Science Translational Medicine AAAS SUPPLEMENTARY MATERIAL for

CIRCADIAN RHYTHM

Timing of expression of the core clock gene Bmal1 influences its effects on aging and survival

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ABSTRACT

The absence of *Bmal1*, a core clock gene, results in a loss of circadian rhythms, an acceleration of aging, and a shortened life span in mice. To address the importance of circadian rhythms in the aging process, we generated conditional *Bmal1* knockout mice that lacked the BMAL1 protein during adult life and found that wild-type circadian variations in wheel-running activity, heart rate, and blood pressure were abolished. Ocular abnormalities and brain astrogliosis were conserved irrespective of the timing of *Bmal1* deletion. However, life span, fertility, body weight, blood glucose levels, and age-dependent arthropathy - which are altered in standard *Bmal1* knockout mice - remained unaltered, while atherosclerosis and hair growth improved, in the conditional adult-life *Bmal1* knockout mice, despite abolition of clock function in both groups. Hepatic RNA-Seq revealed that expression of oscillatory genes was dampened in the adult-life *Bmal1* knockout mice, while overall gene expression was largely unchanged. Thus, many phenotypes in conventional *Bmal1* knockout mice, hitherto attributed to disruption of circadian rhythms, reflect the loss of properties of BMAL1 that are independent of its role in the clock. These findings prompt re-evaluation of the systemic consequences of disruption of the molecular clock.

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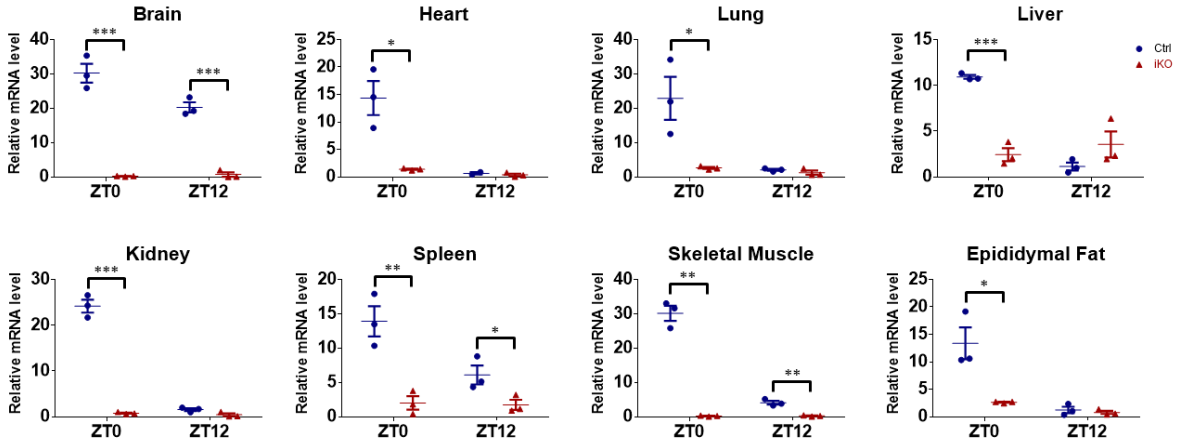
A



B

	Npas2		*Per1*		*Per2*		*Rev-erbα*		*Dbp*	
	Ctrl	iKO	Ctrl	iKO	Ctrl	iKO	Ctrl	iKO	Ctrl	iKO
Fat	10.5*	1.2	5.1**	1.2	10.8**	1.4	3.0*	1.2	52.7***	2.0
Heart	3.4#	1.1	6.5**	1.1	16.5***	1.2	4.0#	1.4	17.3**	6.0
Kidney	27.8***	1.1	5.6*	1.1	19.5**	1.1	2.5*	1.5	61.7***	1.4
Liver	49.2**	1.6	16.0#	1.7	28.2**	4.0#	4.8	2.9	97.5***	2.7
Muscle	13.8*	1.6	5.7#	1.7	12.8***	1.4	3.5**	1.0	21.1**	1.5
Lung	21.0**	1.3	5.7**	1.3	10.7***	1.3	11.0**	1.5	58.1*	2.2

Fig. S1. Validation of *Bmal1* deletion and dampening effect of other clock genes. (**A**) Brain, heart, lung, liver, kidney, spleen, skeletal muscle, and epididymal fat were collected at ZT0 and ZT12. The Bmal1 mRNA levels were determined using qRT-PCR and compared between Ctrls and iKOs (2-way ANOVA; *, P<0.05; **, P<0.01; ***, P<0.001). (**B**) The fold change of clock gene expression levels in various tissues in Ctrl and iKO mice between ZT0 and ZT12 (Student's t-test, ZT0 vs. ZT12; #, P<0.1; *, P<0.05; **, P<0.01; ***, P<0.001; Red, ZT0>ZT12; Green, ZT0<ZT12; Black, no significant difference).

