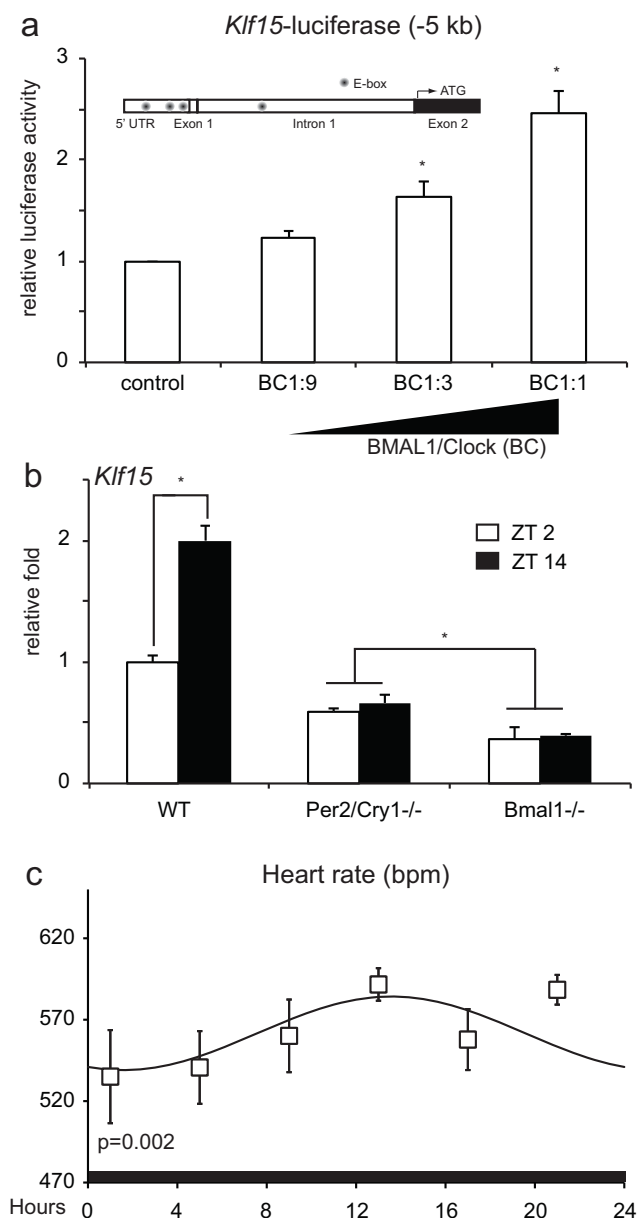
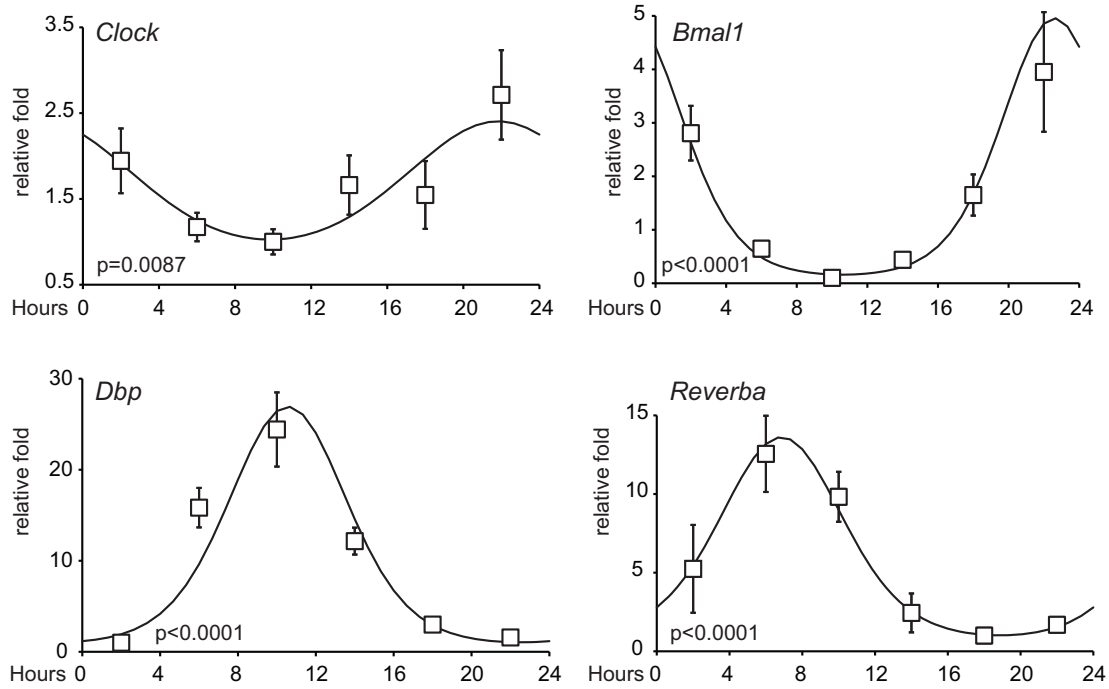


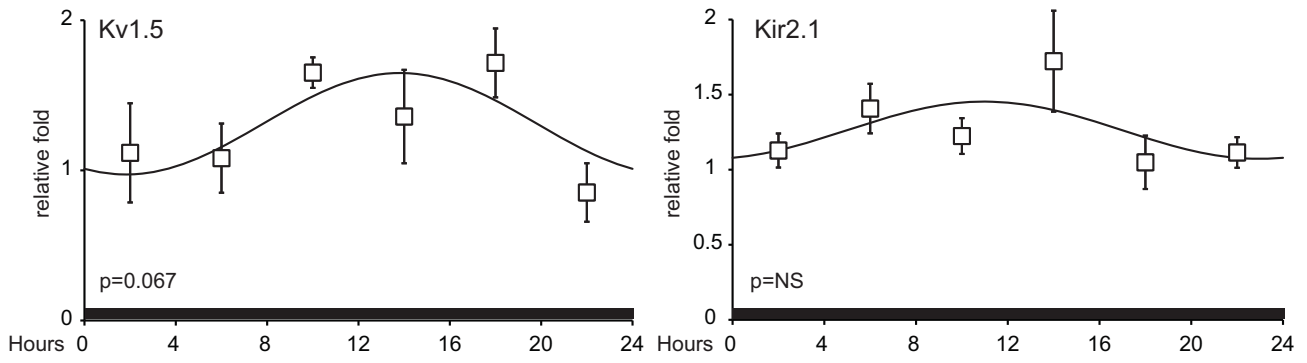
Published in *Nature* (2012), which should be cited to refer to this work.



