

The anti-apoptotic proteins NAF-1 and iASPP interact to drive apoptosis in cancer cells

Anat Iosub-Amir^{a1}, Fang Bai^{b1}, Yang-Sung Sohn^c, Luhua Song^d, Sagi Tamir^c, Henri-Baptiste Marjault^c, Guy Mayer^a, Ola Karmi^c, Patricia A. Jennings^e, Ron Mittler^f, José N. Onuchic^{b2}, Assaf Friedler^{a2}, and Rachel Nechushtai^{c2}

^aInstitute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus at Givat Ram, Jerusalem 91904, Israel; ^bCenter for Theoretical Biological Physics and Department of Physics, Rice University, Houston, TX 77005; ^cThe Alexander Silberman Institute of Life Science, The Hebrew University of Jerusalem, Edmond J. Safra Campus at Givat Ram, Jerusalem 91904, Israel; ^dDepartment of Biological Sciences, University of North Texas, Denton, TX 76203; ^eDepartment of Chemistry & Biochemistry, University of California at San Diego, La Jolla, CA 92093, USA; ^fDepartment of Surgery, University of Missouri School of Medicine. Christopher S. Bond Life Sciences Center University of Missouri. 1201 Rollins St, Columbia, MO 65201.

¹ contributed equally to this work

²corresponding authors:

José N. Onuchic

Center for Theoretical Biological Physics and Department of Physics, Rice University, Houston, TX 77005;

001-713- 3484197. jonuchic@rice.edu

Assaf Friedler

Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus at Givat Ram, Jerusalem

91904, Israel; 972-2-6585746, assaf.friedler@mail.huji.ac.il

Rachel Nechushtai

The Alexander Silberman Institute of Life Science, The Hebrew University of Jerusalem, Givat Ram, Jerusalem

91904;972-2-6585242, rachel@mail.huji.ac.il

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Supporting Information

Supplementary methods

Peptide uptake into cells

MDA-MB-231 cancer cells were incubated with 10 μ M of FL-iASPP 764-780 for 3h and then intensively washed. Cell fluorescence was observed using a semi-confocal microscope Nikon TE2000 microscope equipped with a thermostated stage and a Hamamatsu Orca-Era CCD camera.

Supplementary figures



Figure S1: Mapping the binding sites between iASPP Pro and NAF-1: Peptide array screening. Dark spots indicate binding peptides. Binding of NAF-1 to an array of peptides derived from iASPP Pro. NAF-1 did not bind any peptides derived from iASPP Pro.

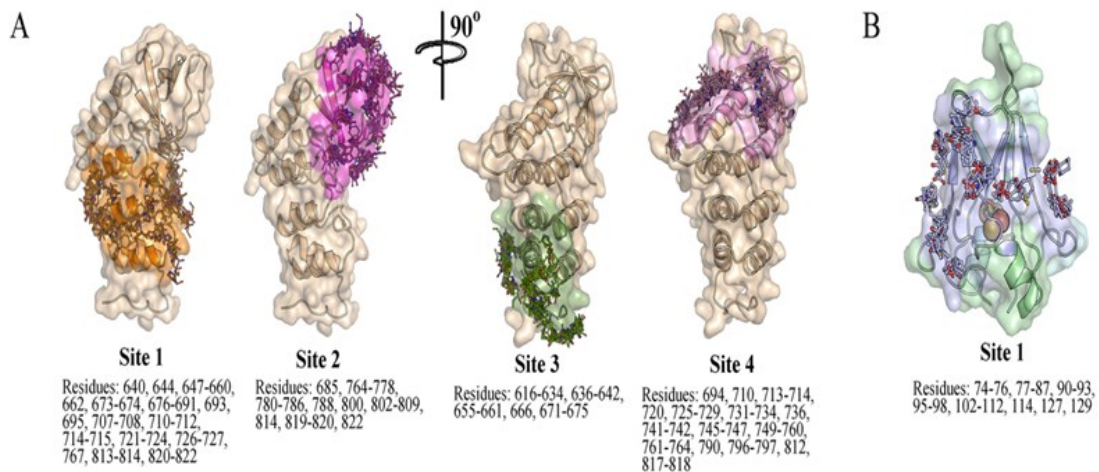


Figure S2: Identifying candidate protein-protein binding sites for iASPP and NAF-1. (A) Four candidate binding sites were obtained on the surface of iASPP and (B) one for NAF-1.

