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Supplementary Materials for

An NF-κB–driven lncRNA orchestrates colitis and circadian clock

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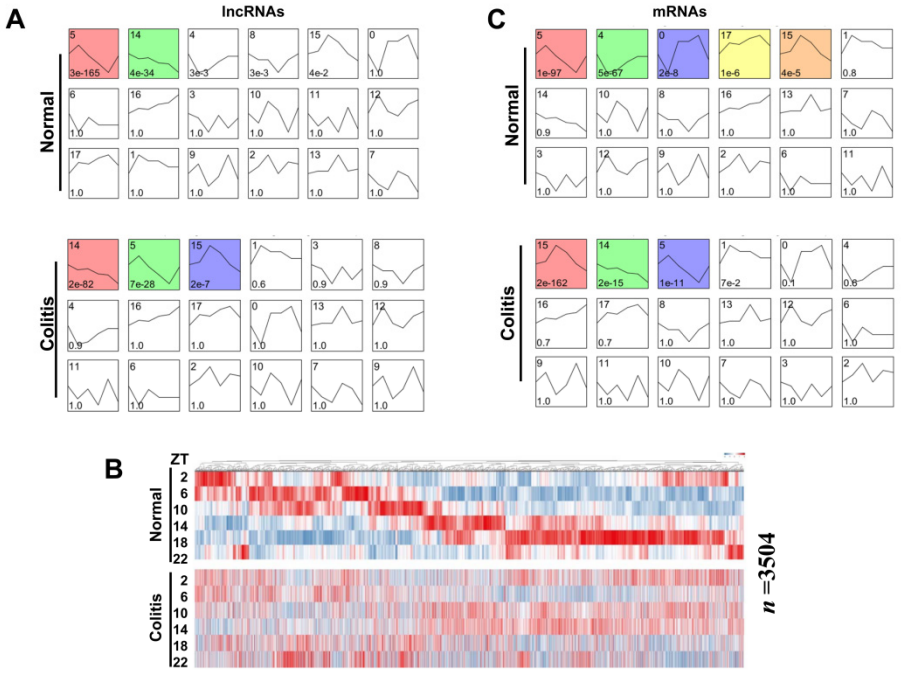


Fig S1. Disrupted rhythmicity in cycling lncRNAs/mRNAs in colitis mice. (A) Clustering analysis of cycling lncRNAs in colons of colitis and normal mice. **(B)** Heatmap for cycling mRNAs in colons of colitis and normal mice at 6 circadian time points. Red indicates high expression and blue indicates low expression of mRNAs as shown in the scale bar. **(C)** Clustering analysis of cycling mRNAs in colons of colitis and normal mice.

