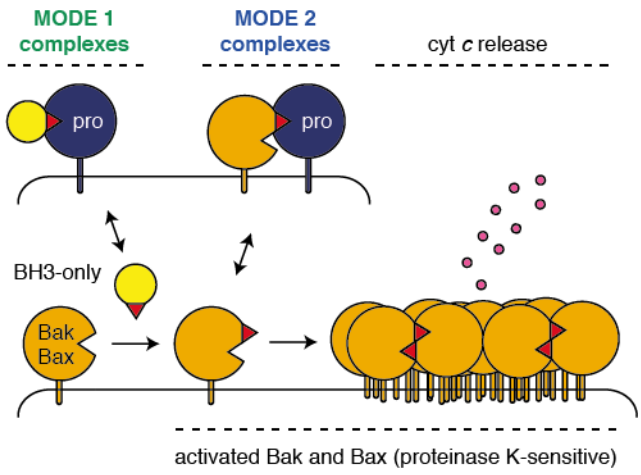
Mcl-1 and Bcl-x_L sequestration of Bak confers differential resistance to BH3-only proteins

A

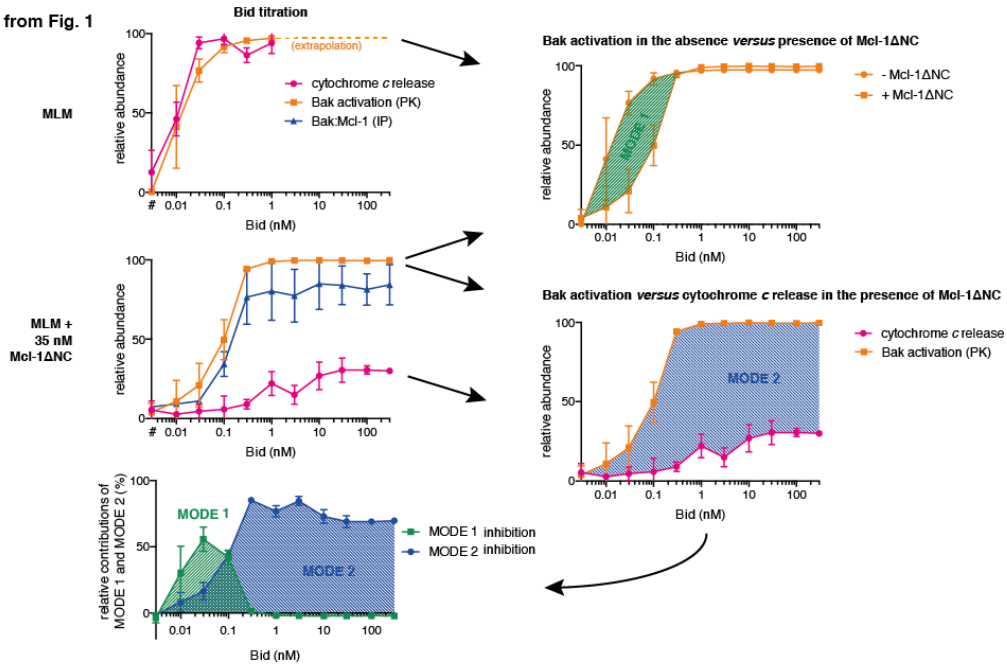


B

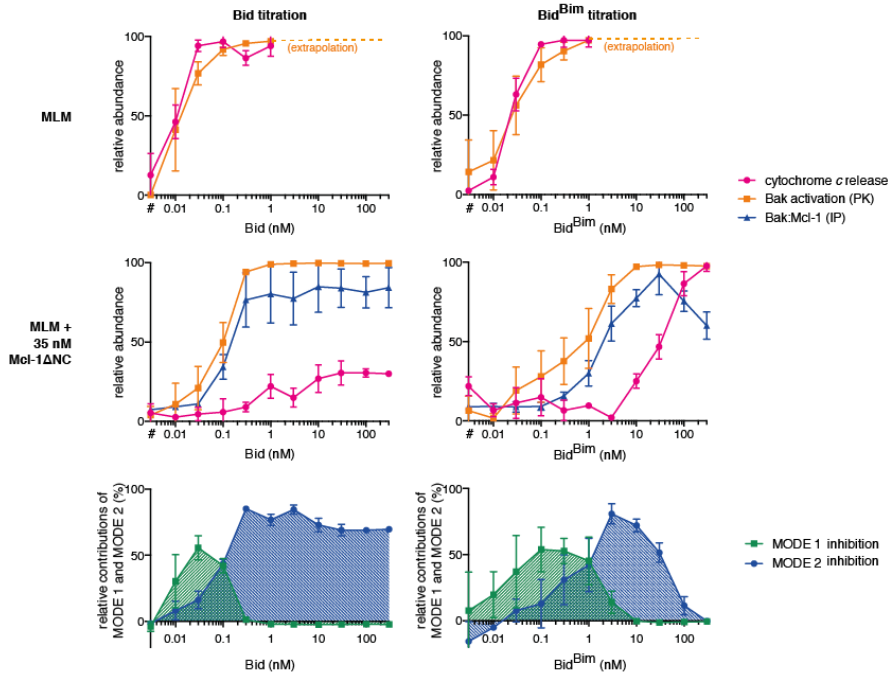
	Bcl-x_L	Mcl-1
BH3 peptide	Half-life (s)	Half-life (s)
Bim	1455.2	2340.4
Bak34	68.0	868.6
Bid	101.8	9.2

