Figure S1, related to figure 4

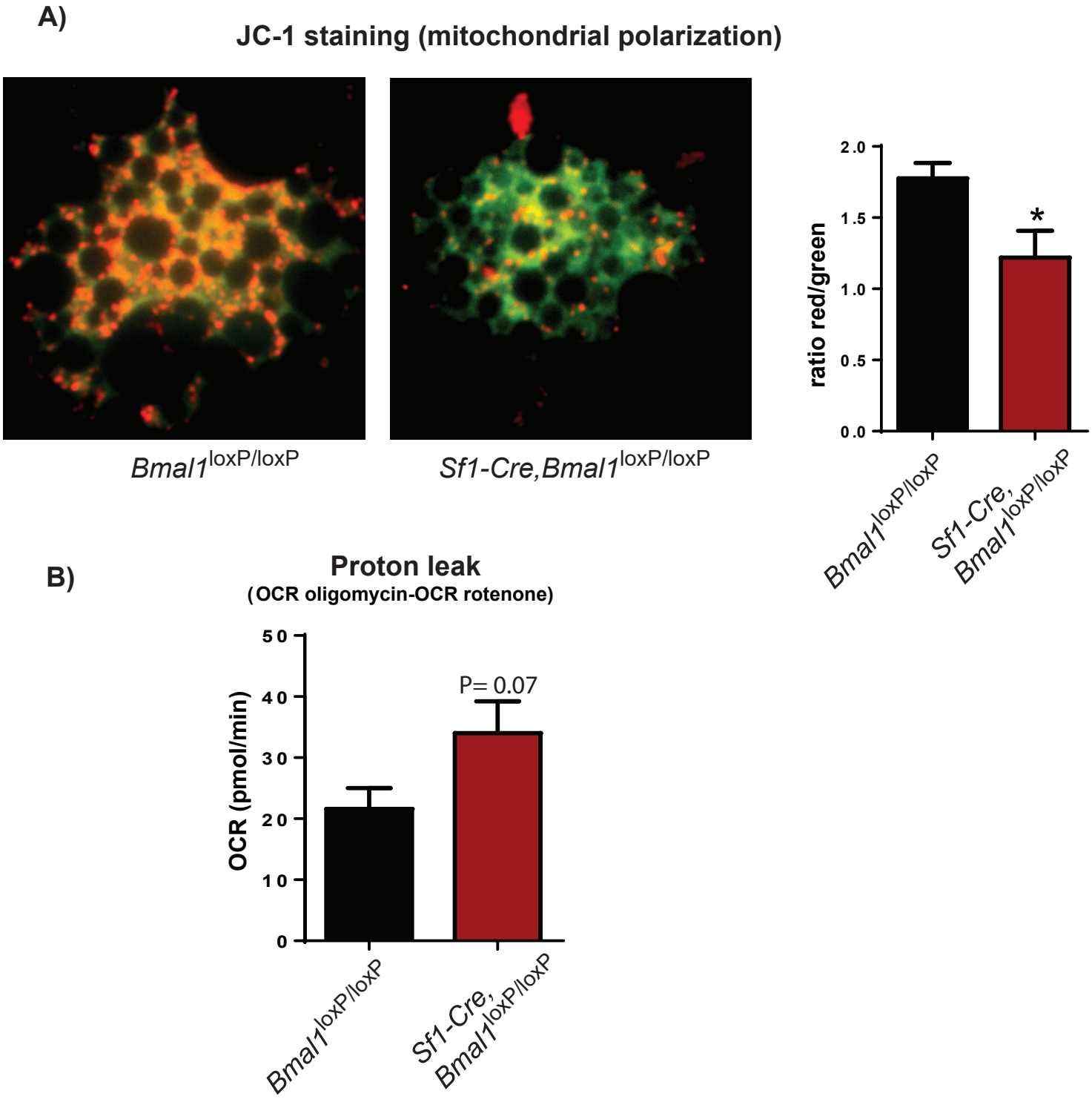

Figure S1, related to figure 4. Membrane potential analysis and proton leak measurements from isolated brown adipocytes
A) Left, representative images of isolated brown adipocytes at various differentiation stages evaluated with JC-1 dye staining; right, histogram depicting the quantification of the red/green signals. Regions of high mitochondrial potentials appear as red fluorescence due to J-aggregate, whereas decreased membrane potential regions are shown by the green fluorescence of the JC-1 monomers, B) Histogram showing proton leak measurements from isolated brown adipocytes (*<0.05; t-test). All plots expressed as means ± SEM. n=4-6 mice per genotype, and 4 technical replicates per mouse. OCR, oxygen consumption rate.

