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Supplemental Material to:

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The RelB subunit of NFκB acts as a negative regulator of circadian gene expression

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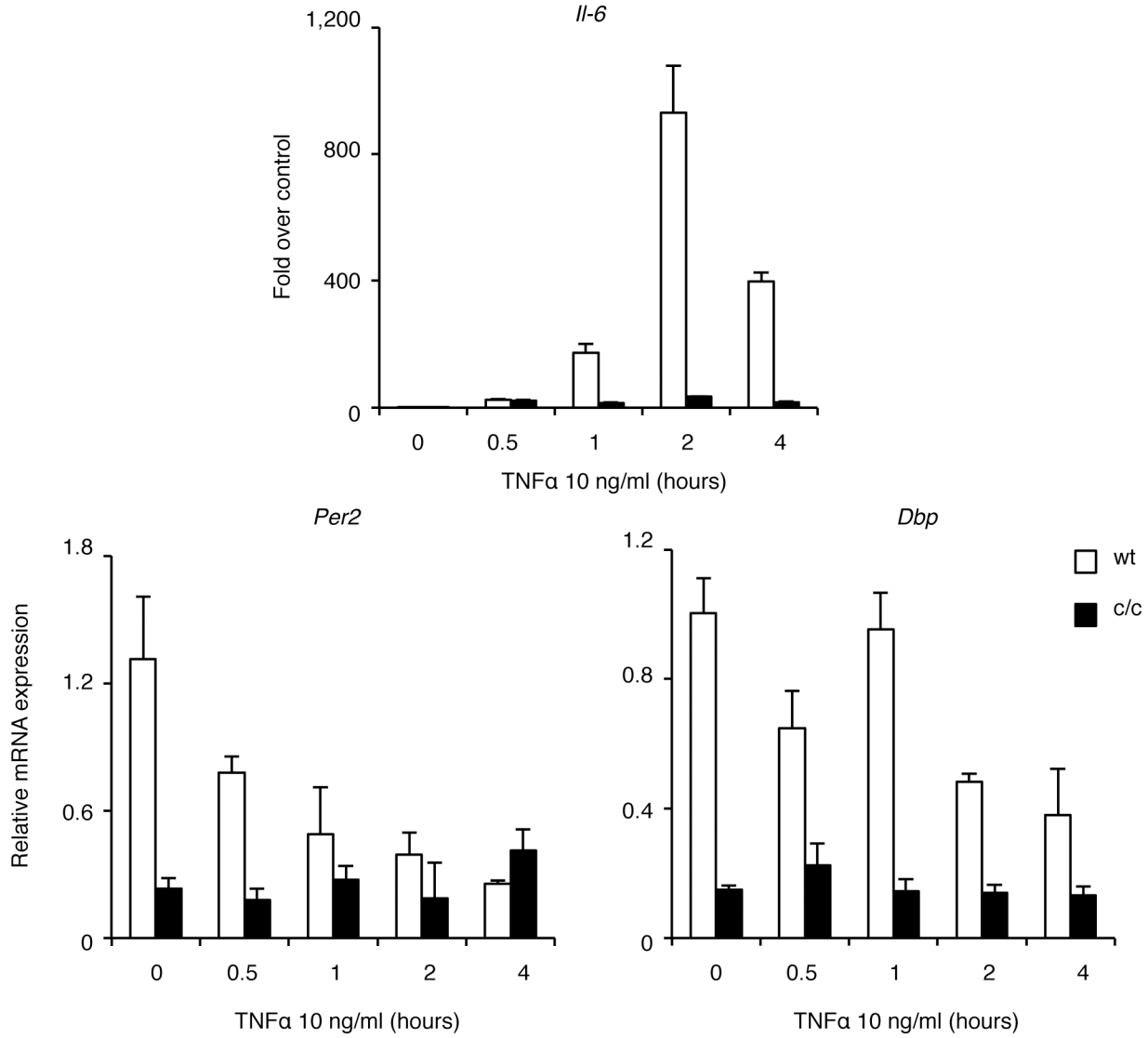

Figure S1.Time course of *Il-6*, *Per2* and *Dbp* mRNA expression after TNF-*α* stimulation (1 µg/ml) of wt and *Clock* mutant (c/c) MEFs, measured by quantitative real time PCR. For *Il-6*, fold changes in gene expression compared to unstimulated cells are shown. For *Per2* and *Dbp*, time 0 (unstimulated cells was set to 1. All the values are the mean ± SD (n=3).

