Supporting Information

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SI Materials and Methods

Animals. Mice with the gene *Bmal1* containing loxP sites at either side of exon 8 were obtained from Jackson Labs (stock no. 007668) and described in further detail below.

Bmal1$^{flox/flox}$ were crossed with *LysM*$^{cre/cre}$, which express Cre recombinase under the control of the *LysozymeM* promoter to produce progeny that have BMAL1 excised in the myeloid lineage (monocytes, macrophages, and granulocytes). *Bmal1*$^{flox/flox}$ *Lys-MCre* were compared with littermate controls, *Bmal1*$^{+/+}$*Lys-MCre*. Offspring were genotyped to confirm the presence of loxP sites and Cre recombinase. The following primers were used: primers for the detection of Cre recombinase—(Cre) 5′ CCC AGA AAT GCC AGA TTA CG 3′, (LysM1) 5′ CTT GGG CTG CCA GAA TTT CTC 3′, and (LysM2) 5′ TTA CAG TCG GCC AGG CTG AC 3′ (WT band 340 bp, CRE band 750 bp); primers for the detection of floxed *Bmal1*—5′ AAT CAC CTT TTG GGG AGG AC-3′, 5′ TCA TCA GAG GAA CCA GGG TAA-3′, and 5′ CCC TGA ACA TGG GAA AGA GA- 3′ (WT band 320 bp, KO band 380 bp).

MiR-155$^{-/-}$ mice, in which exon 2 of MiR-155HG was replaced with a resistance cassette, were used as described (1).

BMDMs. Mice were euthanized by CO_2, and cervical dislocation and the femur and tibia were isolated. BMDMs were isolated and differentiated as described (2). Before the experiment, cells were seeded at 1×10^6 cells per well in 10% (vol/vol) macrophage colony stimulating factor and allowed to rest overnight. LPS was then added at 100 ng/mL for specified times and harvested for RNA or protein analysis, and supernatants were harvested for ELISAs. To synchronize BMDMs, cells were serum-shocked (3). Cells were subjected to 50% (vol/vol) horse serum for 2 h, and then media were replaced with serum-free media and left for 30 h before stimulation with LPS.

Bacterial Infections. Bacterial infections were performed in the macrophage cell line RAW264. Cells were plated at 5×10^5 cells/ml. One day later, cells were infected with *E. coli* (1×10^8/mL) or *Salmonella* U.K.1 (1×10^4/mL). Twenty-four hours after infection, cells were harvested for RNA extraction.

RNA Isolation and Gene Expression Analysis. Total RNA was isolated using the miRNeasy mini kit (Qiagen) and transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For mRNA, 18s ribosomal RNA or the RPS18 gene was used as a housekeeping control. For miRNA analysis, U6SNO was used as an internal control, and expression was analyzed as above. Target gene expression using Taqman probes (Applied Biosystems) was normalized to the housekeeping gene, and relative quantitation values were calculated using the ΔΔCt method.

Protein Isolation, Western Blotting, and Densitometry. Protein was extracted from cells using either T-PER lysis reagent (Peirce) or SDS loading buffer and loaded onto 8–10% (vol/vol) acrylamide gels. Protein was transferred onto a PVDF membrane and blocked with 5% milk for 1 h before incubation with primary antibody. Antibodies used were rabbit anti-BMAL1 (Bethyl Laboratories) and β-actin (4967) (Cell Signaling Technology) or Pan-Actin (4968) (Cell Signaling Technology), anti-Phospho-NF-κB p65 (Ser536) (3031) (Cell Signaling Technology), and total NF-κB p65 (L8F6) (6956), and all were used according to the manufacturers' recommendations. Signal was detected using the enhanced chemiluminescence reagent and imaged using the Geldoc system (Bio-Rad). Densitometry was performed on images using the ImageLab software from Bio-Rad.