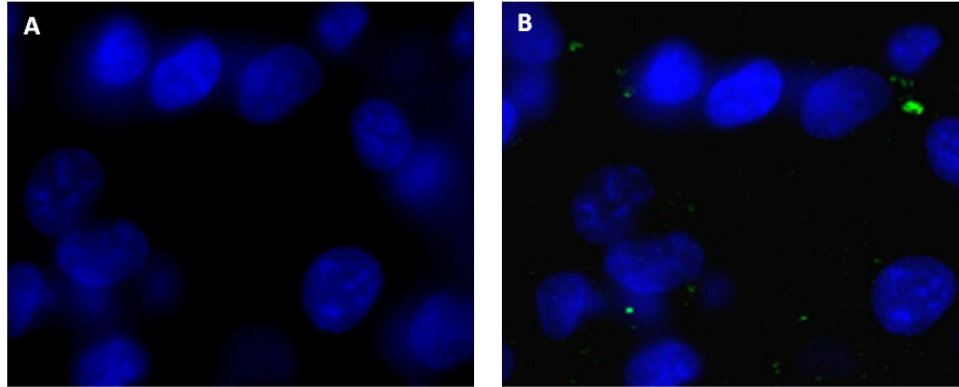
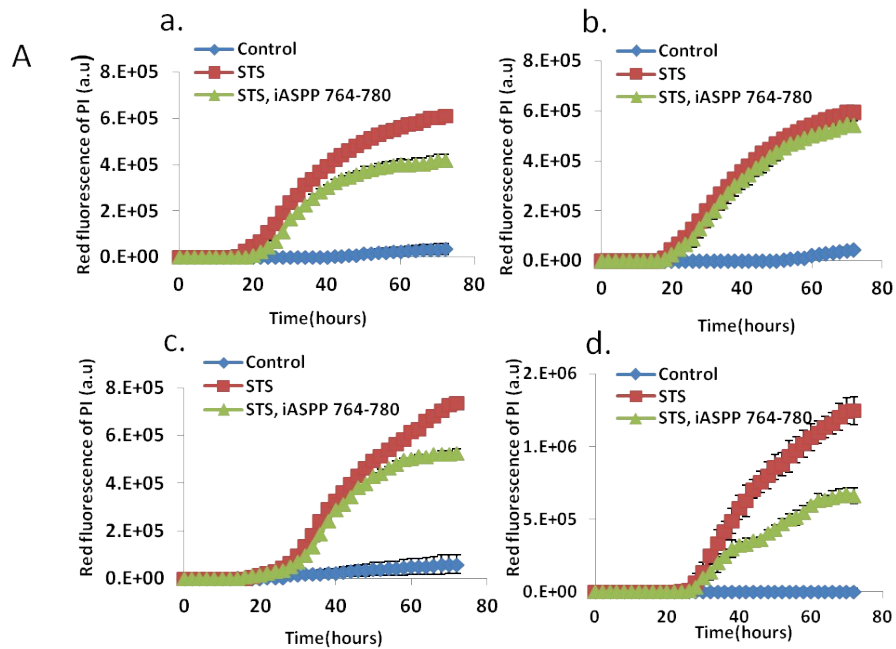


Figure S3: Uptake of FL-peptide (iASPP 764-780) into MDA-MB-231 cancer cells. For cell imaging, cells were incubated with (A) no peptide or (B) 10 μ M of FL-peptides for 3 h and then intensively washed. Cell fluorescence was observed using semi-confocal microscope Nikon TE2000 microscope equipped with a thermostated stage and a Hamamatsu Orca-Era CCD camera.



