Figure S1.

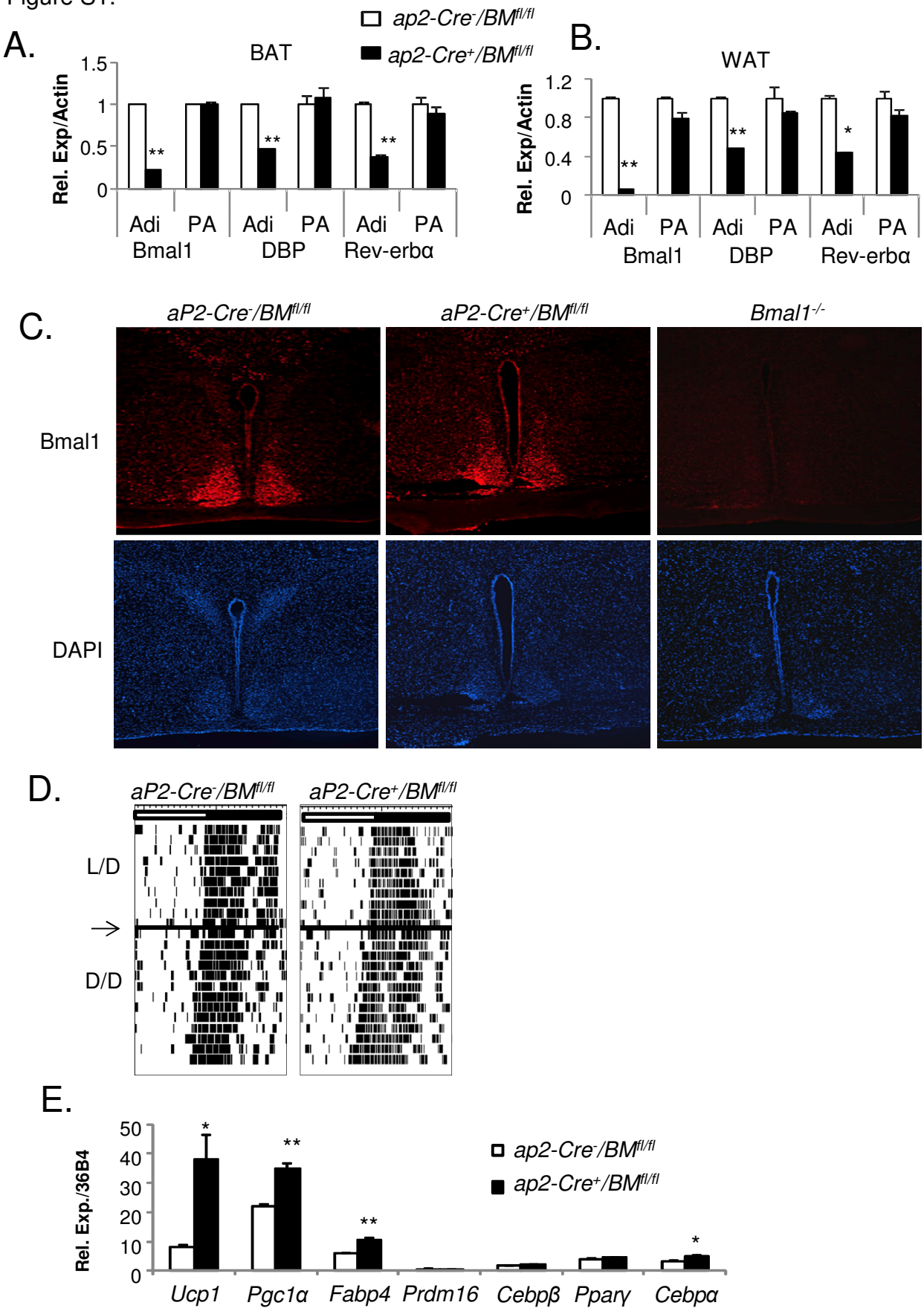

A.
BAT
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl
Rel. Exp/Actin
1.5
1
0.5
0
**
**
**
Adi
PA
Adi
PA
Adi
PA
Bmal1
DBP
Rev-erbα
B.
WAT
Rel. Exp/Actin
1.2
0.8
0.4
0
**
**
*
Adi
PA
Adi
PA
Adi
PA
Bmal1
DBP
Rev-erbα
C.
aP2-Cre-/BMfl/fl
aP2-Cre+/BMfl/fl
Bmal1-/-
Bmal1
DAPI
D.
aP2-Cre-/BMfl/fl
aP2-Cre+/BMfl/fl
L/D
D/D
E.
Rel. Exp./36B4
50
40
30
20
10
0
*
**
**
*
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl
Ucp1
Pgc1α
Fabp4
Prdm16
Cebpβ
Pparγ
Cebpα

Figure S2.

