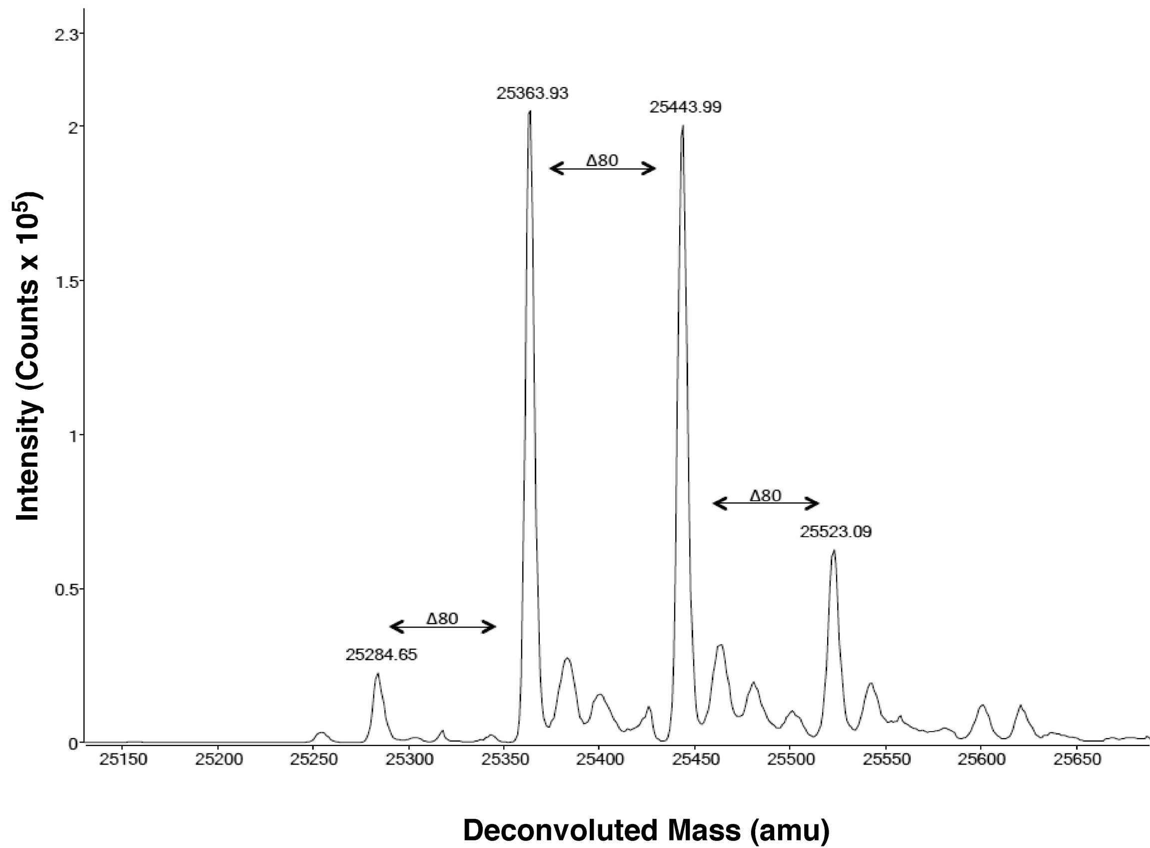
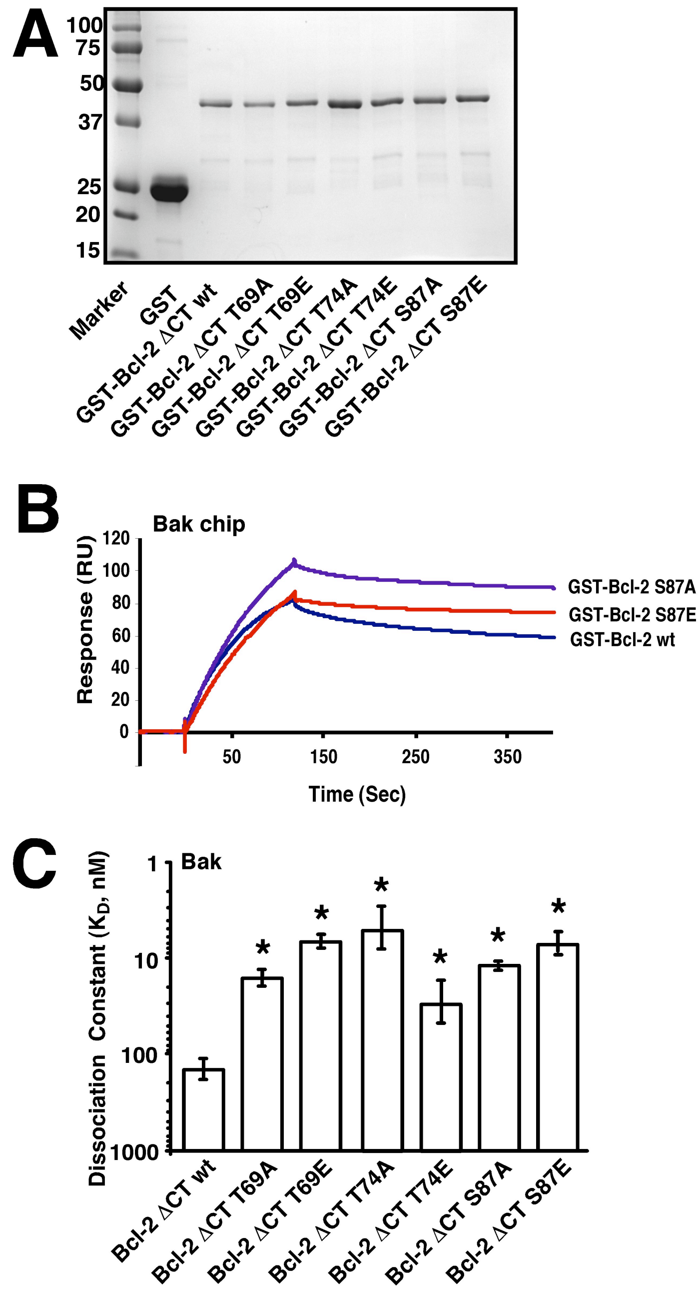