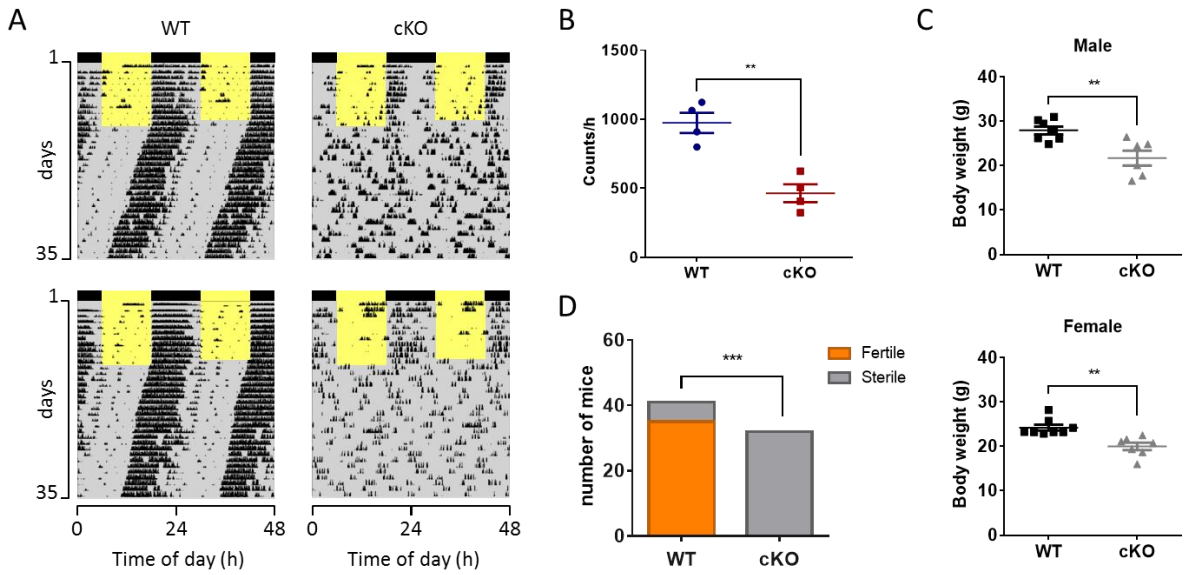


Fig. S2. Wheel-running activity, body weight, and fertility in cKO mice. (**A**) Representative activity records of individual WT and cKO littermates aged 3m are presented in double-plotted format. (**B**) Individual value plot of wheel running activity from WT and cKO mice under DD (Student's t-test; **, $P<0.01$). (**C**) Body weight of 6m old male and female mice (Student's t-test; **, $P<0.01$). (**D**) Fertility analysis of WT and cKO mice (male and female combined, χ^2 test; ***, $P<0.001$).