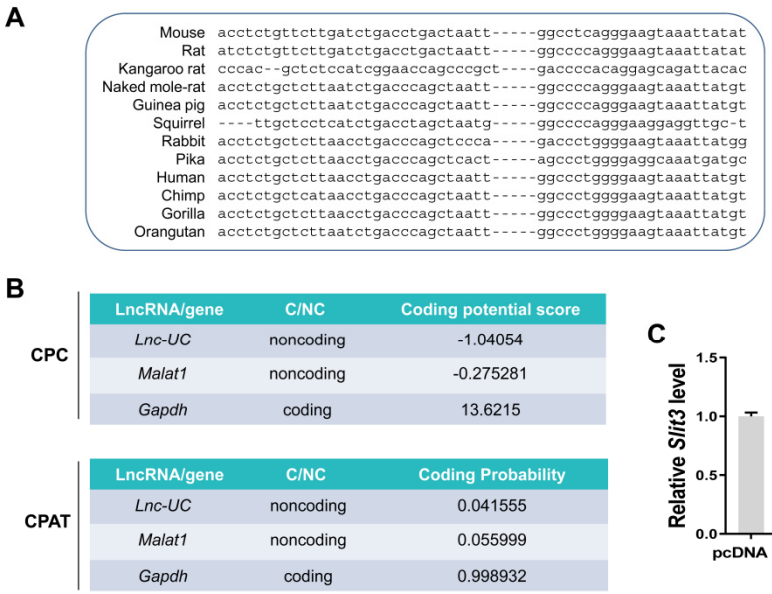


Fig S2. *Lnc-UC* characterization. (A) Sequence alignment of *Lnc-UC* in various species. **(B)** Predictions of protein-coding potential of RNAs based on coding potential calculator (CPC) and coding potential assessing tool (CPAT) software. **(C)** Effect of *Lnc-UC* overexpression on neighboring gene *Slit3* in BMDMs. Data are mean ± SD (n = 5).

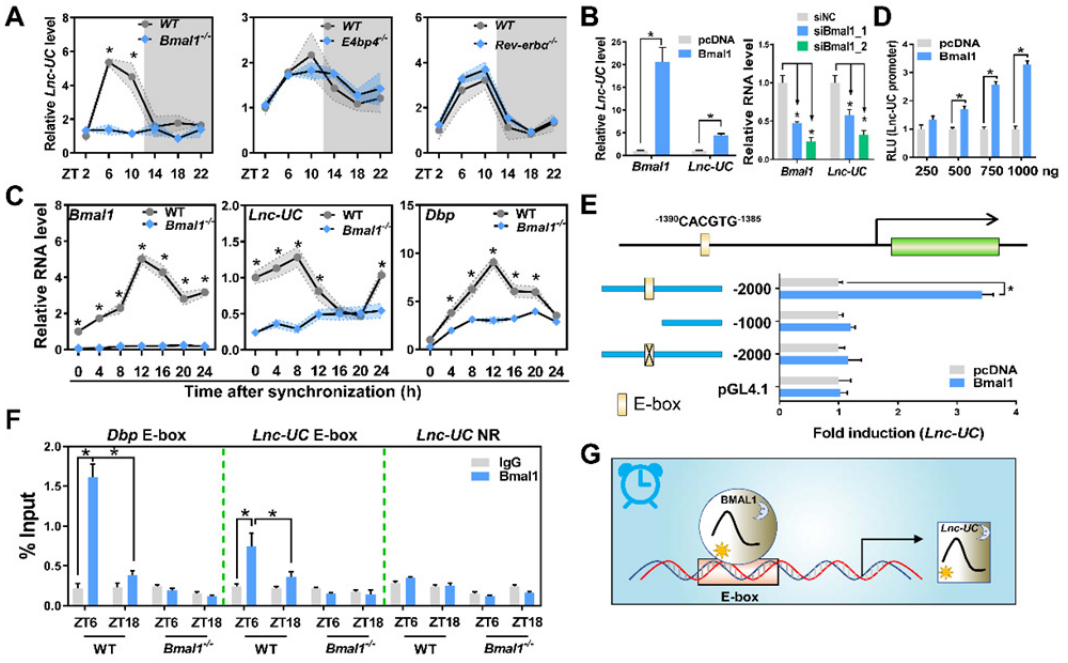


Fig S3. Circadian clock gene *Bmal1* regulates rhythmic expression of *Lnc-UC*. (A) Colonic *Lnc-UC* expression in *Bmal1*$^{-/-}$, *E4bp4*$^{-/-}$, *Rev-erbα*$^{-/-}$ and wild-type mice at 6 circadian time points. Data are mean ± SD (n = 5). **(B)** Effects of Bmal1 overexpression (left panel) and knockdown (right panel) on *Lnc-UC* in BMDMs. Data are mean ± SD (n = 5). **(C)** *Lnc-UC* levels in serum-shocked (synchronized) BMDMs derived from *Bmal1*$^{-/-}$ and wild-type mice. Data are mean ± SD (n = 5). **(D)** Effects of Bmal1 on *Lnc-UC* promoter activity. Data are mean ± SD (n = 6). **(E)** Effects of Bmal1 on different versions of *Lnc-UC* promoters. Data are mean ± SD (n = 6). **(F)** ChIP assays showing enrichment of Bmal1 protein to the E-box sites in *Dbp* and *Lnc-UC* promoters. Data are mean ± SD (n = 5). NR, non-specific region. **(G)** Schematic diagram showing Bmal1 regulation of *Lnc-UC* via the E-box element. In panels A and C, *p < 0.05 at individual time points as determined by two-way ANOVA followed by Bonferroni post hoc test; in panels B (left), D and E, *p < 0.05 as determined by Student's t test; in panel B (right), *p < 0.05 as determined by one-way ANOVA followed by Bonferroni post hoc test; in panel F, *p < 0.05 as determined by two-way ANOVA followed by Bonferroni post hoc test.

