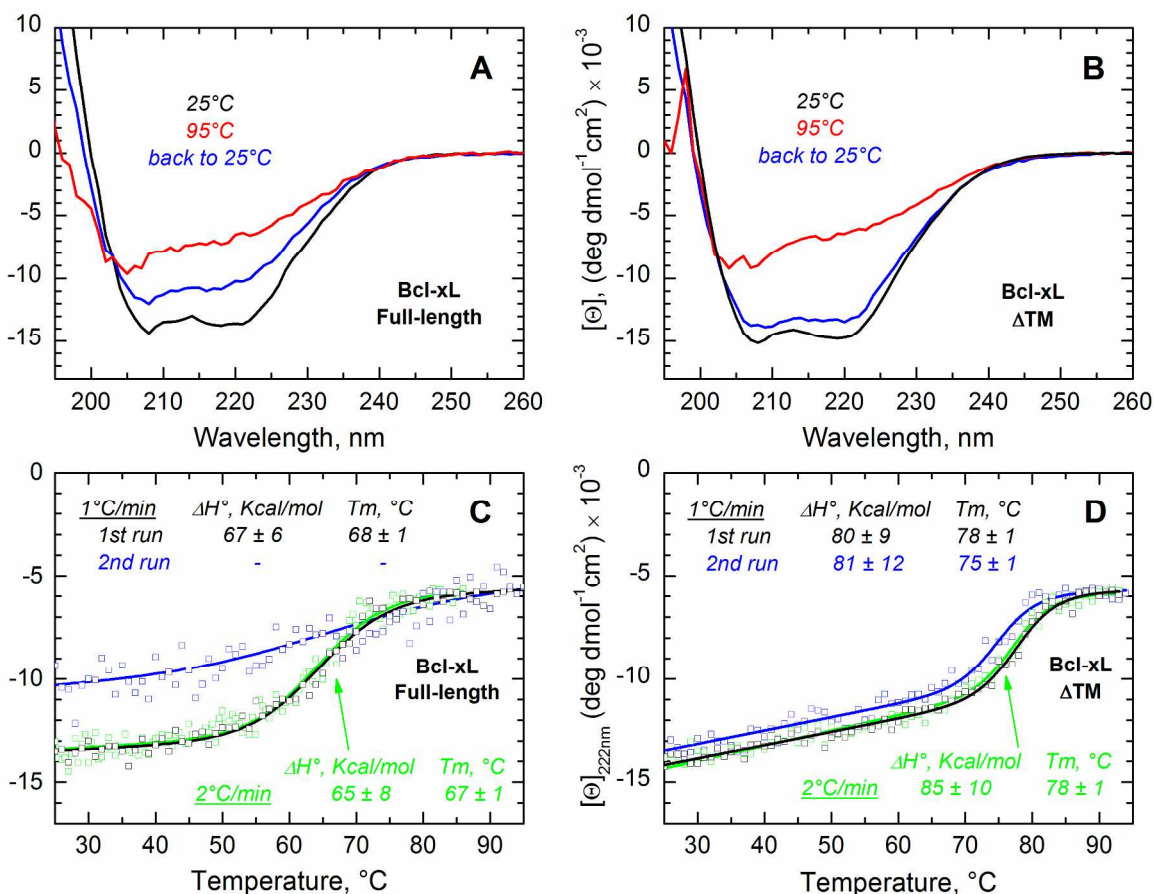
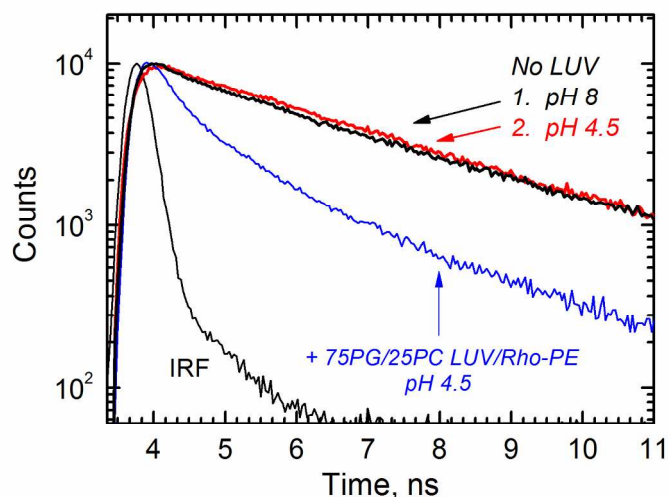