Figure S2, related to figure 5

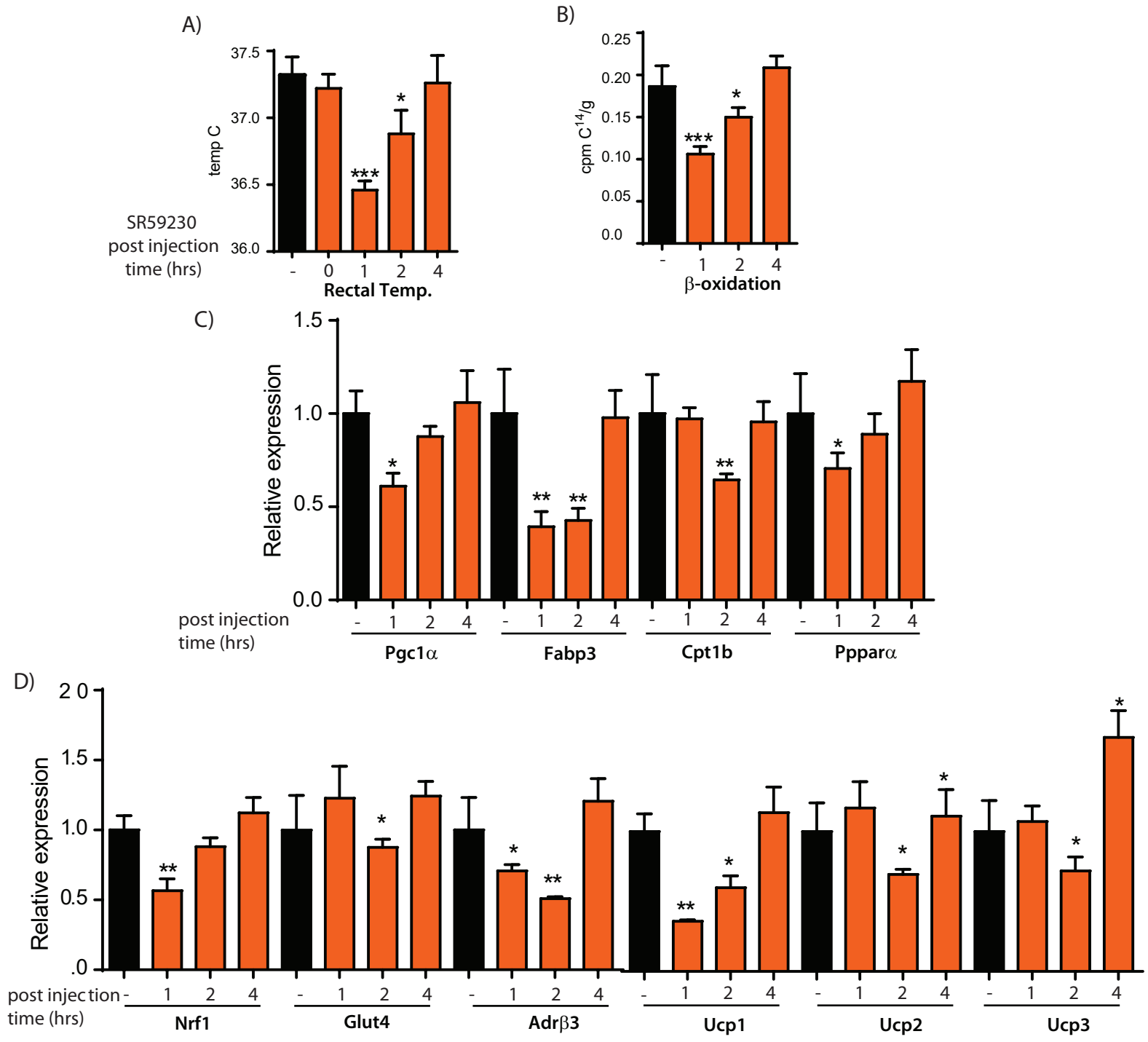

Figure S2, related to figure 5. Effects of the ADRB3 antagonist SR59230A on rectal temperature, b-oxidation and BAT's gene expression after 1, 2 and 4 hrs post injection. A) Rectal temperature, B) b-oxidation, C) Thermogenic genes in BAT. Orange bars, SR59230 intraperitoneal. injection; black bar, intraperitoneal water injection (*<0.05, **<0.001 t-test). All plots expressed as means ± SEM. n=5 per time point.