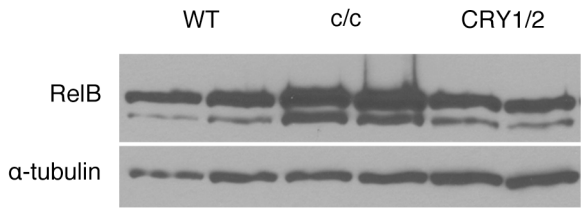

Figure S2 Endogenous RelB expression in wild type (WT), *Clock* mutant (c/c) and CRY1/2 KO (CRY1/2) MEFs was determined by western blot. α-tubulin was used as loading control.

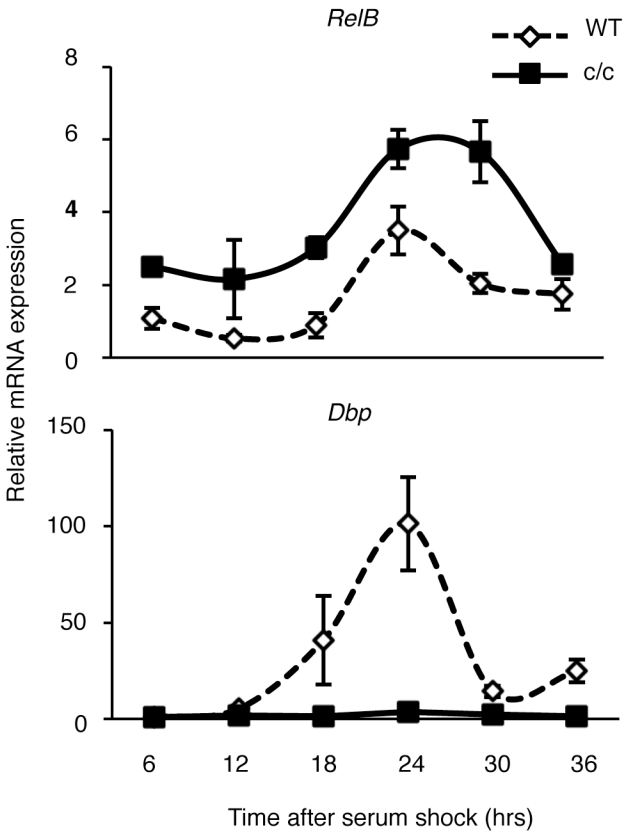

Figure S3. RNA was prepared at the indicated timvs after serum shock synchronization of WT and c/c MEFs, reverse transcribed, and real-time PCR was performed using primers for *RelB*, *Dbp* and *β-actin*. Data is represented as relative levels of RelB and Dbp normalized to β-actin. Time 6 in wt cells was set to 1. All the values are the mean +/- SD (n=3).

