Supplemental Table I. Measured affinities for designed peptides at 37 °C. K_d units are nanomolar. If repeated experiments were performed, the K_d listed is the average value and individual replicates are given in parentheses. A K_d in italics denotes the average with a single outlier removed. A "*" denotes a fitted K_d with R^2 value < 0.8.

	anti-CREBZF	anti-XBP1	OPTanti-XBP1_A	OPTanti-XBP1_B	anti-ATF6	OPTanti-ATF6	anti-FOS	OPTanti-FOS
FOS	1015.6	83.5	2949.8	5000	≥5000	≥5000	≤1 (≤1, ≤1)[1]	≤1 (≤1, ≤1)[1]
FOSL1	39.5*	≥5000 (≥5000, 5000)[1]	≥5000 (≥5000, 5000)[1]	5000	≥5000	≥5000	6.0	2.8
ATF2	≥5000	≥5000 (≥5000, 5000)[1]	≥5000	≥5000	5000 (5000, 5000)[1]	≥5000	5000 (5000, 5000)[1]	400.1
ATF3	≥5000	≥5000	≥5000	≥5000 (≥5000, 5000)[1]	5000	≥5000	300.3	269.5 (392.6)[2]
ATF4	5000	≥5000	≥5000 (≥5000, ≥5000)[1]	≥5000	*≥5000* (705.7, 5000, ≥5000, ≥5000, ≥5000)[1]	≥5000 (5000, 5000, 5000, ≥5000)[1]	(626.7, 5000)[1]	30.5
ATF6	1250.7 (746.1,1755.2)[1]	≥5000	≥5000 (≥5000, 5000)[1]	≥5000	*5000* (103.1, 5000, 5000, 5000, 5000)[1]	13.4 (17.4, 12.4, 11.5, 12.2)[1] (149.5)[2]	≥5000	≥5000
ATF6B	244.7	5000	71.4 (77.7, 65.1)[1]	5000	335.2 (246.3, 378.9, 380.4)[1]	2.9 (5.7, 1.7, 1.3)[1] (7.4)[2]	≥5000	≥5000
CREBZF	1.2 (1.4, 1.0)[1]	≥5000	≥5000	≤1	5000 (5000)[2]	521.3 (274.8, 767.8)[1]	≥5000	≥5000
XBP1	≥5000	5000 (5000, 5000)[1]	91.1 (56.4, 85.4, 131.5)[1]	146.7 (181.4, 112.0)[1]	*4098.9* (342.7, 1395.5, 5000, 5000, 5000)[1]	6.4 (9.5, 4.2, 6.6, 5.3)[1] (34.6)[2]	≥5000	≥5000
JUN	661.8 (698.5, 625.1)[1]	5000	≥5000 (5000, ≥5000)[1]	≥5000	*≥5000* (412.8, 5000, ≥5000, ≥5000)[1]	≥5000	74.0	25.8
JUNB	≥5000	5000	≥5000	≤1 (≤1, ≤1)[1]	≥5000	≥5000	≥5000	≥5000
MAFF	≥5000	≥5000	5000	1.8	4250.3	≥5000	245.0	35.6
MAFG	1474.9	≥5000	5000	≤1	4177.9 (3355.8, 5000)[1]	≥5000	134.5	88.1 (322.0)[2]
MAF	≥5000	5000	≥5000	≥5000	≥5000	≥5000	273.5	34.9
MAFB	≥5000	5000	≥5000	≥5000	≥5000	≥5000	1231.2	65.5
CREB1	≥5000	5000	≥5000	≥5000	≥5000	≥5000	225.7	79.8
CREB3	≥5000	≥5000	5000	≥5000	1704.7	≥5000	≥5000	≥5000
CREB3L1	≥5000	663.9	≥5000	≥5000	≥5000	≥5000	≥5000	≥5000
CREB3L3	≥5000	5000	≥5000	≥5000	5000	≥5000	*5000* (1.1, 5000, 5000)[1]	≥5000
NFE2	763.0	171.3	≥5000	8.5	687.2	≥5000	5000	≥5000
NFE2L1	4911.5 (4822.9, 5000)[1]	≥5000	≥5000	≤1	*5000* (403.1,5000 5000)[1]	≥5000	≥5000	≥5000
NFE2L2	2434.4	5000	≥5000	3714.7*	≥5000	≥5000	5000	364.2
NFE2L3	5000	≥5000	≥5000	≤1	578.8	≥5000	≥5000	≥5000
BACH1	≥5000	≥5000 (≥5000, 5000)[1]	2068.9	160.9	3463.9 (5000, 1927.8)[1]	≥5000	43.5 (67.6)[1]	87.2 (86.3, 88.0)[1]
BACH2	≥5000	5000	5000	16.9	5000	≥5000	5000	202.2 (5000)[2]
BATF2	≥5000 (≥5000, ≥5000)[1]	≥5000	≥5000 (≥5000, 5000)[1]	5000	≥5000	≥5000	97.9	24.4
BATF3	≥5000	≥5000	≥5000	5000	5000	≥5000	17.4 (8.6, 19.5, 24.2)[1]	14.4 (13.5)[2]
HLF	≥5000	5000.0	≥5000	≥5000	5000	≥5000	≥5000	≥5000
DBP	≥5000	≥5000	≥5000	≥5000	≥5000	≥5000	≥5000	≥5000
NFIL3	≥5000	5000	≥5000	≥5000	≥5000	≥5000	5000	1104.3
CEBPG	40.4 (37.3, 43.5)[1]	139.2	≥5000 (≥5000, 5000)[1]	≥5000	2355.5	5000	13.5 (6.5, 18.4, 15.5)[1]	5.4 (3.7, 7.1)[1]
homodimer	5000	≥5000	≥5000	≥5000	≥5000	112.6	85.5	296.6

