SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES

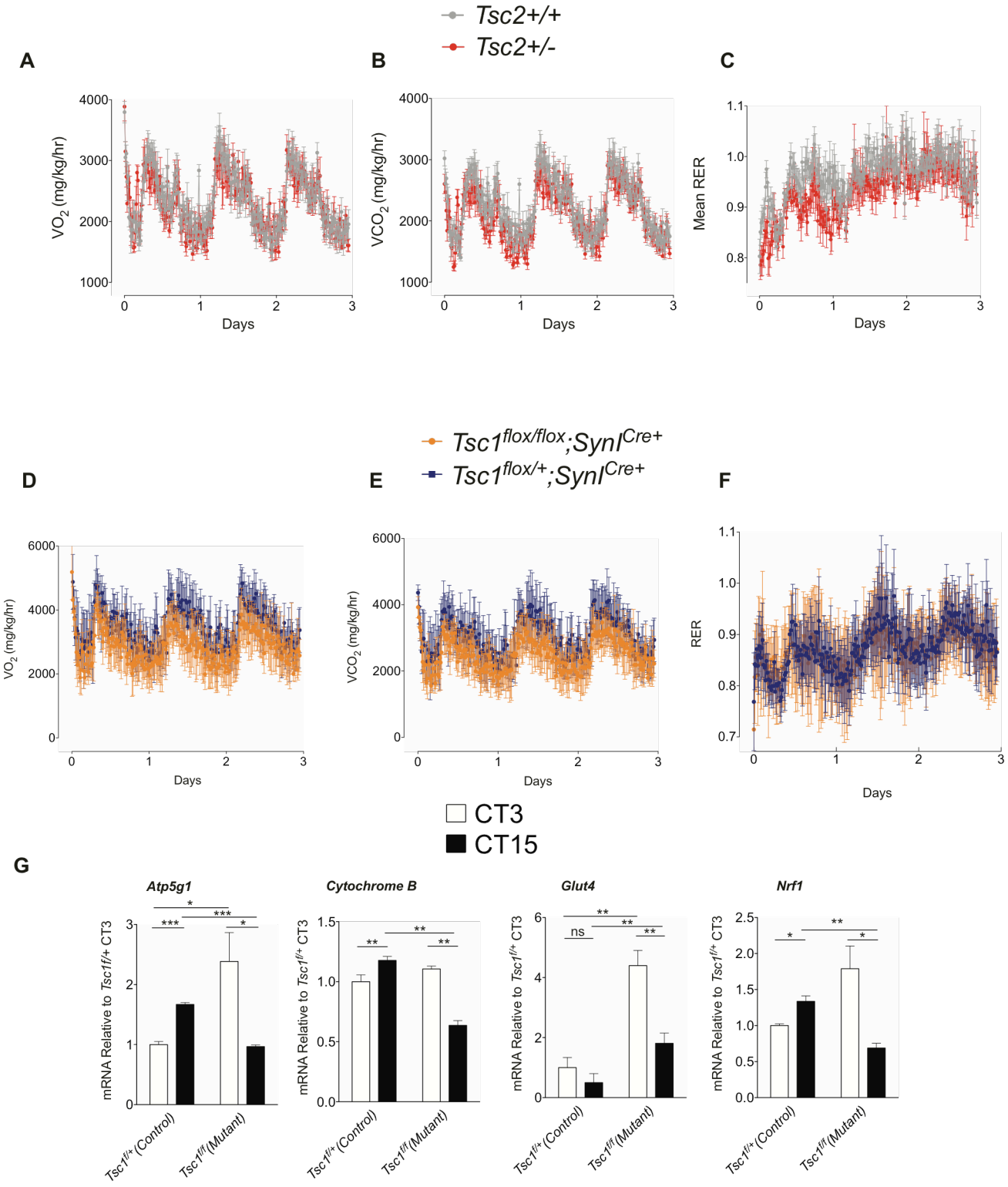

Figure S1. Circadian Respiratory Metabolism and Gene Expression in *Tsc* Mutant Mouse Models. Related to Figures 1 and 2.

