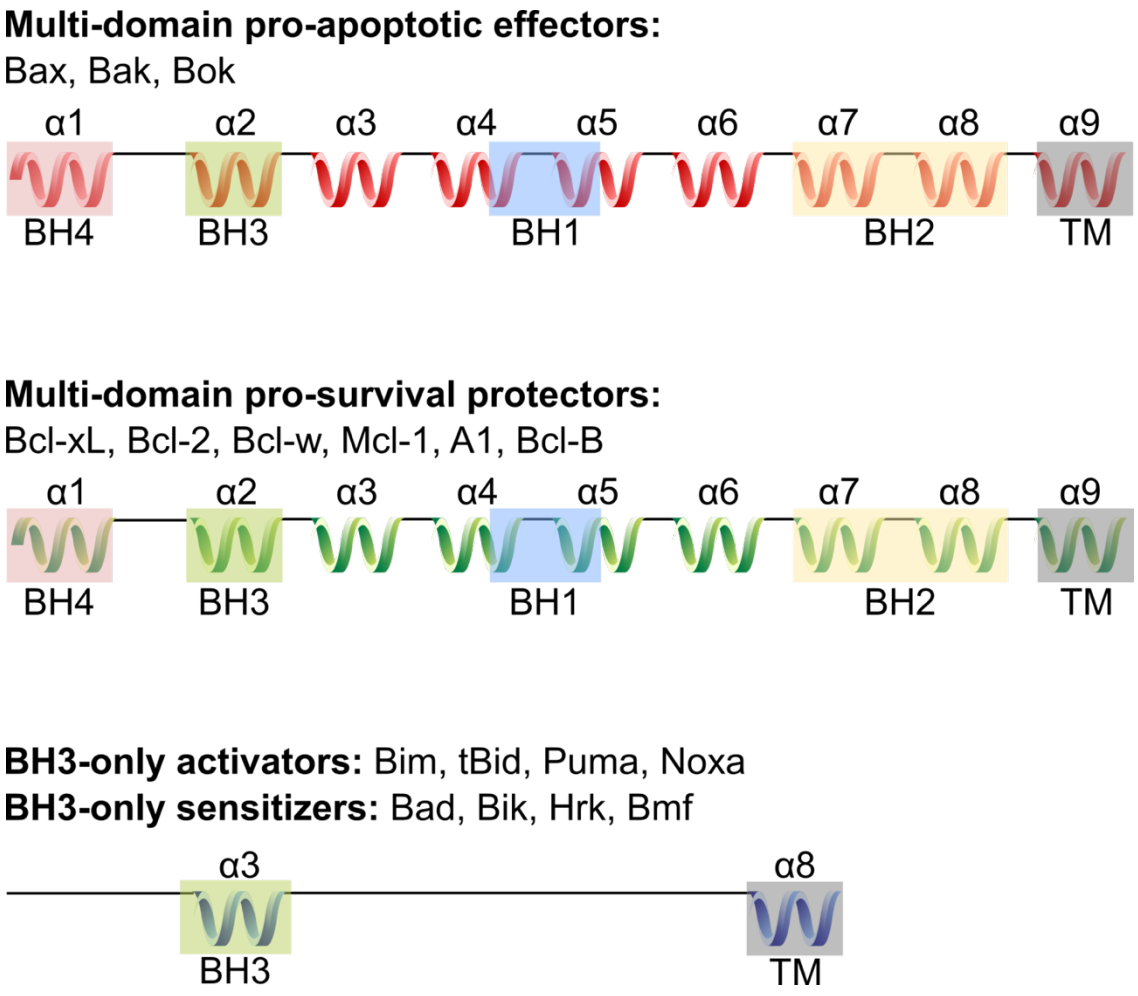
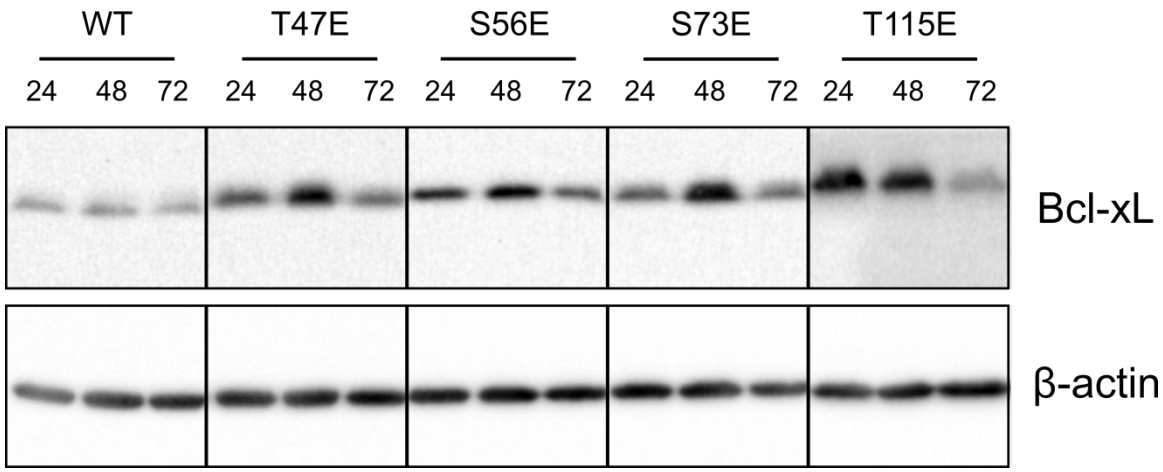
Electronic Supplementary Material (ESI) for Faraday Discussions.

