SUPPLEMENTAL FIGURE LEGENDS

**Figure S1.** LPS-induced NF-κB activation represses circadian clock repressors. (A) Scatter plots depicting log-transformed ChIP-sequencing tag densities for all p65, BMAL1, and CLOCK peaks identified in each replicate (n=2) in saline- (top) and in LPS- (bottom) treated mice. In each condition, there is significant overlap at the 45-degree line which represents equivalent binding between the replicates. P<0.0001 for all comparisons. (B) Table indicating percentage of p65 peaks in both saline and LPS conditions that contained each of the listed consensus motif sequences, as well as the total number of sites in each condition. (C) Strength of binding (reads/bp/peak) at p65 sites that were either lost (left), shared (middle), or gained (right) following LPS treatment for p65 (top) and H3K27Ac (bottom) ChIP-seq. (D) Overlaid representative UCSC genome browser images of p65, H3K27Ac, POL II, BMAL1, and CLOCK ChIP-seq tracks at core clock genes Cry1, Dbp, Rev-erbα, Clock, and Bmal1, as well as at the known NF-κB target Nfkb2. Normalized tag counts are indicated on the y-axis, and for each antibody, maximum track height is the same for all conditions. Orientation for each gene is indicated below each browser track. (n=2 per condition per Ab).

**Figure S2.** NF-κB activation acutely represses circadian clock repressors and is enriched at LPS-induced CLOCK/BMAL1 sites. (A) Browser tracks of p65, BMAL1, and CLOCK ChIP-seq at the Per2 promoter, where validated and putative NF-κB (red and black tick marks, respectively) and E-box (green tick mark) binding motifs are indicated below the browser tracks. Sequence analysis using the promoter prediction and regulatory sequence algorithm (http://www.genomatix.de/matinspector.html) identified putative NF-κB binding sites. (B)
Representative browser tracks of total POL II ChIP-seq following saline or LPS treatment at the positive control *Pai1* (*plasminogen activator inhibitor-1*) and the negative control *Drd2* (*Dopamine receptor D2*) gene promoters. (C) Relative luciferase activity of PER2-LUCIFERASE in the presence of increasing doses of the NF-κB subunits p65 and p50 in HepG2 cells (n=5-11).

(D) Time course of clock gene expression in liver following LPS injection. WT mice were injected with LPS at ZT6, followed by tissue collection for qPCR analysis of clock repressors at indicated times post-injection (p<0.0001, two-way ANOVA, for all clock genes shown) (n=3-4). (E) Histograms showing enrichment of NF-κB p65 and p50 motifs in comparison with LXRE motifs at LPS-induced compared to saline-treated CLOCK/BMAL1 peak centers. Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 unpaired t test.

**Figure S3.** Loss of p65/IKK disrupts molecular clock gene expression and behavioral rhythms. (A) Quantitative RT-PCR analysis of rhythmic expression of core clock genes in forskolin-synchronized WT MEFs (black) compared to p65 KO (green) and *p65*-rescued (gray) MEFs. Cells were harvested at 4 hr intervals for a full circadian cycle, starting 12 hrs following forskolin synchronization. Data are represented as mean ± SEM (n=2-3 independent experiments, each with 4 samples per experiment) (p<0.0001, two-way ANOVA between WT vs. *p65* KO MEFs and *p65* KO vs. *p65*-rescued MEFS for clock genes shown). (B) Change in body weight in *IKKβ*<sup>−/−</sup> and CAGGCre-ER;*IKKβ*<sup>−/−</sup> mice 5 weeks post-injection of either oil or tamoxifen (n=4-15). (C) Period distribution shown for oil- or tamoxifen-treated CAGGCre-ER;*IKKβ*<sup>−/−</sup> mice (n=6-9). (D) Representative actograms showing wheel-running activity from CAGGCre-ER; *IKKβ*<sup>−/−</sup> and control littermate (*IKKβ*<sup>−/−</sup>, CAGGCre-ER, and WT) mice which received either oil (black) or tamoxifen (purple). Mice were injected i.p. at 75 mg/kg body weight of tamoxifen (prepared in
corn oil) once every 24 hrs for a total of 5 consecutive days. Mice were then maintained on a 12:12 LD cycle in wheel cages for 15 days prior to release into DD. (E) Representative diurnal activity profiles during LD are shown. (F-G) Quantitative RT-PCR analysis of (F) IKKβ and Per2 mRNA in CAGGCre-ER; IKKβfx/fx and control littermate (IKKβfx/fx, CAGGCre-ER, and WT) mice which received either oil or tamoxifen (n=3-5) and (G) IKKβ and panel of core clock genes in white adipose tissue (WAT) and hypothalamus of CAGGCre-ER; IKKβfx/fx mice 5 weeks following either tamoxifen or oil injection (n=5-9). (H-I) Quantitative RT-PCR analyses of PAR bZIP genes in (H) liver or WAT of saline- or LPS-treated WT mice (n=5) or (I) liver of oil- or tamoxifen-treated CAGGCre-ER; IKKβfx/fx mice (n=10-11). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 unpaired t test.