Circadian Respiratory Metabolism in *Tsc2* Heterozygote (A-C) or $Tsc1^{flox/flox};Syn1^{Cre}$ (D-F) mice. **(A, D)** Mean oxygen consumption (VO2), **(B, E)** mean carbon dioxide consumption (VCO2), and **(C, F)** respiratory exchange ratio (RER) (2-way ANOVA, Bonferonni post-test not significant for interaction) (n=7-8 per genotype).

(G) Histogram of gene expression measured by quantitative PCR from hippocampi of indicated genotypes at indicated *zeitgeber* times, n=3 per genotype per time point, 2-way ANOVA. *Nrf1*=nuclear respiratory factor 1; *p<0.05, **p<0.01, ***p<0.001.

Error bars indicate mean ± SEM.

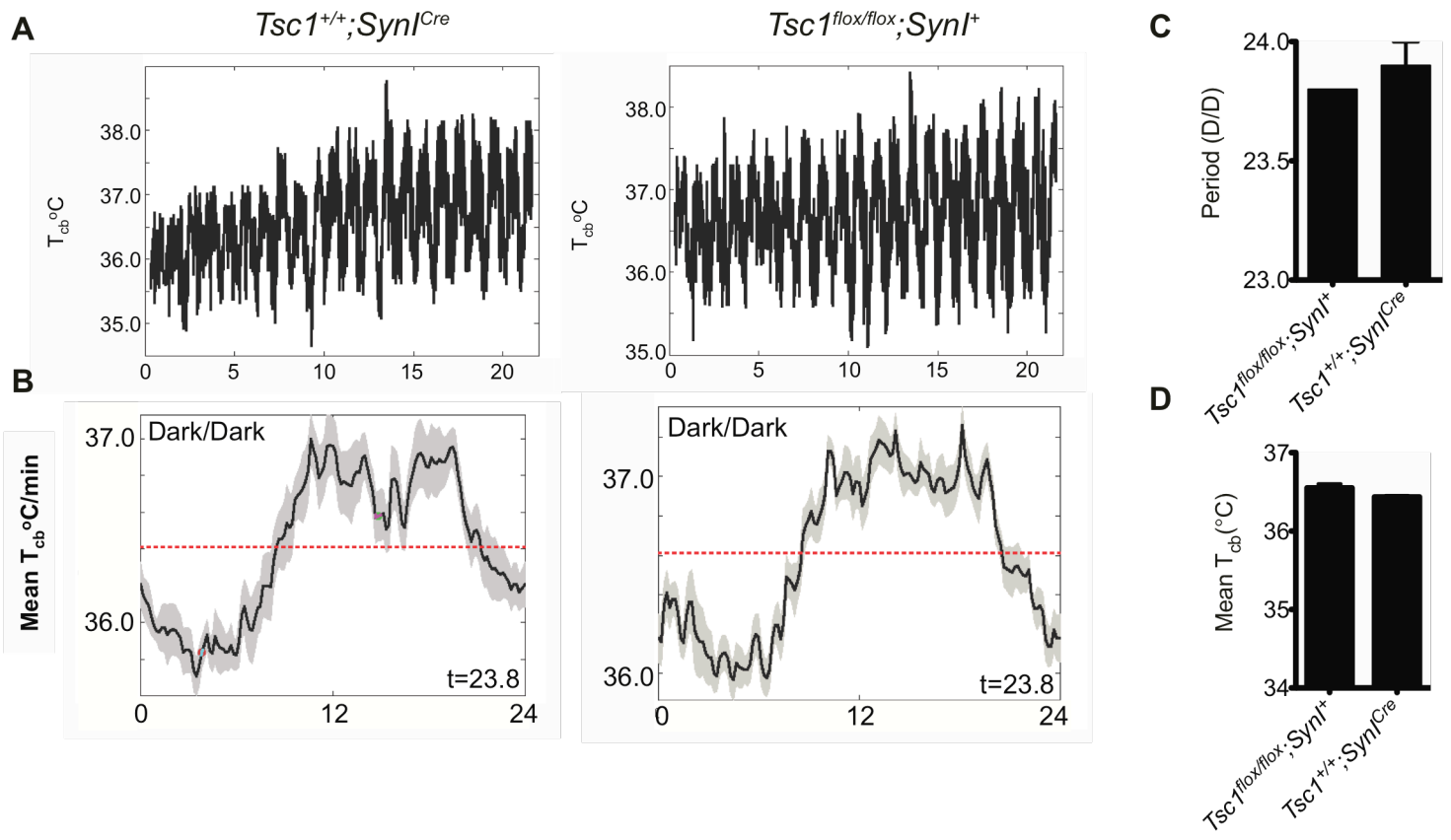

