Supporting Information

Spengler et al. 10.1073/pnas.1206274109

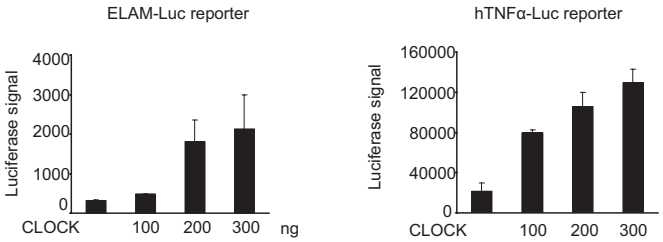

Fig. S1. CLOCK mediates the up-regulation of various NF-κB–responsive genes. 293T cells were cotransfected with increasing amounts of CLOCK-expressing plasmid, as depicted in the figure, and a luciferase reporter that is driven by the NF-κB target promoter ELAM or human TNF (hTNF).

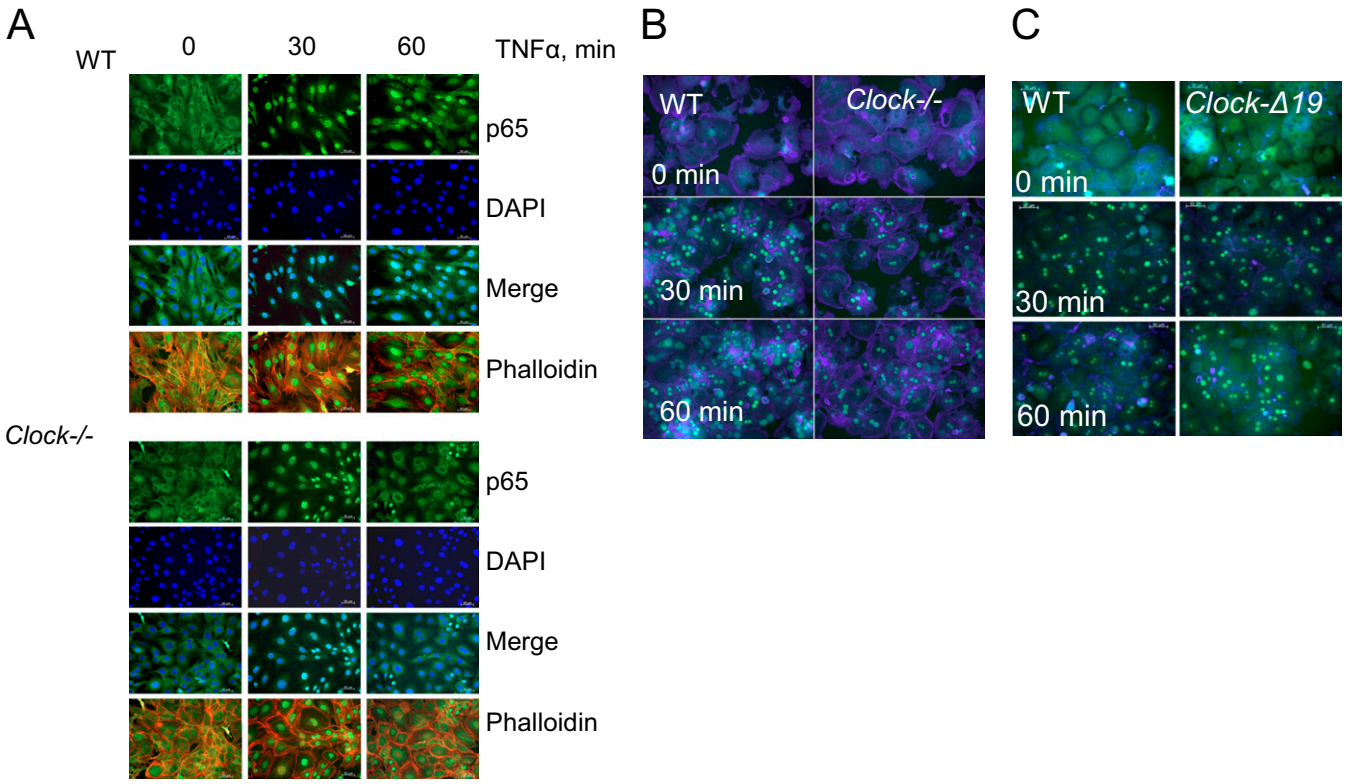

Fig. S2. CLOCK deficiency reduces the abundance of nuclear p65. (*A*) Nuclear translocation of NF-κB/p65 in WT and *Clock*$^{-/-}$ MEFs treated with 1 ng/mL of TNF-α for 30 and 60 min as determined by indirect immunofluorescence using rabbit anti–NF-κB/p65 antibody. The intensity of p65 nuclear staining is lower in *Clock*$^{-/-}$ cells than in WT cells at all time points. Green fluorescence, p65; blue staining, DAPI; red fluorescence, phalloidin (cytoplasmic staining). (*B*) Primary hepatocytes isolated from WT and *Clock*$^{-/-}$ mice were treated with 100 ng/mL CBLB502. Robust nuclear translocation of the p65 NF-κB subunit was observed 30 min after CBLB502 administration. WT hepatocytes displayed a higher intensity of p65 nuclear staining than *Clock*$^{-/-}$ hepatocytes at both times tested. Green fluorescence, p65; purple, phalloidin. (*C*) The *Clock-Δ19* mutation has no effect on p65 nuclear translocation. Nuclear translocation of the p65 NF-κB subunit in primary hepatocytes of WT and *Clock-Δ19* mutant mice treated with 100 ng/mL of CBLB502 for the times indicated. There are no visible differences in the intensity of p65 staining or in the dynamics of p65 nuclear accumulation in WT and *Clock-Δ19* cells. Green fluorescence, p65; purple, phalloidin.

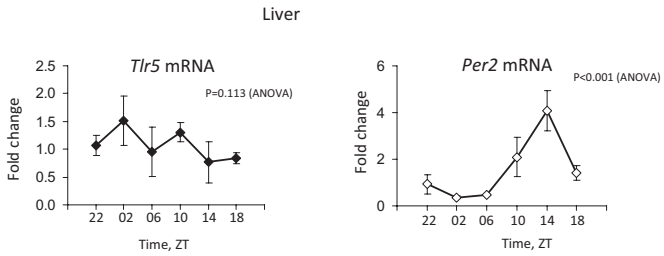

Fig. S3. *Tlr5* mRNA expression in liver is not rhythmic. C57BL/6J male mice were maintained on a 12:12-h light:dark cycle. Liver samples were collected every 4 h starting from ZT22 (ZT0 represents the time lights were turned on). Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA abundance was measured by using an ABI 7300 real-time RT-PCR system and premade primer-probe sets (Applied Biosystems, Inc.). Same samples show prominent mPer2 mRNA oscillation.

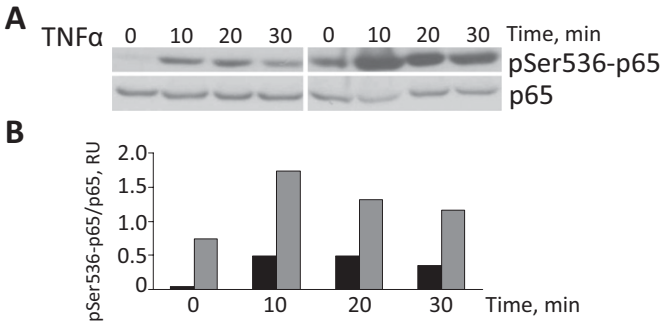

Fig. S4. *Bmal1* deficiency results in increased basal and TNF-α–induced levels of NF-κB. (*A*) Western blot. (*B*) Quantitative analysis. Immortalized WT (black bars) and *Bmal1*$^{-/-}$ (grey bars) MEFs were treated with 1 ng/mL TNF-α for the times indicated and were analyzed by Western blot with p65- and pSer536-p65–specific antibody. Treatment with TNF-α for 10 min induces an approximately eightfold increase in pSer536-p65 in WT MEFs. *Bmal1* deficiency results in an increase in the basal levels of active p65 that were further induced approximately fourfold after 10 min of TNF-α treatment. RU, relative units.