Supplementary Information

Lipid metabolic adaptations during inflammation are controlled by the circadian clock and impaired by light at night

Beata Benedikova, Viera Sebenova Jerigova, Michal Zeman and Monika Okuliarova*

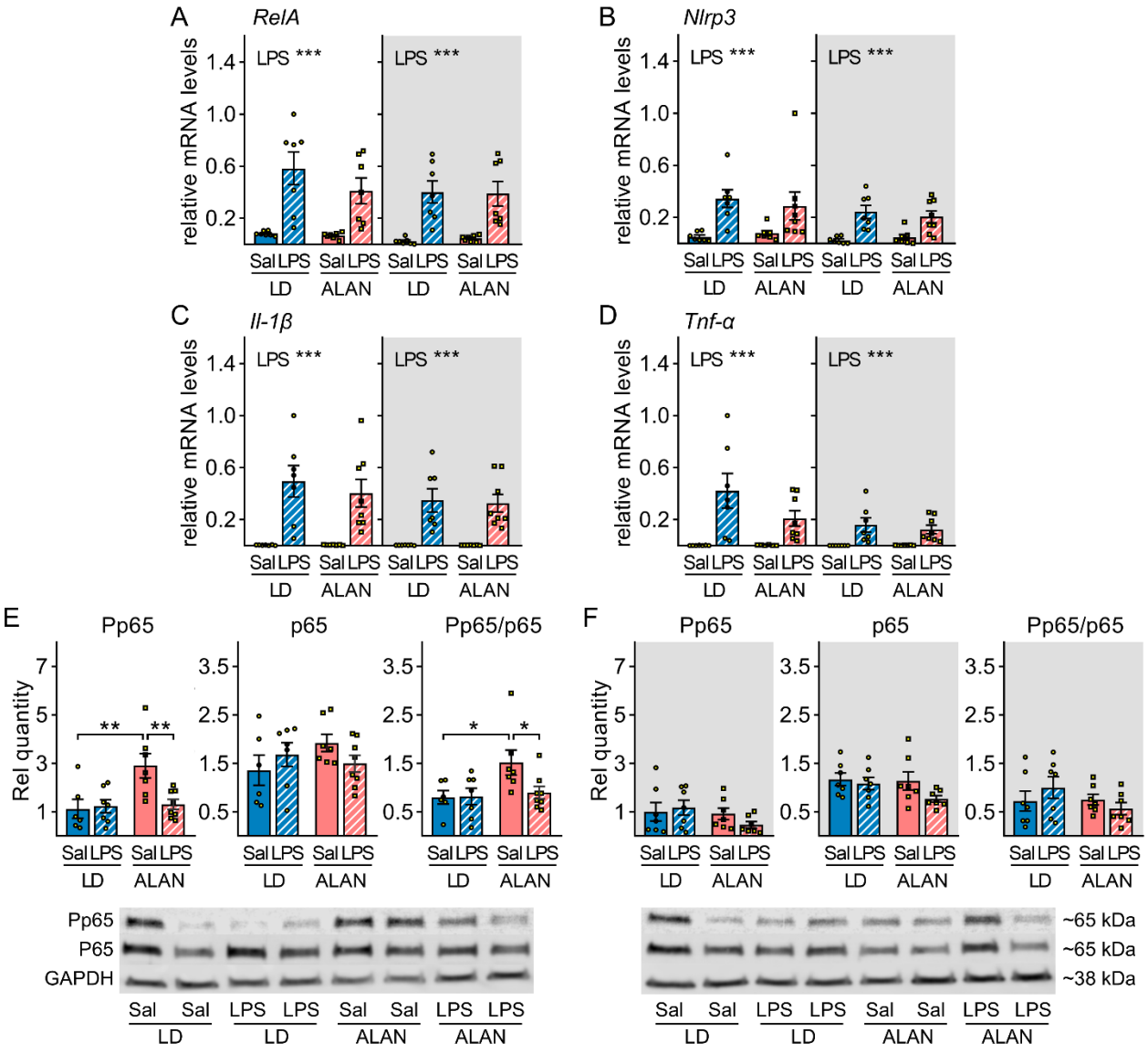
Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Ilkovicova 6, 842 15 Bratislava, Slovakia

*Corresponding author
E-mail: monika.okuliarova@uniba.sk (MO)

