Supplemental Experimental Procedures

Protein Gel Electrophoresis and Immunoblotting

MEF and C2C12 whole cell lysates were prepared in CelLytic™ MT Mammalian Tissue Lysis Reagent (Sigma) supplemented with protease inhibitors. Snap-frozen mouse skeletal muscle tissue was diluted in CelLytic™ MT Mammalian Tissue Lysis Reagent supplemented with protease inhibitors and homogenized using a TissueLyser II apparatus (Qiagen). Protein levels were quantified using DC Protein Assay (Bio-Rad), and protein extracts were subject to SDS-PAGE gel electrophoresis and transferred to nitrocellulose membranes (GE Healthcare). Primary antibodies used were anti-HIF1α (Novus Biologicals) and anti-β-actin (Cell Signaling).

Quantitative Real-Time PCR

Total RNA was extracted from C2C12 and MEF cells and snap-frozen mouse gastrocnemius muscle tissue using Tri-Reagent (Molecular Research Center, Inc). For muscle tissue samples, an entire gastrocnemius muscle from each mouse was homogenized using a TissueLyser II apparatus (Qiagen). cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time PCR was performed and analyzed using a CFX384 (Bio-Rad). PCR conditions were: 10 min at 95°C, then 35 cycles of 10 s at 95°C, 15 s at 60°C. Relative expression levels (normalized to *18S rRNA* (C2C12) or *β-actin* (skeletal muscle and MEF)) were determined using the comparative CT method.

Oxygen Consumption Rate (OCR) and Extracellular Medium Acidification Rate (ECAR) Measurements in Muscle Mitochondria

Whole soleus and gastrocnemius muscles were excised and placed in cold 1XPBS containing 10 mM EDTA. Muscle tissue was minced and incubated 30 minutes on ice in the presence of 0.01% trypsin-EDTA (Gibco) before dounce homogenization and differential centrifugation to isolate mitochondrial fractions. Isolated mitochondria (10 μg protein) were plated on Seahorse Biosciences 96-well culture plates approximately 10 minutes before analysis. OCR and ECAR were measured before and after sequential addition of 10 μM oligomycin and 10 μM

carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (for OCR wells) or 10 mM glucose and 10 μM oligomycin (for ECAR wells).

Luciferase Assays

Undifferentiated C2C12 myoblasts were transfected with plasmids expressing either the PER2:LUCIFERASE (Liu et al., 2008; Welsh et al., 2004) or HRE-luciferase reporters (kindly provided by Dr. Navdeep Chandel) (Emerling et al., 2008), as well as plasmids expressing genes encoding CLOCK, BMAL1, HIF1α, and ARNT (HIF1β). 48 hours after transfection, cells were assayed for luciferase activity using the Dual Luciferase Assay Kit (Promega).

Cycloheximide Experiment

Primary WT and *Bmal1*$^{-/-}$ MEF cells were treated for 6 hours with 100 μM $CoCl_2$ followed by removal of $CoCl_2$ and addition of 100 mg/ml cycloheximide (Sigma). Cells were then collected at 5', 15', 30', 60' and 90' post addition of cycloheximide and protein extracts were subjected SDS-PAGE and immunoblotting analysis.

C2C12 Myotube Synchronization

Confluent dishes of C2C12 myoblasts were first differentiated with 2% horse serum two days prior to cell synchronization. Cells were synchronized by serum shock every 4 hours for 44 hours with 50% horse serum as previously described (Zhang et al., 2012). Twenty-four hours after the final shock, cells were collected and analyzed for mRNA expression and/or analyzed for ECAR. For PER2:LUCIFERASE reporter assays, C2C12 myoblast cells were first infected with PER2:LUC-expressing lentivirus (gift of A. Liu, University of Memphis) (Liu et al., 2008) and maintained in DMEM that included 10% FBS and 2.5 μg/ml blastocidin to select for stable *Per2-luc* integration. Myoblasts were differentiated for 5 days with 2% horse serum, then synchronized in 50% horse serum for 2 hours. Myoblasts were treated with either 125 μM Dimethyloxalylglycine (DMOG) (Sigma) or DMSO (vehicle) in DMEM plus 0.1 mM luciferin and 0.5% horse serum. Luciferase activity was continuously monitored using a LumiCycle apparatus (Actimetrics).