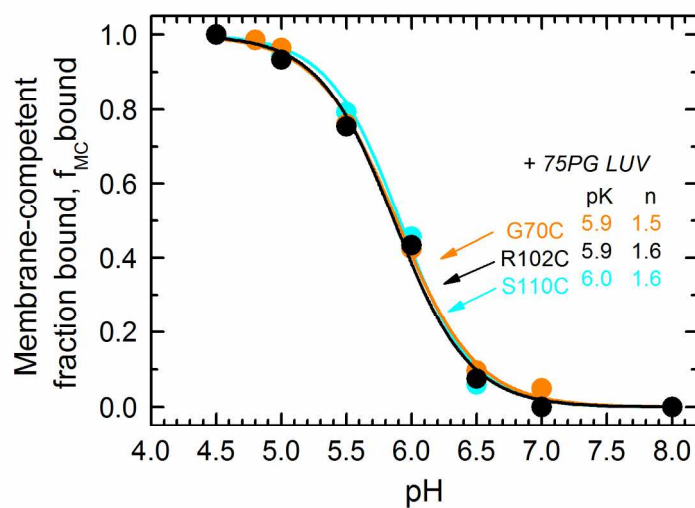
SUPPLEMENTARY INFORMATION



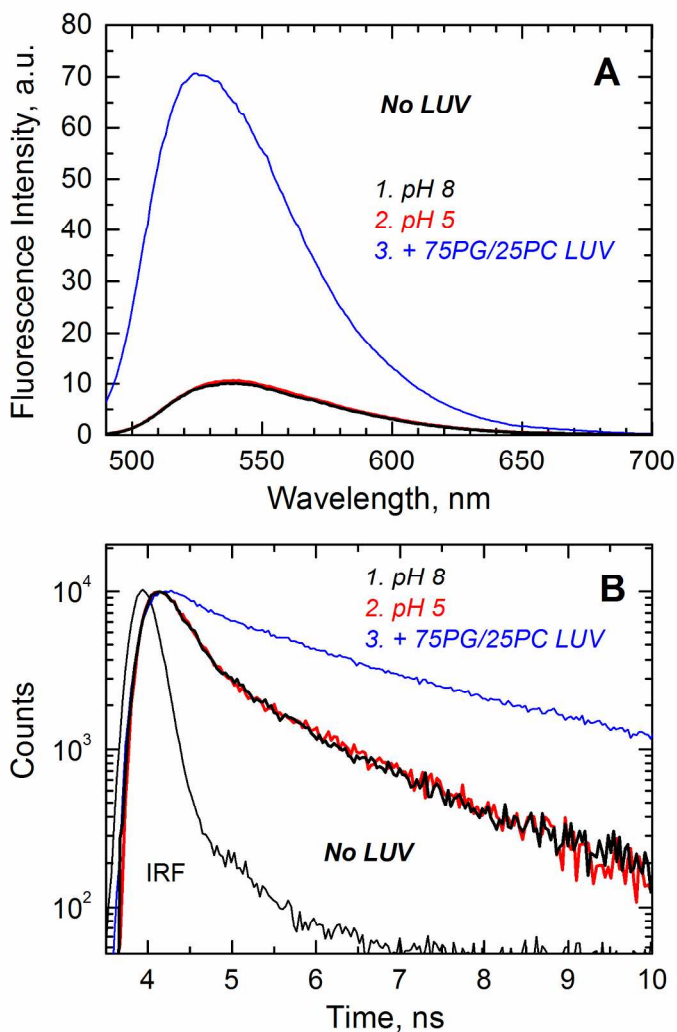
SUPPLEMENTARY FIGURE S1. Thermal unfolding of the full-length Bcl-xL and its truncated versions missing C-terminal hydrophobic TM helix measured by CD in solution at pH 8. Panels A (truncated) and B (full-length) show the CD spectra before (black spectra) and after thermal unfolding (red spectra), and after the rapid cooling to 25°C (blue spectra). Panels C (truncated) and D (full-length) show the thermal unfolding of the folded (1st run, black data) and refolded (2nd run, blue data) Bcl-xL. The latter panels also show the thermal unfolding at double rate of heating (green data). The data suggest that reversible and rate-independent transitions in Bcl-xL, which are the prerequisite to quantitative analysis of thermal unfolding, can be achieved by deletion of the C-terminal TM helix.



SUPPLEMENTARY FIGURE S2. Effect of acidification on the fluorescent signal of the donor attached to Bcl-xL in the absence of acceptor-labeled LUV. Fluorescence decay of Bcl-xL labeled at position R102C with Alexa488 was measured in solution at pH 8 (step 1, black trace). Lowering the pH of the solution to 4.5 causes no changes in the fluorescence decay kinetics (step 2, red trace) in the absence of acceptor-labeled LUV (Compare to the blue trace measured in the presence of acceptor-labeled at low pH). This result indicates that lowering pH in the absence of membranes causes no changes in the fluorescent signal of Alexa488-labeled Bcl-xL, and serves as a control for the binding results presented in Fig. 4.



SUPPLEMENTARY FIGURE S3. Binding of Bcl-xL mutants, labeled at different positions, to 75POPG/25POPC LUV followed as a function of the pH. Measurements performed with labels attached to any of the three single-cysteine mutants, G70C (orange), R102C (black) or S110C (cyan), result in overlapping pH-dependent binding curves. This result suggests that the position of labeling does not affect the membrane binding of Bcl-xL.



SUPPLEMENTARY FIGURE S4. Effect of acidification on the fluorescent signal of NBD attached to Bcl-xL in the absence of membranes. The steady-state spectrum (A) and life-time decay (B) of Bcl-xL labeled at position N175C with NBD were measured in solution at pH 8 (step 1, black). The acidification of the solution to pH 5 causes no changes in the fluorescence intensity, wavelength of maximum emission, and fluorescence decay kinetics (step 2, red). The addition of LUV to the previously acidified sample resulted in increase of emission intensity, blue-shift of the spectrum and longer fluorescence decay (blue), indicating that the insertion is path-independent. This result indicates that lowering pH in the absence of membranes causes no changes in the fluorescent signal of NBD-labeled Bcl-xL, and serves a control for the insertion results presented in Figure 6.