We uncover a cycling and NF-κB–driven IncRNA (named Lnc-UC) that epigenetically modifies transcription of circadian clock gene Rev-erbas, thereby linking circadian clock to colitis. Cycling expression of Lnc-UC is generated by the central clock protein Bmal1 via an E-box element. NF-κB activation in experimental colitis transcriptionally drives Lnc-UC through direct binding to two κB sites. Lnc-UC ablation disrupts colonic expressions of clock genes in mice; particularly, Rev-erbas is down-regulated and its diurnal rhythm is blunted. Consistently, Lnc-UC promotes expression of Rev-erbas (a known dual NF-κB/Nlrp3 repressor) to inactivate NF-κB signaling and Nlrp3 inflammasome in macrophages. Furthermore, Lnc-UC ablation sensitizes mice to experimental colitis and abolishes the diurnal rhythmicity in disease severity. Mechanistically, Lnc-UC physically interacts with Cbx1 protein to reduce its diurnal rhythmicity in symptom severity. However, the role of lncRNAs in the cross-talk of colitis and inflammatory diseases (e.g., IBDs) show disturbed circadian rhythms, highlighting potential mutual interactions between circadian and immune systems (14–16). This bidirectional regulation may be involved in the pathogenesis of IBDs (10). Although how circadian clock affects inflammations is becoming clearer, the molecular mechanisms by which inflammations regulate circadian clock remain elusive.

LncRNAs (long noncoding RNAs), defined as having ≥200 nucleotides, are a class of noncoding RNAs (ncRNAs). LncRNAs regulate gene transcription by interacting physically with DNA, other RNA, and protein via nucleotide base pairing or formation of structural domains generated by RNA folding (17). Several lncRNAs (e.g., lincRNA-EPS, Mirt2, and Arid2-IR) have been implicated in immune responses, playing a role in development of inflammatory diseases such as endotoxemia and renal inflammation (18–20). In addition, lncRNAs have potential impact on circadian biology, and, in turn, they may be under the control of circadian clock (21, 22). However, the role of lncRNAs in the cross-talk of colitis with circadian clock is largely unknown. In this study, we uncover a cycling and colitis-related lncRNA (named Lnc-UC) in mice and in humans. Lnc-UC, driven by NF-κB signaling in colitis, promotes Rev-erbas transcription to alter circadian gene expression. Mechanistically, Lnc-UC interacts with Cbx1 to reduce H3K9me3 at Rev-erbas promoter and to induce Rev-erbas transcription and expression. We therefore propose that Lnc-UC functions as a Rev-erbas modulator, linking circadian clock to colitis.

RESULTS Lnc-UC is a cycling and colitis-related IncRNA
Diurnal expression of IncRNAs was assessed by sampling mouse colon every 4-hour over a 24-hour light-dark cycle. RNA sequencing...
Circadian clock gene Bmal1 regulates rhythmic expression of Lnc-UC

Circadian gene expression is generally produced by a transcriptional mechanism in which core clock genes act on three cis-elements (E-box, D-box, and RevRE or RORE) in target gene promoters (23). We thus first investigated the roles of these three cis-elements in generation of rhythmic Lnc-UC using Bmal1^−/−, E4bp4^−/−, and Rev-erbα^−/− mice. Bmal1, E4bp4, and Rev-erbα are respectively representative cis-acting proteins for E-box, D-box, and RevRE. Lnc-UC expression was down-regulated, and its rhythm was blunted in Bmal1^−/− mice (fig. S3A). By contrast, Lnc-UC remained unaffected in E4bp4^−/− or Rev-erbα^−/− mice (fig. S3A). Supporting an activation effect, Bmal1 overexpression in BMDMs led to increased Lnc-UC and Bmal1 knockdown suppressed Lnc-UC expression (fig. S3B). Consistently, Bmal1 ablation down-regulated Lnc-UC and Dbp (a known Bmal1 target gene) and dampened their rhythms in serum-shocked (synchronized) BMDMs (fig. S3C). Serum shock is a well-recognized method that can synchronize circadian cycles and induce circadian gene expression in cultured cells. Furthermore, Bmal1 dose dependently induced the activity of the luciferase reporter driven by a 2.1-kb Lnc-UC promoter (−2.0/+0.1 kb) (fig. S3D). A canonical E-box (−1390/−1385 bp) in Lnc-UC promoter was identified to be critical for Bmal1 action, by testing Bmal1 effects on different promoter regions with E-box or without E-box site (fig. S3E). Chromatin immunoprecipitation (ChIP) assays showed that colonic Bmal1 protein was recruited to the E-box of Lnc-UC in wild-type (WT) mice in a circadian time-dependent manner (fig. S3F). Together, Bmal1 overexpression in BMDMs led to increased Lnc-UC and Bmal1 knockdown suppressed Lnc-UC expression (fig. S3F). However, Bmal1 recruitment was reduced and its time dependency was abolished in Bmal1^−/− mice (fig. S3F).

