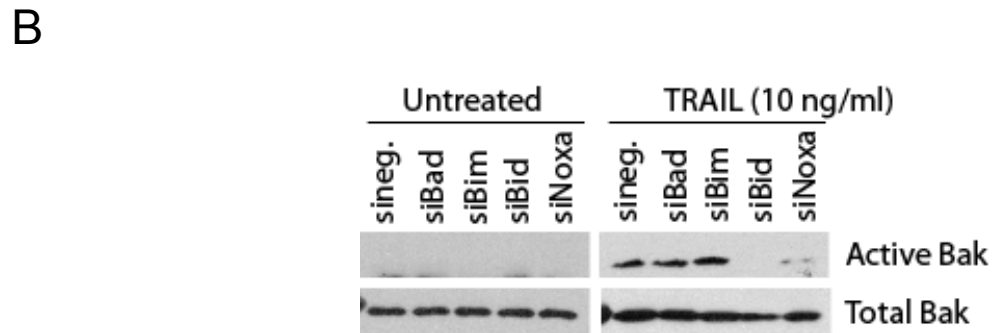
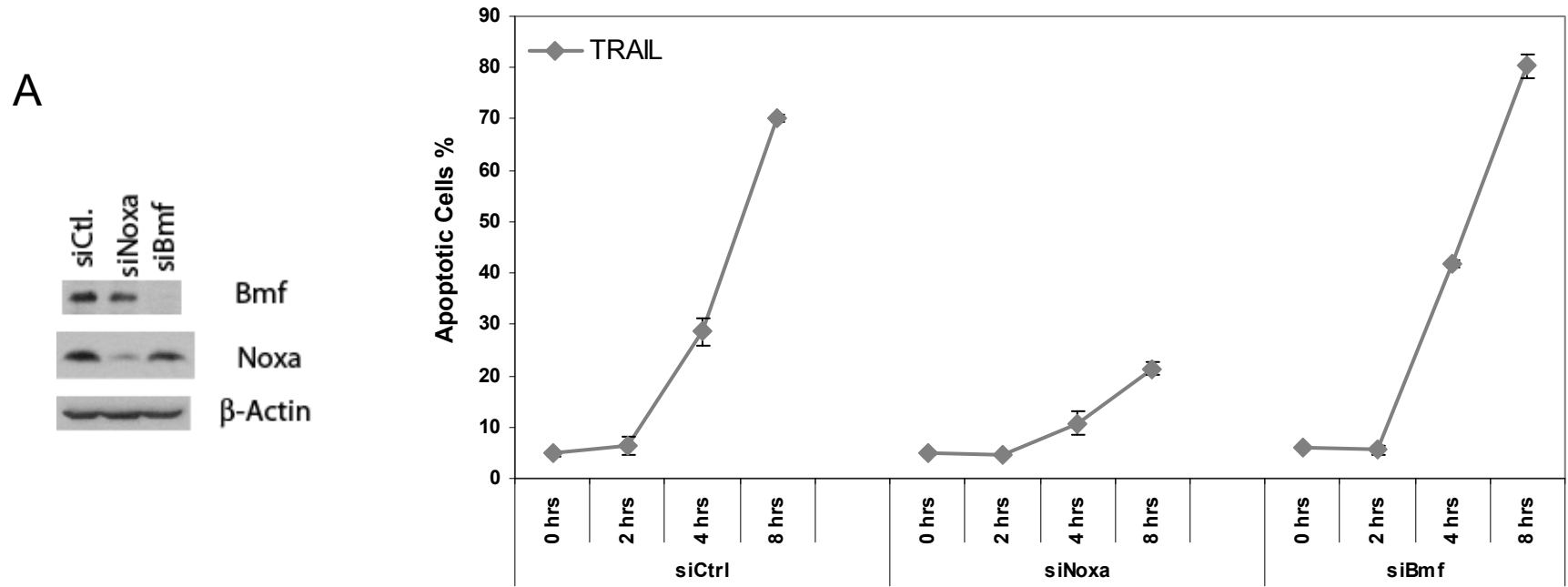


Fig. S1. Effects of siRNA knockdown of select BH3-only proteins on TRAIL-induced Bak activation and apoptosis in HeLa cells