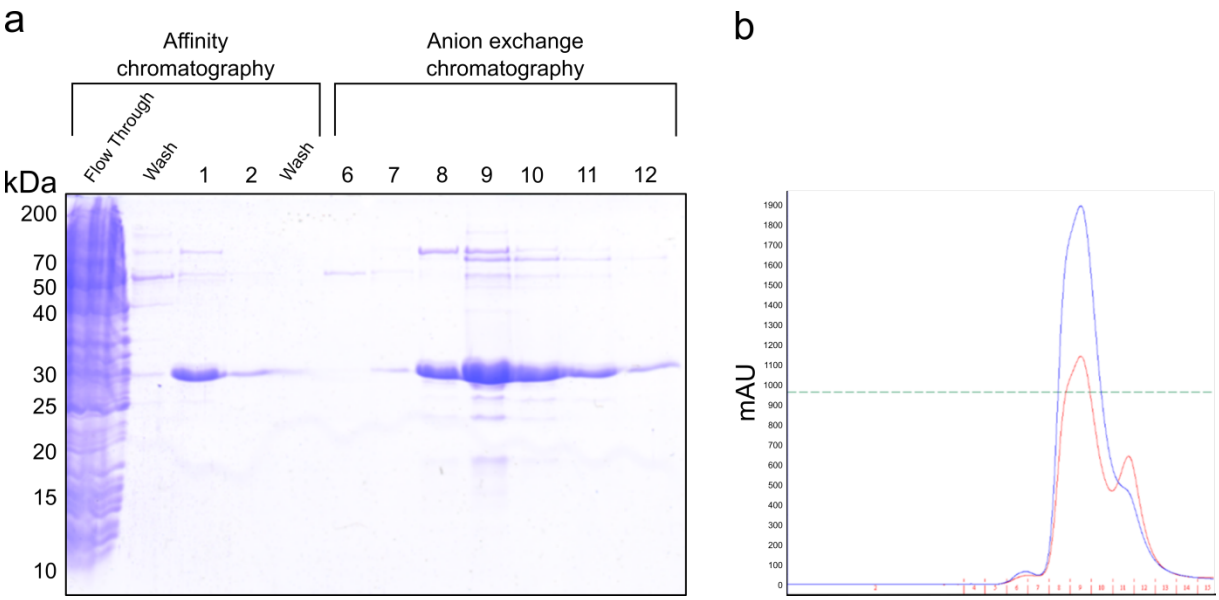

# Supplementary Figures



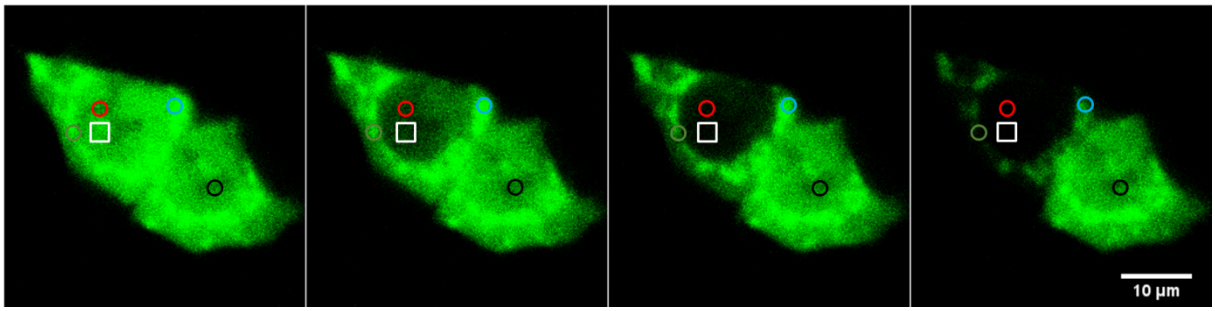

**Supplementary Figure 1. Sequence- and structure-homology in the Bcl-2 protein family.** Based on their pro-apoptotic and pro-survival activities and the Bcl-2 Homology (BH)-domains they contain, the Bcl-2 proteins are divided into 3 sub-groups. Additionally, they have a Transmembrane (TM)-domain at C-terminus for insertion into cellular membranes.



