Cell Reports, Volume *39*

Supplemental information

Flattening of circadian glucocorticoid oscillations drives acute hyperinsulinemia and adipocyte hypertrophy

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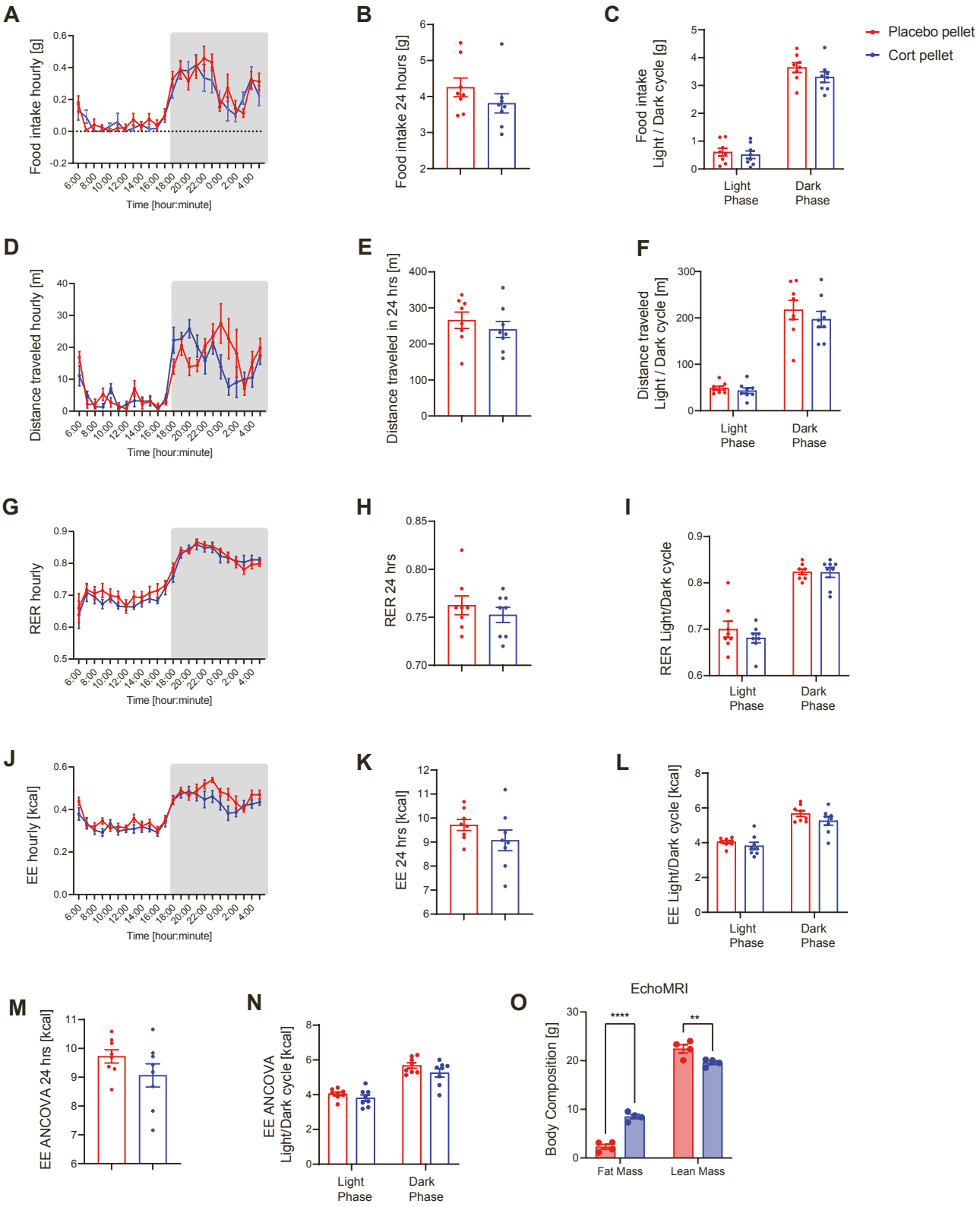


Figure S1. Metabolic cage and EchoMRI measurements in placebo- and cort-pellet mice. Related to Figure 1.
(A) Food intake expressed as total per hour, (B) daily total (24 hrs), and (C) total per day/night cycle (12 hrs).
(D) Distance traveled expressed as total per hour, (E) daily total (24 hrs), and (F) total per day/night cycle (12 hrs).
(G) Respiratory exchange ratio (RER) expressed as hourly averages, (H) daily averages (24 hrs), and (I) day/night cycle averages (12 hrs).
(J) Energy expenditure (EE) expressed as hourly averages, (K) daily total (24 hrs) and (L) total per day/night cycle.
(M) EE daily total (24 hrs) and (N) total per day/night cycle expressed following adjustment for total body mass using analysis of covariance (ANCOVA).
(A-N) Experiments were carried out 18 days after implanting pellets that released 0.25 mg/day placebo- or cort-pellet. Results are presented as mean ± SEM, n = 8. Data of total per hour as well as total per day/night cycle were analyzed using two-way repeated measures ANOVA with Sidak's multiple comparisons test. Daily total (24 hrs) data was analyzed using unpaired t tests.
(O) EchoMRI measurements were carried out 38 days after implanting pellets that released 0.25 mg/day placebo or cort. Results are presented as mean +/- SEM, n = 8. **p<0.01; ****p<0.0001