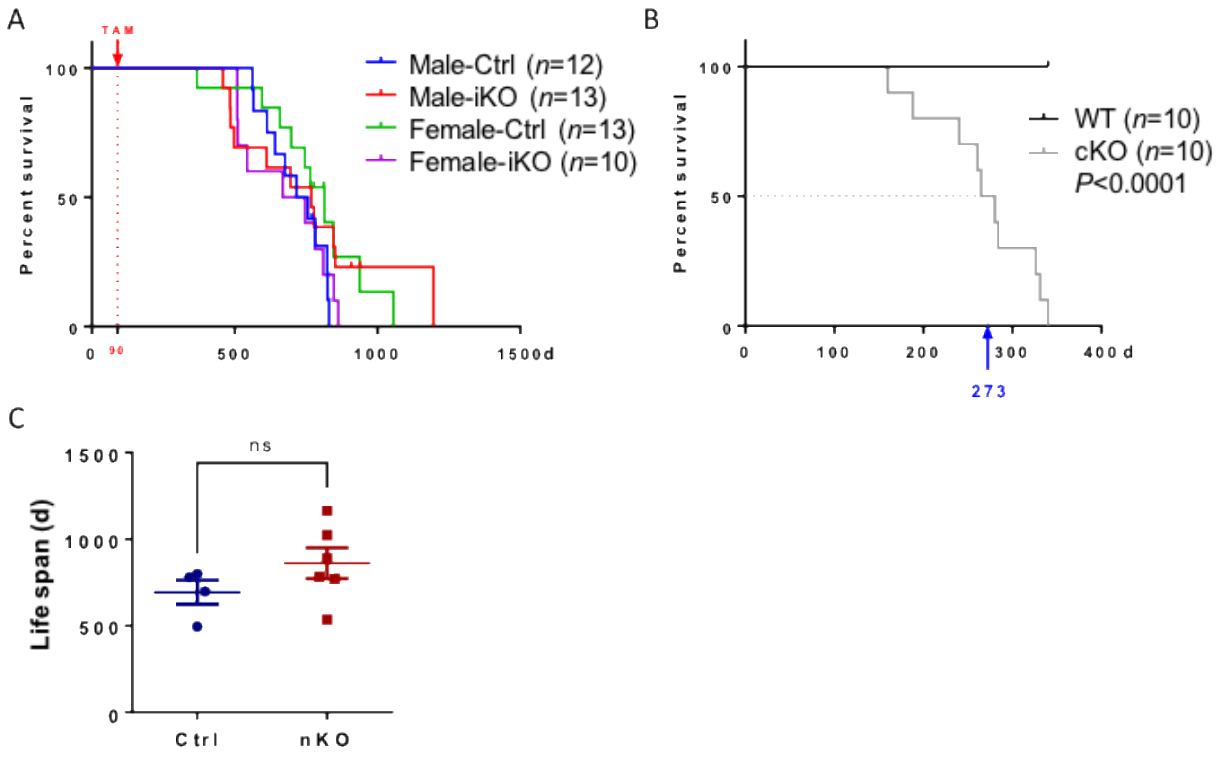


Fig. S3. Life span of iKO, cKO, and nKO mice. (**A**) Both male and female mice at 3 m old were treated with TAM (red arrow) and their life span was monitored for comparison (log-rank test; no significant difference between any 2 groups). (**B**) life span of cKOs. All WT mice at 340 d old were euthanized when last cKO mouse died (log-rank test; $P<0.0001$). (**C**) The life span of nKOs and their littermate controls (Student's t-test; ns, no significant difference).

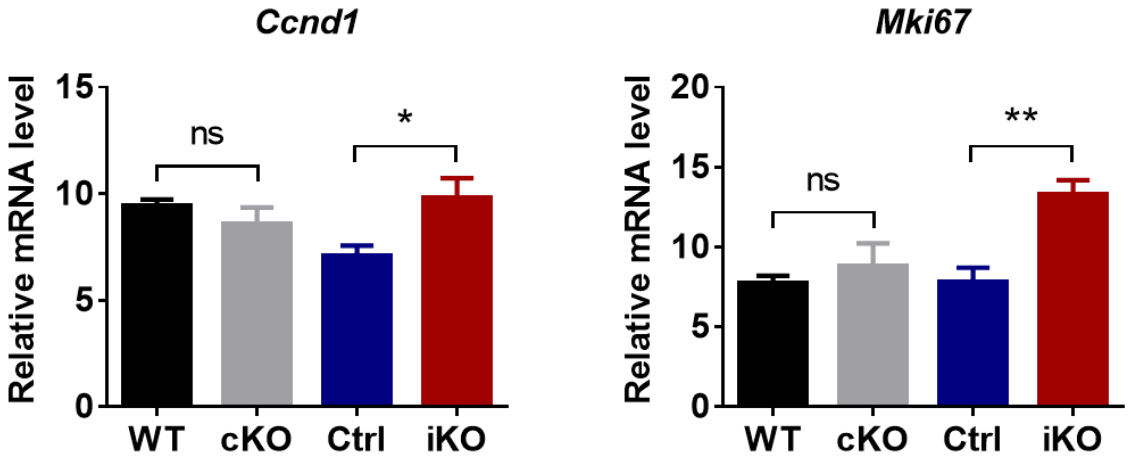


Fig. S4. mRNA levels of *Ccnd1* and *Mki67* in skin. Skin samples from both cKO and iKO and their littermates were used for qRT-PCR (N=6, Student's t-test; ns, no significant difference; *, P<0.05; **, P<0.01).

