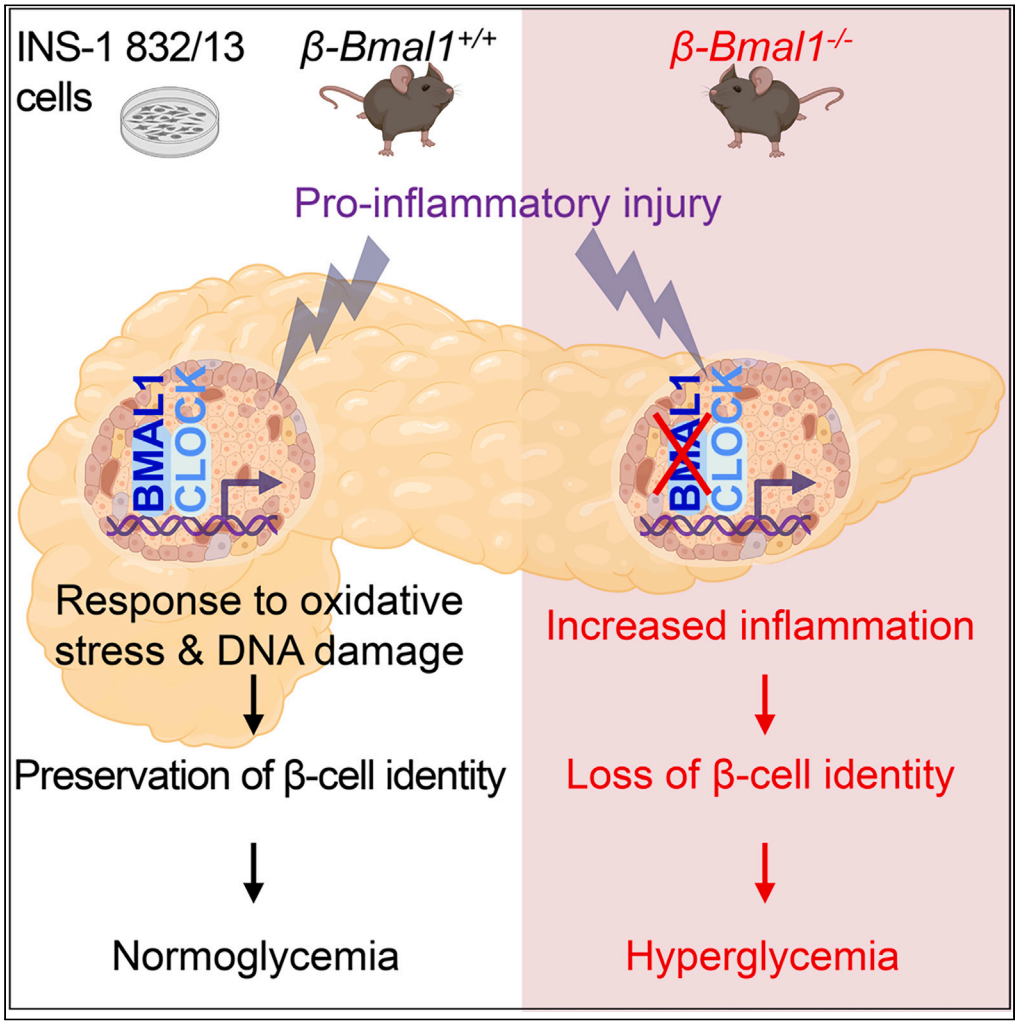
iScience

CellPress
OPEN ACCESS

Article

Core circadian transcription factor Bmal1 mediates β cell response and recovery from pro-inflammatory injury



Kuntol Rakshit, Matthew R. Brown, Naureen Javeed, Jeong-Heon Lee, Tamas Ordog, Aleksey V. Matveyenko

matveyenko.aleksey@mayo.edu

Highlights

IL-1β alters DNA binding of circadian transcription factors BMAL1:CLOCK in β cells

Loss of Bmal1 impairs β cell's ability to recover from pro-inflammatory injury

The circadian clock controls β cell response and recovery from pro-inflammatory injury

Rakshit et al., iScience 27, 111179
November 15, 2024 © 2024 The Author(s). Published by Elsevier Inc.
https://doi.org/10.1016/j.isci.2024.111179

iScience

CellPress
OPEN ACCESS

Article

Core circadian transcription factor Bmal1 mediates β cell response and recovery from pro-inflammatory injury

Kuntol Rakshit,[1] Matthew R. Brown,[1] Naureen Javeed,[1,2] Jeong-Heon Lee,[3,5,6] Tamas Ordog,[1,3,4] and Aleksey V. Matveyenko[1,2,7,*]

SUMMARY

The circadian clock plays a vital role in modulating the cellular immune response. However, its role in mediating pro-inflammatory diabetogenic β cell injury remains largely unexplored. Our studies demonstrate that the exposure of β cells to IL-1β-mediated inflammation alters genome-wide DNA binding of core circadian transcription factors BMAL1:CLOCK enriched for genomic sites important for cellular response to inflammation. Correspondingly, conditional deletion of *Bmal1* in mouse β cells was shown to impair the ability of β cells to recover from streptozotocin-mediated pro-inflammatory injury *in vivo*, leading to β cell failure and the development of diabetes. Further data integration analysis revealed that the β cell circadian clock orchestrates the recovery from pro-inflammatory injury by regulating transcriptional responses to oxidative stress, DNA damage, and nuclear factor κB(NF-κB)-driven inflammation. Our study suggests that the β cell circadian clock mediates β cell response and recovery from pro-inflammatory injury common to the pathogenesis of diabetes mellitus.

INTRODUCTION

Development of type 1 (T1DM) and type 2 (T2DM) diabetes mellitus is mediated through loss of pancreatic β cell survival and function.[1–4] Although there are many distinct pathogenic differences between the two forms of diabetes,[5] β cell stress injury with concurrent activation of the pro-inflammatory molecular signature is a common feature of failing β cells.[6] Emerging evidence suggests that the underlying β cell injury in T1DM is characterized by the induction of endoplasmic reticulum (ER) stress, cellular senescence, and/or DNA damage, which serve as potential triggers for autoimmune assault and corresponding initiation of pro-inflammatory stress.[7–9] In T2DM, β cell injury is driven by chronic exposure to a pro-diabetogenic milieu (e.g., glucolipotoxicity, proinflammatory cytokines, etc.) coupled with proteotoxicity mediated by misfolding of key secretory proteins.[5,10] Thus, the cellular stress response and corresponding unresolved inflammation are commonly observed in β cells of T2DM patients.[6,11–13] Therefore, mechanisms mediating the induction, response, and resolution of inflammation in β cells may shed light on the causes of β cell demise in diabetes.

In recent years, increased attention has been placed on the role of the endogenous circadian clocks as mediators of glucose homeostasis through the regulation of β cell survival, function, regeneration, and inflammation.[14–20] The circadian system is a fundamental component of mammalian homeostasis which coordinates ~24 h periodicity in essential physiological and molecular functions.[21] Cell- and tissue-specific circadian rhythms are generated by a molecular clock driven by a complex transcription factor (TF) network interlocked in positive-negative feedback loops. The core circadian loop is driven by two main TFs (CLOCK and BMAL1), which bind E-box DNA motifs to drive circadian transcription of various genes, including their negative regulators.[22,23] Additional levels of transcriptional rhythmicity arise from clock-regulated TFs that lie "downstream" of CLOCK and BMAL1, such as DBP/NFIL3, REV-ERBα/RORα, and DEC1/2 (BHLHE40/41).[23] This complex molecular network permits temporal regulation of transcription encompassing essential cellular processes such as insulin secretion, proliferation, and response to various intracellular and extracellular stressors.[22]

The circadian clock has emerged as an important component of cellular and organismal immune responses. Circadian oscillations in circulating levels of key hematopoietic cells, cytokines, chemokines, and hormonal factors orchestrate optimal host-pathogen interactions and the activation of innate and adaptive immune response.[24] At the cellular level, circadian TFs (e.g., BMAL1 and REV-ERBα) modulate

[1]Department of Physiology and Biomedical Engineering, Mayo Clinic School of Medicine, Rochester, MN, USA
[2]Department of Medicine, Division of Endocrinology, Metabolism, Diabetes, and Nutrition, Mayo Clinic School of Medicine, Rochester, MN, USA
[3]Epigenomics Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA
[4]Division of Gastroenterology and Hepatology, Department of Medicine, Mayo Clinic College of Medicine and Science, Rochester, MN, USA
[5]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
[6]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine and Science, Rochester, MN, USA
[7]Lead contact
*Correspondence: matveyenko.aleksey@mayo.edu
https://doi.org/10.1016/j.isci.2024.111179

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

the expression of key genes (e.g., *Nrf2*, *Il1β*, *Hspa1b*, etc.) regulating the response to common pro-inflammatory stressors such as lipopolysaccharide (LPS) and TLR4 activation in mice.[25–27] In addition, studies also suggest an important reciprocal relationship between inflammatory responses and regulation of the core circadian clock. For example, in mouse hepatocytes, LPS-mediated inflammation stimulates relocalization of key pro-inflammatory TF nuclear factor κB (NF-κB) to the promoters of core clock genes (e.g., *Per1* and *Cry2*), leading to their repression and compromised circadian function.[28] Moreover, acute hepatic inflammation in mice also results in genome-wide relocalization of BMAL1/CLOCK genomic sites toward genomic loci involved in immune, apoptotic, and metabolic programs.[28] This effect was shown to be dependent on NF-κB expression and enhanced chromatin accessibility, providing evidence for the importance of the core circadian clock in the transcriptional response to inflammation.

Taken together, the circadian clock regulates an array of physiological and molecular responses to cellular stress and inflammation. However, the role of the circadian clock in mediating pro-inflammatory diabetogenic injury in β cells remains unexplored. Here, we report that cytokine-mediated inflammation in β cells alters genome-wide DNA binding patterns of core circadian TFs BMAL1 and CLOCK, resulting in differential binding to sites in proximity of genes controlling the response to inflammation, genotoxic stress, and β cell function. Mechanistically, we found that β cell response to pro-inflammatory injury *in vivo* requires the expression of core clock TF *Bmal1*, as β cell deletion of this gene impairs the response and recovery of β cells to inflammatory injury *in vivo* and results in the development of β cell failure and diabetes mellitus.

RESULTS

IL-1β alters genome-wide DNA binding patterns of core circadian clock transcription factors BMAL1 and CLOCK in INS-1 832/13 cells *in vitro*

We first set out to examine the effects of β cell cytokine-mediated inflammatory response on genome-wide DNA binding patterns of the two core circadian TFs, BMAL1 and CLOCK (Figure 1). To model cytokine-mediated inflammation, we exposed INS-1 832/13 cells to a key pro-inflammatory cytokine IL-1β (2 ng/mL, 24 h) and subsequently performed chromatin immunoprecipitation followed by sequencing (ChIP-seq). The experimental conditions were purposefully chosen as (1) IL-1β is a mediator of islet inflammation under diabetogenic conditions[18,19,29–32] and (2) the concentration and the duration of treatment used in our study models β cell functional failure without affecting cell viability.[18] Intriguingly, IL-1β treatment significantly altered genome-wide DNA binding of circadian TFs by inducing differential (fold change [FC] > 1.5; false discovery rate [FDR]< 0.001) binding unique to either BMAL1 (1,152 differential binding sites), CLOCK (2,615 differential binding sites) and BMAL1:CLOCK co-bound (629 differential binding sites) (Figures 1A–1C). Pathway analysis of BMAL1:CLOCK genomic sites enhanced upon IL-1β treatment revealed enrichment for pathways important for orchestrating the cellular response to pro-inflammatory stress such as cellular senescence,[33] LXR signaling,[34] NRF2 pathways,[35] and apoptosis. (Figure 1D). Consistently, genomic sites co-occupied by BMAL1:CLOCK that displayed enhanced binding upon IL-1β exhibited enrichment for DNA motifs associated with cellular response to inflammatory stress (e.g., CEBP)[36] (Figure 1E). In contrast, evaluation of genomic sites characterized by attenuated BMAL1:CLOCK binding upon IL-1β showed enrichment for pathways involved in the regulation of β cell function and nutrient sensing, such as glucagon/GLP-1 signaling and calcium signaling. (Figure 1D). In accordance, IL-1β-decreased BMAL1:CLOCK binding sites were enriched for regulatory motifs associated with control of β cell function and identity (e.g., PDX1) (Figure 1E). Finally, BMAL1:CLOCK binding sites unaffected by IL-1β exposure were enriched for pathways important in the regulation of the circadian clock, SUMOylation and unfolded protein response (Figures 1D–1F).

