SUPPLEMENTARY FIGURE LEGENDS

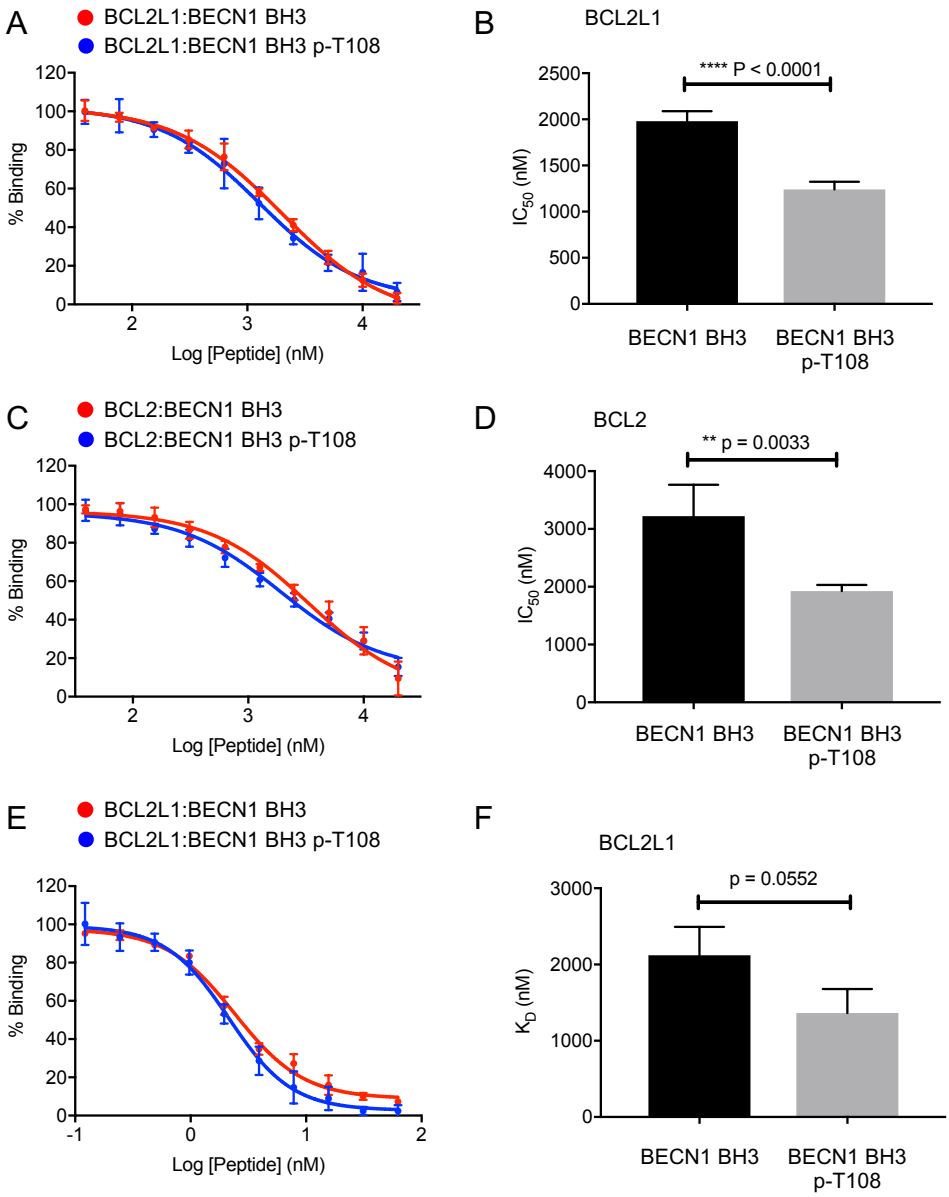
Figure S1. Affinities of wild-type BECN1 BH3 and BECN1 BH3 p-T108 peptides for BCL2 pro-survival proteins. Binding of wild-type and p-T108 BECN1 BH3 peptides to (**A** and **B**) BCL2L1 and (**C** and **D**) BCL2 as determined by solution competition assay using surface plasmon resonance. (**E** and **F**) Direct binding to BCL2L1 was also determined by microscale thermophoresis. The curves and IC_{50}/K_D values represent the average ± SD for n=4-5 separate assays for the SPR experiments and n=3 separate assays for the MST experiments.

Figure S2. Composite omit maps of BCL2L1 complexes**.** (**A**) BECN1 BH3 p-T108 and (**B**) BECN1 BH3 D108. Maps were created using Phenix (contoured at 1 sigma) and indicate no obvious density associated with His 113.