Human Endotoxemia Protocol. As previously described (4–6), healthy volunteers, age 18–40 y, with body mass index of 18–30 kg/m^2 were recruited. Exclusions included inflammatory disease, pregnancy or medication, substance, or supplement use. Subcutaneous adipose samples were collected by core needle aspiration through a 4-mm gluteal incision from distinct sites 30 min before and after i.v. bolus of 3 ng/kg US standard reference endotoxin (LPS, lot no. CC-RE-LOT-1 + 2; Clinical Center, Pharmacy Department, National Institutes of Health) and stored at −80° C. A subset of study participants (n = 14) was used for adipose tissue microarray as described (5, 6).

hPBMC and Human Macrophages. hPBMC isolation and macrophage differentiation was performed as described (7). Macrophages were then washed and treated with 100 ng/mL LPS for 4 h. Cells were then lysed for RNA isolation using TRIzol reagent (Invitrogen). Protein was extracted using RIPA lysis buffer with a proteinase inhibitor.

For transfections in hPBMCs, PBMC were isolated from whole blood using a Ficoll gradient and cultured in RPMI supplemented with 10% FCS, 2 mM L-glutamine, and 1% penicillin/streptomycin solution (vol/vol). Cells (2×10^6) in 100 μL Buffer V (Amaxa Cell Line Nucleofector Kit V) were transfected with indicated amounts of the mir-155 antagomir using Amaxa Nucleofector electroporation (Program Y-001, Version S3.4). Each sample was split into two wells containing prewarmed RPMI medium. After 24 h the cells were stimulated with LPS for 24 h. The cells were spun down and resuspended in 50 μL of sample buffer (50 mM Tris Cl, pH 6.8/10% glycerol (vol/vol)/2% SDS (wt/vol)/0.1% bromophenol blue (wt/vol)/5% 2-mercaptoethanol). The samples were run on a 10% SDS–PAGE gel.

Behavioral Monitoring in Mice. Food and water were available ad libitum throughout all of the experiments, and temperature was 21 ± 1 °C and humidity was 50 ± 10%. For period length measurements, male and female wild-type controls (n = 9) and MiR-155$^{-/-}$ (n = 14; both 12 wk at the start of behavioral monitoring) were singly housed in cages equipped with a running wheel (1-cm diameter) and housed initially under a 12:12 light: dark cycle for 3 wk. After that animals were released in constant darkness for 4 wk, followed by constant light for another 4 wk. The average illumination at the cage level was 250 lx with lights on. Wheel-running activity was recorded with the Chronobiology Kit (Stanford System) and the Kit analyze software was used to run χ^2 periodogram analysis of the free-running rhythms.

1. Rodriguez A, et al. (2007) Requirement of bic/microRNA-155 for normal immune function. *Science* 316(5824):608–611.
2. Sheedy FJ, et al. (2010) Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nat Immunol* 11(2):141–147.
3. Balsalobre A, Damiola F, Schibler U (1998) A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* 93(6):929–937.
4. Anderson PD, et al. (2007) Innate immunity modulates adipokines in humans. *J Clin Endocrinol Metab* 92(6):2272–2279.
5. Shah R, et al. (2012) Adipose genes down-regulated during experimental endotoxemia are also suppressed in obesity. *J Clin Endocrinol Metab* 97(11):E2152–E2159.
6. Shah R, et al. (2009) Gene profiling of human adipose tissue during evoked inflammation in vivo. *Diabetes* 58(10):2211–2219.
7. Ferguson JF, et al. (2012) Translational studies of lipoprotein-associated phospholipase A_2 in inflammation and atherosclerosis. *J Am Coll Cardiol* 59(8):764–772.