Conditional deletion of Bmal1 in β cells impairs functional and morphological recovery of β cells in response to STZ-mediated pro-inflammatory injury

To address whether β cell circadian clock regulates the response to pro-inflammatory stress *in vivo,* we conditionally deleted Bmal*1* in β cells (*β-Bmal1*$^{-/-}$) by crossing mice floxed for *Bmal1*[37] with mice transgenic for inducible Cre recombinase selectively expressed in pancreatic β cells (RIP-CreER)[38] (Figure S1). Of note, deleting *Bmal1* in pancreatic β cells has been shown to be effective in disrupting β cell circadian clock without altering systemic circadian rhythms and/or inducing "off-target" effects.[15,39] Subsequently, *β-Bmal1*$^{-/-}$ and control (*Bmal1*$^{fl/fl}$) mice were administered a submaximal dose of streptozotocin (STZ, 100 mg/Kg body weight) used as a chemical β cell-targeted inducer of inflammatory injury associated with the induction of DNA damage.[7,40] As expected, in control mice, submaximal STZ administration resulted in acute (24 h) reduction in pancreatic insulin expression, which fully recovered within 4 weeks after initial STZ administration, indicative of successful resolution of inflammatory injury (Figures 2A–2D and Figure S2). Correspondingly, control mice demonstrated preservation of glycemic control (Figures 2A–2D and Figure S2). In stark contrast, *β-Bmal1*$^{-/-}$ mice exhibited reduced insulin-positive area at 24 h and 4 weeks after STZ (~75% vs. control, $p < 0.05$, Figures 2C and 2D) and subsequently developed overt hyperglycemia and hypoinsulinemia indicative of failed resolution of STZ-mediated inflammatory injury (Figures 2A–2D and Figure S2). Consistently, examination of β cell turnover in response to acute (24 h) STZ in *β-Bmal1*$^{-/-}$ mice revealed diminished β cell proliferation (~75% vs. control, $p < 0.05$, Figure 3A) and increased β cell apoptosis (~2-fold increase vs. control, $p < 0.05$, Figure 3B). Notably, the effect of STZ on β cell turnover did not depend on time of day of the STZ injection (e.g., $p > 0.05$ for zeitgeber time (ZT) 4 vs. 20) (Figure 3).

Conditional deletion of Bmal1 in β cells modulates transcriptional islet response to STZ-mediated pro-inflammatory injury

To gain insights into the molecular mechanisms underlying islet response to STZ-mediated inflammation, we performed RNA sequencing (RNA-seq) of *β-Bmal1*$^{-/-}$ and control islets isolated after 24 h and 4 weeks following STZ (Figure 4; Figure S3). We identified 564 and 476

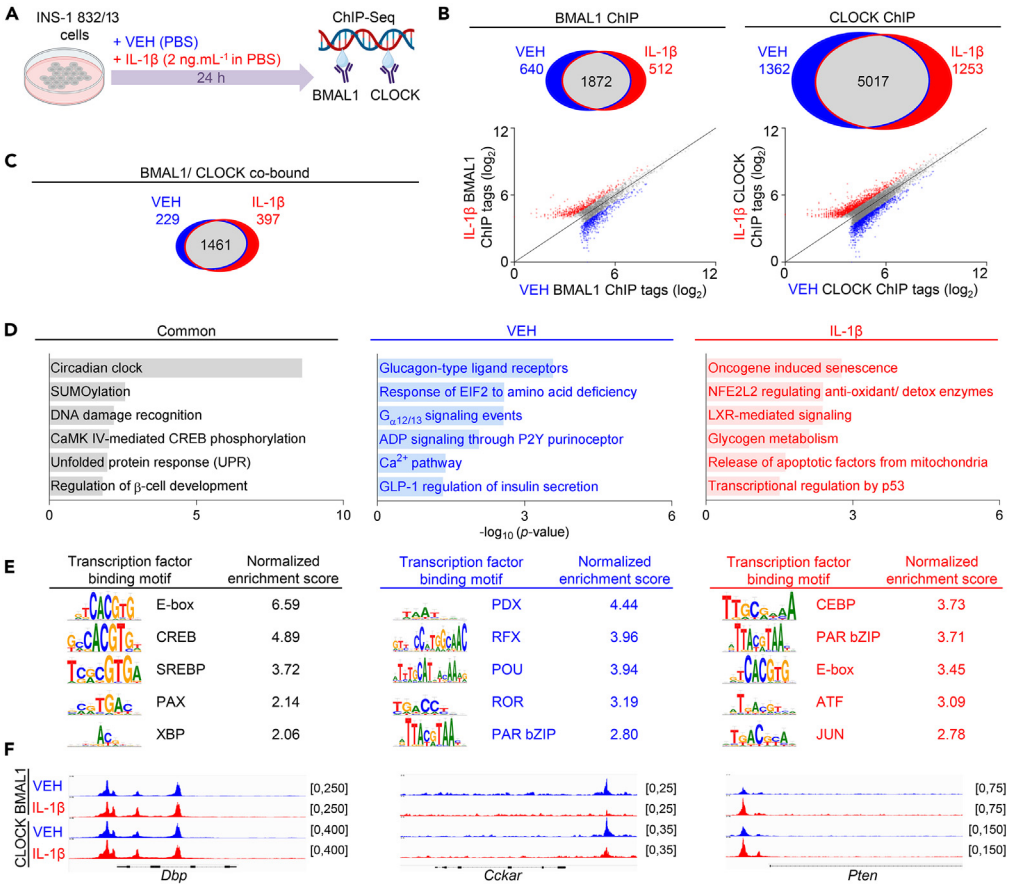


Figure 1. Exposure to IL-1β alters genome-wide DNA binding patterns of core circadian clock transcription factors BMAL1 and CLOCK in β cells

(A) Overview of study design. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) of BMAL1 and CLOCK was performed in INS-1 832/13 β cells upon 24 h exposure to 2 ng/mL of the proinflammatory cytokine IL-1β or vehicle control (n = 2/condition).

(B) Venn diagrams indicating overlapping and significantly differential BMAL1 and CLOCK binding sites in INS-1 832/13 β cells exposed to vehicle or IL-1β treatment (top). Scatterplots at bottom representing ChIP-seq tag density distribution for BMAL1 (left) and CLOCK (right). Gray dots indicate binding sites shared between vehicle and IL-1β conditions, whereas blue and red dots indicate differentially bound regions unique to vehicle or IL-1β treatment (FC > 1.5; $p < 0.05$).

(C) Venn diagram indicating overlapping and significantly differential BMAL1:CLOCK co-bound sites in INS-1 832/13 β cells exposed to vehicle or IL-1β treatment.

(D) REACTOME pathway enrichment analysis of the genomic sites co-occupied by BMAL1:CLOCK with overlapping binding in vehicle or IL-1β treatment (left), decreased binding with IL-1β (center), or enrichment with IL-1β treatment conditions ($p < 0.05$).

(E) Predicted i-Cis target enrichment of regulatory motifs (Z normalized enrichment score) in genomic sites co-occupied by BMAL1:CLOCK with overlapping binding in vehicle or IL-1β treatment (left), decreased binding with IL-1β (center), or enrichment with IL-1β treatment conditions.

(F) Integrative Genomics Viewer browser tracks of representative BMAL1 and CLOCK binding at the *Dbp* (left), *Cckar* (center), and *Pten* (right) genetic loci in vehicle (blue) and IL-1β (red) samples. Signal is normalized to counts per million reads (cpm).

differentially expressed genes at 24 h and 4 weeks following STZ administration (vs. vehicle) in control mice (Figure S3). Strikingly, we noted several-fold increase in the number of differentially expressed transcripts in *β-Bmal1*$^{-/-}$ mice at both 24 h (4,298) and 4 weeks (2,290) in response to STZ (Figure S3). Importantly, the majority (i.e., ~70%) of the STZ-driven differentially expressed transcripts in *β-Bmal1*$^{-/-}$ demonstrated increased expression and were uniquely enriched under Bmal1-deficient conditions (~11% overlap with control) (Figure S3). Specifically, in control mice, gene ontology revealed that acute (24 h) response to STZ was associated with the induction of islet transcripts enriched for pathways regulating metabolic processes, inflammatory response, and cell proliferation (Figure 4). Correspondingly, the islet transcriptome of control mice 4 weeks after STZ was associated with upregulation of genes enriched for negative regulation of inflammation and cell proliferation, and downregulation of genes involved in acute phase response and reactive oxygen species (ROS) metabolism (Figure 4). These observations were consistent with the phenotype of control mice indicative of successful resolution of inflammatory injury and preservation of glycemic control and insulin secretion. In contrast, differential islet transcriptome of STZ-treated *β-Bmal1*$^{-/-}$ mice remained consistent between 24 h and 4 weeks and was markedly enriched for pathways regulating inflammatory response, apoptotic processes, oxidative stress, and negative regulation of cell cycle (Figure 4). In addition, the islet transcriptome of STZ-treated *β-Bmal1*$^{-/-}$ mice was also uniquely

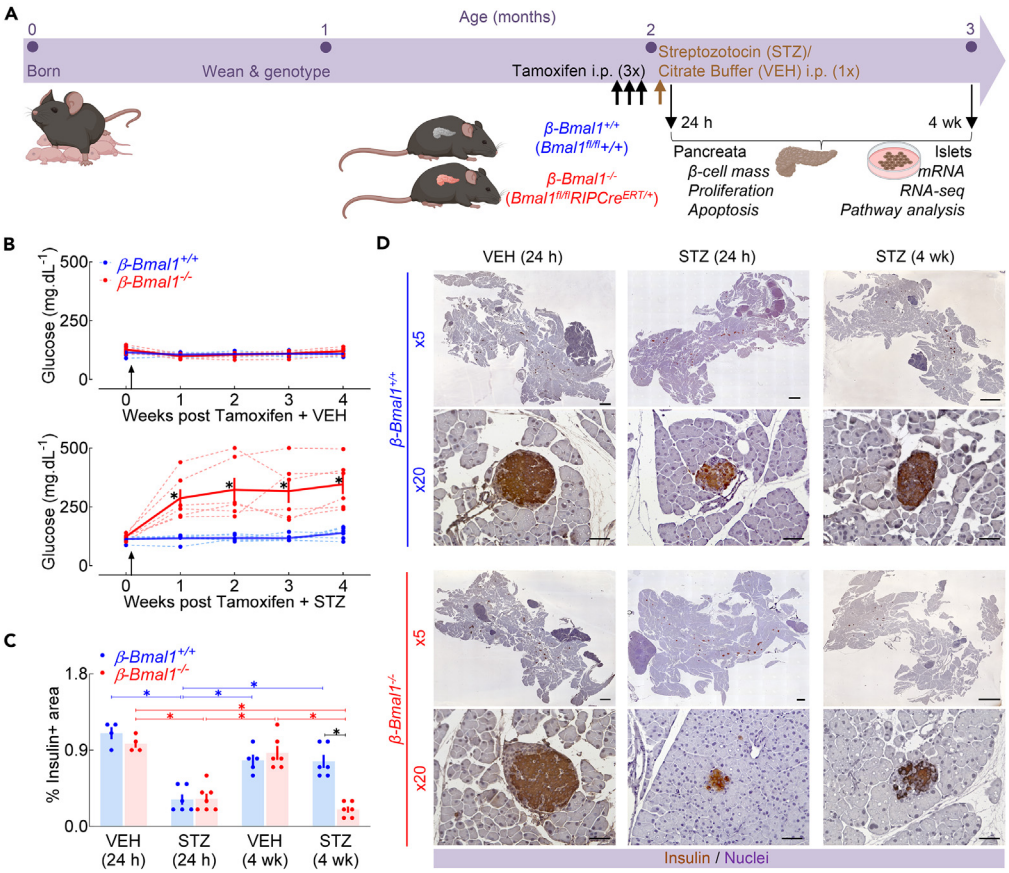


Figure 2. Conditional deletion of *Bmal1* in β cells impairs functional and morphological recovery of β cells in response to STZ-mediated pro-inflammatory injury