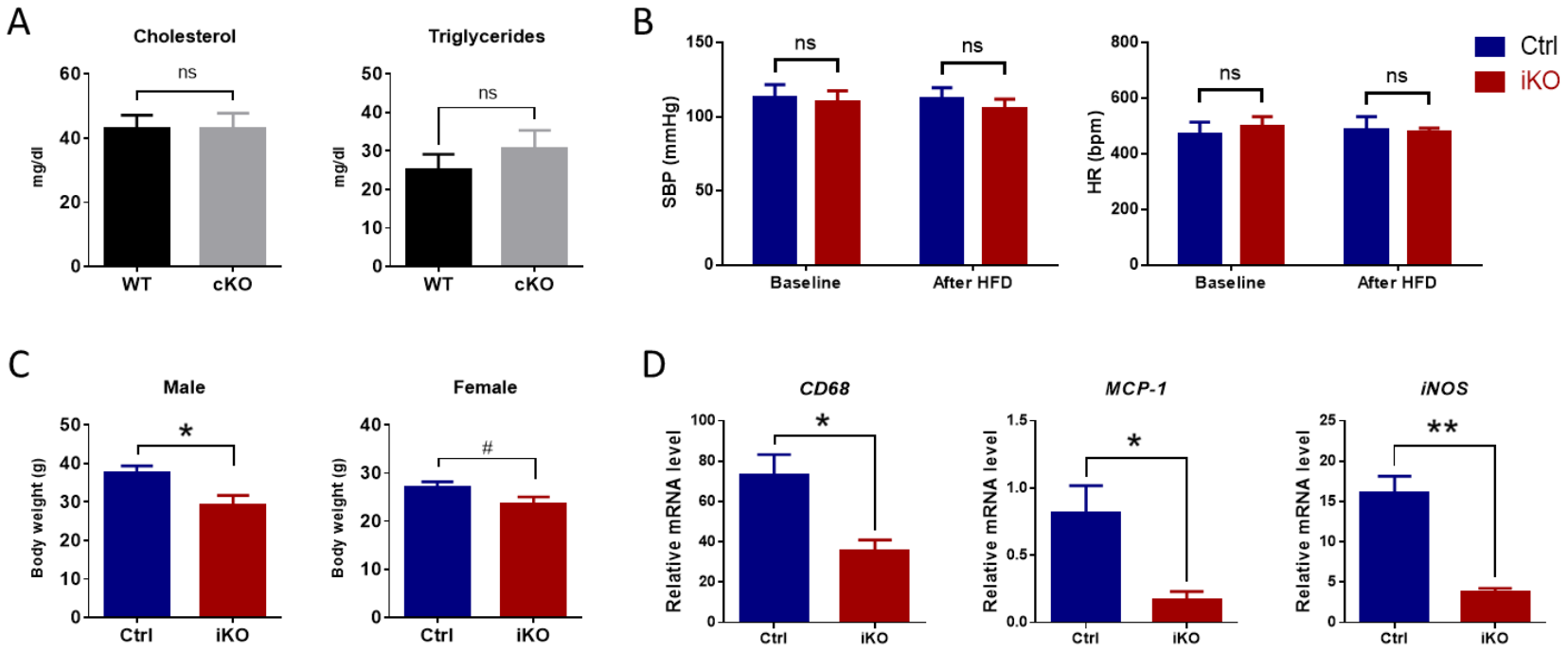


Fig. S5. Other parameters in HFD-fed mice. (**A**) Total cholesterol and triglycerides in cKO plasmas (*N*=5). (**B**) SBP and HR of Ctrls and iKOs were measured by tail cuff at ZT8-9 before or after 2 m HFD treatment (*N*=6 to 8). (**C**) Body weights of both male (*N*=14) and female (*N*=8 to 12) mice. (**D**) qRT-PCR analysis of *CD68*, *MCP-1* and *iNOS* expression in aortas from iKOs (*N*=4). Student's t-test was used for all comparisons (#, *P*<0.1; *, *P*<0.05; **, *P*<0.01).

