



Figure S4. Titration of CLOCK-BMAL1 bHLH domains into tandem E-box DNA. **(A)** Titration into Dbpl2wt DNA. **(B)** Titration into Dbpl2sp10 DNA. $K_D \pm \text{s.d.}$ is shown. Dbpl2wt and Dbpl2sp10 both contain two E-boxes: one is canonical E-box, with sequence of CCACGTGA; the other is non-canonical E-box, with sequence of GCACATTC. The difference between Dbpl2wt and Dbpl2sp10 is mainly the number of spacing base pairs between two E-boxes, which are 7 bp in Dbpl2wt and 10 bp in Dbpl2sp10. The titration results reveal that only one E-box is recognized by proteins, for both of the N values in these two ITC results are approximately 1. Considering the canonical E-box can be well recognized by CLOCK-BMAL1 (Figure 3C), the results indicate non-canonical E-box GCACATTC cannot be recognized.