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(54) **Title**: METHODS FOR MODULATING EXPRESSION OF REV-ERB ALPHA IN BROWN ADIPOSE TISSUE

(57) **Abstract**: The methods and compositions described herein are useful for modulating expression of REV-ERBα and treating conditions associated therewith. In one embodiment, a method of affecting the cold tolerance, and thus, ability of a mammalian subject to survive extreme temperature changes is provided, comprising modulating the expression of REV-ERBα. In one embodiment, an antagonist of REV-ERBα is utilized.

METHODS FOR MODULATING EXPRESSION OF REV-ERB ALPHA IN BROWN ADIPOSE TISSUE

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under Grant Nos. DK44586 and F-32 DK095563 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The molecular clock is an autoregulatory network of core transcriptional machinery orchestrating a fine-tuned balance between behavioral and metabolic programming in the context of a 24-hour light-dark cycle[1,3]. The principle model involves transcriptional activators BMAL1-2 and CLOCK, which induce expression of a multitude of downstream circadian and metabolic effectors including PER1-3, CRY1-2, and REV-ERBa/b that, in turn, feedback negatively to repress BMAL1-2 and CLOCK function[4]. The importance of appropriate synchronization in organismal biology is underscored by the robust correlation between disruption of clock circuitry and development of disease states including obesity, diabetes mellitus, and cancer[5–7]. Tissue-specific clocks are entrained by environmental stimuli, blood-borne hormonal cues, and direct neuronal input from the superchiasmatic nucleus (SCN) located in the hypothalamus[1,4] to ensure coordinated systemic resonance.

One of the defining metrics of circadian patterning is body temperature[8], which is highest in animals while awake and lowest while asleep[1]. A major site of mammalian thermogenesis is brown adipose tissue (BAT), which is characterized by high glucose uptake, oxidative capacity, and mitochondrial uncoupling[2]. BAT activity is influenced through the combined actions of circulating hormones, such as FGF21[9], melatonin[10], and thyroid hormone[11], as well as central nervous system (CNS)-mediated neurotransmitter signaling cascades that culminate in norepinephrine (NE) release at innervated sites within the depot[12]. 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. Clock components that have been

previously linked to the regulation of other homeostatic pathways represent potential points of control. Targeted disruption of the BMAL1/CLOCK heterodimer by BMAL1 deletion had adverse effects on glycaemia and insulin sensitivity but produced no changes in BAT-specific thermogenic programs or whole animal temperature patterns[13], suggesting that the activating arm of the clock machinery was not responsible for mediating diurnal oscillation of BAT activity. Alternatively, the circadian transcriptional repressor REV-ERBα has been implicated in glucose and lipid metabolism in tissues such as skeletal muscle, white adipose, and liver[14–18] but its influence on BAT physiology remains unknown.

BAT is a key thermogenic tissue in rodents and other small mammals, including newborn humans, which defends core body temperature in cold weather. The sensation of cold causes sympathetic nerves to release catecholamines in BAT that stimulate proliferation and heat production by brown fat cells. Studies in rodents have demonstrated that BAT plays an essential role in energy balance and that its activity profoundly influences body weight. Recent reports show that healthy adult humans have significant depots of metabolically active BAT.

Experiments in rodents have shown that BAT is activated and proliferates in response to overfeeding. This so-called "diet-induced adaptive thermogenesis" is an apparent compensatory mechanism to limit excess weight gain and obesity. Overfeeding studies in humans have provided evidence for dramatic individual differences in the energy cost of feeding. It is known that individuals with similar dietary intake and exercise regimes exhibit dramatic differences in their tendency to gain weight. The extent to which BAT-mediated adaptive thermogenesis could account for some of this variability in metabolic efficiency is not known.

SUMMARY OF THE INVENTION

In one aspect, a method of affecting thermogenesis in brown adipose tissue in a subject in need thereof is provided. The method comprises modulating the expression and/or activity of REV-ERBα in the subject.

In one aspect, a method of affecting calorie expenditure in a subject in need thereof is provided. The method comprises modulating the expression and/or activity of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby calorie expenditure in the subject is

increased. In another embodiment, the expression and/or activity of REV-ERBα is increased, whereby the calorie expenditure in the subject is decreased.

In another aspect, a method of affecting the weight of a subject in need thereof is provided. The method comprises modulating the expression and/or activity of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby the weight of the subject is reduced. In another embodiment, the expression and/or activity of REV-ERBα is increased, whereby the weight of the subject is increased.

In yet another aspect, a method of regulating the function of brown adipose tissue (BAT) in a subject in need thereof is provided. The method includes modulating the expression and/or activity of REV-ERBα. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby the function of BAT in the subject is increased. In another embodiment, the expression and/or activity of REV-ERBα is increased, whereby the function of BAT in the subject is decreased.

In another aspect, a method of modulating the cold tolerance of a subject is provided. The method includes modulating the expression and/or activity of REV-ERBα. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby the cold tolerance of the subject is increased. In another embodiment, the expression and/or activity of REV-ERBα is increased, whereby the cold tolerance of the subject is decreased.

In another aspect, a method of treating obesity in a subject is provided. The method includes decreasing the expression and/or activity of REV-ERBα.

In yet another aspect, a method of affecting body temperature of a subject in need thereof is provided. The method includes modulating the expression and/or activity of REV-ERBα. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby the body temperature of the subject is increased. In another embodiment, the expression and/or activity of REV-ERBα is increased, whereby the body temperature of the subject is decreased.

In yet another embodiment, a method of affecting glucose uptake in a subject in need thereof is provided. The method includes modulating the expression and/or activity of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression and/or activity of REV-ERBα, whereby glucose uptake is increased. In one

embodiment, the method includes increasing the expression and/or activity of REV-ERBα, whereby glucose uptake is decreased.

In another embodiment, a method of modulating the expression and/or activity of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression and/or activity of REV-ERBα.

Other aspects and advantages of this invention are disclosed in the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1F demonstrate that REV-ERBa mediates the circadian patterning of cold tolerance. a, *Rev-erba* mRNA and, b, REV-ERBa protein levels in BAT of WT and *Rev-erba* KO mice (n=3 for mRNA and each lane of the western blot represents pooled biological duplicates). c, Cold tolerance tests (CTT) and, d, survival curves for *Rev-erba* KO mice and control littermates from ZT4-10 (11 AM to 5 PM) and, e, CTT and, f, survival curves from ZT16-22 (11 PM to 5 PM). Animals were acclimated to 29°C for at least 2 weeks prior to a 6 h 4°C challenge in which intrascapular surface temperature was measured hourly. The numbers of *Rev-erba* KO 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. *** $p < 0.001$ as analyzed by Student's t-test for the CTT or by Gehan-Breslow-Wilcoxon and Log-rank (Mantel-Cox) tests for the survival curves. Data are expressed as mean ± s.d.

