Insight into Functional Membrane Proteins by Solution NMR: The Human Bcl-2 Protein – A Promising Cancer Drug Target

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Supplementary Information

Supplementary Figure 1: Temperature stability of Bcl-2 and Bcl-2 truncated variants in DPC/NMR buffer monitored by CD.

Supplementary Figure 2: ^{1}H-^{15}N-TROSY spectra of Bcl-2 protein in DPC/NMR buffer above and below CMC.

Supplementary Figure 3: ^{31}P NMR spectra of Bcl-2 protein in DPC/NMR buffer above and below CMC.

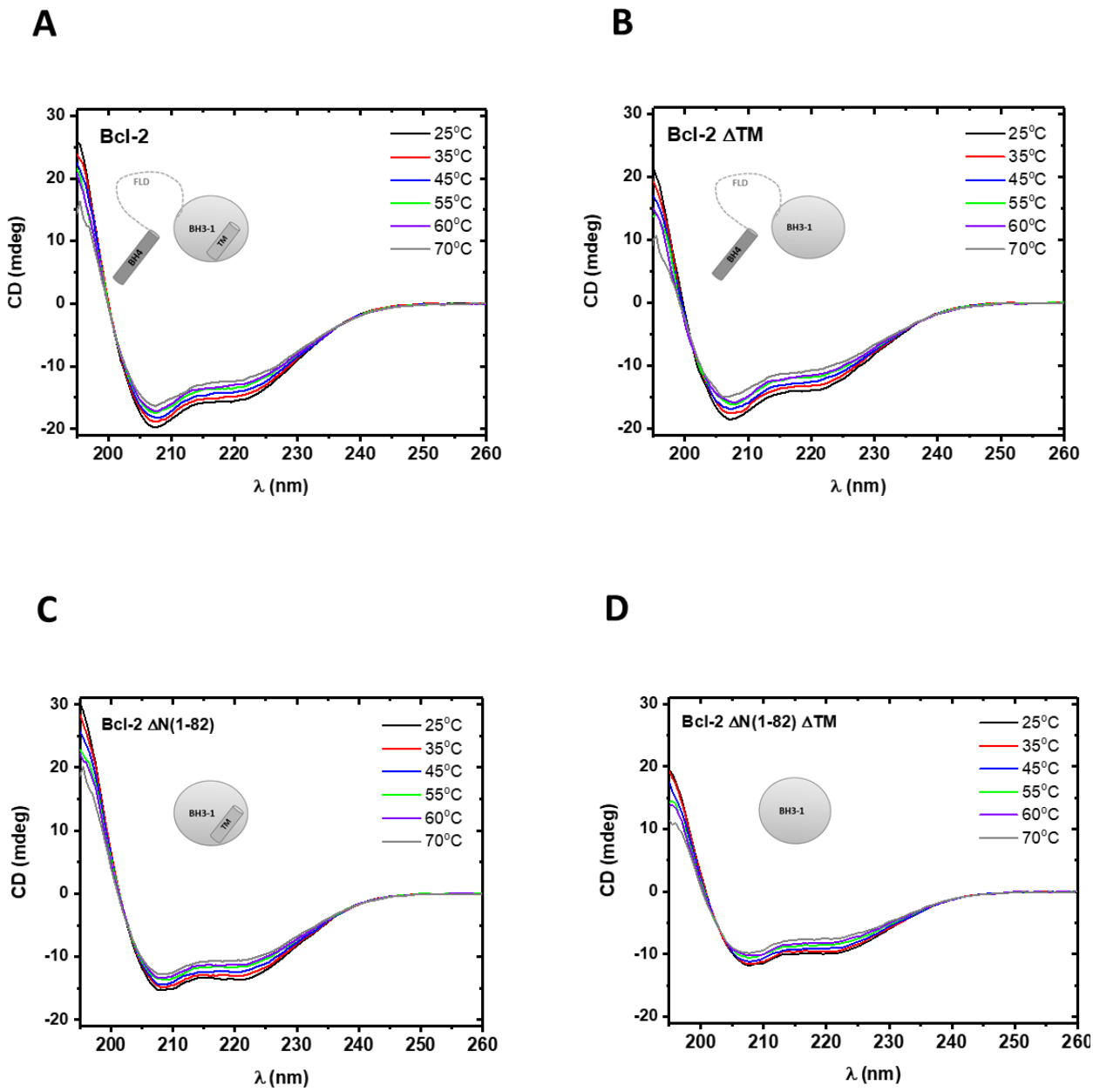
Supplementary Figure 4: NMR spectra of soluble and DPC titrated Bcl-2 ΔTM in NMR buffer

