Supporting information figures

Table S1. Sequences of winner peptides.

position		a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c	
cJun		I	A	R	L	E	E	K	V	K	T	L	K	A	Q	N	S	E	L	A	S	T	A	N	M	L	R	E	Q	V	A	Q	L	K	Q	K	V	M	N	
cJun library	AS-	I	A	R	L	E	E	K	V	K	T	L	K	A	Q	N	Y	E	L	A	S	T	A	N	M	L	R	E	Q	V	A	Q	L	K	Q	K	V	M	N	-GAP
									I						K	I						Q	I						K	I			E							
																						E	T										Q							
																						A	V										V							
																						P																		
																						K																		
1st selection winners																																								
#1									V						K	N						E	T						Q	V			L							
#2									V						Q	N						E	A						K	I			L							
#3									V						H	N					I	E	I						Q	I			Q							
#4									V						N	N					T	E	A						K	I		H	L							
#5									V						Q	N						E	T						Q	I			L							
#6									I						Q	N						T	A						K	V			L							
#7									V						R	N						E	A						K	I			L							
#8									V				R		K	N						Q	T						K	I			L							
major									V						Q	N						Q	T						K	I			L							
2nd selection winners																																								
#1									V						Q	N						E	T						K	V			V							
#2									V				R		Q	N						E	A						K	I			L							
#3									V						K	N						E	T						K	I			L							
#4									V				R		Q	N						E	A						K	I			L							
#5									V						Q	N						Q	A						K	V			L							
#6									V						K	N						E	A						K	V			L							
#7									I						Q	N						E	T						K	I			Q							
#8									I						Q	N						E	T						K	I			Q							
#9									I						Q	N						Q	T						K	I			Q							
#10									V						Q	N						Q	A						K	I			L							
#11									V						K	N						E	T						K	I			L							
#12									V						Q	N						E	A						Q	V			L							
#13									V						K	N						E	A						K	V			L							
#14									V						K	N						E	A						K	V			L							
#15									I						Q	N						Q	A						K	I			V							
#16									V						K	N						Q	T						K	I			L							
#17									V						Q	N						E	A						K	V			L							
#18									V						Q	N						E	T						K	I			M							
#19									V						K	N						E	V						K	I			Q							
#20									I						Q	N						E	A						Q	I			Q							
major									V						Q	N						Q	T						K	I			L							
3rd selection winners																																								
#1									V						Q	N						E	A						K	I			L							
#2									V						Q	N						E	A						K	I			L							
#3									V						Q	N						E	A						K	I			L							
#4									V						Q	N						E	A						K	I			L							
#5									V						Q	N						E	A						K	I			L							
#6									V						Q	N						E	A						K	I			L							
#7									V						Q	N						E	A						K	I			L							
#8									V						Q	N						E	A						K	I			L							
#9									V						Q	N						E	A						K	I			L							
#10									V						Q	N						E	A						K	I			L							
#11									V						Q	N						E	A						K	I			L							
#12									V						Q	N						E	A						K	I			L							
#13									V						Q	N						E	A						K	I			L							
#14									V						Q	N						E	A						K	I			L							
#15									V						Q	N						E	A						K	I			L							
#16									V						Q	N						E	A						K	I			L							
#17									V						Q	N						E	A						K	I			L							
#18									V						Q	N						E	A						K	I			L							
#19									V						Q	N						E	A						K	I			L							
#20									V						Q	N						E	A						K	I			L							
major									V						Q	N						E	A						K	I			L							

