

Supplemental Figure S1

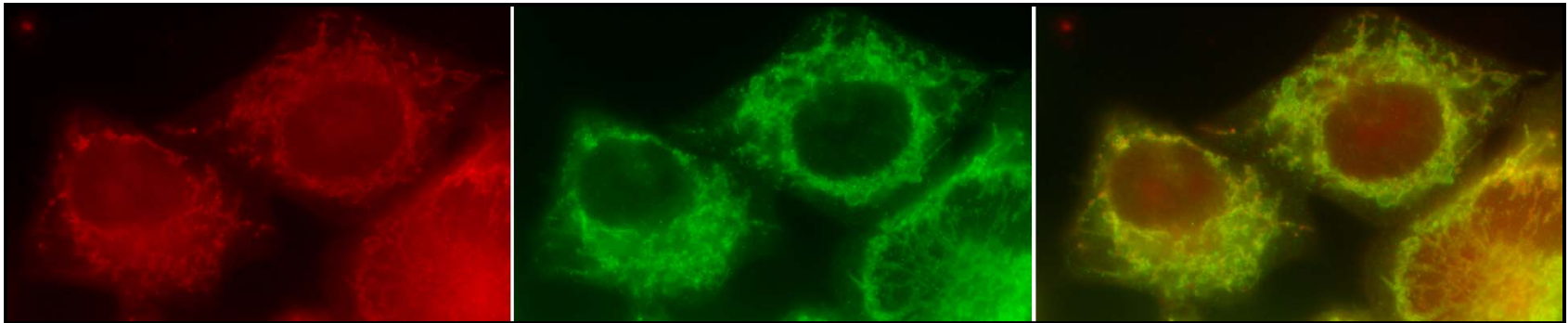


Fig. S1: Immunofluorescent localization of HA-Bcl-xL. KB-3/HA-Bcl-xL cells were fixed and permeabilized. Mitochondria (red signal, left panel) was visualized with Mitotracker Red, and Bcl-xL (green signal, center panel) was localized by probing with rabbit polyclonal Bcl-xL antibody and FITC goat anti-rabbit secondary antibody. Right panel, overlay image.

