Circadian oscillation of body temperature is a basic, evolutionarily conserved feature of mammalian biology. In addition, homeostatic pathways allow organisms to protect their core temperatures in response to cold exposure. However, the mechanism responsible for coordinating daily body temperature rhythm and adaptability to environmental challenges is unknown. Here we show that the nuclear receptor Rev-erbα (also known as Nr1d1), a powerful transcriptional repressor, links circadian and thermogenic networks through the regulation of brown adipose tissue (BAT) function. Mice exposed to cold fare considerably better at 05:00 (Zeitgeber time 22) when Rev-erbα is barely expressed than at 17:00 (Zeitgeber time 10) when Rev-erbα is abundant. Deletion of Rev-erbα markedly improves cold tolerance at 17:00, indicating that overcoming Rev-erbα-dependent repression is a fundamental feature of the thermogenic response to cold. Physiological induction of uncoupling protein 1 (Ucp1) by cold temperatures is preceded by rapid downregulation of Rev-erbα in BAT. Rev-erbα represses Ucp1 in a brown-adipose-cell-autonomous manner and BAT Ucp1 levels are high in Rev-erbα-null mice, even at thermoneutrality. Genetic loss of Rev-erbα also abolishes normal rhythms of body temperature and BAT activity. Thus, Rev-erbα acts as a thermogenic focal point required for establishing and maintaining body temperature rhythm in a manner that is adaptable to environmental demands.

The molecular clock is an autoregulatory network of core transcriptional machinery orchestrating behavioural and metabolic programming in the context of a 24-h light–dark cycle. The importance of appropriate synchronization in organismal biology is underscored by the robust correlation between disruption of clock circuitry and development of disease states such as obesity, diabetes mellitus and cancer. Tissue-specific clocks are entrained by environmental stimuli, blood-borne hormonal cues, and direct neuronal input from the suprachiasmatic nucleus located in the hypothalamus to ensure coordinated systemic responses.

One of the defining metrics of circadian patterning is body temperature, which is highest in animals while awake and lowest while asleep. A major site of mammalian thermogenesis is BAT, which is characterized by high glucose uptake, oxidative capacity and mitochondrial uncoupling. Despite a substantial body of literature examining various regulatory aspects of BAT function and body temperature, little is known about the mechanisms controlling circadian thermogenic rhythms and, more importantly, how this patterning influences adaptability to environmental challenges. The circadian transcriptional repressor Rev-erbα has been previously linked to the regulation of glucose and lipid metabolism in tissues such as skeletal muscle, white adipose and liver.

We investigated the function of Rev-erbα in controlling temperature rhythms and thermogenic plasticity through integration of circadian and environmental signals. All experiments were performed on C57BL/6 mice and, unless otherwise noted, at murine thermoneutrality (29–30 °C) to avoid confounding background contributions from the ‘browning’ of white adipose depots or partial stimulation of BAT activity. At thermoneutrality, the circadian oscillations of Rev-erbα gene expression (Fig. 1a) and protein levels (Extended Data Fig. 1a) in BAT were similar to other tissues, peaking in the light and being nearly absent in the dark. Rev-erbα ablation altered Bmal1 (also known as Arntl) transcription but did not affect the rhythmicity of Rev-erbβ (also known as Nr1d2), Cry1, Cry2, Per1, Per2, Per3 or Clock (Extended Data Fig. 1b), consistent with the mild circadian phenotype observed previously.

To evaluate the role of Rev-erbα in BAT, C57BL/6 wild-type and Rev-erbα knockout mice were subjected to an acute cold challenge from Zeitgeber time (ZT) 4–10 (11:00–17:00) when Rev-erbα levels peak in wild-type mice. In accordance with previous reports that thermoneutrally acclimated C57BL/6 mice fail to thrive during acute cold stresses, body temperatures of wild-type animals dropped markedly when shifted from 29 °C to 4 °C (Fig. 1b), and this inability to maintain body temperature was associated with failure to survive the cold exposure (Fig. 1c). By contrast, Rev-erbα knockout mice maintained body temperature and uniformly survived the ZT4–10 cold challenge.