(A) Diagrammatic representation of study design . Mice were fed standard chow diet throughout. At the age of 2 months, all mice received 3x intraperitoneal (i.p.) injections of tamoxifen for conditional deletion of *Bmal1* in β cells. 1 week post first tamoxifen dose, mice received 1x i.p. injection of streptozotocin (STZ, 100 mg/Kg) or citrate buffer (VEH) at 10:00 h (ZT 4, light cycle) or 02:00 h (ZT 20, dark cycle). Pancreata were collected from mice 24 h or 4 weeks post STZ/VEH injection for assessment of β cell area, proliferation, and apoptosis. Pancreatic islets were also isolated for mRNA extraction and subsequent RNA sequencing.

(B) Graphs showing blood glucose levels in control (β-*Bmal1*$^{+/+}$) and knockout (β-*Bmal1*$^{-/-}$) mice at 0, 1-, 2-, 3-, and 4-weeks post either VEH (top) or STZ (bottom) treatment. Values are mean ± SEM (n = 5–6 per data point). Statistical significance was calculated by two-way ANOVA with Sidak's multiple comparisons test and denoted by *$p < 0.05$. Black arrow represents time of tamoxifen and STZ/VEH administration.

(C) Graph depicting quantification of insulin positive area calculated as the percentage of insulin+ area to total pancreatic area at 24 h or 4 weeks post STZ/VEH treatment. Values are mean ± SEM (n = 4–7 per data point). Statistical significance was calculated by two-way ANOVA with Sidak's multiple comparisons test and is denoted by *$p < 0.05$.

(D) Representative examples of pancreatic islets stained by immunohistochemistry for hematoxylin and insulin imaged at 5× (scale bars, 1,000 μm) and 20× magnification (scale bars, 50 μm) in β-*Bmal1*$^{+/+}$ and β-*Bmal1*$^{-/-}$ mice 24 h and 4 weeks post STZ/VEH injection.

characterized by marked downregulation of genes annotated to pathways essential for the regulation of β cell function and identity, such as insulin secretion, glucose transport, and endocrine pancreas development (Figure 4). It is also important to note that induction of hyperglycemia in STZ-treated β-*Bmal1*$^{-/-}$ mice at 4 weeks likely contributed to the observed differential islet gene expression between control and β cell *Bmal1* deficient mice.

Bmal1 expression in β cell is required for the temporal transcriptional response to STZ-mediated pro-inflammatory injury

We next used Short Time-series Expression Miner (STEM) analysis[41] of islet RNA-seq to get insights into time-dependent transcriptional changes in response to STZ-mediated inflammation in control and *Bmal1* deficient islets (Figures 5 and 6). Whereas in control islets, only 74 genes acutely decreased by STZ at 24 h remained continually reduced at 4 weeks, 1,711 genes remained suppressed throughout the 4 weeks study period in β-*Bmal1*$^{-/-}$ islets (Figure 5A). Importantly, genes with chronically suppressed islet expression after STZ in β-*Bmal1*$^{-/-}$ mice were broadly enriched for processes related to the regulation of insulin secretion, response to DNA damage, response to unfolded proteins, and cell cycle (Figures 5A–5C). Consistently, chronically decreased genes in β-*Bmal1*$^{-/-}$ islets were enriched for DNA regulatory motifs associated with circadian TFs BMAL1 and BHLHE40 as well as β cell stress and DNA damage response

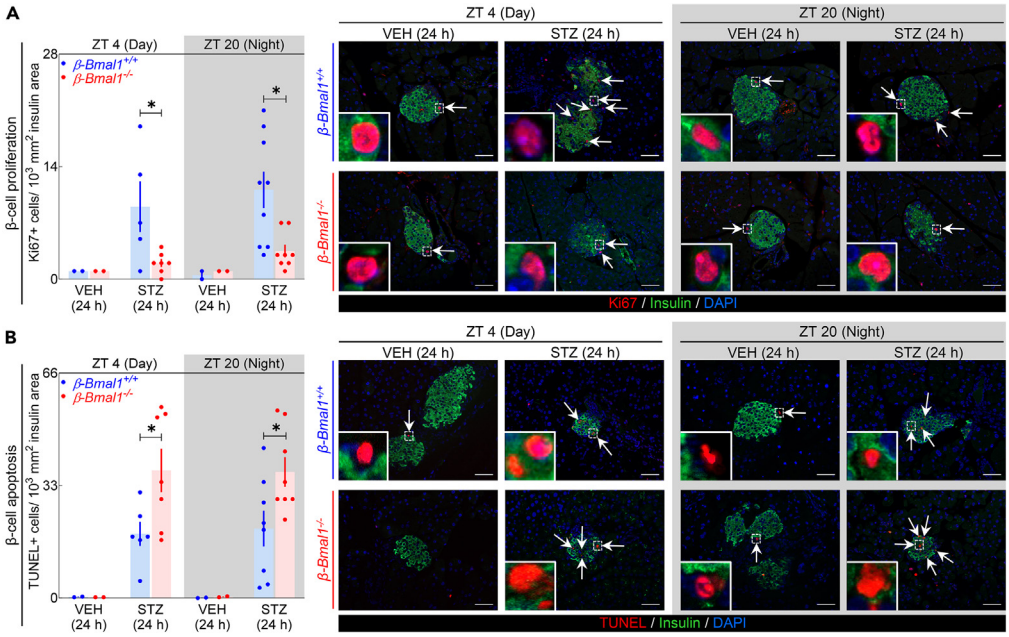


Figure 3. Conditional deletion of *Bmal1* in β cells attenuates β cell's proliferative capacity and promotes apoptosis in response to STZ-mediated pro-inflammatory injury

Bar graphs on left representing β-cell proliferation (A) and apoptosis (B) quantified as the number of Ki-67 or TUNEL$^+$ cells/Insulin$^+$ area in control (*β-Bmal1*$^{+/+}$) and knockout (*β-Bmal1*$^{-/-}$) mice injected with STZ/VEH at 10:00 h (ZT 4, light cycle) or 02:00 h (ZT 20, dark cycle). All values represent 24 h post STZ/VEH time point expressed as mean ± SEM (n = 4–9 per data point). Statistical significance was calculated using unpaired t test and is denoted by *$p < 0.05$. Representative examples of pancreatic islets on right stained by immunofluorescence for insulin (green), proliferation marker Ki-67 or apoptosis marker TUNEL (red), and counterstained with nuclear marker DAPI (blue) imaged at x20 magnification (scale bars, 50 μm) in *β-Bmal1*$^{+/+}$ and *β-Bmal1*$^{-/-}$ mice injected with STZ/VEH at ZT 4 (day) or ZT 20 (night). All images represent 24 h post STZ/VEH time point.

transcriptional regulators, such as Yin Yang 1 (YY1)[42] and nuclear factor erythroid 2-related factor 2 (NRF2).[35] Notably, we observed virtually no overlap in genes chronically decreased by STZ under control vs. *β-Bmal1*$^{-/-}$ conditions (*e.g.*, 5 out of 1,711 genes, Figure 5D). However, a substantial proportion (31%) of chronically decreased genes in *β-Bmal1*$^{-/-}$ STZ-treated islets overlapped with BMAL1/CLOCK target genes previously identified in INS-1 832/13 cells using ChIP-seq (Figure 5E). Importantly, the observed genetic distribution of BMAL1/CLOCK binding sites associated with genes shown to be chronically decreased in STZ-treated *β-Bmal1*$^{-/-}$ islets were localized preferentially to the promoter regions (Figure 5F). Correspondingly, BMAL1 and CLOCK binding to the promoters of genes shown to be chronically suppressed in STZ-treated *β-Bmal1*$^{-/-}$ islets was significantly enhanced in response to IL-1β treatment ($p < 0.001$; Figure 5G). Some noteworthy BMAL1/CLOCK target genes displaying enhanced binding in response to IL-1β-mediated inflammation include genes previously shown to be important for cellular response to DNA damage and inflammation, such as *Yaf2*, *Fbxo3*, *Etv5*, and *Sdf2l1* (Figures 5H and 5I).

Bmal1 expression in β cell is required for the activation of transcriptional programs controlling the restoration of β cell's identity and function in response to STZ-mediated pro-inflammatory injury

Finally, we also focused our attention on transcripts chronically induced in islets following exposure to STZ (Figure 6). STEM analysis revealed comparable induction of ~1,100–1,400 unique genes in response to STZ in either control or *β-Bmal1*$^{-/-}$ islets (Figure 6A). In control islets, chronically induced genes after STZ were broadly enriched for processes related to the regulation of cholesterol metabolism, mitochondrion morphogenesis, and β cell identity (Figure 6B). These observations were reflected by significant enrichment for DNA regulatory motifs associated with key β cell identity TFs such as NKX6.1 and HNF4 (Figures 6A–6C). In contrast, chronically induced genes after STZ in *β-Bmal1*$^{-/-}$ islets were enriched for pathways associated with pro-inflammatory stress, reflected by the enrichment of these genes for DNA motifs of key pro-inflammatory TFs such as NF-κB, STAT, IRF, and SPI (Figures 6A–6C). Interestingly, we observed a significant overlap (30%) between genes induced by STZ in control islets with BMAL1/CLOCK target genes in INS-1 832/13 cells (Figures 6D and 6E). Notably, annotation of BMAL1/CLOCK target genes with significant time-dependent increase in control STZ-treated islets revealed broad enrichment for processes related to the regulation of β cell identity, implying that Bmal1 expression is necessary for successful preservation and/or restoration of β cell transcriptional identity in response to acute pro-inflammatory stress. Consistent with these findings, relative expression of key β cell identity genes was enhanced in control islets and demonstrated striking reduction in response to STZ in islets of β cell *Bmal1*-deficient mice (Figure 6F). Furthermore, we observed restoration of nuclear NKX6.1 expression (marker of β cell functional identity) and absence of ALDH1A3 (marker of