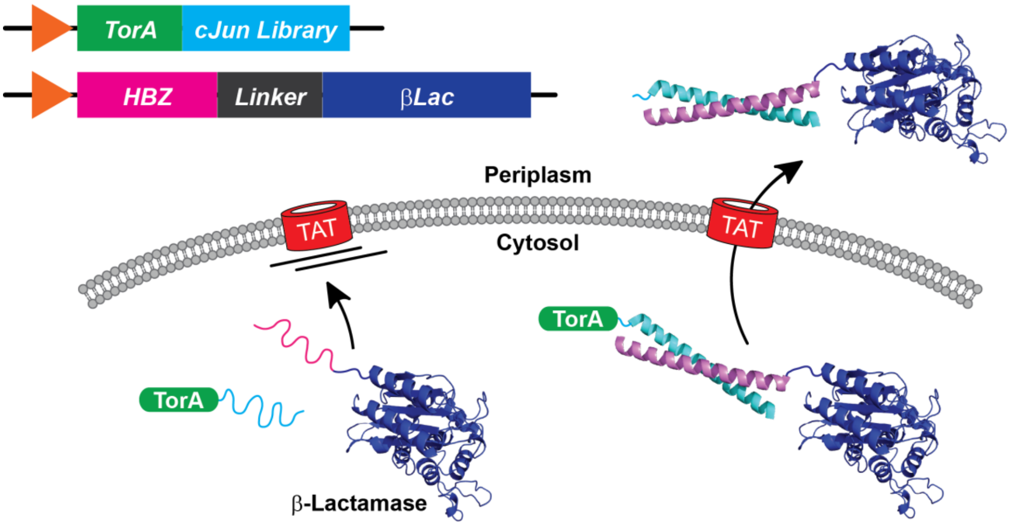


Figure S1. Outline of the TAT Hitchhiker *in vivo* selection system.

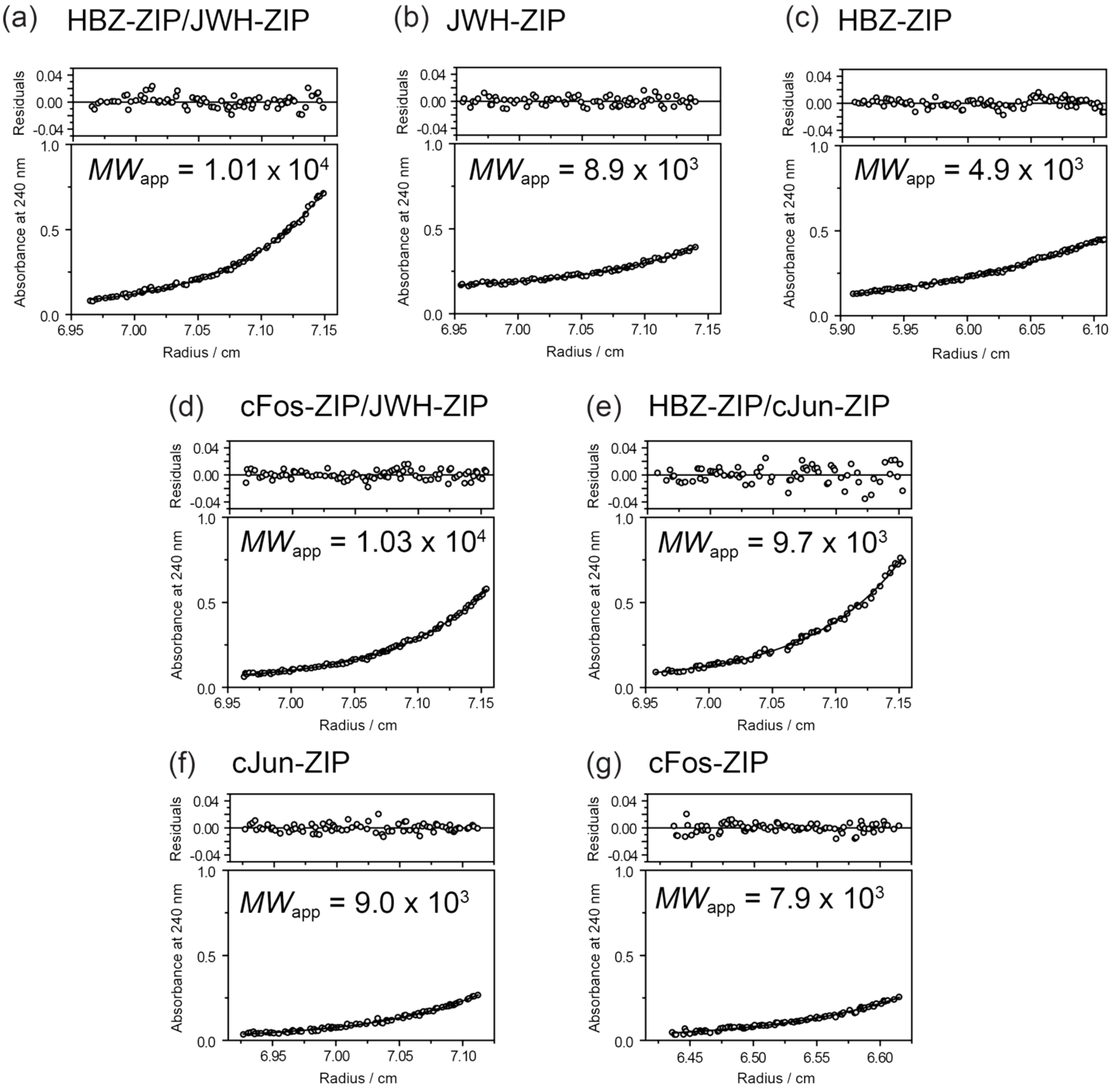
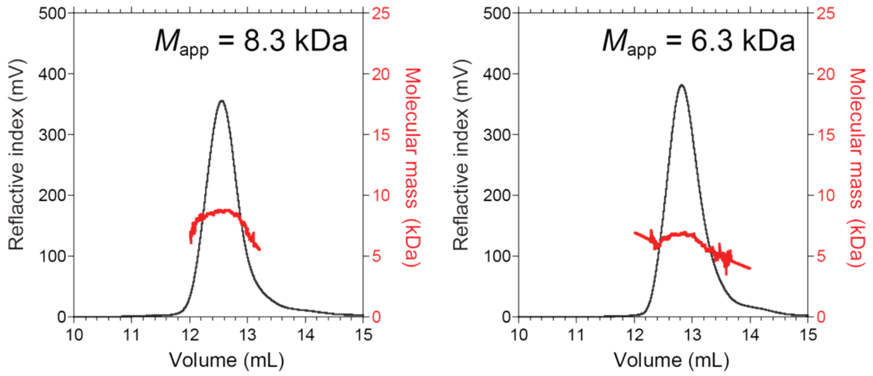