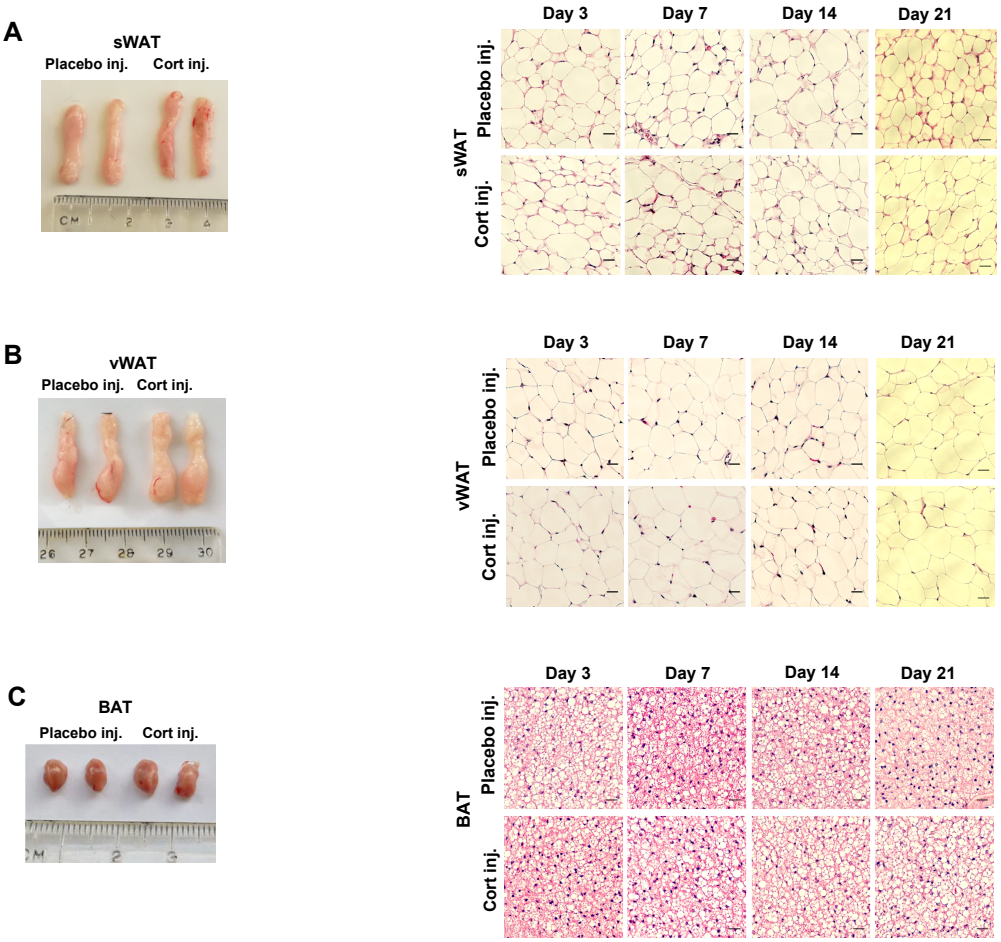


Figure S2. Images of tissue and adipocyte sections in WAT and BAT induced by daily corticosterone injection versus placebo injection. Related to Figure 2.

(A-C) Lack of a visible change in size of WAT and BAT tissue and size of adipocytes in cort-injected mice versus Placebo-injected mice n=3, Scale bar 50 μm, Magnification 20x.