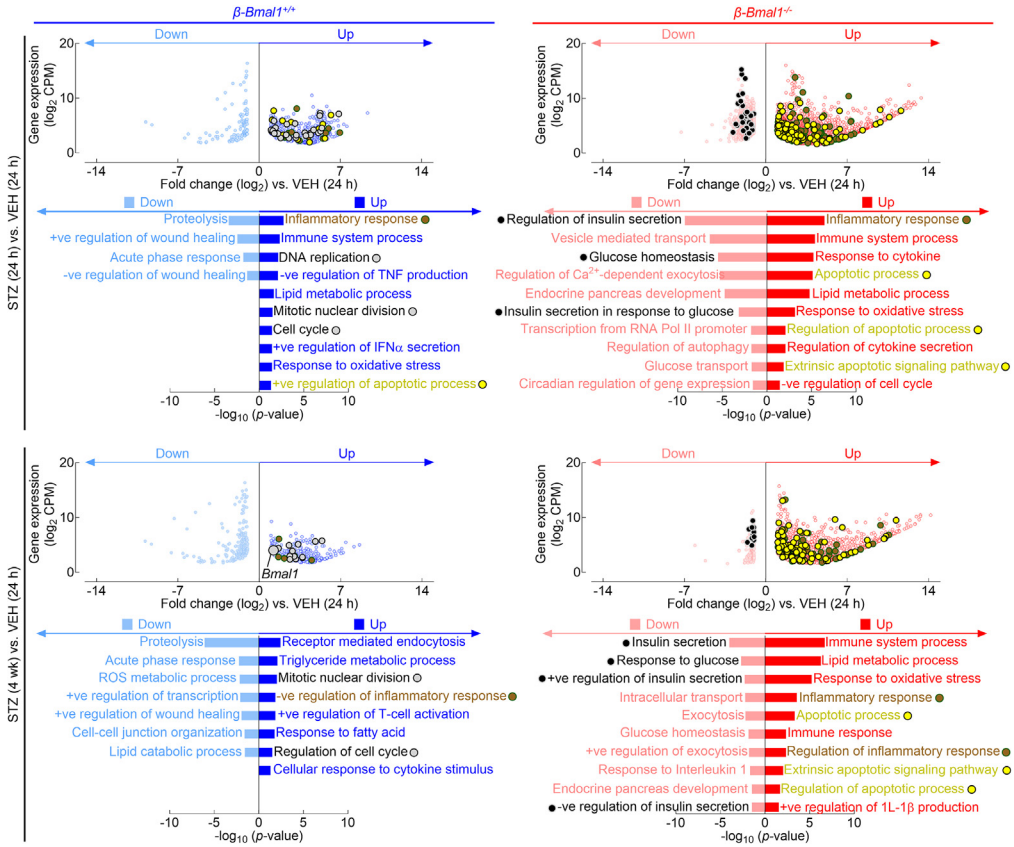


Figure 4. Conditional deletion of *Bmal1* in β cells modulates transcriptional islet response to STZ-mediated pro-inflammatory insult

Volcano plots (top) identifying differentially expressed genes (FC > 2; $p < 0.05$) from RNA sequencing performed on islets isolated from control (*β-Bmal1*$^{+/+}$; blue) and knockout (*β-Bmal1*$^{-/-}$; red) mice at either 24 h and/or 4 weeks post treatment with STZ relative to VEH (each sample represents pooled RNA from at least $n = 4$ mice per genotype). Tables below each volcano plot represent significantly enriched up- and down-regulated Gene Ontology (GO) biological pathways from RNA sequencing performed on islets isolated from control (*β-Bmal1*$^{+/+}$; blue) and knockout (*β-Bmal1*$^{-/-}$; red) mice at either 24 h and/or 4 weeks post treatment with STZ relative to VEH.

β cell dedifferentiation) following STZ in β cells of control mice, and corresponding loss of nuclear NKX6.1 and the induction of ALDH1A3 in islets of β cell *Bmal1* deficient mice (Figure 6G).

DISCUSSION

In the present study, we set out to investigate the role of the circadian clock in the regulation of β cell response, recovery, and resolution of pro-inflammatory injury. We first investigated how inflammation impacts genome-wide binding patterns of core circadian TFs BMAL1 and CLOCK, known to form a heterodimer critical for regulating circadian gene expression. We found that the exposure of β cells to IL-1β *in vitro* leads to enhanced binding of BMAL1:CLOCK to genomic sites enriched for pathways mediating cellular response to inflammation and decreased binding to genomic sites enriched for pathways involved in β cell function and identity. In complementary *in vivo* studies, we demonstrated that inducible deletion of *Bmal1* compromises β cells' ability to respond and recover from the pro-inflammatory insult modeled by submaximal administration of β cell toxin, STZ. Whereas control mice maintained euglycemia and normal β cell function following submaximal STZ administration, *Bmal1* deficiency led to the decompensation of β cell secretory function and the induction of overt hyperglycemia. Mechanistically, our studies revealed that β cell *Bmal1* deficiency was associated with chronic repression of BMAL1:CLOCK target genes involved in the regulation of β cell function and response to intracellular stress, and the upregulation of genes involved in NF-κB-driven pro-inflammatory stress and apoptosis. Taken together, our studies highlight the requirement of the β cell circadian clock in controlling β cell's response and recovery from the inflammatory stress injury common to the pathogenesis of diabetes mellitus.

Inflammation is an essential defense mechanism in response to cellular injury which is critical for the maintenance of tissue homeostasis and repair. However, excessive inflammation (also termed inflammatory stress) compromises normal cellular function and survival thus contributing to the development of chronic diseases including T1DM[43] and T2DM.[11] Although inflammation in diabetes impacts a variety of metabolic tissues, pancreatic β cells are particularly vulnerable to the inflammatory stress due to high secretory burden coupled with low antioxidative capacity.[10] To date, several extra- and intra-cellular triggers of β cell inflammatory stress have been described, such as viral

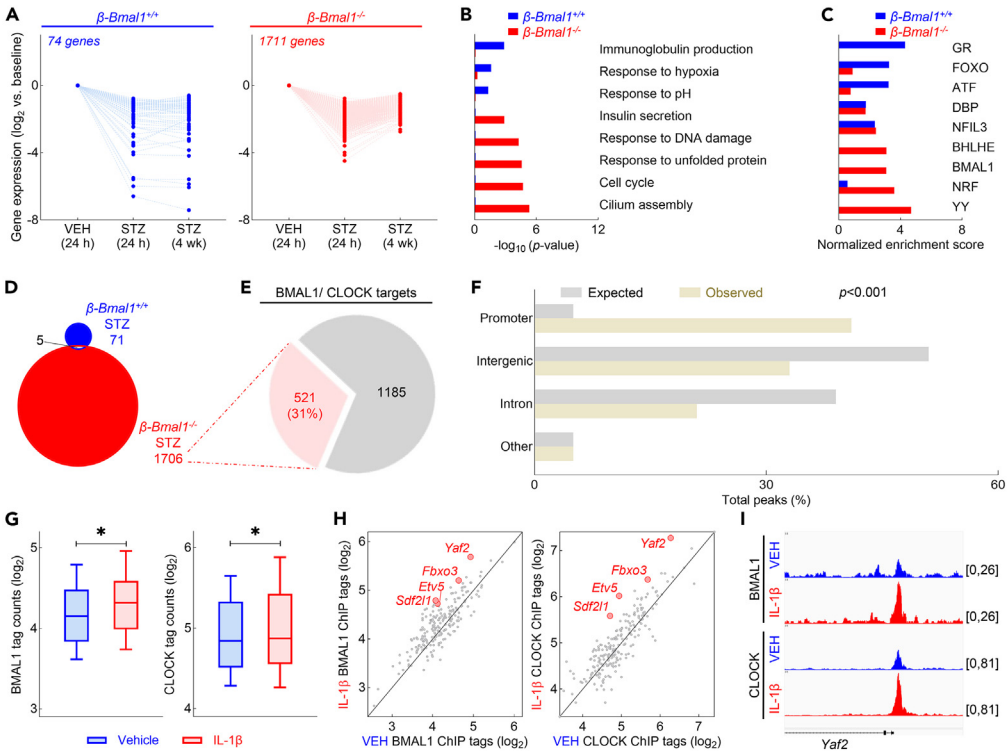


Figure 5. β cell *Bmal1* is required for the transcriptional response to STZ-mediated pro-inflammatory insult

(A) Short Time-series Expression Miner (STEM) analysis of RNA sequencing from *β-Bmal1*$^{+/+}$ (left) or *β-Bmal1*$^{-/-}$ islets (right) collected at 24 h and 4 weeks post STZ injection vs. VEH (set as time 0) (n = 2/condition). Line plots representing genes with significant time-dependant expression decreases (FC > 2; FDR< 0.05).

(B) Enriched GO: Biological Processes annotated from genes with significant time-dependant expression decreases in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) islets.

(C) Predicted i-Cis target enrichment of regulatory motifs (*Z* normalized enrichment score in *cis*-regulatory regions of genes with significant time-dependant expression decreases in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) STZ treated islets.

(D) Venn diagram indicating overlapping and unique genes with a significant time-dependant chronic decrease in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) STZ-treated islets.

(E) Venn diagram of overlap between unique genes with a significant time-dependant chronic decrease in *β-Bmal1*$^{-/-}$ STZ treated islets and BMAL1/CLOCK target genes in INS-1 832/13 cells identified using ChIP-seq.

(F) Bar graph representing the observed genetic distribution of BMAL1/CLOCK binding sites associated with genes chronically suppressed in *β-Bmal1*$^{-/-}$ STZ treated islets relative to an expected peak distribution (n = 50,000; Chi-squared test).

(G) Box and whisker plot representing ChIP-seq tag density in INS-1 832/13 cells for BMAL1 (left) and CLOCK (right), exposed to vehicle or IL-1β, from promoters of target genes chronically suppressed in *β-Bmal1*$^{-/-}$ STZ treated islets ($p < 0.001$; Wilcoxon test).

(H) Scatter plots representing ChIP-seq tag density distribution for BMAL1 and CLOCK in INS-1 832/13 cells, exposed to vehicle or IL-1β, from promoters of target genes chronically suppressed in *β-Bmal1*$^{-/-}$ STZ treated islets. Representative genes associated with proliferation, DNA damage, and inflammatory response are highlighted.

(I) Genome browser track view of representative BMAL1 and CLOCK binding at the *Yaf2* promoter in INS-1 832/13 cells vehicle (blue) and IL-1β-treated (red) samples. Signal is normalized to counts per million reads (cpm).

infections,[44] excessive exposure to pro-inflammatory cytokines,[40] proteotoxicity,[10] glucolipotoxicity,[45] and DNA damage.[7] In addition, several candidate genes have been shown to modulate β cell response to pro-inflammatory insults.[46] Consistent with this, global gene expression analysis in human islets from patients with either T1DM and/or T2DM show commonality in pro-inflammatory gene signature.[6] These observations suggest that successful versus failed recovery from inflammatory insult may underlie susceptibility to β cell failure in patients at risk of developing diabetes.