Figs. 2A-2D demonstrate that cold stress rapidly downregulates REV-ERBa. a, BAT mRNA and, b, protein levels from WT mice following an acute cold timecourse (n=3 for mRNA and each lane of the western blot represents pooled biological duplicates). c, BAT mRNA and, d, protein levels following 3 h of NE administration (1 mg/kg i.p.) or cold exposure in WT mice (n=3). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as determined by one-way ANOVA with multiple comparisons and a Tukey post-test. Data are expressed as mean ± s.d.

Figs. 3A-3G demonstrate that REV-ERBa represses thermogenic programming. a, BAT mRNA and, b, protein from WT and *Rev-erba* KO mice acutely exposed to cold (4°C) for 6 h (n=6). c, BAT mRNA following 3 h of NE administration (1 mg/kg i.p.) in WT and *Rev-erba* KO mice (n=3). d, *Ucp1* mRNA levels in preadipocytes isolated from

Rev-erba KO mice and WT littermates differentiated in culture (n=4). e, REV-ERBa occupancy at the *Ucp1* proximal promoter. REV-ERBa-specific peaks are shaded. f, mRNA levels in preadipocytes isolated from WT mice, differentiated in culture and harvested at the indicated times following synchronization by serum shock (n=4). g, BAT gene expression over a 24 h period (n=3-4). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as determined by Student's t-test (d, g) or one-way ANOVA with multiple comparisons and a Tukey post-test (a, c). Data are expressed as mean ± s.d.

Figs. 4A-4E demonstrate that REV-ERBa orchestrates daily rhythm of body temperature and BAT activity. a, Core (n=3) and, b, BAT (n=10) temperatures measured from subcutaneously implanted thermometers. c, Quantified thermographic measurements of surface temperature (n=3-5) and, d, 18-fluorodeoxyglucose (^{18}FDG) imaging (n=3-4) of *Rev-erba* KO mice and WT littermates during the light and dark phases. Representative coronal and sagittal planes are shown for each group. e, Percent injected dose of ^{18}FDG in the BAT of animals from the study in 4d. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as determined by Student's t-test (a, b) or one-way ANOVA with multiple comparisons and a Tukey post-test (c, e). 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 max to min box-and-whiskers plot; data in 'e' are expressed as a mean ± s.d.

Fig. 5 are line graphs showing BAT mRNA of the indicated genes from WT and *Rev-erbα* KO mice harvested at the indicated times over a 24 h timecourse (n=3-4). *** $p<0.001$ as determined by Student's t-test. Data are expressed as mean ± s.d.

Figs. 6A-6C are bar graphs showing (a) *Rev-erbβ* and (b) *Bmal1* mRNA levels in BAT during an acute cold timecourse (n=3 for mRNA). Fig. 6C: BAT gene expression following moderate (20°C) or acute (4°C) cold challenges (n=3). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as determined by one-way ANOVA with multiple comparisons and a Tukey post-test. Data are expressed as mean ± s.d.

Figs. 7A and 7B show expression of *Bmal1* mRNA and, b, protein from WT and *Rev-erbα* KO mice exposed to cold for 6 h as described in Fig. 3a-b (n=6). ** $p<0.01$, *** $p<0.001$ as determined by one-way ANOVA with multiple comparisons and a Tukey post-test. Data are expressed as mean ± s.d.

Figs. 8A are infrared images from the thermographic surface temperature analysis performed in Fig. 4C. Fig. 8B is a dot plot of BAT and colonic temperatures from WT

and *Rev-erbα* KO mice acclimated to thermoneutrality (n=6). Fig. 8C is a diagram showing how REV-ERBα controls 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. * $p<0.05$, *** $p<0.001$ as determined by Student's t-test. Data are expressed as mean ± s.e.m.

Figs. 9A-9C demonstrate that REV-ERBa mediates the circadian patterning of cold tolerance. a, Line graph showing oxygen consumption rate (n=10) and, b, dot plot showing electromyogram (EMG) (n=4) measurements of cold-challenged Rev-erbα KO mice and WT controls. c, Bar graph showing oxygen consumption rates of BAT isolated from animals exposed to cold for 1 h (n=3). ** $p<0.01$, *** $p<0.001$ as analyzed by two-tailed Student's t-test, one-way ANOVA, or Gehan-Breslow-Wilcoxon and Log-rank (Mantel-Cox) tests for the survival curves. Data are expressed as mean ± s.d.

Fig. 10A is a graph showing food intake from cold-challenged *Rev-erbα* KO mice and control littermates in Fig. 9ba. Fig. 10B shows the root mean squared (RMS) derivation of EMG measurement from Fig. 9B. Fig. 10C shows oxygen consumption rates of *Rev-erbα* KO mice and control littermates following NE administration (1 mg/kg s.c.) (n=6). Figs. 10D-E show RMS derivation of EMG measurements performed on WT and *Rev-erbα* KO mice following NE administration (1 mg/kg s.c.) (n=4). *** $p<0.001$ as determined by Student's t-test. Data are expressed as mean ± s.d.

Fig. 11A and B are graphs showing BAT mRNA (a) and protein (b) from WT and *Rev-erbα* KO mice exposed to cold for 6 h as described in Fig. 3a-b. Fig. 11C is a graph showing mRNA levels in preadipocytes isolated from WT mice, differentiated in culture and harvested at the indicated times following synchronization by serum shock (n=4). ** $p<0.01$, *** $p<0.001$ as determined by one-way ANOVA with multiple comparisons and a Tukey post-test. Data are expressed as mean ± s.d

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein provides novel methods based on the regulation of brown adipose tissue (BAT - also called brown or beige fat) thermogenesis. Daily oscillation in body temperature is one of the most basic and defining characteristics of mammalian circadian biology, with a widespread impact on behavioral and metabolic physiology[7]. The methods disclosed herein are based on discovery of a mechanism

whereby circadian and cold-regulated networks converge on REV-ERBα in BAT to establish and maintain thermogenic rhythmicity. This method provides the mammalian subject with an adaptability to rapidly respond to external temperature stresses. Thus REV-ERBα acts as a focal point, integrating the continuity of circadian rhythms with the variability of environmental challenges. REV-ERBβ is not subject to similar cold-dependent regulation. Unlike control of the molecular clock mechanism and hepatic lipid metabolism where the REV-ERBs have complementary functions[14,16], REV-ERBα singularly modulates brown adipose function, which likely ensures that the homeostatic response to temperature stress does not have a major impact on the BAT core clock.

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application. The following definitions are provided for clarity only and are not intended to limit the claimed invention.

The terms "a" or "an" refers to one or more, for example, "a subject" is understood to represent one or more such subjects. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein. As used herein, the term "about" means a variability of 10% from the reference given, unless otherwise specified.