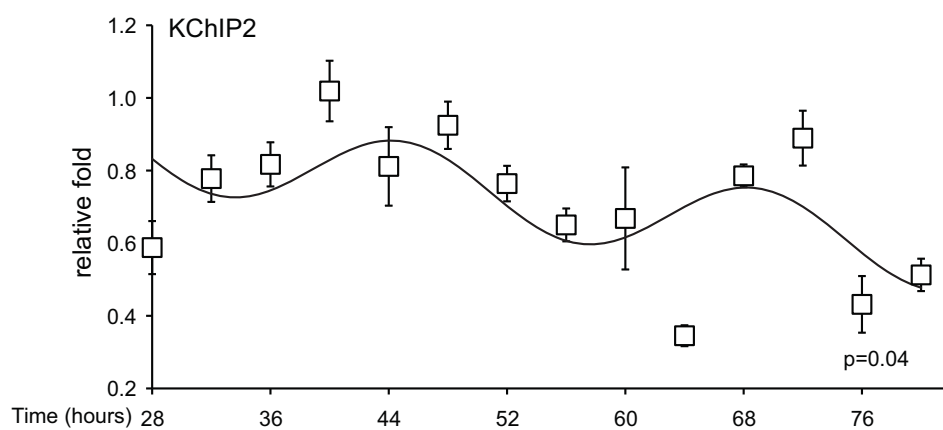
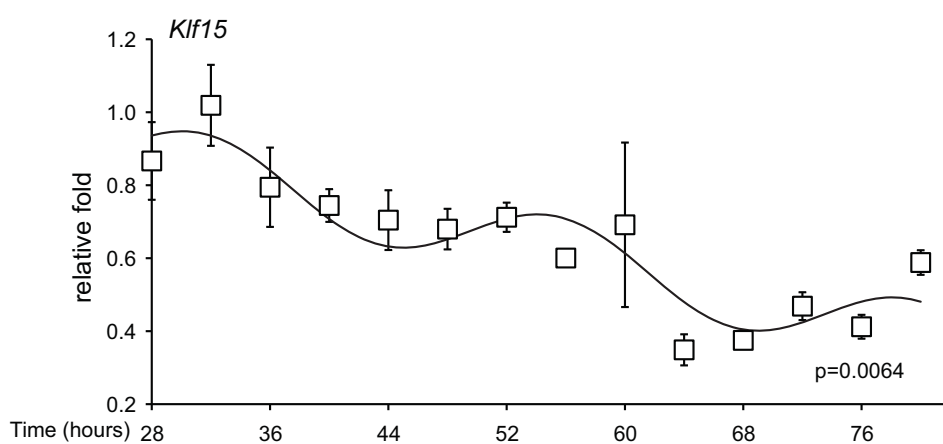
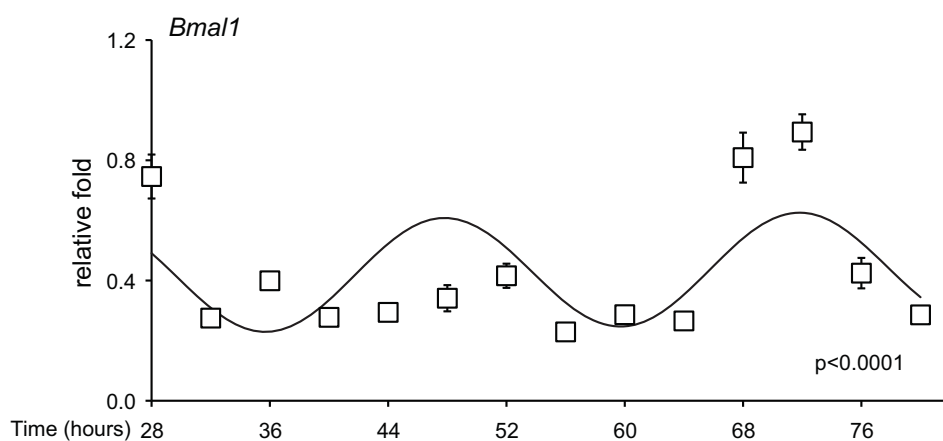
**Supplemental Figure 1.** (a) *Klf15*-luciferase is induced in a dose dependent fashion by CLOCK and BMAL1 (\* $p < 0.05$ ), inset illustrates E-boxes in the *Klf15* promoter region. (b) *Klf15* expression is altered in hearts of *Bmal1*-null and *Per2/Cry1*-null mice with no variation across 12 hours ( $n=4$ , \* $p < 0.05$ ). The expression level of *Klf15* is significantly higher in *Per2/Cry1*-null when compared to *Bmal1*-null hearts at both time points (\* $p < 0.05$ ). (c) Heart rate of WT mice after 36 hours in constant darkness (DD) ( $n=4$ ). Data presented as mean  $\pm$  SEM.



