Supplemental Material and Methods for

Circadian hepatocyte clocks keep synchrony in the absence of a master pacemaker in the suprachiasmatic nucleus or other extrahepatic clocks

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Supplemental materials and methods

Generation of "Hepatocyte clock-only mice"

The generation of the *Bmal1* stopped allele has been performed by modification of the targeting vector for the *Bmal1* floxed allele (Storch et al. 2007). The LoxP-site flanked stop cassette was engineered to contain an engrailed 2-based splice acceptor (Mitchell et al. 2001) followed by *d2EGFP* (Clontech) in-frame, a triple repeat of a SV40 polyA signal and PGKβgeo. The latter represents a pgk-driven neo-β-galactosidase fusion gene that was FRT-flanked. The cassette was inserted into the single *AvrII* site inside the intron between *Bmal1* exon 5 and 6 of the targeting vector. ES cell targeting and mouse blastocyst injections was carried out according to standard methods. Genotyping was performed using multiplex PCR and primers (Supplemental Table S2).

RT-Biolumicorder system

The RT-Biolumicorder (Lesa technology SA) has been designed and engineered as described in (Saini et al. 2013). It consists of a cylindrical cage equipped with a photomultiplier tube, highly light reflective walls, a programmable feeding machine, a water flask equipped with an ultrasonic device measuring water consumption, and an infrared sensor allowing the

simultaneous recording of locomotor activity. Mice were placed into the RT-Biolumicorder a few days before the recordings were initiated, in order to get used to the drinking and food delivery system. For coated mice the bioluminescence recordings were performed after shaving the body part of interest with an electric razor (Phymed) or a depilatory cream (Veet silky fresh). For monitoring water consumption in real time, an ultrasonic sensor has been installed on top of the water flask of the RT-Biolumicorder (SICK, UM12-1172271). It detects the level of the liquid by ultrasound emission and reception at a frequency of 380 kHz with a resolution of 15 µl.

RNA Analysis by Quantitative Real-Time RT-PCR

Between 50 mg to 150 mg of tissue (fresh or kept frozen at −80°C) were ground in 2.5 ml extraction buffer using a Polytron PT 2500 E homogenizer. The extraction buffer was prepared as follows: 250 g of guanidium thiocyanate were dissolved in 320 ml of H2O and 17.6 ml of 0.75 M sodium citrate, pH 7. Just before use, the required volume of extraction buffer was supplemented with 0.1 volume of 2 M ammonium acetate, pH 4, and 0.01 volume of β-mercaptoethanol. Subsequently, 2.5 ml of phenol (saturated with H2O) and 1 ml of chloroform-isoamylalcohol (49:1 volume ratio) were added, and the emulsion was vigorously shaken manually, before the aqueous and organic phases were separated by centrifugation at 4,000 rpm for 20 min at 4°C. RNA was precipitated from the aqueous phase by the addition of an equal volume of isopropanol, and the mixture was kept at −20°C during at least 60 min. The precipitated RNA was pelleted by centrifugation at 4,500 rpm during 15 min at 4°C. The pellet was resuspended in 3 ml of 4 M LiCl to remove traces of DNA, and the RNA was recovered by sedimentation at 4,500 rpm for 15 min at 4°C. The RNA pellet was washed in 75% ethanol, followed by a centrifugation at 4,500 rpm for 15 min at 4°C. The pellet was then dried at room

temperature for approximately 10 min and dissolved in 100 to 400 µl of diethyl dicarbonate (DEPC)-treated H2O. For the DEPC treatment, double-distilled H2O was vigorously mixed with 0.1% volumes of DEPC, before autoclaving. The RNA levels were measured using a ND-1000 NanoDrop machine (Thermo Scientific). In all centrifugation steps, an Eppendorf 5810R tabletop centrifuge was used.

Supplemental Table S2. List of the primers

Primers	**References**	**DNA sequences**	**Targeted regions**
Bmal1 (*Arntl*)	This study	Fw 5'-TGCCCTCTGGAGAAGGTGG-3' Rv 5'-TTCCTCCGCGATCATTCGAC-3'	• NM_007489.4, NM_001357070.1 (Exons16-17) • NM_001243048.1 (Exons 13-15) • NM_001368412.1, NM_001374642.1 (Exons 17-18)
Luciferase	This study	Fw 5'-CCAGGGATTTCAGTCGATGT-3' Rv 5'-AGAATCTCAGCGAGGCAGTT-3'	
Gapdh	(Preitner et al. 2002)	Fw 5'-CATGGCCTTCCGTGTTCCTA-3' Rv 5'-CCTGCTTCACCACCTTCTTGA-3'	
Genotyping primers ("hepatocyte clock-only mice")	This study	sL1 5'-AGCCATCCCTTGACAGTAAG-3' sL2 5'-CAAAGAACGGAGCCGGTTG-3' sR1 5'-AGGATAGACTGGTCAGAGAC-3'	sL1: chr7:113,281,485 sL2: pgk-driven neo-β-galactosidase fusion gene inserted between exon3 and exon4 sR1: chr7:113,281,736

References:

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