Figure S3, related to figure 4

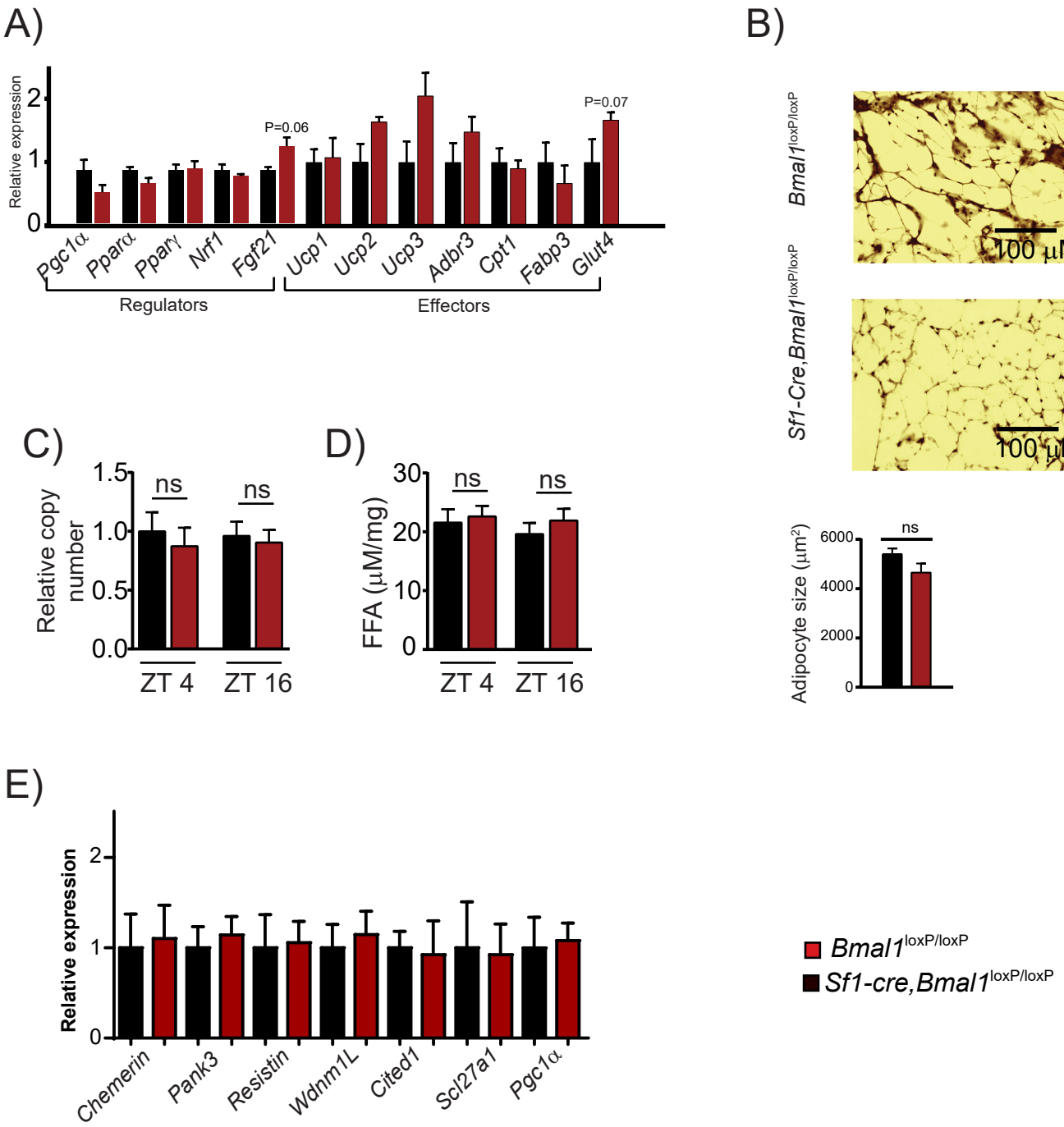

Figure S3, related to figure 4. Lack of BMAL in the SF1-neurons slightly induce WAT activation A) Expression of thermogenic regulators and effectors of wat/beige adipocytes. B) Hematoxylin and eosin (H&E) staining; right plot, measured adipocyte size area C) Mitochondrial DNA copy number (mtDNA) D) Free fatty acid content at ZT 4 and ZT 16. E) Expression of WAT and beige markers (A-D, inguinal WAT; E, subcutaneous WAT), (*<0.05, **<0.001 t-test). All plots expressed as means ± SEM. n=4 per group.