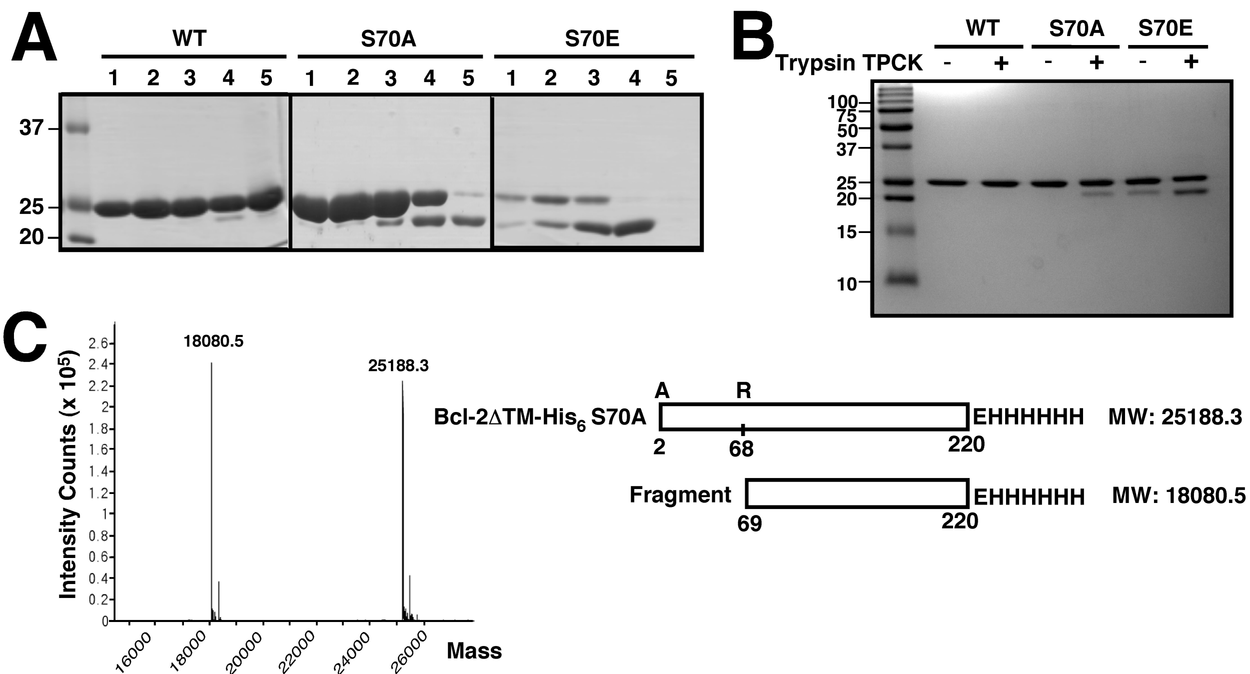