Notably, these studies were all performed during the day, when Rev-erbα peaks in wild-type mice. As Rev-erbα is physiologically nearly absent at night, we next explored whether the circadian expression of Rev-erbα imposed a diurnal variation in cold tolerance. Previous studies of animals exposed to cold at either mid-morning or early afternoon reported modest differences in tolerance, but this effect was believed to be a result of altered vasodilation. Notably, during the dark period, when Rev-erbα levels are at the nadir of their physiological rhythm, wild-type mice were fully able to protect their body temperature and were phenotypically indistinguishable from Rev-erbα knockout mice in both body temperature regulation (Fig. 1d) and survival (Fig. 1e) following cold challenge. These findings implicate Rev-erbα in establishing a circadian rhythm of cold tolerance through suppression of heat-producing pathways.

The increased cold tolerance of Rev-erbα knockout mice was associated with higher oxygen consumption rates compared to wild-type littermates (Fig. 1f). Food intake (Extended Data Fig. 2a), basal muscle activity and cold-induced shivering (Fig. 1g and Extended Data Fig. 2b) were unchanged between genotypes, indicating that the Rev-erbα-dependent differences in oxidative capacity were probably due to alterations in a BAT-driven, non-shivering thermogenic program. Indeed, BAT isolated from cold-challenged Rev-erbα knockout animals consumed more oxygen than BAT from wild-type mice (Fig. 1h). Moreover, BAT
Rev-erbα mediates the circadian patterning of cold tolerance. a, Rev-erbα mRNA (n = 3) in BAT of wild-type (WT) and Rev-erbα knockout (KO) mice. b, c, Cold tolerance tests (CTTs) (b) and survival curves (c) for Rev-erbα knockout mice and control littersmates from ZT4–10 (11:00–17:00). The numbers of Rev-erbα knockout and control mice in the CTT are indicated above or below the first data point, respectively; subsequent designations at data points are made if any animals were removed for having a temperature below 25 °C. f, g, Oxygen consumption rate (n = 10) (f) and Root mean squared (r.m.s.) derivation of electromyogram (EMG) measurements (n = 4) (g) of cold-challenged Rev-erbα knockout mice and wild-type controls. 

Rev-erbα knockout mice exhibited no statistically significant difference in weight or food consumption in Rev-erbα noradrenaline administration induced a larger increase in oxygen consumption in Rev-erbα knockout animals than in control littermates. Enhanced BAT metabolic capacity, Rev-erbα knockout mice exhibited no statistically significant difference in weight or food intake at room temperature (22 °C) and thermoneutrality compared to wild-type controls (data not shown), probably due to counteracting effects of Rev-erbα deletion in other tissues such as increased hepatic lipogenesis or decreased skeletal muscle oxidative capacity.

Given the considerable influence that environmental demands have on BAT-mediated thermogenesis, we investigated whether Rev-erbα was subject to control by temperature in BAT. Rev-erbα levels normally rise between ZT4 and 10 (11:00 and 17:00) in a circadian manner, but cold exposure rapidly attenuated Rev-erbα expression (Fig. 2a), whereas closely related nuclear receptor Rev-erbβ did not undergo a similar cold-dependent decrease (Extended Data Fig. 3a). Cold-mediated reduction of Rev-erbα gene expression occurred in parallel with the induction of Bmal1, an established target of Rev-erbα expression (Extended Data Fig. 3b), as well as the canonical thermogenic regulators Ucp1 (Fig. 2a) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (Pgc-1α, also known as Ppargc1a) (Extended Data Fig. 3c). Rev-erbα expression was attenuated following both moderate (29 °C to 20 °C) and acute (29 °C to 4 °C) cold stresses (Extended Data Fig. 3d). Similarly, Rev-erbα protein levels plummeted when mice were shifted to 4 °C (Fig. 2b). Classically, regulation of brown adipose thermogenesis has been attributed predominantly to sympathetic release of noradrenaline and subsequent activation of adrenergic signalling cascades. We therefore considered whether the cold-induced decrease in Rev-erbα levels was related to the adrenergic pathway. However, whereas the highly cyclic-AMP-sensitive nuclear receptor Nor1 (also known as Nrfα3) (ref. 22) was induced comparably by noradrenaline and cold (Fig. 2c), noradrenaline administration did not mimic the effect of cold exposure on expression of Rev-erbα gene (Fig. 2c) or protein (Extended Data Fig. 3e). This is consistent with reports that pan-sympathomimetic stimulation does not fully recapitulate cold-mediated BAT activation in humans23,24, and suggests that the role of Rev-erbα in thermogenic regulation is independent of sympathetic stimulation.