Figure S4, related to figure S3

A)

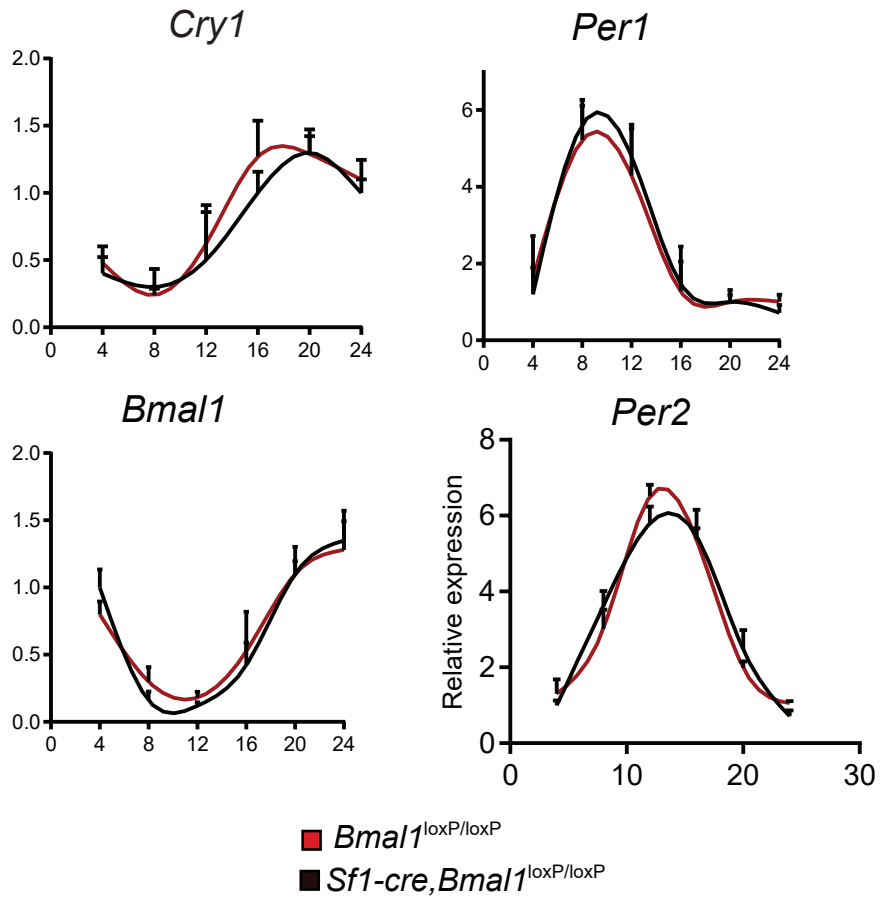

Figure S4, related to figure S3. Lack of BMAL1 in SF1-neurons do not alters the circadian clock in liver.
A) Circadian expression of core-clock genes measured each 4 hrs showing no differences between the genotypes. All plots expressed as means ± SEM. n=4, per time point.