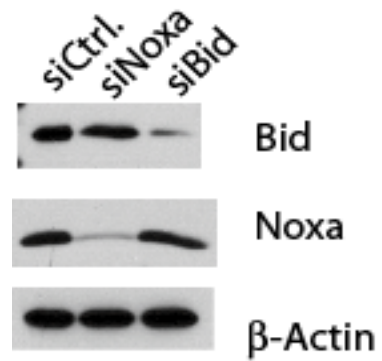
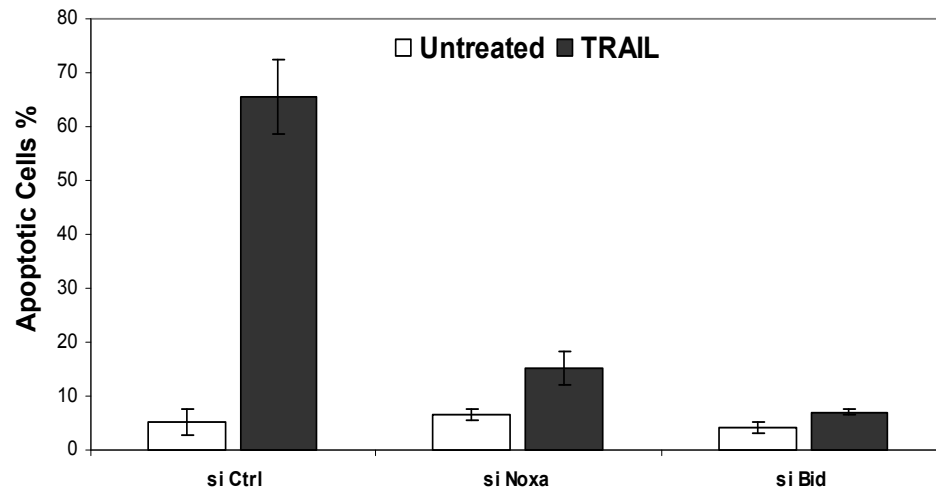


Fig. S2. Requirement of Bid and Noxa for TRAIL-induced apoptosis in H460 cells

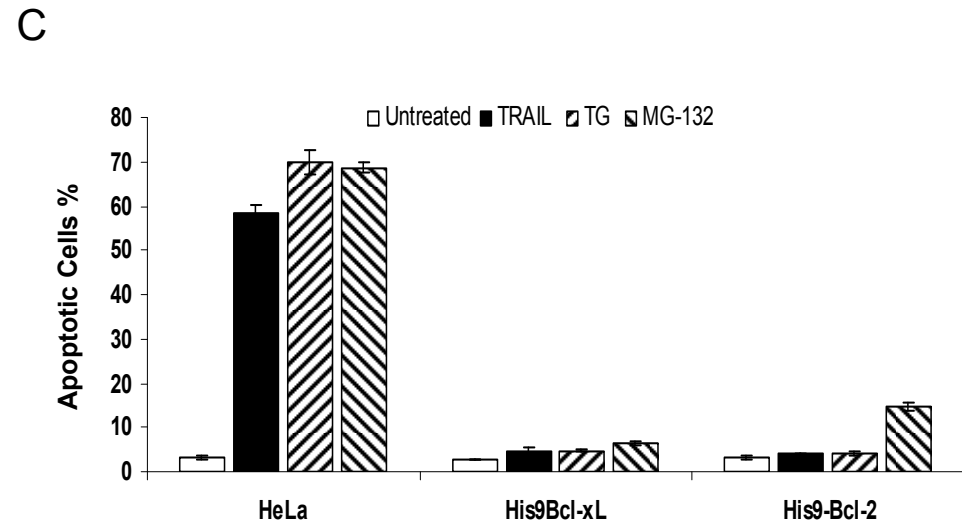
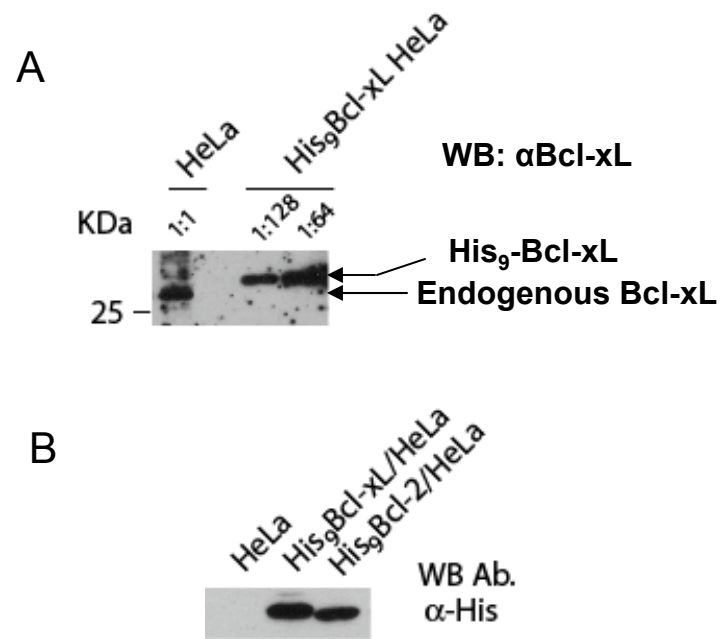