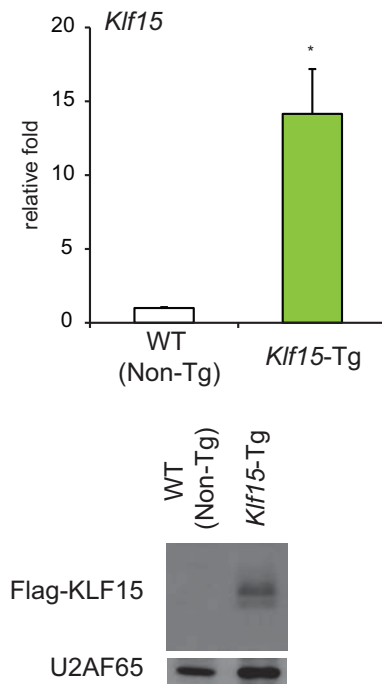
**Supplemental Figure 2.** Expression of core clock machinery components in hearts of WT mice after 36 hours in constant darkness (DD) (n=4 per time point). Data presented as mean  $\pm$  SEM.



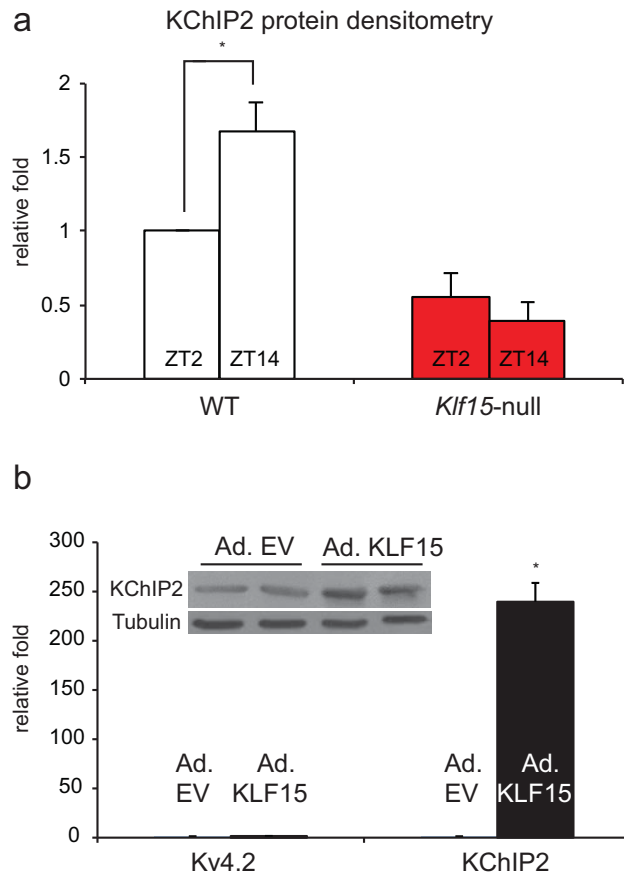
**Supplemental Figure 3.** Transcripts for *Kcna5* (encodes Kv1.5), and *Kcnj2* (encodes Kir2.1) exhibit no significant variation in constant darkness (DD) ( $n=4$  per time point). Data presented as mean  $\pm$  SEM.



**Supplemental Figure 4.** Expression of *Bmal1*, *Klf15* and *KChIP2* in neonatal rat ventricular myocytes following serum shock (n=4 per time point). Data presented as mean  $\pm$  SEM.