The rapidity with which Rev-erbα was reduced in the cold and its inverse relationship with Ucp1 expression suggested that Rev-erbα might elicit thermogenic regulation through active repression of the Ucp1 gene. Indeed, at thermoneutrality Ucp1 mRNA (Fig. 3a) and protein levels (Fig. 3b) were increased in the BAT of Rev-erbα knockout mice.
mice, consistent with the more pronounced metabolic response of these mice to cold exposure or noradrenaline administration. BAT Ucp1 in Rev-erbα knockout mice was only modestly further increased upon cold challenge compared to wild-type animals (Fig. 3a, b), suggesting that Rev-erbα downregulation is an integral component of the physiological Ucp1 induction following cold exposure. Consistent with recent work on the temporal correlation between Ucp1 mRNA and protein levels, we did not observe cold-mediated changes in Ucp1 protein in the acute time frame in which we performed our cold challenges. Increases in Ucp1 were not seen in white adipose depots or skeletal muscle (data not shown), signifying a BAT-specific phenophenon. Bmal1 mRNA and protein followed a similar pattern to Ucp1 (Extended Data Fig. 4a, b), whereas Pgc-1α levels were unchanged between control and Rev-erbα knockout animals at thermoneutrality, and were comparably cold-induced, suggesting Rev-erbα independence (Extended Data Fig. 4a, b). Nevertheless, Rev-erbα controlled the expression of Ucp1, which is critical for non-shivering heat production in BAT23,16.

Ucp1 levels were increased in primary brown adipocytes lacking Rev-erbα and ectopic expression of Rev-erbα restored Ucp1 mRNA to wild-type levels, whereas overexpression of Rev-erbα in wild-type adipocytes caused no further effect (Fig. 3d), illustrating that Rev-erbα represses Ucp1 in a BAT-cell-autonomous manner. Consistent with these findings, Rev-erbα binding was detected at the Ucp1 gene locus, and this binding decreased after cold challenge (Fig. 3e). Ucp1 displayed a rhythmic expression profile anti-phase to Rev-erbα in primary brown adipocytes cultured ex vivo and synchronized by serum shock (Extended Data Fig. 4c). This Ucp1 circadian rhythmicity was completely abolished in Rev-erbα knockout animals (Fig. 3f). These data establish Rev-erbα as a direct, negative regulator of thermogenic transcriptional programs.

The ability of Rev-erbα to repress BAT heat production and impose a circadian pattern of cold tolerance prompted us to investigate whether Rev-erbα influences body temperature rhythm. Rev-erbα ablation considerably altered body temperature oscillation, both of the core (Fig. 4a) and of the interscapular region (BAT) (Fig. 4b). Higher body temperature was maintained by Rev-erbα knockout animals throughout the light phase, indicating that Rev-erbα was required for daily depressions in thermogenic rhythmicity. Indeed, thermographic surface measurements showed that Rev-erbα knockout mice were warmer than wild-type mice from ZT4–10 (11:00–17:00) but not ZT16–22 (23:00–05:00) (Fig. 4c and Extended Data Fig. 5a). Comparison between colonic and interscapular temperatures implicated BAT as the primary source of the genotypic variation (Extended Data Fig. 5a). We note that previous studies of thermoregulation in mice lacking Rev-erbα were performed at room temperature, which could confound the assessment of the role of Rev-erbα in BAT thermogenesis.13,16.

**Figure 3 |** Rev-erbα represses thermogenic programming. a, b, BAT mRNA (n = 6) (a) and protein (b) from wild-type and Rev-erbα knockout mice acutely exposed to cold for 6 h from ZT4–10. c, BAT mRNA following 3 h of noradrenaline administration from ZT7–10 (1 mg kg⁻¹ i.p.) (n = 3). d, Ucp1 mRNA levels in preadipocytes isolated from Rev-erbα knockout mice and wild-type littermates in which either Rev-erbα or vector control has been ectopically expressed (n = 4). e, Rev-erbα occupancy at the Ucp1 proximal promoter. Rev-erbα-specific peaks are shaded. f, Ucp1 gene expression in BAT over a 24-h period (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 as determined by two-tailed Student’s t-test or one-way ANOVA with multiple comparisons and a Tukey’s post-test. Data are expressed as mean ± s.d.