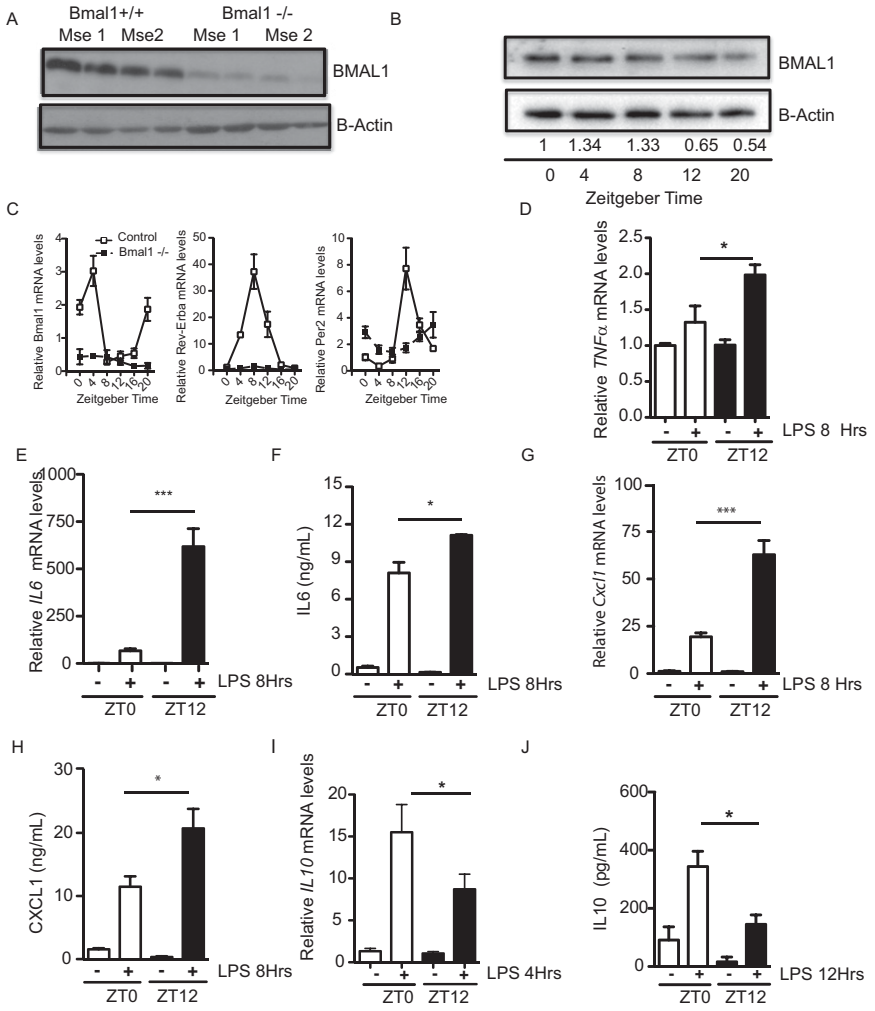


Fig. S1. Clock-gated variation in inflammatory cytokines in response to LPS that is inversely correlated with levels of BMAL1. (*A*) Peritoneal cells harvested from *Bmal1*$^{+/+}$Lys-MCre (mouse 1 and mouse 2) and *Bmal1*$^{-/-}$Lys-MCre (mouse 1 and mouse 2) extracted for protein and analyzed by immunoblot for BMAL1. (*B*) Peritoneal cells were harvested from WT mice in LD conditions at the indicated ZTs, extracted immediately for protein, and analyzed by immunoblot for BMAL1 and β-Actin. Values written underneath the immunoblot are relative band intensity corrected for β-Actin. (*C*) Peritoneal cells were harvested from *Bmal1*$^{+/+}$Lys-MCre and *Bmal1*$^{-/-}$Lys-MCre mice in 12:12 LD conditions at the indicated ZTs beginning at ZT0 and extracted immediately and analyzed for expression of *Bmal1*, *Per2*, and *Rev-Erbα* mRNA. Peritoneal cells harvested at ZT0 and ZT12 (n = 3–4) and treated immediately ex vivo with LPS (100 ng/mL) for indicated times and analyzed for expression levels of (*D*) *Tnfα* mRNA, (*E*) *Il6* mRNA, (*F*) IL6 protein, (*G*) *Cxcl1* mRNA, (*H*) CXCL1 protein, (*I*) *Il10* mRNA, and (*J*) IL10 protein. *$P \leq 0.05$ and ***$P \leq 0.001$.