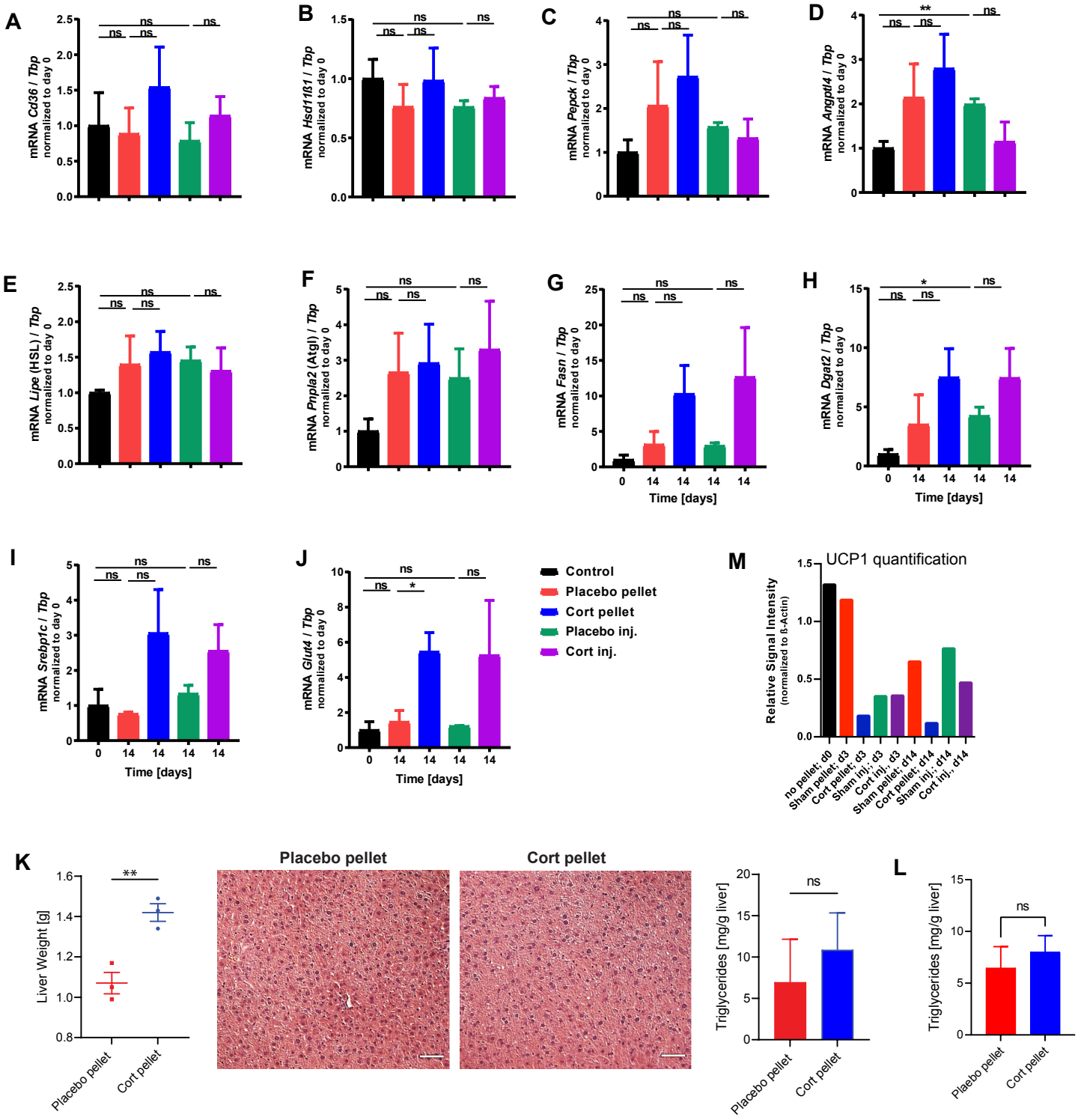


Figure S3. Flattening of corticosterone oscillations does not result in significant gene expression alterations of genes involved in lipid metabolism in liver. Related to Figure 3.

(A-J) Gene expression of different genes measured by qPCR in liver after flattening GCs for 14 days. Gene expression data was normalized to the expression of Tbp and is presented as mean ± SEM, n = 3-4, unpaired t test, *p < 0.05, **p < 0.01, ns = not significant. Control group are mice at beginning of experiment (day 0).

(K) Liver weight, H&E staining, and triglyceride content 35 days after placebo- or cort-pellet implantation; n=3, mean ± SEM, unpaired t tests, **p ≤ 0.01; ns = not significant.

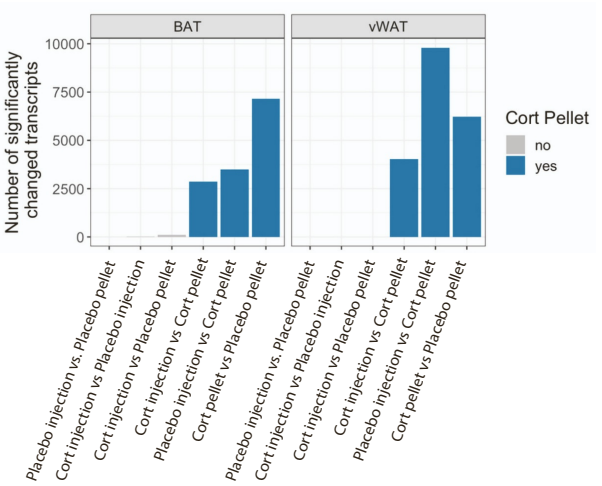
(L) Triglyceride content 21 days after placebo- or cort-pellet implantation; n=3, mean ± SEM, unpaired t tests. ns = not significant.

(M) Quantification of UCP1 western blot in Figure 3F.