Figure S2. Analytical ultracentrifugation (AUC) of equimolar mixtures of HBZ-ZIP, JWH-ZIP and related peptides. A 1:1 mixture of 50 μM peptides or a single peptide of 50 μM was run at 42,000 rpm at 10°C in 10 mM HEPES containing 500mM NaCl (pH 7.4) and absorbance scans were carried out at 240 nm. AUC data for the HBZ-ZIP/JWH-ZIP mixture are best fit to a single-dispersed model with an apparent weight-average molecular weight (MW_{app}) of 10.1×10^4, which corresponds well to the expected MW_{app} of the heterodimer (9.8×10^4). JWH-ZIP, having a high sequence similarity with cJun-ZIP, was also suggested to form a homodimer (observed in AUC: 8.9×10^3; expected as a dimer: 9.6×10^3), as in the case of cJun-ZIP (observed in AUC: 9.0×10^3; expected as a dimer: 9.6×10^3). On the other hand, MW_{app} (5.2×10^3) of HBZ-ZIP suggested that the peptide exists as a monomer (theoretical molecular weight of the monomer, 4.9×10^3). Other expected molecular weight: cFos-ZIP/JWH-ZIP heterodimer, 9.7×10^3; HBZ-ZIP/cJun-ZIP heterodimer, 9.7×10^3; cJun-ZIP homodimer, 9.5×10^3; cFos-ZIP homodimer, 9.6×10^3; cFos-ZIP monomer, 4.8×10^3.

(a) HBZ-ZIP/JWH-ZIP (b) JWH-ZIP



(c) HBZ-ZIP (d) cFos-ZIP/JWH-ZIP

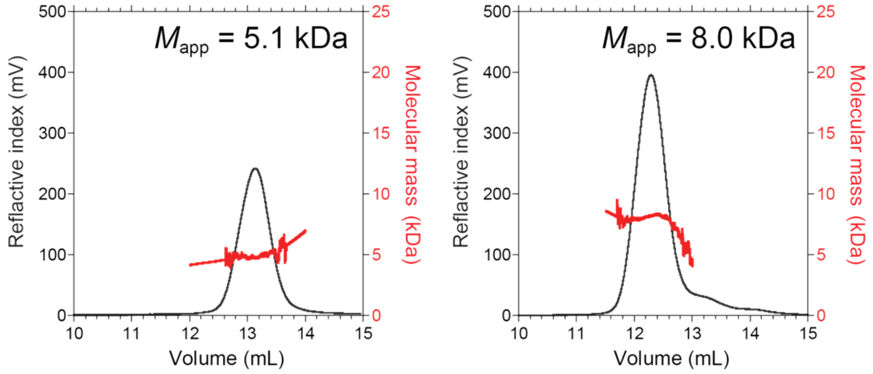


Figure S3. Size-exclusion chromatography with on-line light-scattering (SEC-LS) analysis of (a) an equimolar mixtures of HBZ-ZIP and JWH-ZIP, (b) JWH-ZIP, (c) HBZ-ZIP, (d) an equimolar mixtures of cFos-ZIP and JWH-ZIP. Expected molecular masses; HBZ-ZIP/JWH-ZIP heterodimer, 9.8 kDa; JWH homodimer, 9.6 kDa; JWH-ZIP monomer, 4.8 kDa; HBZ-ZIP homodimer, 9.9 kDa; HBZ-ZIP monomer, 4.9 kDa; cFos-ZIP/JWH-ZIP heterodimer, 9.7 kDa. Total peptide concentration: 1 mg/mL (150 μL) ; eluent: 25 mM HEPES containing 150 mM NaCl (pH 7.4); temperature: 23°C.