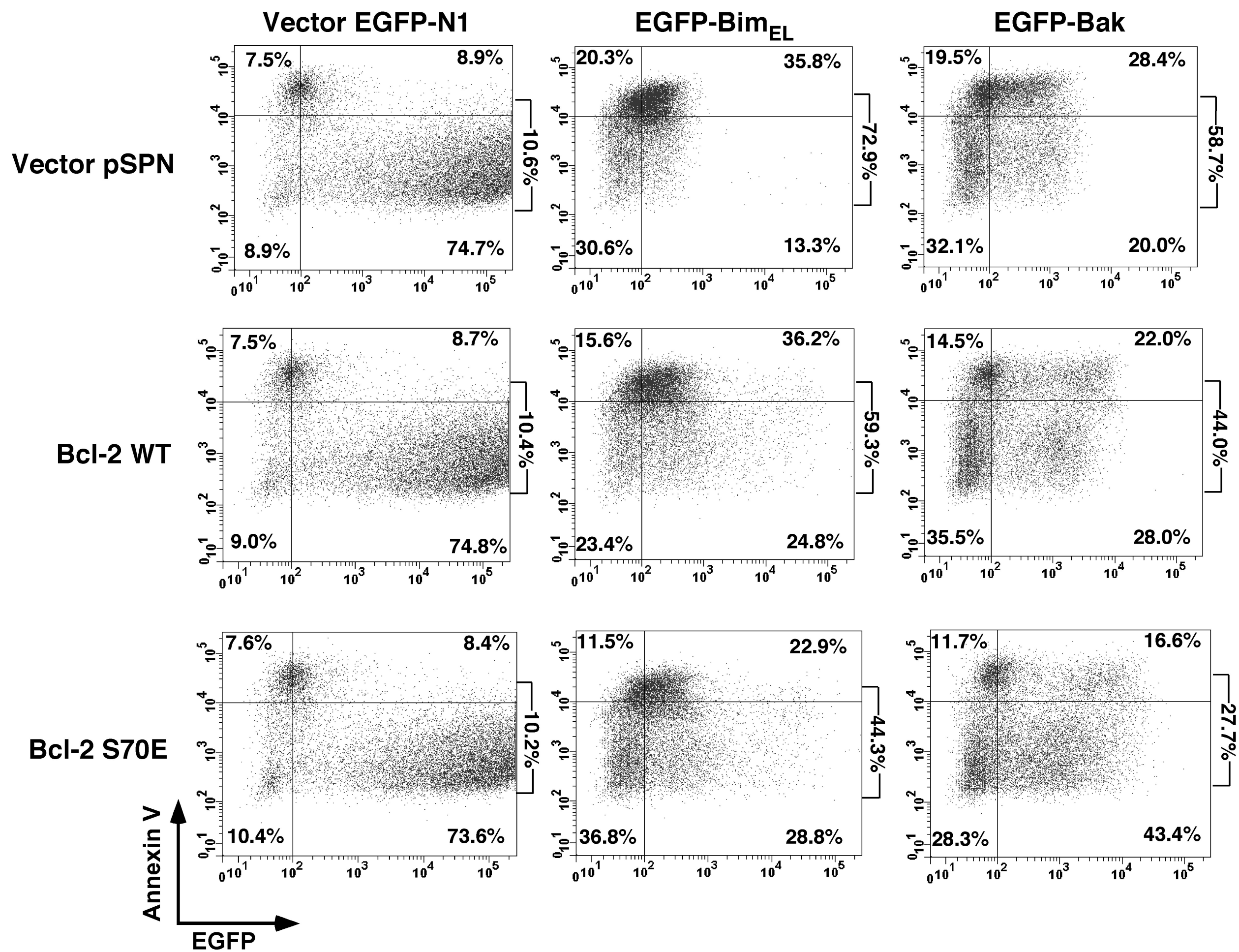

**Supplementary Figure 1. Time-of-flight mass spectrometry of phosphorylated Bcl-2.** After purified Bcl-2ΔTM-$His_6$ was phosphorylated by CDK1/cyclin B *in vitro* as illustrated in Fig. 1A, samples were analyzed by time-of-flight mass spectrometry.