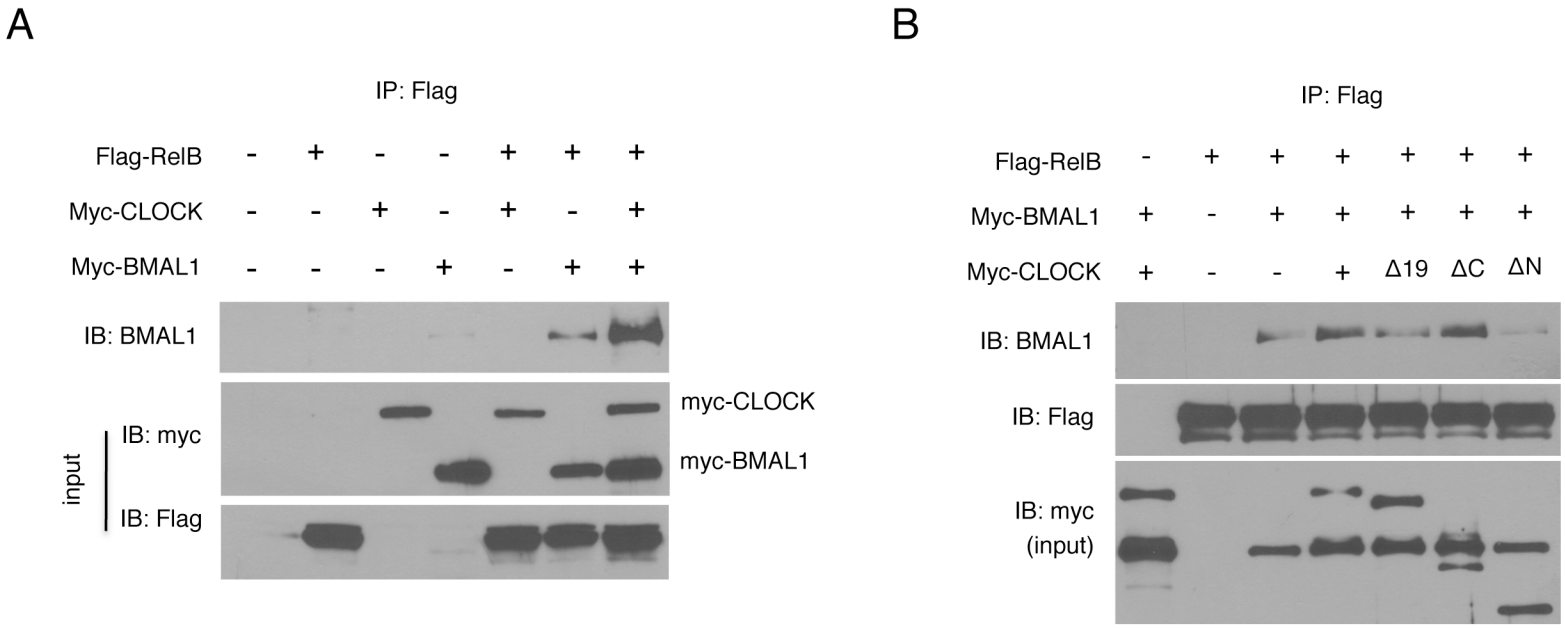

Figure S4 (A) HEK-293 cells were cotransfected with a series of expression vectors as described. After immunoprecipitation using FLAG-Agar, levels of coimmunoprecipitated proteins was detected by western blot analysis with anti-BMAL1 antibody. Lower panel shows the expression of myc-tagged proteins (CLOCK and BMAL1) and Flag-tagged proteins (RelB) in total cell lysates as an input. **(B)** HEK-293 cells were cotransfected with a series of expression vectors as described. After immunoprecipitation using FLAG-Agar, levels of coimmunoprecipitated proteins was detected by western blot analysis with anti-BMAL1 and anti-Flag antibodies. Lower panel shows the expression of myc-tagged proteins (CLOCK and BMAL1) in total cell lysates as an input.