Figure S2. Controls for *SynapsinI*^{Cre} and *Tsc1*^{flox} Alleles. Related to Figure 1.
(A) Mean T_{cb}°C for control mice (with either no *flox* allele or no *Cre* allele).
(B) Mean activity plotted as a function of circadian phase for indicated genotype in constant conditions (dark/dark). Error bars are indicated in grey
(C) Mean circadian period for indicated genotypes.
(D) Mean temperature for indicated genotypes.
Error bars indicate mean ± SEM.

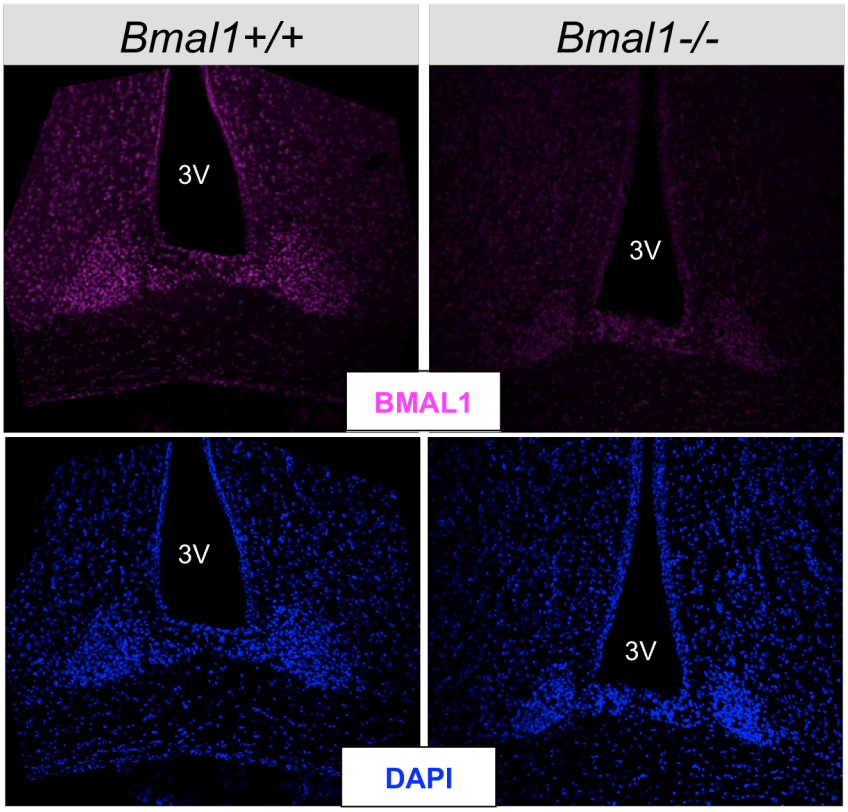

Figure S3. Control Immunohistochemistry for BMAL1 Antibody. Related to Figure 4.
Confocal images of immunohistochemistry of 30μm coronal sections from adult *Bmal1*+/+ and *Bmal1*-/- suprachiasmatic nuclei. 3V= third ventricle. Scale bar = 20μm.