**Figure 4 |** Rev-erbα orchestrates daily rhythms of body temperature and BAT activity. a, b, Core (n = 6) (a) and BAT (n = 10) (b) temperatures measured from subcutaneously implanted thermometers. c, d, Quantified thermographic measurements of surface temperature (n = 5) (c) and ¹⁸FDG imaging (n = 4) (d) of Rev-erbα knockout mice and wild-type littermates during the light and dark phases. Representative coronal planes are shown for each group. e, Per cent injected dose of ¹⁸FDG in the BAT of animals from the study in d. *P < 0.05, **P < 0.01, ***P < 0.001 as determined by two-tailed Student’s t-test or one-way ANOVA with multiple comparisons and a Tukey’s post-test. Data in a are expressed as rolling averages (± 2 time points) ± s.e.m.; data in b are expressed as mean ± s.e.m.; data in c are expressed as a maximum to minimum box-and-whiskers plot; data in e are expressed as a mean ± s.d.
To address the effect of Rev-erbα on the circadian control of BAT function, we measured glucose uptake using 18-fluorodeoxyglucose positron emission tomography (18FDG-PET)20–28. Notably, the diurnal oscillation of BAT glucose uptake29 was abolished by deletion of Rev-erbα (Fig. 4d and Extended Data Fig. 5c). Glucose uptake was higher in Rev-erbα knockout mice than control littermates during the day and did not increase at night as in wild-type animals (Fig. 4e). These results indicate that Rev-erbα is required for the circadian rhythm of body temperature and BAT activity (Extended Data Fig. 6).

Daily oscillation in body temperature is one of the most basic and defining characteristics of mammalian circadian biology1. The present findings suggest a mechanism whereby circadian and cold-regulated networks converge on Rev-erbα in BAT to establish and maintain thermogenic rhythmicity while affording the organism an adaptability to rapidly respond to external temperature stresses. Rev-erbα acts as a focal point, integrating the continuity of circadian rhythms with the variability of environmental challenges. Rev-erbα alone is sufficient to modulate brown adipose function, which is in contrast to the redundancy found between both nuclear receptors Rev-erbα and Rev-erβ in controlling hepatic physiology31,32. The fact that Rev-erβ is not subject to similar cold-dependent regulation ensures that temperature stresses can target appropriate programs without detriment to the BAT core clock machinery.

The function of BAT as a professional heat-producing tissue probably evolved to permit eutherian mammals to survive exposure to an array of environmental demands82. However, from an evolutionary standpoint, constitutive, Ucp1-mediated dissipation of the mitochondrial proton gradient would be wasteful and unfavourable when resources are scarce and increased heat production is unnecessary. Our data are consistent with a model of Rev-erbα-controlled BAT thermogenesis that provides an energetic checks-and-balances system. Circadian rhythm of Rev-erbα imposes an oscillation in brown adipose activity, increasing body temperature when mammals are awake and potentially exposed to harsh environmental conditions and depressing thermogenesis during sleep when mammals are typically in protective shelter and require little facultative heat production. In the event that the animal is confronted by a sudden temperature challenge while sleeping, rapid reduction in Rev-erbα would facilitate appropriate induction of thermogenic programs and organismal survival.

METHODS SUMMARY

Mice were housed on a 12:12-h light–dark cycle (lights on at 07:00, off at 19:00). Gene expression, protein analysis and temperature measurements were carried out on 12–16-week-old male Rev-erbα knockout mice and wild-type littermates. Cold exposure experiments were performed in climate-controlled rodent incubators set on 12–16-week-old male mice. Core and brown adipose temperature measurements were obtained using surgically implanted dataloggers for core (SubCue Dataloggers) and telemetric transmitters for BAT (IPTT 300 transponders, Bio Medic Data Systems) following pentobarbital anaesthesia. Colonico and intercapsular surface measurements were obtained using YSI Precision Thermometers with rectal or banjo probe attachments, respectively. Thermography was performed by the Penn Mouse Phenotyping, Physiology, and Metabolism (MPPM) core using a FLIR SC620 infrared camera. 18FDG imaging was performed in the University of Pennsylvania Small Animal Imaging Facility (SAIF). EMG recordings were performed as described previously29. Chromatin immunoprecipitation of Rev-erbα was performed using the Cell Signaling Technology antibody (no. 2124) as described previously31. Data are presented as means ± s.d. unless otherwise noted.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 15 March; accepted 9 September 2013.