While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

As used herein, the term "mammalian subject" or "subject" includes any mammal in need of these methods of treatment, including particularly humans. Other mammals in need of such treatment or prophylaxis include dogs, cats, or other domesticated animals, horses, livestock, laboratory animals, including non-human primates, etc. The subject may be male or female. In one embodiment, the subject suffers from one or more conditions that could benefit from modulation of BAT thermogenesis. In another embodiment, the subject suffers from or is at risk of developing one or more disease or condition including, without limitation, diabetes, Syndrome X, heart disease, obstructive sleep apnea, polycystic ovarian syndrome, hypertension, abnormal lipids, heart attack and

stroke, cancer, or fatty liver. In one embodiment, the subject is overweight or obese. The terms "overweight" or "obese" refer to body weight that is greater than what is considered healthy for a certain height. In one embodiment, the determination of "overweight" or "obese" is based on known indicators, such as body mass index ("BMI"). In another embodiment, this determination may be made by the subject's health care provider or physician. In another embodiment, the subject suffers from, or is at risk of developing, weight related illness.

By the term "affecting" is meant altering or changing the characteristic, expression, measurement, or biological function in the subject. In one embodiment, the term "affecting" means to increase the characteristic, expression, measurement, or biological function in the subject. In another embodiment, the term "affecting" means to decrease the characteristic, expression, measurement, or biological function in the subject. By the term "increase" is meant an increase of 5% or more from that of the reference level or starting value of the measurement or characteristic. In another embodiment, an increase refers to an increase of 10% or more. In another embodiment, an increase refers to an increase of 20% or more. In another embodiment, an increase refers to an increase of 30% or more. In another embodiment, an increase refers to an increase of 40% or more. In another embodiment, an increase refers to an increase of 50% or more. In another embodiment, an increase refers to an increase of 60% or more. In another embodiment, an increase refers to an increase of 70% or more. In another embodiment, an increase refers to an increase of 80% or more. In another embodiment, an increase refers to an increase of 90% or more. In another embodiment, an increase refers to an increase of 100% or more. By the term "decrease", "reduce" or "reduction" is meant a decrease of 5% or more from that of the reference level or starting value of the measurement or characteristic. In another embodiment, a decrease refers to a decrease of 10% or more. In another embodiment, a decrease refers to a decrease of 20% or more. In another embodiment, a decrease refers to a decrease of 30% or more. In another embodiment, a decrease refers to a decrease of 40% or more. In another embodiment, a decrease refers to a decrease of 50% or more. In another embodiment, a decrease refers to a decrease of 60% or more. In another embodiment, a decrease refers to a decrease of 70% or more. In another embodiment, a decrease refers to a decrease of 80% or more. In another embodiment, a decrease refers to a decrease of 90% or more. In another embodiment, a decrease refers to a decrease of 100% or more.

By the term "modulation of expression" it is meant changing the level of expression of a gene or protein from a reference level or from the starting level in the subject. In one embodiment, the "reference level" is the level of expression seen in a wild type subject or the average, mean or median of a population of wild type subjects. In another embodiment, the reference level is the level seen in the subject prior to treatment. In one embodiment, by modulation of expression is meant increasing expression. In another embodiment, by modulation of expression is meant decreasing expression. "Modulating REV-ERBα" can include binding to REV-ERBα and/or inhibiting the bioactivity of REV-ERBα and/or allosterically regulating the bioactivity of REV-ERBα *in vivo*. By the term "modulation of activity" it is meant changing the level of activity of a protein from a reference level or from the starting level in the subject. In one embodiment, the "reference level" is the level of expression seen in a wild type subject or the average, mean or median of a population of wild type subjects. In another embodiment, the reference level is the level seen in the subject prior to treatment. In one embodiment, by modulation of activity is meant increasing activity. In another embodiment, by modulation of activity is meant decreasing activity.

By "increasing expression" or "increasing activity" is meant an increase of 5% or more from that of the reference level of gene or protein expression or activity. In one embodiment, increasing expression or activity means an increase of 10% or more. In another embodiment, increasing expression or activity means an increase of 20% or more. In another embodiment, increasing expression or activity means an increase of 30% or more. In another embodiment, increasing expression or activity means an increase of 40% or more. In another embodiment, increasing expression or activity means an increase of 50% or more. In another embodiment, increasing expression or activity means an increase of 60% or more. In another embodiment, increasing expression or activity means an increase of 70% or more. In another embodiment, increasing expression or activity means an increase of 80% or more. In another embodiment, increasing expression or activity means an increase of 90% or more. In another embodiment, increasing expression or activity means an increase of 100% or more.

By "decreasing expression and/or activity" is meant a decrease of 5% or more from that of the reference level of gene or protein expression or activity. In another embodiment, decreasing expression or activity means a decrease of 10% or more. In another embodiment, decreasing expression or activity means a decrease of 20% or more.

In another embodiment, decreasing expression or activity means a decrease of 30% or more. In another embodiment, decreasing expression or activity means a decrease of 40% or more. In another embodiment, decreasing expression or activity means a decrease of 50% or more. In another embodiment, decreasing expression or activity means a decrease of 60% or more. In another embodiment, decreasing expression or activity means a decrease of 70% or more. In another embodiment, decreasing expression or activity means a decrease of 80% or more. In another embodiment, decreasing expression or activity means a decrease of 90% or more. In another embodiment, decreasing expression or activity means a decrease of 100% or more.

By "affecting the weight of a subject" is meant to increase or decrease the weight of the subject as compared to the weight of the subject prior to treatment.

"Brown adipose tissue", or "BAT", is sometimes called brown or beige fat. BAT is found in large quantities in newborns, and decreases to a few local depots in adults. Unlike white adipose tissue, which stores and accumulates fat, BAT metabolizes fat, generates heat and increases overall metabolism. For these purposes it contains large amounts of mitochondria and uncoupling protein 1 (UCP-1), which are considered the defining morphological markers for BAT. The function of BAT as a professional heat-producing tissue likely evolved to permit eutherian mammals to survive exposure to an array of environmental demands[29] and perhaps, in more recent history, to cope with the burden of a high caloric diet[30]. However, from an evolutionary standpoint, constitutive, UCP1-mediated dissipation of the mitochondrial proton gradient would be unfavorable when resources are scarce and wasteful when increased heat production is unnecessary.

By "regulating the function of BAT" is meant altering the thermogenic or other functionality of BAT. BAT function can be measured *in vivo* by assessment of maximal thermogenic capacity by indirect calorimetry and the measurement of sympathetic tone of BAT. Additional techniques for assessing BAT function are described in Virtue and Vidal, Assessment of brown adipose tissue function, Front Physiology, 2013 June 4; 4:128, which is incorporated herein by reference.