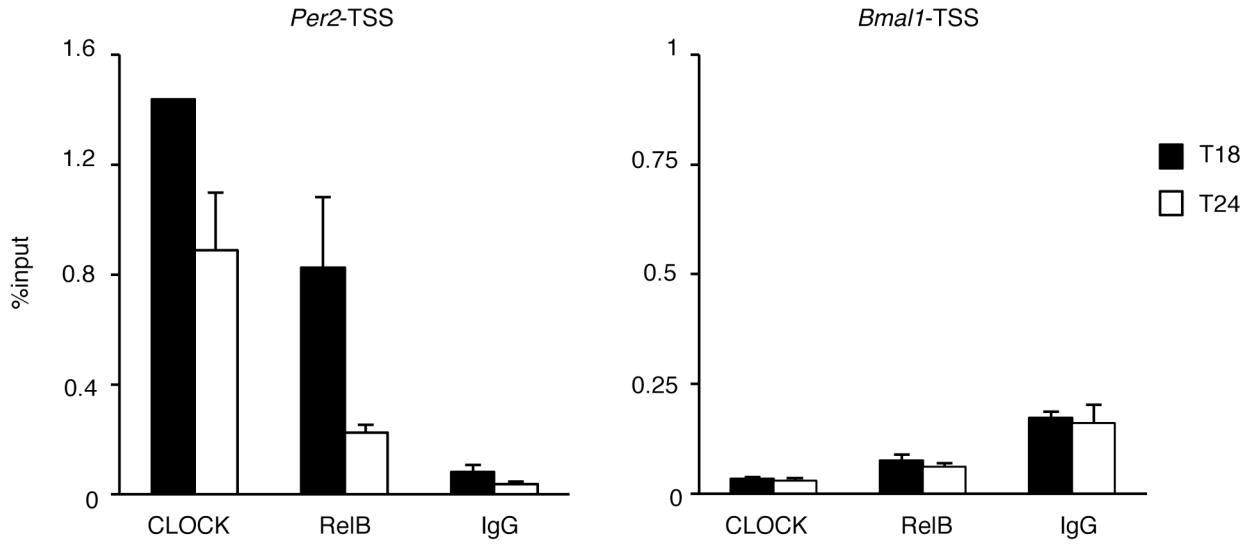

Figure S5 RelB is recruited with CLOCK and BMAL1 at clock-controlled genes (CCGs) promoters. Cross-linked cell extracts were isolated at the indicated time points after serum shock from MEFs. The samples were subjected to ChIP assay with anti-CLOCK, anti-RelB and anti-IgG, and analyzed by quantitative PCR with primers for **(A)** *Per* promoter (*Per2* TSS) and **(B)** *Bmal1* promoter (*Bmal1* TSS). Control IgG were used as control for immunoprecipitation. All the values are the mean +/- SD (n=3).

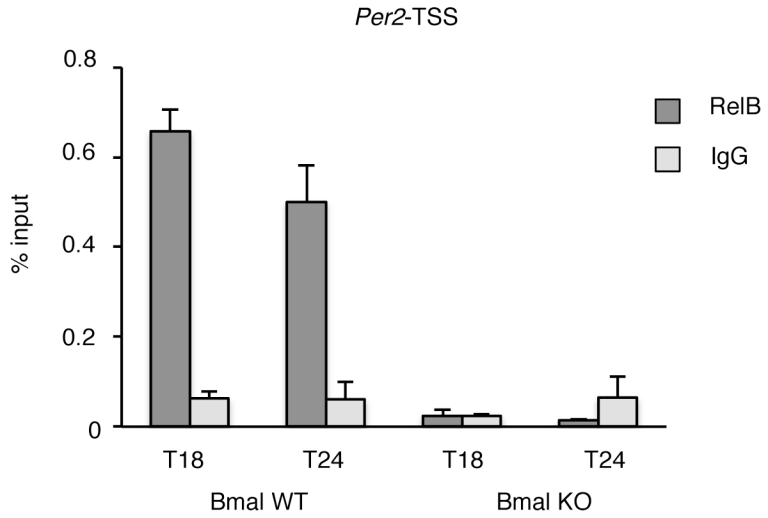

Figure S6. RelB recruitment at CCGs promoters is BMAL1-dependent. Cross-linked cell extracts were isolated at the indicated time points after serum shock from *Bmal1* WT and KO MEFs. The samples were subjected to ChIP assay with anti-RelB and anti-IgG, and analyzed by quantitative PCR with primers for *Per* promoter (*Per2* TSS). Control IgG were used as control for immunoprecipitation.