A

depot	cond_1	cond_2	num_sig
BAT	Cort injection	Cort pellet	2866
BAT	Cort injection	Placebo injection	9
BAT	Cort injection	Placebo pellet	110
BAT	Cort pellet	Placebo pellet	7146
BAT	Placebo injection	Cort pellet	3502
BAT	Placebo injection	Placebo pellet	2
vWAT	Cort injection	Cort pellet	4022
vWAT	Cort injection	Placebo injection	0
vWAT	Cort injection	Placebo pellet	0
vWAT	Cort pellet	Placebo pellet	6233
vWAT	Placebo injection	Cort pellet	9800
vWAT	Placebo injection	Placebo pellet	0

B



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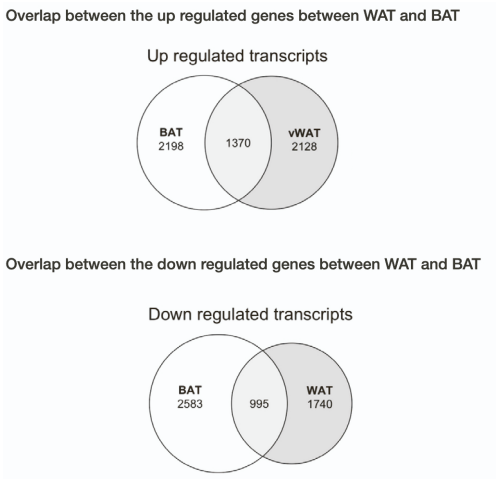

D

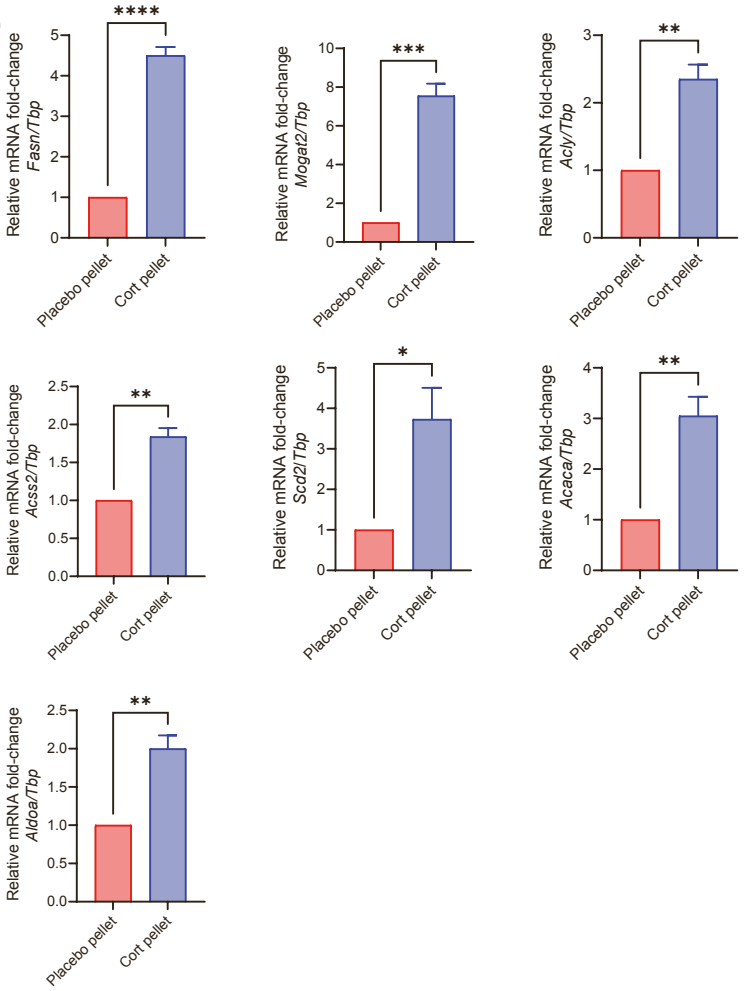

Figure S4. Comparison of BAT and vWAT RNAseq datasets. Related to Figure 4.
(A-B) Number of significantly changed transcripts in BAT and vWAT in different comparisons of treatment methods.
(C) Number of overlapping transcripts in vWAT and BAT. See also Tables S2-S7.
(D) Representative RT-PCR analysis to validate RNAseq analysis carried out in vWAT after 60 days of cort and placebo pellet implantation. In both the RT-PCR and RNAseq analysis, de novo fatty acid synthesis genes such as Fasn, Acaca, and Acly were significantly elevated. Data is normalized to the expression of Tbp and is presented as mean ± SEM (n = 3-4), unpaired t-test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p <0.0001.