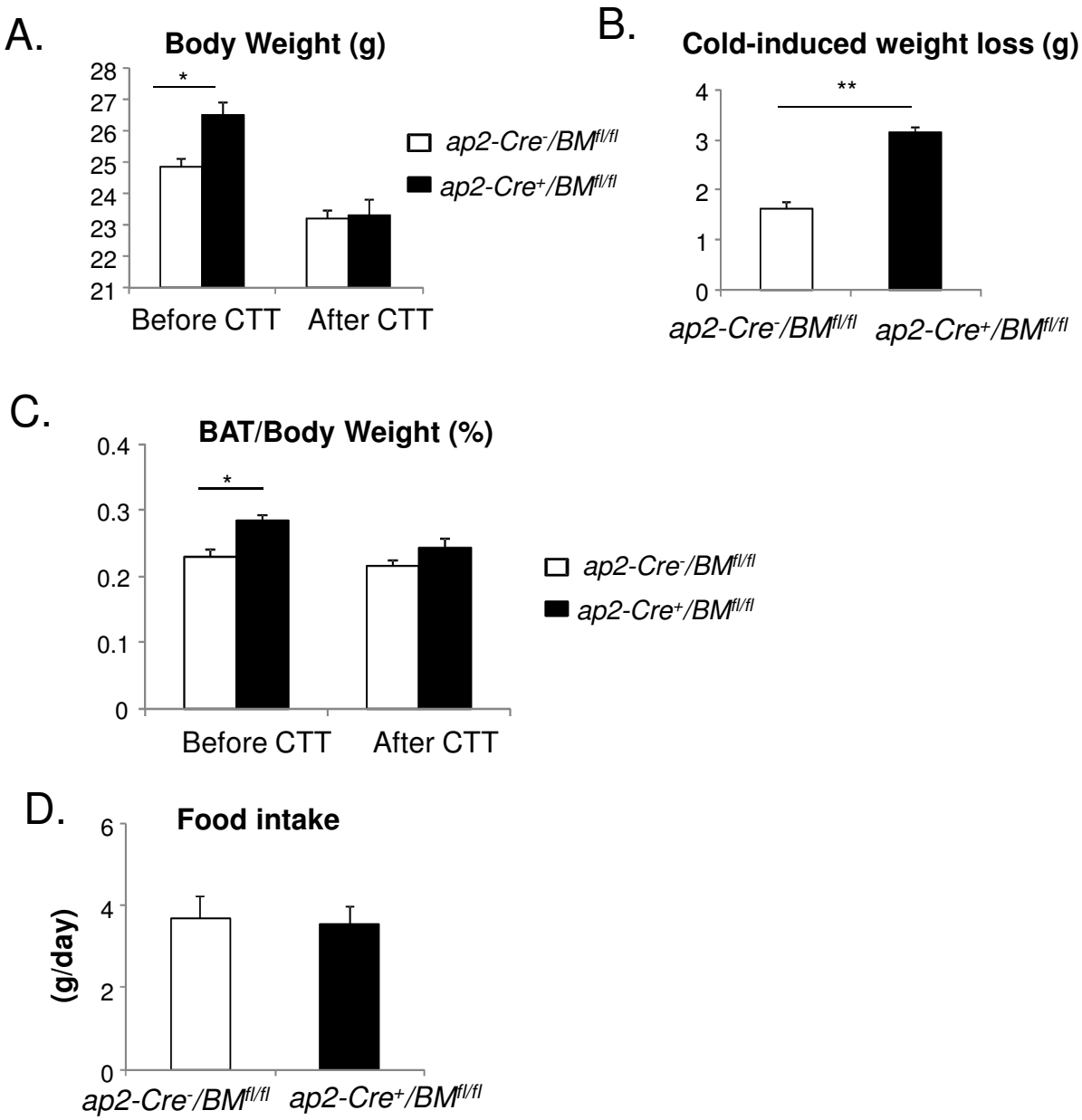