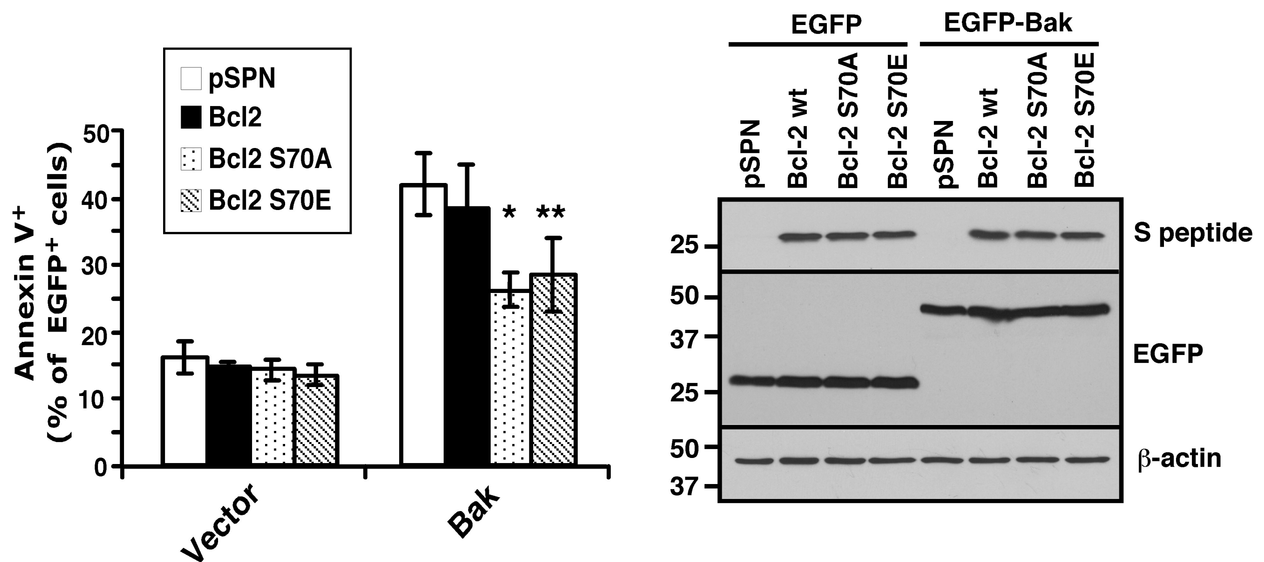

**Supplementary Figure 2. $Thr^{69}$, $Thr^{74}$ and $Ser^{87}$ mutations increase affinity of Bcl-2 for Bak. A,** Coomassie blue-stained gel of the indicated purified, recombinant proteins. **B,** binding of 320 nM GST-Bcl-2ΔTM (wt, S87A, S87E) to immobilized BakΔTM was compared using the same chip. **C,** based on the surface plasmon resonance assay, the affinities of Bcl-2 (wt or mutants) for immobilized BakΔTM were calculated. Error bars, ± SD of three independent experiments. *, $p < 0.002$ vs. wildtype after Bonferronni correction. All other pairwise comparisons failed to reach statistical significance after correction for multiple comparisons.