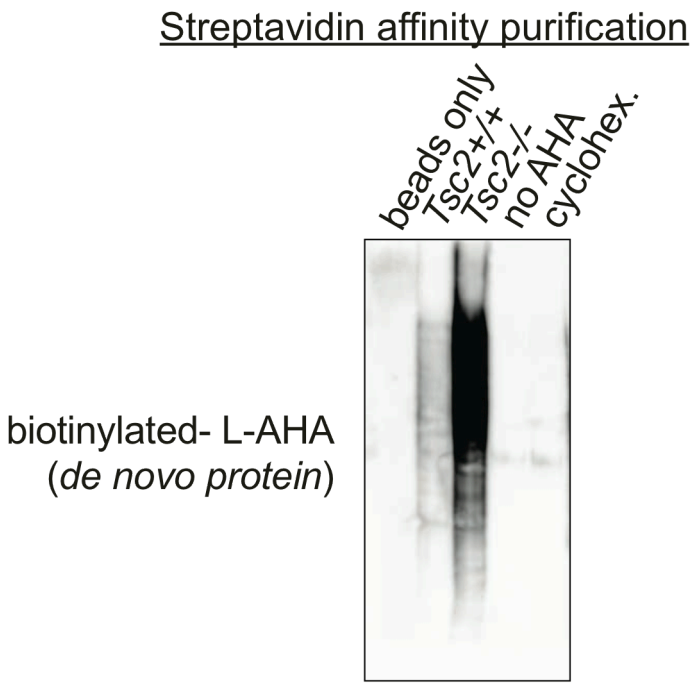

Figure S4. Controls for BONCAT. Related to Figure 5.
Copper-mediated cycloaddition (Click chemistry)-based biotinylation and streptavidin bead affinity purification of *de novo* synthesized proteins in wildtype MEFs treated with cycloheximide or in the absence of non-canonical methionine analog (L-azidohomoalanine).