[1] K_d from repeated experiments.
[2] K_d from an experiment where the designed peptide was labeled with the donor fluorophore and the target was labeled with the acceptor.

Supplemental Table II. Measured affinities for designed peptides at 23 °C. K_d units are nanomolar. If repeated experiments were performed, the K_d listed is the average value and individual replicates are given in parentheses. A K_d in italics denotes the average with a single outlier removed. A "*" denotes a fitted K_d with R^2 value < 0.8.

	anti-CREBZF	anti-XBP1	OPTanti-XBP1_A	OPTanti-XBP1_B	anti-ATF6	OPTanti-ATF6	anti-FOS	OPTanti-FOS
FOS	273.9	42.6	210.1	807.7	≥1000	≥1000	≤1 (≤1)[1]	≤1 (≤1)[1]
FOSL1	1000.0	≥1000 (≥1000, 1000)[1]	693.5 (386.9, 1000)[1]	778.5	≥1000	≥1000	≤1	1.9
ATF2	≥1000	≥1000 (≥1000, 1000)[1]	1000	≥1000	1000 (1000, 1000)[1]	≥1000	1000 (1000, 1000)[1]	65.6
ATF3	≥1000	≥1000	≥1000	≥1000 (≥1000, 1000)[1]	≥1000	≥1000	17.1	28.4 (98.9)[2]
ATF4	1000	≥1000	≥1000 (1000, ≥1000)[1]	≥1000	970.6 (853.2, 1000, 1000, 1000, 1000)[1]	*35.2* (302.4, 33.6, 31.5, 40.5)[1]	80.9 (96.9, 64.9)[1]	7.6
ATF6	609.3 (527.4, 691.2)[1]	≥1000	1000 (1000, 1000)[1]	≥1000	*118.3* (5.4, 102.0, 94.4, 147.7, 129.0)[1]	3.5 (9.2, 1.6, 1.3, 1.7)[1] (13.5)[2]	≥1000	≥1000
ATF6B	134.8	≥1000	13.7 (18.5, 8.9)[1]	86.7	27.4 (35.2, 20.1, 27.0)[1]	≤1 (≤1, ≤1, ≤1)[1] (2.9)[2]	≥1000	≥1000
CREBZF	1.4 (1.8, 1)[1]	≥1000	160.0	1.1	1000 (1000)[2]	11.4 (9.4*, 13.4)	≥1000	≥1000
XBP1	≥1000	1000 (1000, 1000)[1]	11 (9.6, 18.2, 5.2)[1]	25.1 (28.1, 22.1)[1]	165.8 (139.8, 159.3, 197.2, 153.0, 179.9)[1]	3.4 (10.2, 1, 1.2, ≤1)[1] (5.4)[2]	≥1000	835.1
JUN	75.4 (84.2, 66.5)[1]	324.5	128.6 (150.5, 106.6)[1]	238.2*	≥1000 (1000, 1000, ≥1000, ≥1000)[1]	≥1000	16.5	4.9
JUNB	1000	≥1000	867.5	≤1 (≤1, ≤1)[1]	≥1000	≥1000	≥1000	>1000
MAFF	≥1000	≥1000	457.6	≥1000	1000	≥1000	39.7	9.6