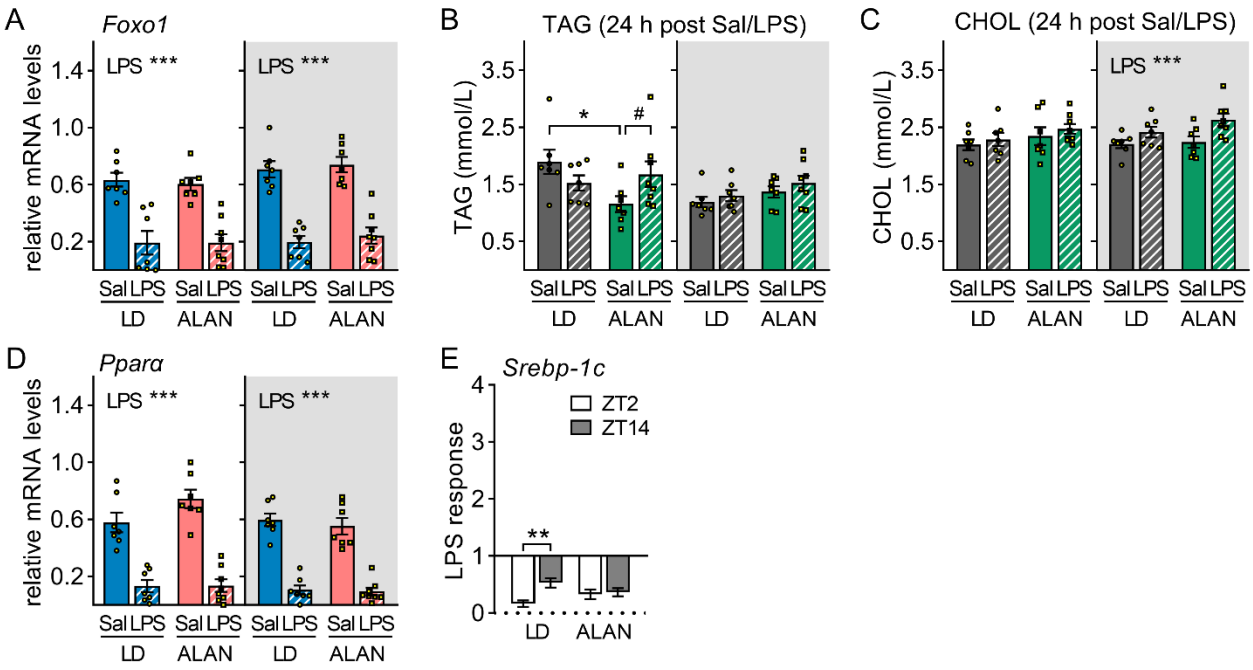
Supplementary Table S1 Primer sequences for real-time PCR