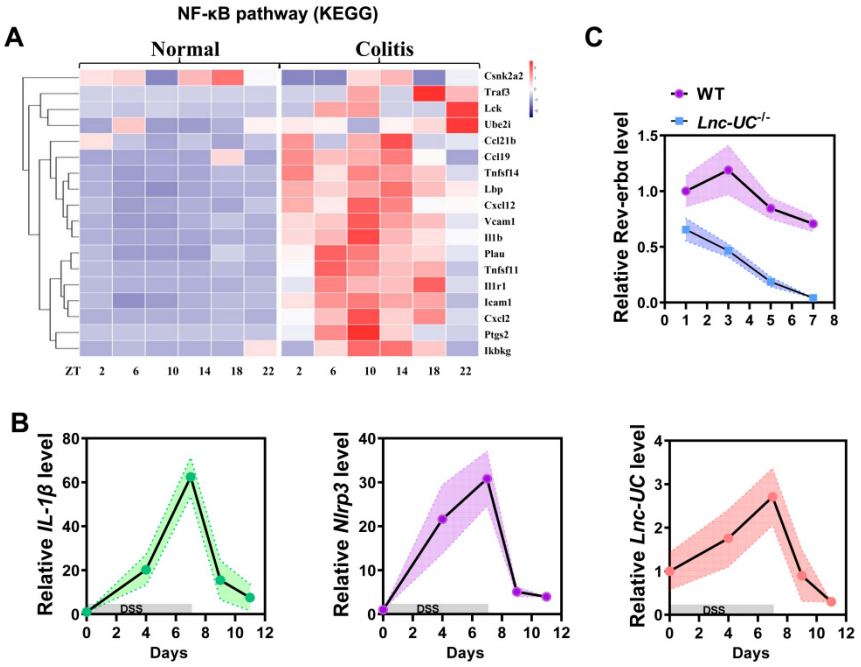


Fig S4. Gene expression changes in colitis model. (A) Heatmap for genes involved in NF-κB signaling pathway in colons of colitis and normal mice at 6 circadian time points. Red indicates high expression and blue indicates low expression as shown in the scale bar. **(B)** *Nlrp3*, *IL-1β,* and *Lnc-UC* levels in colons of wild-type mice during and after DSS treatment. **(C)** Rev-erbα levels in colons of *Lnc-UC*$^{-/-}$ mice and wild-type (WT) mice during DSS treatment. Data are mean ± SD (n = 5).