MAFG	1000	≥1000	476.3	≤1	≥1000 (≥1000, 1000)[1]	≥1000	5.8	71.8* (104.6)[2]
MAF	≥1000	1000	≥1000	≥1000	≥1000	≥1000	28.2	11.1
MAFB	≥1000	1000	≥1000	≥1000	1000	≥1000	84.4	15.7
CREB1	≥1000	≥1000	1000	≥1000	≥1000	≥1000	≥1000	22.2
CREB3	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000
CREB3L1	≥1000	966.8	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000
CREB3L3	≥1000	1000	≥1000	≥1000	1000	≥1000	*1000* (≤1, 1000, 1000)[1]	≥1000
NFE2	124.7	1000	≥1000	3.4	1000	≥1000	≥1000	≥1000
NFE2L1	1000 (1000, 1000)[1]	≥1000	766.8	≤1	902.1 (706.3, 1000, 1000)[1]	≥1000	≥1000	≥1000
NFE2L2	539.2	≥1000	≥1000	≥1000	≥1000	≥1000	1000	453.8
NFE2L3	1000.0	≥1000	≥1000	≤1	1000	≥1000	≥1000	≥1000
BACH1	≥1000	≥1000 (≥1000, 1000)[1]	235.8	70.7	838.1 (≥1000, 676.7)[1]	≥1000	4.5 (9.8)[1]	17.1 (22.7)[1]
BACH2	1000	≥1000	283.3	4.8	≥1000	≥1000	1000	72.0 (263.6)[2]
BATF2	≥1000 (≥1000, ≥1000)[1]	≥1000	935.2 (1000, 870.4)[1]	1000	≥1000	≥1000	15.0	6.5
BATF3	1000	≥1000	485.9	1000	≥1000	≥1000	3.0 (74.4, 2.8, 3.2)[1]	7.1 (4.6)[2]
HLF	≥1000	≥1000	≥1000	≥1000	1000	≥1000	≥1000	≥1000
DBP	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	1000
NFIL3	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	1000	128.3
CEBPG	8.6 (9.5, 7.6)[1]	413.0	≥1000 (≥1000, 1000)[1]	≥1000	1000	26.7	2.1 (1.3, 2.5, 2.5)[1]	≤1 (1.1)[1]
homodimer	420.4	≥1000	≥1000	≥1000	≥1000	9.7	15.8	59.3

[1] K_d from repeated experiments.
[2] K_d from an experiment where the designed peptide was labeled with the donor fluorophore and the target was labeled with the acceptor.

Supplemental Table III. Measured affinities for designed peptides at 4 °C. K_d units are nanomolar. If repeated experiments were performed, the K_d listed is the average value and individual replicates are given in parentheses. A K_d in italics denotes the average with a single outlier removed. A "*" denotes a fitted K_d with R^2 value < 0.8.