Fig. S3. Bcl-xL or Bcl-2 over-expressing cells are resistant to apoptosis

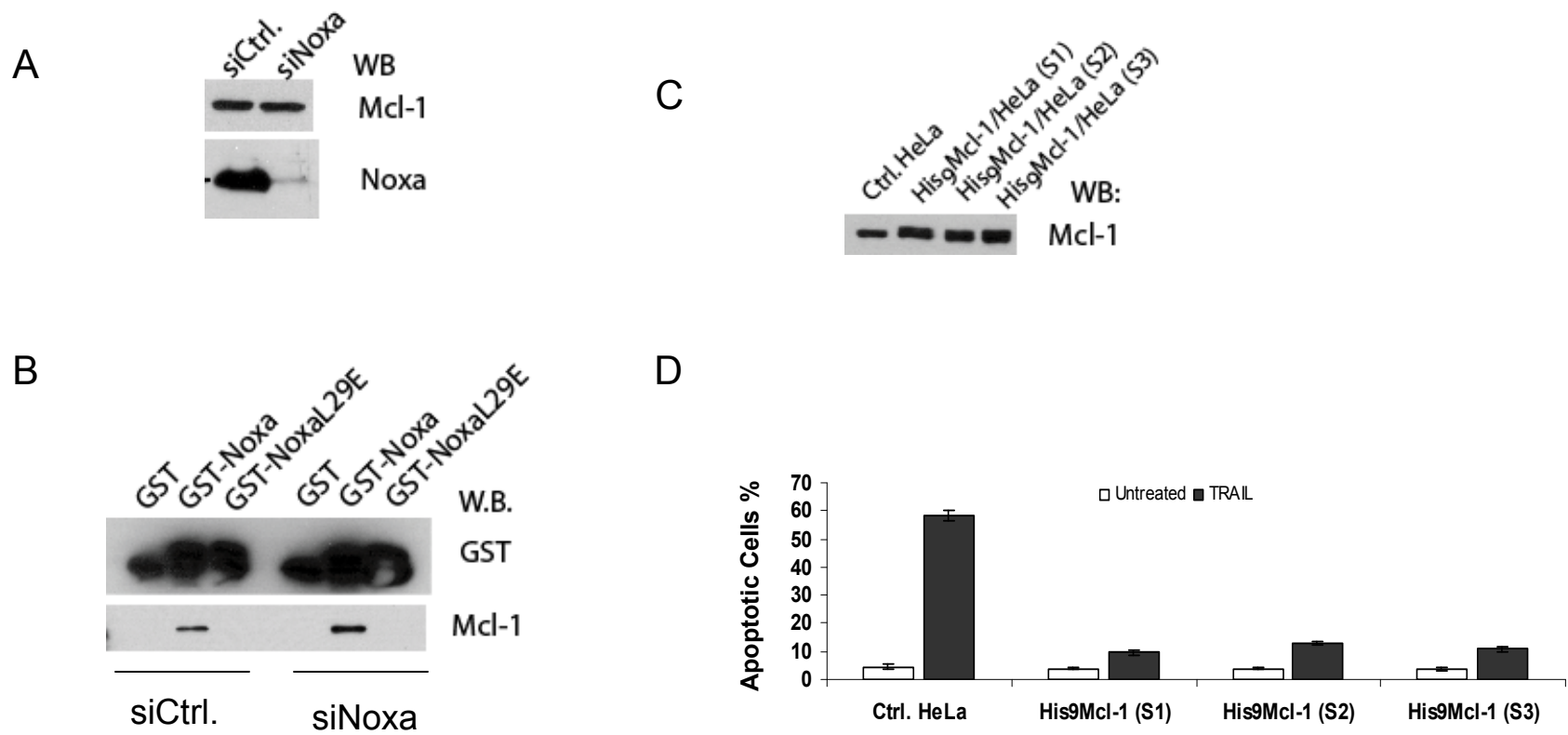