Methods and Components

The methods described herein involve employing and manipulating REV-ERBα-controlled BAT thermogenesis to use the energetic checks-and-balances system to treat

mammalian subjects for a variety of diseases and conditions. Additional methods involve screening assays to obtain new modulators of REV-ERBα.

a. REV-ERBα

REV-ERBα, also known as nuclear receptor subfamily 1, group D, member 1 (NR1D1), or Rev-erba, is encoded by the NR1D1 gene in humans. The REV-ERBα protein is a ligand-sensitive transcription factor that negatively regulates the expression of core clock proteins. In mammals, REV-ERBα is highly expressed in the liver, skeletal muscle, adipose tissue, and the brain, participating in the development and circadian regulation of these tissues. The sequences of REV-ERBα homologs from various species are known. The human sequence can be found at Pubmed accession number CAE75563.1. The mouse sequence can be found at Pubmed accession number NP_633409.2. The human sequence is 614 amino acids in length, while the mouse sequence is 615 amino acids in length. The human and mouse sequences share 95% identity. As used herein, the term REV-ERBα refers to the human sequence or any mammalian ortholog. In another embodiment, the desirable "REV-ERBα" sequences are those sharing 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% or greater identity with a known REV-ERBα sequence.

Circadian rhythm of REV-ERBα imposes an oscillation in brown adipose activity that is highest when mammals are awake and are exposed typically in protective shelter and require little facultative heat production. In the event that the mammalian subject is confronted by a sudden temperature challenge while sleeping, rapid reduction in REV-ERBα would facilitate appropriate induction of thermogenic programs and organismal survival. Mammalian subjects, such as humans and mice, experience peak body temperatures when they active, i.e., when they are awake. As discussed herein, REV-ERBα levels have an inverse relationship with body temperature. That is, when body temperatures reach a trough, REV-ERBα levels peak. Thus, because mice are nocturnal, the experiments described below discussing mice were performed during the day, when REV-ERB is at its peak in WT mice. For humans, the opposite is true. REV-ERBα levels peak at night, during sleep/rest period. In addition, as discussed herein, levels of UCP1 increase and decrease with REV-ERBα levels.

b. Modulators of REV-ERBα

Modulators of REV-ERBα useful in the described methods include any agent which affects the expression or activity of REV-ERBα. Modulators include, without

limitation, both naturally occurring and synthetic compounds, small molecules, peptides, and ligands including antibodies. In one embodiment, the modulator is an agonist of REV-ERBα. In another embodiment, the modulator is an antagonist of REV-ERBα.

Various modulators of REV-ERBα are known in the art and are useful in the methods described herein. Modulators of REV-ERBα are described in Trump et al, J. Med. Chem. 2013, 56, 4729−4737; US 7368286 (Staels); and WO 2013/033310 (Kamenecka), each of which is specifically incorporated by reference in its entirety. A further REV-ERB antagonist is manufactured by Calbiochem as item number SR8278. Further compounds which modulate REV-ERBα and are useful in the methods described herein are described in WO 2011/022619, seliciclib (Iurisci I, et al, Liver circadian clock, a pharmacologic target of cyclin-dependent kinase inhibitor seliciclib, Chronobiol. Int. 2009 Aug; 26(6):1169-88); lithium (Yin L, Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock, Science. 2006 Feb 17; 311(5763):1002-5); dexamethasone (Endocrinology. Circadian and glucocorticoid regulation of Rev-erbalpha expression in liver. Torra, 2000 Oct;141(10):3799-806); GW9662 (Kourtidis A, Peroxisome proliferator-activated receptor-gamma protects ERBB2-positive breast cancer cells from palmitate toxicity. Breast Cancer Res. 2009;11(2):R16. doi: 10.1186/bcr2240. Epub 2009 Mar 19). Each of these documents is hereby incorporated by reference in its entirety.

Antibodies to REV-ERBα are known in the art and available commercially from, e.g., Santa Cruz Biotechnology. In addition, antibodies to REV-ERBα can be generated by one of skill in the art using conventional techniques. The term "antibody" refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE, including antibody fragments. The antibody can be monoclonal or polyclonal and can be of any species of origin, including (for example) mouse, rat, rabbit, horse, goat, sheep, camel, or human, or can be a chimeric antibody. See, e.g., Walker et al., Molec. Immunol. 26:403 (1989). The antibodies can be recombinant monoclonal antibodies produced according to known methods, see, e.g., U.S. Patent Nos. 4,474,893 or 4,816,567, which are incorporated herein by reference. The antibodies can also be chemically constructed according to known methods, e.g., US Patent No. 4,676,980 which is incorporated herein by reference. See also, US Patent No. 8,613,922, which is incorporated herein by reference.

Antibody fragments include, for example, Fab, Fab', F(ab')2, and Fv fragments; domain antibodies, bifunctional, diabodies; vaccibodies, linear antibodies; single-chain

antibody molecules (scFV); and multispecific antibodies formed from antibody fragments. Such fragments can be produced by known techniques.

Antibodies of the invention may be altered or mutated for compatibility with species other than the species in which the antibody was produced. For example, antibodies may be humanized or camelized. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Methods for humanizing non-human antibodies are well known in the art. See, e.g., the method of Winter and co-workers (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), each of which is incorporated herein by reference.

c. Screening for Modulators of REV-ERBα

Prior methods of screening REV-ERBα modulating compounds have been identified based on the tendency of REV-ERBα overexpression to induce adipocyte differentiation. See, e.g., US Patent No. 7,368,286 (Staels), which is incorporated herein by reference. The methods described herein exploit the relationship of REV-ERBα and UCP1. The inventors have observed that a decrease in expression of REV-ERBα correlates with an increase in expression of UCP1. Thus, in one aspect, provided herein are methods of screening compounds to identify those which modulate REV-ERBα expression or activity. In one embodiment, the method includes culturing adipocytes with a test compound. The method further includes measuring the level of expression, or activity, of UCP1. This level is compared with a control level. In one embodiment, an increase in expression or activity of UCP1 corresponds with a decrease in expression or activity of REV-ERBα. In another embodiment, a decrease in expression or activity of UCP1 corresponds with an increase in expression or activity of REV-ERBα. Conventional methods can be used to measure the level of expression or activity of UCP1. The measurement of UCP1 can be accomplished via mRNA or protein.

d. Methods of Treatment

Provided herein are various methods which exploit the relationship of REV-ERBα and BAT function. As described herein, these methods are useful in treatment of various diseases, regulation of temperature and modulation of various biological characteristics.

In one aspect, a method of affecting thermogenesis in brown adipose tissue in a subject in need thereof is provided. The method comprises modulating the expression or activity of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression or activity of REV-ERBα, whereby thermogenesis in BAT of the subject is increased. In another embodiment, the expression or activity of REV-ERBα is increased, whereby thermogenesis in BAT of the subject is decreased. In one embodiment, a method of modulating the expression or activity of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression or activity of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provider given the teachings provided herein.