Fig. 1. Lnc-UC is a cycling and colitis-related IncRNA. (A) Heat map for cycling IncRNAs in colons of colitis and normal mice at six circadian time points. Red indicates high expression, and blue indicates low expression of IncRNAs as shown in the scale bar. (B) Venn diagram showing numbers of cycling, DE (differentially expressed), and CDE (cycling and differentially expressed) IncRNAs. (C) Heat map for CDE IncRNAs in colons of normal mice at six circadian time points. (D) Quantitative polymerase chain reaction measurements of colonic Lnc-UC at six circadian time points. Data are mean ± SD (n = 3). *P < 0.05 at individual time points as determined by two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. (E) CISH analysis of Lnc-UC expression in colon of WT mice. Scale bar, 50 μm. (F) Lnc-UC levels in PMs and various tissues of WT mice. Data are mean ± SD (n = 5). (G) Cytoplasmic and nuclear levels of Lnc-UC in colon and BMDMs derived from WT mice. Data are mean ± SD (n = 5). (H) FISH analysis of subcellular Lnc-UC (green) location in BMDMs. Scale bar, 10 μm.
transcriptionally regulates Lnc-UC and generates its diurnal rhythmicity (fig. S3G).

**NF-κB drives Lnc-UC expression in colitis mice**

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that DSS-induced colitis was associated with activation of NF-κB and NF-κB–related signaling pathways [e.g., tumor necrosis factor (TNF), Rap1, and phosphatidylinositol 3-kinase (PI3K)–Akt signaling] (Fig. 2A and fig. S4A). This was supported by elevated expressions of NF-κB target genes such as Ptgs2, IL-1β, and Cxcl2 (Fig. 2B). Because Lnc-UC was induced in colitis mice (Fig. 1, C and D), it was of interest to test whether NF-κB played a role in regulation of Lnc-UC. Lipopolysaccharide (LPS; an NF-κB activator) dose and time dependently increased Lnc-UC expression in BMDMs (Fig. 2, C and D). Similar induction effects of LPS on Lnc-UC were observed in serum-shocked BMDMs (Fig. 2E) and in PMs (Fig. 2F). Fluorescence in situ hybridization (FISH) assays confirmed an increased nuclear Lnc-UC expression in LPS-stimulated BMDMs (Fig. 2G). Also, both recombinant Tnfα protein (an NF-κB activator) and p65 (an NF-κB subunit) overexpression plasmid induced Lnc-UC expression in BMDMs (Fig. 2I). Furthermore, Lnc-UC expression was correlated with NF-κB activity (reflected by IL-1β and Nlrp3 levels) during colitis development (fig. S4B). On the basis of luciferase reporter analyses, we identified two κB sites (i.e., −137/-127 bp and −108/-98 bp) in Lnc-UC promoter for transcriptional action of p65 (Fig. 2J). Recruitment of p65 to these two κB sites was confirmed by ChIP assays (Fig. 2K). p65 recruitment was enhanced by recombinant Tnfα protein (Fig. 2K). Collectively, NF-κB drives expression of Lnc-UC via a transcriptional activation mechanism.
Lnc-UC acts as an inflammatory regulator in vitro

Lnc-UC overexpressed and control BMDMs were subjected to RNA-seq analyses. Lnc-UC induced notable expression changes in genes involved in immune responses (NF-κB and related signaling pathways) (Fig. 3, A and B, and fig. S5A). In line with RNA-seq data, Lnc-UC was a negative regulator of NF-κB target genes (including Nlrp3, IL-6, Tnfa, IL-1β, and IL-18) in BMDMs and in PMs based on gain- and loss-of-function experiments (Fig. 3, C and D, and fig. S5, B to D). Lnc-UC knockdown–induced changes in expressions of NF-κB target genes can be restored by Lnc-UC overexpression, confirming a specific effect of Lnc-UC on NF-κB (Fig. 3E).