Figure S4. High fat diet represses clock repressors. (A) Representative UCSC genome browser images of p65 ChIP-seq tracks at the core clock activator genes Clock and Bmal1 in RC and HFD. Normalized tag counts indicated on y-axis. Orientation for each gene is indicated below each browser track. (B) Quantitative RT-PCR analysis of core clock genes in liver and white adipose tissue of mice at ZT8 following either RC (black) or HFD (orange) feeding (n=5-11). Data are represented as mean ± SEM. *p<0.01, **p<0.01, ***p<0.001 (C) KEGG pathways analysis reveals CLOCK/BMAL1 enrichment at both lipid metabolism and immune pathways following high fat feeding.

Figure S5. p65 binds core clock genes across multiple cell types. Comparative analysis of GEO repository NF-κB p65 ChIP-seq data for TNFα-stimulated human lymphoblasts, human umbilical vein endothelial cells (HUVECs), HELA B2, and A549 lung carcinoma cells. Representative
ChIP-seq browser tracks depict NF-κB p65 binding in the promoter regions of the canonical circadian clock repressor genes (*Per1, Per2, Rev-erba*) in each of these cell types. Vertical green tick marks indicate locations of predicted E-box motifs that aligned with the p65 peaks (Nakahata et al. 2008; Heinz et al. 2010).

**Table S1.** Macronutrient composition of high fat diet. Nutrient composition (% kcal) of the regular chow diet (RC, Harlan Teklad 7102) compared to the high fat diet (HFD, Research Diet, Inc, Custom Diet # D06022405) which was custom made (*) in this study.

**Table S2.** Primer names and sequences for quantitative real-time PCR used in this study.
B | Percentage of peaks containing consensus motif sequence

| Motif name          | Saline (% total sites) | LPS (% total sites) |
|---------------------|------------------------|---------------------|
| NFκB-p65/NFκB-p50   | 7.9                    | 44.7                |
| Ronin-Thap11/GFY    | 31.3                   | 9.3                 |
| ZNF143              | 35.9                   | 13.7                |
| CEBP                | 15.2                   | 29.6                |
| STAT                | 18.3                   | 26.8                |
| HNF6                | 8.1                    | 12.3                |
| FOXA                | 14.5                   | 22.8                |
| JUN-AP1             | 5.1                    | 11.7                |
| E-box               | 21.5                   | 21.9                |
| Total number of sites | 3535                  | 12052               |

C | Strength of binding at p65 sites that were lost, shared or gained with LPS

D | Activated p65 inhibits clock repressors but not activators

|   | Cry1 | Dbp | Rev-erbβ | Clock | Bmal1 | Nfkb2 |
|---|------|-----|----------|-------|-------|-------|
|   | Saline 1' | LPS 1' | Saline 5' | LPS 5' | Saline 10' | LPS 10' | Saline 1' | LPS 1' | Saline 5' | LPS 5' | Saline 10' | LPS 10' |
| p65 |   |     |          |       |       |       |   |     |        |     |        |       |
| H3K27Ac |     |     |          |       |       |       |   |     |    |       |       |       |
A E-box and NF-κB motifs overlap at the *Per2* promoter

B Positive and negative controls for POL II binding following LPS

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C NF-κB represses *Per2* transcription

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D Clock gene expression following LPS injection

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E Enrichment of NF-κB motifs at LPS-induced CLOCK/BMAL1 peaks
A. p65 rescues clock gene expression in p65 KO MEFs