Gene	Accession Number	Forward primer	Reverse primer
Acat1	NM_017075.2	5′-GTCTACCCATTGCCACTCCG-3′	5′-TGACATGCTCTCCATTCCGC-3′
Acly	NM_016987.2	5′-ATGGGCTTCATCGGGCACT-3′	5′-GGCTGCTGGCTCGGTTACAT-3′
Actb	NM_031144.3	5′-GATCAAGATCATTGCTCCTCCTG-3′	5′-AGGGTGTAAAACGCAGCTCA-3′
Adn	NM_144744.3	5′-GCCGTTCTCTTCACCTACGA-3′	5′-CCCCTTCCCCATACACTTGG-3′
Bmal1	NM_024362.2	5′-CACCTTGCGGAATGTCACAG-3′	5′-TACTTCCTTGGTCCACGGGT-3′
Cd36	NM_031561	5′-CGGCGATGAGAAAGCAGAAA-3′	5′-GGCTCATCCACTACTTATTTTCC-3′
Cd68	NM_001031638.1	5′-CTGGGGCCTCTCTGTATTGA-3′	5′-TGATGTCGGTCCTGTTTGAAT-3′
Cpt1b	NM_013200.2	5′-TAAGTGACTGGTGGGAAGAGT-3′	5′-TGCTTGTTGGCTCGTGTTC-3′
Dbp	NM_012543.3	5′-TTGCCCTGTCAAGCATTCCA-3′	5′-ACTTCTCATCCTTCTGTTCCTCG-3′
Fasn	NM_017332.1	5′-GAGTCTGTCTCCCGCTTGAC-3′	5′-TTGCCTTGCTCACCTTCGAG-3′
Foxo1	NM_001191846.2	5′-CAGCAAATCAAGTTATGGAGGA-3′	5′-TATCATTGTGGGGAGGAGAGTC-3′
Glut1	NM_138827.1	5′-GCCGCTTCATCATTGGAGTG-3′	5′-CGAACACCTGGGCAATAAGGA-3′
Glut2	NM_012879.2	5′-GAAGGATCAAAGCCATGTTGG-3′	5′-CCTGATACGCTTCTTCCAGCA-3′
Glut4	NM_012751.1	5′-TATGTTGCGGATGCTATGGGT-3′	5′-AATGTCCGGCCTCTGGTTTC-3′
Hif-1α	NM_024359.1	5′-CAGTTGCCACTTCCCCACAA-3′	5′-TCAATGTCAAGATCACCAGCACC-3′
Hsl	NM_012859.1	5′-TCACGCTACATAAAGGCTGCT-3′	5′-CCACCCGTAAAGAGGGAACT-3′
IL-1β	NM_031512.2	5′-GCCAACAAGTGGTATTCTCCA-3′	5′-GCCGTCTTTCATCACACAGG-3′
Insr	NM_017071.2	5′-CAGTGCCAGTGATGTCTTTCCA-3′	5′-ATTGACCGTCTTCACCGCAA-3′
Lep	NM_013076.3	5′-GACCCCAGCGAGGAAAATGT-3′	5′-GGATACCGACTGCGTGTGT-3′
Lpl	NM_012598.2	5′-TCATCAACTGGTTGGAGGAAG-3′	5′-ACGAAATCCGCATCATCAGG-3′
Mcad	NM_016986.2	5′-GGAGCCGGGACTAGGGTTTA-3′	5′-CTGGCAAACTTCCGAGCAAT-3′
Nampt	NM_177928.3	5′-AAGTTGCTGCCACCTTACCTT-3′	5′-TTCATTCCCTCGACAATCTCCT-3′
Nfil3	NM_053727.2	5′-TTTGACCTGGAGAAGCATGG-3′	5′-GCCTCGGAAACCTTATAGCC-3′
Nlrp3	NM_001191642.1	5′-TCCAGTGTGTTTTCCCAGACC-3′	5′-ACTTGAGAAGAGACCTCGGC-3′
Nr1d1	NM_145775.2	5′-TCCCACATACTTCCCACCATCA-3′	5′-CACTCGGCTGCTGTCTTCCAT-3′
Pck1	NM_198780.3	5′-TTCGGAAGCGGATACGGTG-3′	5′-GCCAGGTTGGTTTTCCCACA-3′
Per2	NM_031678.1	5′-TGGCAACCTTGAAGTACGCT-3′	5′-GCTGGCTCTCACTGGACATT-3′
Pparα	NM_013196.1	5′-GACTAGCAACAATCCGCCTT-3′	5′-GAAGAATCGGACCTCTGCCT-3′
Pparγ	NM_013124.3	5′-TCCAAGAATACCAAAGTGCGA-3′	5′-CCATGAGGGAGTTTGAAGGC-3′
Pygl	NM_022268.1	5′-ATCCACTCGGACATCGTGAA-3′	5′-CCTGGGTTGCAGAGTAAGAG-3′
RelA	NM_199267.2	5′-ATTGTGTTCCGAACTCCCCC-3′	5′-TTTCTTCAATCCGGTGGCGA-3′
Rps29	NM_012876.1	5′-GCTGAACATGTGCCGACAGT-3′	5′-GGTCGCTTAGTCCAACTTAATGAA-3′
Sirt1	NM_012818.2	5′-TTATGCTCGCCTTGCTGTGG-3′	5′-CTGTCCGGGATATATTTCCTTTGC-3′
Srebp-1c	NM_001276707.1	5′-GCCATCGACTACATCCGCTT-3′	5′-CCAGGTCTTTCAGTGATTTGCT-3′
TNF-α	NM_012675.3	5′-TCCGAGATGTGGAACTGGCA-3′	5′-CATTTGGGAACTTCTCCTCCTTGT-3′