	anti-CREBZF	anti-XBP1	OPTanti-XBP1_A	OPTanti-XBP1_B	anti-ATF6	OPTanti-ATF6	anti-FOS	OPTanti-FOS
FOS	27.2	48.1	12.5	59.9	≥1000	≥1000	≤1 (≤1)[1]	≤1 (≤1)[1]
FOSL1	98.7	≥1000 (1000, 1000)[1]	15.1 (11.0, 19.1)[1]	39.5	≥1000	≥1000	2.2	1.7
ATF2	≥1000	≥1000 (1000, 1000)[1]	128.9	445.6	163.3 (141.7)[1]	≥1000	1000 (1000,1000)[1]	13.0
ATF3	≥1000	≥1000	1000	≥1000 (≥1000, 1000)[1]	≥1000	≥1000	12.6	16.0* (16.6)[2]
ATF4	1000	≥1000	67.9 (80.1, 55.6)[1]	≥1000	10.0 (5.3, 13.9, 8.3, 11.3, 11.2)[1]	*12.4* (96.2, 11.9, 10.0, 15.4)[1]	21.0 (16.0)[1]	1.4
ATF6	154.7 (118.9, 190.4)[1]	≥1000	74.0 (78.9, 87.6, 55.6)[1]	≥1000	*15.2* (≤1, 12.6, 14.6, 20.3, 13.2)[1]	1.3 (≤1, 1.1, 1.6, 1.5)[1] (≤1)[2]	1000	≥1000
ATF6B	1000	≥1000	22.8 (30.3, 15.2)[1]	167.6	38.6 (37.2, 39.8, 38.9)[1]	1.0 (≤1, 1.1, ≤1)[1] (≤1)[2]	≥1000	≥1000
CREBZF	1.5 (1.9, ≤1)[1]	≥1000	14.2	≤1	43.6 (1000)[2]	2.7 (≤1, 4.4)[1]	≥1000	≥1000
XBP1	1000	449.8 (415.7, 483.9)[1]	3.7 (2.9, 6.9, 1.3)[1]	5.6 (5.5, 5.6)[1]	*35.9* (≤1, 41.4, 40.9, 31.2, 30.1)[1]	1.1 (≤1, ≤1, 1.2, ≤1)[1] (≤1)[2]	≥1000	35.3
JUN	6.4 (8.6, 4.2)[1]	1000	3 (4.9, 1.1)[1]	1.6	276.1 (218.4, 276.1, 251.2, 358.8)[1]	≥1000	4.9	≤1
JUNB	44.3	69.1	9.2	1.3 (1.5, ≤1)[1]	≥1000	≥1000	12.9*	7.6*
MAFF	≥1000	≥1000	47.1	≥1000	102.6	≥1000	18.4	2.4
MAFG	1000	≥1000	53.2	≤1	722.4 (444.7, 1000)[1]	≥1000	5.7	28.4* (31.9)[2]
MAF	≥1000	≥1000	35.2*	691.5	94.5	≥1000	44.0	4.3
MAFB	≥1000	≥1000	≥1000	≥1000	195.6	≥1000	21.2	2.9
CREB1	≥1000	≥1000	135.0	≥1000	635.1	≥1000	≥1000	18.5*
CREB3	≥1000	520.9	≥1000	≥1000	520.7	≥1000	≤1	≥1000
CREB3L1	≥1000	≥1000	≥1000	≥1000	1000	≥1000	≥1000	≥1000
CREB3L3	≥1000	378.9	≥1000	≤1	104.8	≥1000	*≥1000* (≤1, ≥1000, ≥1000)[1]	≥1000
NFE2	6.8	1000	293.9	1.5	≥1000	≥1000	≥1000	≥1000
NFE2L1	1000 (1000, 1000)[1]	≥1000	96.1	≤1	≥1000 (1000, ≥1000, 1000)[1]	≥1000	1000	≥1000
NFE2L2	25.0	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	1000
NFE2L3	≥1000	≥1000	564.3	≤1	1000	≥1000	≥1000	≥1000
BACH1	1000	1000 (1000, 1000)[1]	16.3	5.1	1000 (1000, 1000)[1]	≥1000	1.6 (3.2)[1]	7.3 (6.7)[1]
BACH2	42.4	338.0	6.2	3.7	416.8	≥1000	83.9	8.1 (13.4)[2]
BATF2	1000 (1000, 1000)[1]	≥1000	168.2 (192.0, 144.4)[1]	≥1000	≥1000	≥1000	1.9	1.3
BATF3	1000	≥1000	21.5	115.0	≥1000	≥1000	1.4 (1.4, 1.4, 1.5)	5.0 (1.7)[2]
HLF	≥1000	≥1000	≥1000	509.9*	158.9	≥1000	≥1000	≥1000
DBP	≥1000	≥1000	≥1000	≥1000	112.2	≥1000	≥1000	≥1000
NFIL3	≥1000	≥1000	1000	≥1000	1000	≥1000	583.5	15.1
CEBPG	2.5 (2.9, 2.0)[1]	1000	≥1000 (≥1000, 1000)[1]	≥1000	253.8	4.2*	1.5 (≤1, 1.9, 1.7)[1]	≤1 (≤1)[1]
homodimer	16.1	≥1000	≥1000	≥1000	1000	≤1	14.3	8.8

[1] K_d from repeated experiments.
[2] K_d from an experiment where the designed peptide was labeled with the donor fluorophore and the target was labeled with the acceptor.