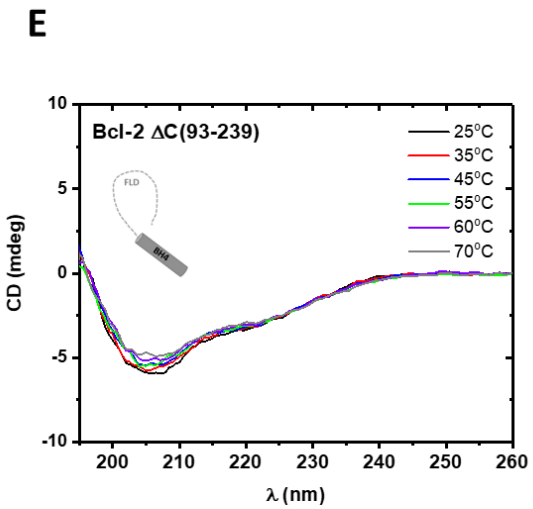
Supplementary Figure 5: (A-E): *In vitro* binding assays on full-length Bcl-2, Bcl-2 ΔTM, Bcl-2 ΔN (1-82) and Bcl-2 ΔN (1-82) ΔTM constructs**.**

Supplementary Figure 6: Schematic diagram of Bcl-2 ΔTM showing truncation constructs.

Supplementary Table I: Binding fragments identified by ^{19}F NMR screening.

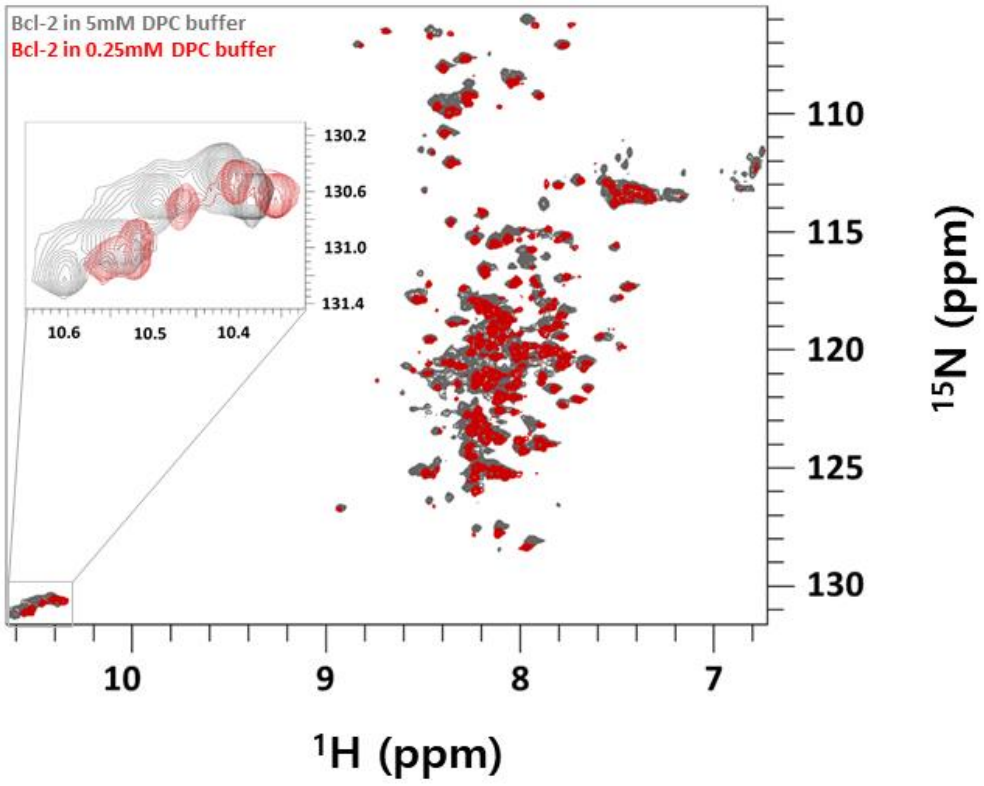
Supplementary Figure 1. Temperature stability of Bcl-2 and Bcl-2 truncated variants in DPC monitored by CD.