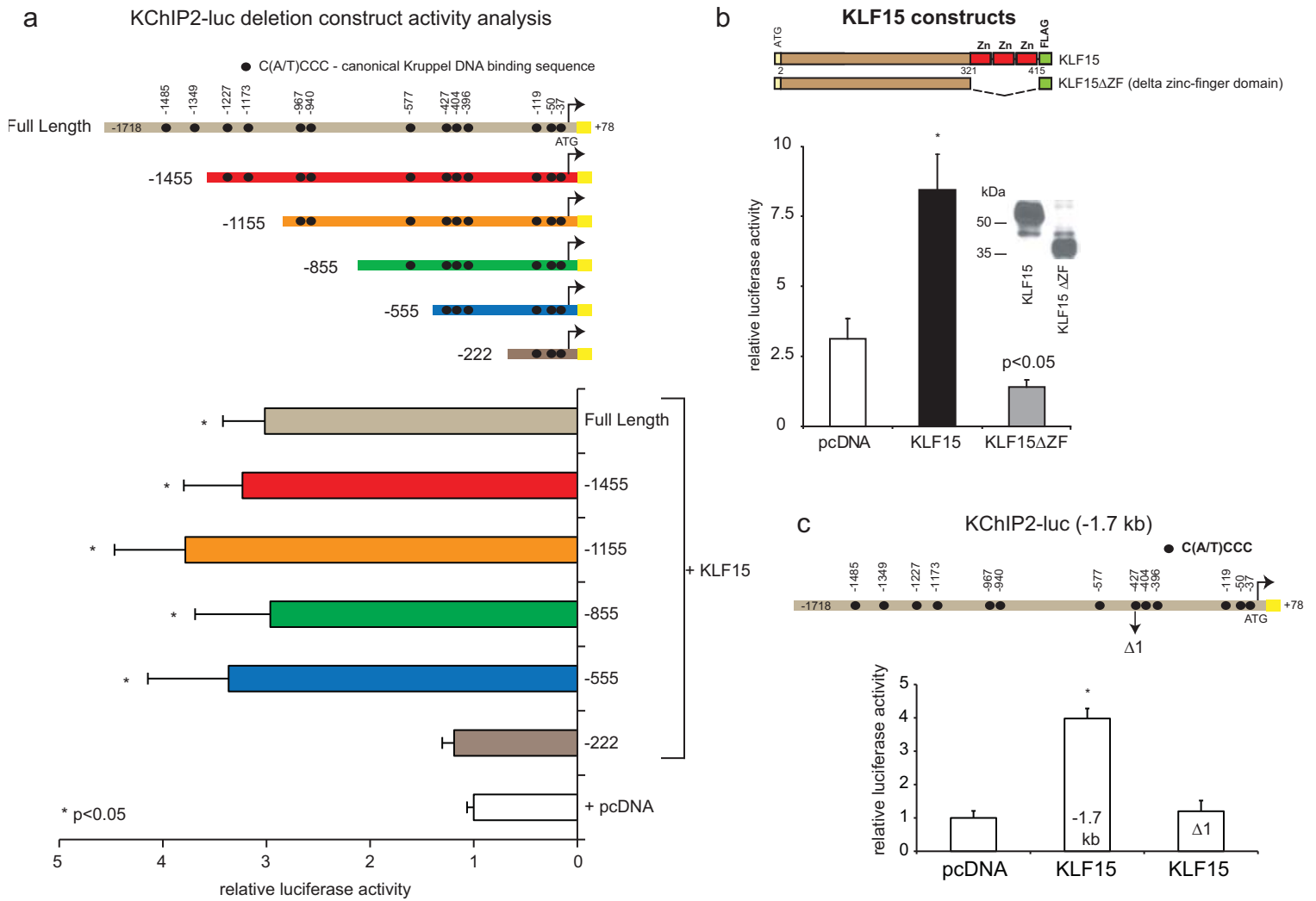


**Supplemental Figure 5.** Real-Time PCR for Klf15 illustrating ~15 greater expression of Klf15 in transgenic hearts compared to WT (Non-Tg) littermate controls (n=3 per group, \* p<0.05). Western blot for Flag from nuclear lysates illustrating Flag-KLF15 in Klf15-Tg hearts. Data presented as mean  $\pm$  SEM.

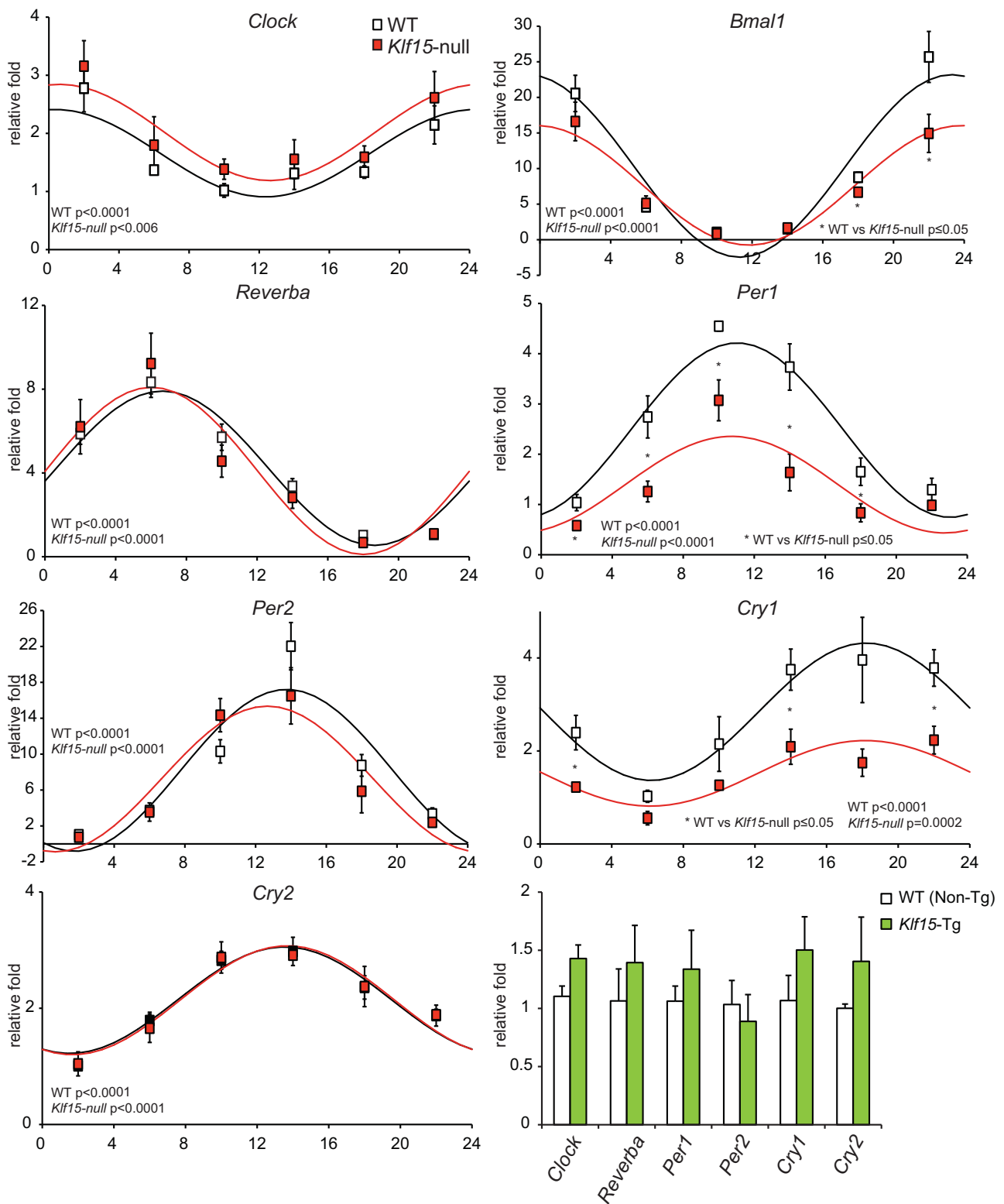


**Supplemental Figure 6.** (a) KChIP2 protein expression using densitometry exhibits no variation across 12 hours in *Klf15*-null hearts (\* $p < 0.05$ ).

(b) Adenoviral overexpression of KLF15 induces KChIP2 expression, but has no effect on Kv4.2 expression ( $n = 3$  per group, \*  $p < 0.05$ ). Data presented as mean  $\pm$  SEM.