Figure S5, related to figure 6

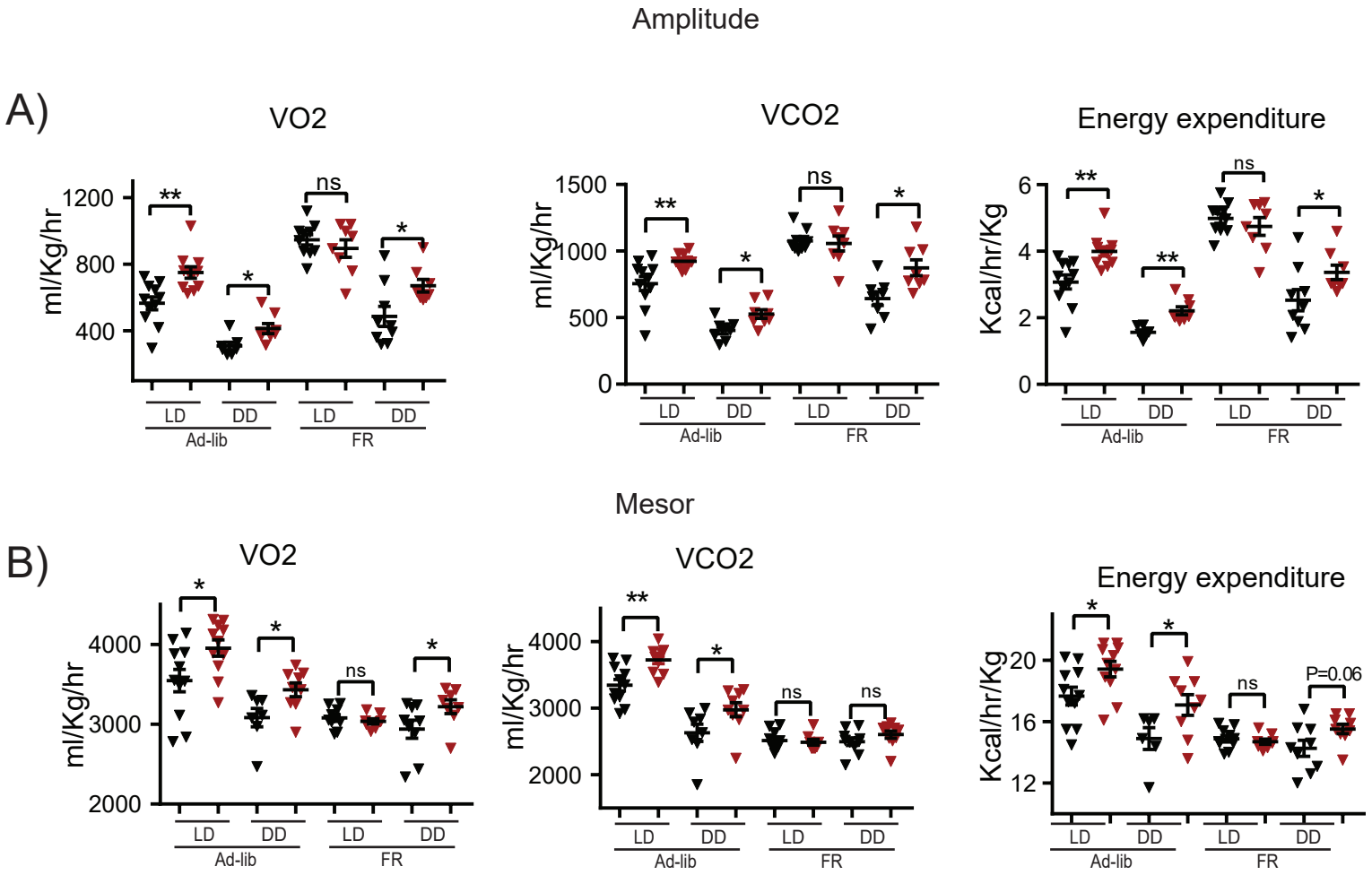

Figure S5, related to figure 6. Effects on feeding and light conditions on the Amplitude and MESOR, on the circadian respiratory cycles. The circadian parameters Amplitude and MESOR were computed from the measured values of O2 consumption, CO2 production and energy expenditure. A) Amplitude, B) Mesor. LD, Light-dark; DD, constant darkness; Ad-lib, ad-libitum feeding; FR, feeding restriction feeding (from ZT13 to ZT17). (*<0.05, **<0.001 t-test). All plots expressed as means ± SEM. n=8-11 per group.

Table S1, related to Figure 1

Effect of genotype and time on the circadian gene expression in the SCN and the VMH.

2 way ANOVA	Gene		Interaction	Time	Genotype
SCN	Bmal1	F	(5, 42) = 0.4420	(5, 42) = 3.335	(1, 42) = 0.5220
		P value	0.8166	0.0126	0.474
		Significance	ns	*	ns
	Cry1	F	(5, 48) = 0.3817	(5, 48) = 12.15	(1, 48) = 0.007155
		P value	0.8589	P < 0.0001	0.9329
		Significance	ns	****	ns
	Per2	F	(5, 48) = 0.3255	(5, 48) = 12.18	(1, 48) = 0.03808
		P value	0.8952	P < 0.0001	0.8461
		Significance	ns	****	ns
	Per1	F	(5, 48) = 0.2497	(5, 48) = 19.91	(1, 48) = 0.3547
		P value	0.9379	P < 0.0001	0.5542
		Significance	ns	****	ns
VMH	Bmal1	F	(5, 48) = 2.626	(5, 48) = 2.804	(1, 48) = 160.5
		P value	0.0354	0.0267	P < 0.0001
		Significance	*	*	****
	Cry1	F	(5, 48) = 2.193	(5, 48) = 3.524	(1, 48) = 119.1
		P value	0.0705	0.0086	P < 0.0001
		Significance	ns	**	****
	Per2	F	(5, 48) = 21.01	(5, 48) = 12.93	(1, 48) = 22.34
		P value	P < 0.0001	P < 0.0001	P < 0.0001
		Significance	****	****	****
	Per1	F	(5, 48) = 2.736	(5, 48) = 6.177	(1, 48) = 7.386
		P value	0.0297	0.0002	0.0091
		Significance	*	***	**