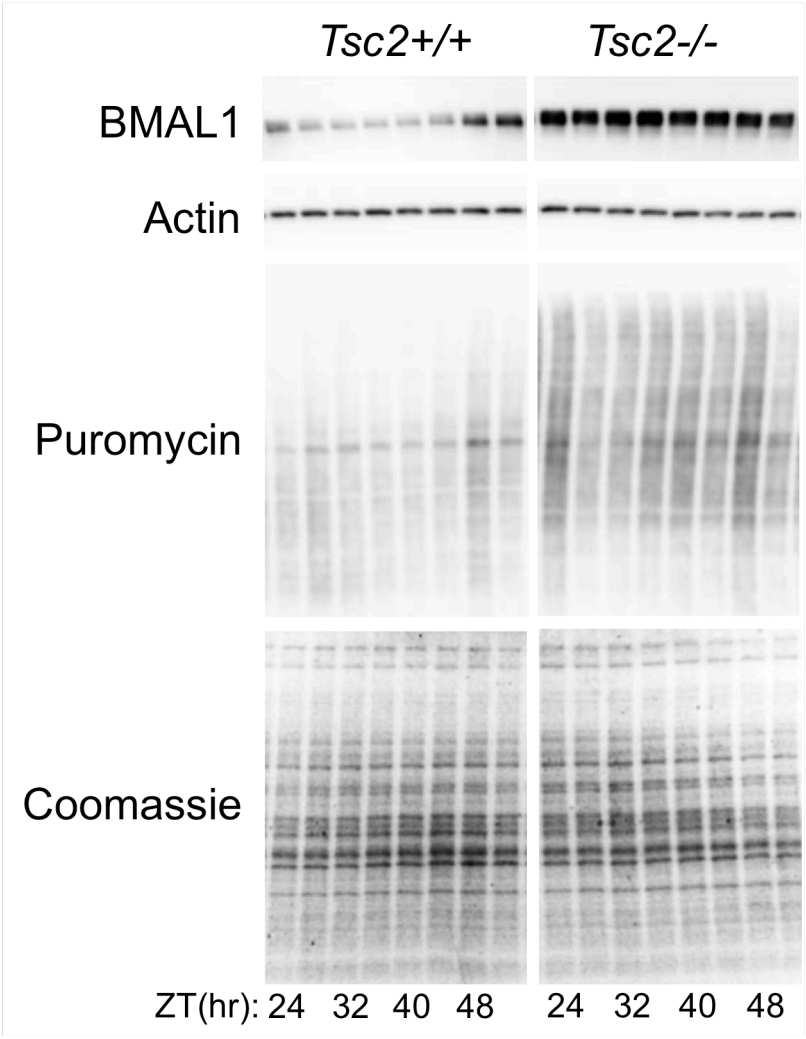

Figure S5. Protein Synthesis in Synchronized *Tsc2* MEFs. Related to Figures 4 and 5.
Circadian rhythm in *de novo* protein synthesis demonstrated by puromycin incorporation (SUnSET assay) into *Tsc2*+/+ or *Tsc2*-/- cells serum-synchronized and immunoblotted with anti-puromycin antibody every 4 hours.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

RNA isolation and qPCR

For cells lines, cells were washed twice with cold PBS and then lysed in 1mL of Trizol reagent (Invitrogen). Lysates were then either used immediately or stored for up to 2 weeks at -80°C. cDNA reactions were performed using the High Capacity cDNA kit (Applied Biosystems). 1-3μg of total RNA was used per reaction and a 100% conversion of mRNA to cDNA was assumed for downstream dilutions. 10ng of cDNA was used per qPCR reaction. Because we made no *a priori* assumptions about the circadian properties of control transcripts, for most qPCR reactions, 5ng of uncapped luciferase RNA (Promega) was spiked-in for normalization unless otherwise noted. qPCR was performed with SYBR green (Invitrogen) as a detector with an Applied Biosystems Prism 7300 cycler. All reactions were performed in duplicate or triplicate and in simultaneity with control. Primers were obtained from IDT DNA (Coralville, Iowa) and used at a final concentration of 300nM. RNA harvested from tissue was processed similarly with the exception that tissue was progressively triturated with 18G and then 22G needles prior to chloroform extraction.

Immunofluorescence

Cells were plated on pre-rinsed coverslips overnight, washed twice with PBS, fixed with 4% PFA for 15 minutes at room temperature, washed twice in PBS, permeabilized with 0.2% triton in PBS for 5 minutes at room temperature, washed twice with PBS, blocked in 0.25% BSA/PBS for 30 minutes at room temperature followed by incubation in primary antibody (1:1000 BMAL1 or 1:500 CLOCK) overnight at 4ºC. Cells were then washed four times with PBS and incubated in fluorescently conjugated secondary anitbodies (1:1000) followed by four washes with PBS. DAPI at 1:20,000 was included during the penultimate wash. Cells were mounted onto glass coverslips with 10μL FluoroMount and imaged. Quantification was performed with a customized CellProfiler (MIT) pipeline as previously described(Lipton et al., 2015).

Puromycin Incorporation Assay (SUnSET Assay)

SUnSET assays were performed as described and recommended by manufacturer (Kerafast)(Schmidt et al., 2009). We incubated cells with 1μM puromycin (Sigma) for 30 minutes followed by washing twice with ice cold PBS and lysing with modified RIPA buffer. We normalized lysates by Bradford assay and loaded onto SDS-PAGE and performed western blots with mouse anti-puromycin monoclonal antibody (Kerafast) at 1:1000 in milk overnight. Signal was normalized against Coomassie blue staining.