Fig. S4. Increase of “Noxa free” Mcl-1 after Noxa knockdown.

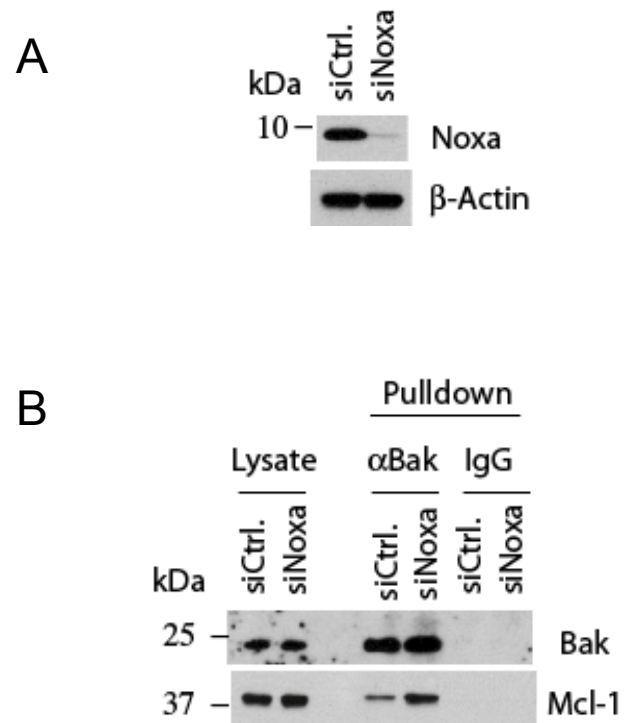
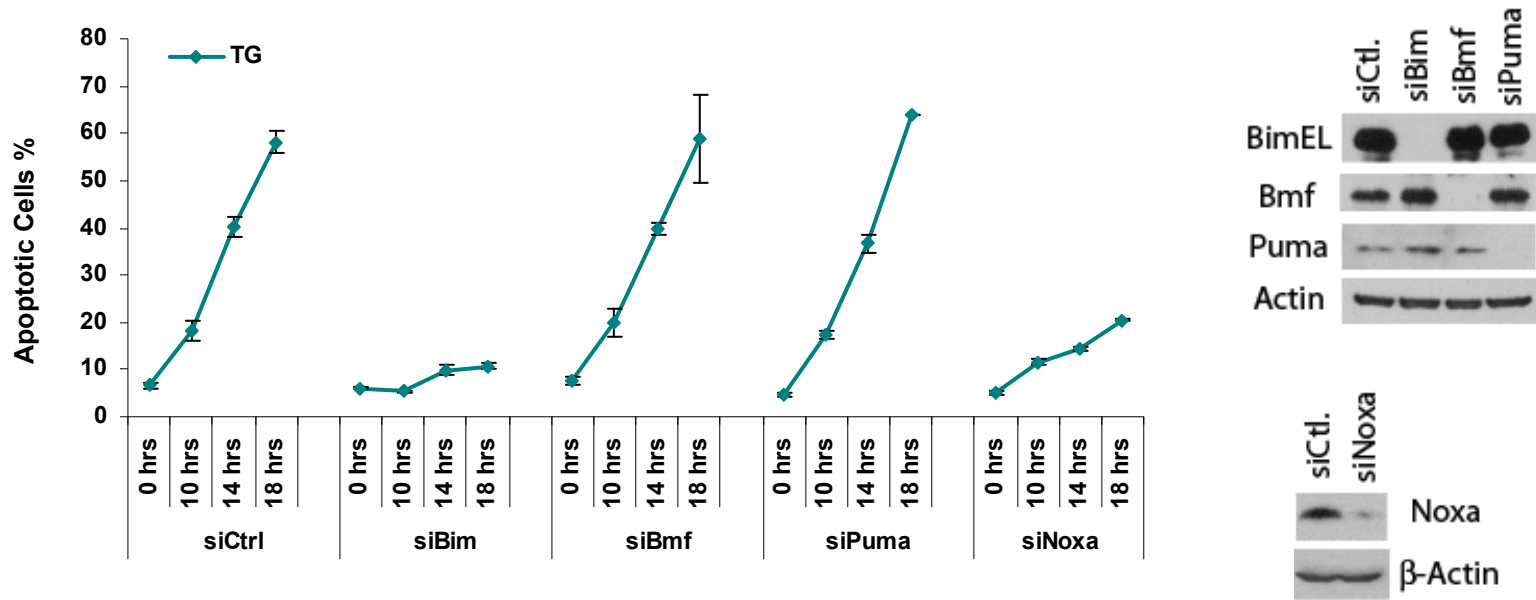


