Peptide ligands for pro-survival protein Bfl-1 from computationally guided library screening

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(Supporting Information)

Supplemental Table 1. Half lives for dissociation of fluoresceinated peptides from different pro-survival proteins.

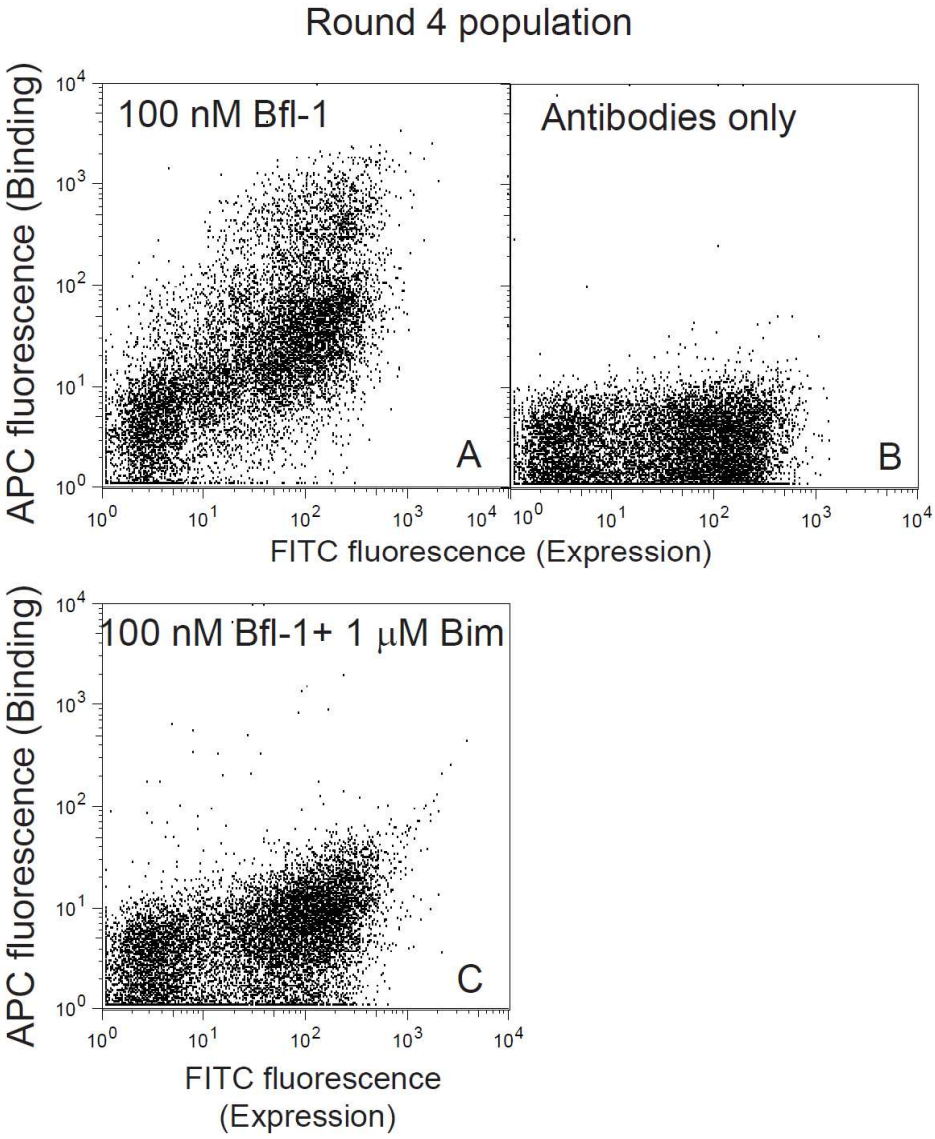
Fluoresceinated peptides	Half life (minutes)				
	Bfl-1	Mcl-1	Bcl-x_L	Bcl-2	Bcl-w
Bim (18-mer)	40	14	2	14	<1
Bim (23-mer)	> 2000	>2000		55	
FA1 (23-mer)	> 2000	243		<1	
FA1_D3fK (23-mer)	5				
Bim_D3fK (23-mer)	<1	<1			
Bim_D3fKF4aL (23-mer)	15	3			
Bim_D3fKE3gV (23-mer)	<1	<1			
Bim_I3dAD3fK (23-mer)	<1				
FW1 (23-mer)	37	197		2	

[a]Concentration of fluorescent peptide was 10-20 nM in all experiments.
[b]Concentration of pro-survival protein ranged from 50-100 nM.

Supplemental Table 2. Sequences of BH3 peptides selected for binding to Bfl-1 preferentially over Bcl-w.