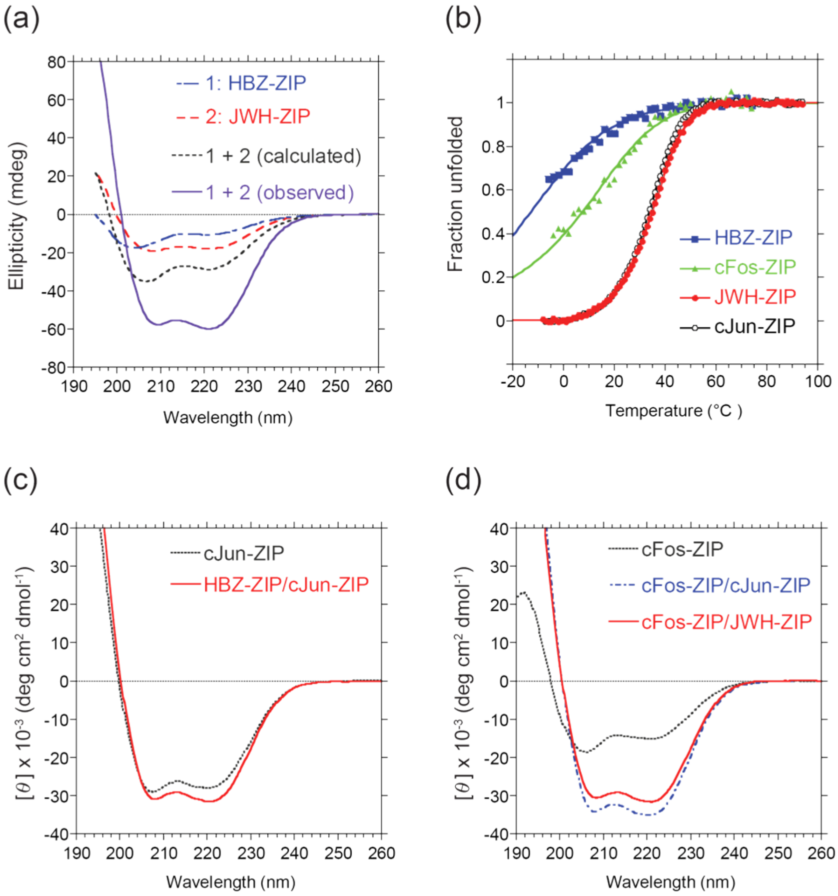
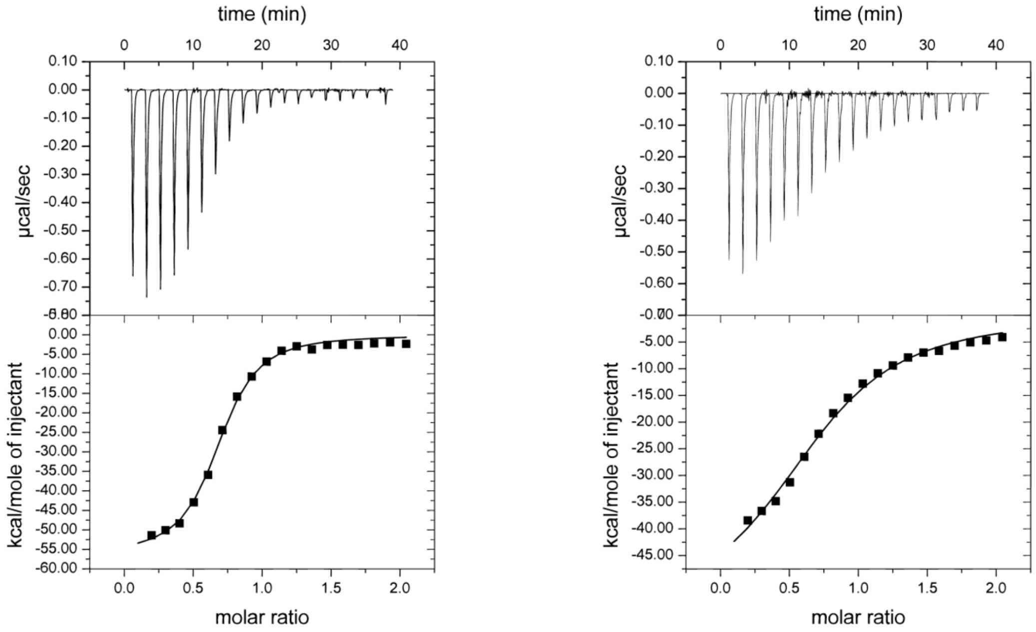
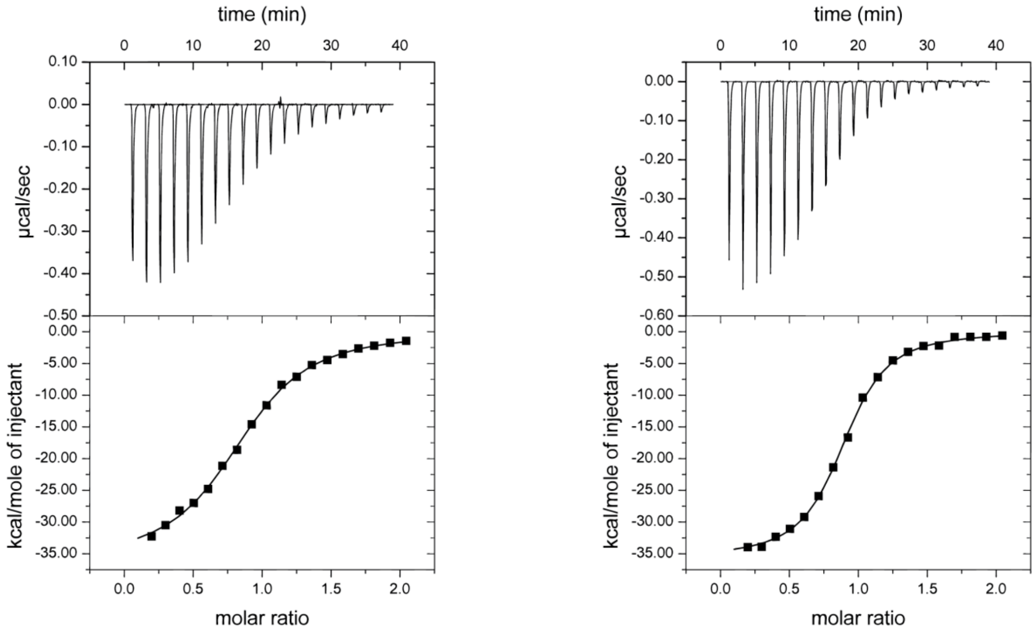