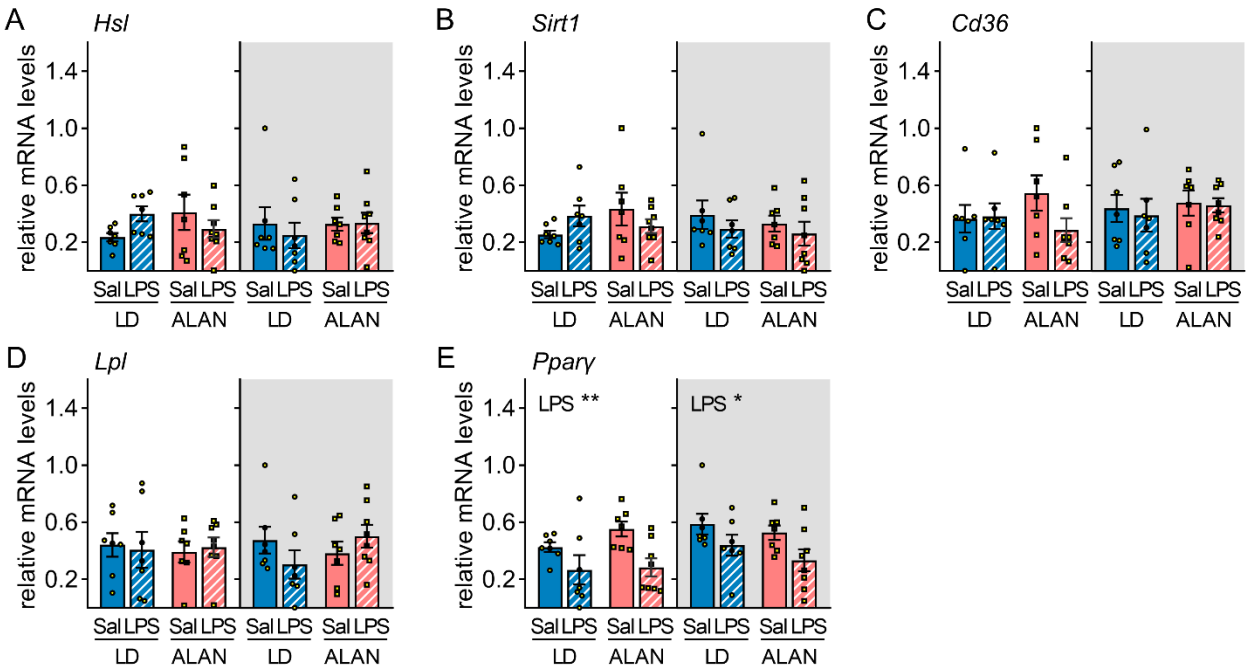
Abbreviations: *Acat1*, acetyl-CoA acetyltransferase 1; *Acly*, ATP citrate lyase; *Actb*, actin, beta; *Adn*, adiponectin; *Bmal1*, brain and muscle Arnt-like protein-1; *Cd36*, fatty acid translocase; *Cd68*, lysosome-associated macrophage receptor; *Cpt1b*, carnitine palmitoyltransferase 1b; *Dbp*, D-box binding PAR bZIP transcription factor; *Fasn*, fatty acid synthase; *Foxo1*, forkhead box O1; *Glut*, glucose transporter; *Hif-1α*, hypoxia inducible factor 1 subunit alpha; *Hsl*, hormone sensitive lipase; *Il-1β*, interleukin 1 beta; *Insr*, insulin receptor; *Lep*, leptin; *Lpl*, lipoprotein lipase; *Mcad*, medium chain acyl-CoA dehydrogenase; *Nampt*, nicotinamide phosphoribosyltransferase; *Nfil3*, nuclear factor, interleukin 3 regulated; *Nlrp3*, NLR family, pyrin domain containing 3; *Nr1d1*, nuclear receptor subfamily 1, group D, member 1 (Reverbα); *Pck1*, phosphoenolpyruvate carboxykinase 1; *Per2*, period 2; *Pparα*, peroxisome proliferator activated receptor alpha; *Pparγ*, PPAR gamma; *Pygl*, glycogen phosphorylase L; *RelA*, NF-kB subunit (p65); *Rps29*, ribosomal protein S29; *Sirt1*, sirtuin 1; *Srebp-1c*, sterol regulatory element binding protein 1c; *TNF-α*, tumor necrosis factor alpha