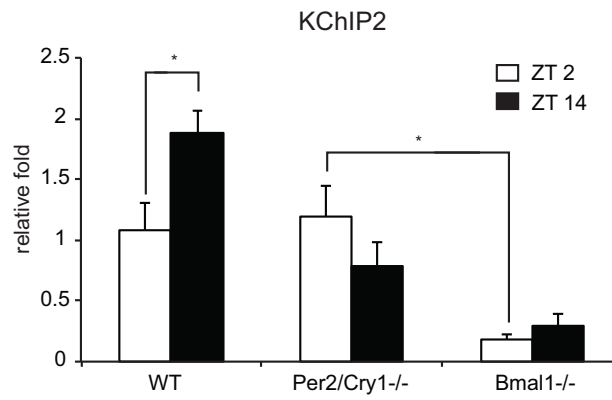


**Supplemental Figure 7.** (a) -1.7kb fragment of the rat KChIP2 promoter reveals numerous consensus Kruppel binding sites, i.e., C(A/T)CCC. Deletion constructs of KChIP2-luc are illustrated. The luciferase activity is completely lost in the -222kb construct. All other constructs exhibit similar activity in response to KLF15 (\* P<0.05). (b) KChIP2-luc is activated by full-length Flag-KLF15, but not by a mutant that lacks the zinc-finger DNA binding domain (\* p<0.05). Inset illustrates Western blot against Flag for the full length and the mutant ( $\Delta$ ZF) without the zinc-finger domain. (c) KChIP2-luciferase is induced by KLF15, and the activity is significantly reduced in the  $\Delta$ 1 mutant, that has one mutated C(A/T)CCC site (-427) (\*p<0.05). Data presented as mean  $\pm$  SEM.

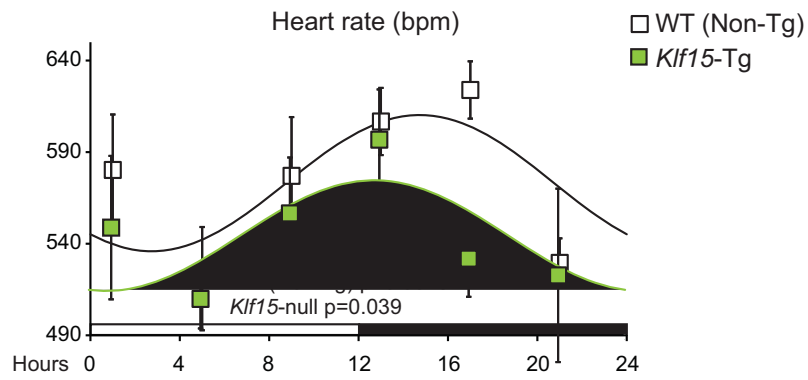
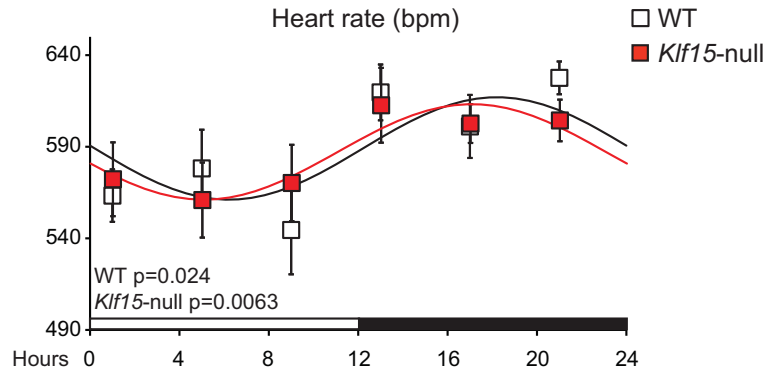


**Supplemental Figure 8.** Transcripts for core clock machinery components in hearts of WT and Klf15-null mice (n=4 per time point per group), and at ZT6 in Klf15-Tg mice (n=4 per group). Data presented as mean  $\pm$  SEM.