Supplemental Figure S2

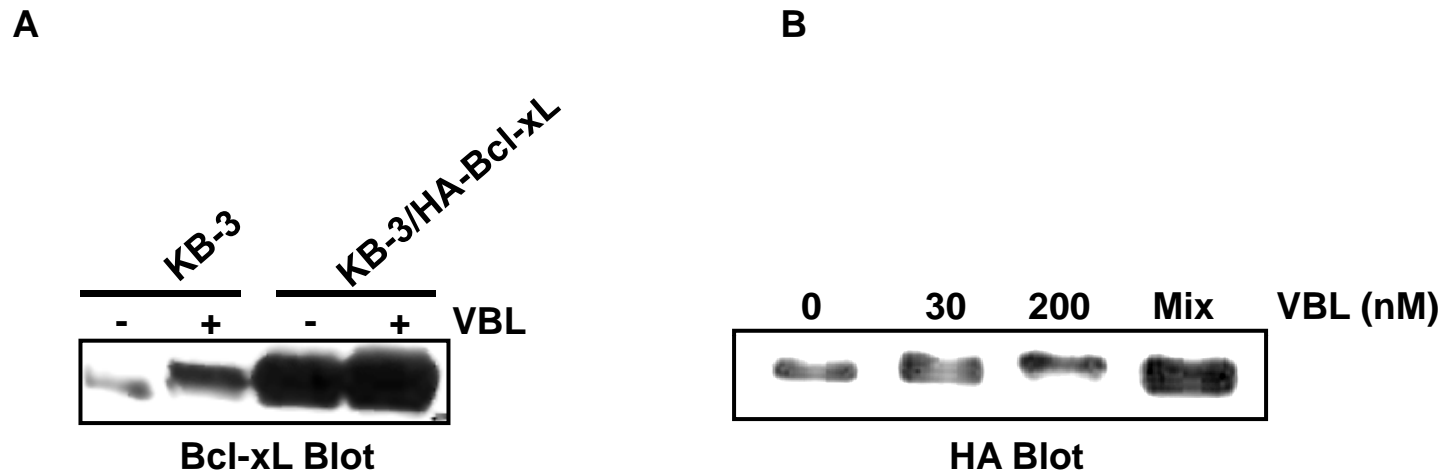


Fig. S2:HA-Bcl-xL undergoes vinblastine-induced phosphorylation. **A**, KB-3 or KB-3/HA-Bcl-xL cells were untreated or treated with vinblastine (VBL; 30 nM) and extracts (50 μ g) subjected to immunoblotting with Bcl-xL antibody. **B**, KB-3/HA-Bcl-xL