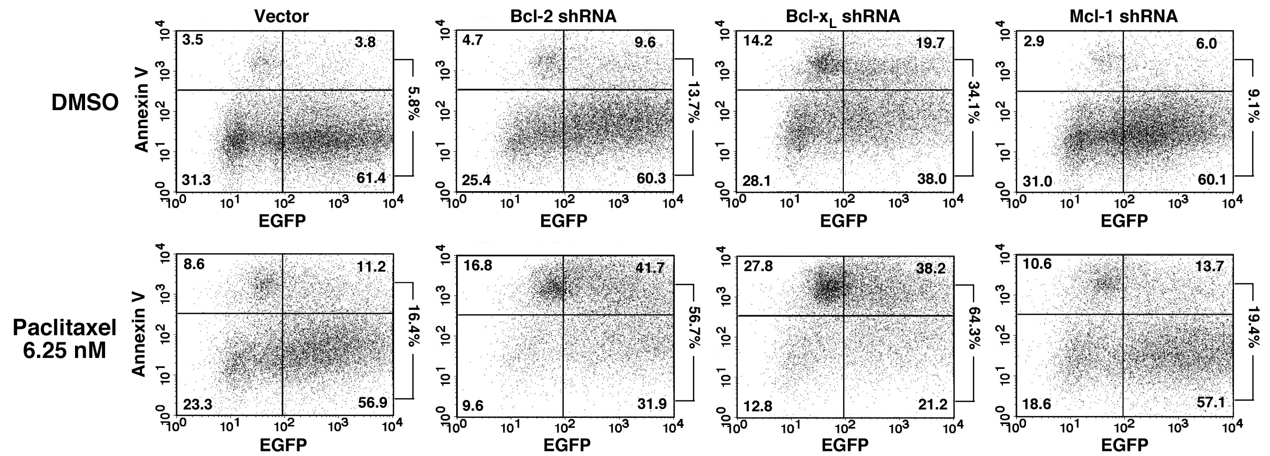

**Supplementary Figure 3. Ser[70] mutations alter Bcl-2 conformation. A,** after isolation on $Ni^{2+}$-NTA beads, Bcl-2ΔTM-$His_6$ (wt, S70A or S70E) was further purified by FPLC on a mono Q column. Fractions (identified as 1-5) were separated by SDS-PAGE and stained with Coomassie blue. **B,** after 2.5 µg purified Bcl-2ΔTM-$His_6$ (wt, S70A, or S70E) was incubated with (+) or without (-) 5 ng trypsin-TPCK at 30°C for 30 min, reaction products were subjected to SDS-PAGE and stained with Coomassie blue. **C,** mass spectrum of Bcl-2ΔTM-$His_6$ S70A after partial digestion with trypsin. The mass of the major fragment is consistent with cleavage at Arg[68].



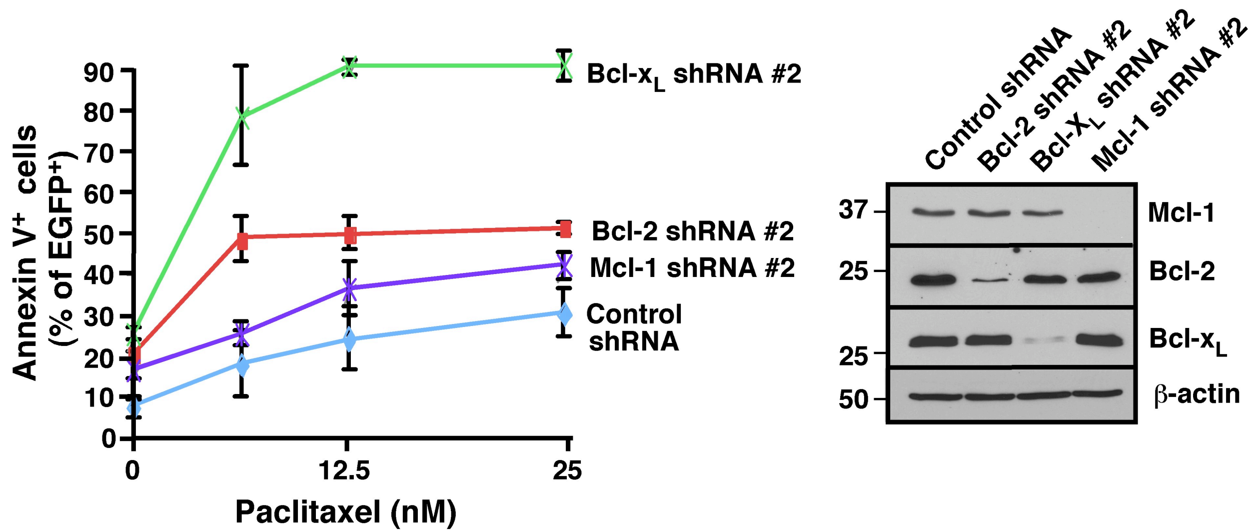

**Supplementary Figure 4. Effect of various Bcl-2 constructs on apoptosis induction by EGFP-Bim$_{EL}$ and EGFP-Bak.** After transfection with 10 µg empty vector, EGFP-Bim$_{EL}$ or EGFP-Bak together with 40 µg pSPN or S peptide-tagged Bcl-2 (wt, S70A or S70E), Jurkat cells were incubated for 24 h in the presence or absence of 5 µM QVD-OPh, sedimented and stained with APC-annexin V. Dot plots from a representative experiment are shown. Summarized results are presented in Fig. 2C.