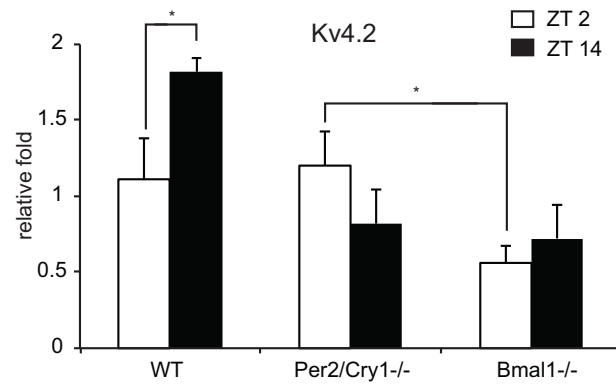




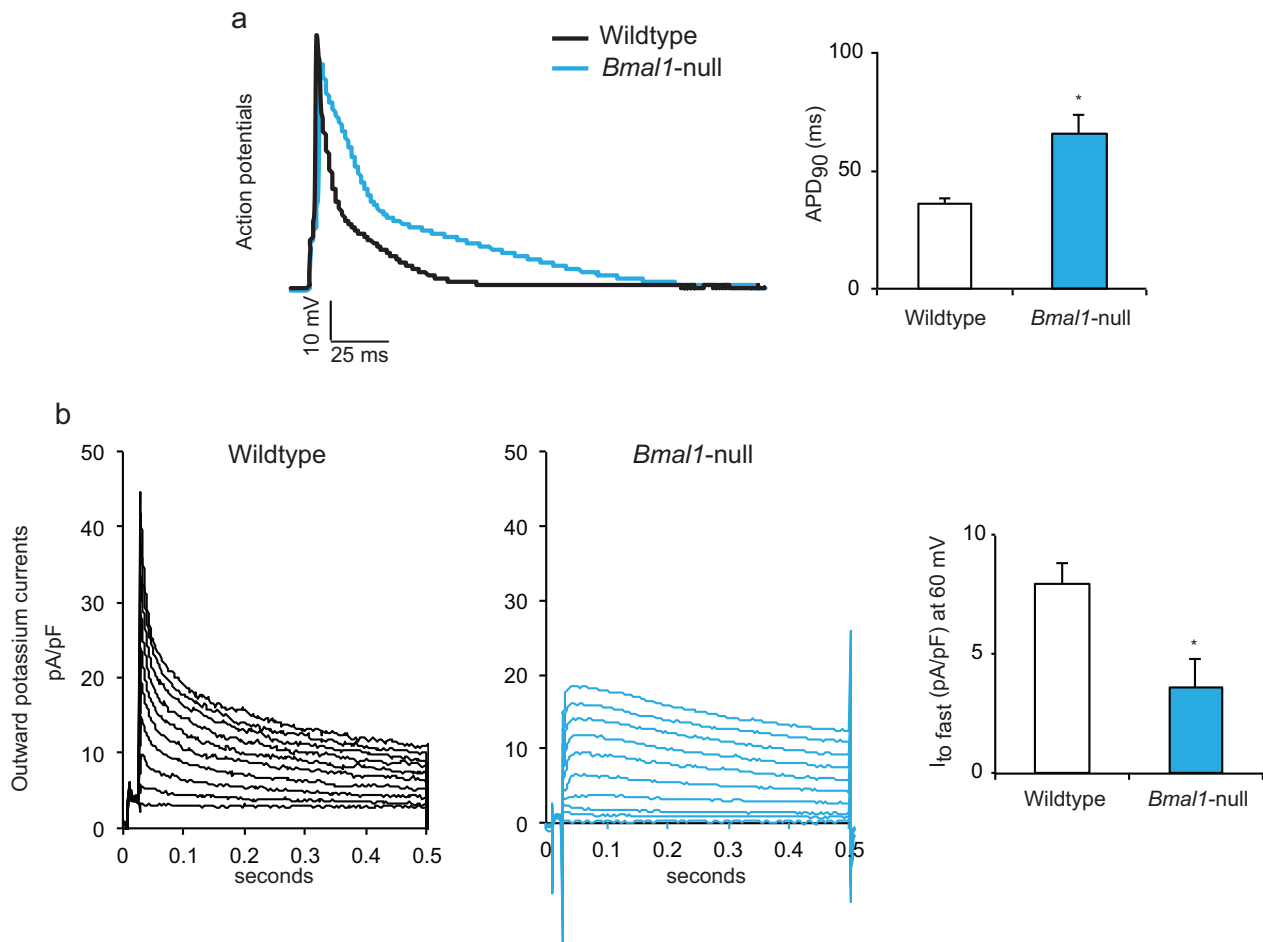
**Supplemental Figure 9.** KChip2 expression is altered in hearts of Bmal1-null and Per/Cry1-null mice with no variation across 12 hours. The expression level at ZT2 is modestly higher in Per2/Cry1-null when compared to Bmal1 (n=4 per group, \* p<0.05). Data presented as mean  $\pm$  SEM.



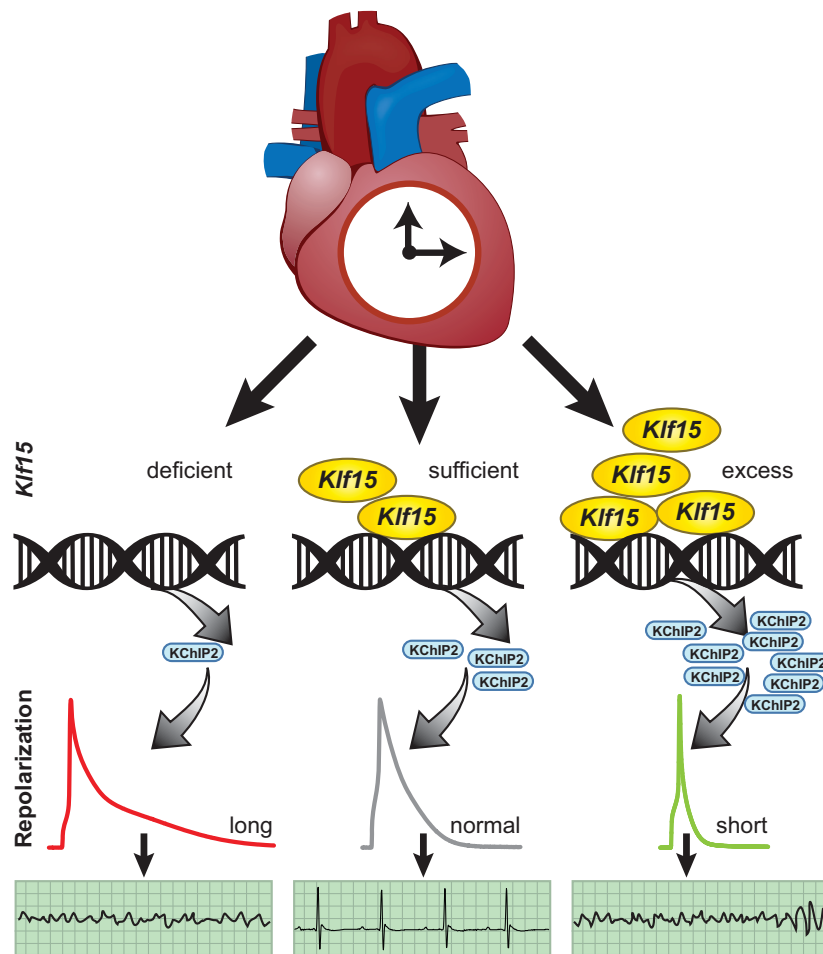
**Supplemental Figure 10.** Heart rate over a 24-hour period in WT (n=4), *Klf15*-null (n=4), WT (Non-Tg) (n=3) and *Klf15*-Tg mice (n=4). Data presented as mean  $\pm$  SEM.