Published online 27 October 2013.

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Acknowledgements We thank the Functional Genomics Core (J. Schug) and the Mouse Phenotyping, Physiology, and Metabolism Core (R. Ahima and R. Dhir) of the Penn Diabetes Research Center (DK51259). We also thank the Small Animal Imaging Facility of the Perelman School of Medicine at the University of Pennsylvania (E. Blankemeyer). This work was supported by NIH grants R01 DK45586 (M.A.L.) and F32 DK095563 (Z.G.H.) and the JPB Foundation. A.B. was funded by the Novo Nordisk STAR postdoctoral program.

Author Contributions D.F., M.J.E., L.J.E., E.R.B., A.B. and C.F. performed key experiments/data analysis and read the manuscript. P.S. provided advice and read the manuscript. E.L. and T.S.K. designed, performed and analysed EMG studies and read the manuscript. C.H. and D.A.P. designed, performed and analysed 18FDG scans and read the manuscript. Z.G.H. performed many of the experiments, and Z.G.H. and M.A.L. conceived the project, designed experiments, analysed all results and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper.

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METHODS
Animal studies. All animal studies were performed with an approved protocol from the University of Pennsylvania Perelman School of Medicine Institutional Animal Care and Use Committee. The Rev-erba knockout mice were obtained from B. Vennström and backcrossed seven or more generations with C57BL/6 mice. Mice were housed on a 12:12-h light–dark cycle (lights on at 07:00, lights off at 19:00). Gene expression, protein analysis and temperature measurements were carried out on 12–16-week-old male Rev-erba knockout mice and wild-type littermates. Cold-exposure experiments were performed in climate-controlled rodent incubators set to 29 °C and 40% relative humidity. All wild-type and Rev-erba knockout mice used in the studies were first placed in individual cages with access to food and water and allowed to acclimate to 29 °C for 2 weeks before cold challenge. For noradrenaline administration experiments, thermoneutrally acclimated wild-type and Rev-erba knockout mice were given 1 mg kg⁻¹ L(-)-noradrenaline-bitartrate salt monohydrate (Sigma). Mice were injected subcutaneously for noradrenaline-induced oxygen-consumption assays but intraperitoneally for all other procedures.

Whole-animal oxygen-consumption rate. Oxygen-consumption rates were measured using Comprehensive Laboratory Animal Monitoring System (CLAMS) metabolic cages contained within temperature-controlled rodent incubators. Cold-induced oxygen consumption rates were assessed on singly-housed, unaesthetized wild-type and Rev-erba knockout mice. Temperature of the housing unit was transitioned from 29 °C to 4 °C over the course of 20–30 min, and mice were then cold-challenged for an additional 2 h. Noradrenaline-induced oxygen consumption rates were assessed as described previously. In brief, mice were anaesthetized with 75 mg kg⁻¹ pentobarbital intraperitoneally and placed in a CLAMS unit set to 33 °C to maintain body temperature. One mg kg⁻¹ noradrenaline was administered subcutaneously once a baseline oxygen consumption rate had been obtained (approximately 20 min after pentobarbital injection). Noradrenaline-induced oxygen consumption was then measured until rates had plateaued and started declining (approximately 90 min after noradrenaline administration).

Temperature measurements. Core and brown adipose temperature measurements were obtained using surgically implanted dataloggers for core (SubCue Dataloggers) and telemetric transmitters for BAT (IPPT 300 transponders, BioMed Data Systems) following pentobarbital anesthesia. Mice were maintained at 29 °C and monitored daily and surgical sites were treated with bacitracin to prevent discomfort. Following a week of convalescence, temperature measurements were recorded. Colonics and interscapular surface measurements were obtained using YSI Precision Thermometers with rectal or banjo probe attachments, respectively.