cells were either untreated or treated with 30 or 200 nM VBL (16 h) and 3 μ g protein/lane analyzed for HA immunoreactivity by blotting. “Mix” indicates a mixture of samples prepared from cells at 0 and 200 nM VBL.

Supplemental Figure S3

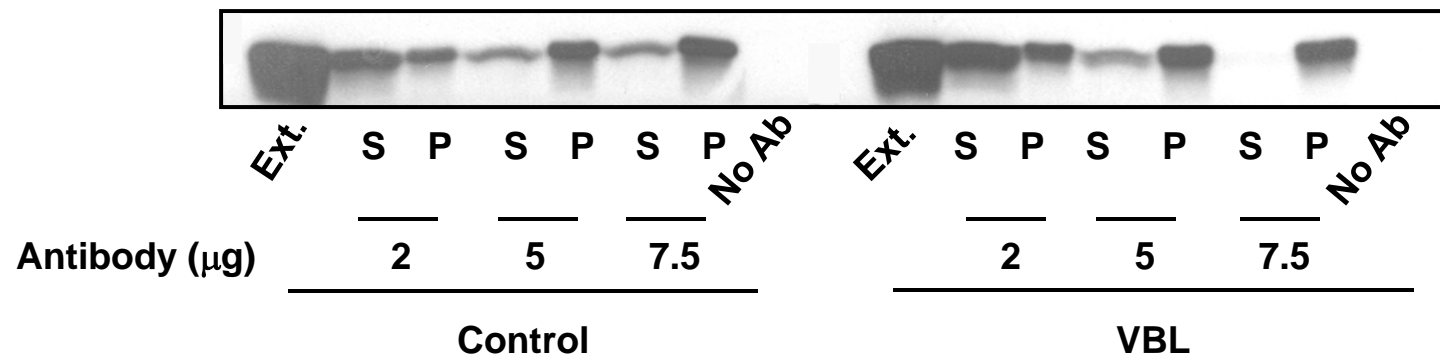
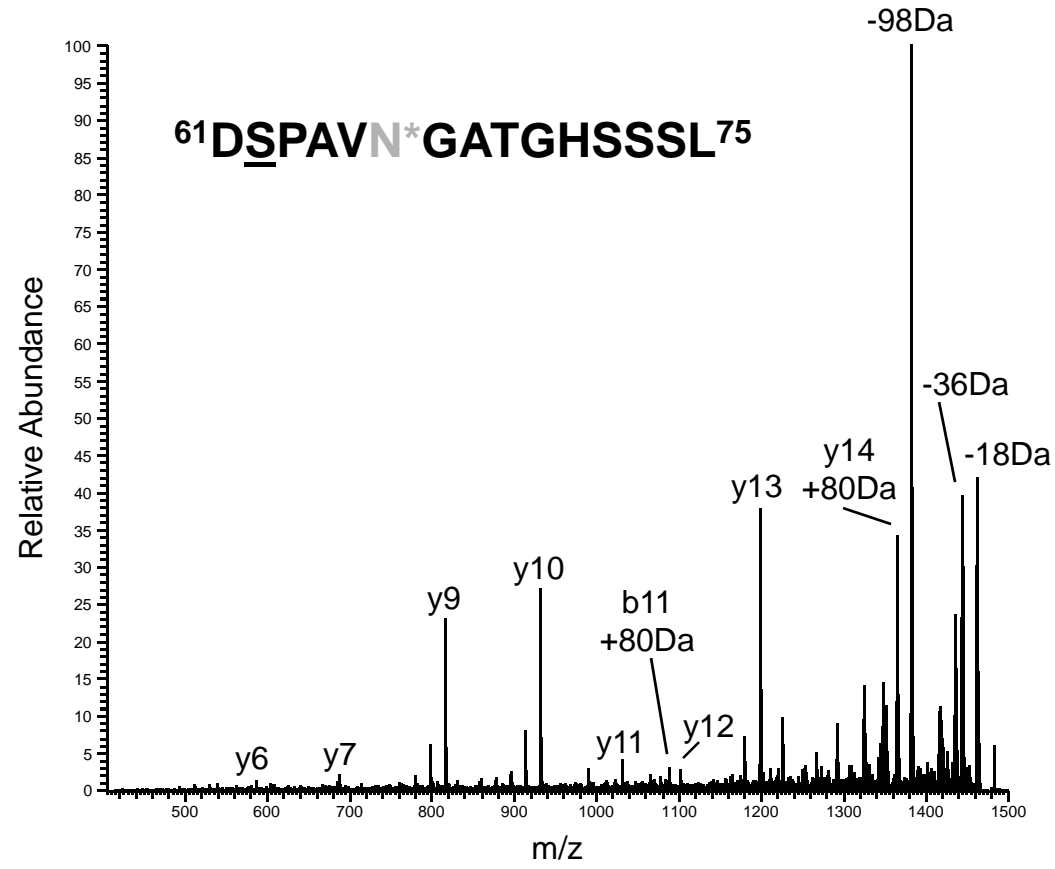


