SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure 1 A short variant BMAL2b weakly inhibits transactivation induced by BMAL1:CLOCK. *A,* Structures of mouse BMAL2a (579 amino acids) and BMAL2b (199 amino acids) are shown schematically. Except the C-terminal residue 199 (Lys), the amino acid sequence of BMAL2b is identical to the N-terminal sequence of BMAL2a at positions 1-198. *B,* Transactivation of 2.1-kb *mPer1* promoter by BMAL1:CLOCK or BMAL2:CLOCK was examined in the presence or absence of BMAL2b. HEK293 cells were transfected with a combination 250 ng of CLOCK expression plasmid and 50 ng of FLAG-BMAL1 or FLAG-BMAL2 expression plasmid, with or without 250 ng of FLAG-BMAL2b expression plasmid. Relative luciferase activities were shown by mean fold increase from the value of the control sample that was co-transfected with 2.1-kb *mPer1* reporter and pcDNA3.1/V5-His empty vector. Data are means ± SEM from three independent experiments (Student's *t* test, #: $p < 0.05$).

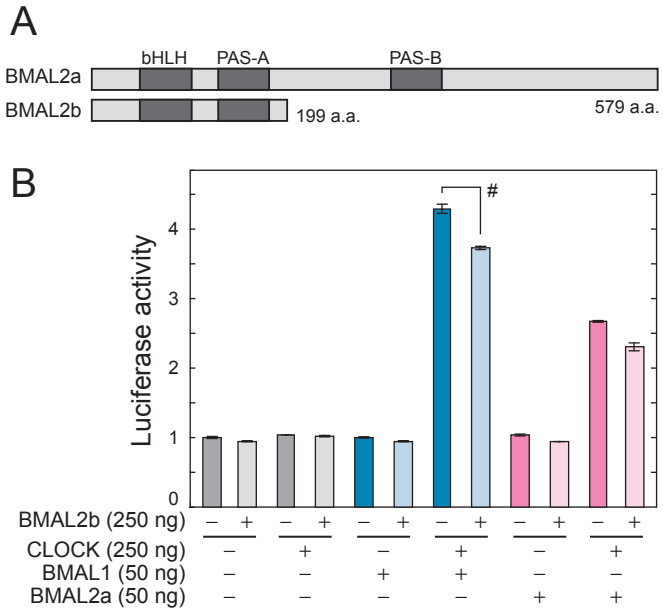
Supplemental Figure 2 Immunoblot analysis of the protein levels of BMAL1 and BMAL2 exogenously expressed in NIH3T3 cells for bioluminescence rhythm monitoring. The lysates of NIH3T3 cells used in the experiments in Fig. 2, panel *A* (lane 1), *C* (lane 2), *D* (lane 3) and *F* (lane 4) were subjected to immunoblot analysis with affinity purified anti-BMAL1 antibody (lanes 1 and 2) and affinity purified anti-BMAL2 antibody (lanes 3 and 4) to verify high level expression of the exogenously expressed BMAL1 and BMAL2. The anti-BMAL1 and BMAL2 antibodies were raised in Wister rats (male, 7 week old; Clea Japan Inc.) against peptides of mBMAL1b (amino acid 446-479) and mBMAL2a (amino acid 18-49).

Supplemental Figure 3 Endogenous expression of *Bmal1* and *Bmal2* mRNA in NIH3T3 cells. To verify expression of *Bmal1* and *Bmal2* mRNA in NIH3T3 cells, reverse transcriptase-PCR (RT-PCR) was performed on total RNA extracted from NIH3T3 cells using LA-Taq polymerase (Takara Bio Inc.) with a pair of primers for BMAL1 (rmBmal1-F1 primer 5'-TGGTA CCAAC ATGCA ATGC and rmBmal1-R1 primer 5'-AGTGT CCGAG GAAGA TAGCT G) or BMAL2 (mBMAL2-F2 primer 5'-TGGTT GGATG CGAAA GAGG and mBMAL2-R4 primer 5'-AGGTT TCTCT CTTGG TGAAC C). The PCR products were subjected to 6% polyacrylamide gel electrophoresis, stained with SYBR Green I (Takara Bio Inc.), and then detected with an image analyzer FLA-2000 (Fuji Film).

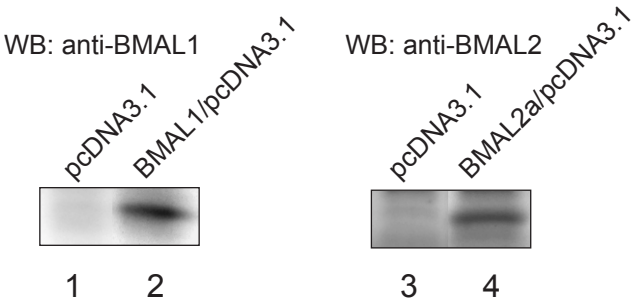
Supplemental Figure 4 Immunoblot analysis of the protein levels of BMAL1 and BMAL2 exogenously expressed in NIH3T3 cells. The lysates of NIH3T3 cells transfected with indicated amounts of FLAG-BMAL1 or FLAG-BMAL2 expression plasmid (ng) in combination with 250 ng of CLOCK expression plasmid were subjected to immunoblot analysis with anti-FLAG antibody to verify expression levels of FLAG-tagged BMAL1 and BMAL2. The asterisks indicate non-specific bands.

Supplemental Figure 5 Immunoblot analysis of the protein levels of BMAL1 and BMAL2. Shown is a part of immunoblot image of BMAL1 and BMAL2 detected under the same condition as described in Fig. 4*A* and Fig. 5*A*. The asterisks indicate non-specific bands.

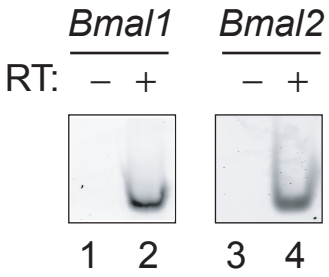
Supplemental Figure 1



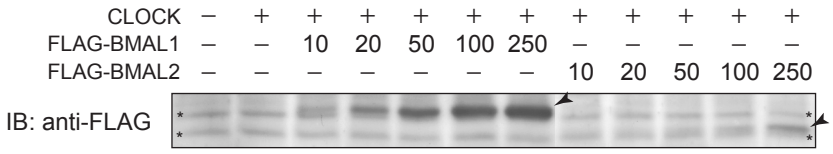
Supplemental Figure 2

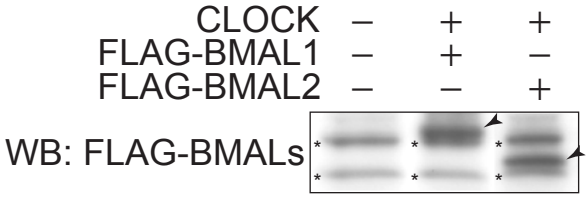


Supplemental Figure 3



Supplemental Figure 4




CLOCK – + +
FLAG-BMAL1 – + –
FLAG-BMAL2 – – +
WB: FLAG-BMALs