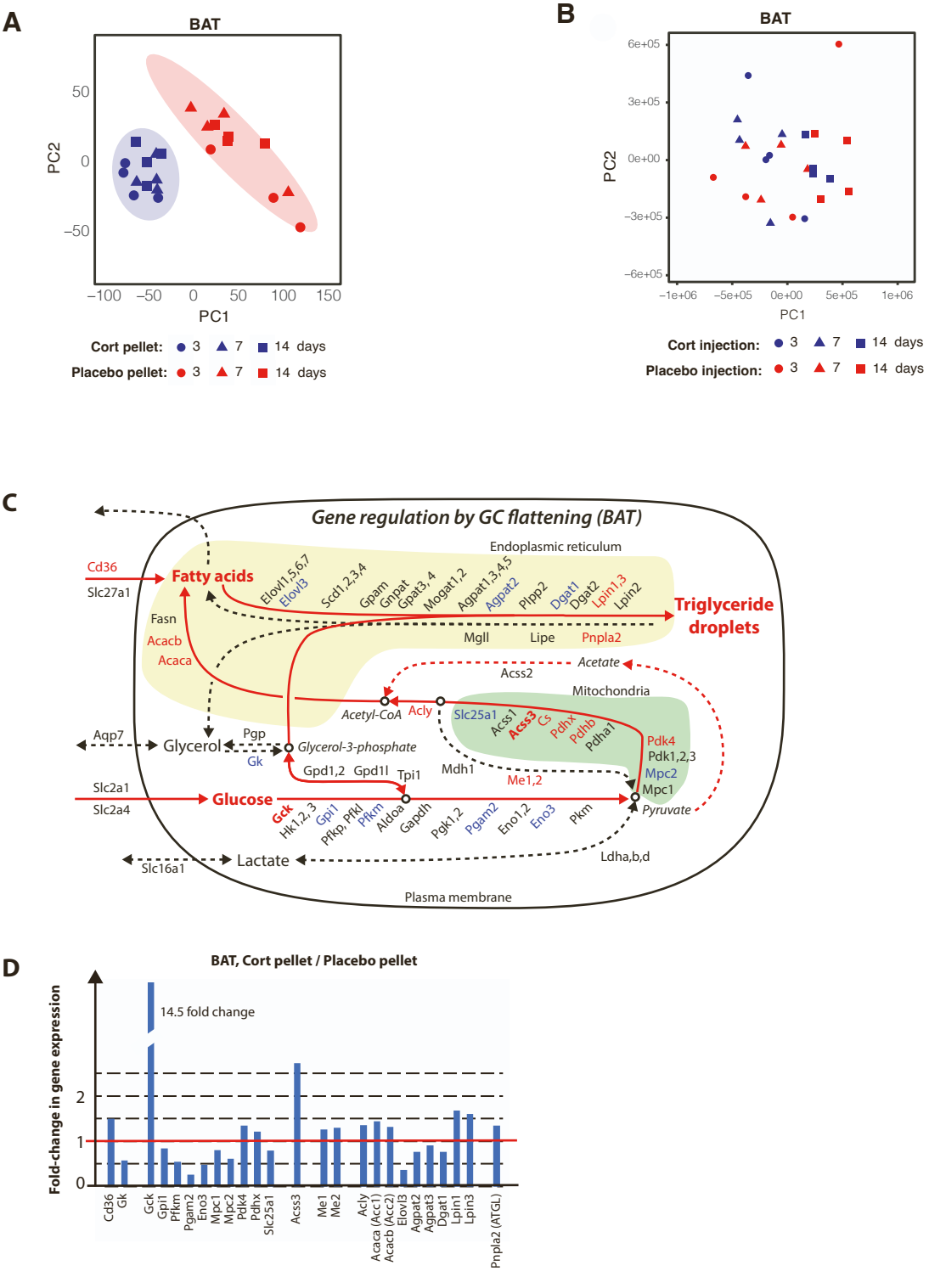

Figure S5. Analysis of BAT RNAseq dataset. Related to Figure 4.

(A) Principal component analysis of BAT from placebo versus cort-pellet mice obtained at 3, 7, and 14 days after pellet implantation.

(B) Principal component analysis of BAT from corticosterone and placebo injected mice obtained at 3, 7, and 14 days after the start of injections.

(C) Schematic representation of gene expression changes in triglyceride synthesis pathways in BAT from placebo versus cort-pellet implanted mice. Upregulated, unchanged, and downregulated genes are marked in red, black, and blue, respectively. Genes with a more than 2-fold change in gene expression are marked bold. See also Tables S2-S7.

(D) Barplots showing fold-change of significantly changed BAT genes in placebo versus cort-pellet. See also Tables S2-S7.