Preparation of Cell Lysates and Western Blotting

Cells lysates were prepared with Laemmli SDS sample buffer. Lysates were sonicated on ice for 3-5 seconds at lowest setting with a handheld sonicator. Lysates were boiled at 95°C for five minutes, placed on ice, vortexed at high speed, and centrifuged. Lysates were normalized with either Bradford reagent or bicinchonic acid assay (Thermo). For SDS-PAGE, lysates were loaded onto 4-20% gradient gel and electrophoresed, transferred to PVDF, blocked in 5% powdered milk/TBST for one hour, followed by incubation with primary antibody overnight at 4°C.

Antibodies

The following primary antibodies were used: Phospho-S6 (S240/244) (Cell Signaling #2215, 1:1000), BMAL1 (Bethyl, 1:500), S6K1 (Santa Cruz #230, 1:2500), CLOCK (Bethyl A302-618A, 1:500), Phospho-S6K1(Thr389) (Cell Signaling #9234, 1:1000), TSC2 (Cell Signaling, 1:1000), anti-biotin-HRP (Cell Signaling 1:2000-5000), phospho-BMAL1 (Cell Signaling), Actin (Abcam), Tubulin (Abcam, 1:50,000), Ubiquitin, (Cell Signaling #3936 1:1000), UBE3A/E6AP (Bethyl A300-352A, 1:1000).

Tissue Harvesting

Immediately after dissection, brains were flash frozen on liquid nitrogen and maintained at -80°C until further processing. Protein lysates were prepared by adding 0.5mL of protein lysis buffer (modified RIPA buffer: 50 mM Tris-HCl, 150mM NaCl, 0.5g sodium deoxycholate, 1% NP-40, 0.1% SDS, freshly supplemented with Complete and PhosStop) in a Dounce homogenizer followed by 5-6 thrusts with the loose pestle followed by 5-6 thrusts with the tight pestle. Lysates were then incubated on ice for 10 minutes followed by centrifugation at 16,000xg for 15 minutes at 4°C. Supernatants were collected and then normalized with lysis buffer to a final concentration of 1mg/mL and kept at -20°C.

Preparation of Cytoplasmic Brain Lysates
Freshly harvested brain was washed twice in PBS and flash frozen in liquid nitrogen. ~100g of tissues was cut with an ice cold razor blade and placed in a Dounce homogenizer containing 1mL cold lysis buffer (50mM Tris-HCl, pH 7.4, 3mM calcium chloride, 1mM magnesium chloride, 0.25M sucrose, PhosStop and EDTA-free Complete inhibitors). Tissue was homogenized with 6-8 turns of a loose pestle a 6-8 turns of the tight pestle. The lysate was transferred to a 15mL conical tube containing another 3mL of lysis buffer and briefly vortexed at low setting. The sample was centrifuge at 4°C at 1000G. Supernatant was retrieved and saved as the cytoplasmic portion.

Preparation of Cytoplasmic Brain Lysates from *Tsc1-SynCre* Mice
After euthanasia with inhaled CO2, mouse heads were removed and the skull opened with dissecting scissors. The brain has removed and cut in the sagittal plane. After removal of the basal ganglia, the hippocampus was identified and isolated and flash frozen in liquid nitrogen. Just prior to use, the tissue was warmed on ice briefly and placed in 300µL of ice cold buffer (30mM Tris-HCl, pH 7.5, 15mM magnesium chloride, 0.4% Nonidet P-40, 2mM EGTA, freshly supplemented with EDTA-free Complete protease inhibitor mini tabs (Roche), PhosStop phosphatase inhibitor tabs (Roche)). Samples were individually passaged through a 1mL syringe using a 18G needle followed by a 22G needle. Samples were incubated on ice for 10 minutes and then centrifuged at 16000G for 10 minutes. Supernatants were removed, normalized with Bradford reagent, and used for Western blotting.