In one aspect, a method of modulating the thermogenic properties, e.g., cold tolerance, of a subject is provided. This method is useful in promoting survival of the mammalian subject exposed to extreme temperatures or temperature changes. The method includes modulating the expression of REV-ERBα. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby the cold tolerance of the subject is increased. In another embodiment, the expression of REV-ERBα is increased, whereby the cold tolerance of the subject is decreased. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In one aspect, a method of affecting calorie expenditure in a subject in need thereof is provided. The method comprises modulating the expression of REV-ERBα in

the subject. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby calorie expenditure in the subject is increased. In another embodiment, the expression of REV-ERBα is increased, whereby the calorie expenditure in the subject is decreased. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. An effective amount means an amount sufficient to produce a selected effect, such as decreasing expression of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In another aspect, a method of affecting the weight of a subject in need thereof is provided. The method comprises modulating the expression of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby the weight of the subject is reduced. In another embodiment, the expression of REV-ERBα is increased, whereby the weight of the subject is increased. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In yet another aspect, a method of regulating the function of brown adipose tissue (BAT) in a subject in need thereof is provided. The method includes modulating the expression of REV-ERBα. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby the function of BAT in the subject is increased. In another embodiment, the expression of REV-ERBα is increased, whereby the function of

BAT in the subject is decreased. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In another aspect, a method of treating obesity in a subject is provided. The method includes decreasing the expression of REV-ERBα. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In yet another aspect, a method of affecting body temperature of a subject in need thereof is provided. The method includes modulating the expression of REV-ERBα. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby the body temperature of the subject is increased. In another embodiment, the expression of REV-ERBα is increased, whereby the body temperature of the subject is decreased. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In yet another embodiment, a method of affecting glucose uptake in a subject in need thereof is provided. The method includes modulating the expression of REV-ERBα in the subject. In one embodiment, the method includes decreasing the expression of REV-ERBα, whereby glucose uptake is increased. In one embodiment, the method includes increasing the expression of REV-ERBα, whereby glucose uptake is decreased. In one embodiment, a method of modulating the expression of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In one embodiment, decreasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an antagonist of REV-ERBα. In one embodiment, increasing the expression and/or activity of REV-ERBα is accomplished by administering an effective amount of an agonist of REV-ERBα. Regimens of administration and dosages of the selected compounds may be selected by the subject's health care provide given the teachings provided herein.

In one embodiment of any of the methods described herein, modulation of REV-ERBα is provided only during the inactive or rest period of the subject. In one embodiment, in humans, the modulation occurs only at night. In another embodiment, the modulation occurs only or primarily while the subject is asleep. By targeting REV-ERBα for modulation only during the rest period, several benefits are provided. Because the body temperature is normally lower than average during sleep, reduction of activity of REV-ERBα, and subsequent rise in body temperature is better tolerated. Thus, fever is not induced. In addition, because REV-ERBα levels peak during the rest period, and trough during the wakeful period, treatment only during rest period would avoid or reduce side effects caused by lack of the REV-ERBα target. In another embodiment, the modulation occurs when the subject's body temperature is at or about a daily trough, or low point.

In one embodiment, any of the methods herein described further includes adjusting the environmental temperature to which the subject is exposed. In certain embodiments, adjustment of the environmental temperature to which the subject is exposed, enhances the modulation of REV-ERBα. In one embodiment, when the environmental temperature to which the subject is exposed is decreased, the dosage and/or exposure time of the antagonist may be increased to enhance the effect on the subject. In one embodiment, when the environmental temperature to which the subject is

exposed is increased, the dosage of the agonist is able to be increased to enhance the effect on the subject.

In another embodiment, the methods described herein are useful in treating subjects who have, or are at risk of developing, certain disorders. In one embodiment, the disorder is related to excessive weight or obesity. In another embodiment, the disorder is related to insufficient body weight. In another embodiment, the subject has, or is at risk of developing obesity, diabetes, Syndrome X, heart disease, obstructive sleep apnea, polycystic ovarian syndrome, hypertension, abnormal lipids, heart attack and stroke, cancers, fatty liver, anorexia, or bulimia. In yet another embodiment, the disorder is related to excessively high or low body temperature. In one embodiment, the disorder is hypothermia or hyperthermia.

In another embodiment, a method of modulating the expression or activity of REV-ERBα includes administering any of the compounds described herein. In another embodiment, modulating REV-ERBα includes administering an agent that modulates expression of REV-ERBα. In another embodiment, modulating REV-ERBα includes administering an agent that modulates activity of REV-ERBα The compounds described herein may be administered as pharmaceutical compositions as further described below.

In still other embodiments, REV-ERBα may be modulated using biomolecular or biological means, such as by administering to a subject in need thereof a therapeutic reagent that down-regulates the expression or activity of REV-ERBα. In one embodiment, such a therapeutic reagent includes a short nucleic acid molecule comprising a nucleotide sequence that is complementary to at least a portion of the nucleotide sequence encoding REV-ERBα. In certain embodiments, this short nucleic acid molecule is a short hairpin RNA (shRNA) or a short interfering RNA (siRNA). In another embodiment, the method employs as the therapeutic agent a plasmid or viral vector that comprises the short nucleic acid molecule, e.g., an shRNA, that comprises a sequence that is complementary to at least a portion of the nucleotide sequence encoding REV-ERBα, under the control of regulatory sequences. In another embodiment, the viral vector is complexed with a polymer to create a nanoparticle. In yet another embodiment, the therapeutic reagent is an antibody.

In another aspect, use of an agent which modulates REV-ERBα is provided. In one embodiment, the agent is used for affecting the calorie expenditure in a subject in need thereof. In one embodiment, the agent increases expression or activity of REV-

ERBα, which decreases the calorie expenditure in the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα, which increases the calorie expenditure in the subject. In another embodiment, the agent is used for regulating the function of BAT in a subject in need thereof. In one embodiment, the agent increases expression or activity of REV-ERBα, which decreases the function of BAT in the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα, which increases the function of BAT in the subject. In another embodiment, the agent is used to modulate the cold tolerance of a subject in need thereof. In one embodiment, the agent increases expression or activity of REV-ERBα, which decreases the cold tolerance of the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα, which increases the cold tolerance of the subject. In another embodiment, the agent is used to affect the body temperature of a subject. In one embodiment, the agent increases expression or activity of REV-ERBα, which decreases the body temperature of the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα, which increases the body temperature of the subject. In yet another embodiment, the agent is used to affect glucose uptake in a subject. In one embodiment, the agent increases expression or activity of REV-ERBα, which decreases the glucose update of the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα, which increases the glucose uptake of the subject.

In another aspect, use of an agent which modulates REV-ERBα is provided, for affecting the weight of a subject in need thereof. In another aspect, use of an agent which modulates REV-ERBα is provided for reducing weight in the subject. In one embodiment, the agent decreases expression or activity of REV-ERBα. In another aspect, use of an agent which modulates REV-ERBα is provided for increasing weight in the subject. In one embodiment, the agent increases expression or activity of REV-ERBα.

In another aspect, use of an agent which modulates REV-ERBα is provided, for treating obesity in a subject in need thereof.

