

Figure S1: Alternative view of the data presented in Figure 3, broken down based on protein concentration. A: protein concentration of 400 nM, B: 1600 nM

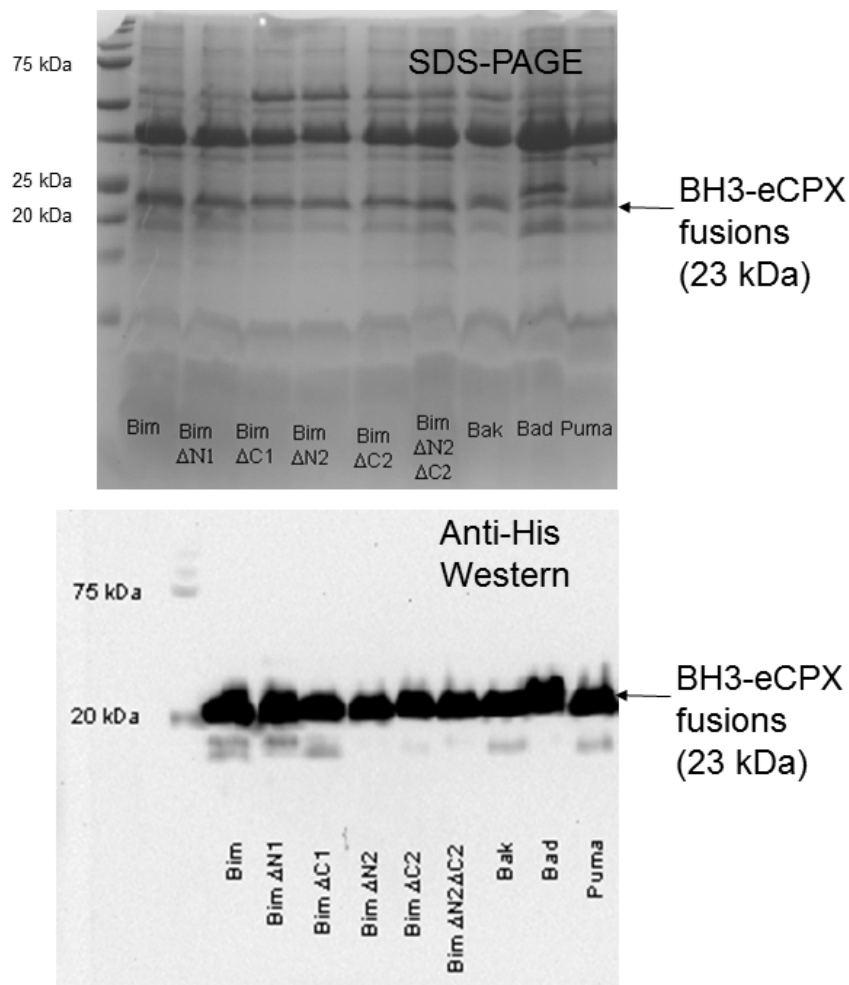


Figure S2: SDS-PAGE (top) and Western blot (bottom) of outer membrane protein preparations (OMPs) from cells expressing different BH3-eCPX fusion constructs. A C-terminal His-tag was added to these constructs to allow for Western blot detection. Detailed protocols for isolation of outer membrane fractions can be found in reference 46. The gel and the Western blot demonstrate that different BH3-eCPX constructs express and are targeted to the outer membrane at similar levels.