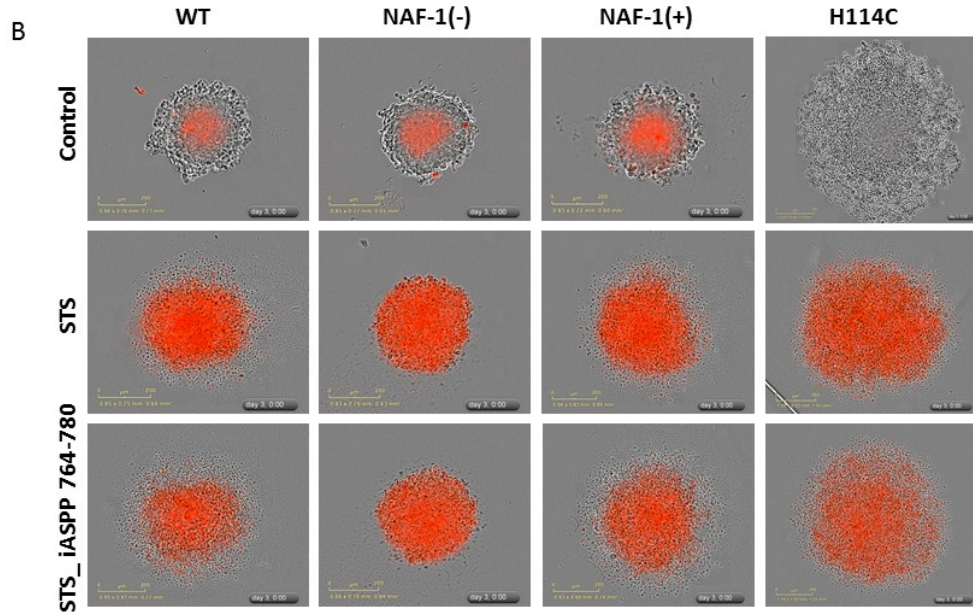


Figure S4: The effect of iASPP 764-780 on the viability of MDA-MB-231 spheroids with altered NAF-1 expression/function. Four different cell lines were used: WT, NAF-1(-), NAF-1(+), and H114C. (A) Time-dependent effect of iASPP 764-780 on cytotoxicity in spheroids of MDA-MB-231 cancer cells. Cytotoxicity was measured using propidium iodide. (a) WT cells, (b) NAF-1 suppressed cells, (c) NAF-1 over-expressed cells (d) H114C mutant cells. The graphs show untreated cells (blue), cells treated with STS (red), and cells treated with STS and iASPP 764-780 (green); (B) Representative spheroid images taken at day 3: WT NAF-1 expression, NAF-1 (-) with suppressed NAF-1 protein levels, NAF-1(+), with over-expressed NAF-1 protein levels, and H114C with H114C mutant in NAF-1 protein. Cells were treated with or without iASPP 764-780 in the presence of STS.

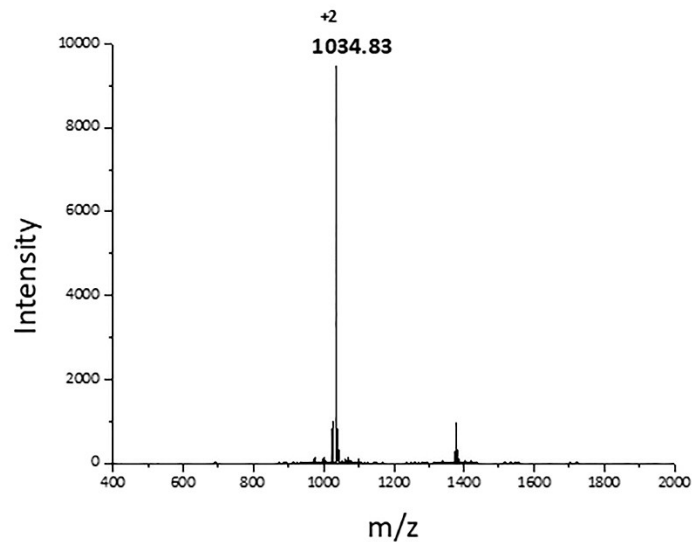


Figure S5: ESI-MS of iASPP 764-780. The calculated Mass is 2068.21 Da and the observed mass is 2067.64 Da.