Supplemental Table IV. Measured affinities for OPTanti-XBP1_A at 37, 23, and 4 °C in 0.4 M KCl. K_d units are nanomolar.

	37 °C	23 °C	4 °C
XBP1	98.8	6.6	2.0
ATF6B	64.0	15.4	17.6
CREBZF	1028.8	20.0	12.7
BACH1	1075.3	140.5	9.6
BACH2	1623.1	92.3	2.2
FOS	≥5000	279.9	14.4
JUNB	≥5000	258.5	1.5
MAFG	1539.9	255.5	24.0
NFE2	≥5000	≥1000	≥1000
NFE2L1	≥5000	≥1000	≥1000
NFE2L3	≥5000	≥1000	≥1000

Supplemental Table V. Measured affinities for OPTanti-XBP1_B at 37, 23, and 4 °C in 0.4 M KCl. K_d units are nanomolar.

	37 °C	23 °C	4 °C
XBP1	145.6	6.1	1.6
ATF6B	99.9	24.5	31.6
CREBZF	3852.4	29.5	17.8
BACH1	≥5000	199.1	7.8
BACH2	3253.6	118.2	4.4
FOS	≥5000	271.8	9.7
JUNB	≥5000	137.9	1.0
MAFG	≥5000	223.9	17.8
NFE2	≥5000	≥1000	≥1000
NFE2L1	≥5000	≥1000	≥1000
NFE2L3	≥5000	≥1000	≥1000

Supplemental Table VI. Measured affinities for OPTanti-XBP1_B-GLN at 37, 23, and 4 °C. K_d units are nanomolar.

	37 °C	23 °C	4 °C
XBP1	93.7	6.5	1.6
ATF6B	109.5	26.1	34.2
CREBZF	≥5000	76.7	11.1
BACH1	≥5000	331.6	11.7
BACH2	≥5000	323.5	33.5
FOS	1354.5	153.5	6.9
JUNB	54.5	182.8	2.2
MAFG	1721.9	670.2	54.0
NFE2	≥5000	≥1000	≥1000
NFE2L1	≥5000	≥1000	≥1000
NFE2L3	≥5000	≥1000	≥1000

Supplemental Table VII. Measured affinities for anti-FOS at 37, 23, and 4 °C in buffer with 3 M urea. K_d units are nanomolar. If repeated experiments were performed, the K_d listed is the average. A K_d in italics denotes the average with an outlier removed. A "*" denotes a fitted K_d with R^2 value < 0.8.

	37 °C	23 °C	4 °C
FOS	*259.1* (30.2*, 164.3, 193.4, 298.1, 380.7)[1]	*33.4* (6.3, 16.7, 36.5, 46.9)[1]	4.8 (1.8, 3.5, 6.5, 7.4)[1]
BATF3	≥5000	≥1000	727.9
BACH1	≥5000	≥1000	≥1000
CEBPG	≥5000	939.4	212.3

[1] K_d from repeated experiments.

Supplemental Table VIII. Measured affinities for OPTanti-FOS at 37, 23, and 4 °C in buffer with 3 M urea. K_d units are nanomolar. If repeated experiments were performed, the K_d listed is the average.

	37 °C	23 °C	4 °C
FOS	19.8 (11.6, 23.0, 24.8)[1]	3.0 (2.0, 3.4, 3.6)[1]	1.1 (1.0, 1.2, 1.0)[1]
BATF3	5000	$\geq$1000	150.9
BACH1	5000	$\geq$1000	$\geq$1000
CEBPG	5000	1000	542.1

[1] K_d from repeated experiments.