Supporting this, Lnc-UC inhibited the transcriptional activity of NF-κB according to luciferase reporter assay and electrophoretic mobility shift assay (fig. S6, A to D). Moreover, Lnc-UC inactivated Nlrp3 inflammasome (whose activity depends on NF-κB signaling) as evidenced by reduced maturation of and release of proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 (fig. S6, E and F). Overall, these data indicated a repressive role of Lnc-UC in regulation of NF-κB signaling and inflammations.

Lnc-UC ablation sensitizes mice to experimental colitis

We established Lnc-UC–deficient (Lnc-UC−/−) mice by deleting ENSMUSG00000086311 sequence (fig. S7, A and B). Both Lnc-UC−/− and WT mice were subjected to colitis inducement with DSS. Lnc-UC−/− mice showed an increased sensitivity to colitis development, as evidenced by more extensive weight loss, higher disease activity index (DAI), shorter colons, and higher myeloperoxidase (MPO) activity (Fig. 3, H to K). More severe colitis in Lnc-UC−/− mice was confirmed.
by histological examinations (Fig. 3, L and M). Lnc-UC deficiency was also associated with an increased number of macrophages, aggravated cell apoptosis, and reduced cell proliferation (fig. S7, C to E). In addition, colonic protein levels of phosphorylated p65 and mature caspase-1 as well as proinflammatory cytokines (i.e., IL-1β and IL-6) were higher in Lnc-UC−/− mice than in WT mice, indicating exacerbated activation of NF-κB signaling and Nlrp3 inflammasome in the genetically modified mice (fig. S7, F and G). Overall, these data supported that Lnc-UC regulated the development of experimental colitis in mice. Because Lnc-UC is diurnally rhythmic, it was of interest to test circadian time-varying severity in colitis. Mice were treated with oxazolone to induce colitis at six different circadian points. Colitis severity (reflected by the mortality rate or survival time) displayed a significant diurnal rhythm (most severe at ZT22 and least severe at ZT6) (Fig. 3N and O). However, the time difference in disease severity was lost in Lnc-UC−/− mice (Fig. 3P), supporting circadian regulation of colitis by Lnc-UC.

**Lnc-UC regulates circadian clock gene Rev-erbα and inflammations**

Lnc-UC ablation led to disrupted expressions of circadian clock genes (Fig. 4A and fig. S7H). In particular, Rev-erbα was markedly down-regulated (Fig. 4A and B). Rev-erbα has been established as an integrator of circadian clock and colonic inflammation via inactivation of NF-κB signaling and Nlrp3 inflammasome (19, 20, 22). We thus investigated a potential role of Rev-erbα in Lnc-UC regulation of inflammation. As expected, overexpression of Rev-erbα in BMDMs induced expression changes in genes involved in immune responses (NF-κB and related signaling pathways) (Fig. 4C). Rev-erbα ablation led to increased levels of NF-κB–related inflammatory factors, confirming Rev-erbα as a negative regulator of inflammatory responses (Fig. 4D). We observed a high overlap (~50%) between Lnc-UC– and Rev-erbα–associated enriched pathways (including inflammatory responses based on KEGG analysis) in BMDMs (Fig. 4E). In addition, Lnc-UC reduced the mRNA level of Nlrp3 (a direct Rev-erbα target gene), and this inhibitory effect was further demonstrated by RIP assays showing no interaction between Rev-erbα protein and Lnc-UC in WT mice (Fig. 4G). The KEGG pathways of Lnc-UC and Rev-erbα-associated pathways were then analyzed by using the gene sets with high overlap. As expected, the expression of Rev-erbα in WT mice was increased by Lnc-UC knockdown by ASO1 and ASO2 in PMs and BMDMs (Fig. 4J).