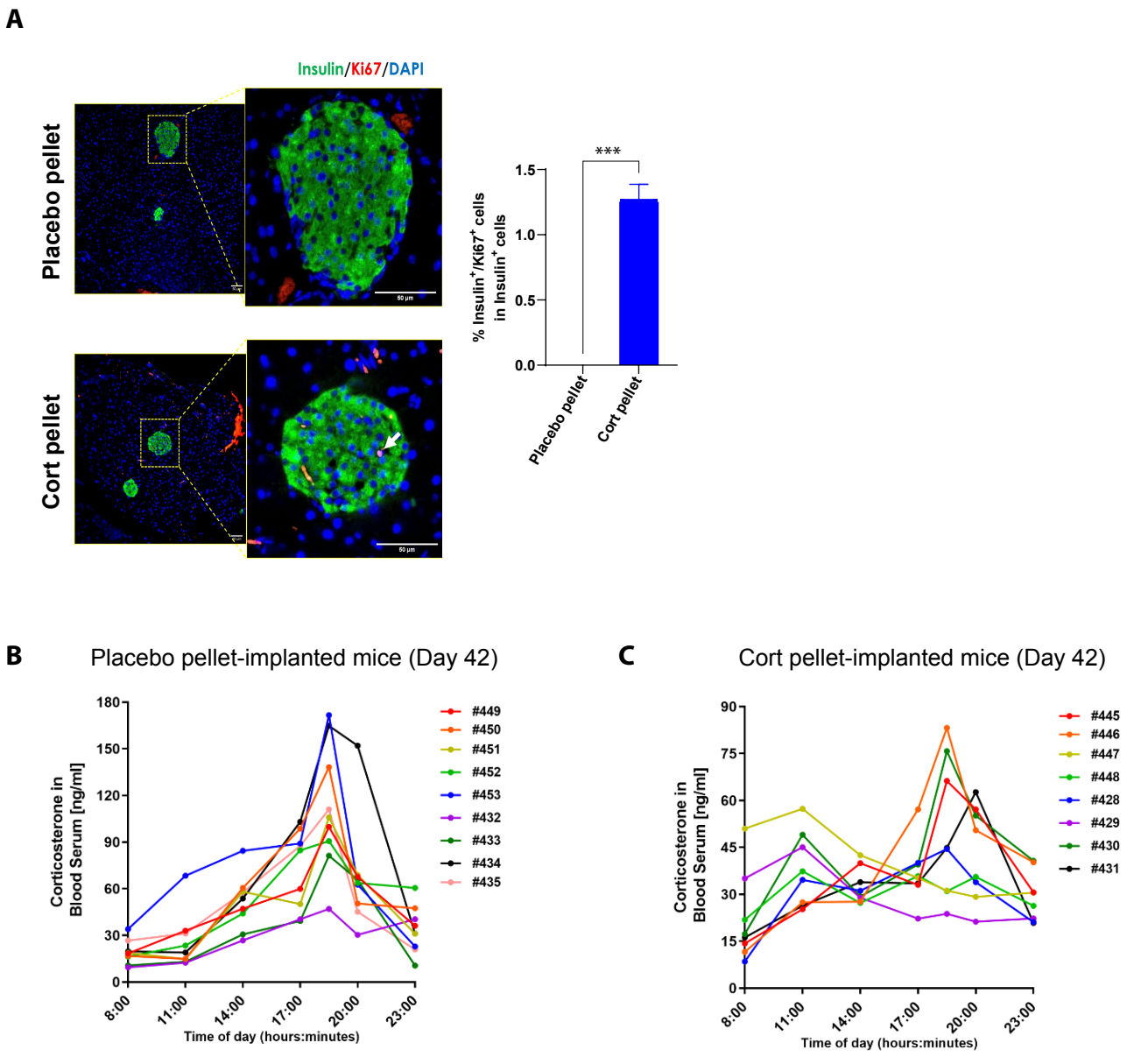

Figure S6. Assessment of pancreatic beta-cell proliferation after 35 days of GC-flattening, and measurements of circulating GC levels in mice which have undergone 21 days of GC flattening, followed by 21 days with no GC flattening. Related to Figures 5 and 6.

(A) Left, Representative immunofluorescence images of pancreatic β-cell proliferation in mice implanted with placebo or cort pellets for 35 days. DAPI-blue, Insulin-green, Ki67-red. White arrow shows a cell positive for both insulin and Ki67. Scale bar 50 μm. Right, Barplot showing immunofluorescence analysis for percent pancreatic β-cell proliferation 35 days after implanting placebo- or cort-pellets; n=3, mean ± SEM, ***p > 0.001.

(B-C) Mice were implanted with either (B) a 21-day placebo pellet or (C) a 21-day cort pellet, and then blood serum corticosterone levels were measured at Day 42 (21 days after pellet stopped releasing corticosterone). While the healthy low trough GC levels were restored by 3 weeks after the pellet stopped releasing GC, the peak levels during waking remained reduced in most of the mice, suggesting that the 3-week GC flattening has a long-term impact on the circadian control of GC secretion from the adrenal cortex.