Chromatin Immunoprecipitation (ChIP)

ChIP methods were adapted from previously described experimental procedures (Barish et al., 2010). Briefly, C2C12 myotubes were dual-crosslinked with Disuccinimidyl glutarate followed by 1% formaldehyde. Nuclei were isolated via needle lysis in IP buffer, and chromatin was sheared by sonication followed by incubation overnight with either anti-HIF1α antibody (Novus Biologicals) or anti-BMAL1 antibody (Perelis et al., 2015) followed by 2 hours with protein A Agarose beads (Millipore). Beads were washed 6 times followed by de-crosslinking using Chelex beads (Sigma) and proteinase K (Invitrogen) digestion. Eluted immunoprecipitated DNA and input DNA were purified by Qiaprep mini-elute reagents and subjected to qPCR analysis using primers specific to known CLOCK/BMAL1 E-box target sites.

Mouse Embryonic Fibroblast (MEF) Isolation

MEFs were isolated as previously described (Peek et al., 2013). Pregnant mice were sacrificed at pcd 14-15, and embryos were removed and placed in 1XPBS. Heads and internal organs were removed, and blood was washed away from the remaining carcasses with 1XPBS. Tissue was minced in Trypsin-EDTA, followed by incubation with stirring for 30 minutes. Trypsin was neutralized with heat-inactivated FBS, and cells were collected by centrifugation then resuspended in DMEM containing 15% FBS and plated at $1x10^6$ cells per 100 mm dish. Media was changed 24 hours after plating. For *CAG-Cre-ER; Vhl*$^{fx/fx}$ and control MEFs, cells were treated with 1 μM tamoxifen (Sigma) for 24 hours followed by 24 hours without tamoxifen before collection.

Mouse Treadmill Exercise Experiments

3-4 month old male C57B6L/J mice were maintained on a 12:12 light-dark cycle and fed a regular chow diet *ad libitum* throughout their lives. During the exercise experiment, both the exercised mice and control mice did not have access to food. Each mouse was familiarized with the treadmill (Columbus Instruments) by acclimation at a low running speed for a total of 15 min (starting at 3 m/min, accelerated 1 m/min until 6 m/min were reached, which was then maintained for an additional 10 min) on three consecutive days prior to our experiments. On the fourth day, mice were placed on the treadmill for 3 min at 6 m/min. After 3 min, the treadmill speed was increased by 1 m/min every minute until exhaustion, which was defined as more than 10 falls onto the stimulus grid per minute. Control mice were placed in a new cage while exercised mice were on the treadmill. At exhaustion, mice were immediately

removed from the treadmill and euthanized by CO_2 inhalation that took approximately 5 minutes, followed by cervical dislocation. Whole gastrocnemius muscle was then excised within 3-5 minutes and snap-frozen in liquid nitrogen. All mice were exposed to equal lengths of time in CO_2 prior to tissue collection to control for effects of CO_2 exposure during euthanasia on HIF activity and oxygenic metabolism.

References Cited:

Barish, G.D., Yu, R.T., Karunasiri, M., Ocampo, C.B., Dixon, J., Benner, C., Dent, A.L., Tangirala, R.K., and Evans, R.M. (2010). Bcl-6 and NF-kappaB cistromes mediate opposing regulation of the innate immune response. Genes Dev *24*, 2760-2765.

Edgar, R.C. (2004a). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC bioinformatics *5*, 113.

Edgar, R.C. (2004b). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res *32*, 1792-1797.

Emerling, B.M., Weinberg, F., Liu, J.L., Mak, T.W., and Chandel, N.S. (2008). PTEN regulates p300-dependent hypoxia-inducible factor 1 transcriptional activity through Forkhead transcription factor 3a (FOXO3a). Proc Natl Acad Sci U S A *105*, 2622-2627.

Liu, A.C., Tran, H.G., Zhang, E.E., Priest, A.A., Welsh, D.K., and Kay, S.A. (2008). Redundant function of REV-ERBalpha and beta and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms. PLoS Genet *4*, e1000023.

Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C.*, et al.* (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science *342*, 1243417.

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.

Welsh, D.K., Yoo, S.H., Liu, A.C., Takahashi, J.S., and Kay, S.A. (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. Curr Biol *14*, 2289-2295.

Zhang, X., Patel, S.P., McCarthy, J.J., Rabchevsky, A.G., Goldhamer, D.J., and Esser, K.A. (2012). A non-canonical E-box within the MyoD core enhancer is necessary for circadian expression in skeletal muscle. Nucleic Acids Res *40*, 3419-3430.

Supplemental Figure Legends

Fig S1 (related to Figure 1). CRISPR-Cas9 gene editing eliminates *Bmal1* in C2C12 myotubes. (A) Immunoblot of BMAL1 and β-actin from WT and *Bmal1*$^{-/-}$ C2C12 myotubes (n=2). **(B)** Expression of *Bmal1* mRNA and BMAL1 target genes in WT vs *Bmal1*$^{-/-}$ C2C12 myotubes (n=7-14). **(C)** OCR in mitochondria isolated from soleus and gastrocnemius muscles of WT and *Bmal1*$^{-/-}$ mice in the presence of palmitoyl-carnitine treated with FCCP ZT8 (n=5-6). **(D)** Immunoblots of HIF1α and β-actin in WT vs *Bmal1*$^{-/-}$ and schematic of experimental design (left). Quantification of relative HIF1α remaining after removal of $CoCl_2$ and addition of cycloheximide (right). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

Fig S2 (related to Figure 1). *In vitro* circadian oscillations of ECAR with glucose in synchronized C2C12 myotubes. (A) Differentiated C2C12 myotubes were synchronized with 50% horse serum and ECAR was measured every 4 hours from 24-48 hours after serum treatment. At each time point, ECAR was measured after addition of glucose (red) followed by the respiratory chain inhibitor oligomycin (green) (n=7-9). **(B)** Relative gene expression of *Bmal1* and canonical BMAL1 target genes in adult-life inducible skeletal muscle *Bmal1* knockout mice (*ACTA-rtTA-TRE-Cre;Bmal*$^{fx/fx}$) and controls (*ACTA-rtTA-TRE-Cre* and *Bmal*$^{fx/fx}$) (n=4-5). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

Fig S3 (related to Figures 1-2). Protein sequence alignment of mouse BMAL1 and mouse HIF1β (ARNT). (A) Full-protein sequences were aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE) software available through the European Bioinformatics Institute (EMBL; http://www.ebi.ac.uk) (Edgar, 2004b)(Edgar, 2004a). bHLH and PAS domains are highlighted. Consensus symbols indicate complete identity (*), strong similarity (scoring >0.5 in the Gonnet PAM 250 matrix) (:), and weak similarity (scoring <0.5 in the Gonnet PAM 250 matrix) (.) (http://www.ebi.ac.uk/Tools/msa/clustalw2/help/index.html). **(B)** Schematic of BMAL1 and HIF1β (ARNT) proteins with % identity in the bHLH and PAS domains regions (not to scale).

Fig S4 (related to Figure 2). Hypoxic stress disrupts circadian gene expression in mouse embryonic fibroblasts. Relative gene expression of core clock target genes in MEFs exposed to 100 μM $CoCl_2$ for 6 hours vs untreated conditions (n=6). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

