

Table S1 Summary of ITC data

Titration of CLOCK bHLH domain into BMAL1 bHLH domain					
Sample in cell	Sample in syringe	$K_D \pm$ s.d. (μ M)	N	$\Delta H \pm$ s.d. (kcal/mol)	- ΔS (kcal/mol)
CLOCK	BMAL1	0.37 \pm 0.13	1.09 \pm 0.01	-5.37 \pm 0.09	-3.40
CLOCK (F50M)	BMAL1	0.33 \pm 0.08	0.96 \pm 0.01	-7.18 \pm 0.09	-1.65
CLOCK	BMAL1 (M88F)	0.27 \pm 0.08	0.93 \pm 0.01	-6.27 \pm 0.09	-2.69
CLOCK (H84L)	BMAL1	56.18 \pm 10.79	0.11 \pm 0.06	13.46 \pm 7.60	-19.25
CLOCK	BMAL1 (L125H)	64.94 \pm 30.37	2.49 \pm 0.19	1.20 \pm 0.08	-6.91
Titration of CLOCK and BMAL1 bHLH homo- / heterodimer in to WT E-box DNA					
Protein		$K_D \pm$ s.d. (μ M)	N	$\Delta H \pm$ s.d. (kcal/mol)	- ΔS (kcal/mol)
CLOCK-CLOCK homodimer		8.93 \pm 0.48	0.95 \pm 0.01	-18.55 \pm 0.42	11.65
BMAL1-BMAL1 homodimer		14.49 \pm 1.92	0.91 \pm 0.04	-4.65 \pm 0.33	-1.95
CLOCK-BMAL1 heterodimer		1.52 \pm 0.10	0.82 \pm 0.01	-15.96 \pm 0.18	8.02
Titration of CLOCK-BMAL1 bHLH domains into WT E-box DNA in the presence of NAD cofactors					
NAD cofactors (10 mM)		$K_D \pm$ s.d. (μ M)	N	$\Delta H \pm$ s.d. (kcal/mol)	- ΔS (kcal/mol)
NAD ⁺		1.18 \pm 0.01	0.82 \pm 0.01	-13.32 \pm 0.01	5.24
NADH		2.13 \pm 0.27	0.86 \pm 0.01	-12.57 \pm 0.31	4.83
NADP ⁺		2.54 \pm 0.11	0.87 \pm 0.01	-12.76 \pm 0.12	5.13
NADPH		2.74 \pm 0.16	0.88 \pm 0.01	-12.04 \pm 0.15	4.44
Titration of CLOCK-BMAL1 bHLH domains into tandem E-boxes DNA					
DNA name	DNA sequence (33bp)	$K_D \pm$ s.d. (μ M)	N	$\Delta H \pm$ s.d. (kcal/mol)	- ΔS (kcal/mol)
Dbpl2wt	GAGTTGCACATTCCTC GCCACGTGAGTGGTAC	1.42 \pm 0.06	0.88 \pm 0.01	-12.47 \pm 0.08	4.50
Dbpl2sp10	GTGCACATTCCTAGTCG CCACGTGAGTGGTAC	1.23 \pm 0.07	0.85 \pm 0.01	-12.47 \pm 0.11	4.41

Titration of CLOCK-BMAL1 bHLH domains into WT or single-mutated E-box DNA					
DNA name	DNA sequence (16bp)	$K_D \pm$ s.d. (μ M)	N	$\Delta H \pm$ s.d. (kcal/mol)	- ΔS (kcal/mol)
WT	AGGAAC <u>CACGTG</u> ACCCA	1.52 \pm 0.10	0.82 \pm 0.01	-15.96 \pm 0.18	8.02
1CAAd	AGGAA <u>A</u> ACGTGACCCA	4.44 \pm 0.24	0.83 \pm 0.01	-10.83 \pm 0.15	3.52
1CTz	AGGAA <u>T</u> ACGTGACCCA	ND	ND	ND	ND
1CGd	AGGAA <u>G</u> ACGTGACCCA	ND	ND	ND	ND
2AGz	AGGAA <u>C</u> GCGTGACCCA	8.70 \pm 1.03	1.05 \pm 0.03	-2.87 \pm 0.12	-4.05
2ATd	AGGAA <u>CT</u> CGTGACCCA	11.35 \pm 2.06	1.20 \pm 0.04	-2.21 \pm 0.13	-4.53
2ACd	AGGAA <u>CC</u> CGTGACCCA	11.26 \pm 1.08	1.30 \pm 0.02	-2.01 \pm 0.07	-4.74
3CTz	AGGAA <u>CA</u> TGTGACCCA	5.52 \pm 0.49	0.75 \pm 0.02	-9.74 \pm 0.31	2.57
3CAAd	AGGAA <u>CA</u> AGTGACCCA	ND	ND	ND	ND
3CGd	AGGAA <u>CA</u> GGTGACCCA	ND	ND	ND	ND
4GAz	AGGAA <u>CAC</u> ATGACCCA	ND	ND	ND	ND
5TCz	AGGAA <u>CACG</u> CGACCCA	14.99 \pm 3.50	0.99 \pm 0.07	-3.27 \pm 0.37	-3.31
5TAd	AGGAA <u>CACG</u> AGACCCA	12.66 \pm 2.16	0.94 \pm 0.05	-3.81 \pm 0.31	-2.87
5TGd	AGGAA <u>CACG</u> GGACCCA	21.32 \pm 12.91	1.62 \pm 0.13	-1.37 \pm 0.24	ND
6GAz	AGGAA <u>CACGT</u> AACCCA	ND	ND	ND	ND
6GTd	AGGAA <u>CACGT</u> TACCCA	ND	ND	ND	ND
6GCd	AGGAA <u>CACGT</u> CACCCA	ND	ND	ND	ND
7ATd	AGGAA <u>CACGTG</u> TCCCA	2.57 \pm 0.17	0.78 \pm 0.01	-16.64 \pm 0.22	9.00

ND, not detected.

The six bp E-box sequences are underlined.

The single-mutated nucleotides different from WT 16 bp canonical E-box DNA are in red.