**Fig. 4. Lnc-UC regulates circadian clock gene Rev-erbα and inflammations.** (A) Expression of Lnc-UC and Rev-erbα mRNA in the colons of Lnc-UC−/− and WT mice at six circadian time points. (B) Protein expression of Rev-erbα in the colons of Lnc-UC−/− and WT mice at six circadian time points. (C) KEGG analysis of Rev-erbα–induced differentially expressed genes in BMDMs. (D) Rev-erbα ablation up-regulates NF-κB target genes in BMDMs. (E) Venn diagram showing an extensive overlap of Lnc-UC– and Rev-erbα–associated pathways. (F) mRNA levels of Nlrp3 in BMDMs derived from Rev-erbα−/− and WT mice. (G) RIP assays showing no interaction between Rev-erbα protein and Lnc-UC in WT mice. (H) Effects of Lnc-UC overexpression on mRNA levels of Rev-erbα and Bmal1 in PMs and BMDMs. (I) Effects of Lnc-UC overexpression on protein levels of Rev-erbα and Bmal1 in PMs and BMDMs. (J) Effects of Lnc-UC knockdown by ASO1 and ASO2 on mRNA levels of Rev-erbα and Bmal1 in PMs and BMDMs. In (H) to (J), cells were transfected with overexpression plasmid or ASO for 24 hours before harvest. Data are mean ± SD (n = 5). In (A), *P < 0.05 as determined by one-way ANOVA followed by Bonferroni post hoc test; in (D) and (H), *P < 0.05 as determined by Student’s t test; in (F) and (J), *P < 0.05 as determined by one-way ANOVA followed by Bonferroni post hoc test.
was lost in Rev-erba−/− mice (Fig. 4F). All these data indicated a mediating role for Rev-erba in Lnc-UC regulation of inflammation.

We next explored the mechanisms by which Lnc-UC regulated Rev-erba. Because there were no interactions between Lnc-UC and Rev-erba according to RNA immunoprecipitation (RIP) assay (Fig. 4G), we tested whether Lnc-UC regulated the expression of Rev-erba. Overexpression of Lnc-UC led to an increase in cellular Rev-erba expression and therefore to a decrease in Bmal1 (a Rev-erba target gene) expression (Fig. 4, H and I). Consistently, Lnc-UC knockdown resulted in reduced Rev-erba expression and in increased Bmal1 expression (Fig. 4J). The data suggested that the anti-inflammatory effect of Lnc-UC was attained probably by regulating Rev-erba expression.

**Lnc-UC epigenetically regulates Rev-erba by interacting with Cbx1**

Lnc-UC contains four stem loop structures [i.e., 1–74, 123–193, 213–267, and 326–432 nucleotides (nt)] that may bind to target proteins to affect their functions (Fig. 5A). RNA pull-down assays followed by silver staining, mass spectrometric analyses, and Western blotting identified Cbx1 (about 25 kDa, also known as HP1β) as a nuclear protein interacting with Lnc-UC (Fig. 5B and C). A direct interaction of Lnc-UC with Cbx1 was further confirmed by RIP assays (Fig. 5D). Moreover, Lnc-UC and Cbx1 colocalized in the nuclei of BMDMs (Fig. 5E). Truncation analyses suggested that the fragments of 1–74 nt and 326–432 nt were responsible for Cbx1 binding (Fig. 5C). Supporting this, Lnc-UC carrying mutations of both 1–74 nt and 326–432 nt fragments failed to induce Rev-erba expression or to suppress inflammatory responses (Fig. 5F and fig. S8, A and B).

Functionally, Cbx1 binds to sites of histone H3 lysine 9 (H3K9) methylation and recruits histone methyltransferases such as Suv39h1 (a histone-lysine N-methyltransferase), playing an important role in gene silencing and formation of heterochromatin (24–26). Lnc-UC knockdown enhanced the trimethylation of H3K9 in BMDMs, raising a possibility for epigenetic regulation of Rev-erba by the Lnc-UC.
lncRNA (Fig. 5G). ChIP assays showed significant enrichments of Cbx1, Suv39h1, and H3K9me3 to Rev-erba promoter (Fig. 5H). Enrichments of these three proteins were enhanced when Lnc-UC was knocked down (Fig. 5H). In addition, knockdown of Cbx1 reduced enrichment of H3K9me3 to Rev-erba promoter, promoting Rev-erba transcription and expression (Fig. 5I and fig. S8C). Supporting this, knockdown of Cbx1 suppressed the expression of inflammatory factors in LPS-stimulated BMDMs (Fig. 5J). Lnc-UC failed to increase Rev-erba expression in Cbx1-silenced BMDMs (fig. S8D). Collectively, Lnc-UC regulates Rev-erba transcription through attenuation of Cbx1-mediated trimethylation of H3K9 (Fig. 5K). To be specific, Lnc-UC may act as a decoy and titrate away Cbx1 and its partners such as Suv39h1 from the Rev-erba promoter (Fig. 5K). Thereby, trimethylation of H3K9 is diminished and Rev-erba transcription is activated (Fig. 5K).