Figure S1

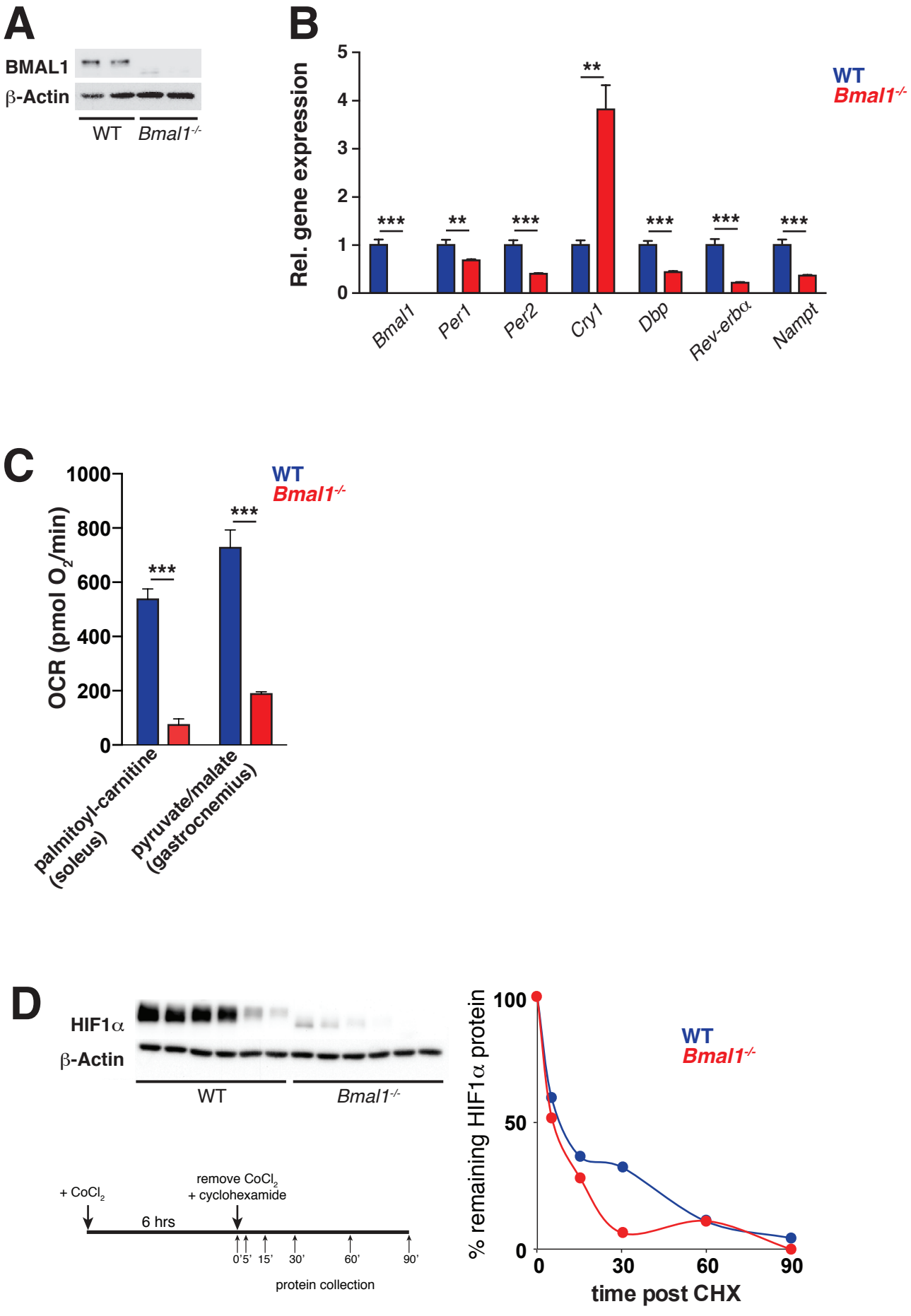
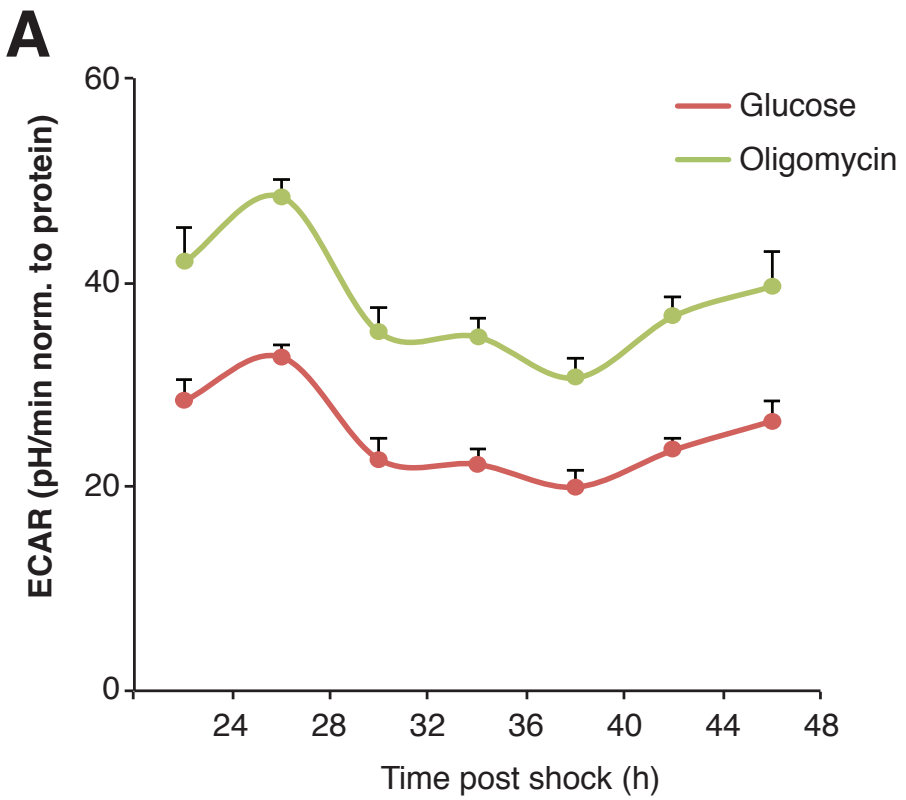