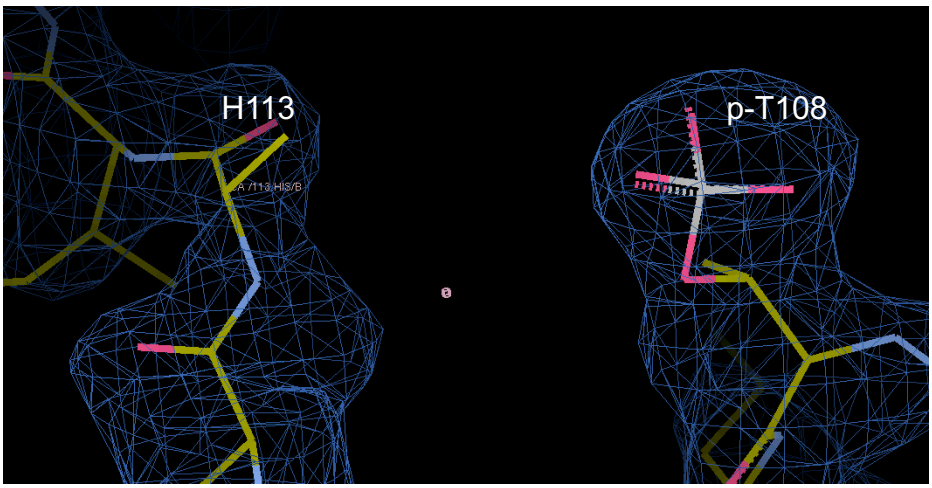
Figure S3. Comparison of BCL2 and BCL2L1 BH3 protein complexes. (**A**) Overlay of the NMR structure of apo-BCL2 (white, PDB code 1GJH) and the crystal structure of BCL2 (yellow) in complex with the BECN1 BH3 domain (orange) (PDB code 5VAU). Binding of BECN1 BH3 causes a widening of the hydrophobic binding groove on BCL2 as a consequence of movement of the α3 helix. (**B**) This is in contrast to when BECN1 BH3 (orange) binds to BCL2L1 (white) (PDB code 2P1L) where both the α3 and α4 helices move to the BECN1 BH3 ligand as compared to apo-BCL2L1 (white, PDB code 1PQ0).

Figure S4. Interaction maps of all hydrophobic and polar interactions between residues in different pro-survival proteins: BH3 domain complexes. (**A**) BECN1 BH3:BCL2 (PDB code 5VAU) (**B**) BECN1 BH3:BCL2L1 (PDB code 2P1L) and (**C**) BAX BH3:BCL2 (PDB code 2XA0). Maps were created with LIGPLOT. Interactions shown are for residues within 4 Å of each other. Red lines: electrostatic interactions, black lines: hydrophobic interactions, green lines: hydrogen bonds. Residues in yellow are the conserved hydrophobic residues found in all BH3 domains, whilst the conserved aspartic acid is indicated in red.

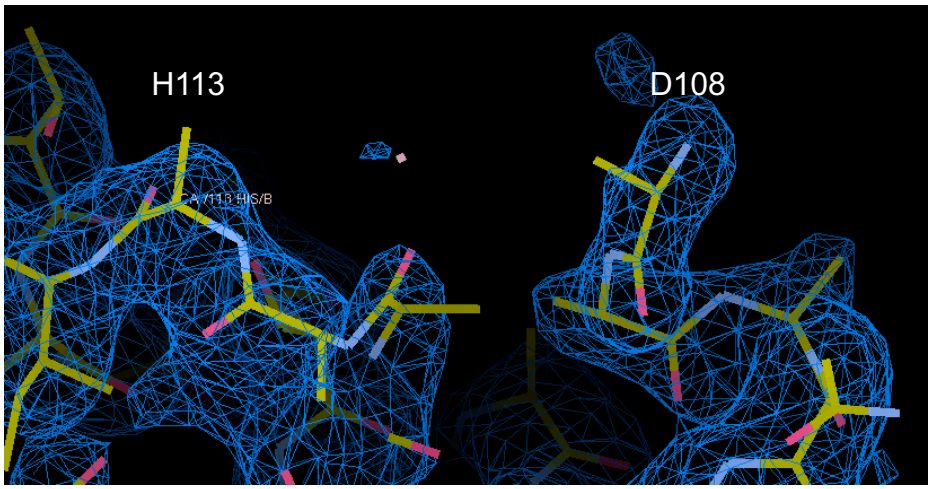
Figure S5. Binding of wild-type and BECN1 BH3 p-T108 peptides to BCL2L1 in the presence and absence of detergents. Binding of wild-type and p-T108 BECN1 BH3 peptides to BCL2L1 as determined by microscale thermophoresis either (**A** and **B**) without detergent, or in the presence of (**C** and **D**) Triton X-100 or (**E** and **F**) CHAPS. The assays all use fluorescently-labelled BCL2L1 protein. The curves and K_D values represent the average ± SD for n=3 separate assays.


