

1 **Methods**

2 **Animals**

3 *Bmal1* KO mice were obtained from C. Bradfield (University of Wisconsin, Madison, WI). Control mice
4 used in this study were WT littermate controls to the *Bmal1* KO mice and were generated by in house
5 breeding. Male animals (6-10 week old) were entrained to a 12 hour light, 12 hour dark light cycle for a
6 minimum of 2 weeks prior to release into constant darkness for harvesting brain tissue (under dim red
7 light). All animals in this study were housed according to the guidelines of the Institutional Animal Care
8 and Use Committee (IACUC) of the University of Pennsylvania. All experimental protocols were
9 approved by IACUC.

10 **qPCR**

11 Total RNA from male brain hypothalamus was isolated using the Qiagen RNeasy Kit. Hypothalamic
12 sections were taken from freshly isolated brains with five cuts made using a razor blade. Viewing the
13 ventral side of the brain, two coronal cuts were placed at the apex of the optic chiasm and the rostral
14 margin of the mammillary bodies. This slab was then placed flat, and two cuts were placed on either
15 side of the optic chiasm. A third cut was placed just above the third ventricle. All cuts from brains were
16 approximately the same size. Reverse transcription was performed using an RNA-cDNA kit (Applied
17 Biosystems). Real-time PCR was performed using ABI TaqMan primers and reagents on an ABI Prism
18 7500 thermocycler according to the manufacturer's instructions. All mRNA measurements were
19 normalized to *Gapdh* mRNA levels. Average expression of hypothalamic *Fabp3*, *Fabp5* and *Fabp7* mRNA
20 was calculated across all 6 circadian timepoints and a ratio calculated (KO/WT) for values depicted in
21 Figure 1A.

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23 **Primers:**

24 **Fabp3 Forward** CTG-ACT-CTC-ACT-CAT-GGC-AGT-GT

25 **Fabp3 Reverse** GCC-AGG-TCA-CGC-CTC-CTT

26 **Fabp5 Forward** CGA-CAG-CTG-ATG-GCA-GAA-AAA

27 **Fabp5 Reverse** GAC-CAG-GGC-ACC-GTC-TTG

28 **Fabp7 Forward** CTC-TGG-GCG-TGG-GCT-TT

29 **Fabp7 Reverse** TTC-CTG-ACT-GAT-AAT-CAC-AGT-TGG-TT

30 **Gapdh Forward primer** CAT-GGC-CTT-CCG-TGT-TCC-T

31 **Gapdh Reverse primer** GCG-GCA-CGT-CAG-ATC-CA

32 **Data analysis**

33 Statistical analysis included student's t-test, two-way ANOVA, and Bonferroni post-hoc analysis

34 GraphPad Prism software was used for the analysis. Sample sizes were based on variability of the test

35 measurement and the desire to detect a minimal 10% difference in the variables assessed with $\alpha = 0.05$

36 and power $(1-\beta) = 0.8$.

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