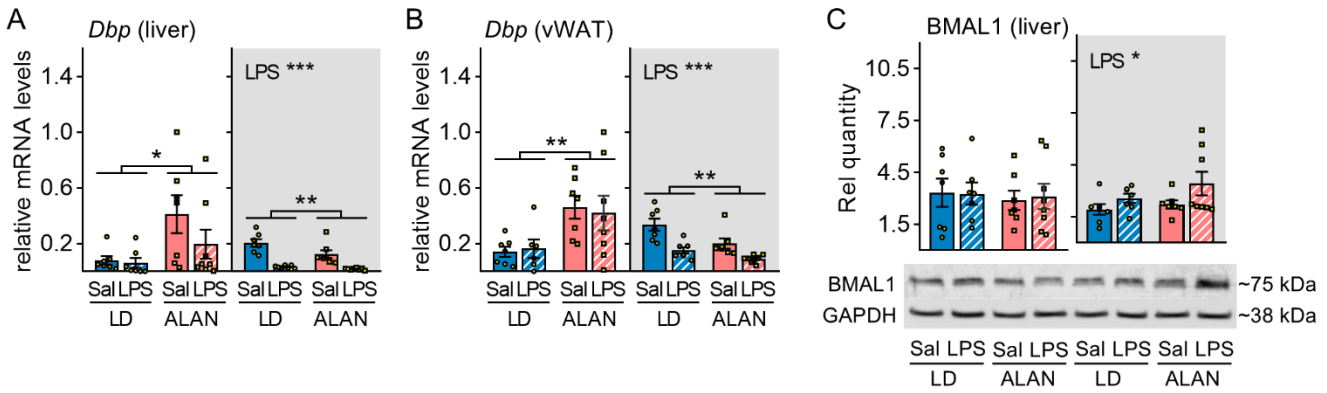
Supplementary Fig. S1 Hepatic inflammatory response to daytime/nighttime lipopolysaccharide (LPS) injection under ALAN. Rats were exposed to either the control 12/12 h light/dark regime (LD) or dim ALAN (~2 lx) and injected with saline (Sal) or LPS at either ZT2 (white background) or ZT14 (shaded background). Zeitgeber time (ZT) 0 = lights on. Data were analysed 3 h post-injection. **A–D** Relative mRNA levels (normalized to *Rps29* and *Actb*) of hepatic *RelA*, NLR family pyrin domain containing 3 (*Nlrp3*), interleukin *Il-1β* and tumour necrosis factor-α (*Tnf-α*). **E–F:** Representative immunoblots and quantification of phosphorylated p65 (Pp65), total p65 and the Pp65/p65 ratio 3 h after daytime (**E**) or nighttime injection (**F**). Bars represent means ± SE (n = 6–8 rats per group). Data were evaluated by two-way ANOVA with Bonferroni's multiple comparisons test at **P* < 0.05, ***P* < 0.01 and ****P* < 0.001



Supplementary Fig. S2 Hepatic metabolic response to daytime/nighttime lipopolysaccharide (LPS) injection under ALAN. Rats were exposed to either the control 12/12 h light/dark regime (LD) or dim ALAN (~2 lx) and injected with saline (Sal) or LPS at either ZT2 (white background) or ZT14 (shaded background). Zeitgeber time (ZT) 0 = lights on. **A** Relative mRNA levels of transcription factor *Foxo1* in the liver 3h post-injection. **B, C** Triacylglycerol (TAG) and total cholesterol (CHOL) plasma levels 24 h post-injection. **D** Relative mRNA levels of peroxisome proliferator-activated receptor-α (*Pparα*) in the liver 3h post-injection. **E** The LPS response (fold change relative to the mean of the saline-injected group) for sterol regulatory element binding protein-1c (*Srebp-1c*). Bars represent means ± SE (n = 6–8 rats per group). Data were evaluated by two-way ANOVA with Bonferroni's multiple comparisons test at **P* < 0.05, ***P* < 0.01 and ****P* < 0.001



Supplementary Fig. S3 Adipose metabolic response to daytime/nighttime lipopolysaccharide (LPS) injection under ALAN. Rats were exposed to either the control 12/12 h light/dark regime (LD) or dim ALAN (~2 lx) and injected with saline (Sal) or LPS at either ZT2 (white background) or ZT14 (shaded background). Zeitgeber time (ZT) 0 = lights on. Data were analysed in visceral fat (vWAT) 3 h post-injection. Relative mRNA levels of hormone-sensitive lipase (*Hsl*) (**A**), sirtuin 1 (*Sirt1*) (**B**), *Cd36* (**C**), lipoprotein lipase (*Lpl*) (**D**) and peroxisome proliferator-activated receptor-γ (*Pparγ*) (**E**). Bars represent means ± SE (n = 6–8 rats per group). Data were evaluated by two-way ANOVA with Bonferroni's multiple comparisons test at **P* < 0.05 and ***P* < 0.01



Supplementary Fig. S4 Time-of-day-dependent response of peripheral clock components to LPS-induced inflammation. Rats were exposed to either the control 12/12 h light/dark regime (LD) or dim ALAN (~2 lx) and injected with saline (Sal) or lipopolysaccharide (LPS) at either ZT2 (white background) or ZT14 (shaded background). Zeitgeber time (ZT) 0 = lights on. Relative mRNA levels of D-box binding transcription factor (*Dbp*) in the liver (**A**) and visceral fat (vWAT) (**B**) 3 h post-injection. **C** Protein levels of BMAL1 in the liver and representative immunoblot. Bars represent means ± SE (n = 6–8 rats per group). Data were evaluated by two-way ANOVA with Bonferroni's multiple comparison test at **P* < 0.05, ***P* < 0.01 and ****P* < 0.001