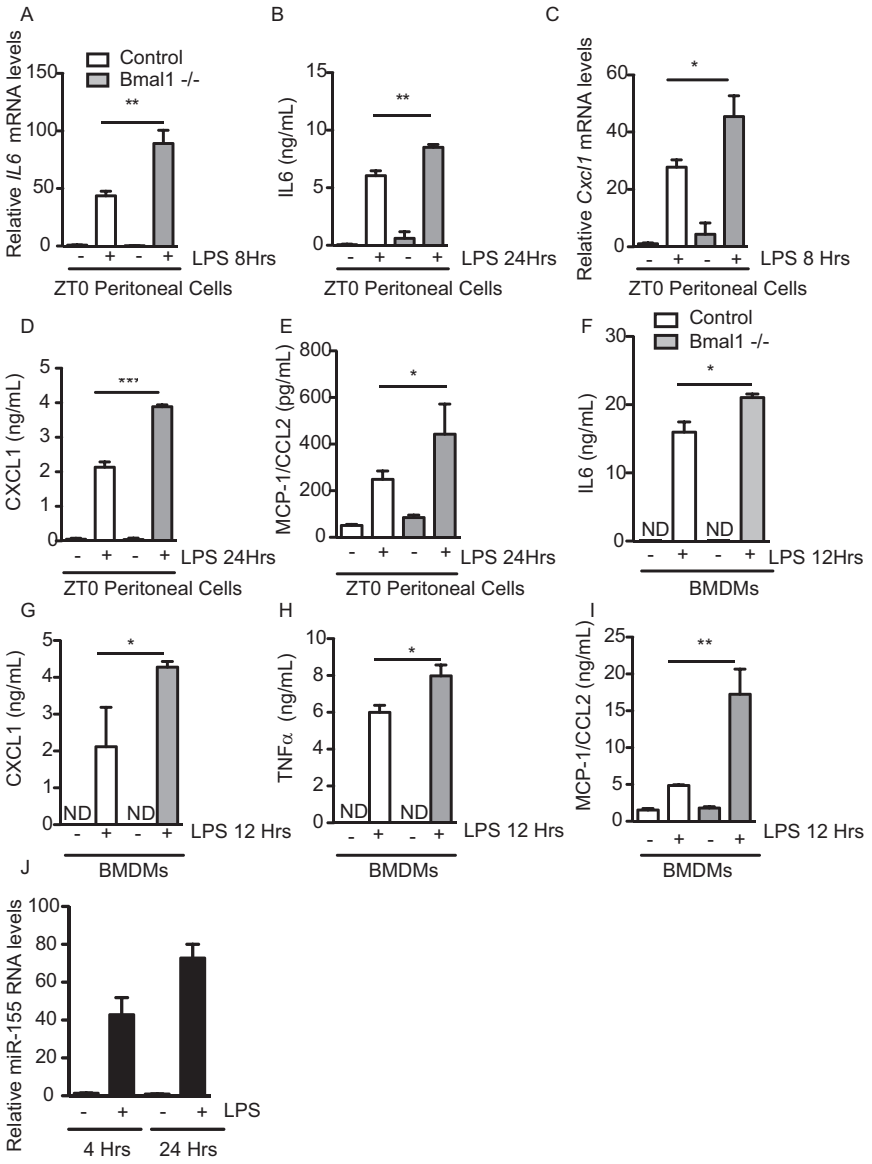


Fig. S2. Peritoneal cells and BMDMs deficient in BMAL1 display heightened proinflammatory responses. Peritoneal cells were harvested from *Bmal1*$^{+/+}$Lys-MCre and *Bmal1*$^{-/-}$Lys-MCre mice ($n = 3$–4) and treated immediately ex vivo with LPS (100 ng/mL) for the indicated times and analyzed for expression levels of (*A*) *Il6* mRNA, (*B*) IL6 protein, (*C*) *Cxcl1* mRNA, (*D*) CXCL1 protein, and (*E*) MCP-1/CCL2 protein. Bone marrow was harvested from *Bmal1*$^{+/+}$Lys-MCre and *Bmal1*$^{-/-}$Lys-MCre mice and allowed to differentiate into BMDMs in culture. Cells were then stimulated for the indicated times with LPS, and supernatants were harvested and analyzed for protein levels of (*F*) IL6, (*G*) CXCL1, (*H*) TNFα, and (*I*) MCP-1/CCL2. (*J*) BMDMs stimulated with LPS for the indicated times and analyzed for expression of mature miR-155. *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$.