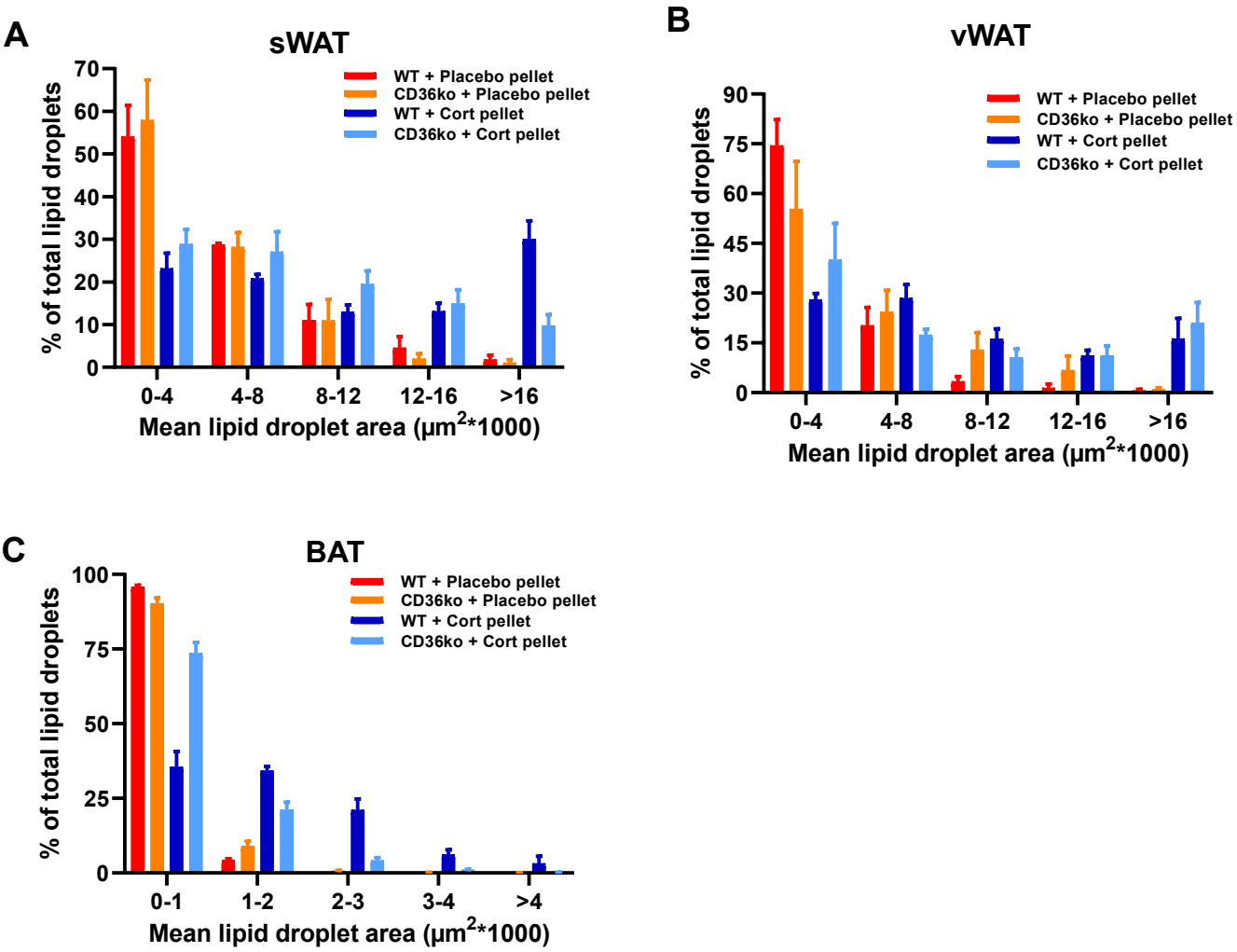

Figure S7. Quantification of lipid droplet size in WT and CD36KO mice implanted with corticosterone or placebo pellets for 21 days. Related to Figure 7.

(A-C) Frequency distributions of mean lipid droplet area in sWAT, vWAT, and BAT as analyzed using Adiposoft (mean + SEM; n=3 mice per treatment, 25 images per mouse (20x)).