Immunoblotting. BAT samples were homogenized in tissue lysis buffer (137 mM NaCl, 0.1% SDS, 0.5% sodium-deoxycholate, 1% NP-40, 20 mM NaF and 20 mM β-glycophosphate in 1× PBS, pH 7.4, supplemented with Complete protease inhibitor (Roche)) using a TissueLyser (Qiagen) for 1.5 min at a frequency of 20 Hz. BAT samples were homogenized in tissue lysis buffer (137 mM NaCl, 0.1% Triton X-100, 0.11% sodium-deoxycholate, complete protease inhibitor cocktail, pH 7.4) and 20 nM insulin) for 36 h. After induction, cells were cultured in maintenance media supplemented with 25 mM glucose and 1 mM sodium pyruvate and adjusted to pH 7.4. Subsequently, the BAT pieces were placed individually in the centre of a well of a Seahorse XF24 islet capture microplate and held in place by overlaying a capture screen followed by addition of 675 μl of the supplemented Seahorse XF assay media. The oxygen-consumption rate of each well was measured three times for 2 min after 3 min of mixing and a 2-min wait on the Seahorse XF24 analyser ( Seahorse Bioscience). The results from the 11 wells of each genotype were averaged and normalized to total mg of tissue.

EMG. EMG recordings were made essentially as described previously. Three 29-gauge needle electrodes (two recording electrodes 4 mm apart and 3 mm deep, and one reference electrode) were inserted subcutaneously without a 6-h cold challenge as described previously. After EMG signal from the scapular muscles. For optimal stability, recording electrodes were placed into 4-mm diameter plastic tubes (1 ml serological pipettes) and juxtaposed using polyolefin tubing. The entire electrode set was introduced into the scapular region of prone mice using a micromanipulator (WPI). The EMG signal was processed (low-pass filter, 3 kHz; high-pass filter, 10 Hz; notch filter, 60 Hz) and amplified 1,000× with a P55 differential amplifier (Grass Instruments).

Cell culture. Preadipocytes were collected from BAT depots of pups that were between postnatal days 1–3. Depots were minced finely using spring scissors (Roboz) in DMEM/F-12 GlutaMax (Invitrogen) before addition of 1.5 U ml⁻¹ penicillin/streptomycin (Invitrogen)). Adipocyte differentiation was induced upon addition of 137 mM NaCl, 0.1% SDS, 0.5% sodium-deoxycholate, 125 mM indomethacin, 0.5 mM IBMX, 1 nM rosiglitazone, 1 mM T3 and 20 nM insulin) for 36 h. After induction, cells were cultured in maintenance media supplemented with 1 mM T3 and 20 mM insulin) for 1,000× with a P55 differential amplifier (Grass Instruments).

ChIP. Murine BAT was collected immediately after euthanasia. It was quickly minced and cross-linked in 1% formaldehyde for 20 min, followed by quenching with 1/20 volume of 2.5 M glycine solution and two washes with ice-cold PBS. Chromatin fragmentation was performed by sonication in ChIP SDS lysis buffer (50 mM HEPES, 1% SDS, 10 mM EDTA, pH 7.5) using probe sonication. Proteins were immunoprecipitated in ChIP dilution buffer (50 mM HEPES, 155 mM NaCl, 1% Triton X-100, 0.11% sodium-deoxycholate, complete protease inhibitor tablet, pH 7.5). Crosslinking was reversed overnight at 65 °C in elution buffer (50 mM Tris–HCl, 10 mM EDTA, 1% SDS, pH 8.0) and DNA was isolated using phenol/chloroform/isomyl alcohol. Precipitated DNA was analysed by quantitative PCR. ChIP experiments were performed independently on BAT samples collected at 5 min post CHOP administration as described previously. ChIP of Rev-erba was performed using the Cell Signaling Technology antibody. Deep sequencing was carried out by the Functional Genomics Core (J. Schug and K. Kaestner) of the Penn Institute for Diabetes, Obesity, and Metabolism using the Illumina Genome Analyzer IIx and Illumina HiSeq 2000 and sequenced and obtained using the Solexa Analysis Pipeline.

18FDG imaging. 18FDG imaging was performed in the University of Pennsylvania Small Animal Imaging Facility (SAIF). Doses of saline containing 300 μCi 18FDG were administered through the lateral tail vein under constant isoflurane anaesthesia (1–2%, 1 L O₂ min⁻¹). Mice were scanned on a Philips Mosaic HP 1 h after injection. Per cent injected dose was calculated by assessing the ratio of radioactive counts in the region of interest for brown adipose to the total counts for the animal using Amide medical imaging software.