Table S2, related to figure 2

Effects on respiratory rate analyzed by 2-way ANOVA and ANCOVA statistical methods

LD *Ad-libitum*		**Energy expenditure (EE)**		**VO2**		**VCO2**	
		P	**F**	**P**	**F**	**P**	**F**
2-way ANOVA	**Time**	<0.0001	F (1, 9) = 640.7	<0.0001	F (1, 9) = 511.8	<0.0001	F (1, 9) = 177.4
	Genotype	0.011	F (1, 9) = 10.38	0.017	F (1, 9) = 8.526	0.011	F (1, 9) = 10.06
ANCOVA	**Genotype ZT 4**	0.17	2.07	0.31	1.12	0.59	0.31
(covariate factor BW)	**Genotype ZT 16**	0.048	4.536	0.020	6.643	0.029	5.664

Table S3, related to figure 3

Analysis of the effect of genotype and time on the circadian gene expression in the BAT

2 way ANOVA	Gene		Interaction	Time	Genotype
BAT	Bmal1	F	(5, 48) = 0.1249	(5, 48) = 6.169	(1, 48) = 0.09915
		P value	0.9861	0.0002	0.7542
		Significance	ns	***	ns
	Cry1	F	(5, 48) = 0.3705	(5, 48) = 31.57	(1, 48) = 0.1824
		P value	0.8663	P < 0.0001	0.6712
		Significance	ns	****	ns
	Per2	F	(5, 48) = 0.3709	(5, 48) = 36.41	(1, 48) = 0.6657
		P value	0.8661	P < 0.0001	0.4186
		Significance	ns	****	ns
	Per1	F	(5, 48) = 0.2114	(5, 48) = 25.93	(1, 48) = 0.04639
		P value	0.9561	P < 0.0001	0.8304
		Significance	ns	****	ns
	Reberbα	F	(5, 48) = 0.1352	(5, 48) = 8.630	(1, 48) = 1.404
		P value	0.9834	P < 0.0001	0.2419
		Significance	ns	****	ns
	Rorα	F	(5, 48) = 0.7000	(5, 48) = 6.509	(1, 48) = 0.6631
		P value	0.6261	0.0001	0.4195
		Significance	ns	***	ns
	Ucp1	F	(5, 48) = 0.5448	(5, 48) = 3.122	(1, 48) = 18.89
		P value	0.7413	0.0161	P < 0.0001
		Significance	ns	*	****
	Ucp2	F	(5, 48) = 4.655	(5, 48) = 7.448	(1, 48) = 10.29
		P value	0.0015	P < 0.0001	0.0024
		Significance	**	****	**
	Ucp3	F	(5, 48) = 1.431	(5, 48) = 8.831	(1, 48) = 34.98
		P value	0.2301	P < 0.0001	P < 0.0001
		Significance	ns	****	****
	Adrb3	F	(5, 48) = 1.151	(5, 48) = 7.531	(1, 48) = 1.862
		P value	0.3468	P < 0.0001	0.1787
		Significance	ns	****	ns
	Nrf1	F	(5, 48) = 4.853	(5, 48) = 38.58	(1, 48) = 3.200
		P value	0.0011	P < 0.0001	0.08
		Significance	**	****	ns
	Cpt1b	F	(5, 48) = 3.316	(5, 48) = 4.997	(1, 48) = 7.832
		P value	0.0119	0.0009	0.0074
		Significance	*	***	**
	Fabp3	F	(5, 48) = 2.123	(5, 48) = 23.19	(1, 48) = 0.6570
		P value	0.0787	P < 0.0001	0.4216
		Significance	ns	****	ns