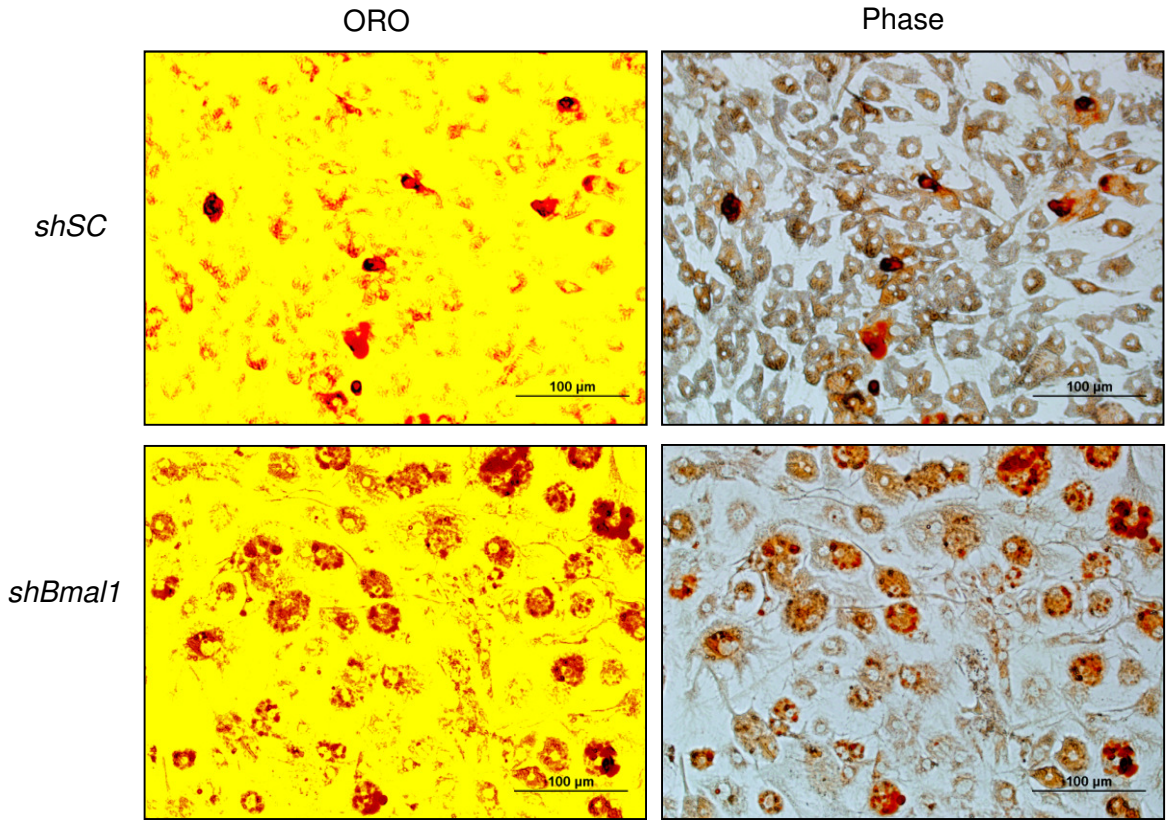