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	Wild-type mice				Cd36ko mice	
	Placebo pellet	Cort pellet	Placebo injection	Cort injection	Placebo pellet	Cort pellet
Body weight gain [g]	2.7 ± 0.5	4.8 ± 0.5	1.0 ± 0.3	1.1 ± 0.3	1.7 ± 0.4	3.3 ± 0.4
Body weight gain [% of initial body weight]	11.0 ± 0.8	20.1 ± 1.5	4.4 ± 1.4	4.4 ± 1.0	7.8 ± 1.0	14.7 ± 1.9
sWAT mass [mg]	296.2 ± 27.4	721.2 ± 35.1	326.0 ± 72.5	329.0 ± 6.4	264.0 ± 23.6	557.3 ± 49.9
vWAT mass [mg]	448.8 ± 37.6	1250.0 ± 45.5	456.3 ± 105.7	496.0 ± 27.8	468.2 ± 60.3	676.3 ± 48.8
BAT mass [mg]	148.8 ± 12.5	321.4 ± 11.5	123.7 ± 16.6	151.0 ± 10.6	98.0 ± 10.2	178.3 ± 12.6
Insulin [ng/ml]; fasted	0.52 ± 0.06	1.53 ± 0.16	0.56 ± 0.08	0.54 ± 0.04	n.d.	n.d.
Insulin [ng/ml]; not fasted	0.61 ± 0.06	2.60 ± 0.72	n.d.	n.d.	1.52 ± 0.22	7.43 ± 1.35
Blood glucose [mg/dl]; fasted	131.0 ± 22.1	139.8 ± 24.0	161.0 ± 15.1	160.7 ± 14.7	n.d.	n.d.
Blood glucose [mg/dl]; not fasted	189.1 ± 11.0	146.3 ± 8.3	n.d.	n.d.	171.5 ± 8.4	140.0 ± 5.0
Liver [mg]	971.3 ± 48.5	974.0 ± 9.5	977.0 ± 51.4	1027.3 ± 38.5	n.d.	n.d.
Food intake [g/mouse/day]	3.5 ± 0.1	3.8 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	n.d.	n.d.
FFA [mM]	0.84 ± 0.13	1.65 ± 0.08	1.21 ± 0.24	1.16 ± 0.15	n.d.	n.d.
Glycerol [mg/ml]	0.29 ± 0.05	0.48 ± 0.05	0.35 ± 0.03	0.46 ± 0.04	n.d.	n.d.
Triglycerides [mg/dl]	46.2 ± 6.8	72.6 ± 6.0	84.8 ± 6.5	79.1 ± 7.6	n.d.	n.d.

Table S1: Summary of mouse experiments. All data was acquired after a treatment period of 21 days, except data for blood glucose (not fasted), FFA, glycerol, and triglyceride levels, which were determined 14 days after beginning of treatment. Values are presented as mean +/- SEM; n.d. = no data.

Table S8: RT-PCR Primers

Primer	Sequence (5' to 3')
Acaca Fwd	ATGGGCGGAATGGTCTCTTTC
Acaca Rev	TGGGGACCTTGTCTTCATCAT
Acly Fwd	CAGCCAAGGCAATTTCAGAGC
Acly Rev	CTCGACGTTTGATTAACTGGTCT
Acss2 Fwd	CACGGGAGTTTTGGGGAAACA
Acss2 Rev	GTTGCTCCTTTCATCCACTCA
Aldoa Fwd	CGTGTGAATCCCTGCATTGG
Aldoa Rev	CAGCCCCTGGGTAGTTGTC
Angptl4 Fwd	CATCCTGGGACGAGATGAACT
Angptl4 Rev	TGACAAGCGTTACCACAGGC
Atgl (Pnpla2) Fwd	ATGTTCCCGAGGGAGACCAA
Atgl (Pnpla2) Rev	GAGGCTCCGTAGATGTGAGTG
Cd36 Fwd	ATGGGCTGTGATCGGAACTG
Cd36 Rev	TTTGCCACGTCATCTGGGTTT
Dgat2 Fwd	GCGCTACTTCCGAGACTACTT
Dgat2 Rev	GGGCCTTATGCCAGGAAACT
Fasn Fwd	GGAGGTGGTGATAGCCGGTAT
Fasn Rev	TGGGTAATCCATAGAGCCCAG
Glut4 Fwd	ATCATCCGGAACCTGGAGG
Glut4 Rev	CGGTCAGGCGCTTTAGAC TC
Hsd11ß1 Fwd	GGAGCCCATGTGGTATTGACT
Hsd11ß1 Rev	CCGCAAATGTCATGTCTTCCAT
Lipe (Hsl) Fwd	GATTTACGCACGATGACACAGT
Lipe (Hsl) Rev	ACCTGCAAAGACATTAGACAGC
Mogat2 Fwd	TGGGAGCGCAGGTTACAGA
Mogat2 Rev	CAGGTGGCATACAGGACAGA
Pepck Fwd	CTGCATAACGGTCTGGACTTC
Pepck Rev	GCCTTCCACGAACTTCCTCAC
Scd2 Fwd	GCATTTGGGAGCCTTGTACG
Scd2 Rev	AGCCGTGCCTTGTATGTTCTG
Srebp1 Fwd	TGACCCGGCTATTCCGTGA
Srebp1 Rev	CTGGGCTGAGCAATACAGTTC
Tbp Fwd1	GAAGCTGCGGTACAATTCCAG
Tbp Rev1	CCCCTTGTACCCTTCACCAAT
Tbp Fwd2	CTTCCTGCCACAATGTCACAG
Tbp Rev2	CCTTTCTCATGCTTGCTTCTCTG
Ucp1 Fwd	AGCCGGCTTAATGACTGGAG
Ucp1 Rev	TCTGTAGGCTGCCCAATGAAC