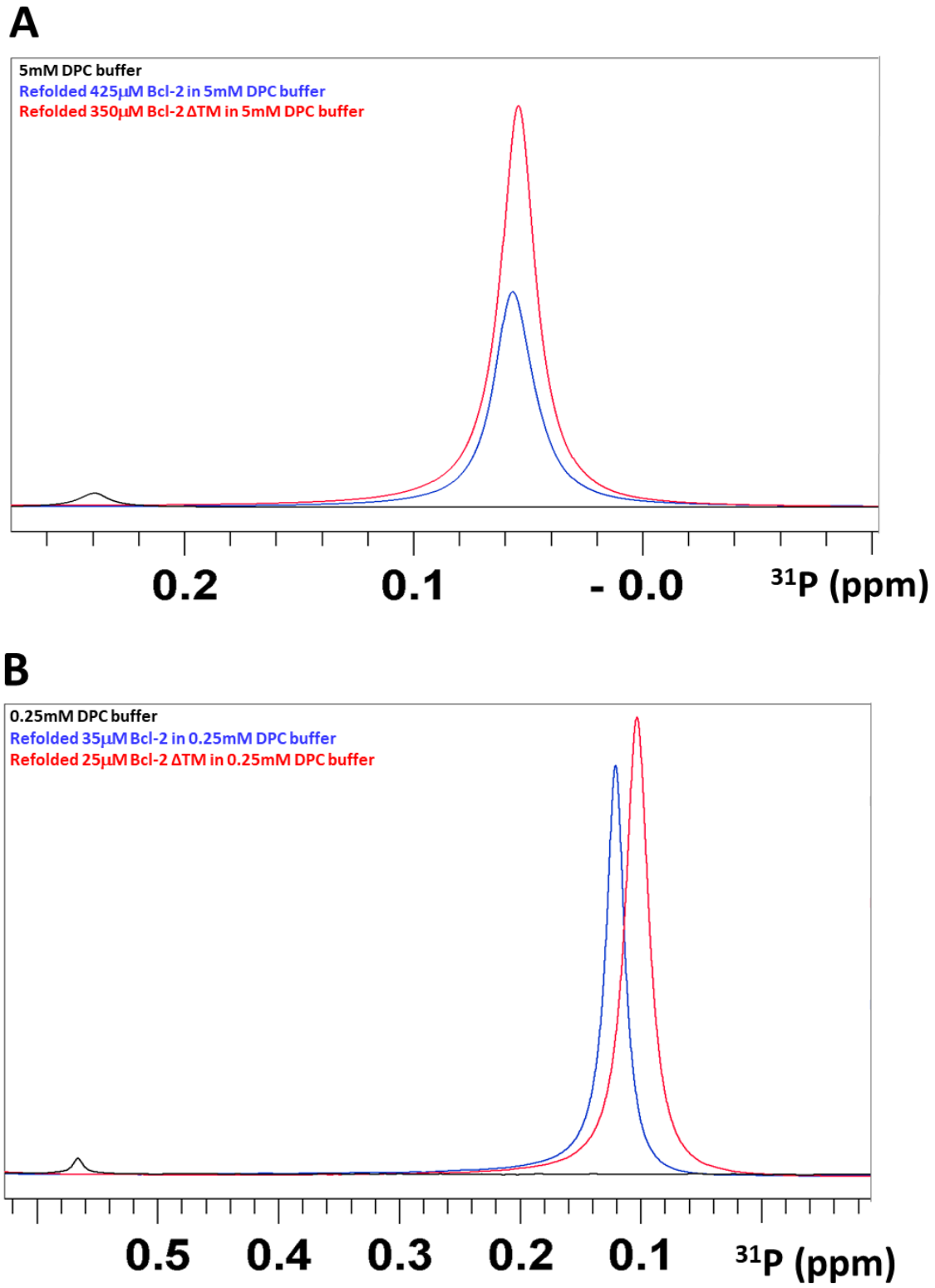
Supplementary Figure 1. **A-E)** Far-UV CD spectra of full-length Bcl-2 protein compared to truncated variants in 5mM DPC in NMR buffer (20 mM NaPi, 20 mM NaCl, 2 mM TCEP, pH 6.0), recorded at increasing temperatures between 25°C and 70°C. **A)** CD spectra of 5 µM full-length Bcl-2, **B)** 5 µM Bcl-2 ΔTM, **C)** 5 µM Bcl-2 ΔN(1-82), **D)** 5 µM Bcl-2 ΔN(1-82) ΔTM, and **E)** 5 µM Bcl-2 ΔC(93-239). All shown spectra are averages of 10 individual scans with subtracted buffer backgrounds.