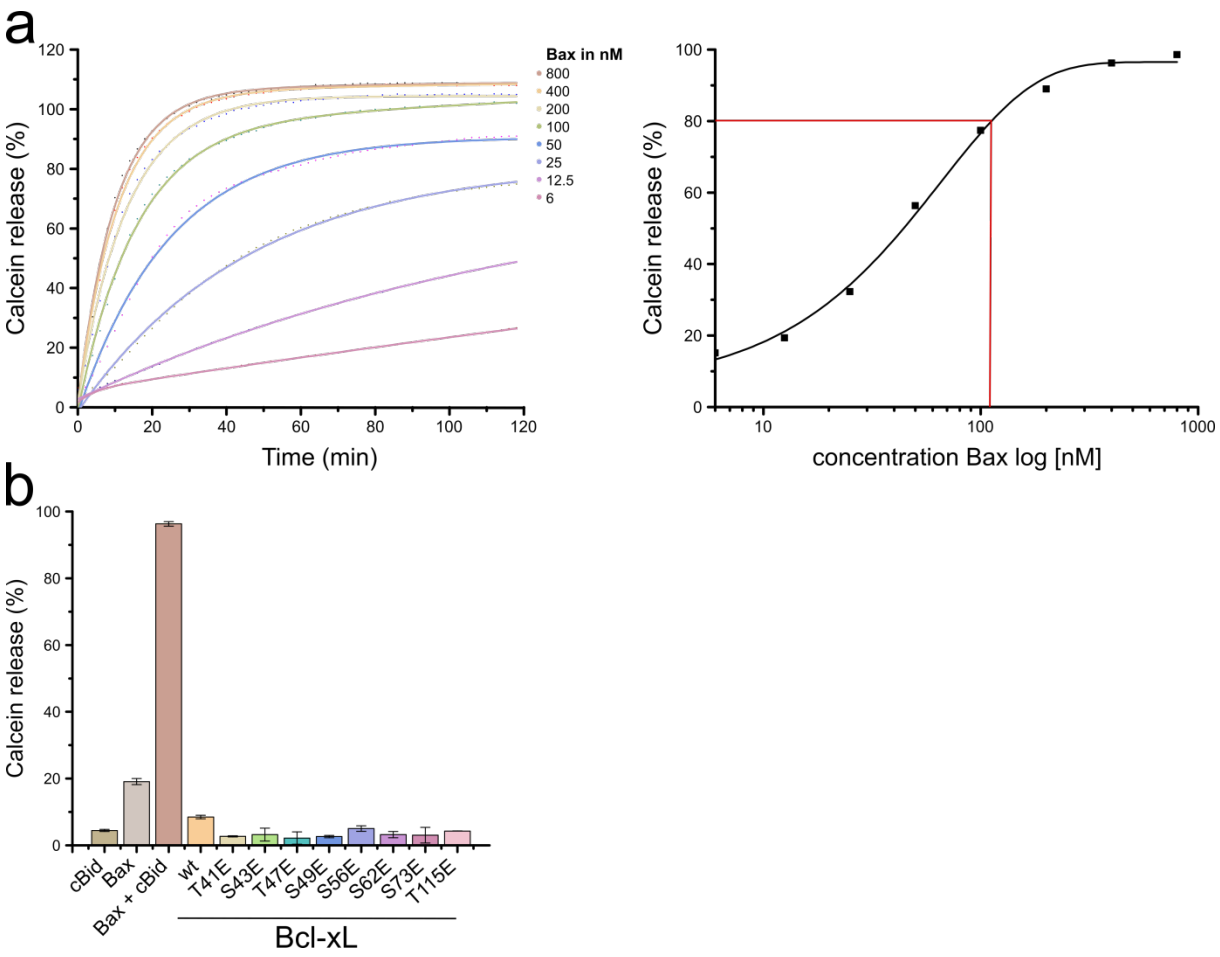

**Supplementary Figure 2. Protein levels of Bcl-xL-wt and phospho-mimetic variants.** HCT116 allBcl-2 KO cells were transfected transiently with Bcl-xL-wt or the phospho-mimetic Bc-xL variants and the expression levels at 24, 48 and 72 hours were analyzed by western blot. Protein lysates were immunoblotted for Bcl-xL and β-Actin.




**Supplementary Figure 3. Purification of recombinant Intein-tagged Bcl-xL. (a)** Analysis of total protein by SDS-page and coomassie staining at various stages of purification using chitin-binding beads for affinity chromatography followed by anion exchange chromatography **(b)** Chromatogram of the purification of recombinant Bcl-xL. Blue: UV280 absorbance. Red: UV260 absorbance.




**Supplementary Figure 4. FLIP analysis to compare the mitochondrial/cytosolic shuttling of proteins.** The fluorescence of tBid-GFP transiently expressed in HCT116 allBcl-2 KO cells repeatedly bleached and monitored in the cytoplasm for the whole time of measurement represented by the white square. The changes in fluorescence intensities of tBid-GFP in mitochondrial compartments are represented by red, green and blue circles and analyzed based on their distances to the bleaching area. The black circle represents the control fluorescence intensity in a neighboring, non-bleached cell.




**Supplementary Figure 5. In vitro analysis of pore forming activity of recombinant Bax. (a)** Left panel: % Calcein release from LUVs incubated with different concentrations of recombinant Bax and activated by cBid. Right panel: Effective Concentration of Bax required for 80% calcein release from LUVs. **(b)** Calcein release from LUVs incubated with Bax and cBid (positive control) and the single Bcl-xL variants (negative controls).