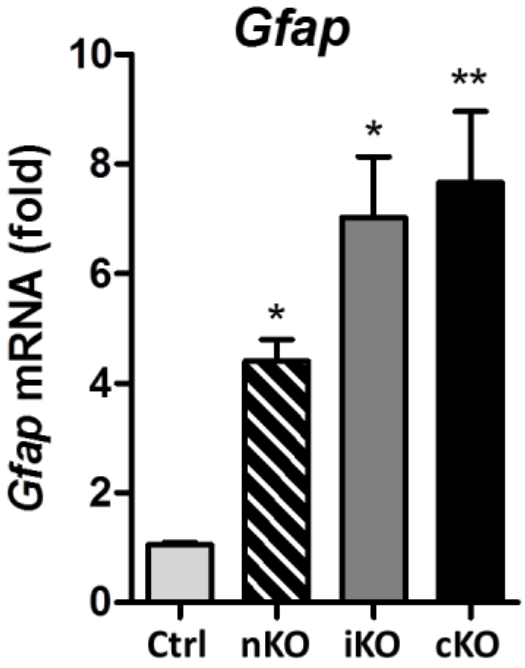
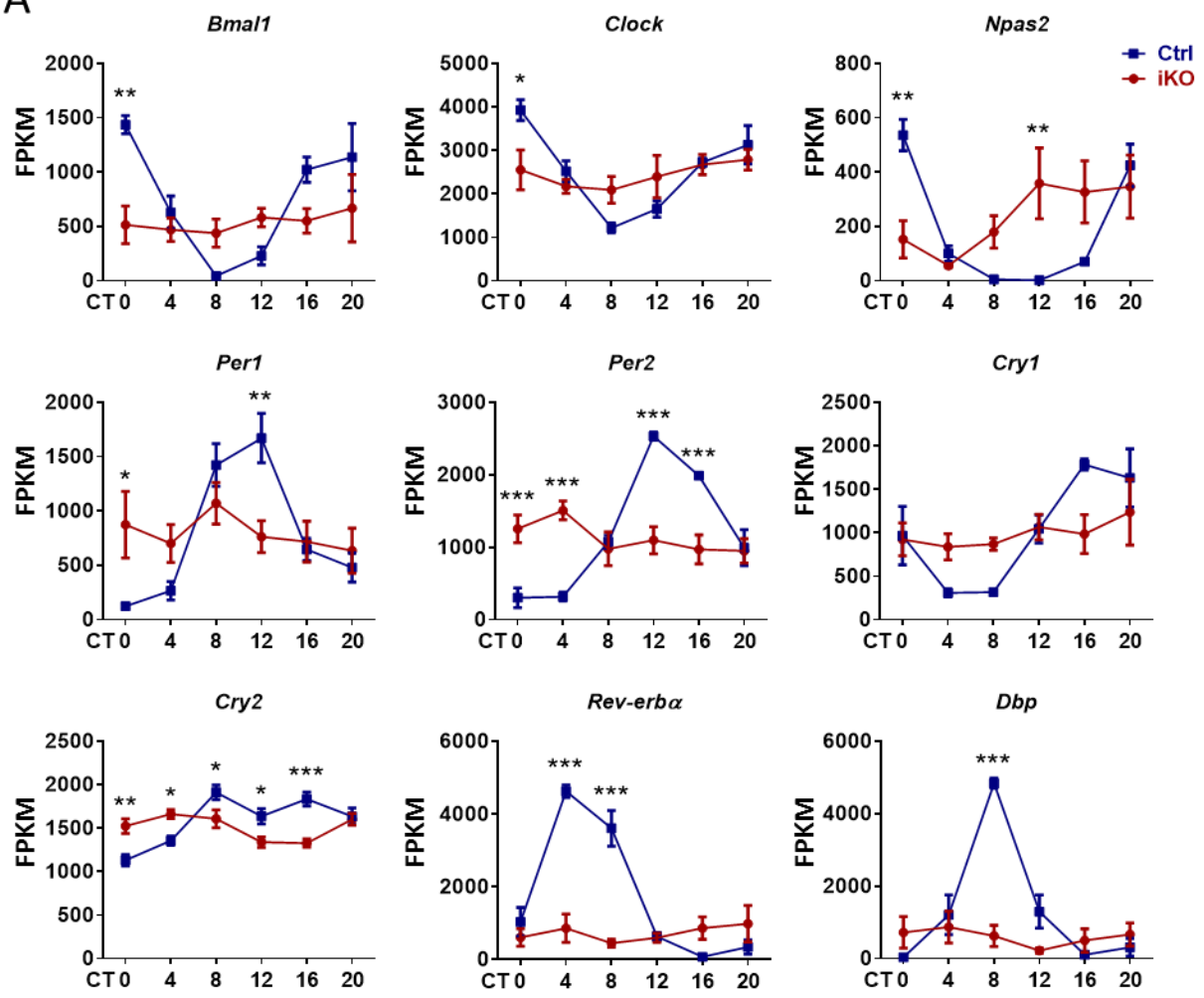


