

## Supporting Information for World Wide Web Edition:

**Table S1.** Thermodynamic parameters <sup>a</sup>

RNA	N Peptide					
	$\lambda_{N11}$	$\lambda_{N22}$	P22 <sub>N11</sub>	P22 <sub>N21</sub>	$\phi 21_{N11}$	$\phi 21_{N22}$
Dissociation Constant (nM)						
$\lambda$ boxB <sub>L</sub> -2	650 ± 385	0.76 ± 0.36 †	390 ± 140	0.6 ± 0.07 †	1,100 ± 1,000	1,300 ± 650
$\lambda$ boxB <sub>L</sub> -3		0.99 ± 0.41 †				1,800 ± 360 ‡
$\lambda$ boxB <sub>R</sub> -2	1,200 ± 260	1.9 ± 0.69 †	750 ± 280	0.85 ± 0.28 †	1,800 ± 920	1,800 ± 710
$\lambda$ boxB <sub>R</sub> -3		1.0 ± 0.34 †				1,900 ± 210 ‡
$\lambda$ boxB <sub>R</sub> -4		1.2 ± 0.59 †				
P22boxB <sub>L</sub> -2		260 ± 81	840 ± 710	0.005 ± 0.002 †	1,100 ± 540	800 ± 380
P22boxB <sub>L</sub> -4	55,000 ± 1,900 ‡	110 ± 96	750 ± 80 ‡	0.004 ± 0.002 †		
P22boxB <sub>R</sub> -2		530 ± 320				
P22boxB <sub>R</sub> -4	313,000 ± 33,000 ‡	820 ± 710	970 ± 390	0.005 ± 0.002 †	1,500 ± 860	
$\phi$ 21boxB <sub>L</sub> -3		9,100 ± 5,400	16,000 ± 1,500 ‡	340 ± 240	3,700 ± 3,200	160 ± 95
$\phi$ 21boxB <sub>L</sub> -4		9,900 ± 1500				200 ± 180
$\phi$ 21boxB <sub>R</sub> -3		5,000 ± 1,800	13,000 ± 1,900 ‡	320 ± 210	2,800 ± 220 ‡	120 ± 80
$\phi$ 21boxB <sub>R</sub> -4		4,200 ± 800			1,200 ± 70 ‡	65 ± 52
Salt-Dependence ( $-\partial \log K_{\text{obs}} / \partial \log [M^+]$ ) <sup>-1</sup>						
$\lambda$ boxB <sub>L</sub> -2	2.6 ± 0.2	3.6 ± 0.3	3.7 ± 0.1	4.3 ± 0.1	5.1 ± 0.4	4.0 ± 0.2
$\lambda$ boxB <sub>L</sub> -3		3.3 ± 0.3				
$\lambda$ boxB <sub>R</sub> -2	2.6 ± 0.1	3.2 ± 0.2	3.9 ± 0.1	4.1 ± 0.2	5.3 ± 0.2 §	4.5 ± 0.2 §
$\lambda$ boxB <sub>R</sub> -3		3.3 ± 0.2				
$\lambda$ boxB <sub>R</sub> -4		3.0 ± 0.2 §				
P22boxB <sub>L</sub> -2		3.3 ± 0.1	3.9 ± 0.3	5.2 ± 0.3	4.9 ± 0.2	3.5 ± 0.2
P22boxB <sub>L</sub> -4		3.1 ± 0.3 §		5.0 ± 0.3		
P22boxB <sub>R</sub> -2		3.6 ± 0.2				
P22boxB <sub>R</sub> -4		3.4 ± 0.2	3.6 ± 0.1	5.2 ± 0.2	3.4 ± 0.2 §	
$\phi$ 21boxB <sub>L</sub> -3		3.4 ± 0.2		4.3 ± 0.2	4.5 ± 0.3	3.9 ± 0.2
$\phi$ 21boxB <sub>L</sub> -4						3.6 ± 0.3
$\phi$ 21boxB <sub>R</sub> -3		3.2 ± 0.1		5.1 ± 0.3		3.7 ± 0.3
$\phi$ 21boxB <sub>R</sub> -4						3.8 ± 0.3

<sup>a</sup> Dissociation constants listed for standard conditions: 50 mM KOAc, 20 mM Tris•OAc; 20° C; pH 7.5.  $K_d$  values were calculated from salt-dependence data and deviate < 15% from experimental values determined at standard conditions.