A.
Body Weight (g)
28
27
26
25
24
23
22
21
*
Before CTT
After CTT
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl
B.
Cold-induced weight loss (g)
4
3
2
1
0
**
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl
C.
BAT/Body Weight (%)
0.4
0.3
0.2
0.1
0
*
Before CTT
After CTT
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl
D.
Food intake
(g/day)
6
4
2
0
ap2-Cre-/BMfl/fl
ap2-Cre+/BMfl/fl

Figure S3.



Supplemental Figure Legends

Figure S1. Adipose-specific ablation of *Bmal1* in *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice with intact central clock function. (A, B) Characterization of *Bmal1* deletion in brown and white adipose tissue of *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ and littermate *ap2-Cre*$^{-}$*/BM*$^{fl/fl}$ mice. Loss of *Bmal1* expression in mature adipocytes but not preadipocytes isolated from *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ brown (A), or white adipose tissue (B), as analyzed by RT-qPCR. Adi: adipocytes; PA: preadipocytes. (C, D) Intact central clock in *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice. Representative images of Bmal1 immunostaining in the central clock, suprachiasmatic nuclei (C), and circadian locomotor activity as monitored by wheel-running actograms to assess central clock function (D) in *ap2-Cre*$^{-}$*/BM*$^{fl/fl}$ and *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice. *Bmal1*$^{-/-}$ mice were included as negative controls for the immunostaining. Actograms were performed on mice kept in normal light/dark cycles for 10 days before switching to dark/dark cycles for 14 days (n=6/group). (E) RT-qPCR analysis of brown adipogenic gene expression in BAT of *ap2-Cre*$^{-}$*/BM*$^{fl/fl}$ and *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice at ZT10 (5PM) (n=6/group). *, **: P<0.05 and 0.01 *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ *vs. ap2-Cre*$^{-}$*/BM*$^{fl/fl}$.

Figure S2. Body weight and brown fat weight change before and after cold tolerance test in *ap2-Cre*$^{-}$*/BM*$^{fl/fl}$ and *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice. (A-C) Body weight (A), body weight loss after cold tolerance (B) and brown fat to body weight ratio (C) before and after cold tolerance test at 4^{0}C for 8 hours (CTT). N=6-7/group, *,**:P<0.05 and 0.01 *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ *vs. ap2-Cre*$^{-}$*/BM*$^{fl/fl}$. D. Average daily food intake as measured by CLAMS unit monitoring of *ap2-Cre*$^{-}$*/BM*$^{fl/fl}$ and *ap2-Cre*$^{+}$*/BM*$^{fl/fl}$ mice. Food intake is monitored for 5 days under ambient temperature (22^{0}C) and average daily intake was calculated (n=6-7/group).

Figure S3. Representative images of Oil-Red-O staining (left panels) and phase-contrast (right panels) of day 10-differntiated shSC and shBmal1 C3H10T1/2 cells (20X).

Table S1 Complete dataset of KEGG pathway analysis of microarray results of differentially regulated genes in *Bmal1* KD vs. SC C3H10T1/2 cells with adjusted P value $\leqslant$ 0.05.

Download Table S1.

Table S2. Bmal1 E-box elements identified on TGF-β pathway gene promoters.

Gene Name	Chromosome	E-box Sequence	TSS Distance
Rev-erbα	Chr11	CACGTG	-17
TGFb1	Chr7	CACGTG	-642
TGFb2	Chr1	CACGTG	-50
TGFbr2	Chr9	CACGTG	-785
Smad3	Chr9	CACGTG	-323

Table S3. Primer sequences for ChIP analysis of candidate Bmal1 targets.

Genes		Sequences
Tbp	Forward	CCACACCCGCCACCAGTTCG
	Reverse	TACAGCCCGGGGAGCATCGT
Rev-erba	Forward	TTTCCCTGGGACAGAGGGCTCT
	Reverse	TACAAATCCCAACAATCCTGGCGGT
TGFb1	Forward	AGTGGAGTGTTGAGGGACTT
	Reverse	TGATCAGGACTGACAGTCTC
TGFb2	Forward	TTCTCTCTTTCTCTCCCACTC
	Reverse	AACAGAGGACTTCTGACTGT
TGFbr2	Forward	TTTGCTGGGTGAAGCTCTGT
	Reverse	TGCCCTGGAACTCACTCTGTA
Smad3	Forward	ACAGACTCTCAGATACCGTTGCA
	Reverse	TTCCTGATCTCACCCAGCAGGAA

Table S4. Primary antibodies used in the study.

Antibody	Host	Vendor	Dilution
Bmal1	Rabbit	Abcam	1:1,000
UCP-1	Rabbit	Millipore	1: 5,000
Smad3	Rabbit	Cell Signaling	1:1,000
P-Smad3	Rabbit	Cell Signaling	1:1,000
Smad5	Rabbit	Cell Signaling	1:1,000
P-Smad5	Rabbit	Cell Signaling	1:1,000
b-actin	Mouse	Sigma-Aldrich	1:10,000
TBP	Rabbit	Santa Cruz Biotechnology	1:1,000