A
BMAL1
β-Actin
WT
Bmal1-/-
B
Rel. gene expression
WT
Bmal1-/-
Bmal1
Per1
Per2
Cry1
Dbp
Rev-erbα
Nampt
C
OCR (pmol O2/min)
WT
Bmal1-/-
palmitoyl-carnitine (soleus)
pyruvate/malate (gastrocnemius)
D
HIF1α
β-Actin
WT
Bmal1-/-
+ CoCl2
remove CoCl2
+ cyclohexamide
6 hrs
0'
5'
15'
30'
60'
90'
protein collection
% remaining HIF1α protein
WT
Bmal1-/-
time post CHX

Figure S2


A
ECAR (pH/min norm. to protein)
60
40
20
0
24
28
32
36
40
44
48
Time post shock (h)
Glucose
Oligomycin

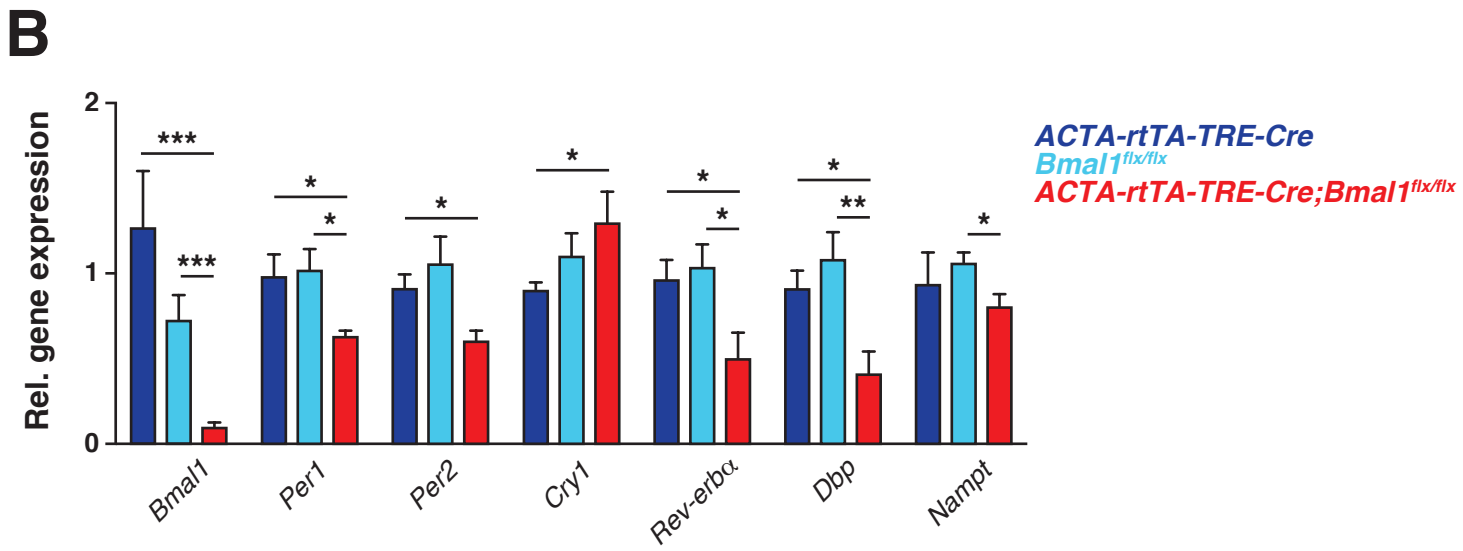

B
Rel. gene expression
2
1
0
***
***
*
*
*
*
*
*
*
**
*
Bmal1
Per1
Per2
Cry1
Rev-erbα
Dbp
Nampt
ACTA-rtTA-TRE-Cre
Bmal1flx/flx
ACTA-rtTA-TRE-Cre;Bmal1flx/flx

Figure S3

A

```
mBMAL1   MADQRMDISSTISDFMSPGPTDLLSGSLGT----SGVDCNRK-RKGSATDYQESMDTDKD
mARNT    MAATTAN-PEMTSDVPSLGPT-IASGNPGPGIQGGGAVVQRAIKRRSGLDFDDEVEVNTK
         **    : ..  **. * *** : **. *.    .*.  :*  .. *. *:::.::.:..

mBMAL1   ----------DPHGRLEYAEHQ----GRIKNAREAHSQIEKRRRDKMNSFIDELASLVPT
mARNT    FLRCDDDQMCNDKERFARSDDEQSSADKERLARENHSEIERRRRNKMTAYITELSDMVPT
                  : : *:  :: :    .. . *** **:**.***:**.::* **:.:***

mBMAL1   CNAMSRKLDKLTVLRMAVQHMKTLRGATNPYTEANYKPTFLSDDELKHLILRAADGFLFV
mARNT    CSALARKPDKLTILRMAVSHMKSLRGTGNTSTDGSYKPSFLTDQELKHLILEAADGFLFI
         *.*::** ****:*****.***:***: *. *:..***:**:*:******* *******:

mBMAL1   VGCDRGKILFVSESVFKILNYSQNDLIGQSLFDYLHPKDIAKVKEQLSSSDTAPRERLID
mARNT    VSCETGRVVYVSDSVTPVLNQPQSEWFGSTLYDQVHPDDVDKLREQLSTSENALTGRVLD
         *.*: *.:::**:**  :** .*.: :*.:*:* :**.*: *:.****:*:.*   *::*

mBMAL1   AKTGLPVKTDITPGPSRLCSGARRSFFCRMKCNRPSVKVEDKD----FASTCSKK----K
mARNT    LKTG-TVKKEGQQSSMRMCMGSRRSFICRMRCGTSSVDPVSMNRLSFLRNRCRNGLGSVK
          *** .**.:   .. *:* *:****:***.*. .**.  . :    : . * :     *