Supplementary Tables

Table S1: Peptides derived from iASPP Ank-SH3 included in the peptide array

peptide array position	Peptide sequence	Peptide name
A/B 1	PQSMEMRSVLRKAGS	iASPP 602-616
A/B 2	KAGSPRKARRARLNP	iASPP 613-627
A/B 3	ARRARLNPLVLLLLDA	iASPP 620-634
A/B 4	LLDAALTGELEVQ	iASPP 631-645
A/B 5	GELEVQAVKEMND	iASPP 638-652
A/B 6	EMNDPSQPNEEGITA	iASPP 649-663
A/B 7	PNEEGITALHNAICG	iASPP 656-670
A/B 8	AICGANYSIVDFLIT	iASPP 667-681
A/B 9	SIVDFLITAGANVNS	iASPP 674-688
A/B 10	NVNSPDSHGWTPLHC	iASPP 685-699
A/B 11	HGWTPLHCAASCNDT	iASPP 692-706
A/B 12	CNDTVICMALVQHGA	iASPP 703-717
A/B 13	MALVQHGAEIFATTL	iASPP 710-724
A/B 14	ATTLSDGATAFEKCD	iASPP 721-735
A/B 15	ATAFEKCDPYREGYA	iASPP 728-742
A/B 16	EGYADCATYLADVEQ	iASPP 739-753
A/B 17	TYLADVEQSMGLMNS	iASPP 746-760
A/B 18	LMNSGAVYALWDYSA	iASPP 757-771
A/B 19	YALWDYSAEFGDELS	iASPP 764-778
A/B 20	DELSFREGESVTVLR	iASPP 775-789
A/B 21	GESVTVLRDGPPEET	iASPP 782-796
A/B 22	PEETDWWAALHGQE	iASPP 793-807
A/B 23	WAALHGQEGYVPRNY	iASPP 800-814
A/B 24	YFGLFPRVKPQRSKV	iASPP 814-828

Table S2: Peptides derived from iASPP Pro included in the peptide array

	Peptide name	Peptide sequence
A 6	iASPP 1-15	ADSEAFQSARDFLDM
A 7	iASPP 8-22	SARDFLDMNFQSLAM
A 8	iASPP 19-33	SLAMKHMDLKQMELD
A 9	iASPP 26-40	DLKQMELDTAAAKVD
A10	iASPP 37-51	AKVDELTKQLESLWS
A11	iASPP 44-58	KQLESLWSDSPAPPG
A12	iASPP 55-69	APPGPQAGPPSRPPR
A13	iASPP 62-76	GPPSRPPRYSSSSIP

A14	iASPP 73-87	SSIPEPFGSRGSPRK
A15	iASPP 80-94	GSRGSPRKAATDGAD
A16	iASPP 91-105	DGADTFGRSESAPT
A17	iASPP 98-112	GRSESAPTLHPYSPL
A18	iASPP 109-123	YSPLSPKGRPSSPRT
A19	iASPP 116-130	GRPSSPRTPLYLQPD
A20	iASPP 127-141	LQPDAYGSLDRATSP
A21	iASPP 134-148	SLDRATSPRPRAFDG
A22	iASPP 145-159	AFDGAGSSLGRAPSP
A23	iASPP 152-166	SLGRAPSPRPGGPL
A24	iASPP 163-177	PGPLRQQGPPTPFD
B 1	iASPP 170-184	GPPTPFDFLGRAGSP
B 2	iASPP 181-195	AGSPRGSPLAEGPQA
B 3	iASPP 188-202	PLAEGPQAFFPERGP
B 4	iASPP 199-213	ERGSPRPPATAYDA
B 5	iASPP 206-220	PPATAYDAPASAFGS
B 6	iASPP 217-231	AFGSSLLGSGGSAFA
B 7	iASPP 224-238	GSGGSAFAPPLRAQD
B 8	iASPP 235-249	RAQDDLTLRRRPPKA
B 9	iASPP 242-256	LRRRPPKAWNESDLD
B10	iASPP 253-267	SDLDVAYEKKPSQTA
B11	iASPP 260-274	EKKPSQTASIERLDV
B12	iASPP 271-285	RLDVFARPASPSLQL
B13	iASPP 278-292	PASPSLQLLPWRESS
B14	iASPP 289-303	RESSLDGLGGTGKDN
B15	iASPP 296-310	LGGTGKDNLTSATLP
B16	iASPP 307-321	ATLPRNYKVSPLASD
B17	iASPP 314-328	KVSPLASDRRSDAGS
B18	iASPP 325-339	DAGSYRRSLGSAGPS
B19	iASPP 332-346	SLGSAGPSGTLPRSW
B20	iASPP 343-357	PRSWQPVSRIPMPPS
B21	iASPP 350-364	SRIPMPPSSPQPRGA
B22	iASPP 361-375	PRGAPRQRP IPLSMI
B23	iASPP 368-382	RPIPLSMIFKLQNAF
B24	iASPP 379-393	QNAFWEHGASRAMLP
C 1	iASPP 386-400	GASRAMLPGSPLFTR
C 2	iASPP 397-411	LFTRAPPPKLQPQPQ
C 3	iASPP 404-418	PKLQPQPQPQPQS
C 4	iASPP 415-429	QPQSQPQPQLPPQPQ
C 5	iASPP 422-436	PQLPPQPQTQPQTPT
C 6	iASPP 433-447	QTPTPAPQHQPQTWP
C 7	iASPP 440-454	QHPQQTWPPVNEGPP
C 8	iASPP 451-465	EGPPKPPTELEPEPE
C 9	iASPP 458-472	TELEPEPEIEGLLTP