A
BCL2L1:BECN1 BH3
BCL2L1:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
B
BCL2L1
**** P < 0.0001
IC50 (nM)
BECN1 BH3
BECN1 BH3 p-T108
C
BCL2:BECN1 BH3
BCL2:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
D
BCL2
** p = 0.0033
IC50 (nM)
BECN1 BH3
BECN1 BH3 p-T108
E
BCL2L1:BECN1 BH3
BCL2L1:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
F
BCL2L1
p = 0.0552
KD (nM)
BECN1 BH3
BECN1 BH3 p-T108

A



B



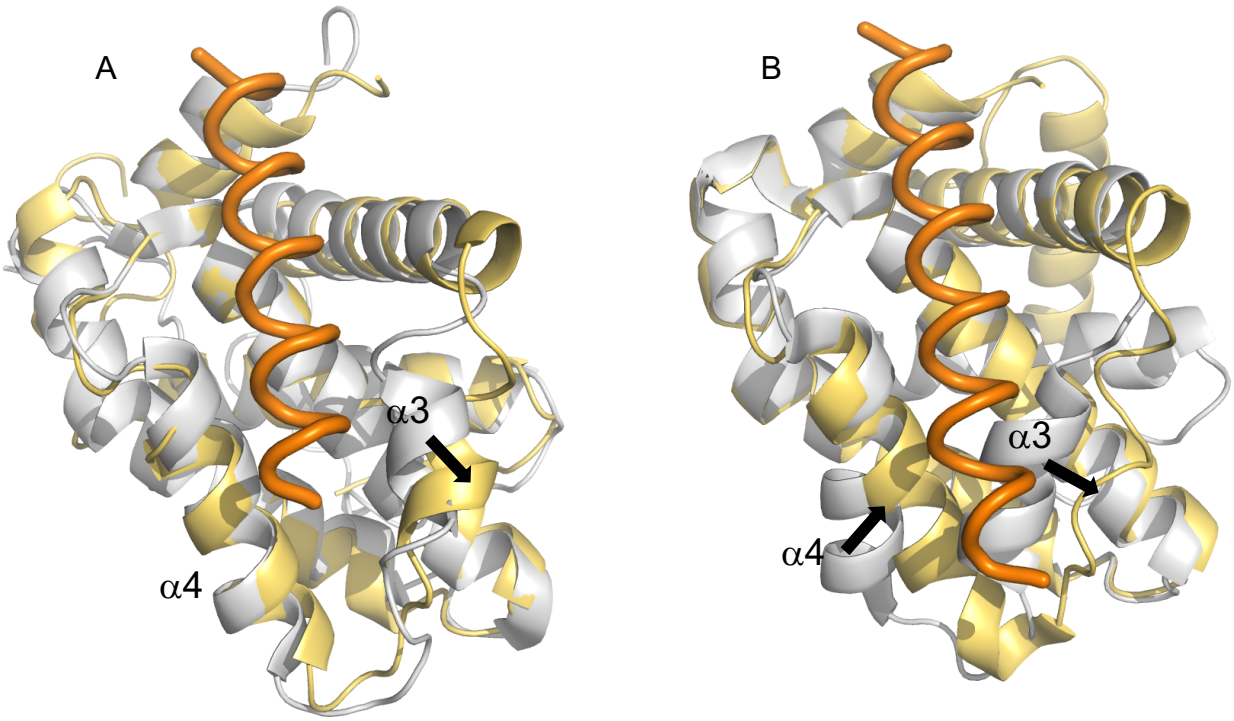
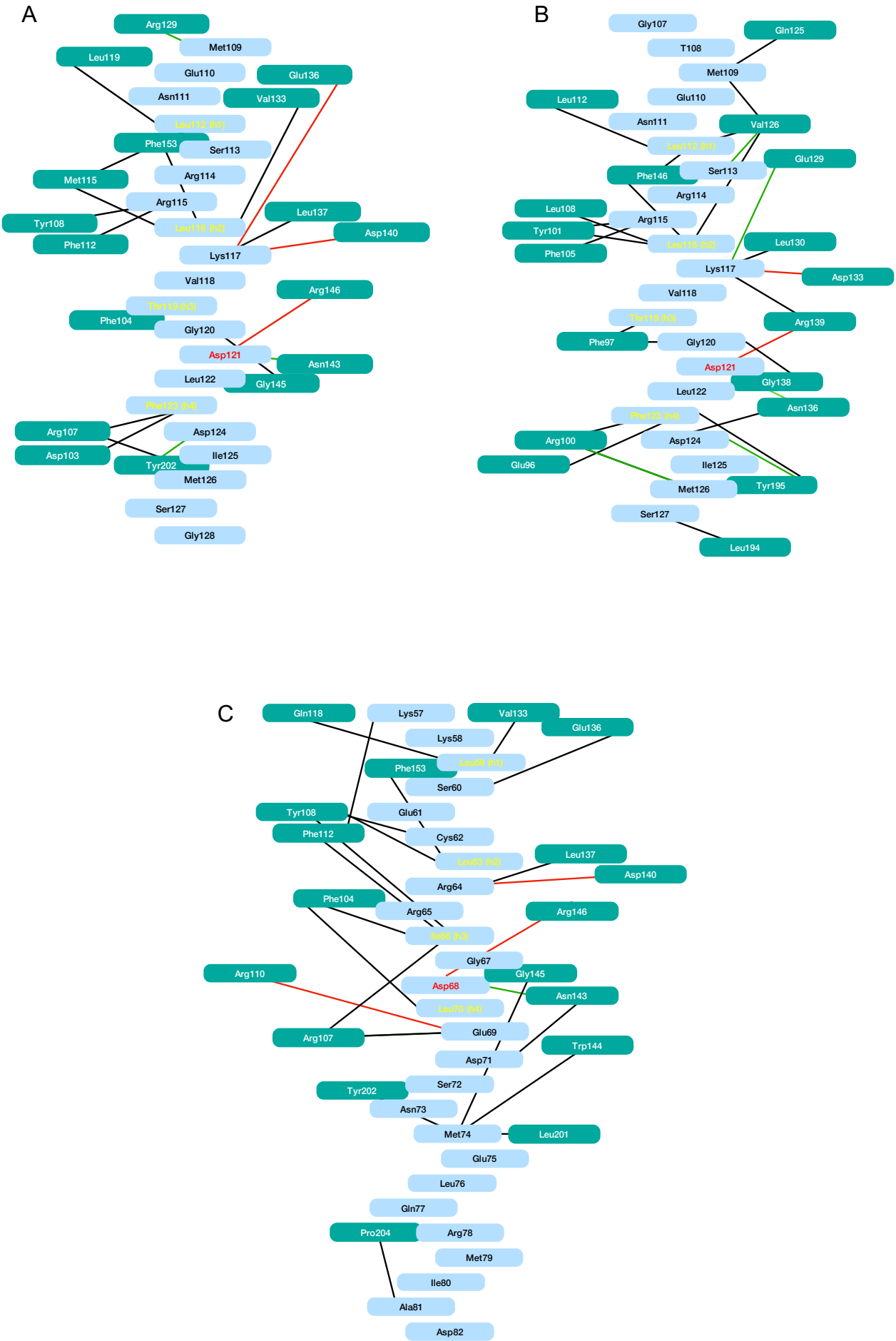
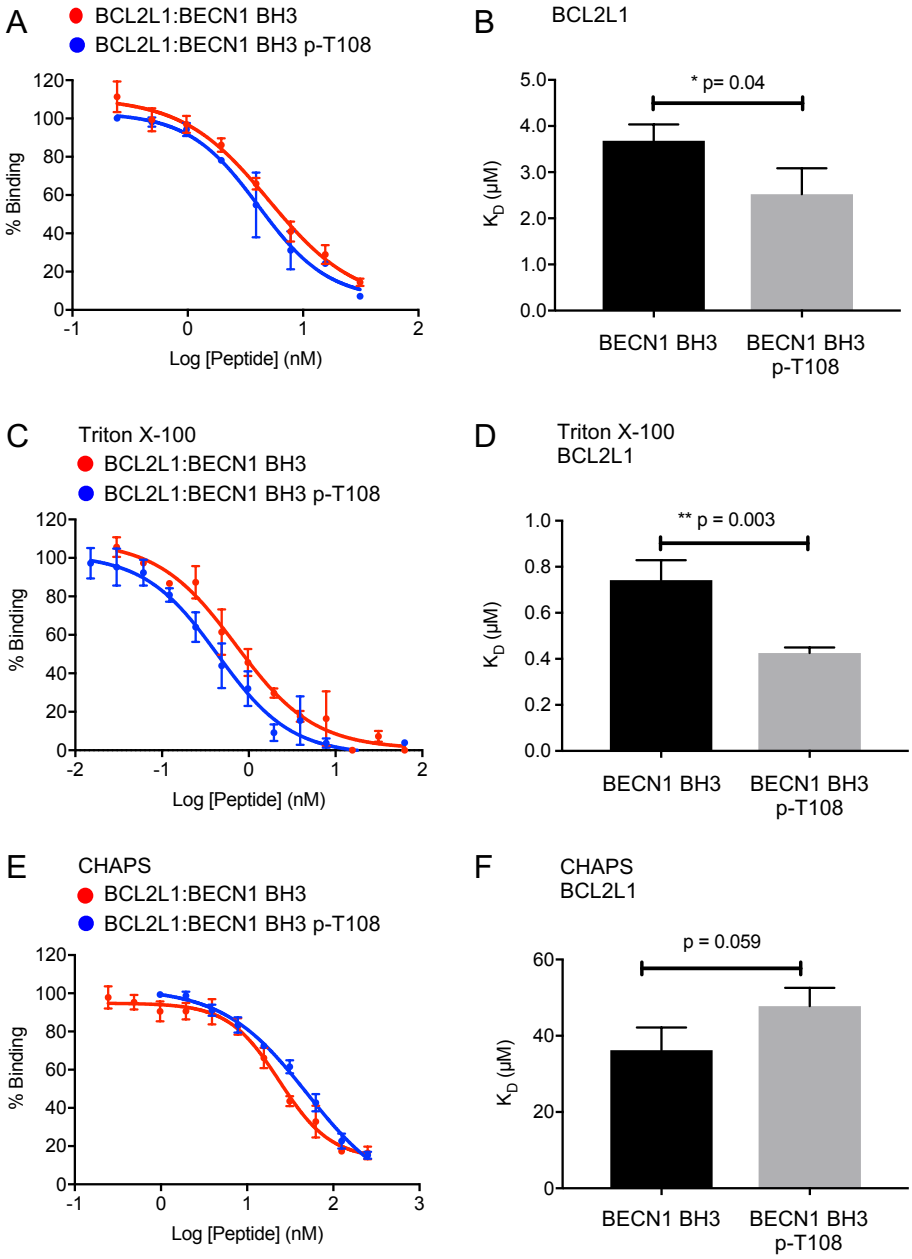