Fig. S6. iKO mice display similar *Gfap* induction in the brain as cKO. Cerebral cortex samples harvested from Ctrl, nKO, iKO, and cKO mice. *Gfap* mRNA level was quantified by qRT-PCR (N=4 to 12, 1-way ANOVA with Bonferroni posttest analysis, as compared to Ctrl group; *, P<0.05, **, P<0.01).

A



B

Gene Symbol	Ctrl	iKO
Arntl	3.22e-06	1
Clock	3.45e-05	1
Npas2	1.49e-06	0.3044
Nr1d1	7.31e-06	1
Per1	9.19e-05	1
Per2	4.59e-06	1
Cry1	0.00014	1
Cry2	0.01628	0.43426
Dbp	3.45e-05	1

Fig. S7. RNA-Seq results revealed dampening effect in core clock genes in iKO mice. (**A**) Expression of core clock genes in Ctrl and iKO mice (*N*=4/genotype/time point, 2-way ANOVA; *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001). (**B**) JTK_CYCLE *q*-values of core clock genes in Ctrl and iKO mice.

Table S1. Top 20 oscillating hepatic genes (JTK_CYCLE *q*-value) in Ctrl mice show no circadian pattern in iKO mice

Genes	Ctrl	iKO
Tars	1.76e-07	0.5649
Igfbp4	1.49e-06	0.6630
Hnrnpl	1.49e-06	1.0000
Ptprk	1.49e-06	0.9546
Blcap	1.49e-06	1.0000
Npas2	1.49e-06	0.3044
Cald1	2.18e-06	0.4950
Rfxank	2.18e-06	1.0000
Gde1	2.36e-06	0.1105
Spata22	2.36e-06	0.8014
Crot	2.36e-06	1.0000
Slc9a3r1	2.36e-06	0.2826
Chka	2.36e-06	1.0000
St3gal1	2.36e-06	1.0000
Nr1d2	3.14e-06	1.0000
Lasp1	3.22e-06	1.0000
Arntl	3.22e-06	1.0000
Pitpnb	3.22e-06	1.0000
Mdfic	3.22e-06	1.0000
Rnf125	4.59e-06	1.0000

Table S2. The ratio of differentially expressed gene numbers in cKO strain to the numbers in iKO strain

q-value	cKO	iKO	Fold (cKO/iKO)
0.01	234	3	*78*
0.05	**462**	**3**	***154***
0.1	656	9	*73*
0.2	998	15	*67*

Table S3. Summary of phenotypes of cKO and iKO mice

Phenotypes	cKO	iKO	Consistence
Circadian rhythm	Loss	Loss	√
Life span	↓	-	×
Fertility	↓	-	×
Body weight	↓	-[2]	×
Organ weight	↓[1]	-	×
Arthropathy	+	-	×
Astrogliosis	+	+	√
Eye pathologies	+[3]	+	√
Hair growth	↓	↑	××
Atherogenesis	↑	↓	××

↑, increased; ↓, decreased; +, positive; -, negative/no change; √, yes; ×, no; ××, opposite

1, experiment was not performed in the current study; 2, lower body weight seen in HFD fed mice; 3, histology was not studied, but the ocular abnormalities can be easily seen.

Data file S1. Circadian transcriptome. [Excel file]

Data file S2. Differentially expressed genes irrespective of time points. [Excel file]

Data file S3. Phenotype enrichment analysis of differentially expressed genes. [Excel file]