† Values listed were determined by extrapolation from salt-dependence data and were found to be within 0.5 kcal mol<sup>-1</sup> agreement with  $K_d$  values calculated from stopped-flow kinetic data ( $k_{\text{off}}/k_{\text{on}}$ ).

‡ Error estimates indicate the precision of single fits

§ Data deviates from the Poisson-Boltzmann salt-dependence correlation ( $\partial \log K_{\text{obs}} / \partial \log [M^+]$ )<sup>-1</sup> = -0.9(± 0.1)z

Supporting Information for World Wide Web Edition:

**Table S2.** BoxB RNA substitutions<sup>a</sup>

	A A A G A U A C G C G C G G C g c	A A A G A U A C G G C C G G C g c	C A A G A U A C G C G C G G C g c	C A A G A U A C G G C C G G C g c
	$\lambda$ boxB <sub>R</sub>	$\lambda$ boxB <sub>R</sub> (C3G,G3C)	$\lambda$ boxB <sub>R</sub> (A8C)	$\lambda$ boxB <sub>R</sub> (C3G,G3C) (A8C)
	$\Delta G^\circ$ (kcal mol <sup>-1</sup> )			
$\lambda_{N11}$ MDAQTRRRERRgy	-7.9	-8.3	-5.7	-5.7
P22 <sub>N11</sub> (K4Q,H8R) GNAQTRRRERRgy	-9.6	-9.9	-7.4	-7.4

<sup>a</sup> Free energies listed for standard conditions: 50 mM KOAc, 20 mM Tris•OAc; 20° C; pH 7.5. Substituted residues in **bold**

**Table S3.** Reciprocal mutant peptides, thermodynamic and fluorescent parameters<sup>a</sup>

Peptides (11mers)		RNA					
		$\lambda$ boxB <sub>R</sub>			P22boxB <sub>L</sub>		
		K <sub>d</sub>	F		K <sub>d</sub>	F	
		2AP-2	2AP-2	2AP-4	2AP-2	2AP-2	2AP-4
$\lambda_{N11}$	MDAQTRRRERRgy	1,200 ± 32	1.50	2.20	54,000 ± 1,500	0.90	1.30
	GDAQTRRRERRgy	730 ± 3.6	1.60		28,000 ± 2,500	0.90	1.40
	MNAQTRRRERRgy	120 ± 10	1.60		6,600 ± 370	0.90	1.70
	MDAKTRRRERRgy	250 ± 12	1.70		2,300 ± 300	0.90	1.60
	MDAQTRRHERRgy	410,000 ± 53,000	1.70		nd		
	MNAKTRRRERRgy	45 ± 2.6	1.70		210 ± 18	0.90	1.90
	MNAQTRRHERRgy	23,000 ± 380	1.70		nd		
	MDAKTRRHERRgy	28,000 ± 560	1.70		43,000 ± 4,100	1.20	0.80
	GDAQTRRHERRgy	200,000 ± 11,000	2.00		nd		
	GDAKTRRRERRgy	360 ± 9	1.60		1,700 ± 180	0.90	1.50
	GNAQTRRRERRgy	66 ± 3.8	1.60		2,700 ± 240	0.90	1.70
	GNAKTRRRERRgy	41 ± 5.7	1.80		79 ± 8.6	0.90	1.80
	GNAQTRRHERRgy	7,000 ± 140	1.70		36,000 ± 2,500	1.10	1.00
	GDAKTRRHERRgy	17,000 ± 590	1.60		22,000 ± 1,700	1.20	0.90
MNAKTRRHERRgy	1,900 ± 48	1.70		2,800 ± 110	1.20	0.90	
P22 <sub>N11</sub>	GNAKTRRHERRgy	750 ± 55	1.70	1.80	840 ± 51	1.20	0.90

<sup>a</sup> Measurements were performed at standard conditions: 50 mM KOAc, 20 mM Tris•OAc; 20° C; pH 7.5.