A
α3
α4
B
α3
α4

FIGURE S4


A
Arg129
Met109
Leu119
Glu110
Glu136
Asn111
Val133
Leu112 (h1)
Phe153
Ser113
Met115
Arg114
Arg115
Tyr108
Leu137
Leu116 (h2)
Asp140
Phe112
Lys117
Val118
Arg146
Thr119 (h3)
Phe104
Gly120
Asp121
Asn143
Leu122
Gly145
Phe123 (h4)
Arg107
Asp124
Asp103
Ile125
Tyr202
Met126
Ser127
Gly128
B
Gly107
Gln125
T108
Met109
Leu112
Glu110
Asn111
Val126
Leu112 (h1)
Glu129
Phe146
Ser113
Arg114
Leu108
Arg115
Tyr101
Leu116 (h2)
Leu130
Phe105
Lys117
Asp133
Val118
Thr119 (h3)
Arg139
Phe97
Gly120
Asp121
Gly138
Leu122
Asn136
Phe123 (h4)
Arg100
Asp124
Glu96
Ile125
Met126
Tyr195
Ser127
Leu194
C
Gln118
Lys57
Val133
Glu136
Lys58
Leu59 (h1)
Phe153
Ser60
Tyr108
Glu61
Phe112
Cys62
Leu63 (h2)
Leu137
Asp140
Arg64
Phe104
Arg65
Arg146
Ile66 (h3)
Gly67
Arg110
Gly145
Asp68
Asn143
Leu70 (h4)
Glu69
Arg107
Trp144
Asp71
Ser72
Tyr202
Asn73
Met74
Leu201
Glu75
Leu76
Gln77
Pro204
Arg78
Met79
Ile80
Ala81
Asp82


A
BCL2L1:BECN1 BH3
BCL2L1:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
B
BCL2L1
* p= 0.04
K_D (μM)
BECN1 BH3
BECN1 BH3 p-T108
C
Triton X-100
BCL2L1:BECN1 BH3
BCL2L1:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
D
Triton X-100
BCL2L1
** p = 0.003
K_D (μM)
BECN1 BH3
BECN1 BH3 p-T108
E
CHAPS
BCL2L1:BECN1 BH3
BCL2L1:BECN1 BH3 p-T108
% Binding
Log [Peptide] (nM)
F
CHAPS
BCL2L1
p = 0.059
K_D (μM)
BECN1 BH3
BECN1 BH3 p-T108