Biology oxygen-consumption rate. Mice were housed at thermoneutrality for 1 week and subjected to a 1-h cold challenge (4 °C) starting at 13:00. The interscapular BAT depot of each mouse was collected and divided into 11 pie pieces weighing between 1.5 and 2 mg, and washed three times in Seahorse XF assay media supplemented with 25 mM glucose and 1 mM sodium pyruvate and adjusted to pH 7.4. Subsequently, the BAT pieces were placed individually in the centre of a well of a Seahorse XF24 islet capture microplate and held in place by overlaying a capture screen followed by addition of 675 μl of the supplemented Seahorse XF assay media. The oxygen-consumption rate of each well was measured three times for 2 min after 3 min of mixing and a 2-min wait on the Seahorse XF24 analyser ( Seahorse Bioscience). The results from the 11 wells of each genotype were averaged and normalized to total mg of tissue.

Statistics. Data are presented as means ± s.d. unless otherwise noted. Statistical analysis was performed using Student's t-test for comparisons between two groups, one-way ANOVA with multiple comparisons for assessment of more than two groups on GraphPad Prism software. Comparisons among specific groups were done using post-tests as indicated in the respective figure legends.

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Extended Data Figure 1 | The BAT core clock is largely unaffected by Rev-erbα deletion. a, Rev-erbα protein levels in BAT of wild-type and Rev-erbα knockout mice (n = 2; each lane of the western blot represents pooled biological duplicates). b, BAT mRNA for indicated genes from wild-type and Rev-erbα knockout mice collected at the indicated times over a 24-h time course (n = 3).
Extended Data Figure 2 | Rev-erbα controls cold and noradrenaline-induced oxidative metabolism independently of skeletal muscle metabolism. a, Food intake from cold-challenged Rev-erbα knockout mice and control littermates in Fig. 1f. b, r.m.s. derivation of EMG measurement from Fig. 1g. c, Oxygen consumption rates of Rev-erbα KO mice and control littermates following noradrenaline administration (1 mg kg⁻¹ s.c.) (n = 6). d, e, r.m.s. derivation of EMG measurements performed on wild-type and Rev-erbα knockout mice following noradrenaline administration (1 mg kg⁻¹ s.c.) (n = 4). ***P < 0.001 as determined by Student’s t-test. Data are expressed as mean ± s.d.
Extended Data Figure 3 | *Rev-erba*, but not *Rev-erbβ*, is decreased in a cold-dependent manner. a–c, *Rev-erba* (a), *Bmal1* (b) and *Pgc1a* (c) mRNA levels in BAT during a cold-exposure time course (n = 3 for mRNA). d, BAT gene expression following moderate (20 °C) or acute (4 °C) cold challenges (n = 3). e, BAT protein levels after 3 h noradrenaline administration (1 mg kg⁻¹ i.p.) or cold exposure (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 as determined by one-way ANOVA with multiple comparisons and a Tukey’s post-test. Data are expressed as mean ± s.d.
**Extended Data Figure 4 | Rev-erba negatively regulates Ucp1.** a, b, BAT mRNA (a) and protein (b) from wild-type and Rev-erba knockout mice exposed to cold for 6 h as described in Fig. 3a, b. c, mRNA levels in preadipocytes isolated from wild-type mice, differentiated in culture and collected at the indicated times after synchronization by serum shock (n = 4). **P < 0.01, ***P < 0.001 as determined by one-way ANOVA with multiple comparisons and a Tukey’s post-test. Data are expressed as mean ± s.d.
Extended Data Figure 5 | Rev-erbα controls circadian oscillation of surface temperature and BAT activity. 

a, Infrared images from the thermographic surface temperature analysis performed in Fig. 4c. 
b, Genotypic differences between BAT and core temperatures from wild-type and Rev-erbα knockout mice acclimated to thermoneutrality (n = 6). 
c, 18FDG imaging (n = 4) of Rev-erbα knockout mice and wild-type littermates during the light and dark phases. Representative sagittal planes are shown for each group. *P < 0.05, Δcore temperature versus ΔBAT temperature; †P < 0.05, core temperature versus Rev-erbα knockout core temperature; ‡P < 0.001, wild-type BAT temperature versus Rev-erbα knockout BAT temperature as determined by Student’s t-test. Data are expressed as mean ± s.e.m.
Extended Data Figure 6 | The nuclear receptor Rev-erbα controls circadian thermogenic plasticity. Rev-erbα regulates the circadian rhythm of body temperature through direct suppression of thermogenesis and BAT activity. Cold exposure during the light phase rapidly overrides Rev-erbα-dependent repression to induce thermogenic programs.