C10	iASPP 469-483	LLTPVLEAGDVDEGP
C11	iASPP 476-490	AGDVDEGPFVARPLSP
C12	iASPP 487-501	PLSPTRLQPALPPEA
C13	iASPP 494-508	QPALPPEAQSVPELE
C14	iASPP 505-519	PELEEVARVLAIEIPR
C15	iASPP 512-526	RVLAEIPRPLKRRGS
C16	iASPP 523-537	RRGSMEQAPAVALLPP
C17	iASPP 530-544	APAVALLPPTHKKQYQ
C18	iASPP 541-555	KQYQQIISRLFHRHG
C19	iASPP 548-562	SRLFHRHGGPGGGP
C20	iASPP 559-573	PGGPEPELSPITEGS
C21	iASPP 566-580	LSPITEGSEARAGPP
C22	iASPP 577-591	AGPPAPAPPAPIPPP
C23	iASPP 584-598	PPAPIPPPAPSPQSSP
C24	iASPP 595-609	QSSPPEQPQSMEMRS

Table S3: Peptide array used for alanine scan of iASPP 764-778

peptide array position	Peptide sequence	Peptide name
C1	AALWDYSAEFGDELS	iASPP 764-778 Y764A
C2	YAAWDYSAEFGDELS	iASPP 764-778 L766A
C3	YALADYSAEFGDELS	iASPP 764-778 W767A
C4	YALWAYS EFGDELS	iASPP 764-778 D768A
C5	YALWDASAEFGDELS	iASPP 764-778 Y769A
C6	YALWDYAAEFGDELS	iASPP 764-778 S770A
C7	YALWDYSAAEFGDELS	iASPP 764-778 E772A
C8	YALWDYSAEAGDELS	iASPP 764-778 F773A
C9	YALWDYSAEFADELS	iASPP 764-778 G774A
C10	YALWDYSAEFGAELS	iASPP 764-778 D775A
C11	YALWDYSAEFGDALS	iASPP 764-778 E776A
C12	YALWDYSAEFGDEAS	iASPP 764-778 L777A
C13	YALWDYSAEFGDELA	iASPP 764-778 S778A

Table S4: Top 20 DI-ranked residue pairs computed with DCA for interaction between NAF-1 and iASPP.

Residue in NAF-1	Residue in IASSP	MI	DI
78	709	0.441383	1.00764
80	814	0.279379	0.994566
76	780	0.435899	0.937921
94	655	0.583294	0.903548
97	781	0.311647	0.881439
94	774	0.440141	0.880342
80	774	0.432062	0.867212
94	773	0.56617	0.855003
115	773	0.4334	0.835885
76	773	0.386364	0.834587
80	653	0.413315	0.833971
94	804	0.489086	0.825877
84	767	0.359676	0.822661
74	651	0.333779	0.821456
74	802	0.293356	0.816544
80	682	0.596889	0.815193
131	681	0.363302	0.8124
97	804	0.237167	0.809175
106	652	0.231318	0.80807
86	804	0.360061	0.807675

DCA, direct coupling analysis; DI, direct information; MI, mutation information.
 Data in red represent co-evolving residue pairs.