In one embodiment, the agent which modulates REV-ERBα is an agonist or antagonist of REV-ERBα. In another embodiment, the agent is one described herein. In another embodiment, the subject has, or is at risk of developing obesity, diabetes, syndrome x, heart disease, obstructive sleep apnea, polycystic ovarian syndrome, hypertension, abnormal lipids, heart attack and stroke, cancers, fatty liver, anorexia, or bulimia.

e. Pharmaceutical Compositions

In various embodiments, the invention provides the use of pharmaceutical compositions comprising a compound which modulates REV-ERBα and a pharmaceutically acceptable excipient. Such suitable compositions are described in WO 2013/033310 which is incorporated herein by reference. In one embodiment, the composition is used alone or in combination with another medicament which is useful for treatment of the indicated disease or condition. Pharmaceutical compositions can be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995, incorporated by reference herein. The compositions can appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

In one embodiment, pharmaceutical compositions include a compound which modulates REV-ERBα and a pharmaceutically acceptable excipient which can be a carrier or a diluent. The active compound may be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which can be in the form of an ampoule, capsule or other container. Some examples of suitable carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatin, lactose, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. The carrier or diluent can include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

The compositions can be mixed with additional agents that do not negatively react with the active compounds. Such additives can include wetting agents, emulsifying and suspending agents, salt for influencing osmotic pressure, buffers and/or coloring substances preserving agents, sweetening agents or flavoring agents. The compositions can also be sterilized if desired.

The route of administration can be any route which effectively transports the active compound of the invention to the appropriate or desired site of action. In one embodiment, the route of administration is oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal or parenteral, e.g., rectal, depot, subcutaneous, intravenous,

intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment. In one embodiment, administration is oral.

Injectable dosage forms generally include aqueous suspensions or oil suspensions which can be prepared using a suitable dispersant or wetting agent and a suspending agent injectable forms can be in solution phase or in the form of a suspension, which is prepared with a solvent or diluent. Acceptable solvents or vehicles include sterilized water, Ringer's solution, or an isotonic aqueous saline solution. Alternatively, sterile oils can be employed as solvents or suspending agents. Preferably, the oil or fatty acid is non-volatile, including natural or synthetic oils, fatty acids, mono-, di- or tri-glycerides.

For injection, the formulation can also be a powder suitable for reconstitution with an appropriate solution as described above. The compounds can be formulated for parenteral administration by injection such as by bolus injection or continuous infusion. A unit dosage form for injection can be in ampoules or in multi-dose containers.

The formulations of the invention can be designed to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. Thus, the formulations can also be formulated for controlled release or for slow release.

The compositions can be compressed into pellets or cylinders and implanted intramuscularly or subcutaneously as depot injections. Such implants can employ known inert materials such as silicones and biodegradable polymers, e.g., polylactide-polyglycolide. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides).

The compounds and/or pharmaceutical compositions described herein which are known modulators of REV-ERBα as referenced herein or newly identified compounds identified as REV-ERBα agonists or antagonists are likely effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.005 to about 5000 mg, preferably from about 1 to about 2000 mg, and more preferably between about 2 and about 2000 mg per day of the selected compound can be used. A typical dosage is about 10 mg to about 1000 mg per day. In choosing a regimen for subjects, on of skill in the art can frequently begin with a higher dosage and adjust or reduce dosage or frequency of administration when the condition is under control, e.g., obesity. The exact dosage will depend upon the condition being treated, the activity of the compound, mode of administration, on the therapy desired, form in which administered, the subject to be

treated and the body weight of the subject to be treated. Such dosages can be determined by the subject's health care provider.

Dosage forms suitable for oral, nasal or transdermal administration include from about 125 μg to about 1250 mg, preferably from about 250 g to about 500 mg, and more preferably from about 2.5 mg to about 250 mg, of the compounds admixed with a pharmaceutically acceptable carrier or diluent.

Dosage forms can be administered daily, or more than once a day, such as twice or thrice daily. Alternatively dosage forms can be administered less frequently than daily, such as every other day, weekly, or monthly.

Examples

The following examples demonstrate the function of REV-ERBα in controlling temperature rhythms and thermogenic plasticity through integration of circadian and environmental signals using a genetic null model (*Rev-erbα* KO). All experiments were performed in mice on a C57Bl/6 background and, unless otherwise noted, at murine thermoneutrality (~29-30°C) in order to avoid confounding background contributions from the "browning" of white adipose depots or partial, cold-induced adrenergic stimulation of BAT activity[19]. At thermoneutrality, the circadian oscillations of *Rev-erbα* gene expression (Fig. 1A) and protein levels (Fig. 1B) in BAT were similar to other tissues[14,20], peaking in the light and being nearly absent in the dark, and REV-ERBα ablation altered *Bmal1* transcription but did not affect the rhythmicity of *Rev-erbα*, *Cry1-2*, *Per1-3*, nor *Clock* (Fig. 5A), consistent with the mild circadian phenotype previously observed[20].

Example 1: Materials and Methods

A. Animal Studies: All animal studies were performed with approval from the University of Pennsylvania Perelman School of Medicine Institutional Animal Care and Use Committee. The *Rev-erba* KO mice[20] 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 7 AM, lights off at 7 PM). Gene expression, protein analysis, and temperature measurements were carried out on 12-16 week old male *Rev-erba* KO mice and WT littermates. Cold exposure experiments were performed in climate controlled rodent incubators set to 29°C and 4°C. All WT and *Rev-erba* KO 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 prior to cold challenge. Core abdominal and brown adipose measurements were obtained using surgically implanted dataloggers for core (SubCue Dataloggers) and telemetric transmitters for BAT (IPTT 300 transponders, Biomedic data systems) following pentobarbital anesthetization. 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. Colonic and intrascapular surface measurements were obtained using YSI Precision Thermometers with rectal or banjo probe attachments, respectively. Thermoneutrally-acclimated WT and *Rev-erba* KO mice were intraperitoneally injected with 1 mg/ml L-(−)-NE-bitartrate salt monohydrate (Sigma) and harvested at ZT10.

B. Immunoblotting: BAT samples were homogenized in RIPA (137 mM NaCl, 0.1% SDS, 0.5% Na-deoxycholate, 1% NP-40, 20 mM NAF, and 20 mM G2P in 1X PBS pH 7.4, supplemented with Complete protease inhibitors (Roche)) using a Tissuelyser (Qiagen) for 1.5 min at a frequency of 20 s^{-1} followed by sonication using a Bioruptor (Diagenode) for 30 sec on the "high" setting. SDS-PAGE was performed using 50 mg of protein loaded onto a 10% Tris-glycine gel (Invitrogen), followed by transfer to a PVDF membrane (Invitrogen). After antibody incubation, blots were developed using the SuperSignal West Dura chemiluminescence kit from Pierce.