Immunohistochemistry
Mice were deeply anesthetized with ketamine by intraperitoneal injection. A 26G needle was inserted into the left ventricle and at least 30mL of cold PBS was perfused with a peristaltic pump (VWR) over 5 minutes followed by 40mL of freshly prepared 4% paraformaldehyde/PBS over 7 minutes. After removing the eyes and cutting the optic nerves the brain was removed and post-fixed in 4% paraformaldehyde/PBS overnight at 4ºC. The brain was serially dehydrated with 10%, 20%, 30% sucrose (w/v) PBS solution for 12 hours each at 4ºC. Brains were then cryopreserved in OCT media and flash frozen with a dry ice/ethanol slurry. Blocks were sectioned at 30μm on a Leica CM3050S cryostat and floating sections were placed in PBS. Sections were then washed twice with PBS/0.05% Triton X-100 (PBST), blocked with 5% normal goat serum/2% BSA/PBST for one hour followed by incubation in primary antibody (BMAL1, Bethyl A302-616A, 1:1000) in PBST/2% BSA overnight at 4ºC. Sections were then washed three times with PBST. Sections were incubated with 1:1000 Alexfluor-568 goat anti-rabbit antibody in PBST for one hour at room temperature and then washed three times in PBST. In dim light, sections were mounted onto slides, air dried in the dark, and prepared with glass coverslips in Aqau-Mount (Thermo) for imaging.

Animal Recordings
Core Body Temperature
Male mice were communally housed (5/cage) and maintained from birth in a 12hour/12hour light/dark cycle (LD) according to standard animal facility protocols. Food and water were provided *ad libitum*. At P21, animals were treated with intraperitoneal rapamycin 3mg/kg times weekly until about P50. For data logger implantation, adult animals were anesthetized with halothane. Using sterile techniques, a midline abdominal incision was made with a razor blade to open the peritoneal cavity. SubCue mini-dataloggers (SubCue, Calgary, Alberta) pre-programmed to record temperature every 15 minutes, were sterilized by soaking overnight in 100% ethanol washed repeatedly with sterile saline and then inserted into the peritoneum. The peritoneum was closed with absorbable Chromic gut sutures and the skin closed with Nylon sutures. Animals were treated with Meloxicam for at least three consecutive days following surgery for analgesia. Rapamycin treatment was continued during the post-operative period and recovery period. During the entire post-operative and experimental period, mice were individually housed in a temperature-controlled light-dark box maintained on a 12hour/12hour light/dark schedule with *ad libitum* access to food. After two weeks of recovery, mice were placed in constant darkness (DD). After two weeks of DD, a 12h:12h light/dark cycle was continued for another 7-10 days. Mice were then sacrificed by CO_2 inhalation followed by cervical dislocation. Dataloggers were retrieved and tissues were harvested for further processing. Temperature data was obtained with SubCue software (Calgary, Alberta) and analyzed with ClockLab (Actimetrics).

Wheel Running
Adult male (P50-60 at the start of each experiment) 2-5 month old *Tsc2*+/- and *Tsc2*+/+ male mice were placed in individual cages, provided with *ad libitum* food and water, equipped with running wheels coupled to mechanical

switches (Colbourn Instruments). Cages were placed in a temperature-controlled, ventilated light/dark box. LD or DD was implemented with the use of a timer mechanism. Computer clocks were synchronized with ambient time weekly. Wheel running was recorded in 6 minute bins and analyzed using ClockLab software (Actimetrics). Period was determined with ClockLab software. For data logger experiments, recorded files were converted to a ClockLab-compatible files and period, FFT, and mean counts/circadian time were determined and plotted.

Phase shifting
Adult male mice aged 3-6 months were individually housed in a temperature and humidity controlled box with control of light dark cycles equipped with running wheels coupled to ClockLab recording software. Food and water were available *ad libitum*. After two weeks of 12 hour light and 12 hour dark schedule, the dark cycle was advanced by 6 hours starting at ZT8-9.

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