**Human Lnc-UC (hLnc-UC) is a cycling and anti-inflammatory lncRNA**

It is of interest to test whether function of Lnc-UC in mice can be translated to humans due to high species conservation (fig. S2A). Full length of human Lnc-UC (hLnc-UC) was identified by performing rapid amplification of complementary DNA (cDNA) ends (RACE) and mapped to the region of Chr5:169,052,253-169,052,777 (Fig. 6A and fig. S9, A and B). CISH assays confirmed the presence of hLnc-UC in human colons (Fig. 6B). Patients with colitis showed increased hLnc-UC as well as NLRP3 and IL-1β in colonic mucosae as compared to healthy individuals (Fig. 6C). This lncRNA was rhythmically expressed in serum-shocked THP-1 cells with a pattern similar to BMAL1 (Fig. 6D). Moreover, overexpression of hLnc-UC in THP-1 cells resulted in increased REV-ERBa expression and in reduced levels of REV-ERBa–dependent inflammatory factors (Fig. 6, E to G). Similar observations were noted in monocyte-derived macrophages (MDMs) (Fig. 6, H and I). Together, these data suggest that colitis-related hLnc-UC may promote REV-ERBa expression to restrain inflammations as its counterpart does in mice.

**DISCUSSION**

We have uncovered an NF-κB–driven lncRNA (named Lnc-UC) that epigenetically modifies transcription of circadian clock genes.
in colonic Rev-erbα expression in WT mice during the induction of colitis (fig. S4C). This seems to occur in concert with an elevation in Lnc-UC, a Rev-erbα activator, in colitis development. Colitis-associated down-regulation of Rev-erbα was much more extensive in Lnc-UC+/- mice (fig. S4C), supporting a role for Lnc-UC in resisting down-regulation of Rev-erbα by colitis (i.e., a Rev-erbα-promoting effect). It is therefore speculated that a down-regulation mechanism should be involved in the colitis-caused reduction of Rev-erbα; however, it was unaddressed in the current study. This down-regulation mechanism, probably involving NF-κB–mediated transcriptional repression (41, 42), overwhelms the activation effect of Lnc-UC, resulting in an “apparent” reduction in Rev-erbα expression.

In summary, NF-κB–driven Lnc-UC functions as an epigenetic regulator of Rev-erbα, thereby orchestrating circadian clock and colitis. Lnc-UC might be targeted to intervene in communications between inflammation and circadian clock to manage colitis.

**MATERIALS AND METHODS**

**Materials**

Human and murine macrophage colony-stimulating factor (CSFs) were purchased from PeproTech (Rocky Hill, NJ). LPS, oxazolone, phorbol 12-myristate 13-acetate (PMA), adenosine triphosphate (ATP), and collagenase I were purchased from Sigma-Aldrich (St. Louis, MO). JSH-23 was purchased from Selleck (Houston, TX). DSS (molecular weight of 36 to 50 kDa) was obtained from MP Biomedicals (Irvine, CA). Murine Tnfα, BAY 11-7082, and NF-κB-Luc were purchased from Beyotime (Shanghai, China). Cytoplasmic/nuclear RNA purification kit was purchased from Norgen Biotek (Burlington, CA). FISH kit was purchased from GenePharma (Shanghai, China). RNAiso Plus reagent and PrimeScript RT Master Mix were purchased from Takara (Shiga, Japan). ChamQ Universal SYBR qPCR Master Mix was purchased from Vazyme (Nanjing, China). Dual-Luciferase Reporter System was purchased from Promega (Madison, WI). Pierce magnetic RNA-protein pull-down kit was purchased from Thermo Fisher Scientific (San Jose, CA). Murine IL-1β and IL-18 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Neobioscience (Shenzhen, China). JetPrime transfection kit was purchased from POLYPLUS Transfection (Illkirch, France). ChIP kit was purchased from Cell Signaling Technology (Beverly, MA).