In this regard, our studies suggest that the endogenous β cell circadian clock, and in particular *Bmal1* expression, is important for the successful resolution of acute inflammatory stress. These results provide a mechanistic underpinning behind clinical and epidemiological observations linking environmental and/or genetic circadian rhythm disruption and impairments in glucose homeostasis.[47] Indeed, we previously reported that when exposed to pro-diabetogenic conditions *in vivo* (e.g., high fat diet) β cell-specific *Bmal1* loss-of-function mice exhibit key features of β cell failure such as loss of glucose-stimulated insulin secretion, increased β cell apoptosis, and islet transcriptional profiles characteristic of diabetogenic injury.[15] In contrast, previous work from our lab also shows that *Bmal1* gain-of-function mice exhibit protection from

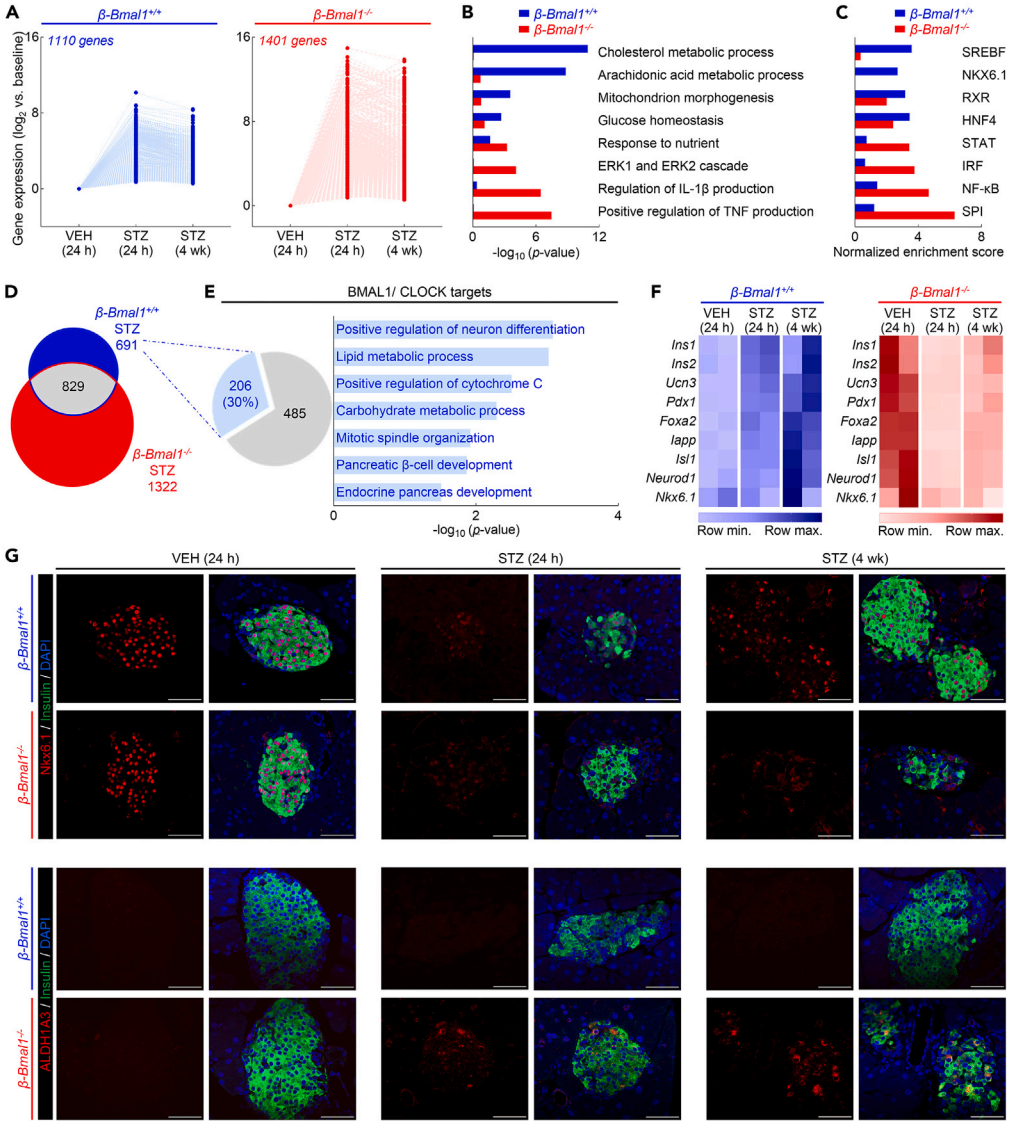


Figure 6. β cell *Bmal1* is required for the activation of transcriptional programs controlling restoration of β cell's identity and function in response to STZ-mediated pro-inflammatory insult

(A) Short Time-series Expression Miner (STEM) analysis of RNA sequencing from *β-Bmal1*$^{+/+}$ (left) or *β-Bmal1*$^{-/-}$ islets (right) collected at 24 h and 4 weeks post STZ injection vs. vehicle (set as time 0) (n = 2/condition). Line plots representing genes with significant time-dependant expression increases (FC > 2; FDR< 0.05).

(B) Enriched GO: Biological Processes annotated from genes with significant time dependant expression increases in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) islets.

(C) Predicted i-Cis target enrichment of regulatory motifs (Z normalized enrichment score in *cis*-regulatory regions of genes with significant time-dependant expression increases in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) STZ treated islets.

(D) Venn diagram indicating overlapping and unique genes with a significant time dependant increase in *β-Bmal1*$^{+/+}$ (blue) or *β-Bmal1*$^{-/-}$ (red) STZ treated islets.

(E) Venn diagram of overlap between unique genes with a significant time dependant increase in *β-Bmal1*$^{+/+}$ STZ treated islets and BMAL1/CLOCK target genes in INS-1 832/13 cells identified using ChIP-seq (left). Bar graph (right) representing enriched GO: Biological Processes annotated from BMAL1/CLOCK target genes in INS-1 832/13 cells with significant time-dependant increase in control *β-Bmal1*$^{+/+}$ STZ-treated islets.

(F) Heatmaps demonstrating temporal changes in the relative expression of key β-cell identity genes from *β-Bmal1*$^{+/+}$ (left) or *β-Bmal1*$^{-/-}$ islets (right) collected at 24 h and 4 weeks post STZ injection vs. vehicle.

(G) Representative examples of pancreatic sections immunostained for insulin (green), Nkx6.1 and/or ALDH1A3 (red), and DAPI (blue) imaged at 20× (scale bars, 50 μm) obtained from control (*β-Bmal1*$^{+/+}$) and knockout (*β-Bmal1*$^{-/-}$) mice at either 24 h and/or 4 weeks post treatment with STZ vs. VEH. Images are representative of n = 2–3 independent mice per group.

glucose intolerance and β cell failure concomitant with islet transcriptional profiles characterized by the induction of stress-resistant gene expression.[48] These observations are particularly relevant given the reported loss of β cell *Bmal1* expression and transcriptional activity in humans with type 2 diabetes.[18,49] Intriguingly, other chronic conditions associated with unresolved inflammatory stress (e.g., neurodegenerative disorders, atherosclerosis, and chronic obstructive pulmonary disease)[50,51] are also marked by loss of cellular *Bmal1* expression. Consistently, pharmacological and nutritional approaches aiming at augmenting *Bmal1* expression are effective in attenuating β cell failure in human islets[48] and diabetogenic phenotypes in rodent models.[50]

To obtain insights into BMAL1-mediated transcriptional response to pro-inflammatory stress, we performed RNA-seq with concurrent temporal analysis (STEM) of gene expression 24 h and 4 weeks after STZ administration. Notably, a substantial number (1,711) of transcripts unique to *Bmal1*-deficient islets remained decreased even after 4 weeks post STZ administration. These repressed transcripts were enriched for DNA regulatory motifs associated with BMAL1, and its target ancillary clock component BHLHE40, which was consistent with ChIP-seq analysis in INS-1 832/13 cells. Importantly, both BMAL1 and BHLHE40 are important for maintenance of β cell identity and preferentially target genes enriched for components of the insulin secretory apparatus and energy metabolism.[52] In addition, *Bmal1*-deficient islets also exhibited suppression of genes enriched for DNA motifs associated with oxidative stress and DNA damage response TFs, YY1, and NRF2. These observations are noteworthy given recent publications highlighting NRF2 as a key transcriptional regulator of β cell protection from oxidative stress under T2DM and T1DM conditions,[35,53] and YY1 as a transcriptional regulator of β cell response to DNA damage.[42] Moreover, BMAL1 modulates IL-1β production via NRF2 in macrophages which can further contribute to pathophysiology of β cell failure in diabetes.[26] Importantly, mice with β cell-specific depletion of either NRF2 and/or YY1 display diabetes phenotype characterized by loss of insulin expression, diminished secretory function, and increased β cell apoptosis, reminiscent of the phenotype observed in STZ-treated *Bmal1*-deficient mice.[42,53] Correspondingly, our studies suggest that β cell *Bmal1* is required to mount an appropriate transcriptional response to acute inflammatory stress by (1) maintaining β cell's functional identity and (2) activating response to oxidative stress and DNA damage.

CLOCK:BMAL1 have been shown to regulate β cell circadian gene expression through preferential binding to genomic sites colocalized with a core pancreatic lineage factor, PDX1[14]; an observation consistent with our ChIP-seq results in INS-1 832/13 cells. Notably, we additionally observed that IL-1β-treated β cells displayed diminished CLOCK:BMAL1 binding to the sites enriched for PDX1 regulatory motifs and enhanced binding to the sites enriched for pathways regulating cellular response to stress and inflammation such as NRF2 signaling. Indeed, previous studies provided evidence for (1) circadian control of NRF2 expression through direct E-box binding by CLOCK:BMAL1[54] and (2) cooperative regulation of antioxidant gene expression by BMAL1 and NRF2 through binding to E-Box and NRF2/ARE elements.[55] Moreover, work in mouse hepatocytes reported that exposure to inflammatory stimuli (e.g., LPS) leads to preferential binding of CLOCK and BMAL1 to sites exhibiting inducible binding by key transcriptional regulator of inflammation, NF-κB, and enriched for genes involved in the regulation of cellular response to inflammation and apoptosis.[28] In concert with these findings, Weidmann et al.,[56] recently demonstrated the existence of a unique subpopulation of human β cells exhibiting genome-wide enrichment for NF-κB pro-inflammatory activity and reduced activity of PDX1 (CLOCK:BMAL1). The same investigators also reported that experimental ablation of *Bmal1* in β cells impairs NF-κB-mediated regulation of anti-apoptotic gene expression and compromises PDX-1-mediated control of genes regulating insulin secretion and β cell identity, thus highlighting multidirectional relationship between the circadian clock, PDX1, and NF-κB.

In summary, our results provide evidence supporting the notion that the circadian clock in β cells plays an important role in orchestrating transcriptional and physiological response to pro-inflammatory injury common to the pathogenesis of diabetes mellitus. Specifically, our data imply that the expression of BMAL1 is important for the β cell to withstand and successfully recover from an inflammatory insult. This is particularly notable given evidence pointing to (1) suppression of *Bmal1* expression in islets of patients with diabetes,[18] and (2) perturbations of circadian gene expression in islets following environmental disruption of circadian rhythms.[57] Future studies are warranted to elucidate the role of the circadian clock in human β cell response to inflammatory stress and whether therapeutic strategies to enhance circadian clock function can provide protection from inflammation-mediated β cell failure in diabetes mellitus.

Limitations of the study

Due to technical difficulties in performing ChIP-seq experiments in primary β cells, we utilized a commonly used rat β cell line INS-1 832/13. Although INS-1 832/13 cells display many functional features of primary β cells,[58] there are clear functional and molecular differences that distinguish these cells from primary rodent and human β cells. We also utilized 24 h exposure to IL-1β to model a diabetogenic pro-inflammatory environment previously shown to promote β cell dysfunction and alter the circadian clock.[18,59] In fact, recent studies highlight the importance of β cell IL-1β signaling in the regulation of β cell's functional and transcriptional identity.[56,60] However, exposure to IL-1β alone does not fully recapitulate cytokine exposure common to the development of diabetes.[61] Furthermore, the duration of cytokine exposure in our study (24 h) potentially did not account for early immediate inflammatory response genes. Finally, it is also important to acknowledge the limitation of using STZ-based injury to model β cell DNA damage and pro-inflammatory insult in our study. STZ-based injury is associated with alkylation of DNA and the corresponding formation of toxic superoxide radicals and nitric oxide which can promote necrosis in affected β cells.[62]

RESOURCE AVAILABILITY

Lead contact

Further information and resource requests should be directed to and will be fulfilled by the lead contact, Aleksey V. Matveyenko (Matveyenko.Aleksey@mayo.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

RNA-seq data have been deposited at NCBI GEO. Accession number is listed in the key resources table and is publicly available as of the date of publication. No original code was generated for this study. All code used for analysis was properly cited in the STAR methods. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

We acknowledge funding support from the National Institutes of Health (R01DK098468 to A.V.M.), J.W. Kiekeffer Foundation, and the Center for Regenerative Biotherapeutics (Mayo Clinic, Rochester, MN).

