mice, and the summary data are provided (*p<0.05). (b) Occurrence of spontaneous ventricular arrhythmia in *Klf15*-Tg mice, and summary data are provided (*p<0.05).

SUPPLEMENTAL METHODS:

Mice:

All animal studies were done with permission, and in accordance to animal care guidelines from the Institutional Animal Care Use Committee (IACUC) at Case Western Reserve University, Cleveland, OH and at collaborating facilities. Wildtype male mice on C57BL6/J background (Jackson Laboratory, Bar Harbor, ME) bred in our facility were used for circadian studies. Mice were housed under strict light/dark conditions (6 AM lights on and 6PM lights off), and had free access to standard chow/water, and were minimally disturbed for four to six weeks prior to the final experiment. Generation of systemic *Klf15*-null mice was previously described, and *Klf15*-null mice have been backcrossed into the C57BL6/J background for over 10 generations, [18] and the BMAL1 mice were previously described [19]. Generation of *Klf15*-Tg mice was by cloning Flag-KLF15 downstream of an attenuated α-myosin heavy chain promoter as previously described [20]. This construct was injected into FVB oocytes, following germline transmission the mice were examined for expression of the transgene, WT (non-Tg) littermates served as controls. For Light dark (LD) experiments, mice were euthanized with $C0_2$ inhalation or isoflurane every four hours for 24 hours. For constant dark (DD) experiments, mice were placed in complete darkness for 36 hours (starting at the end of light phase ZT12) followed by harvest every four hours over a 24-hour period.

RNA isolation and Real-Time PCR analysis:

Following euthanasia, hearts were harvested, washed in cold phosphate buffered saline, the atria removed and the ventricle dissected to apical/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, Carlsbad, CA) by following manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA was reverse transcribed following DNAse treatment (New England Biolabs, Ipswich, MA). Real-time PCR was performed using LNA based Taqman approach with primers/probes designed and

efficiency tested from the Universal Probe Library (Roche, Indianapolis, IN), and Beta actin used as the normalizing gene.

Cell culture studies:

Neonatal rat ventricular myocytes were isolated from 1-2 day old rat pups and cultured under standard conditions. Adenoviral overexpression was performed for 24 hours, and harvested for mRNA and protein analysis. For synchronization, the myocytes were starved in media containing insulin, transferrin & selenium (ITS supplement, Sigma-Aldrich, St. Louis, MO) for 48 hours. Following this, the myocytes were synchronized with 50% horse serum for 30 minutes, washed twice with no serum media and replenished with ITS containing media. The mouse *Klf15* promoter (-5kb) was cloned into PGL3-basic (Promega, Madison, WI), and the rat KChIP2-luciferase was a generous gift from Dr. Peter H. Backx (Univ. of Toronto, Canada). Mutant constructs of rat KChIP2-luc was generated by PCR-based Topo cloning (Invitrogen, Carlsbad, CA), and site-directed mutagenesis was performed using Quikchange II mutagenesis kit (Agilent technologies, Santa Clara, CA), and confirmed by sequencing. *Klf15* and KChIP2 luciferase studies were conducted in NIH3T3 cells, and luciferase activity was normalized to protein concentration.

Western immunoblotting:

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

Chromatin immunoprecipitation:

Chromatin immunoprecipitation was performed from hearts as previously described [21, 22]. Briefly, hearts were fixed with fresh 1.11% formaldehyde for 10 minutes, followed by chromatin preparation and sonication (Diagenode, Sparta, NJ). The sonicated chromatin was immunoprecipitated using BMAL1 or Flag antibody bound to Dynabeads (Invitrogen, Carlsbad, CA). The relative abundance was normalized to abundance of 28S between the input and immunoprecipitated samples as previously described [21]. Primers used for BMAL1 ChIP on the *Klf15* promoter were F gcctgagcatcctccccatca, R ggggccacctctctggactt, and probe cccgcccagtgaccatgtctgcctgt (FAM/BHQ1), and non-target primers were F gccaattcacatttcaacca, R gacacaaggcatttcaa, and probe tgcaaagggctggacatggg (FAM/BHQ1). Primers used for ChIP of Flag-KLF15 on the KChIP2 promoter were F gctccgctctcacttgct and R ggctggcaaggcttttct.

Telemetry ECG and interval analysis:

Mice were implanted with telemetry devices (ETA F20, Data Sciences International, Minnesota, MN) and allowed to recover for at least two weeks. Conscious mice ECGs were recorded continuously in their native environment and digital data stored for future analysis (Data Sciences International, Minnesota, MN). Due to rapid changes in the mouse heart rate, a weighted heart rate approach was used to assess rhythmic changes in QT interval, and measurements were made every two hours over a 24-hour period. First, the average heart rate was calculated for each hour by digital tracking of the RR intervals. 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 (QTc) using a previously validated formula for conscious mice $QT/(RR/100)^{1/2}$ [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[24]. Myocytes were re-suspended in media 199, allowed to recover and recordings conducted within hours on

the same day. The conventional whole cell mode was used to record action potentials and transient outward potassium current (I_{to}). Briefly, myocytes were bathed in a chamber continuously perfused with Tyrode's solution of the following composition (in mmol/L): NaCl 137, KCl 5.4, $CaCl_2$ 2.0, $MgSO_4$ 1.0, Glucose 10, HEPES 10 (pH 7.35). Patch pipettes (0.9-1.5 MΩ) were filled with electrode solution composed of (in mmol/L): aspartic acid 120, KCl 20, NaCl 10, $MgCl_2$ 2, 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, of amplitude 1.5 - 2 times threshold. Action potential duration (APD) was measured at 90% repolarization (APD_{90}). To measure I_{to}, cells were placed in above Tyrode's solution containing 1 uM nisoldipine, to block calcium current and calcium-activated chloride current and tetrodotoxin (100 µmol/l) to block sodium current. Cells were brought from a holding potential of –70 to –25 mV for 25 ms. To isolate the fast component of the outward current, the outward potassium current traces were fitted to a biexponential function. The decay phases of outward potassium current were fitted by the sum of two exponentials using the expression: $y(t) = A_1*\exp(-t/tau_1) = A_2*\exp(-t/tau_2) + A_{ss}$, where Tau_1 is the time constant of decay of the fast component of outward potassium current, $I_{to,fast}$, A_1 is the amplitudes of $I_{to,fast}$. Consistent with previous studies[25], the time constant of decay of the fast component was 46±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, Foster City, CA).

Programmed electrical stimulation:

Intra-cardiac programmed electrical stimulation was performed as previously described [26]. Briefly, mice were anesthetized using 1.5 % isofluorane in 95% O_2 following an overnight fast. ECG channels were amplified (0.1 mV/cm) 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 4 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, Germany). Standard clinical electrophysiologic pacing protocols were used to determine all basic electrophysiologic parameters. Overdrive pacing and single, double, and triple extrastimuli, as well as ventricular burst pacing, were delivered to determine inducibility of VT, which were tested twice.

Statistical analysis:

A cosinor model was adopted to determine whether or not there is significant 24-hour 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 [27]. To determine the coefficients (amplitude and phase) of the sinusoidal function and the significant level of the rhythm, a mixed model ANOVA was performed using standard least square regression and the restricted maximum likelihood method (JMP 8.0, SAS Institute Inc, North Carolina) as previously described [28]. Data are presented as mean ± SEM, Student's T-test was used for assessing difference between individual groups and $p \leq 0.05$ was considered statistically significant.

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