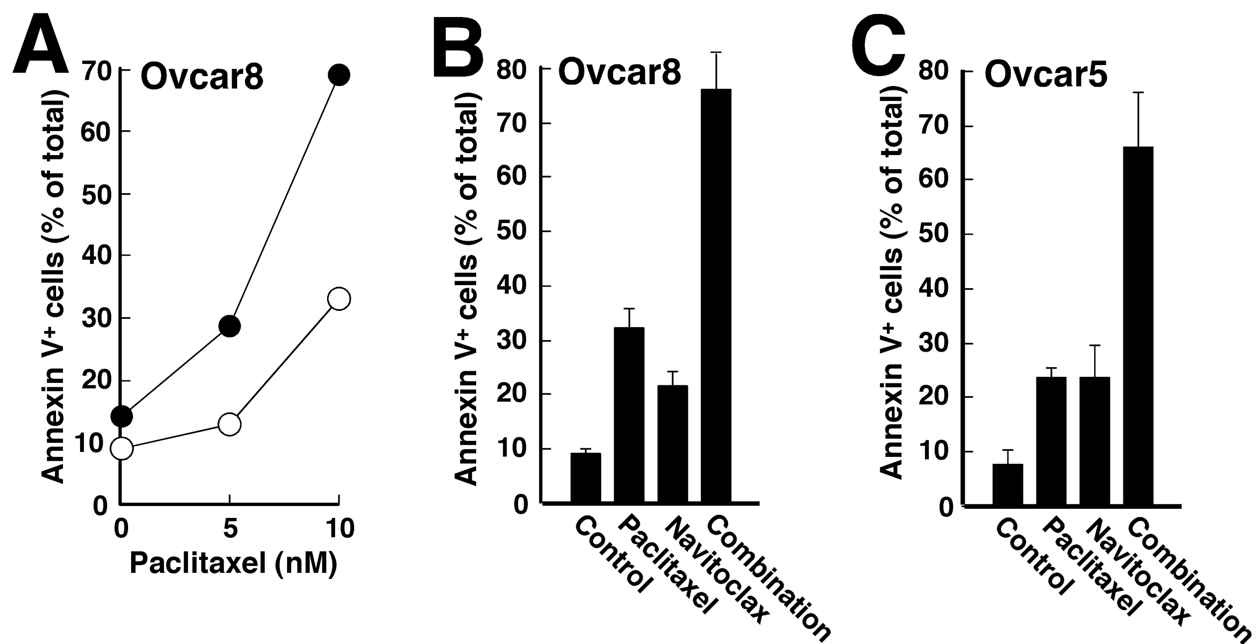

**Supplementary Figure 5. Bcl-2 Ser$^{70}$ modifications increase MEF cell resistance to Bak-induced apoptosis.** After transfection with 20 µg EGFP-C1 (control) or EGFP-Bak together with 20 µg pSPN or pSPN encoding Bcl-2 (wt, S70A or S70E), MEFs were incubated for 24 h and stained with APC-annexin V as illustrated in Figs. 2 and S4. **Graph at left:** percentage of EGFP$^+$ cells that are also annexin V$^+$. Error bars, ± SD from three independent experiments. *, **, $p$ = 0.03 and 0.06, respectively, vs. empty vector after Bonferronni correction. **Blot at right:** After transfection, MEFs were incubated for 24 h in the presence of 10 µM Q-VD-OPh. Cell lysates were then subjected to SDS-PAGE and probed with antibodies to the indicated antigens to confirm equal transgene expression.