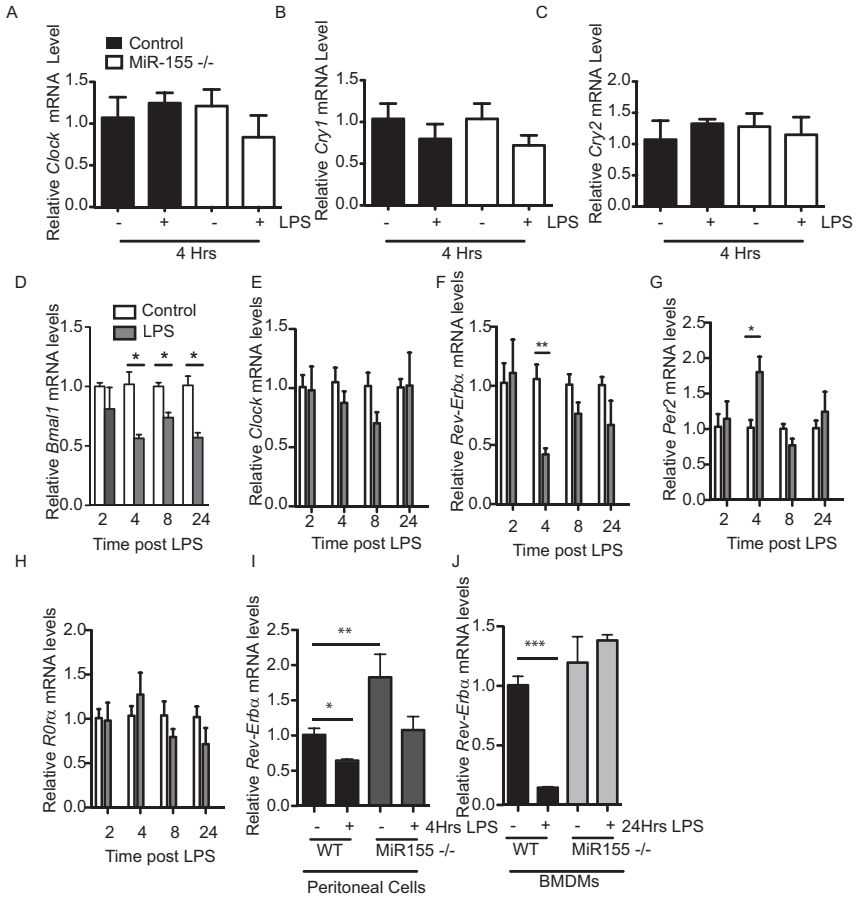
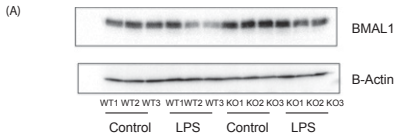


Fig. S3. Bone marrow was harvested from control and miR-155$^{-/-}$ mice and allowed to differentiate into BMDMs in culture. Cells were then stimulated for the indicated times with LPS and RNA was harvested and analyzed for mRNA levels of (*A*) *Clock*, (*B*) *Cry1*, and (*C*) *Cry2*. Peritoneal cells harvested at ZT0 and treated immediately ex vivo with or without LPS (100 ng/mL) for indicated times and analyzed for mRNA expression levels of (*D*) *Bmal1*, (*E*) *Clock*, (*F*) *Rev-Erbα*, (*G*) *Per2*, and (*H*) *RoRα*. (*I*) Peritoneal cells harvested from control and miR-155$^{-/-}$ mice, treated immediately ex vivo with LPS (100 ng) for the indicated time, and analyzed for expression of *Rev-Erbα*. (*J*) BMDMs harvested from control and miR-155$^{-/-}$ mice and treated immediately ex vivo with LPS (100 ng) for the indicated time and analyzed for expression of *Rev-Erbα*. $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$.