Figure S4. CD spectra and denaturation curves. (a) Complex formation of JWH-ZIP with HBZ-ZIP. CD spectrum of the 1:1 mixture of HBZ-ZIP and JWH-ZIP (solid line, purple) was suggestive of a significantly more stabilized helical structure than the added curves of JWH-ZIP (25 µM) and HBZ-ZIP (25 µM) (dashed line, black). (b) Thermal denaturation curves for HBZ-ZIP (Tm=-1°C, squares, blue), cFos-ZIP (Tm=7°C, triangles, green), cJun-ZIP (Tm=34°C, open circles, black), and JWH-ZIP (Tm=35°C, closed circles, red). (c) CD spectra of cJun-ZIP alone (dashed line, black) and 1:1: mixtures of cJun-ZIP with HBZ-ZIP (solid line, red). (d) CD spectra of cFos-ZIP (dashed line, black), mixture of cFos-ZIP/cJun-ZIP (dashed dotted line, blue), and mixture of cFos-ZIP/JWH-ZIP (solid line, red). For (b), (c) and (d) total peptide concentration is 50 µM.


(a) HBZ-ZIP/JWH-ZIP

(b) HBZ-ZIP/cJun-ZIP


(c) cFos-ZIP/JWH-ZIP

(d) cFos-ZIP/cJun-ZIP

Figure S5. ITC measurements. (a) HBZ-ZIP titrated into JWH-ZIP; (b) HBZ-ZIP titrated into JWH-ZIP; (c) cFos-ZIP titrated into JWH-ZIP; and (d) cFos-ZIP titrated into cJun-ZIP.