C. Cell Culture: Preadipocytes were harvested 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 Collagenase D (Roche) and 2.4 U/ml Dispase II (Roche) and incubation in a 37°C shaking water bath for 45 minutes. Cells were purified through 100 mm filters (Millipore), pelleted and resuspended in Growth media (DMEM/F-12 GlutaMax supplemented with 10% Fetal Bovine Serum (Tissue Culture Biologicals), HEPES pH 7.2 (Invitrogen), and Penicillin/Streptomycin (Invitrogen)). Adipocyte differentiation was induced upon confluence with Induction media (Growth media supplemented with 500 nM Dexamethasone, 125 nM Indomethacin, 0.5 mM IBMX, 1 nM Rosiglitazone, 1 nM T3, and 20 nM Insulin) for 36 h. Following induction cells were cultured in Maintenance media (Growth media supplemented with 1 nM T3 and 20 nM Insulin). Serum synchronization was performed by incubating differentiated adipocytes overnight in starvation media (DMEM/F-12 GlutaMax containing 0.5% Fetal Bovine Serum) and then

replacing the starvation media with DMEM/F-12 GlutaMax containing 50% Horse Serum for 2 h. Following two washes in PBS, cells were placed in DMEM/F-12 GlutaMax containing 0.5% Fetal Bovine Serum, 1 nM T3, and 20 nM Insulin and total RNA was harvested at the indicated time points.

D. Thermographic Imaging: Thermography was performed by the Penn Mouse Phenotyping, Physiology, and Metabolism (MPPM) core during the light and dark phases using a FLIR SC620 infrared camera on WT and *Rev-erba* KO mice acclimated at thermoneutrality for 2 weeks. No anesthesia was used in order to avoid confounding effects on body temperature.

E. Fluorodeoxyglucose Imaging: ^{18}Fluorodeoxyglucose (^{18}FDG) uptake was performed. Doses of saline containing 300 μCi ^{18}FDG were administered through the lateral tail vein under constant isoflurane anesthesia (1-2%, 1 L O_2/min). Mice were scanned on a Philips Mosaic HP 1 h after injection. Percent injected dose was calculated by assessing the ratio of radioactive counts in the region of interest (ROI) for brown adipose to the total counts for the animal using Amide medical imaging software.

F. ChIP: Murine BAT was harvested 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 at pH 7.5) using probe sonication. Proteins were immunoprecipitated in ChIP dilution buffer (50 mM HEPES, 155 mM NaCl, 1.1% Triton X-100, 0.11% Na-deoxycholate, Complete protease inhibitor tablet at pH 7.5). Cross-linking was reversed overnight at 65°C in elution buffer (50 mM Tris-HCL, 10 mM EDTA, 1% SDS at pH 8), and DNA was isolated using phenol/chloroform/isoamyl alcohol. Precipitated DNA was analyzed by quantitative PCR.

G. ChIP-seq: ChIP experiments were performed independently on BAT samples from three mice harvested at 5 PM with or without a 6 h cold challenge as previously described[14]. ChIP of REV-ERBa was performed using the Cell Signaling Technology antibody (#2124). 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 sequences were obtained using the Solexa Analysis Pipeline.

H. RNA: Total RNA was isolated from BAT tissue by Trizol (Invitrogen) extraction and 1.5 mg of total RNA was used for cDNA synthesis using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Relative mRNA levels were determined using quantitative PCR and normalization to housekeeping gene 36B4. Primer sequences are available upon request.

I. 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 analysis of variance (ANOVA) for 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.

J. Whole animal oxygen consumption rate: Oxygen consumption rates were measured using comprehensive lab animal monitoring system (CLAMS) metabolic cages contained with temperature-controlled rodent incubators. Cold-induced oxygen consumption rates were assessed on singly-housed, unanaesthetized WT and Rev-erbα KO 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. NE-induced oxygen consumption rates were assessed as previously described16. Briefly, mice were anaesthetized with 75 mg/kg pentobarbital intraperitoneally and placed in a CLAMS unit set to 33°C to maintain body temperature. One mg/kg NE was administered subcutaneously once a baseline oxygen consumption rate had been obtained (approximately 20 min after pentobarbital injection). NE-induced oxygen consumption was then measured until rates had peaked and started declining (approximately 90 min after NE administration).

K. Temperature measurements: Core and brown adipose temperature measurements were obtained using surgically implanted dataloggers for core (SubCue Dataloggers) and telemetric transmitters for BAT (IPTT 300 transponders, Biomedic data systems) following pentobarbital anesthetization. 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. Colonic and interscapular surface measurements were obtained using YSI Precision Thermometers with rectal or banjo probe attachments, respectively.

L. Electromyogram (EMG): EMG recordings were made essentially as previously described (Golozoubova V, et al. Only UCP1 can mediate adaptive nonshivering

thermogenesis in the cold. FASEB J. 2001; 15:2048–2050. [PubMed: 11511509]. Three 29 gauge needle electrodes (2 recording electrodes 4 mm apart and 3 mm deep and 1 reference electrode placed distally) were fixed transcutaneously for acquiring the 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 1000X with a P55 differential amplifier (Grass Instruments, Quincy, MA). Data were A/D converted and recorded with a PowerLab 8SP at a sampling frequency of 10 kHz (ADInstruments, Colorado Springs, CO). The signal was acquired and Root Mean Square (RMS) of the EMG signal was calculated with LabChart 7 (ADInstruments).

For cold induced shivering, mice were exposed to 4°C for 1 h, quickly anesthetized with isoflurane and placed on a temperature controlled pad maintained at 15°C. EMG signals were recorded for 15 min and the data collected between minutes 2 and 7 were used for the analyses. Mice were allowed to recover for one day and then subjected to EMG measurement at thermoneutrality, maintaining the temperature controlled pad at 33°C.

For recording norepinephrine (NE)-induced EMGs, mice were anesthetized with an IP injection of 75 mg/kg pentobarbital. The temperature controlled pad was maintained at 33°C. After obtaining 5 min of basal EMG recordings, 1 mg/kg NE was injected subcutaneously on the back of the mouse and the recording continued for 20 min. All RMS calculations were made from 2 min of data collected prior to NE administration as well as 5, 10 and 15 min after NE administration.

Example 2: Discussion of Experiments of Example 1

To evaluate the role of REV-ERBα in BAT, C57Bl/6 wild type (WT) and *Rev-erbα* KO mice were subjected to an acute cold challenge from ZT4-10 (11 AM to 5 PM) when REV-ERBα levels peak in WT animals. Consistent with previous reports that thermoneutrally-acclimated C57Bl/6 mice fail to thrive during acute cold stresses[19,21,22], body temperatures of WT animals dropped markedly when shifted from 29°C to 4°C (Fig. 1C), and this inability to maintain body temperature was associated with failure to survive the cold exposure (Fig. 1D). By contrast, *Rev-erbα* KO mice were fully capable

of maintaining body temperature and uniformly survived the ZT4-10 cold challenge suggesting that REV-ERBα represses the thermogenic program.