mBMAL1   ADRKSFCTIHSTGYLKSWPPTKMGLDEDNEPDNEGCNLSCLVAIGRLHSHMVPQPANGEI
mARNT    EGEPHFVVVHCTGYIKAWPPAGVSLPDDDPEAGQGSKF-CLVAIGRLQVTSSPNCTDMSN
          .   * .:*.***:*:***: :.* :*:   .:*.:: ********:    *: :: .

mBMAL1   RVKSMEYVSRHAIDGKFVFVDQRATAILAYLPQELLGTSCYEYFHQDDIGHLAECHRQVL
mARNT    ICQPTEFISRHNIEGIFTFVDHRCVATVGYQPQELLGKNIVEFCHPEDQQLLRDSFQQVV
           :. *::*** *:* *.***:*..* :.* ******..  *: * :*   * :...**:

mBMAL1   QTREKITTNCYKFKIKDGSFITLRSRWFSFMNPWTKEVEYIVSTNTVV----------LA
mARNT    KLKGQVLSVMFRFRSKTREWLWMRTSSFTFQNPYSDEIEYIICTNTNVKNSSQEPRPTLS
         : . :: :  :.*. *  .:: :*:  *:* **::.*:***:.*** *          *:

mBMAL1   NV-------------LEGGDPTFPQ-----------------LTAPPHSMDSMLPSGEG
mARNT    NTIPRSQLGPTANLSLEMGTGQLPSRQQQQQHTELDMVPGRDGLASYNHSQVSVQPVASA
         *.             ** *   :*.                 *::  **  *: * ...

mBMAL1   GPKRTHP---------------------------------TVPG----------IPGGT
mARNT    GSEHSKPLEKSEGLFAQDRDPRFPEIYPSITADQSKGISSSTVPATQQLFSQGSSFPPNP
         *.:.::*                                  ***.          :* ..

mBMAL1   RAG-----------------AGKIGRMIAEEIMEIHRIRGSSPSSCGSSPLNITSTPPPD
mARNT    RPAENFRNSGLTPPVTIVQPSSSAGQILAQISRHSNPAQGSAPTWTSSSRPGFAAQQVPT
         *..                :.. *.::*:     :  .**:*:  .**  .:::   *

mBMAL1   ASS-----------------------PGGKKILNGGT--PDIPSTG--LLPGQAQETPG
mARNT    QATAKTRSSQFGVNNFQTSSSFSAMSLPGAPTASSGTAAYPALPNRGSNFPPETGQTTGQ
          ::                       **. .  .* :  * :*. *  : *  .* *

mBMAL1   YPYSDSSSI------LGENPHIGIDMIDN--DQGSSSPSNDEAAMAVIMSLLEADAGLGG
mARNT    FQARTAEGVGVWPQWQGQQPHHRSSSSEQHVQQTQAQAPSQPEVFQEMLSMLGDQSNTYN
         :    :..:       *::**   .  ::  :* .:....:  .:  ::*:*  ::.  .

mBMAL1   PVDFSDL----PWPL
mARNT    NEEFPDLTMFPPFSE
           :*.**    *:.
```