Antibodies: Anti-Nlrp3 and anti–IL-18 were purchased from Abcam (Cambridge, UK). Anti-p65, anti–Rev-erbα, anti-p–p65, anti–p–IKBα, and anti-rabbit immunoglobulin G (IgG) were obtained from Cell Signaling Technology (Danvers, MA). Anti-mouse IgG was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Histone H3 was purchased from Proteintech (Wuhan, China).
Mice
WT C57BL/6 mice were obtained from HFK Biotechnology (Beijing, China). Ekbpa−/− (a C57BL/6 background) was obtained from M. Kubo at RIKEN Institute in Japan (43). Bmal1−/− and Rev-erba−/− mice (a C57BL/6 background) have been established and validated in our laboratory (13, 44). Lnc-UC−/− mice were generated by deleting ENSMUSG00000086311 gene with the aid of Cyagen Biosciences Inc. (Guangzhou, China). All mice were maintained under a 12-hour light/12-hour dark cycle [light on 7:00 a.m. (= ZT0) to 7:00 p.m. (= ZT12)] with ad libitum access to food and water. Mice with age of 6 to 12 weeks were used for experiments. All experiments were performed using protocols approved by the Institutional Animal Care and Use Committees of Jinan University.

Colitis models
DSS-induced colitis was established by feeding mice with 2.5% (w/v) DSS in drinking water for 7 days. Mice were sacrificed on day 7, and colons were collected for biochemical analyses (colonic IL-1β, IL-18, IL-6, Tnfr levels, and MPO activity). DAI was scored based on body weight loss, occult blood, and stool consistency as described previously (13). In addition, colon tissues were fixed in 4% paraformaldehyde and embedded in paraffin, followed by hematoxylin and eosin staining. Histological damage was scored based on goblet cell loss, mucosa thickening, inflammatory cells infiltration, submucosa cell infiltration, ulcers, and crypt abscesses (13). A score of 1 to 3 or 1 to 4 was given for each parameter with a maximal total score of 20.

Oxazolone-induced colitis was established with mice as previously described (45). In brief, a small area (approximately 2 × 2 cm) of dorsal skin was shaved, and 200 μl of 3% oxazolone was applied (presensitization). Seven days later, mice were challenged intrarectally with 180 μl of 2% oxazolone in 50% ethanol under light anesthesia. Animal survival was recorded in the following 24 hours.

Human specimens
Colonic specimens were obtained from the First Affiliated Hospital of Jinan University. Biopsies were obtained from inflamed areas of colonic tissues of patients with UC. Colonic mucosae were collected from patients with UC and healthy individuals. Research protocol was approved by the Medical Ethical Committee of the First Affiliated Hospital of Jinan University.

Isolation of macrophages
Primary PMs and BMDMs were isolated from mice as previously described (13). In brief, peritoneal fluid was collected and cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Two hours later, adherent cells (PMs) were obtained and used for further experiments. Tibias were separated from the femurs, and the bones were washed using 75% ethanol. Bone marrow cavities were rinsed with RPMI 1640 medium using a syringe. The rinsing solutions were collected and centrifuged at 1000 rpm for 5 min. The pellet cells (BMDMs) were collected and cultured in RPMI 1640 medium containing 10% FBS, 1% penicillin-streptomycin, and murine CSF (20 ng/ml). MDMs were prepared as previously described (32). Human peripheral blood mononuclear cells were first isolated from the blood of healthy donors by Ficoll density gradient centrifugation. After 7 days of culture in RPMI 1640 with 10% FBS, 1% penicillin-streptomycin, and human CSF (20 ng/ml), monocytes were differentiated into macrophages.

Isolation of CECs
Mouse colons were dissected and flushed with phosphate-buffered solution containing 1 mM dithiothreitol (DTT) to remove fecal contents. Colons were digested by incubation with a solution containing 0.1% collagenase I at 37°C for 30 min. CECs were recovered by centrifugation at 1000 rpm for 5 min and suspended in Dulbecco’s modified Eagle’s medium (DMEM)/F12 medium containing 10% FBS.

Serum shock
BMDMs, MDMs, and THP-1 cells were cultured in RPMI medium containing 10% FBS and 1% penicillin-streptomycin. On the next day, the culture medium was replaced with serum-free RPMI medium. Twelve hours later, 50% FBS was added for 2 hours and the medium was changed back to serum-free RPMI medium. Cells were harvested for RNA extraction at 0, 4, 8, 12, 16, 20, and 24 hours after serum shock. In particular, BMDMs were primed with LPS (500 ng/ml) for 3 hours before harvest.

Quantitative polymerase chain reaction
Total RNA from colon samples or cells was isolated and reversely transcribed to cDNA. Polymerase chain reaction (PCR) amplification procedures were as follows: initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Mouse Hmb s or human GAPDH were used as internal controls. Relative mRNA or lncRNA levels were determined using the 2−ΔΔCT method. Primers are provided in table S1.