Fig. S3: Quantitative immunoprecipitation of Bcl-xL. Extracts (Ext.) were prepared from untreated or vinblastine (VBL, 200 nM) treated KB-3/HA-Bcl-xL cells and 1 mg aliquots were subjected to incubation with increasing concentrations of rabbit Bcl-xL antibody as indicated. Immunoprecipitates (P) and supernatants (S) were immunoblotted with mouse monoclonal Bcl-xL antibody. “No Ab” indicates immunoprecipitation conducted in the absence of antibody.

Supplemental Figure S4

A



Supplemental Figure S4

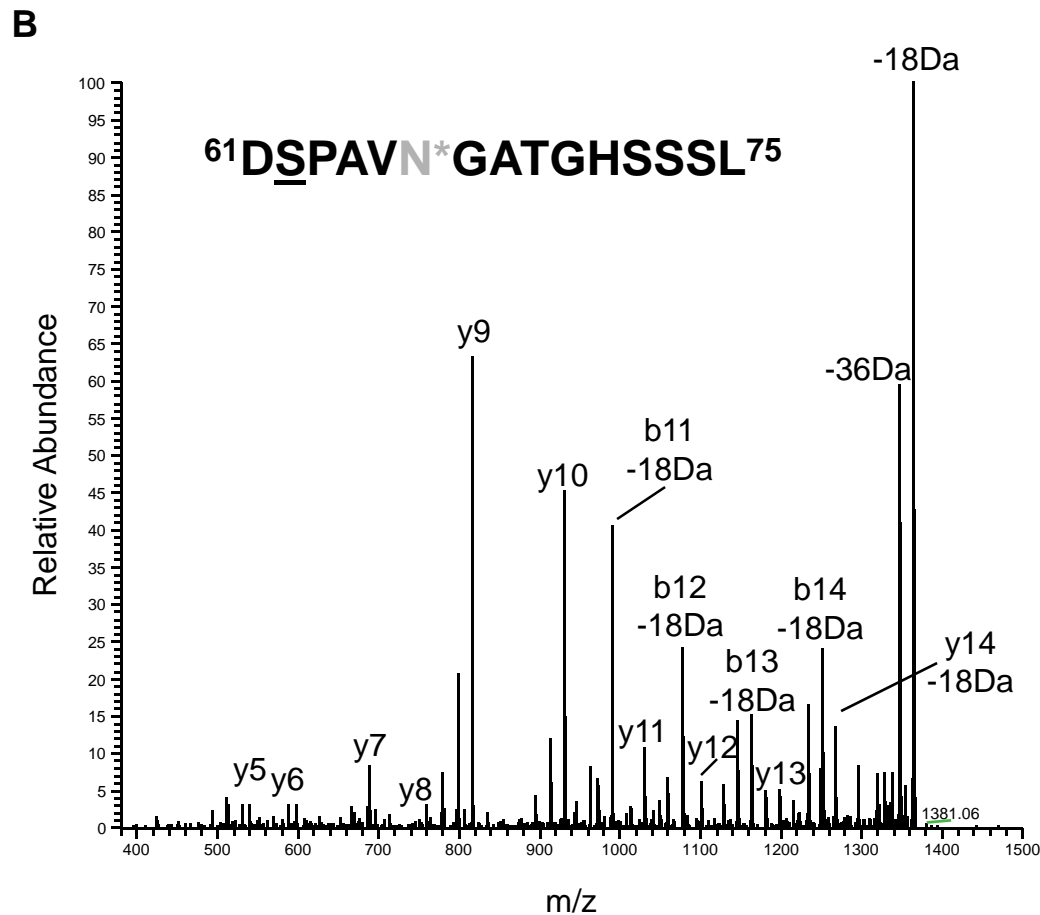


Fig. S4: Tandem mass spectrometric analysis mapping the single phosphorylation site Ser62 (underlined) in phosphopeptide 61-75. **A**, The signature phosphopeptide neutral loss of 98 Da from the parent ion is indicated. Fragment ions containing the phosphorylated amino acid will have a mass addition of 80 Da. Peptide deamidation was mapped to Asn66. **B**, MALDI-MS3 analysis of the -98Da fragment from vinblastine-treated HA-Bcl-xL phosphopeptide 61-75 provided further confirmation that Ser62 was phosphorylated and Asn66 was deamidated. Fragment ions that contained the phosphorylated amino acid will show an 18 Da mass loss.

Supplemental Figure S5

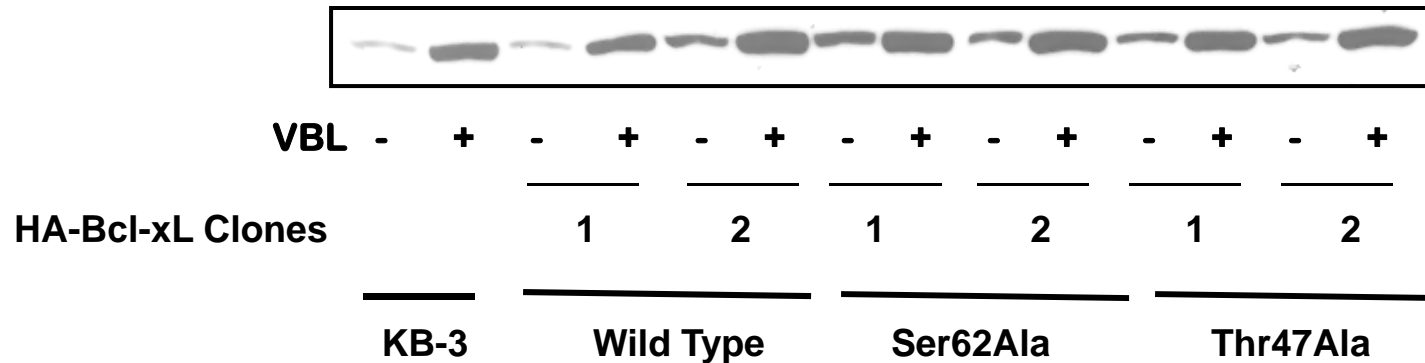


Fig. S5: Ser62 to Ala mutation of Bcl-xL does not alter mitotic arrest caused by vinblastine treatment. KB-3 cells or cell lines overexpressing wild-type Bcl-xL, Bcl-xL(S62A) or Bcl-xL(T47A) (2 clones of each) were untreated or treated with 30 nM vinblastine (VBL) for 16 h and cell extracts subjected to immunoblotting for cyclin B