(B)

Coordinates Chromosome 7, 120457132-57636
BMAL 3′UTR
MiR-155 binding sites { }
Morpholino against Site 1- 22 nucleotides : ATTAATGCACTTTGATACTGCA
Morpholino against Site 2- 25 nucleotides : ACAATTAATGCAAGCTATCCACAGC

5′CTCGAGACACTACATTTGCTTTGGCAACAGC TGCAGTATCAAAGT{GCATTAA}T GGTGAAGTTTTACAGTCTGT

GAAGCTTACTGGATAGAGAGAGAACAGCTTTTATGTACTGACTCCATAAAAGCCACCTCAGAGCCATTGATACAA
Morpholino to Site 1
GTCAATCTACCATGTGTAACTTCAGACAAAGTGGAACTAAACCTGCTCCAGTGTTTCCTCATCATTGAGTTTGGGC

TA GCTGTGGATAGCTT{GCATTAA}TTGT ATATTTTGGATTTTGTTTGTGTTGAATTTTTTAATCATTGTGCACAGAAG

CATCATTGGTAGCTTTTATATGCAAAATGGTCATTTCAGATGTATGGTGTTTTTACACTACAAAGAAGTCCCCCATGT
Morpholino to Site 2
GGATATTTCTTATACTAATTGTATCATAAAGCTGTTTATTCTTCCTTGTAAGAATCCTTTACTATAAATATGGGTTAAAG

TATAATGTATTAGACAGTTAAATATTTTTAATAAATGTTTCCCTTGTTCTGCGGCCGC 3′

Fig. S4. (*A*) Immunoblot from WT and miR-155$^{-/-}$ BMDMs exposed to LPS (100 ng/mL) for 24 h. This along with immunoblot from Fig. 3*G* was used to calculate densitometry values for Fig. 3*H*. As WT1 did not show the characteristic reduction in BMAL1 protein, this mouse was excluded from the densitometry analysis. (*B*) Position of two miR-155–binding sites in the Bmal1 3′ UTR along with design and position of morpholino sequences against the two MiR-155–binding sites in the Bmal1 3′ UTR.

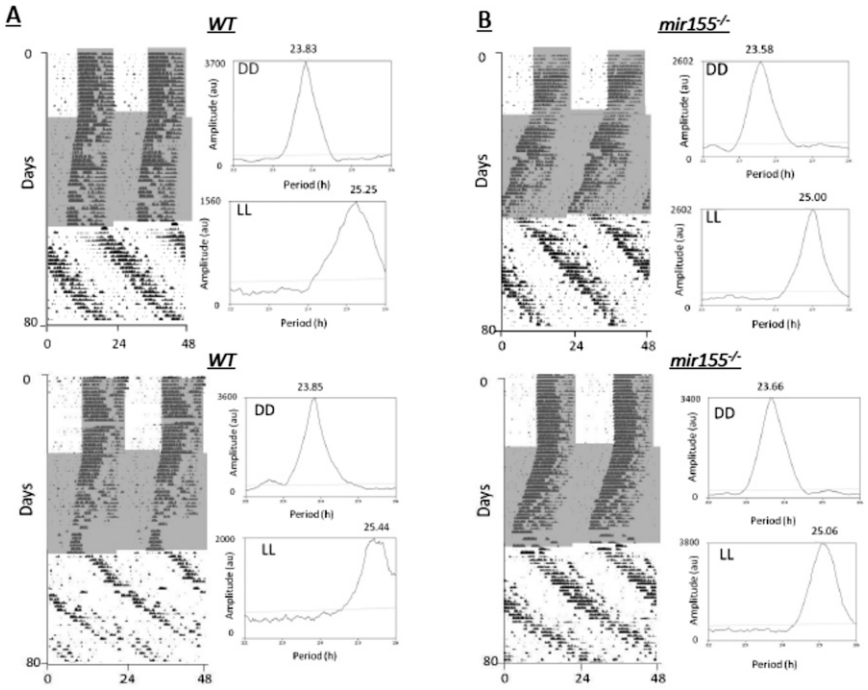


Fig. S5. Sample double-plotted actograms for (*A*) two wild-type and (*B*) two mir155 $^{-/-}$ mice, as well as the appropriate sample periodograms for periods in constant darkness (DD) and constant light (LL). Shaded portions on the actograms indicate lights off; unshaded portions indicate lights on.