Table S4, related to figure 5

Effect on genotype and SR59230A on Sf1.cre;Bmal1$^{loxP/loxP}$ and Bmal$^{loxP/loxP}$ mice

2-way ANOVA	Temperature		β-oxydation		Cpt1b		Fabp3		Nrf1		Pgc1α	
	P value		P value		P value		P value		P value		P value	
Interaction	0.2886	ns	0.0981	ns	0.0003	***	0.0259	*	0.0039	**	0.0012	**
Genotype	< 0.0001	****	0.0018	**	< 0.0001	****	< 0.0001	****	< 0.0001	****	< 0.0001	****
SR59230A	0.0131	*	< 0.0001	****	< 0.0001	****	0.0452	*	0.0039	**	0.0039	**
	Pparα		**Adrβ3**		**Glut4**		**UCP1**		**UCP2**		**UCP3**	
	P value		P value		P value		P value		P value		P value	
Interaction	0.0028	**	< 0.0001	****	0.0005	***	0.7486	ns	0.0059	**	0.0033	**
Genotype	< 0.0001	****	< 0.0001	****	< 0.0001	****	< 0.0001	****	< 0.0001	****	< 0.0001	****
SR59230A	< 0.0001	****	0.0491	*	0.0005	***	0.0486	*	0.0382	*	0.0025	**

Table S5, related to figures 1, 3, 4, 5, S2, S3, S4

Primers used in this study

Gene name	Symbol	Primer forward	Primer reverse
18S ribosomal RNA	18S	TGGCTCATTAAATCAGTTATGGT	GTCGGCATGTATTAGCTCTAG
Adrenoceptor beta 3	Adrb3	TTCTGTGTAGCTACGGTGG	CCATCAAACCTGTTGAGCG
Aryl hydrocarbon receptor nuclear translocator-like (ARNTL)	Bmal ex4	AGGCCCACAGTCAGATTGAA	GCTGAACAGCCATCCTTAGC
Beta actine	Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Carnitine palmitoyltransferase I	Cpt1b	GTCGCTTCTTCAAGGTCTGG	AAGAAAGCAGCACGTTCGAT
Cbp/p300-interacting transactivator 1	Cited1	ATGCCAACCAGGAGATGAAC	AGGATGCAGGTTGAAGGATG
Cell death activator	Cidea	GCAGCCTGCAGGAACTTATC	CCATTTCTGTCCCTTTTCCA
Chemerin	Rarres2	TACAGGTGGCTCTGGAGGAGTTC	CTTCTCCCGTTTGGTTTGATTG
Cryptochrome 1	Cry1	CAGACTCACTCACTCAAGCAAGG	TCAGTTACTGCTCTGCCGCTGGAC
Cytochrome c oxidase polypeptide 7A1	Cox7a1	AAAACCGTGTGGCAGAGAAG	CCAGCCCAAGCAGTATAAGC
Cytochrome c oxidase subunit 1	Cox1	ACCATCATTTCTCCTTCTCCTA	TAGATTTCCGGCTAGAGGTG
cytochrome c oxidase subunit VIIIb	Cox8b	TGCGAAGTTCACAGTGGTTC	TGCTGCGGAGCTCTTTTTAT
Epithelial stromal interaction 1	Epsti1	AGAACTGGCAGACCTGGAGA	GCTTCCTCAGCTTCCTTCCT
Fatty acid binding protein	Fabp3	GACGAGGTGACAGCAGATGA	TGCCATGAGTGAGAGTCAGG
Fatty acid transport protein 1 (Fatp1), Scl271a	Scl27a1	GCTTCAACAGCCGTATCCTC	TCTTCTTGTTGGTGGCACTG
Fibroblast growth factor 21	Fgf21	AGATCAGGGAGGATGGAACA	TCAAAGTGAGGCGATCCATA
Forkhead box protein C2	Foxc2	ATGTTCGAGAATGGCAGCTT	GACTTTCTTCTCGGCCTCCT
Glucose transporter type 4	Glut4	CGTCATTGGCATTCTGGTTG	CGGATGATGTAGAGGTATCTGG
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	CACTGAGCATCTCCCTCACA	GTGGGTGCAGCGAACTTTAT
LIM homeobox protein 8	Lhx8	CGTCAGTCCCAACCATTCTT	CATTGGATGGGGTAACAAGG
Lipase, hormone-sensitive	Lipe	AAGATCAAAGCCTCAGCGT	CATATTGTCTTCTGCGAGTGTC
Nuclear receptor subfamily 1, group D, member 1 (Nr1d1)	Reverba	GGGCACAAGCAACATTACCA	CACGTCCCCACACACCTTAC
Nuclear respiratory factor 1	Nrf1	CAACAGGGAAGAAACGGAAA	GCACCACATTCTCCAAAGGT
Pantothenate kinase 3	Pank3	TGCTGTAGTGTCCCATTTCTGCCT	AGCTGGAACAGCAACACCTAGGAA
Period 1	Per1	ACCAGCGTGTCATGATGACATAC	GTGCACAGCACCCAGTTCCC
Period 2	Per2	CGCCTAGAATCCCTCCTGAGA	CCACCGGCCTGTAGGATCT
Peroxisome proliferator-activated receptor alpha	Ppara	ACAAGGCCTCAGGGTACCA	GCCGAAAGAAGCCCTTACAG
Peroxisome proliferator-activated receptor gamma	Pparg	CAAGAATACCAAAGTGCGATCAA	GCCGAAAGAAGCCCTTACAG
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	Pgc1a	AACCACACCCACAGGATCAGA	TCTTCGCTTTATTGCTCCATGA
PR domain containing 16	Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Pyruvate dehydrogenase kinase, isozyme 4	Pdk4	GAGCTGGTATATCCAGAGCCTGAT	CGAACTTTGACCAGCGTGTCT
Resistin	Retn	AAGAACCTTTCATTTCCCCTCCT	GTCCAGCAATTTAAGCCAATGTT
Thyroid hormone receptor beta	Thrb	AAGTGCCCAGACTTTCCAGA	TGCGGGTGACTTTGTCTATG
Type II iodothyronine deiodinase	Dio2	CCACCTGACCACCTTTCACT	TGGTTCCGGTGCTTCTTAAC
Ubiquitin carboxy-terminal hydrolase L1	Uchl1	GATTAACCCCGAGATGCTGA	CCTGTCCCTTCAGTTCCTCA
Uncoupling protein 1	Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Uncoupling protein 2	Ucp2	GCGTTCTGGGTACCATCCTA	GCTCTGAGCCCTTGGTGTAG
Uncoupling protein 3	Ucp3	ATGAGTTTTGCCTCCATTCG	GGCGTATCATGGCTTGAAAT
West-mead DMBA-8 non-metastatic-like	Wdnm1L	AGTGAGACCTCTGCAGCTTTTAGG	CAACTGTTTTCTTGGTCACAGAGC
Zic family member 1	Zic1	CAGAGCAGAGCAACCACATC	CTCCCCTGTGTGTGTCCTTT