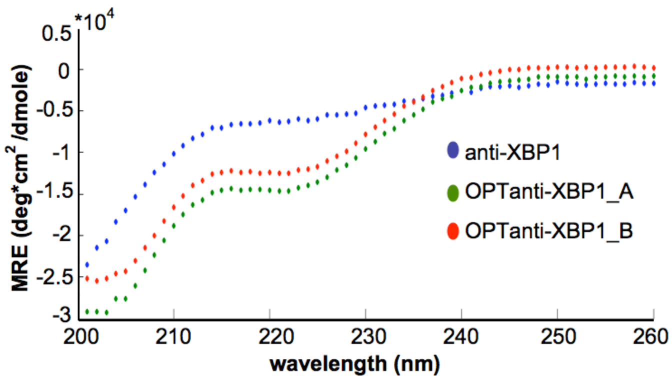
Supplemental Table IX. Fitted masses of design-target complexes from analytical ultracentrifugation.

design	target	Expected Mass	Fitted Mass	Ratio Fitted:Expected	Fit RMSD[1]
anti-CREBZF	CREBZF	15265.1	15465.3	1.0	0.015
OPTanti-XBP1_A	XBP1	14636.6	14846.7	1.0	0.021
OPTanti-ATF6	ATF6	14612.4	15986.2	1.1	0.008

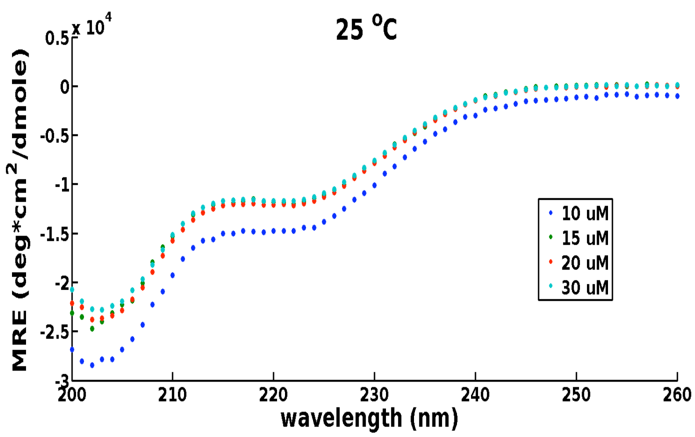
[1] RMSD describes the deviation between the experimental data and the fit. The fits reported are for mixtures of 20 μM design peptide + 20 μM coiled-coil target.

Supplemental Figure 1. CD spectra of the designed peptides anti-XBP1, OPTanti-XBP1_A, and OPTanti-XBP1_B. (A) Scans were performed at 25 °C with 10 µM peptide. Helical content calculated by the Baldwin method (1) was determined to be 20% for anti-XBP1, 44.3% for OPTanti-XBP1_A and 38.3% for OPTanti-XBP1_B. (B) Scans of OPTanti-XBP1_B performed at 25 °C and (C) 37 °C.

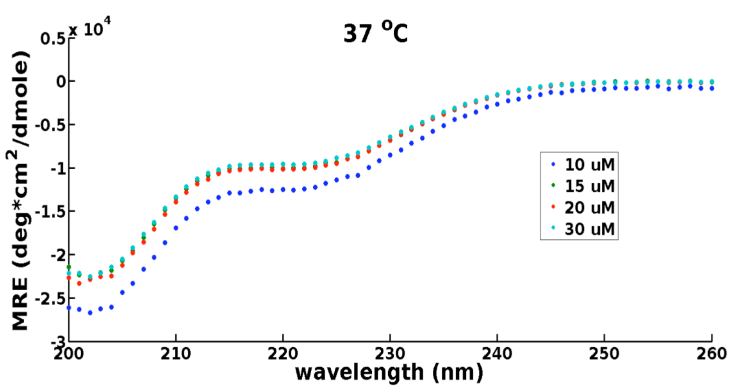
(A)