- p65
- Per2
- Dbp
- Cry2

B. IKK loss reduces body weight

C. Loss of IKK alters period

D. Inducible loss of IKK in vivo disrupts circadian behavior

E. Inducible loss of IKK in vivo disrupts circadian activity

F. Loss of IKK alters Per2

G. IKK loss alters peripheral clock expression

H. LPS alters bZIP expression

I. Loss of IKK alters bZIP expression in liver
A. HFD does not affect p65 binding at *Clock* and *Bmal1*

![Image showing p65 binding at Clock and Bmal1](image)

B. HFD represses clock repressors

![Image showing miRNA expression in Liver and WAT](image)

C. CLOCK/BMAL1 binding in HFD enriched at immune system and lipid and fatty acid metabolism pathways

- Metabolism of lipids/lipoproteins
- Fatty acid, triacylglycerol, ketone metabolism
- Immune system
- HIF-1α transcription factor network
- PPARα signaling pathway
- Phospholipid metabolism
- Unfolded protein response
- Glycerophospholipid biosynthesis
- FOXA2 and FOXA3 transcription factor networks
- Adaptive immune system
- Cytokine signaling in immune system

![Bar chart showing -Log10 p-value](image)
p65 binds core clock repressors across multiple cell types

|        | Lymphoblast | HUVEC | HELA B2 | A549 |
|--------|-------------|-------|---------|------|
| PER1   |             |       |         |      |
| PER2   |             |       |         |      |
| CRY2   |             |       |         |      |
| REV-ERBα |         |       |         |      |

* E-box motif
| Kcal (%)          | RC  | HFD  |
|-------------------|-----|------|
| Protein           | 25  | 20   |
| Carbohydrate      | 58  | 35   |
| Fat               | 17  | 45   |
| Saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0) | 4.4 | 26.3 |
| Monounsaturated fatty acids (C14:1, C16:1, C18:1, C20:1) | 2.7 | 13.5 |
| Polyunsaturated fatty acids (C18:2, C18:3) | 9.9 | 5.2 |
| Total kcal/g      | 3.1 | 4.73 |
| Primer name | Primer sequence |
|-------------|----------------|
| Per1 F      | CCCAGCTTTAACCTGCAGAAG |
| Per1 R      | ATGGTCGAAAGGAAGCCTCT |
| Per2 F      | TGTGCGATGATGATTCGTGA |
| Per2 R      | GGTAGAAGTGATAGTTTGGTTG |
| Per3 F      | GTGATATTTACCCGACGTCT |
| Per3 R      | CACTGCGATCTCGAGATT |
| Cry1 F      | TGAGGCAAGACAGACTGAATATTG |
| Cry1 R      | CCTCTGTACCAGGAAGCAAGC |
| Cry2 F      | CTGGCGAGAAGGTAGTG |
| Cry2 R      | GACGCAGAGATTACTGGCTTTG |
| Clock F     | ACCACAGCAACAGCAACAAC |
| Clock R     | GGCTGCTAAACTGAAGGAAAG |
| Bmal1 F     | AGGCCACAGTCGATTGAA |
| Bmal1 R     | TGTTACCAAAGAAGCCAATTCAT |
| Rev-erbα F  | CAGTGCTCTGCGCATGACTA |
| Rev-erbα R  | CCACATCCCAACAGACTTTA |
| Rorα F      | ACGCCCACTCAAACATCTC |
| Rorα R      | TGCCCATCCATATAGGTGCT |
| Dbp F       | GCAAGTGCTGTGCTTGCT |
| Dbp R       | CTGCTATGACGTGCTTG |
| Tef F       | ATCTTTCAGCCCTCGGAAA |
| Tef R       | GGTCTCTGCCTCCTTTTCA |
| E4BP4 F     | CGGAGCTTGAAATCGGCCC |
| E4BP4 R     | GGGTTATCGTGTTTCTGCTCCCTG |
| Ikkβ F      | CAGCCTAAAAAGACAGAACC |
| Ikkβ R      | ACCACATTGGGATGGTTCAG |
| p65 F       | ATCTTCTTGGCTGTGCAGAAG |
| p65 R       | TGGTCCCTGAAATACACCT |
| Gapdh F     | CAAGAGTAAGAAACCTGGACC |
| Gapdh R     | CGAGTTGGGATAGGGCCTT |
|              |              |