Supplementary Figure 2. NMR spectra dependence on CMC of DPC: spectra above and below CMC of DPC.



Supplementary Figure 2. Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra of 0.36mM ^{15}N-labeled Bcl-2 in 5 mM DPC micelles in NMR buffer (gray) and 35 µM ^{15}N-labeled Bcl-2 in 0.25 mM DPC in NMR buffer (red); all at 310 K.

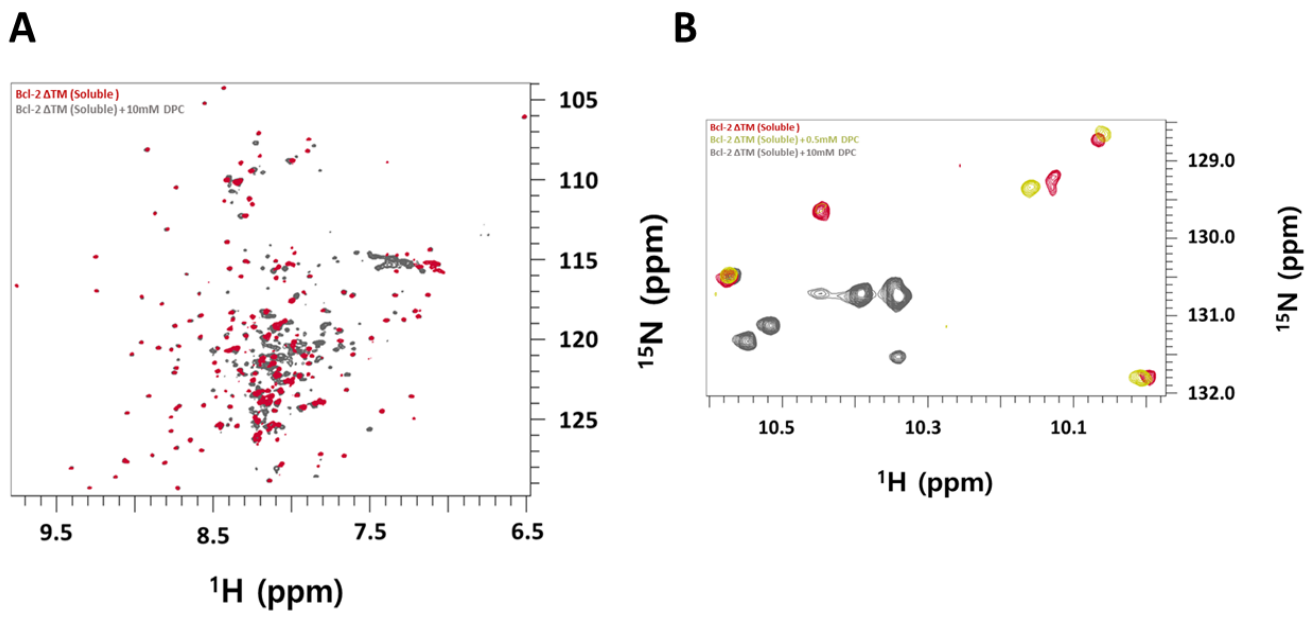
Supplementary Figure 3. ***^{31}P NMR spectra of Bcl-2 protein in DPC micelles above and below CMC.***



Supplementary Figure 3. **(A)** ^{31}P NMR spectra of reference 5 mM DPC micelles in NMR buffer(black), 425μM Bcl-2 and 350 μM Bcl-2 ΔTM in 5 mM DPC micelles in NMR buffer (in blue and red, respectively) at 298 K.