Luciferase reporter assay
Cells were cotransfected with luciferase reporter, pRL-TK, and overexpression plasmid (Lnc-UC, p65, or Bmal1 plasmid). After 24-hour transfection, cells were lysed and luciferase activities were determined using Dual-Luciferase Reporter Assay System and GloMax 20/20 luminometer (Promega). Firefly luciferase activity was normalized to renilla luciferase activity and expressed as relative luciferase unit. For assessment of Lnc-UC effect on NF-κB transcription, BAY 11-7082 was added for 1 hour and LPS for 12 hours after cotransfection. Cells were then collected for measurements of luciferase activities.

Chromatin immunoprecipitation
ChiP assays were performed using BMDMs or colon tissues from mice with the Enzymatic Chromatin IP kit as previously described (13). In brief, cells or tissues were cross-linked in 37% formaldehyde for 10 min and the reaction was quenched by glycine. Cells were lysed, and nuclei were digested with micrococcal nuclease. Sheared chromatin was immunoprecipitated with antibodies against p65, Bmal1, Cbx1, H3K9me3, Suv39h1, or normal IgG. Immunoprecipitated chromatin was decross-linked at 65°C for 4 hours and purified with spin columns. Purified DNAs were analyzed by quantitative PCR (qPCR) with specific primers.

Electrophoretic mobility shift assay
Nuclear proteins were prepared from BMDMs using a cytoplasmic/nuclear protein extraction kit (Beyotime, Shanghai, China). Nuclear proteins were incubated with biotin-labeled oligonucleotide probes containing NF-κB binding site in a binding buffer containing poly(dI-dC), MgCl2, NaCl, EDTA, DTT, and glycerol. The mixture was loaded onto a 4% nondenaturing polyacrylamide gel. After electrophoresis, the products were transferred onto a Hybond-N+ membrane and visualized.
Cells were fixed in 4% paraformaldehyde, permeated with 4′,6-diamidino-2-phenylindole (DAPI) and the images were captured by using a laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

**Western blotting**

Protein samples were subjected to SDS–polyacrylamide gel electrophoresis (10% acrylamide gels). Products were transferred onto a polyvinylidene difluoride membrane, followed by incubation with primary antibody and with horseradish peroxidase–conjugated secondary antibody. The blots were visualized by using enhanced chemiluminescence and Omega Lum G imaging system (Aplegen, Pleasanton, CA).

**RNA sequencing**

Eighteen colitis mice and 18 normal mice were used for RNA-seq experiments. Three colitis mice and three normal mice were sacrificed at each circadian time point (ZT2, ZT6, ZT10, ZT14, ZT18, and ZT22), and the colons were collected, snap-frozen, and stored at −80°C. RNA was extracted using RNAiso Plus reagent and analyzed for quality using Agilent 2100 BioAnalyzer Expert (Agilent Technologies, Santa Clara, CA). Ribosomal RNA (rRNA) was depleted using a Ribo-zero Gold rRNA Removal kit (Illumina, San Diego, CA, USA). Strand-specific libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (Ipswich, MA, USA). The libraries were sequenced on Illumina HiSeq X Ten to generate 2 × 150–base pair (bp) paired-end reads. Reads were aligned to mouse GRC38/mm10 genome with HISAT2 (v2.0.4). FPKMs (fragments per kilobase of transcript per million mapped reads) were calculated using the StringTie-eB software. Ensembl (GENCODE) transcripts were used for annotation. Differential gene expression analysis was performed using Ballgown software. Genes were defined as differentially expressed when \( P < 0.05 \) and fold change >1.5. Cycling mRNAs and lncRNAs were determined by the JTK-cycle algorithm with adjusted \( P < 0.05 \). In addition, RNA-seq was performed with BMDMs (Lnc-UC–transfected and LPS-stimulated BMDMs, Rev-erbα–transfected and LPS-stimulated BMDMs, and control BMDMs). Poly(A) RNA was extracted using poly-T oligo-attached magnetic beads. Poly(A) RNA was used for construction of libraries using NEBNext Ultra RNA Library Prep Kit for Illumina (Ipswich, MA, USA). Gene set enrichment analysis was performed using the GSEA software (Broad Institute). KEGG pathways fulfilling the criterion of a hypergeometric \( P < 0.05 \) were defined as significantly enriched in differentially expressed genes. In bubble plots (Figs. 2A and 4C and fig. S5A), GeneRatio is the number of observed divided by the number of expected genes from each KEGG term.