bHLH domain
PAS domain

* Complete identity
: Strong similarity
. Weak similarity

B

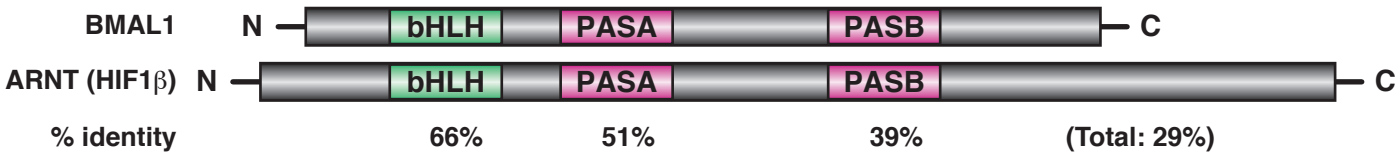
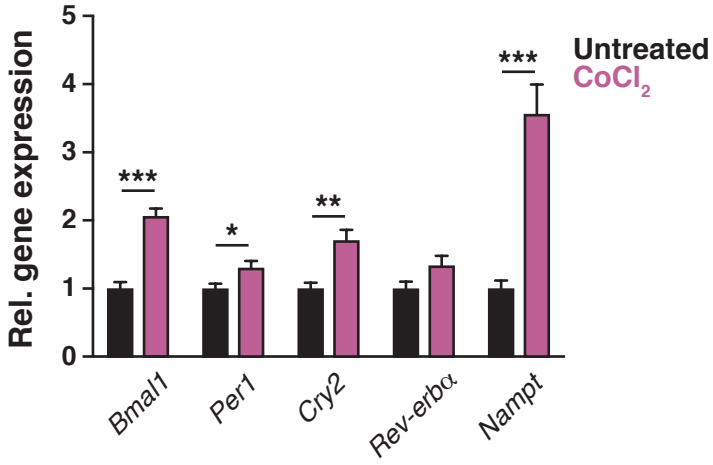


Figure S4


Rel. gene expression
5
4
3
2
1
0
***
*
**
***
Untreated
CoCl2
Bmal1
Per1
Cry2
Rev-erbα
Nampt