AUTHOR CONTRIBUTIONS

K.R. contributed to study design, conducted experiments, assisted with the data analysis, interpretation, and preparation of the manuscript. M.R.B., N.J., and J.H.L. conducted experiments, assisted with the data analysis, interpretation, and preparation of the manuscript. T.O. contributed to study design, data analysis, interpretation, and preparation of the manuscript. A.V.M. designed, interpreted the studies, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Cell models
  - Animal models
  - *In vivo* study design
- METHOD DETAILS
  - Immunofluorescence and endocrine pancreas morphology
  - RNA sequencing
  - Chromatin immunoprecipitation-sequencing (ChIP-seq)
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Analytical methods
  - Informatics, statistical analysis, and calculations

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.111179.

Received: February 5, 2024
Revised: July 18, 2024
Accepted: October 14, 2024
Published: October 16, 2024

REFERENCES

1. Kahn, S.E., Zraika, S., Utzschneider, K.M., and Hull, R.L. (2009). The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. Diabetologia *52*, 1003–1012.
2. DiMeglio, L.A., Evans-Molina, C., and Oram, R.A. (2018). Type 1 diabetes. Lancet *391*, 2449–2462. https://doi.org/10.1016/S0140-6736(18)31320-5.
3. Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A., and Butler, P.C. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes *52*, 102–110.
4. Meier, J.J., Bhushan, A., Butler, A.E., Rizza, R.A., and Butler, P.C. (2005). Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia *48*, 2221–2228. https://doi.org/10.1007/s00125-005-1949-2.
5. Eizirik, D.L., Pasquali, L., and Cnop, M. (2020). Pancreatic beta-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat. Rev. Endocrinol. *16*, 349–362. https://doi.org/10.1038/s41574-020-0355-7.
6. Yi, X., Marmontel de Souza, B., Sawatani, T., Szymczak, F., Marselli, L., Marchetti, P., Cnop, M., and Eizirik, D.L. (2022). Mining the transcriptome of target tissues of autoimmune and degenerative pancreatic beta-cell and brain diseases to discover therapies. iScience *25*, 105376. https://doi.org/10.1016/j.isci.2022.105376.
7. Horwitz, E., Krogvold, L., Zhitomirsky, S., Swisa, A., Fischman, M., Lax, T., Dahan, T., Hurvitz, N., Weinberg-Corem, N., Klochendler, A., et al. (2018). beta-Cell DNA Damage Response Promotes Islet Inflammation in Type 1 Diabetes. Diabetes *67*, 2305–2318. https://doi.org/10.2337/db17-1006.
8. Thompson, P.J., Shah, A., Ntranos, V., Van Gool, F., Atkinson, M., and Bhushan, A. (2019). Targeted Elimination of Senescent Beta Cells Prevents Type 1 Diabetes. Cell Metabol. *29*, 1045–1060.e10. https://doi.org/10.1016/j.cmet.2019.01.021.
9. Tersey, S.A., Nishiki, Y., Templin, A.T., Cabrera, S.M., Stull, N.D., Colvin, S.C., Evans-Molina, C., Rickus, J.L., Maier, B., and Mirmira, R.G. (2012). Islet beta-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. Diabetes *61*, 818–827. https://doi.org/10.2337/db11-1293.
10. Costes, S., Langen, R., Gurlo, T., Matveyenko, A.V., and Butler, P.C. (2013). beta-Cell failure in type 2 diabetes: a case of asking too much

of too few? Diabetes *62*, 327–335. https://doi.org/10.2337/db12-132662/2/327.
11. Donath, M.Y., Böni-Schnetzler, M., Ellingsgaard, H., and Ehses, J.A. (2009). Islet inflammation impairs the pancreatic beta-cell in type 2 diabetes. Physiology *24*, 325–331. https://doi.org/10.1152/physiol.00032.2009.
12. Camunas-Soler, J., Dai, X.Q., Hang, Y., Bautista, A., Lyon, J., Suzuki, K., Kim, S.K., Quake, S.R., and MacDonald, P.E. (2020). Patch-Seq Links Single-Cell Transcriptomes to Human Islet Dysfunction in Diabetes. Cell Metabol. *31*, 1017–1031.e4. https://doi.org/10.1016/j.cmet.2020.04.005.
13. Blencowe, M., Furterer, A., Wang, Q., Gao, F., Rosenberger, M., Pei, L., Nomoto, H., Mawla, A.M., Huising, M.O., Coppola, G., et al. (2022). IAPP-induced beta cell stress recapitulates the islet transcriptome in type 2 diabetes. Diabetologia *65*, 173–187. https://doi.org/10.1007/s00125-021-05569-2.
14. Perelis, M., Marcheva, B., Ramsey, K.M., Schipma, M.J., Hutchison, A.L., Taguchi, A., Peek, C.B., Hong, H., Huang, W., Omura, C., et al. (2015). Pancreatic beta cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. Science *350*, aac4250. https://doi.org/10.1126/science.aac4250.
15. Rakshit, K., Hsu, T.W., and Matveyenko, A.V. (2016). Bmal1 is required for beta cell compensatory expansion, survival and metabolic adaptation to diet-induced obesity in mice. Diabetologia *59*, 734–743. https://doi.org/10.1007/s00125-015-3859-2.
16. Lee, J., Liu, R., de Jesus, D., Kim, B.S., Ma, K., Moulik, M., and Yechoor, V. (2015). Circadian control of beta-cell function and stress responses. Diabetes Obes. Metabol. *17*, 123–133. https://doi.org/10.1111/dom.12524.
17. Petrenko, V., Stolovich-Rain, M., Vandereycken, B., Giovannoni, L., Storch, K.F., Dor, Y., Chera, S., and Dibner, C. (2020). The core clock transcription factor BMAL1 drives circadian beta-cell proliferation during compensatory regeneration of the endocrine pancreas. Genes Dev. *34*, 1650–1665. https://doi.org/10.1101/gad.343137.120.
18. Javeed, N., Brown, M.R., Rakshit, K., Her, T., Sen, S.K., and Matveyenko, A.V. (2021). Proinflammatory Cytokine Interleukin 1beta Disrupts beta-cell Circadian Clock Function and Regulation of Insulin Secretion. Endocrinology *162*, bqaa084. https://doi.org/10.1210/endocr/bqaa084.
19. Turek, F.W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D.R., et al. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. Science *308*, 1043–1045. https://doi.org/10.1126/science.1108750.
20. Marcheva, B., Ramsey, K.M., Buhr, E.D., Kobayashi, Y., Su, H., Ko, C.H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M.H., et al. (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature *466*, 627–631. https://doi.org/10.1038/nature09253.
21. Allada, R., and Bass, J. (2021). Circadian Mechanisms in Medicine. N. Engl. J. Med. *384*, 550–561. https://doi.org/10.1056/NEJMra1802337.
22. Bass, J., and Lazar, M.A. (2016). Circadian time signatures of fitness and disease. Science *354*, 994–999. https://doi.org/10.1126/science.aah4965.
23. Kim, Y.H., and Lazar, M.A. (2020). Transcriptional Control of Circadian Rhythms and Metabolism: A Matter of Time and Space. Endocr. Rev. *41*, 707–732. https://doi.org/10.1210/endrev/bnaa014.
24. Scheiermann, C., Kunisaki, Y., and Frenette, P.S. (2013). Circadian control of the immune system. Nat. Rev. Immunol. *13*, 190–198. https://doi.org/10.1038/nri3386.
25. Gibbs, J.E., Blaikley, J., Beesley, S., Matthews, L., Simpson, K.D., Boyce, S.H., Farrow, S.N., Else, K.J., Singh, D., Ray, D.W., and Loudon, A.S.I. (2012). The nuclear receptor REV-ERBalpha mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. Proc. Natl. Acad. Sci. USA *109*, 582–587. https://doi.org/10.1073/pnas.1106750109.
26. Early, J.O., Menon, D., Wyse, C.A., Cervantes-Silva, M.P., Zaslona, Z., Carroll, R.G., Palsson-McDermott, E.M., Angiari, S., Ryan, D.G., Corcoran, S.E., et al. (2018). Circadian clock protein BMAL1 regulates IL-1beta in macrophages via NRF2. Proc. Natl. Acad. Sci. USA *115*, E8460–E8468. https://doi.org/10.1073/pnas.1800431115.
27. Oishi, Y., Hayashi, S., Isagawa, T., Oshima, M., Iwama, A., Shimba, S., Okamura, H., and Manabe, I. (2017). Bmal1 regulates inflammatory responses in macrophages by modulating enhancer RNA transcription. Sci. Rep. *7*, 7086. https://doi.org/10.1038/s41598-017-07100-3.
28. Hong, H.K., Maury, E., Ramsey, K.M., Perelis, M., Marcheva, B., Omura, C., Kobayashi, Y., Guttridge, D.C., Barish, G.D., and Bass, J. (2018). Requirement for NF-kappaB in maintenance of molecular and behavioral circadian rhythms in mice. Genes Dev. *32*, 1367–1379. https://doi.org/10.1101/gad.319228.118.
29. Spranger, J., Kroke, A., Möhlig, M., Hoffmann, K., Bergmann, M.M., Ristow, M., Boeing, H., and Pfeiffer, A.F.H. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes *52*, 812–817. https://doi.org/10.2337/diabetes.52.3.812.
30. Grossmann, V., Schmitt, V.H., Zeller, T., Panova-Noeva, M., Schulz, A., Laubert-Reh, D., Juenger, C., Schnabel, R.B., Abt, T.G.J., Laskowski, R., et al. (2015). Profile of the Immune and Inflammatory Response in Individuals With Prediabetes and Type 2 Diabetes. Diabetes Care *38*, 1356–1364. https://doi.org/10.2337/dc14-3008.
31. Westwell-Roper, C.Y., Ehses, J.A., and Verchere, C.B. (2014). Resident macrophages mediate islet amyloid polypeptide-induced islet IL-1beta production and beta-cell dysfunction. Diabetes *63*, 1698–1711. https://doi.org/10.2337/db13-0863.
32. Masters, S.L., Dunne, A., Subramanian, S.L., Hull, R.L., Tannahill, G.M., Sharp, F.A., Becker, C., Franchi, L., Yoshihara, E., Chen, Z., et al. (2010). Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. Nat. Immunol. *11*, 897–904. https://doi.org/10.1038/ni.1935.
33. Munoz-Espin, D., and Serrano, M. (2014). Cellular senescence: from physiology to pathology. Nat. Rev. Mol. Cell Biol. *15*, 482–496. https://doi.org/10.1038/nrm3823.
34. Schulman, I.G. (2017). Liver X receptors link lipid metabolism and inflammation. FEBS Lett. *591*, 2978–2991. https://doi.org/10.1002/1873-3468.12702.
35. Yagishita, Y., Uruno, A., Chartoumpekis, D.V., Kensler, T.W., and Yamamoto, M. (2019). Nrf2 represses the onset of type 1 diabetes in non-obese diabetic mice. J. Endocrinol. *240*, 403–416. https://doi.org/10.1530/JOE-18-0355.
36. Poli, V. (1998). The role of C/EBP isoforms in the control of inflammatory and native immunity functions. J. Biol. Chem. *273*, 29279–29282. https://doi.org/10.1074/jbc.273.45.29279.
37. Storch, K.F., Paz, C., Signorovitch, J., Raviola, E., Pawlyk, B., Li, T., and Weitz, C.J. (2007). Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell *130*, 730–741. https://doi.org/10.1016/j.cell.2007.06.045.
38. Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature *429*, 41–46. https://doi.org/10.1038/nature02520.
39. Rakshit, K., Qian, J., Gaonkar, K.S., Dhawan, S., Colwell, C.S., and Matveyenko, A.V. (2018). Postnatal Ontogenesis of the Islet Circadian Clock Plays a Contributory Role in beta-Cell Maturation Process. Diabetes *67*, 911–922. https://doi.org/10.2337/db17-0850.
40. Nordmann, T.M., Dror, E., Schulze, F., Traub, S., Berishvili, E., Barbieux, C., Böni-Schnetzler, M., and Donath, M.Y. (2017). The Role of Inflammation in beta-cell Dedifferentiation. Sci. Rep. *7*, 6285. https://doi.org/10.1038/s41598-017-06731-w.
41. Ernst, J., and Bar-Joseph, Z. (2006). STEM: a tool for the analysis of short time series gene expression data. BMC Bioinf. *7*, 191. https://doi.org/10.1186/1471-2105-7-191.
42. Martins Pecanha, F.L., Jaafar, R., Werneck-de-Castro, J.P., Apostolopolou, C.C., Bhushan, A., and Bernal-Mizrachi, E. (2022). The Transcription Factor YY1 Is Essential for Normal DNA Repair and Cell Cycle in Human and Mouse beta-Cells. Diabetes *71*, 1694–1705. https://doi.org/10.2337/db21-0908.
43. Eizirik, D.L., Colli, M.L., and Ortis, F. (2009). The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nat. Rev. Endocrinol. *5*, 219–226. https://doi.org/10.1038/nrendo.2009.21.
44. Dotta, F., Censini, S., van Halteren, A.G.S., Marselli, L., Masini, M., Dionisi, S., Mosca, F., Boggi, U., Muda, A.O., Del Prato, S., et al. (2007). Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc. Natl. Acad. Sci. USA *104*, 5115–5120. https://doi.org/10.1073/pnas.0700442104.
45. Poitout, V., and Robertson, R.P. (2007). Glucolipotoxicity: Fuel Excess and {beta}-Cell Dysfunction. Endocr. Rev. *29*, 351–366.
46. Santin, I., and Eizirik, D.L. (2013). Candidate genes for type 1 diabetes modulate pancreatic islet inflammation and beta-cell apoptosis. Diabetes Obes. Metabol. *15*, 71–81. https://doi.org/10.1111/dom.12162.
47. Javeed, N., and Matveyenko, A.V. (2018). Circadian Etiology of Type 2 Diabetes Mellitus. Physiology *33*, 138–150. https://doi.org/10.1152/physiol.00003.2018.
48. Rakshit, K., and Matveyenko, A.V. (2021). Induction of Core Circadian Clock Transcription Factor Bmal1 Enhances beta-Cell Function and Protects Against Obesity-Induced Glucose Intolerance. Diabetes *70*, 143–154. https://doi.org/10.2337/db20-0192.