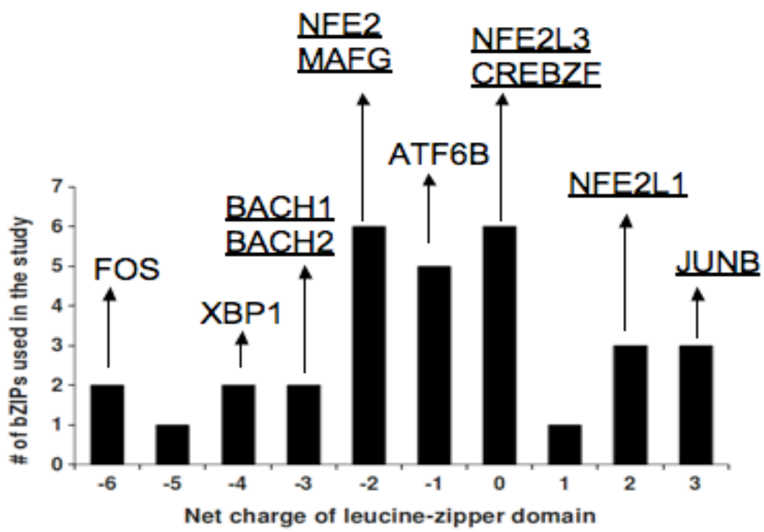
(B)



(C)

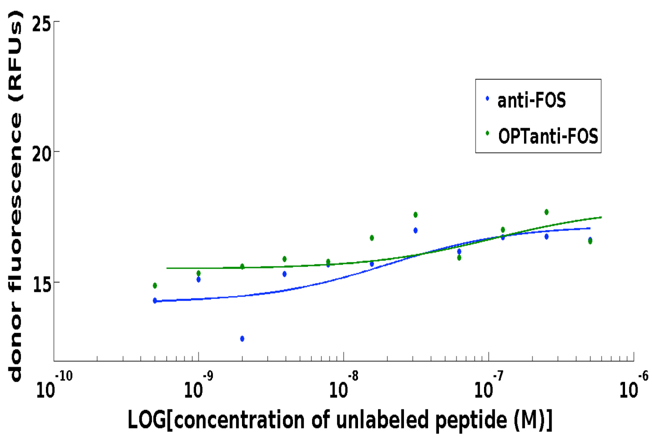


Supplemental Figure 2. Net charges of bZIPs used in the study. A histogram showing the distribution of bZIP leucine-zipper domains having a particular net charge. Underlined bZIPs interacted more tightly with OPTanti-XBP1_B than with OPTanti-XBP1_A.

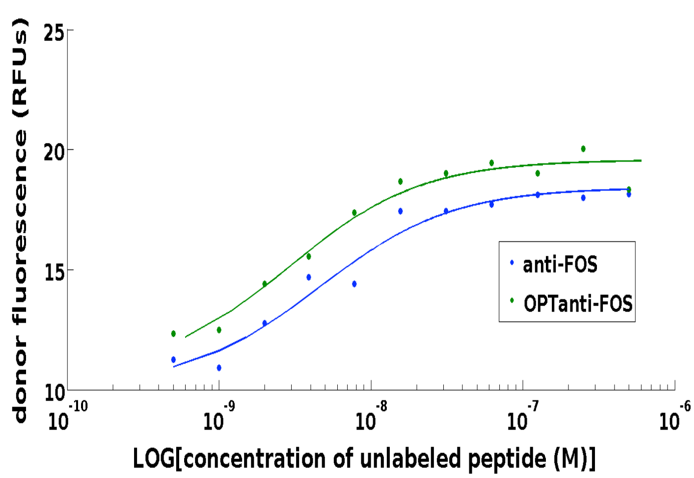


Supplemental Figure 3. Controls for the anti-FOS and OPTanti-FOS inhibition experiments. (A) Lack of inhibition by anti-FOS and OPTanti-FOS of JUN homodimerization (total JUN concentration 20 nM) in the presence of 40 nM of a consensus AP1 site. (B) Inhibition of FOS-JUN (20 nM total) by anti-FOS and OPTanti-FOS. Lack of a lower baseline and larger fluorescent signal detected at the lowest inhibitor concentrations compared to when DNA is present (Figure 5B) suggests the complex being inhibited in Figure 5b is FOS-JUN bound to DNA.

(A)



(B)



References

1. Scholtz JM, Qian H, York EJ, Stewart JM, and Baldwin RL. (1991). Biopolymers 31:1463-1470.