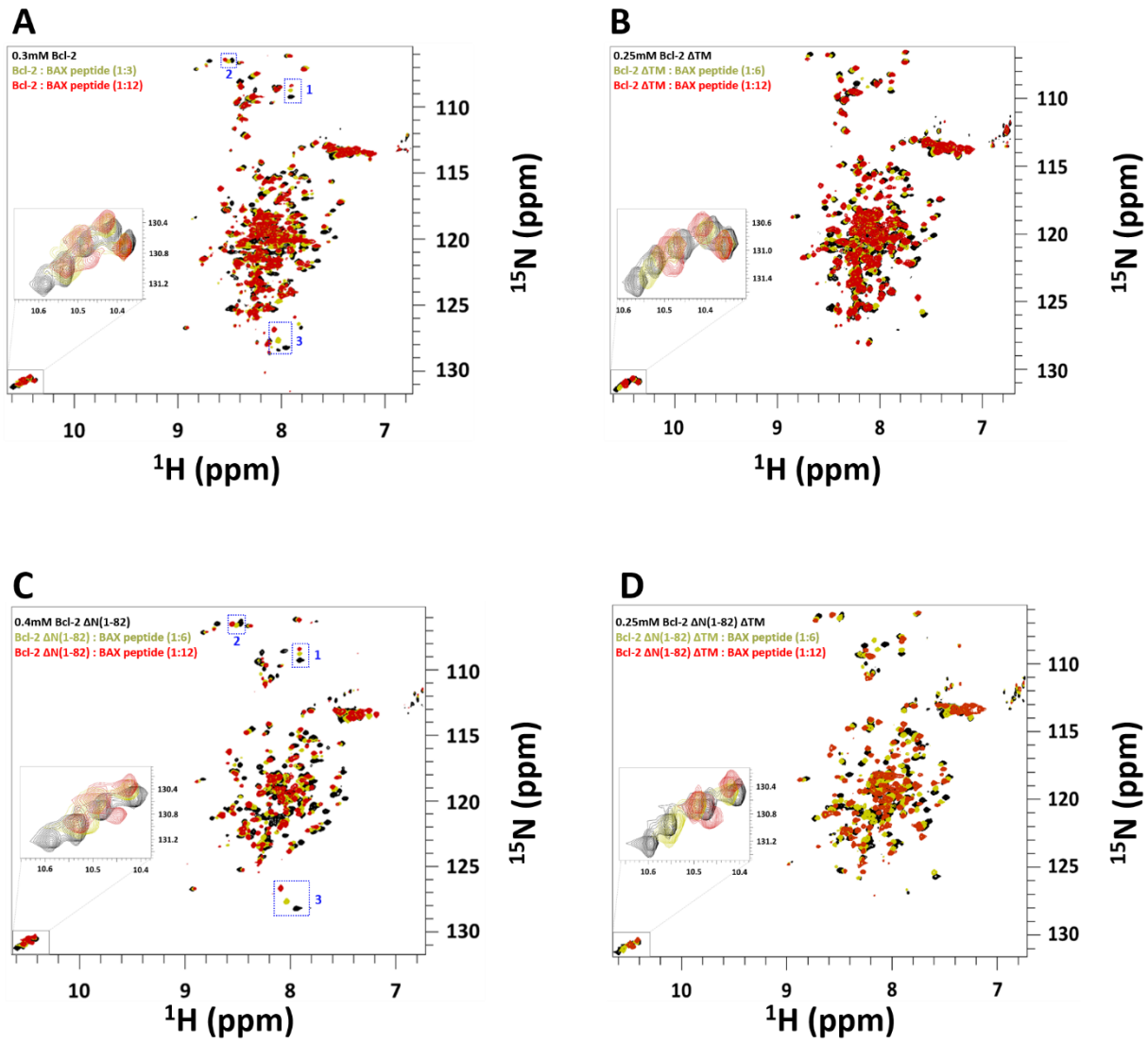
(B) ^{31}P NMR spectra of reference 0.25 mM DPC in NMR at pH 6.0 (black), 35μM Bcl-2 and 25 μM Bcl-2 ΔTM in 0.25 mM DPC in NMR buffer (in blue and red, respectively). NMR spectra were acquired under proton decoupling using a ^{31}P pulse width of 12 μs duration and inter-scan delay of 5 s.