BH3 peptide	Sequence-[a]		
	2	3	4
	defgabcdefga		
FW1	IAQ**G**LRRIGD**TW**		
FW2	**L**AQ**G**LRR**V**GD**KW**		
FW3	IAQELRRIGD**KI**		
FW4	IAQ**C**LRR**V**GD**KW**		
FW5	IAQELRRIGDE**W**		
FW6	IAQELRRIGD**K**F		

Supplemental Figure 1. Analysis of the population selected after 4 rounds of positive screening for binding to 1 μM Bfl-1. Shown are FACS dot plots for binding to (A) 100 nM Bfl-1 (B) only antibodies and (C) 100 nM Bfl-1 in the presence of 1 μM unlabeled Bim BH3 peptide.



Supplemental Figure 2. Fluorescence polarization competition assay for Bim and FD3 binding to Bfl-1. The concentrations of fluorescent Bim and the prosurvival proteins were 10 nM and 50 nM, respectively. The sequences of Bim and FD3, with heptad numbering, are shown at the top.

```
          ---2------3------4---
          abcdefgabcdefgabcdefg
Bim       RPEIWIAQELRRIGDEFNAYYAR
FD3       RPEIWLYQVLRRYGDLWNAYYAR
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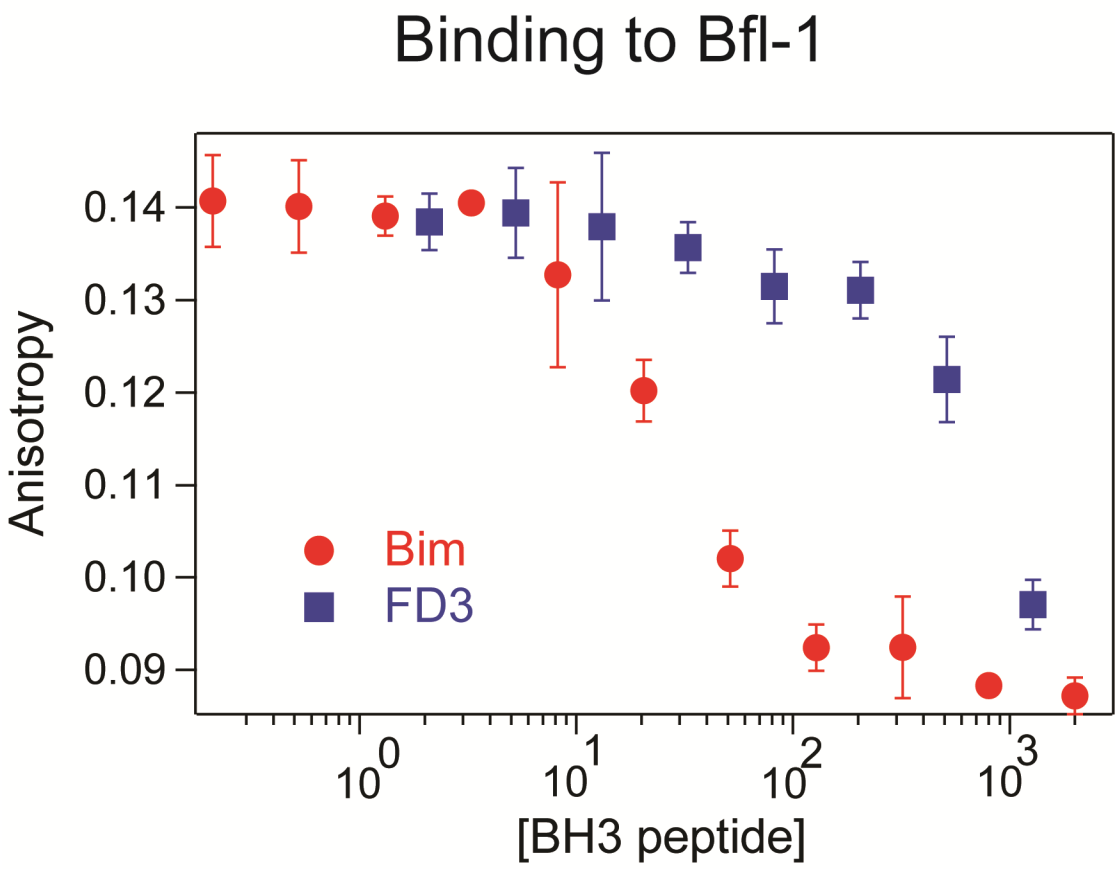
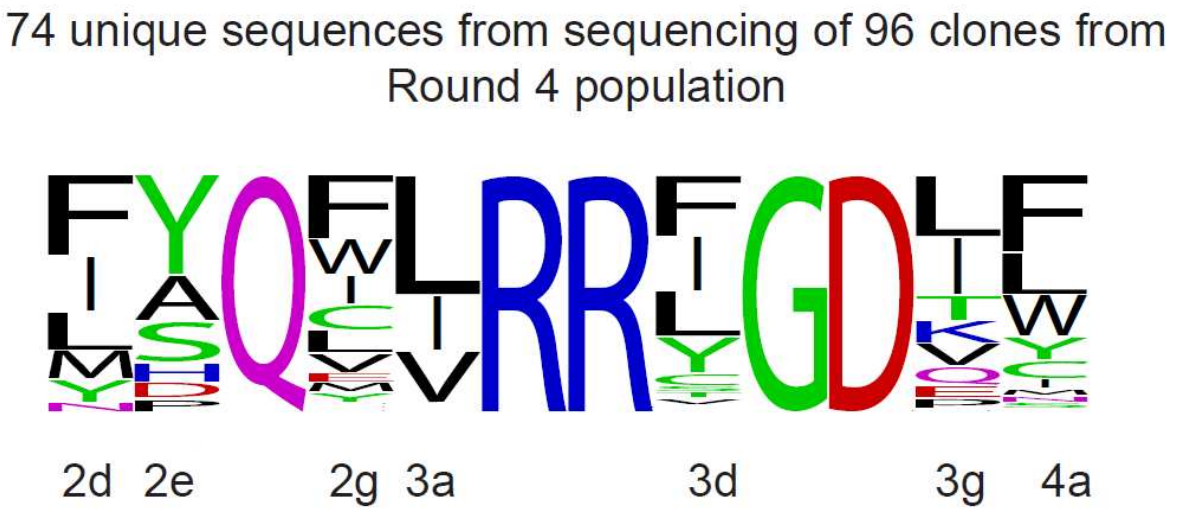
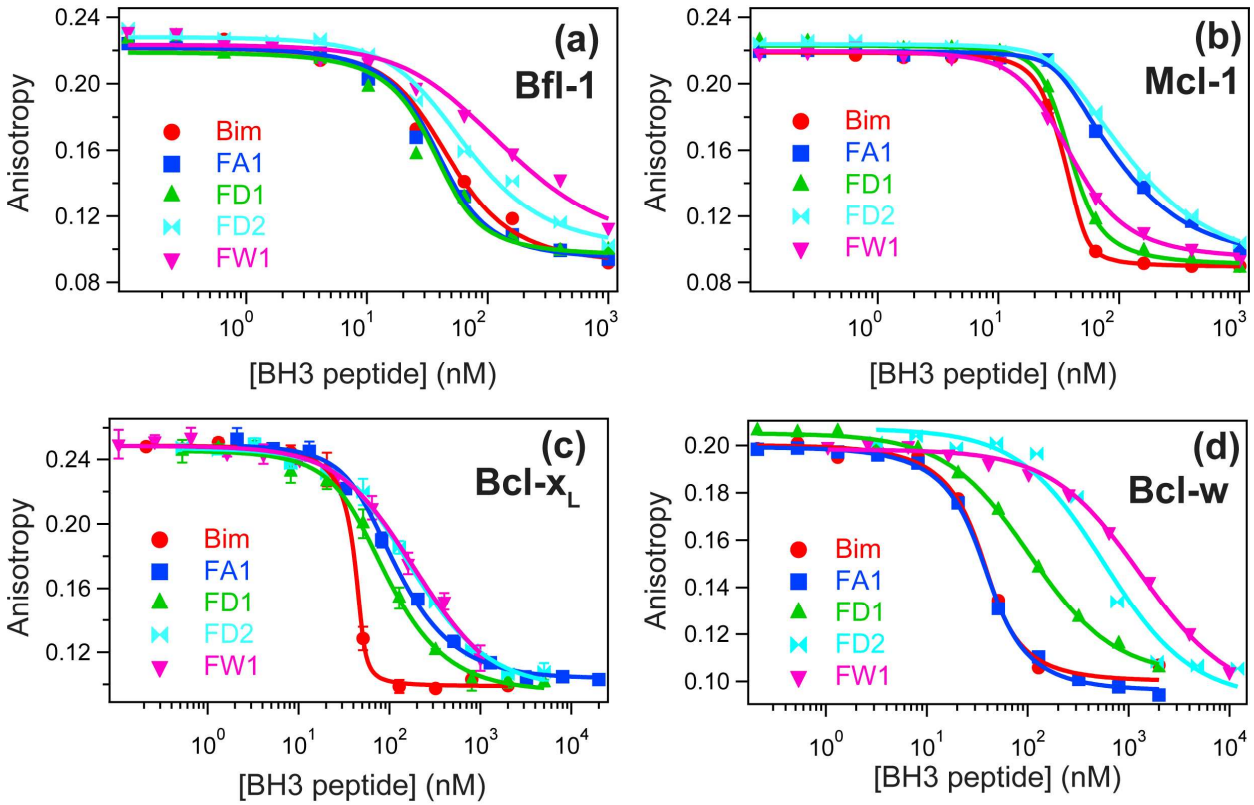


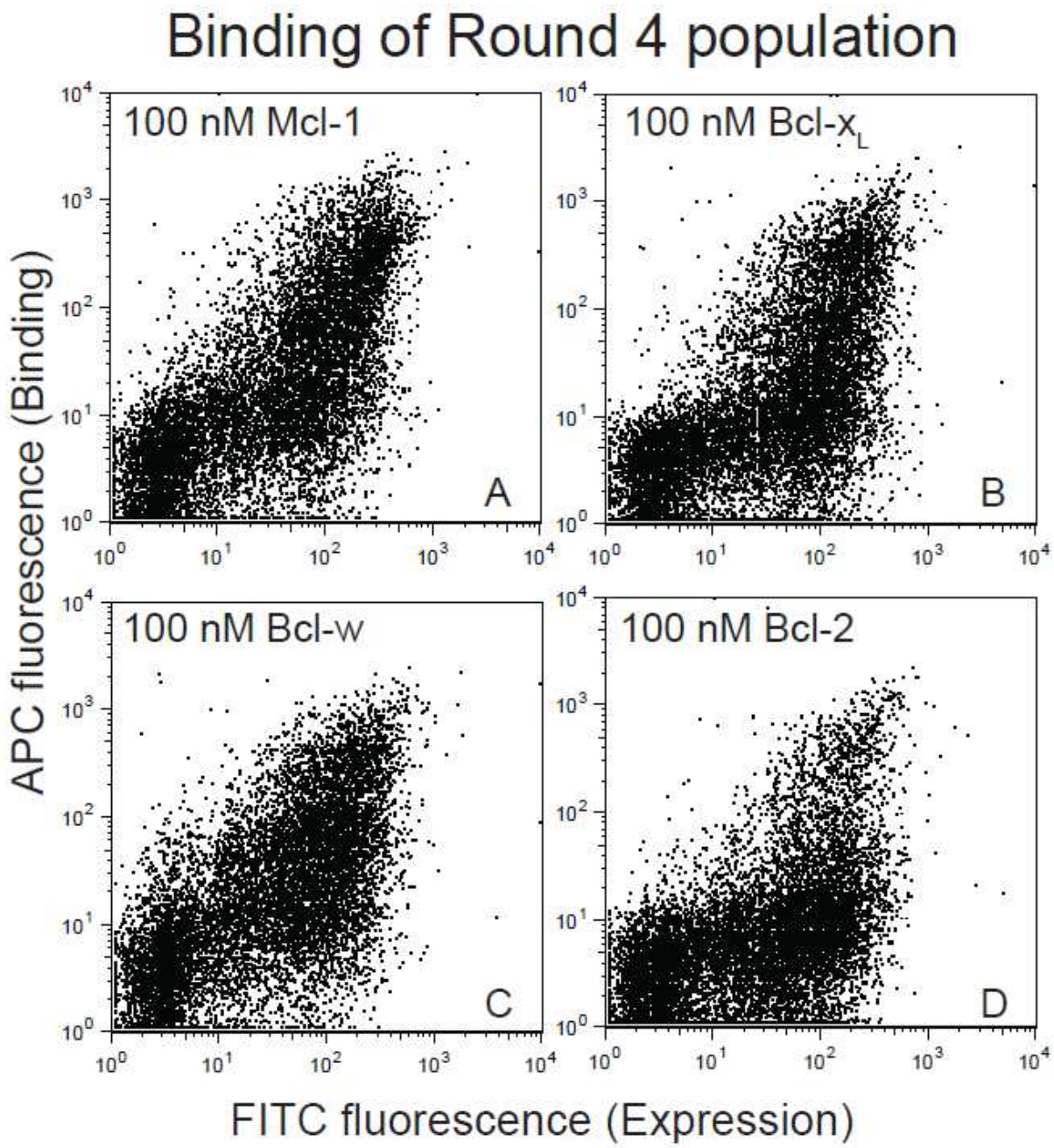
Supplemental Figure 3. Sequence logo generated from 74 unique sequences obtained from conventional sequencing of 96 clones from the yeast population after 4 rounds of positive selection against 1 μM Bfl-1. The positions randomized in the library are shown at the bottom.