Supplementary Figure 1. Regulation of apoptosis by Bcl-2 protein interactions.
A. Unified model of Bcl-2 family function. A prosurvival protein can sequester BH3-only proteins (MODE 1 complexes) or activated Bak and Bax (MODE 2 complexes), as previously described [14]. Note that once Bak is activated, any MODE 1 complexes will compete with MODE 2 complexes, and thus promote cytochrome *c* release.
B. Prosurvival protein:BH3 peptide complex half-lives were determined in direct binding assays using a Biacore S51 instrument exactly as described previously. Half-life calculations were made according to the formula $t_{1/2}=\ln 2/K_d$.

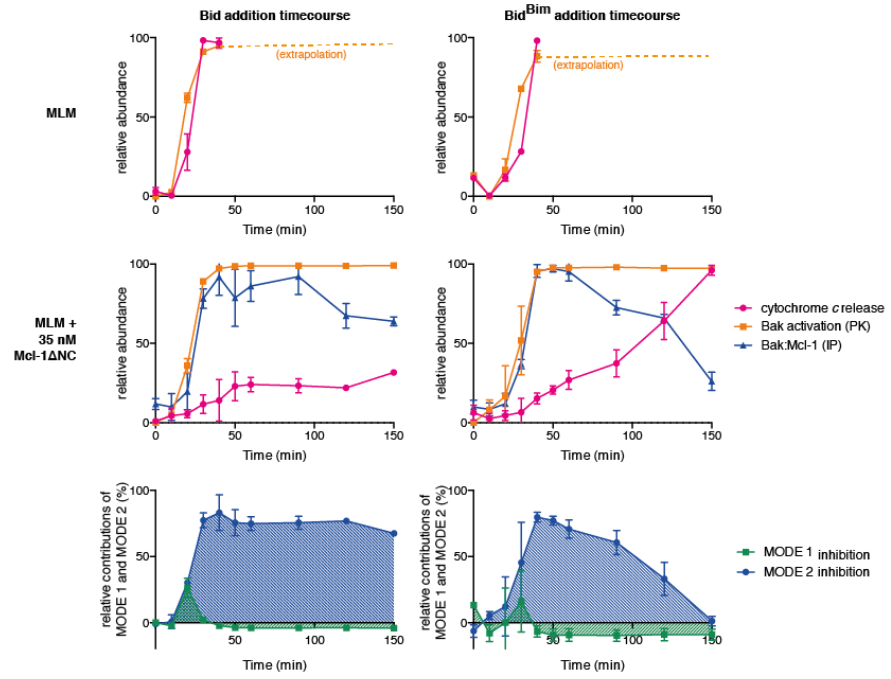
A Based on Bid samples from Fig. 1

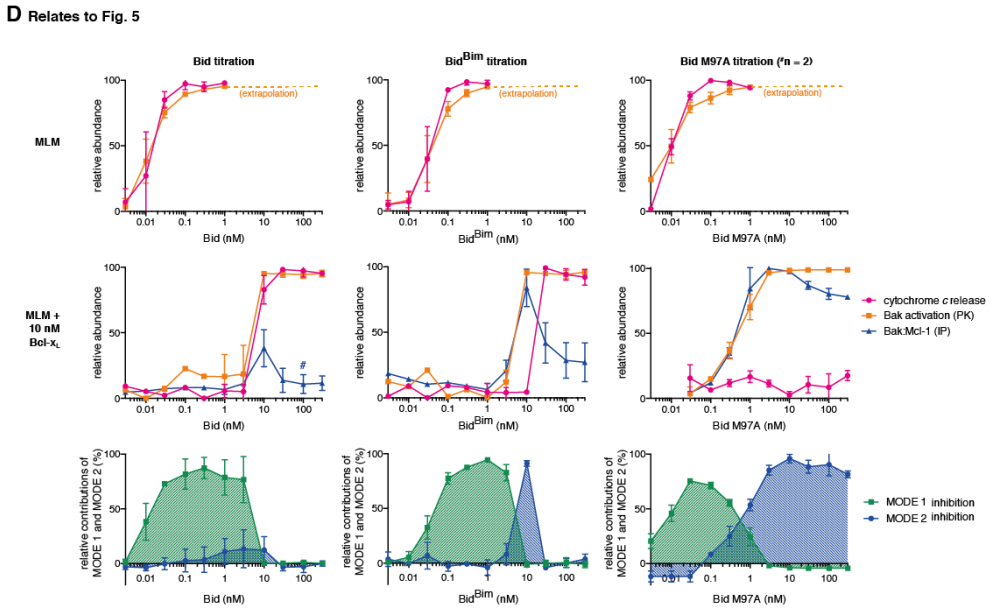


B Relates to Fig. 1



C Relates to Fig. 2





Supplementary Figure 2. Quantitation of western blots to estimate the relative contributions of MODE 1 and MODE 2 inhibition in particular mixtures of Bcl-2 proteins