Supplementary Figure 4. Bcl-2 ΔTM soluble and DPC titrated (membrane-mimic environment) NMR spectra.



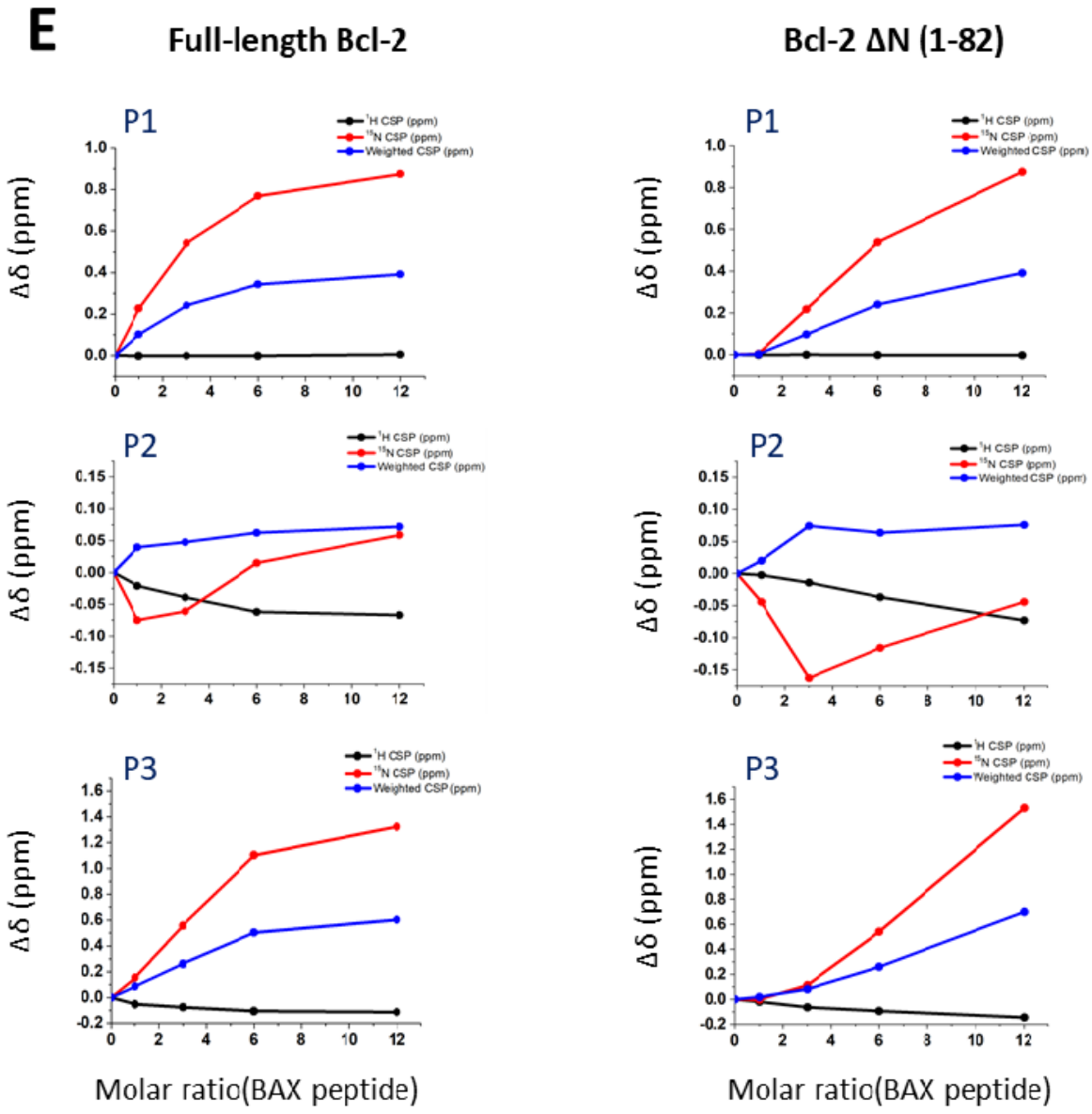
Supplementary Figure 4. (A) Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra of 40 μM ^{15}N-labeled Bcl-2 ΔTM in 20 mM NaPi, 20 mM NaCl, 2 mM TCEP at pH 6.0 (red), 40 μM ^{15}N-labeled Bcl-2 ΔTM in 10 mM DPC micelles in NMR buffer(gray). **(B)** Overlay of ^{1}H-^{15}N TROSY-HSQC spectra showing side-chain tryptophan $^{15}N\varepsilon^{1}H$ of 40 μM ^{15}N-labeled Bcl-2 ΔTM in NMR buffer (red), 40 μM ^{15}N-labeled Bcl-2 ΔTM in 0.5 mM DPC in NMR buffer (olive yellow), 40 μM ^{15}N-labeled Bcl-2 ΔTM in 10 mM DPC micelles in NMR buffer (gray). All spectra acquired at 298 K.