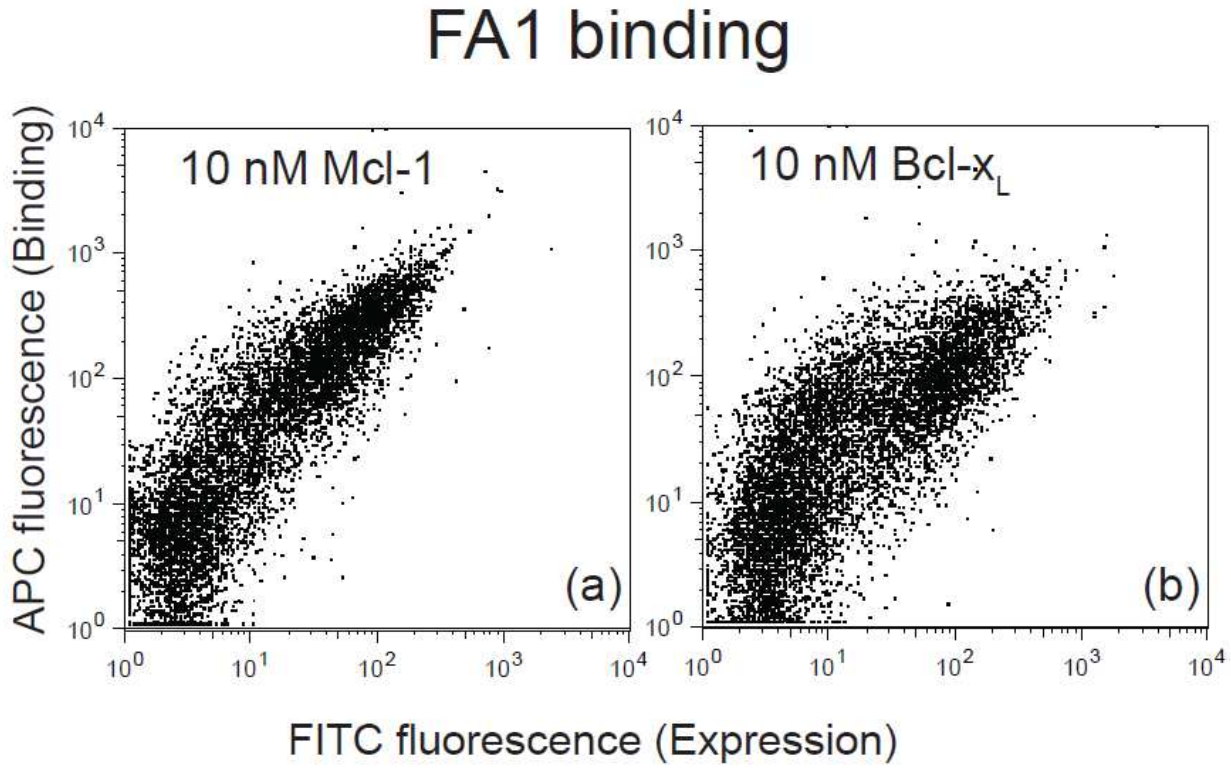
Supplemental Figure 4. Competition of designed unlabeled peptides with fluorescently labeled Bim BH3 for binding to Bfl-1 (a), Mcl-1 (b), Bcl-x_L and (c) Bcl-w (d). The concentrations of fluorescent Bim and the prosurvival proteins were 10 nM and 50 nM, respectively. Measurements for panels were made after 20 hours. Note that Bim binding to Bfl-1 and Mcl-1, and FA1 binding to Bfl-1, were probably not yet equilibrated at this time point.



Supplemental Figure 5. The Bfl-1 binding population after 4 rounds of positive selection was assayed for binding to (A) 100 nM Mcl-1 (B) 100 nM Bcl-x_L (C) 100 nM Bcl-w and (D) 100 nM Bcl-2.



Supplemental Figure 6. Binding of yeast displaying FA1 to 10 nM Mcl-1 (A) or 10 nM Bcl-x_L (B) monitored using FITC fluorescence (expression axis) and APC fluorescence (binding axis).



Supplemental Figure 7. Competition of unlabeled FA1_D3fK with fluorescently labeled Bim BH3 for binding to Bfl-1, Mcl-1 and Bcl-x_L.