49. Petrenko, V., Gandasi, N.R., Sage, D., Tengholm, A., Barg, S., and Dibner, C. (2020). In pancreatic islets from type 2 diabetes patients, the dampened circadian oscillators lead to reduced insulin and glucagon exocytosis. Proc. Natl. Acad. Sci. USA *117*, 2484–2495. https://doi.org/10.1073/pnas.1916539117.

50. Fan, R., Peng, X., Xie, L., Dong, K., Ma, D., Xu, W., Shi, X., Zhang, S., Chen, J., Yu, X., and Yang, Y. (2022). Importance of Bmal1 in Alzheimer's disease and associated aging-related diseases: Mechanisms and interventions. Aging Cell *21*, e13704. https://doi.org/10.1111/acel.13704.

51. Yao, H., Sundar, I.K., Huang, Y., Gerloff, J., Sellix, M.T., Sime, P.J., and Rahman, I. (2015). Disruption of Sirtuin 1-Mediated Control of Circadian Molecular Clock and Inflammation in Chronic Obstructive Pulmonary Disease. Am. J. Respir. Cell Mol. Biol. *53*, 782–792. https://doi.org/10.1165/rcmb.2014-0474OC.

52. Alvarez-Dominguez, J.R., Donaghey, J., Rasouli, N., Kenty, J.H., Helman, A., Charlton, J., Straubhaar, J.R., Meissner, A., and Melton, D.A. (2020). Circadian Entrainment Triggers Maturation of Human In Vitro Islets. Cell Stem Cell *26*, 108–122.e10. https://doi.org/10.1016/j.stem.2019.11.011.

53. Baumel-Alterzon, S., Katz, L.S., Brill, G., Jean-Pierre, C., Li, Y., Tse, I., Biswal, S., Garcia-Ocaña, A., and Scott, D.K. (2022). Nrf2 Regulates beta-Cell Mass by Suppressing beta-Cell Death and Promoting beta-Cell Proliferation. Diabetes *71*, 989–1011. https://doi.org/10.2337/db21-0581.

54. Pekovic-Vaughan, V., Gibbs, J., Yoshitane, H., Yang, N., Pathiranage, D., Guo, B., Sagami, A., Taguchi, K., Bechtold, D., Loudon, A., et al. (2014). The circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant defense pathway to modulate pulmonary fibrosis. Genes Dev. *28*, 548–560. https://doi.org/10.1101/gad.237081.113.

55. Chhunchha, B., Kubo, E., and Singh, D.P. (2020). Clock Protein Bmal1 and Nrf2 Cooperatively Control Aging or Oxidative Response and Redox Homeostasis by Regulating Rhythmic Expression of Prdx6. Cells *9*, 1861. https://doi.org/10.3390/cells9081861.

56. Weidemann, B.J., Marcheva, B., Kobayashi, M., Omura, C., Newman, M.V., Kobayashi, Y., Waldeck, N.J., Perelis, M., Lantier, L., McGuinness, O.P., et al. (2024). Repression of latent NF-kappaB enhancers by PDX1 regulates beta cell functional heterogeneity. Cell Metabol. *36*, 90–102. https://doi.org/10.1016/j.cmet.2023.11.018.

57. Brown, M.R., Sen, S.K., Mazzone, A., Her, T.K., Xiong, Y., Lee, J.H., Javeed, N., Colwell, C.S., Rakshit, K., LeBrasseur, N.K., et al. (2021). Time-restricted feeding prevents deleterious metabolic effects of circadian disruption through epigenetic control of beta cell function. Sci. Adv. *7*, eabg6856. https://doi.org/10.1126/sciadv.abg6856.

58. Hohmeier, H.E., Mulder, H., Chen, G., Henkel-Rieger, R., Prentki, M., and Newgard, C.B. (2000). Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. Diabetes *49*, 424–430.

59. Ohara-Imaizumi, M., Cardozo, A.K., Kikuta, T., Eizirik, D.L., and Nagamatsu, S. (2004). The cytokine interleukin-1beta reduces the docking and fusion of insulin granules in pancreatic beta-cells, preferentially decreasing the first phase of exocytosis. J. Biol. Chem. *279*, 41271–41274. https://doi.org/10.1074/jbc.C400360200.

60. Burke, S.J., Batdorf, H.M., Burk, D.H., Martin, T.M., Mendoza, T., Stadler, K., Alami, W., Karlstad, M.D., Robson, M.J., Blakely, R.D., et al. (2018). Pancreatic deletion of the interleukin-1 receptor disrupts whole body glucose homeostasis and promotes islet beta-cell de-differentiation. Mol. Metabol. *14*, 95–107. https://doi.org/10.1016/j.molmet.2018.06.003.

61. Nunemaker, C.S. (2016). Considerations for Defining Cytokine Dose, Duration, and Milieu That Are Appropriate for Modeling Chronic Low-Grade Inflammation in Type 2 Diabetes. J. Diabetes Res. *2016*, 2846570. https://doi.org/10.1155/2016/2846570.

62. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. *50*, 537–546.

63. Zhong, J., Ye, Z., Lenz, S.W., Clark, C.R., Bharucha, A., Farrugia, G., Robertson, K.D., Zhang, Z., Ordog, T., and Lee, J.H. (2017). Purification of nanogram-range immunoprecipitated DNA in ChIP-seq application. BMC Genom. *18*, 985. https://doi.org/10.1186/s12864-017-4371-5.

64. Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. *7*, 562–578. https://doi.org/10.1038/nprot.2012.016.

65. Pertea, M., Pertea, G.M., Antonescu, C.M., Chang, T.C., Mendell, J.T., and Salzberg, S.L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat. Biotechnol. *33*, 290–295. https://doi.org/10.1038/nbt.3122.

66. Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140.

67. Rakshit, K., Qian, J., Ernst, J., and Matveyenko, A.V. (2016). Circadian variation of the pancreatic islet transcriptome. Physiol. Genom. *48*, 677–687. https://doi.org/10.1152/physiolgenomics.00019.2016.

68. Huang, D.W., Sherman, B.T., Tan, Q., Collins, J.R., Alvord, W.G., Roayaei, J., Stephens, R., Baseler, M.W., Lane, H.C., and Lempicki, R.A. (2007). The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol. *8*, R183. https://doi.org/10.1186/gb-2007-8-9-r183.

69. Huang, D.W., Sherman, B.T., Tan, Q., Kir, J., Liu, D., Bryant, D., Guo, Y., Stephens, R., Baseler, M.W., Lane, H.C., and Lempicki, R.A. (2007). DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res. *35*, W169–W175. https://doi.org/10.1093/nar/gkm415.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
ALDH1A3 Antibody, Rabbit polyclonal (IF 1:200)	Novus Biologicals	Cat# NBP2-15339; RRID: AB_3262785
BMAL1 Antibody, Rabbit polyclonal (IF 1:1000)	Abcam	Cat# ab3350; RRID: AB_303729
CLOCK Antibody, Rabbit polyclonal	Abcam	Cat# ab3517; RRID: AB_303866
CRE Antibody, Rabbit purified (IF 1:1000)	Novagen	Cat# 69050; RRID: AB_2314229
Insulin Antibody, Guinea pig polyclonal (IF 1:100)	Abcam	Cat# ab7842; RRID: AB_306130
Insulin Antibody, Rabbit monoclonal (IF 1:100)	Cell Signaling	Cat# 3014; RRID: AB_2126503
Insulin Antibody, Rat monoclonal (IF 1:100)	R&D Systems	Cat# MAB1417; RRID: AB_2126533
Ki-67 Antibody, Mouse monoclonal (IF 1:40)	BD Pharmingen	Cat# 550609; RRID: AB_393778
NKx-6.1 Antibody, Mouse monoclonal (IF 1:100)	DSHB	Cat# F55A12; RRID: AB_532379
Chemicals, reagents, and recombinant proteins		
Citric acid monohydrate	Sigma-Aldrich	Cat# C1909
Liberase™ TL Research Grade	Roche	Cat# 05401020001
Recombinant Human IL-1β/IL-1F2 Protein	R&D Systems	Cat# 01-LB-005/CF
RPMI 1640 Medium	Gibco	Cat# 11875-093
Sodium citrate	Sigma-Aldrich	Cat# C8532
Streptozotocin	Sigma-Aldrich	Cat# S0130
Sunflower seed oil	Sigma-Aldrich	Cat# S5007
Tamoxifen	Sigma-Aldrich	Cat# T5648
Vectashield® Antifade Mounting Medium with DAPI	Vector Laboratories	Cat# H-1200-10
Critical commercial assays		
DAB Substrate Kit, Peroxidase (HRP), with Nickel, (3,3′-diaminobenzidine)	Vector Laboratories	Cat# SK-4100
In Situ Cell Death Detection Kit, TMR red	Roche	Cat# 12156792910
Mouse Ultrasensitive Insulin ELISA	Alpco	Cat# 80-INSMSU-E10
RNeasy Mini Kit	Qiagen	Cat# 74106
RNase-Free DNase Set	Qiagen	Cat# 79254
TruSeq Stranded mRNA Library Prep Kit v2	Illumina	Cat# RS-122-2001
Deposited data		
RNA-Seq raw and analyzed data	This paper	GEO: GSE278711
Experimental models: Cell lines		
INS-1 832/13	Christopher Newgard Lab	Hohmeier et al.[58]
Experimental models: Organisms/strains		
(Cg)-*Arntl*^{tm1Weit}/J (*Bmal1*$^{fl/fl}$)	Jackson Lab	B6.129S4; RRID:IMSR_JAX:007668
STOCK Tg(Ins2-cre/ERT)1Dam/J (*RIPCre*^{ERT})	Jackson Lab	008122; RRID:IMSR_JAX:008122
Bmal1$^{fl/fl}$ crossed with *RIPCre*$^{ERT/+}$	in house	Rakshit et al.[15]
Software and algorithms		
cisTarget	https://resources.aertslab.org/cistarget/	RRID:SCR_024808
DAVID	https://david.ncifcrf.gov/	RRID:SCR_001881