Supplementary Figure 5 (A-E). In vitro binding assay on full-length Bcl-2, Bcl-2 ΔTM, Bcl-2 ΔN (1-82) and Bcl-2 ΔN (1-82) ΔTM constructs.



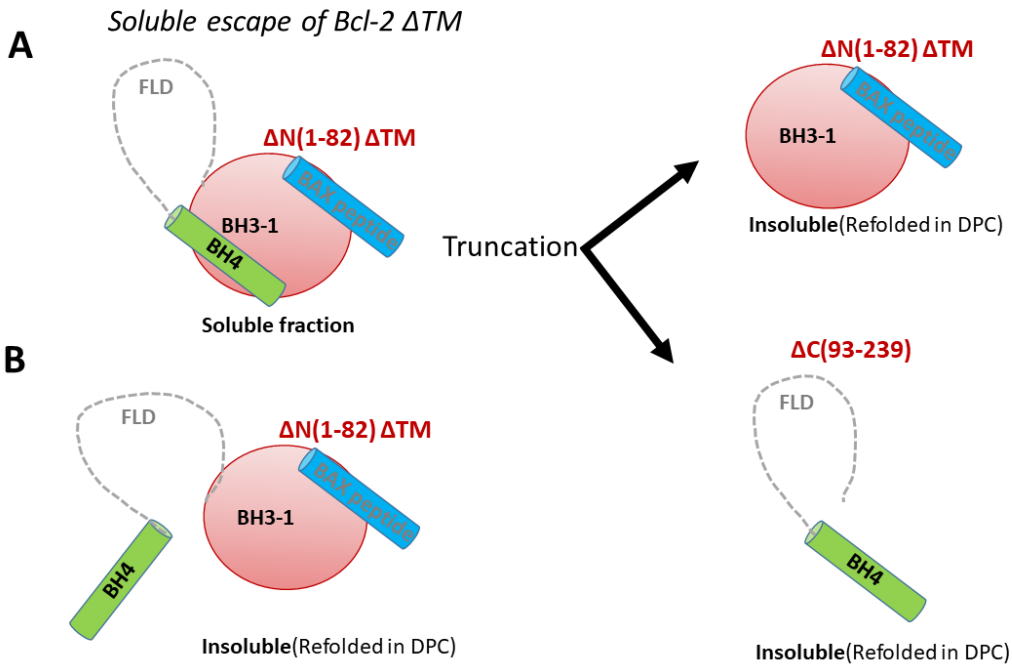
Supplementary Figure 5 (A-D). **(A)** Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra showing chemical shift perturbations (CSP's) observed in ^{15}N-labeled Bcl-2 upon titration with the Bax-BH3 peptide at 310K. In black is the spectrum of 0.3 mM Bcl-2 in 5 mM DPC micelles in NMR buffer. Coloured in olive, yellow and red are the spectra of Bcl-2 with Bax-BH3 peptide added at 1:3 and 1:12 stoichiometry. **(B)** Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra showing chemical shift perturbations (CSP's) observed in the ^{15}N-labeled Bcl-2 ΔTM when titrated with Bax-BH3 peptide at 310 K. In black is the spectrum of 0.25 mM Bcl-2 ΔTM in 5 mM DPC micelles