Fig. S5. Effect of Noxa knockdown on the association between Mcl-1 and active Bak

A



B

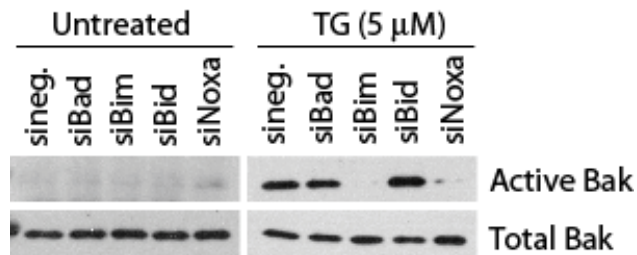


Fig. S6. Effects of siRNA knockdown of select BH3-only proteins on TG-induced Bak activation and apoptosis in HeLa cells

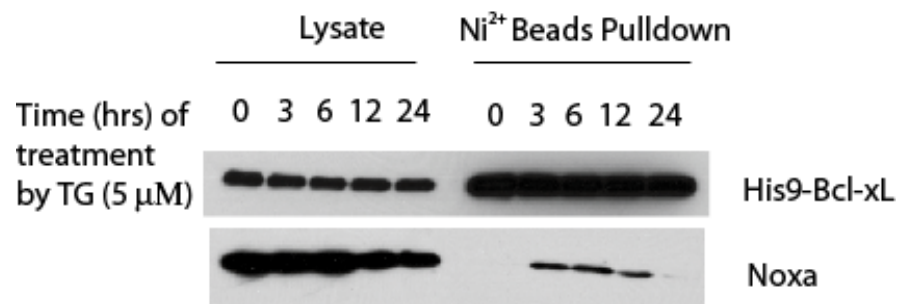
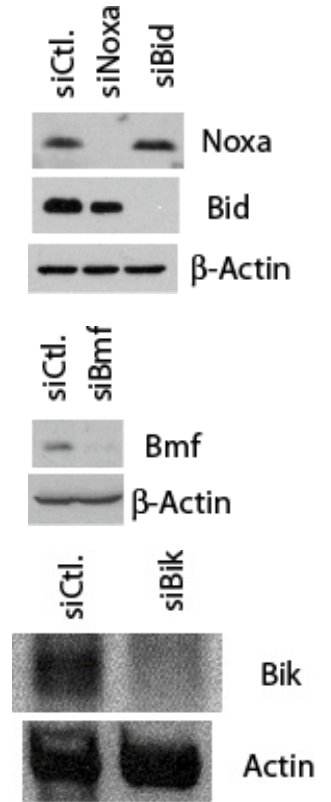
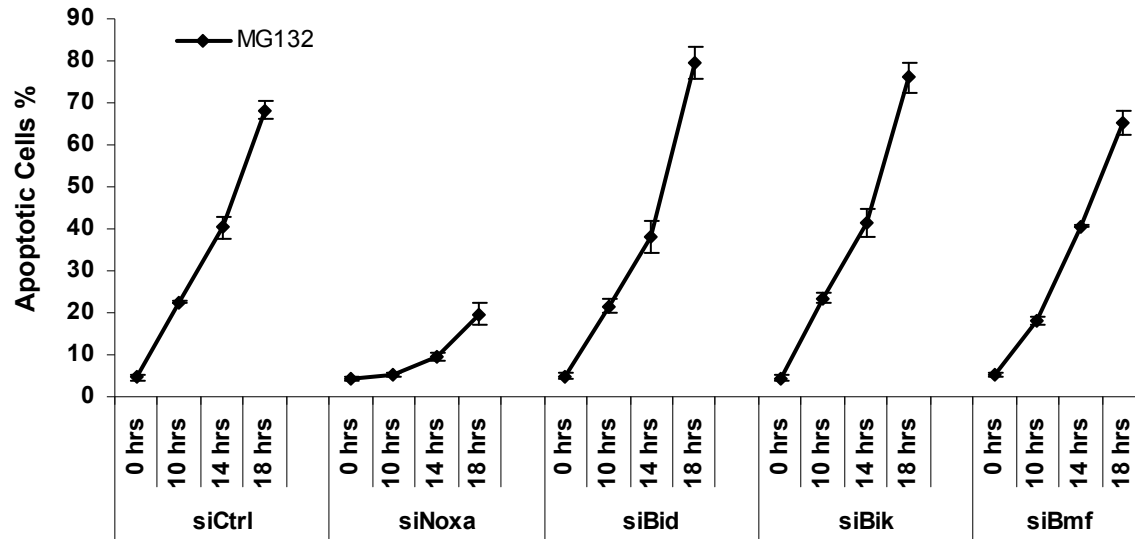