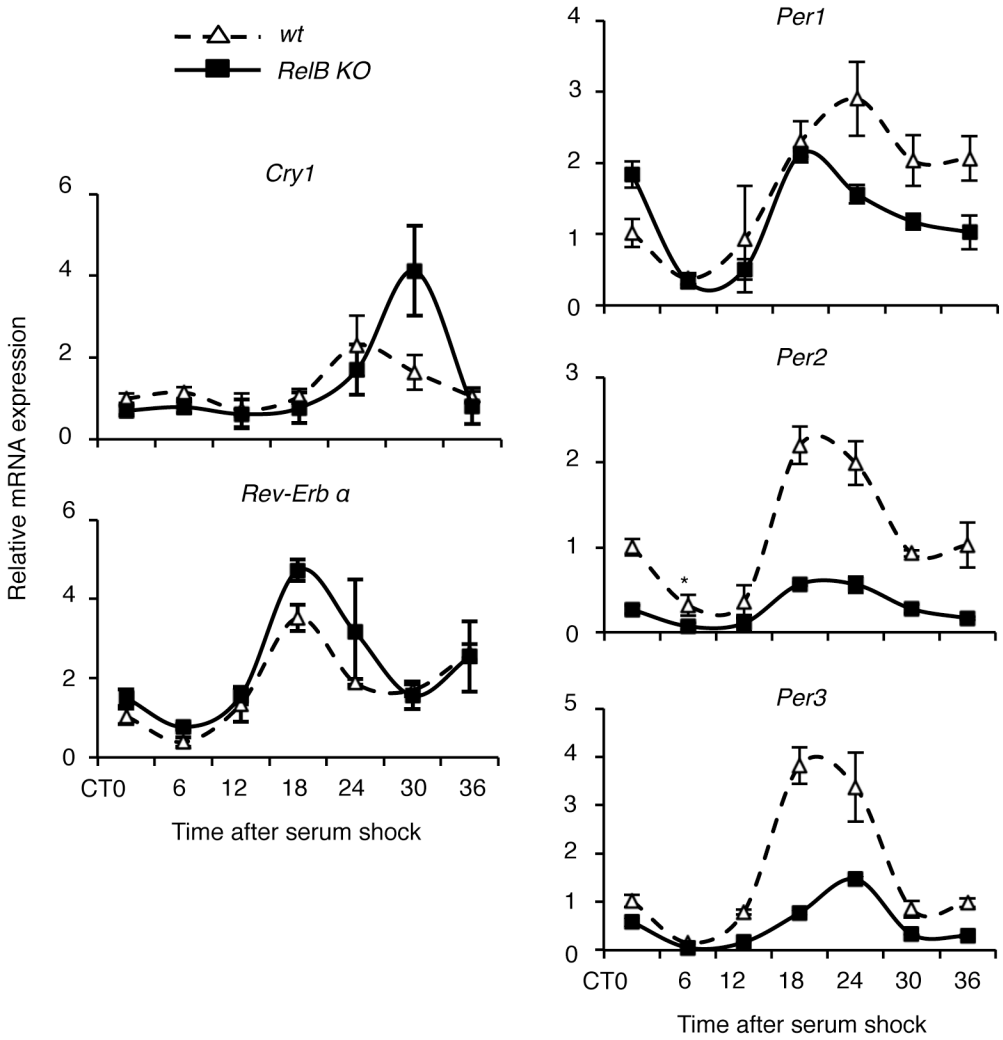

Figure S7. Altered circadian expression of clock genes and CCGs in WT and RelB KO MEFs. RNA was prepared at the indicated times after serum shock synchronization, reverse transcribed, and real-time PCR was performed using primers for *Cry1*, *Rev-erbα*, *Per1*, *Per2*, *Per3* and *β-actin*. The values are relative to those of *β-actin* mRNA levels at each CT (circadian time). Time 0 (unsynchronized cells, CT0) in wt cells was set to 1. All the values are the mean +/- SD (n=3).