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
edgeR v.3.1.1	http://bioconductor.org/packages/release/bioc/html/edgeR.html	RRID:SCR_012802
Gene Ontology	https://www.geneontology.org/	RRID:SCR_002811
GraphPad Prism v8.4	https://www.graphpad.com	RRID:SCR_002798
HOMER	http://homer.ucsd.edu/	RRID:SCR_010881
Illumina Real Time Analysis v.2.7.7	https://www.illumina.com/informatics/sequencing-data-analysis.html	RRID:SCR_014332
KEGG curated pathways	https://www.kegg.jp/	RRID:SCR_012773
Reactome	https://reactome.org/	RRID:SCR_003485
Short Time-series Expression Miner v.1.13.13	https://www.cs.cmu.edu/~jernst/stem/	https://doi.org/10.1186/1471-2105-7-191
StringTie v.2.1.3	https://ccb.jhu.edu/software/stringtie/	RRID:SCR_016323
WebGestalt: WEB-based GEne SeT AnaLysis Toolkit	https://www.webgestalt.org/	RRID:SCR_006786
ZEISS ZEN Microscopy Software	https://www.zeiss.com/microscopy/en/products/software/zeiss-zen.html	RRID:SCR_013672

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Cell models

INS-1 832/13 cells were generously provided by Dr. Christopher Newgard (Duke University, Durham, NC). We have authenticated that this cell line exhibits glucose-stimulated insulin secretion, and robust insulin expression by qRT-PCR, western blot, and immunohistochemistry. INS-1 832/13 cells were cultured in the presence or absence of 2 ng mL^{-1} of IL-1β (201-LB-005/CF; R&D Systems, Minneapolis, MN) in RPMI media with L-glutamine, 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 10 mM HEPES, and 50 μM β-mercaptoethanol.

Animal models

Mice homozygous for the floxed *Bmal1* gene (B6.129S4 (Cg)-*Arntl*^{tm1Weit}/J, Jackson Lab, Bar Harbor, ME) were crossed to mice with a tamoxifen-inducible Cre-mediated recombination system driven by rat *Insulin2* promoter (STOCK Tg (Ins2-cre/ERT) 1Dam/J, Jackson Lab, Bar Harbor, ME). Resulting off-springs were genotyped to identify mice that are homozygous for the deletion of *Bmal1* gene in β-cells (*β-Bmal1*$^{-/-}$), or littermate controls with functional *Bmal1* (*β-Bmal1*$^{+/+}$). In total, 100 mice were used in this study including 46 control (34 males +12 females *β-Bmal1*$^{+/+}$) and 54 knockout (41 males +13 females *β-Bmal1*$^{-/-}$). Mice were housed at Mayo Clinic, Rochester animal facilities under standard 12 h light: 12 h dark (LD) cycle. By convention, start of the "lights on" period is denoted as zeitgeber time (ZT) 0 and "lights off" as ZT 12. All experimental procedures were approved by Mayo Clinic Institutional Animal Care and Use Committee (IACUC).

In vivo study design

Mice were fed standard Chow diet (24.7 kcal% protein, 62.1 kcal% carbohydrate, 13.2 kcal% fat; #5053; LabDiet, St. Louis, MO) throughout. For conditional deletion of *Bmal1* gene in β-cells, all experimental mice including control received 3 intraperitoneal (i.p.) injections each containing 4 mg tamoxifen (Sigma-Aldrich, St. Louis, MO) dissolved in sunflower oil (Sigma-Aldrich, St. Louis, MO) on alternate days at 2 months age. To model inflammatory stress in β-cells, a subset of mice from each genotype (32 controls and 40 *β-Bmal1*$^{-/-}$) were given single i.p. injection of 100 mg. Kg^{-1} body weight Streptozotocin (STZ, S0130, Sigma-Aldrich) in 0.1 M Citrate Buffer pH 4.0, freshly prepared by mixing 0.1 M sodium citrate (C8532, Sigma-Aldrich) and 0.1 M citric acid (C1909, Sigma-Aldrich) in 2:3 ratio. The other subset of mice (14 controls and 14 *β-Bmal1*$^{-/-}$) received an equivalent dose of 0.1 M Citrate Buffer alone, referred to as vehicle (VEH).

METHOD DETAILS

Immunofluorescence and endocrine pancreas morphology

Mice were euthanized and pancreas immediately harvested and fixed in 4% paraformaldehyde overnight. To quantify β-cell area, paraffin-embedded pancreatic sections were stained for hematoxylin, insulin (ab7842, 1:100, Abcam, Cambridge, MA, USA) and DAB (SK-4100, Vector Laboratories, Burlingame, CA). For quantification of β-cell proliferation and apoptosis, pancreatic sections were stained with insulin (ab7842, 1:100, Abcam, Cambridge, MA, USA), replication marker Ki67 (550609; 1:40; BD Pharmingen, Franklin Lakes, NJ) and apoptosis marker TUNEL (Roche, Indianapolis, IN). Pancreatic sections were also immunostained with NKX6.1 (F55A12-SN, 1:100, Developmental Studies Hybridoma Bank, Iowa City, IA), BMAL1 (ab3350, 1:1000, Abcam), ALDH1A3 (NBP2-15339, 1:200, Novus Biologicals, Centennial CO), Cre recombinase (69050, 1:1000, Novagen, Madison, WI) and Insulin (C27C9, 1:100, Cell Signaling Technology, Danvers, MA; MAB1417, 1:100, R&D Systems, Minneapolis, MN). Slides were viewed using Axio Observer Z1 microscope (Carl Zeiss Microscopy, White Plains, NY) and LSM 980 confocal

microscope with Airyscan 2 (Carl Zeiss Microscopy, White Plains, NY). Images were acquired and quantified using ZEN Pro software (Carl Zeiss Microscopy).

RNA sequencing

Pancreatic islets were isolated from mice using standard collagenase method and total islet mRNA extracted using RNeasy Kit (Qiagen, Germantown, MD). RNA quality was initially assessed using Qubit fluorometry (ThermoFisher Scientific, Waltham, MA) and the Agilent Fragment Analyzer (Santa Clara, CA). cDNA libraries were prepared using 150 ng of total RNA according to the manufacturer's instructions for the TruSeq Stranded mRNA Sample Prep Kit (Illumina, San Diego, CA). The concentration and size distribution of completed libraries were determined using an Agilent Bioanalyzer DNA 1000 chip and Qubit fluorometry. Libraries were sequenced at six samples per lane following Illumina's standard protocol using the Illumina cBot and HiSeq 3000/4000 PE Cluster Kit. The flow cells were sequenced as 100 x 2 paired end reads on an Illumina HiSeq 4000 using HiSeq 3000/4000 sequencing kit and HD 3.4.0.38 collection software. Base-calling was performed using Illumina Real Time Analysis version 2.7.7.

Chromatin immunoprecipitation-sequencing (ChIP-seq)

INS-1 832/13 cells were cross-linked with 1% formaldehyde for 10 min, followed by quenching with 125 mM glycine for 5 min at room temperature. Fixed cells were washed with TBS and the cell pellets were frozen at −80°C. ChIP-seq was performed.[63] Briefly, cells were resuspended in cell lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 0.5% NP-40) and incubated on ice for 10 min. The lysates were washed with MNase digestion buffer (20 mM Tris-HCl, pH 7.5, 15 mM NaCl, 60 mM KCl, 1 mM $CaCl_2$) and incubated for 20 min at 37°C in the presence of MNase. After adding the same volume of sonication buffer (100 mM Tris-HCl, pH 8.1, 20 mM EDTA, 200 mM NaCl, 2% Triton X-100, 0.2% Sodium deoxycholate), the lysate was sonicated for 5 min (30 sec-on/30 sec-off) in Diagenode bioruptor and centrifuged at 15,000 rpm for 10 min. The cleared supernatant equivalent to about 40×10^6 cells was incubated with 5 μg of anti-BMAL1 (Abcam, ab3350) or anti-CLOCK (Abcam, ab3517) antibodies overnight. After adding 30 μL of prewashed protein G agarose beads, the reactions were further incubated for 3 h. The beads were extensively washed with ChIP buffer, high salt buffer, $LiCl_2$ buffer, and TE buffer. Bound chromatins were eluted and reverse-crosslinked at 65°C overnight. DNAs were purified using Min-Elute PCR purification kit (Qiagen) after treatment of RNase A and proteinase K. The enrichment was analyzed by targeted real-time PCR in *Per2* and negative genomic loci. For next-generation sequencing, ChIP-seq libraries were prepared from 5 to 10 ng of ChIP and input DNAs with the ThruPLEX DNA-seq Kit V2 (Rubicon Genomics, Ann Arbor, MI). The ChIP-seq libraries were sequenced to 51 base pairs from both ends using the Illumina HiSeq 4000 in the Mayo Clinic Center for Individualized Medicine Medical Genomics Facility. Paired-end reads were mapped to the rat reference genome (release rn6) by BWA with default settings, and only uniquely mapped reads remained for further analysis. Peaks were called and annotated using the HOMER algorithm for CLOCK and BMAL1 using default settings. Differential analysis was performed using HOMER's getDifferentialPeaks function. Differentially enriched loci were identified using a fold change >1.5, false discovery rate <0.001, and cumulative Poisson *p*-value < 0.05. Functional enrichment analysis was performed using DAVID and WebGestalt to determine enrichment of Biological Process terms and Reactome pathways of identified gene targets, respectively. Motif analysis of binding sites and/or identified gene targets was performed using iCisTarget using default settings.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analytical methods

Blood glucose was measured with FreeStyle Lite Blood Glucose measuring system (Abbott Laboratories, Abbott Park, IL). Plasma insulin was measured using Mouse Insulin ELISA (Alpco Diagnostics, Salem, NH) as per manufacturer's instructions.

Informatics, statistical analysis, and calculations

RNA-seq libraries were aligned to the mouse genome mm10 using TopHat[64] and assembled using StringTie.[65] Gene expression was normalized to counts per million (CPM) and genes that were expressed ≥1 CPM in at least 2 replicates were filtered prior to differential analysis. Differentially expressed genes were identified using default settings in edgeR.[66] Fold change cut off was set at 2 ($log_2 = 1$, $p < 0.05$). Short Time-series expression miner (STEM) analysis of temporal RNA-seq data was performed to identify statistically significant chronically regulated gene expression profiles.[41,67] Briefly, log ratios of gene expression data relative to vehicle (t = 0) was calculated. Using default settings, significantly time-dependant transcripts were identified and assigned to a corresponding temporal profile model by STEM (FC > 2, FDR <0.05, R > 0.7). Differentially expressed and time-dependant gene lists were subjected to Gene Ontology (GO) enrichment analysis using DAVID.[68,69] GO pathways with $p < 0.05$ were considered to be significantly enriched. Statistical analysis was performed using ANOVA with post hoc tests wherever appropriate (GraphPad Prism v.8.4, San Diego, CA). Data in graphs are presented as means ± SEM and assumed statistically significant at $p < 0.05$.