Fig. S7. ER stress-induced interaction between Noxa and Bcl-xL in CHAPS buffer

A



B

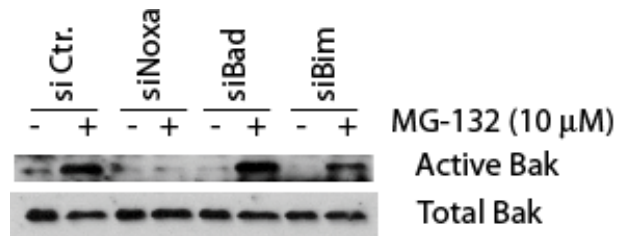


Fig. S8. Effects of siRNA knockdown of select BH3-only proteins on MG-132-induced Bak activation and apoptosis

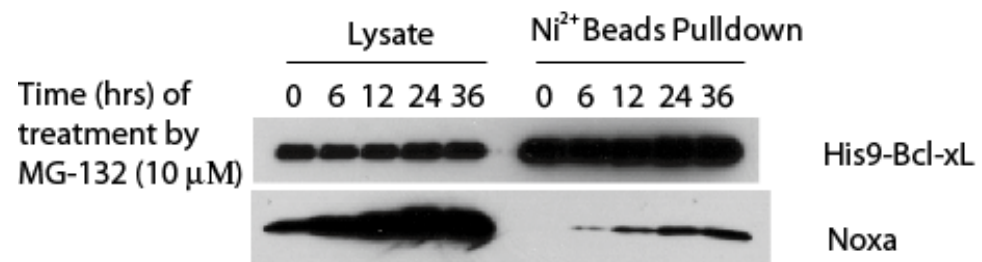
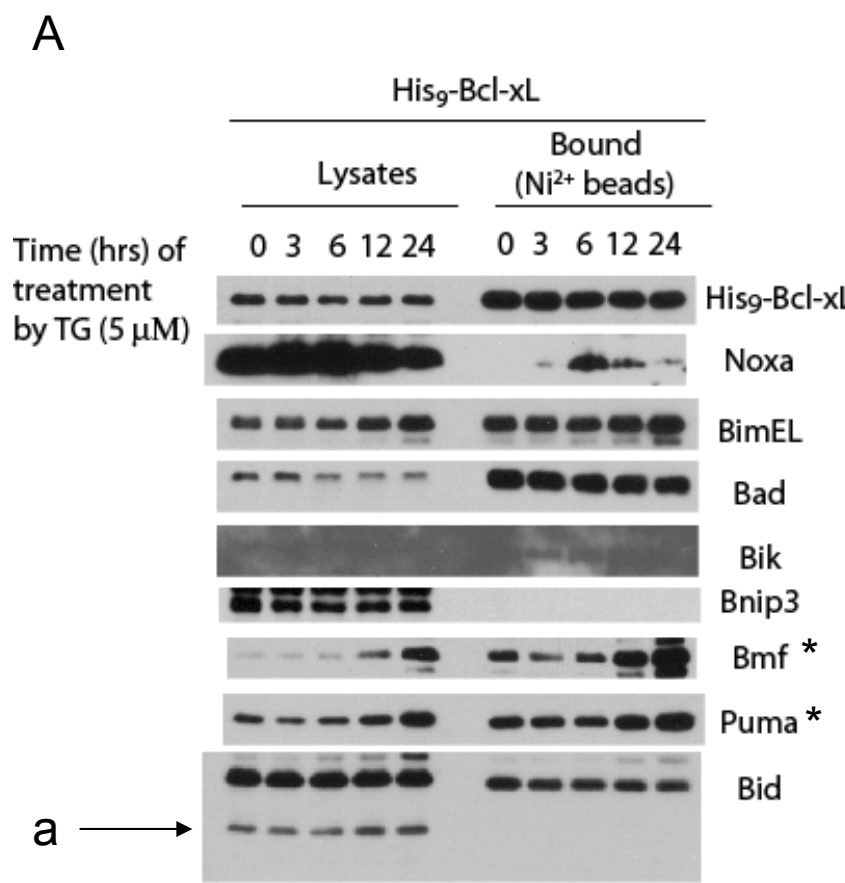