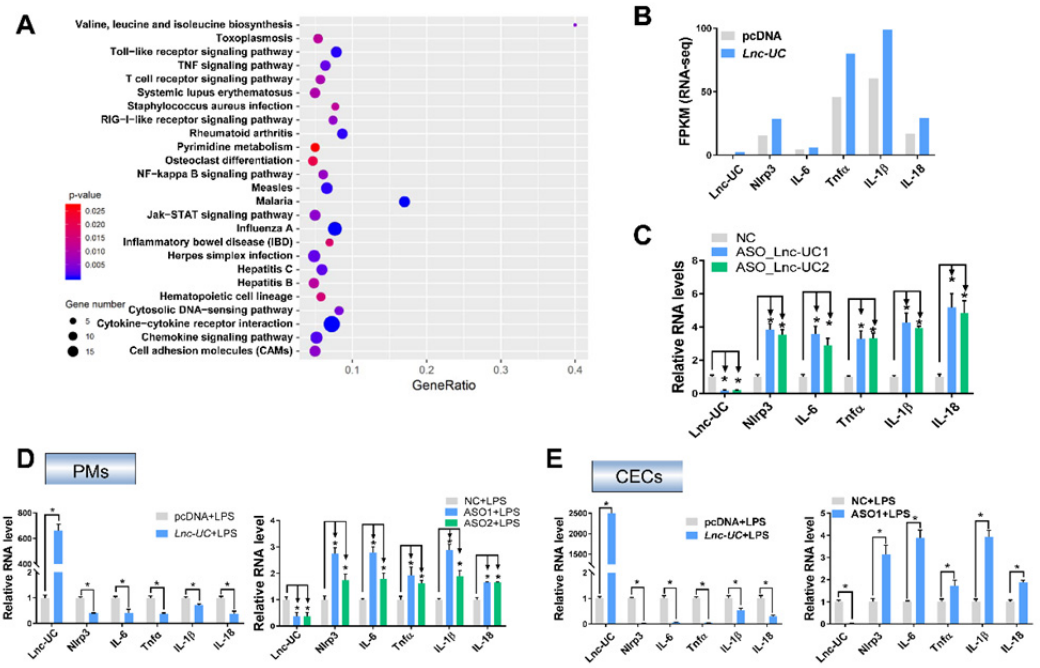


Fig S5. *Lnc-UC* acts as an inflammatory regulator in BMDMs, PMs and CECs. (A) KEGG analysis of *Lnc-UC*-induced differentially expressed genes (*n* = 582) in BMDMs. **(B)** RNA-seq FPKM values of *Lnc-UC* and selected inflammatory factors. **(C)** *Lnc-UC* knockdown by ASO-Lnc-UC1 (ASO1) or ASO-Lnc-UC2 (ASO2) up-regulates NF-κB target genes in BMDMs. **(D)** *Lnc-UC* overexpression down-regulates (left panel), whereas *Lnc-UC* knockdown by ASO1 or ASO2 up-regulates (right panel), NF-κB target genes in LPS-treated PMs. **(E)** *Lnc-UC* overexpression down-regulates (left panel), whereas *Lnc-UC* knockdown by ASO1 or ASO2 up-regulates (right panel), NF-κB target genes in LPS-treated CECs. Data are mean ± SD (n = 5). In panels C and D (right), *p < 0.05 as determined by one-way ANOVA followed by Bonferroni post hoc test; in panels D (left) and E, *p < 0.05 as determined by Student's t test.