in NMR buffer. In olive yellow and red are the spectra of Bcl-2 with Bax-BH3 peptide added at 1:6 and 1:12 stoichiometry. **(C)** Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra showing chemical shift perturbations (CSP's) observed in the ^{15}N-labeled Bcl-2 ΔN(1-82) when titrated with Bax-BH3 peptide at 310K. In black is the spectrum of 0.4 mM Bcl-2 ΔN(1-82) in 5 mM DPC micelles in NMR buffer. In olive yellow and red are the spectra of Bcl-2 with Bax-BH3 peptide added at 1:6 and 1:12 stoichiometry. **(D)** Overlay of ^{1}H-^{15}N-TROSY-HSQC NMR spectra showing chemical shift perturbations (CSP's) observed in the ^{15}N-labeled Bcl-2 ΔN(1-82) ΔTM when titrated with Bax-BH3 peptide at 310 K. In black is the spectrum of 0.25 mM Bcl-2 ΔN(1-82) ΔTM in 5 mM DPC micelles in NMR buffer. In olive yellow and red are the spectra of Bcl-2 with Bax-BH3 peptide added at 1:6 and 1:12 stoichiometry. The Figure inserts (zoomed boxes) show the tryptophan side-chain region of the respective NMR spectrum.



Supplementary Figure 5E. Plots showing the Chemical Shift Perturbation's (CSP's) of the ^{1}H-N protons (δH), ^{15}N amide nitrogen's(δN) and weighted ^{1}H-^{15}N chemical-shift differences ($\Delta\delta_{1H\text{-}15N}$ (ppm) = [$(\delta H)^2 + 0.2 \times (\delta N)^2]^{1/2}$) of the backbone amide peaks (P1-3 highlighted as dashed boxes in Supplementary Figures 5A and 5C) of the Bcl-2 and Bcl-2 ΔN(1-82) upon titration with BH3 Bax peptide at Bax peptide/protein molar ratios of 0, 1, 3, 6 and 12, respectively.

Supplementary Figure 6. Schematic diagram of Bcl-2 ΔTM showing truncation constructs



Supplementary Figure 6. **(A)** Schematic diagram showing the truncation of **(A)** soluble and **(B)** insoluble Bcl-2 ΔTM, soluble Bcl-2 ΔTM fraction showing BH4 and BH3-1 domains packed together to form a soluble globular fold.

Supplementary Table 1. Binding fragments found in the ^{19}F screening

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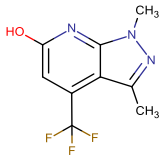
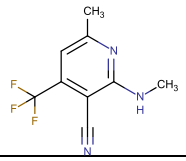
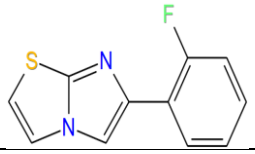
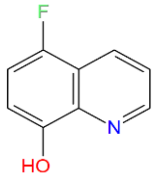
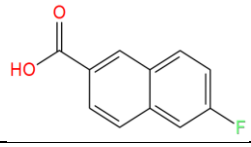
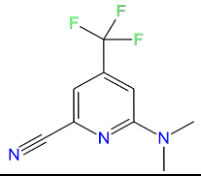
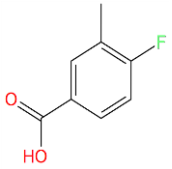
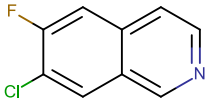
1		45
2		55
3		30
4		30
5		50
6		30
7		30
8		25

[a]The observed signal reduction in the spin-echo experiment in samples with Bcl-2 compared to reference samples.