Fig. S9. MG-132-induced interaction between Noxa and Bcl-xL in CHAPS buffer



a: Non-specific

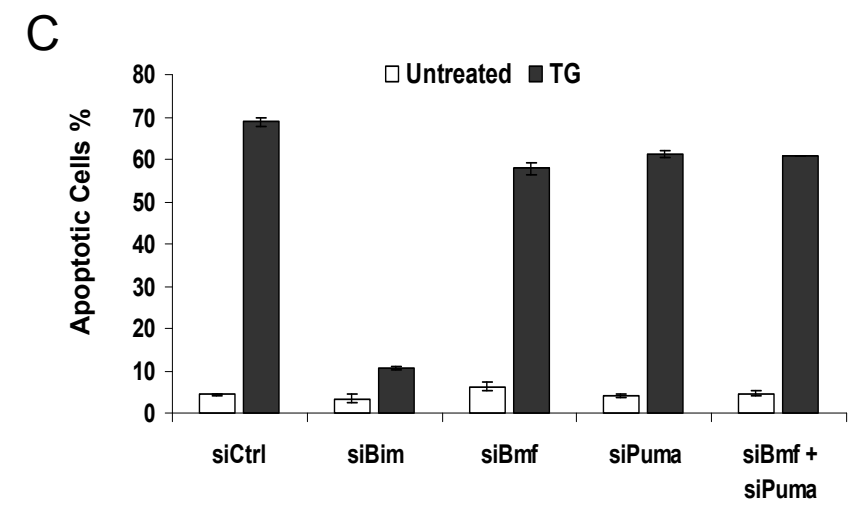
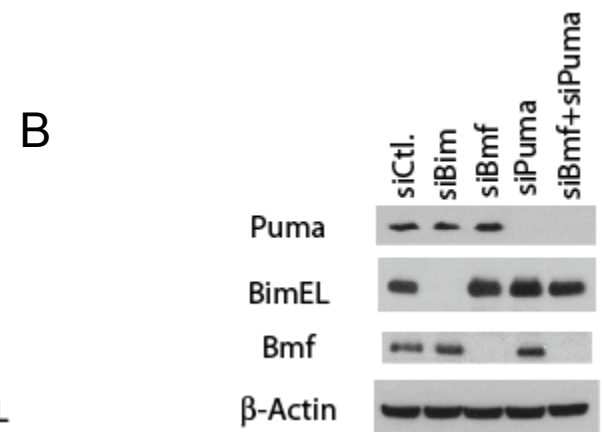
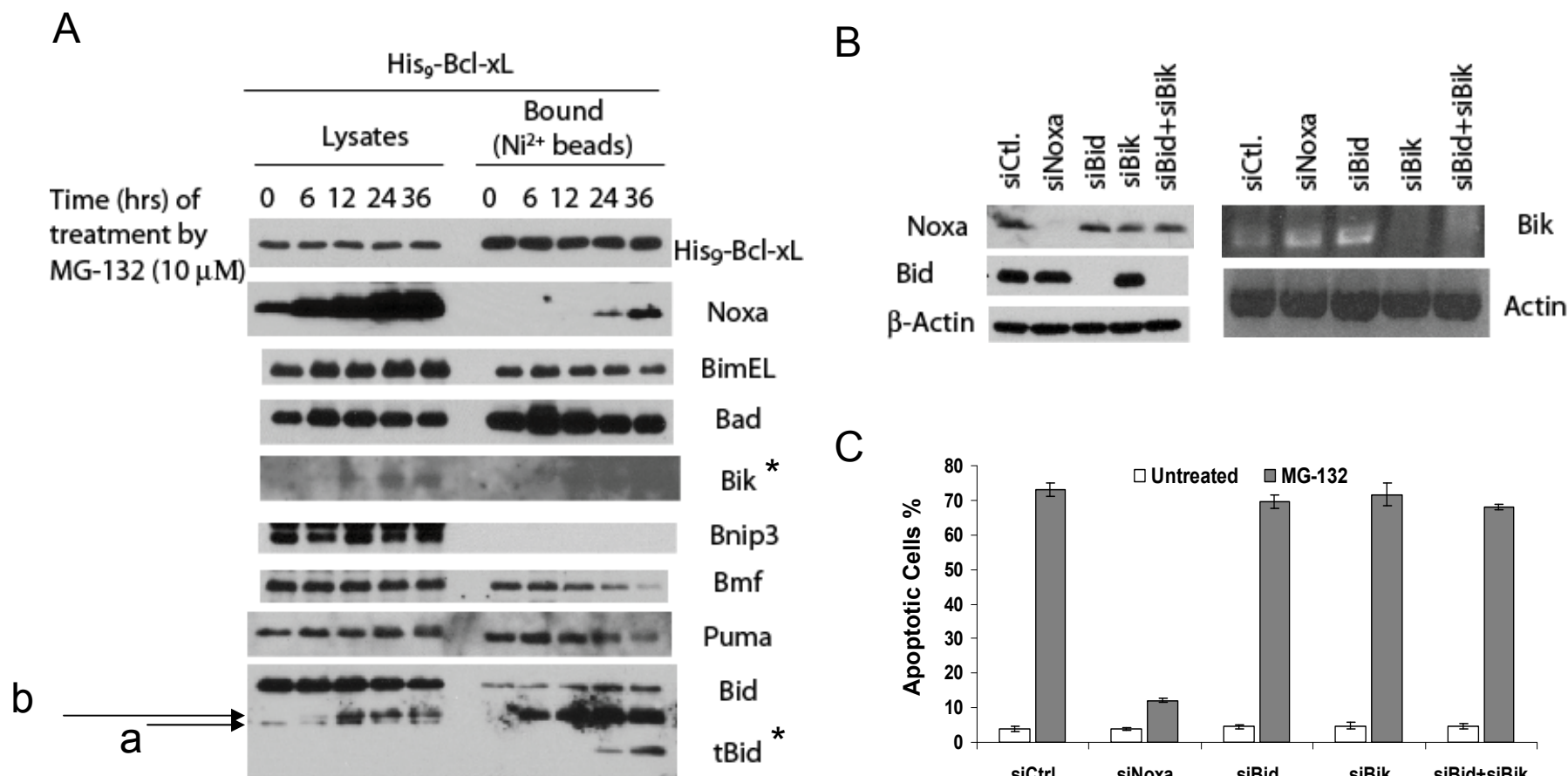


Fig. S10. Binding of BH3-only proteins to Bcl-xL following TG treatment



a, non-specific; b, unknown.

Fig. S11. Binding of BH3-only proteins to Bcl-xL following MG-132 treatment