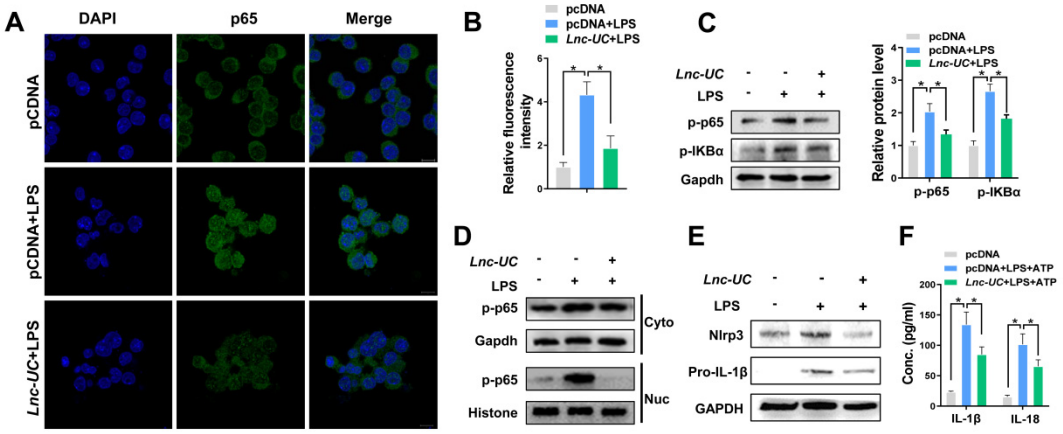


Fig S6. Repressive role of *Lnc-UC* in regulation of NF-κB signaling and NLRP3 inflammasome. **(A)** Immunofluorescence images showing *Lnc-UC* suppresses nuclear translocation of p65 in BMDMs. Scale bar = 10 μm. **(B)** Quantification data on panel **A**. Data are mean ± SD (n = 6). **(C)** *Lnc-UC* reduces protein levels of phosphorylated (p)-p65 and phosphorylated (p)-IKBα in BMDMs. **(D)** *Lnc-UC* reduces both cytoplasmic and nuclear p-p65 in BMDMs. **(E)** *Lnc-UC* reduces protein levels of Nlrp3 and pro-IL-1β in BMDMs. **(F)** *Lnc-UC* reduces IL-1β and IL-18 in BMDM incubation medium. Data are mean ± SD (n = 5). NC, negative control. Cyto, cytoplasmic. Nuc, nuclear. In panels B, C and F, *p < 0.05 as determined by one-way ANOVA followed by Bonferroni post hoc test.