**Fluorescence in situ hybridization**

Subcellular localization of Lnc-UC was determined using a FISH kit according to the manufacturer’s protocol (GenePharma, Shanghai, China). In brief, BMDMs were fixed in 4% paraformaldehyde and hybridized with Lnc-UC probe (10 nM) in a hybridization buffer. After washing with saline sodium citrate, cells were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and the images were captured by using a laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

**Immunofluorescence**

Cells were fixed in 4% paraformaldehyde, permeated in 0.1% Triton X-100, and blocked with 5% bovine serum albumin. Thereafter, cells were incubated with primary antibody against p65 or Cbx1 overnight at 4°C, followed by incubation with an Alexa Fluor 488–conjugated anti-rabbit antibody or Alexa Fluor 594–conjugated anti-rabbit antibody for 1 hour. Cells were then stained with DAPI, and the images were captured by using a laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

**Chromogenic in situ hybridization**

CISH assay was performed using a CISH kit according to the manufacturer’s protocol (BersinBio, Guangzhou, China). In brief, mouse and human colon samples were fixed in 4% paraformaldehyde. Digoxin-labeled probe targeting Lnc-UC was obtained from BersinBio. Samples were incubated with proteinase K at 37°C for 10 min, followed by prehybridization in hybridization buffer for 3 hours, and hybridization with denatured probes overnight at 42°C. The images were acquired using the streptavidin–biotin complex method with a Nikon Eclipse Ci-S optical microscope (Tokyo, Japan).

**RNA pull-down**

Nuclear proteins were prepared from BMDMs using a nuclear protein extraction kit (Beyotime, Shanghai, China). Full-length Lnc-UC and four truncated versions of Lnc-UC were cloned into T7 promoter–based vector and transcribed using TranscriptAid T7 High Yield Transcription kit (Thermo Fisher Scientific, Madison, WI). Purified RNA was biotinylated using Magnetic RNA-Protein Pull-down kit (Thermo Fisher Scientific, Madison, WI). Biotinylated RNAs (50 pmol) were incubated with 30 μl of streptavidin magnetic beads at room temperature for 30 min and then incubated with 300 μg of nuclear proteins at 4°C for 1 hour. Proteins were eluted from the beads with elution buffer. An aliquot of proteins was subjected to mass spectrometric analysis, while remaining proteins were separated using SDS-PAGE (polyacrylamide gel electrophoresis) and silver staining was subsequently performed using a fast silver stain kit (Beyotime, Shanghai, China) and Western blotting.

**RNA immunoprecipitation**

RIP assays were performed using a Magna RIP (RNA binding protein immunoprecipitation) kit according to the manufacturer’s protocol (Millipore, Bedford, MA). Colons were homogenized and lysed in RIP lysis buffer supplemented with a ribonuclease (RNase) inhibitor and protease inhibitor cocktail. The lysate was centrifuged at 14,000 rpm for 10 min, and supernatant was incubated overnight with magnetic beads conjugated with anti-Cbx1 (5 μg) or IgG in immunoprecipitation buffer at 4°C. Beads were then incubated with RIP washing buffer containing proteinase K, and immunoprecipitates were collected. RNA was extracted from the immunoprecipitates. Purified RNA was quantified by qPCR analysis.

**Rapid amplification of cDNA ends**

Total RNA was extracted from human colon. RNA with poly(A) was isolated from total RNA using Library Preparation VAHTS mRNA Capture Beads and reversely transcribed using SMARTer RACE 5’/3’ Kit. PCR amplification was performed using Phusion Hot Start II DNA Polymerase with 5’ or 3’ RACE specific primers. Products of PCR were recovered from the gel using gel extraction kit and subjected to sequencing. The full length of hLnc-UC was obtained by integrating sequencing data of 5’ and 3’ RACE.
Statistical analyses
Data are presented as mean ± SD. Statistical significance was determined using Student’s t test or analysis of variance (ANOVA) (one- or two-way) with Bonferroni post hoc test. Statistical analysis for survival curves was performed with the log-rank test. The level of significance was set at *P < 0.05.

SUPPLEMENTARY MATERIALS
Material supplementary for this article is available at http://advances.sciencemag.org/cgi/content/full/6/42/eaab5202/DC1

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