Supporting Information

hBfl-1/hNOXA interaction studies provide new insights on the role of Bfl-1 in cancer cell resistance and for the design of novel apoptosis-based therapeutics

Elisa Barile,[1] Guya D. Marconi,[1] Surya K. De,[1] Carlo Baggio,[1] Luca Gambini,[1] Ahmed F. Salem, [1] Manoj K. Kashyap,[2,3] Januario E. Castro,[2,3] Thomas J. Kipps,[2,3] and Maurizio Pellecchia[1,*]

1. Division of Biomedical Sciences, School of Medicine, University of California Riverside, 900 University Avenue, Riverside, CA 92521, USA.
2. Moores Cancer Center, University of California, San Diego, La Jolla, CA, 92093, USA
3. CLL Research Consortium, and Department of Medicine, University of California, San Diego, La Jolla, CA, 92093, USA

* Corresponding author: Maurizio Pellecchia, phone number: **(951)-827-7829**; email address: maurizio.pellecchia@ucr.edu

Table S1: Aminoacid sequences, and corresponding molecular weights of hNOXA and hBIM derived peptides reported in Tables 1 and 2.

Compd ID	aa sequence	MW
hNOXA	Ac-CATQLRRFGDKLNFRQKLLN-NH_2	2463.02
homo_hNOXA	Ac-*hC*ATQLRRFGDKLNFRQKLLN-NH_2	2476.77
λ_hNOXA	Ac-λATQLRRFGDKLNFRQKLLN-NH_2	2521.07
hNOXAs	Ac-CATQLRRFGDKLN-NH_2	1561.83
NOXA A	Ac-AELPPEFAAQLRKIGDKVYC-NH_2	2288.19
NOXA B	Ac-PADLKDECAQLRRIGDKVNL-NH_2	2294.21
C1A_hNOXAs	Ac-AATQLRRFGDKLN-NH_2	1529.84
cyc_hNOXA	Ac-*C*ATQLRRFGDKLNFRQKLLNLISKLF*C*-NH_2	3366.94
hBIM	Ac-IWIAQELRRIGDEFNAYYARR-NH_2	2682.15
130E7	Ac-λIAQELRRIGDEFNAYYARR-NH_2	2545.51
130D11	Ac-λAQELRX_iIGDX_{i+4}FNAYYARR-NH_2	2511.53
130G4	Ac-$(PRR)_5$λIAQELRRIGDEFNAYYARR-NH_2	4593.32

hC = homocysteine; λ =Dap(2-chloroacetamide); *C1-C27* in cyc-NOXA = disulfide bridge

Table S2: *Kd* values derived by Isothermal Titration Calorimetry (ITC) measurements for the selected peptides against hBcl-xL, hMcl-1 and hBfl-1. Isothermal titration calorimetry measurements were performed using a VP-ITC calorimeter from Microcal (Northampton, MA, USA). Measurements were performed in a reverse fashion (i.e., the protein was titrated into the compound solution). All titrations were performed at 25°C in PBS buffer supplemented with 10% DMSO. Experimental data were analyzed using Microcal Origin software provided by the ITC manufacturer (Microcal).

Compd ID	ITC K_D(nM)		
	hBcl-X_L	hMcl-1	hBfl-1
130E7	25.5	2.0	2.6
130D11	No Binding	No Binding	No Binding
130G4	12.7	11.6	23.0

Figure S1: Dose-response DELFIA assay curves used to characterize the binding affinities of hNOXAs and hNOXAs_C1A peptides for their ability to displace a BID peptide from hBfl-1. Dose response curves are the results of duplicate measurements. The relative IC_{50} values are reported in Table 1.

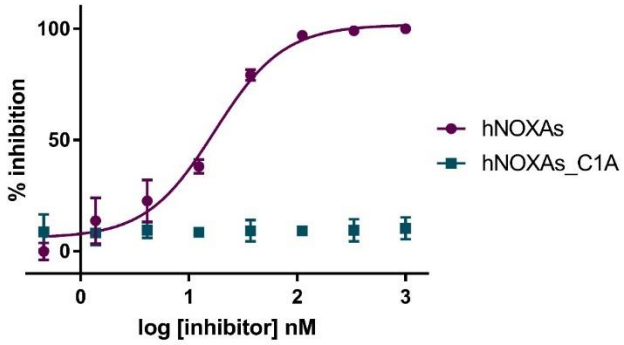

Figure S2: SDS-PAGE gel electrophoresis followed by Coomassie staining of hBfl-1 (10 μM) in absence and presence of equimolar concentrations of homo_hNOXA (Panel A, Table 1, Table S1) λ_hNOXA (Panel B, Table 1, Table S1) and 130G4 (Panel C, Table 1, Table S1) at different time points (15', 30', 1h, 2h).