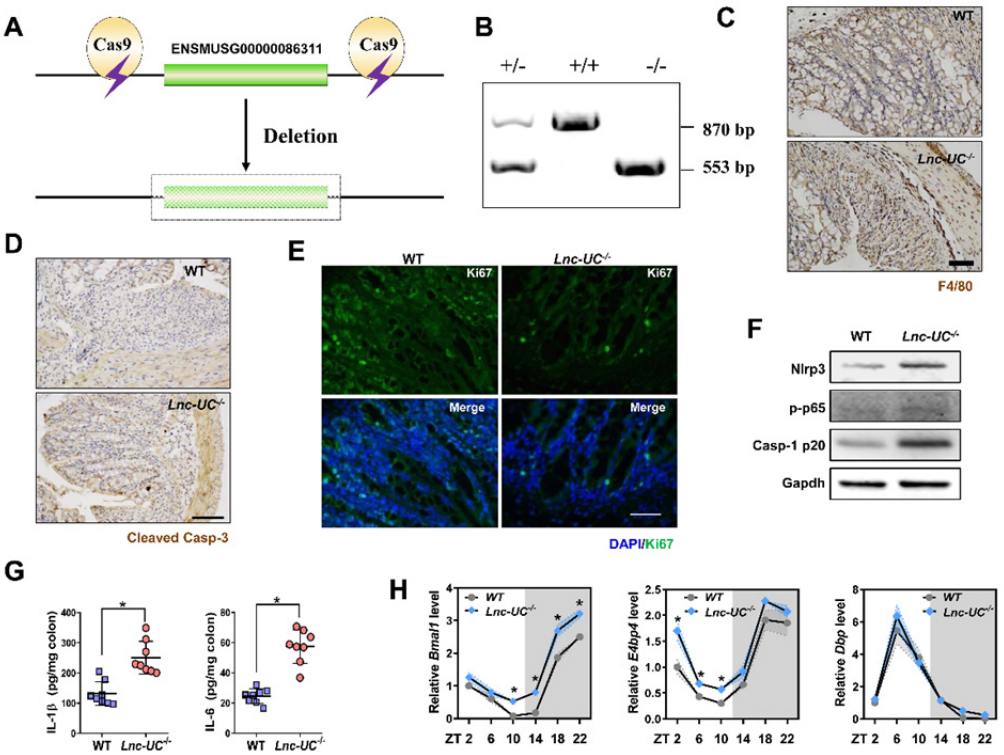


Fig S7. *Lnc-UC* ablation sensitizes mice to experimental colitis and leads to disrupted expressions of circadian clock genes. **(A)** Schematic of CRISPR/Cas9-mediated genome-editing procedure for *Lnc-UC* knockout mice. **(B)** Mouse genotyping image generated from agarose gel electrophoresis. **(C)** Immunohistochemistry for F4/80 in colons of *Lnc-UC*$^{-/-}$ and WT mice. **(D)** Immunohistochemistry for cleaved caspase-3 (casp-3) in colons of *Lnc-UC*$^{-/-}$ and WT mice. **(E)** Immunofluorescence for Ki67 in colons of *Lnc-UC*$^{-/-}$ and WT mice. **(F)** Protein expressions of Nlrp3, phosphorylated (p)-p65, and mature caspase-1 (caspase-1 p20) in colons of *Lnc-UC*$^{-/-}$ and WT mice. Caspase-1; Casp-1. **(G)** Expressions of inflammatory cytokines in colons of *Lnc-UC*$^{-/-}$ and WT mice. Data are mean ± SD (n = 8). **(H)** Expression of clock genes in the colons of *Lnc-UC*$^{-/-}$ and wild-type (WT) mice at 6 circadian time points. Data are mean ± SD (n = 5). In panel G, *p < 0.05 as determined by Student's t test; in panel H, *p < 0.05 at individual time points as determined by two-way ANOVA followed by Bonferroni post hoc test.

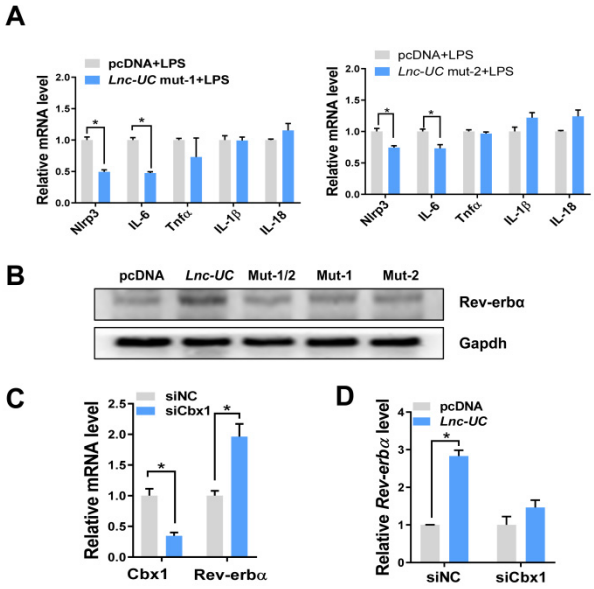
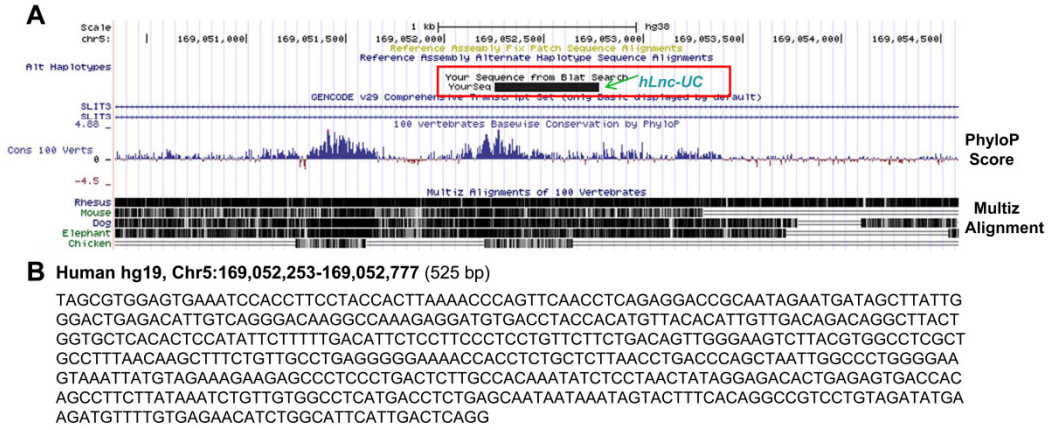