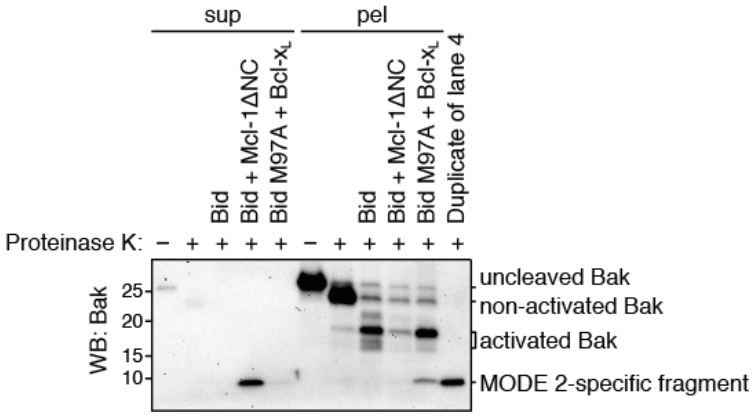
A. Graphs showing how the contributions of MODE 1 inhibition and MODE 2 inhibition have been estimated in B,C and D. Western blots from three independent experiments in Figs 1B and 1C were quantitated by densitometry and the percentage cytochrome *c* release, Bak activation, and Bak:Mcl-1 complex formation estimated as outlined in Methods. MODE 1 inhibition (green shaded area) is the difference in Bak activation when prosurvival protein (Mcl-1) is absent or present. MODE 2 (blue shaded area) is the difference between Bak activation and cytochrome *c* release when prosurvival protein (Mcl-1) is present. (Bak activation at doses higher than those tested experimentally were extrapolated by assuming they were equal to the highest dose tested.)

B. Analysis of western blots from Fig 1. Values are mean +/- SEM of three experiments ($^{\#}$two experiments for 0.003 nM Bid or Bid^{Bim}).

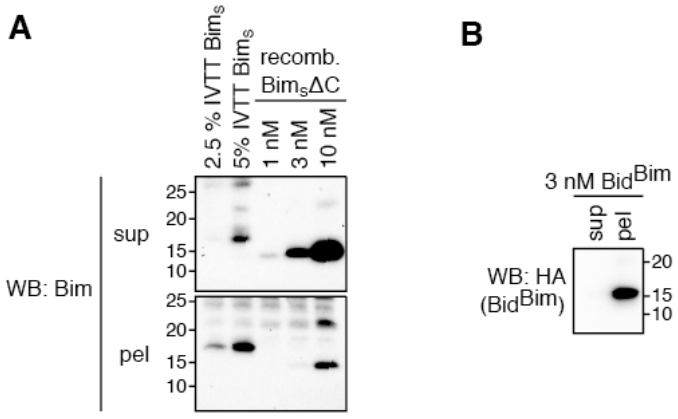
C. Analysis of western blots from Fig 2. Values are mean +/- SEM of two experiments.

D. Analysis of western blots from Fig 5. Values are mean +/- SEM of three experiments. ($^{\#}$two experiments for the Bid M97A titrations and for 100 nM Bid + Bcl-x_L immunoprecipitation)

Note that in each case, when Bak had become mostly activated, MODE 1 inhibition was minimal.



Supplementary Figure 3. Proteinase K cleavage of Bak resolves different conformations
Mouse liver mitochondria (MLM) supplemented with or without 35 nM Mcl-1 and 10 nM Bcl-x_L were treated with 1 nM Bid or 3 nM Bid M97A (samples from Figs 1 and 5). Samples were incubated with proteinase K and western blotted with the BakBH3-specific antibody 4B5 [31]. Non-activated Bak undergoes only a small 'clip', while activated and dimerised Bak is cleaved to a ~17 kD band. When activated Bak is in a MODE 2 complex with Mcl-1ΔNC or Bcl-x_L, Bak is cleaved to a ~10 kDa MODE 2-specific fragment. Note also that this fragment locates to the supernatant if Bak is bound by truncated Mcl-1, and in the pellet if bound by full-length Bcl-x_L, likely due to greater membrane-insertion of full-length Bcl-x_L compared to C-terminally truncated Mcl-1. (Samples were from the experiment in Fig 1, except for Bcl-x_L samples which were from the experiment in Fig 5.)



Supplementary Figure 4. Full-length versions of Bim mostly associate with the membrane fraction
Mouse liver mitochondria (MLM) were treated with three variants of Bim at the indicated concentrations, as in Fig. 4A. Supernatant (sup) and pellet (pel) fractions were western blotted for Bim (A), or for HA (B) to detect HA-tagged Bid^{Bim}. Blots are representative of two (A) or more (B) independent experiments.