**Supplemental Figure 11.** Kv4.2 expression is altered in hearts of Bmal1-null and Per/Cry1-null mice with no variation across 12 hours. The expression level at ZT2 is modestly higher in Per2/Cry1 when compared to Bmal1 (n=4 per group, \* p<0.05). Data presented as mean  $\pm$  SEM.



**Supplemental Figure 12.** a) Action potential recordings from WT (n=18), and *Bmal1*-null (n=18) cardiomyocytes illustrate prolongation of action potential duration (APD90) in the *Bmal1*-deficient state. Summary data shown in bar graph, \*p<0.01. b) Outward potassium currents measured either in WT (n=13) or *Bmal1*-null (n=18) cardiomyocytes. The fast component of the transient outward current was not detectable in 14 of 18 *Bmal1*-null myocytes measured. In the remainder four myocytes  $I_{to,fast}$  was markedly reduced compared to WT. Summary data shown in bar graph, \* p<0.05. Data presented as mean  $\pm$  SEM.



**Supplemental Figure 13.** Schematic illustrating link between circadian rhythms and heart rhythms.

## Mice

All animal studies were carried out with permission, and in accordance with, animal care guidelines from the Institutional Animal Care Use Committee (IACUC) at Case Western Reserve University and at collaborating facilities. Wild-type male mice on C57BL6/J background (Jackson Laboratory) were bred in our facility and used for circadian studies. Mice were housed under strict light–dark conditions (lights on at 6:00 and lights off at 18:00) and had free access to standard chow and water, and were minimally disturbed for 4–6 weeks before the final experiment. Generation of systemic *Klf15*-null mice was as described previously<sup>18</sup>. *Klf15*-null mice have been backcrossed into the C57BL6/J background for over ten generations<sup>18</sup> and the BMAL1 mice were bred as previously described<sup>19</sup>. For *Klf15*-Tg mice, Flag–KLF15 was cloned downstream of an attenuated  $\alpha$ -myosin heavy-chain promoter as previously described<sup>20</sup>. This construct was injected into FVB (friend leukemia virus B mouse strain) oocytes, and after germline transmission the mice were examined for expression of the transgene. Wild-type (non-Tg) littermates served as controls. For light–dark experiments, mice were killed with CO<sub>2</sub> inhalation or isoflurane every 4 h for 24 h. For constant dark experiments, mice were placed in complete darkness for 36 h (starting at the end of light phase at ZT12) and hearts were collected every 4 h over a 24-h period.

## RNA isolation and RT–PCR analysis:

After euthanasia, hearts were collected, washed in cold phosphate buffered saline, the atria removed and the ventricles dissected to the apical and basal regions, and flash frozen in liquid nitrogen. RNA was isolated from the apical regions of frozen heart samples by homogenization in Trizol reagent (Invitrogen) by following the manufacturer's instructions (Invitrogen). RNA was reverse transcribed after DNase treatment (New England Biolabs). RT–PCR was performed using locked nucleic acid (LNA)-based TaqMan approach with primers and probes designed, and their efficiency tested, at the Universal Probe Library (Roche), and with  $\beta$ -actin used as the normalizing gene.

## Cell-culture studies

Neonatal rat ventricular myocytes were isolated from 1–2-day-old rat pups and grown under standard conditions<sup>18</sup>. Adenoviral overexpression was performed for 24 h and myocytes were then collected for mRNA and protein analysis. For synchronization, the myocytes were starved in media containing insulin, transferrin and selenium (ITS supplement, Sigma-Aldrich) for 48 h. After this, the myocytes were synchronized with 50% horse serum for 30 min, washed twice with no-serum media and replenished with ITS-containing media. The mouse *Klf15* promoter (approximately 5 kb) was cloned into PGL3-basic (Promega). The rat *KChIP2* luciferase was a gift from P. H. Backx. Mutant constructs of rat *KChIP2* luciferase were generated by PCR-based TOPO cloning (Invitrogen), and site-directed mutagenesis was performed using Quikchange II mutagenesis kit (Agilent Technologies) and confirmed by sequencing. *Klf15* and *KChIP2* luciferase studies were conducted in NIH3T3 cells, and luciferase activity was normalized to protein concentration.

## Western immunoblot analysis

For detecting Flag–KLF15, nuclear lysates were prepared using the NE-PER kit following manufacturer's instructions (Thermo Scientific) and probed with anti-Flag antibody (Sigma). For KChIP2 analysis, whole-cell lysates were prepared by homogenizing the basal regions of the hearts in buffer containing Tris-HCl (50 mM, pH 7.4), NaCl (150 mM), NP-40 (1%), sodium deoxycholate (0.25%), EDTA (1 mM), and supplemented with protease and phosphatase inhibitors (Roche). The blots were probed with a mouse monoclonal antibody against KChIP2 (NIH Neuromab), normalized to tubulin (Sigma-Aldrich) and quantified using Quantity One software (Bio-Rad).

## ChIP

ChIP was performed with hearts as previously described<sup>21, 22</sup>. In brief, hearts were fixed with fresh 1.11% formaldehyde for 10 min, and then by chromatin preparation and sonication (Diagenode). The sonicated chromatin was immunoprecipitated using BMAL1 or Flag antibody bound to Dynabeads (Invitrogen). The relative abundance was normalized to abundance of 28S between the input and immunoprecipitated samples as previously described<sup>21</sup>. Primers that were used for BMAL1 ChIP on the *Klf15* promoter were; forward, 5'-GCCTGAGCATCCTCCCCATCA-3'; reverse, 5'-GGGGCCACCTCTCTGGACTT-3'; and probe, 5'FAM-CCCGCCAGTGACCATGTCTGCCTGT-3'BHQ1. Non-target primers were; forward, 5'-GCCAATTCACATTTCAACCA-3'; reverse, 5'-GACACAAGGCATTTCAA-3'; and probe, 5'FAM-TGCAAAGGGCTGGACATGGG-3'BHQ1. Primers that were used for ChIP of Flag–KLF15 on the *KChIP2* promoter were; forward, 5'-GCTCCGCTCTCACTTGCT-3'; and reverse, 5'-

GGCTGGCAAGGCTTTTCT-3'.