Supplementary Methods

Brown adipocytes isolation

Brown adipose tissue dissected from interscapular fat pad was minced in PBS, and a equal amount of isolation buffer (123 mM NaCl, 5mM KCl, 1.3 mM CaCl2, 5mM Glucose, 100 mM HEPES) with collagenase (1.5mg/ml) and dispase (2.0 mg/ml) was added. Samples were incubated in 37^{o}C water bath and vortexed for 20 seconds every 5 min during 45 minutes. Digested tissues were filtered through a cell strainer (100 μM) and centrifuged at 800 g for 5 min. Mature adipocytes on the surface and adipocyte precursor cells in the precipitates were collected as dispersed brown adipocyte suspension, washed 2 times with DMEM, and immediately used for subsequent proton leak experiments and membrane potential assay (JC-1 staining).

JC-1 Staining

Isolated brown adipocytes were incubated with 20 μM JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) for 30 min before observation at green and red emission wavelengths using fluorescence microscope. Images were analyzed using the ImageJ software.

Proton leak measurements

Metabolic rates of cells were measured using a Seahorse Bioscience XF24 Extracellular Flux Analyzer following manufacturer's protocol. Briefly, dispersed brown adipocytes were plated in a 0.2% gelatin coated 24-well Seahorse XF-24 assay plate at 5 × 104 cells/well, centrifuged at 400G and pre-incubated in DMEM at 37^{o}C for 1 hr before measurements. The oxygen consumption rate (OCR) values were measured following sequential injection of oligomycin (1.0 μg/ml), carbonyl cyanide p-trifluoromethoxyphenylhydrazone (1.4 μM), and rotenone (5 μM). The proton leak was calculated by subtracting OCR values after rotenone injection from those values after oligomycin injection.