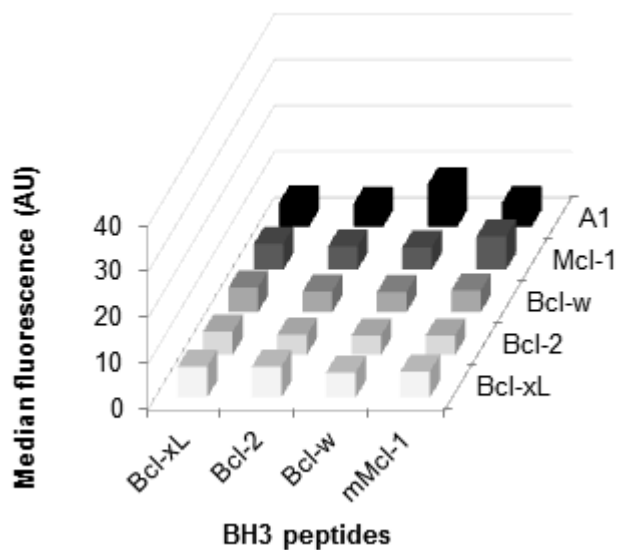
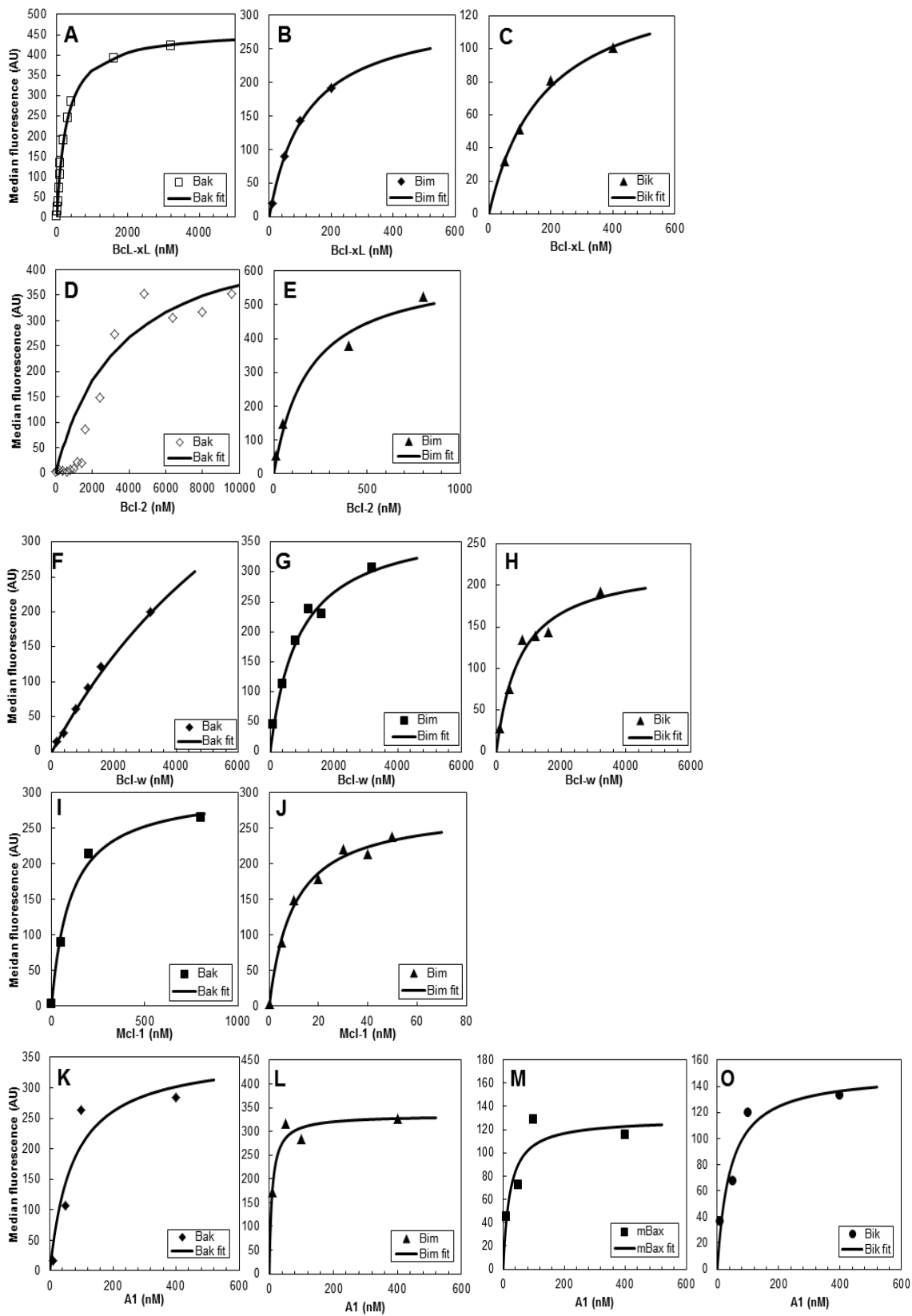


Figure S3: Interactions between anti-apoptotic BH3 peptides and anti-apoptotic proteins (1600 nM). In general these interactions result only in background fluorescence due to no or very weak binding. Signal above background is observed for the mMcl-1 BH3:Mcl-1 interaction and the Bcl-w BH3:A1 interaction.



Caption on the next page

Figure S4: Saturation binding curves for determination of apparent K_d values found in Table 1. A-C: Binding of Bcl-x_L to the Bak, Bim, and Bik BH3 peptides. D,E: Bcl-2 binding to the Bak and Bim peptides. F-H: Bcl-w binding to Bak, Bim, and Bik BH3 peptides. I,J: Mcl-1 binding to Bak and Bim. K-O: A1 binding to Bak, Bim, mBax, and Bik.

Table S1: Apparent K_d (nM) values of Bim 16-mer peptide binding each anti-apoptotic proteins measured in this study, compared with IC_{50} (nM) values of Bim 26-mer peptide determined by Chen *et al.* using competition SPR assays. As discussed in the text, Bcl-w used by Chen *et al.* was 24 aa shorter at the C-terminus than what was used in our study. The Bim 26-mer exhibited significantly higher binding affinity to nearly all anti-apoptotic protein targets when compared with the 16-mer.

	Bcl-x _L	Bcl-2	Bcl-w	Mcl-1	A1
Bim 16-mer	120	190	902	9.78	8.57
Bim 26-mer	4.6	2.6	4.3	2.4	4.3