### Telemetry ECG and interval analysis

Mice were implanted with telemetry devices (ETA F20, Data Sciences International) and allowed to recover for at least 2 weeks. ECGs were recorded from conscious mice continuously in their native environment and digital data (PhysioTel, Data Sciences International) were stored for future analysis. Owing to rapid changes in the mouse heart rates, a weighted heart-rate approach was used to assess rhythmic changes in QT interval, and measurements were made every 2 h over a 24-h period. First, the average heart rate was calculated for each hour by digital tracking of the ECG RR intervals (time interval between two consecutive R waves) using the Dataquest analysis software (Data Sciences International). Then, during the first instance within each hour when the average heart rate was present, the QT interval was measured using electronic calipers from two consecutive beats. The QT interval was corrected for heart rate using a previously validated formula for conscious mice  $QT/(RR/100)^{1/2}$  (ref. 23). A Cosinor model was applied to assess the 24-h rhythm in QT using a sinusoidal regression function and raw data presented in four hourly blocks for visualization purposes.

### Electrophysiological studies in myocytes

Murine ventricular myocytes were isolated using a standard enzymatic dispersion technique following overnight fast as previously described<sup>24</sup>. Myocytes were re-suspended in media 199, allowed to recover and recordings were conducted within several hours on the same day. The conventional whole-cell mode was used to record action potentials and  $I_{to}$ . In brief, myocytes were bathed in a chamber that was continuously perfused with Tyrode's solution of the following composition (in  $\text{mmol l}^{-1}$ ): NaCl, 137; KCl, 5.4;  $\text{CaCl}_2$ , 2.0;  $\text{MgSO}_4$ , 1.0; glucose, 10; and HEPES, 10 (pH 7.35). Patch pipettes (0.9–1.5 M $\Omega$ ) were filled with electrode solution composed of (in  $\text{mmol l}^{-1}$ ): aspartic acid, 120; KCl, 20; NaCl, 10;  $\text{MgCl}_2$ , 2; and HEPES, 5 (pH 7.3). Action potentials were elicited in current-clamp mode by injection of a square pulse of current of 5 ms duration and 1.5–2 times the threshold amplitude. APD was measured at 90% repolarization. To measure  $I_{to}$ , cells were placed in Tyrode's solution (as described earlier) containing 1  $\mu\text{M}$  nisoldipine to block calcium current and calcium-activated chloride current, and tetrodotoxin (100  $\mu\text{mol l}^{-1}$ ) to block sodium current. Cells were brought from a holding potential of  $-70$  mV to  $-25$  mV for 25 ms. To isolate the fast, transient component of the outward currents,  $I_{to \text{ fast}}$ , the decay phase of outward potassium currents was fit by the exponential functions of the form:

$$y(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_{ss}$$

where  $\tau_1$  is the time constant of decay of the fast, transient component of outward potassium currents;  $A_1$  is the amplitude coefficient of  $I_{to \text{ fast}}$ ;  $\tau_2$  is the time constant of decay of the slow, transient component of the outward currents;  $A_2$  is the amplitude of  $I_{to \text{ slow}}$ ; and  $A_{ss}$  is the amplitude coefficient of the non-inactivating steady-state outward potassium current  $I_{ss}$ . Consistent with previous studies<sup>25</sup>, the time constant of decay of the fast, transient component  $I_{to \text{ fast}}$  was  $46 \pm 5$  ms. The measured current amplitudes were normalized to cell capacitance and converted into current densities. All experiments were conducted at 36 °C. Cell capacitance and series resistance were compensated electronically at ~80%. Command and data acquisition were operated with an Axopatch 200B patch-clamp amplifier controlled by a personal computer using a Digidata 1200 acquisition board driven by pCLAMP 7.0 software (Axon Instruments).

### Programmed electrical stimulation

Intracardiac programmed electrical stimulation was performed as previously described<sup>26</sup>. In brief, mice were anaesthetized using 1.5% isoflurane in 95% O<sub>2</sub> after an overnight fast. ECG channels were amplified (0.1 mV  $\text{cm}^{-1}$ ) and filtered between 0.05 and 400 Hz. A computer-based data acquisition system (Emka Technologies) was used to record a 3-lead body surface ECG, and up to four intracardiac bipolar electrograms. Bipolar right atrial pacing and right ventricular pacing were performed using 2-ms current pulses delivered by an external stimulator (STG-3008, MultiChannel Systems; Reutlingen). Standard clinical electrophysiologic pacing protocols were used to determine all basic electrophysiologic parameters. Overdrive pacing, single, double and triple extrastimuli, as well as ventricular burst pacing, were delivered to determine the inducibility of ventricular arrhythmias, which was tested twice.

### Statistical analysis

A cosinor model was adopted to determine whether there is a substantial 24-h rhythm in each physiological and molecular variable of interest. By pooling data points of all mice, the model fits data to a fundamental sinusoidal function<sup>27</sup>. To determine the coefficients (amplitude and phase) of the sinusoidal function and to see whether there were significant relationships, a mixed model analysis of variance was performed using standard least-square regression and the restricted maximum likelihood method (JMP 8.0, SAS Institute) as previously described<sup>28</sup>. Data are presented as mean  $\pm$  s.e.m., the Student's *t*-test was used for assessing the difference between individual groups and  $P \leq 0.05$  was considered statistically significant.