Fig S8. *Lnc-UC* regulates *Rev-erbα* transcription through attenuation of Cbx1-mediated tri-methylation of H3K9. (A) Effects of single-site mutated *Lnc-UC* on NF-κB target genes in LPS-treated BMDMs. **(B)** Effects of mutated versions of *Lnc-UC* on Rev-erbα protein in LPS-treated BMDMs. *Lnc-UC* mut-1 [*Lnc-UC* with mutated site (1-74 nt)], *Lnc-UC* mut-2 [(*Lnc-UC* with mutated site (326-432 nt)], *Lnc-UC* mut-1/2 [*Lnc-UC* with mutated sites of (1-74 nt) and (326-432 nt)]. **(C)** Cbx1 knockdown by siRNA up-regulates *Rev-erbα* in BMDMs. **(D)** Effects of *Lnc-UC* on *Rev-erbα* expression in BMDMs transfected with siCbx1 or siNC (siRNA for negative control). Data are mean ± SD (n = 5). In panels A, C and D, *p < 0.05 as determined by Student's t test.



B **Human hg19, Chr5:169,052,253-169,052,777** (525 bp)

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AGATGTTTTGTGAGAACATCTGGCATTCATTGACTCAGG

Fig S9. *Lnc-UC* is conserved in humans. (A) UCSC genome browser showing location of *hLnc-UC* in human genome. **(B)** Full-length cDNA sequence of human *Lnc-UC* (525 bp).

Name	Forward (5'-3' sequence)	Reverse (5'-3' sequence)
mLnc-UC	GAGGGGGTGTAAGGGGGTAA	ACTTAGGGATGAACGCAGCA
mNeat1	TTGGGACAGTGGACGTGTGG	TCAAGTGCCAGCAGACAGCA
mE4bp4	CTTTCAGGACTACCAGACATCCAA	GATGCAACTTCCGGCTACCA
mHmbs	CCGAGCCAAGGACCAGGATA	CTCCTTCCAGGTGCCTCAGA
mBmal1	CTCCAGGAGGCAAGAAGATTC	ATAGTCCAGTGGAAGGAATG
mDbp	ACATCTAGGGACACACCCAGTC	AAGTCTCATGGCCTGGAATG
mRev-erbα	TTTTTCGCCGGAGCATCCAA	ATCTCGGCAAGCATCCGTTG
mIL-1β	AATGCCACCTTTTGACAGTGATG	AGCTTCTCCACAGCCACAAT
mNlrp3	ATTACCCGCCCGAGAAAGG	TCGCAGCAAAGATCCACACAG
mIL-6	ATCCAGTTGCCTTCTTGGGACTGA	TAAGCCTCCGACTTGTGAAGTGGT
mTnfα	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
mIL-18	TCAAAGTGCCAGTGAACCCC	GGTCACAGCCAGTCCTCTTAC
mSlit3	CCACCAAGTGTACCTGCTCC	GCCAGCGAAGTCCATTTTGG
mCbx1	TGATCGGCGAGTTGTCAAGG	TCCCAAGTGTTGTCCTCATCTG
hLnc-UC	CCTGAGTCAATGAATGCCAGATG	TCTGTTGTGGCCTCATGACC
hREV-ERBα	GACATGACGACCCTGGACTC	GCTGCCATTGGAGTTGTCAC
hBMAL1	ACTTCCCCTCTACCTGCTCA	ATCCAGCCCCATCTTTGTGG
hNLRP3	CCGCTATCTTTCTATTAACTGACCA	TCAACCAGCTACAAAAAGCATGG
hIL-1β	GCTCGCCAGTGAAATGATGG	GGTGGTCGGAGATTCGTAGC
hIL-6	ACCCCCAGGAGAAGATTCCA	GATGCCGTCGAGGATGTACC
hTNFα	TGGGATCATTGCCCTGTGAG	GGTGTCTGAAGGAGGGGGTA
hIL-18	GCTGAAGATGATGAAAACCTGGA	GAGGCCGATTTCCTTGGTCA
hGAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT

m: mouse h:human

Table S1. Sequences of primers for qPCR analysis