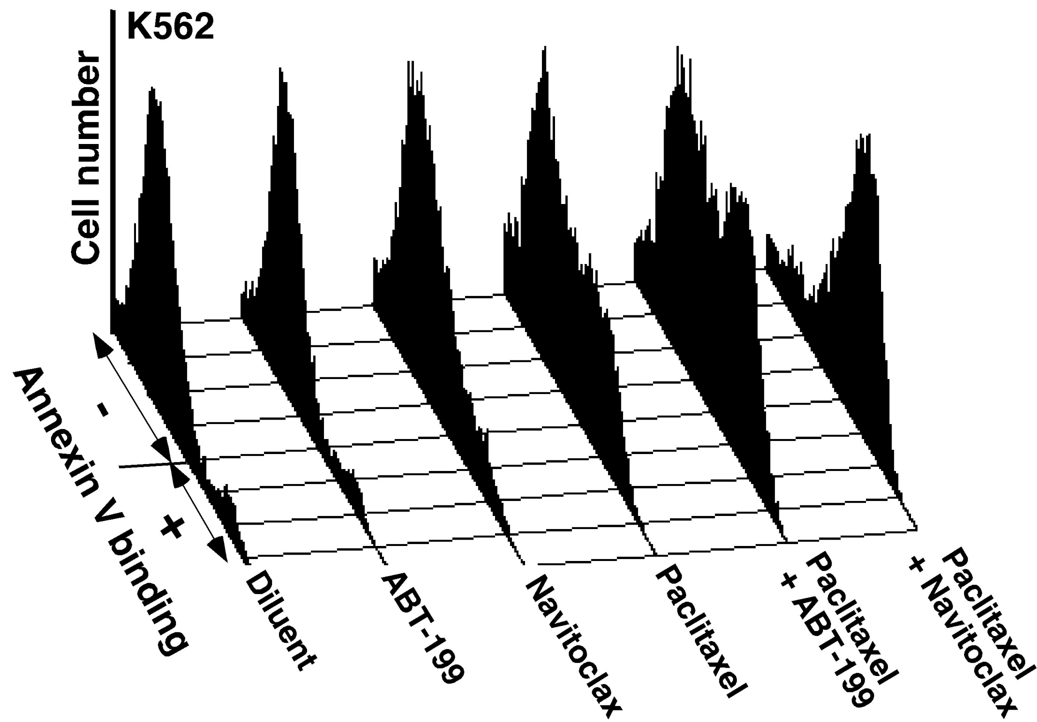

**Supplementary Figure 6. Effect of RNA interference for Bcl-2, Bcl-$x_L$ and Mcl-1 on induction of apoptosis by paclitaxel.** Beginning 24 h after K562 cells were transiently transfected with empty vector (PCMS5A), PCMS5A-Bcl-2 shRNA, PCMS5A-Bcl-$x_L$ shRNA or PCMS5A-Mcl-1 shRNA (all with EGFP-histone H2B expression), cells were treated with paclitaxel for another 48 h, and stained with APC-annexin V. Dot plots from a representative experiment are shown. Summarized results are presented in Fig. 4D.




**Supplementary Figure 7. Effects of a second set of shRNAs targeting Bcl-2, Bcl-$x_L$ or Mcl-1 on paclitaxel-induced apoptosis. Left,** 24 h after K562 cells were transiently transfected with empty vector, Bcl-2 shRNA #2, Bcl-$x_L$ shRNA #2 or Mcl-1 shRNA #2 (all cotransfected with EGFP-histone H2B), cells were treated with paclitaxel for another 48 h, stained with APC-annexin V and analyzed as illustrated in Fig. S6. The percentage of $EGFP^+$ cells that stained with annexin V is indicated. **Right,** immunoblots showing knockdown of the targeted protein.




**Supplementary Figure 8. Effect of navitoclax on paclitaxel-induced apoptosis in additional ovarian cancer cell lines. A,** after Ovcar8 cells were treated for 48 h with the indicated concentrations of paclitaxel in the presence of diluent (open circles) or 1 µM navitoclax (closed circles), cells were stained with APC-annexin V and subjected to flow microfluorimetry as illustrated in Figs. 5A and S9. **B, C,** Ovcar8 (B) or Ovcar 5 cells (C) were treated diluent, 10 nM paclitaxel, 1 µM navitoclax or 10 nM paclitaxel + 1 µM navitoclax (combination) for 48 h, stained with APC-annexin V and subjected to flow microfluorimetry. Bars, mean ± SD of 3 independent assays.




**Supplementary Figure 9. Comparison of ability of ABT-199 and navitoclax to sensitize cells to paclitaxel.** K562 cells treated with diluent or paclitaxel (25 nM) with or without ABT-199 (1 μM) or navitoclax (1 μM) for 48 h were stained with APC-annexin V and subjected to flow microfluorimetry.