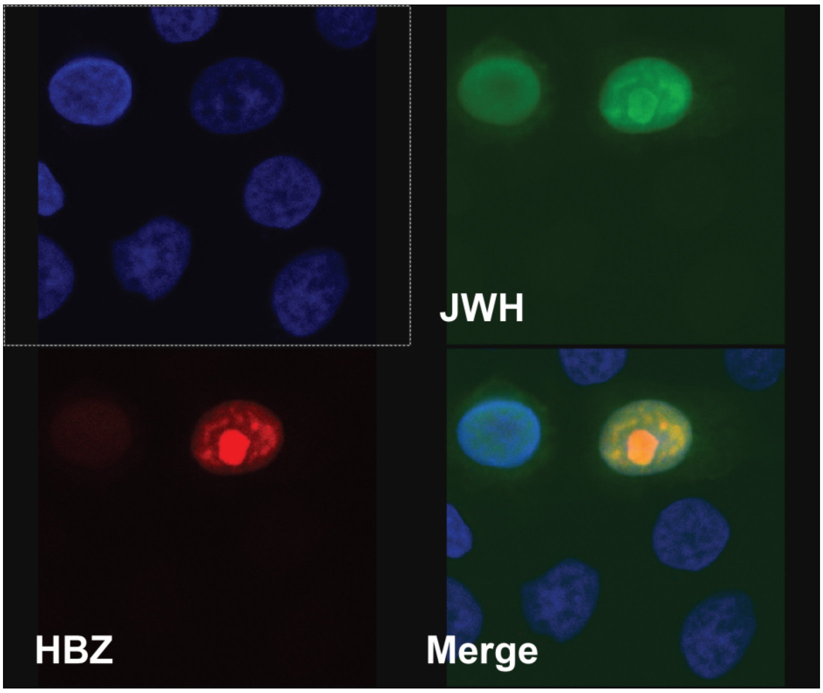


Figure S6. Co-localization of HA-JHW (red) and Myc-HBZ (green).

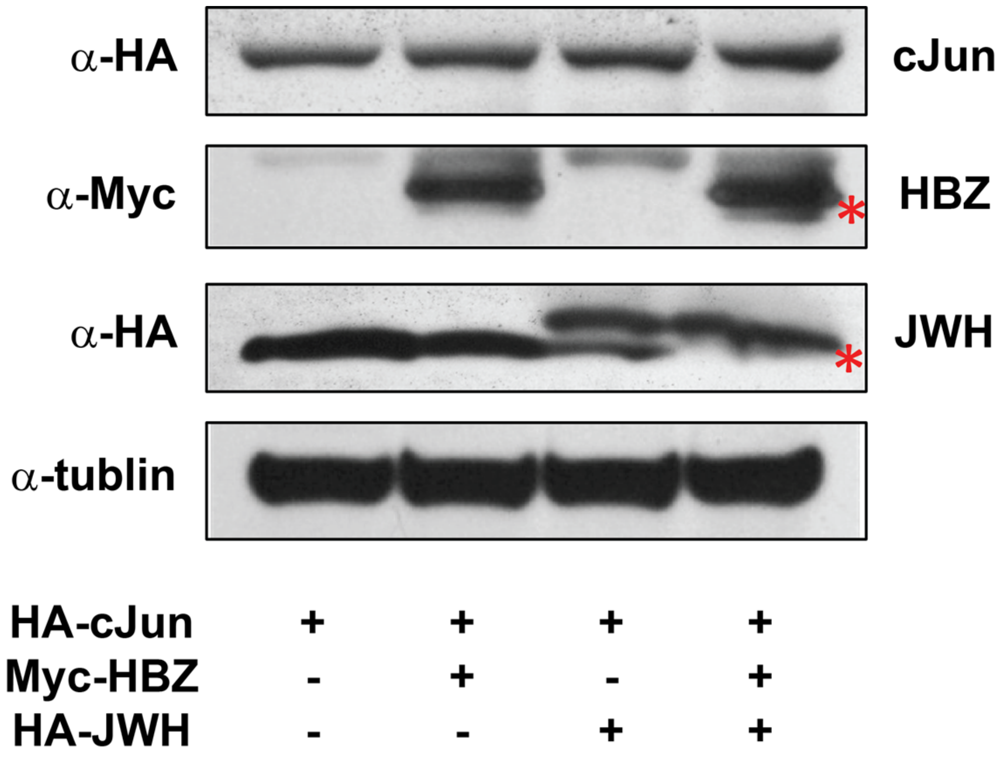


Figure S7. Expression check of the HA-cJun, Myc-HBZ, and HA-JWH. *protein from control vector pCAG-HA.