These experiments were performed during the day, when REV-ERBα is at its peak in WT mice. Since REV-ERBα is physiologically nearly absent in the 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[23]; importantly, these analyses were all carried out within the light phase when murine REV-ERBα is highest.

Remarkably, during the dark period, when REV-ERBα levels are at the nadir of their physiological circadian rhythm, WT mice were much better able to protect their body temperature and were indistinguishable from *Rev-erbα* KO mice in both body temperature regulation (Fig. 1E) and survival (Fig. 1F) 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 KO mice was associated with higher oxygen consumption rates compared to WT littermates (Fig. 9a). Food intake (Fig. 10a), basal muscle activity, and cold-induced shivering (Fig. 9b, Fig. 10b) were unchanged between genotypes indicating that the Rev-erbα-dependent differences in oxidative capacity were likely due to alterations in BAT-driven, nonshivering thermogenic program. Indeed, brown adipose isolated from cold-challenged Rev-erbα KO animals consumed more oxygen than BAT from WT mice (Fig. 9c). Moreover, NE administration induced a larger increase in oxygen consumption in Rev-erbα KO animals than in control littermates (Fig. 10c) with no genotypic difference in muscle activity (Fig. 10d-e) further suggesting that Rev-erbα modulates heat production and cold susceptibility through BAT thermogenic pathways. Despite enhanced BAT metabolic capacity, Rev-erbα KO mice exhibited no significant difference in weight or food intake at room temperature and thermoneutrality compared to WT controls (data not shown) likely 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 in a circadian

manner but cold exposure rapidly attenuated *Rev-erbα* expression whereas closely-related nuclear receptor *Rev-erbα* did not undergo a similar cold-dependent decrease (Fig. 2A, Fig. 6A). Cold-mediated reduction of *Rev-erbα* gene expression occurred in parallel with the induction of *Bmal1*, an established target of REV-ERBα repression (Fig. 6B), as well as the canonical thermogenic regulators uncoupling protein 1 (*Ucp1*) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Pgc-1α*)[24] (Fig. 2A). *Rev-erbα* expression was attenuated following both moderate (29°C to 20°C) and acute (29°C to 4°C) cold stresses (Fig. 6C). Similarly, REV-ERBα protein plummeted when mice were shifted to 4°C (Fig. 2B).

Classically, regulation of brown adipose thermogenesis has been attributed predominantly to sympathetic release of NE and subsequent activation of adrenergic signaling cascades[2]. We therefore considered whether the cold-induced decrease in REV-ERBα levels was related to the adrenergic pathway. However, whereas the highly cAMP-sensitive nuclear receptor NOR1[25] was induced comparably by NE and cold, NE administration did not mimic the effect of cold exposure on expression of *Rev-erbα* gene (Fig. 2C) or REV-ERBα protein (Fig. 2D). These data support the role of REV-ERBα in thermogenic regulation as being independent of sympathetic stimulation.

The rapidity with which REV-ERBα was reduced in the cold and its inverse relationship to *Ucp1* expression suggested that REV-ERBα might actively repress the *Ucp1* gene. Indeed, at thermoneutrality BAT *Ucp1* mRNA (Fig. 3A) and protein levels (Fig. 3B) were significantly higher in *Rev-erbα* KO mice than WT littermates, and exhibited only a modest further induction in response to cold challenge. *Bmal1* mRNA and protein followed a similar pattern (Fig. 7A-B) whereas *Pgc-1α* expression was similar between control and *Rev-erbα* KO animals at thermoneutrality and induced comparably in the cold (Fig. 3A-B), suggesting *Rev-erbα*-independence. Nevertheless, REV-ERBα controlled *Ucp1*, which is critical for mammalian heat production and BAT function[2,22]. The elevated *Ucp1* levels in saline-injected *Rev-erbα* KO mice exceeded those of NE-treated WT animals and did not increase further when NE was administered to the *Rev-erbα* KOs (Fig. 3C). This data supports that overriding REV-ERBα-dependent control is an integral feature of the complete thermogenic response. Furthermore, genetic ablation of *Rev-erbα* caused a similar increase in *Ucp1* in primary brown adipocytes illustrating that the effect was cell-autonomous and is the result of direct REV-ERBα repression (Fig. 3D). Consistent with this observation, chromatin immunoprecipitation followed by deep

sequencing (ChIP-seq) assessment of REV-ERBα genomic occupancy revealed two strong binding events in the proximal *Ucp1* promoter that were significantly reduced 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 (Fig. 3F). This *Ucp1* circadian rhythmicity was abolished in *Rev-erbα* KO animals (Fig. 3G). These data establish REV-ERBα as a direct, negative regulator of thermogenic transcriptional programs.

Ucp1 was elevated in primary brown adipocytes lacking Rev-erbα and ectopic expression of Rev-erbα restored Ucp1 mRNA to WT levels, whereas overexpression of Rev-erbα in WT 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 (Fig. 11c). This Ucp1 circadian rhythmicity was completely abolished in Rev-erbα KO 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 influenced body temperature rhythm. *Rev-erbα* ablation dramatically altered body temperature oscillation, both of the core (Fig. 4A) and intrascapular region (BAT) (Fig. 4B). Higher body temperature was maintained by *Rev-erbα* KO 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* KO mice were warmer than WT mice from ZT4-10 but not ZT16-22 (Fig. 4C, Fig. 8A). Comparison between colonic and intrascapular temperatures implicated BAT as the primary source of the genotypic variation (Fig. 8B). Most strikingly, the diurnal oscillation of BAT glucose uptake[28] was absent in *Rev-erbα* KO mice as evidenced by 18-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) (Fig. 4D). Glucose uptake was higher in mice lacking *Rev-erbα* than WT littermates during the day and did not increase at night as in WT animals (Fig. 4E). These results indicate that REV-ERBα is required for the circadian rhythm of body temperature and BAT activity (Fig. 8C).

Example 3: Screening

Brown adipocytes are isolated according to known procedures. See, e.g., Cigolini et al, Isolation and ultrastructural features of brown adipocytes in culture, J Anat. 1986 April; 145: 207–216 which is hereby incorporated by reference herein. The cells are cultured using appropriate medium such as Eagle's medium modified with Earle's salts, glutamine and 20 mm HEPES buffer, supplemented with 20% pooled fresh human serum, 80mg/l non-essential amino acids, 10mU/ml pork insulin, l00µg/ml streptomycin and 100 IU/ml penicillin. Test compounds in DMSO are diluted to suitable concentration. Adipocytes are cultured with test compounds for 1, 2, 8, 12 and 24 hours. Expression of UCP1 is measured either via mRNA (see, e.g., Chen et al, Synergism between cAMP and PPARγ Signalling in the Initiation of UCP1 Gene Expression in HIB1B Brown Adipocytes. PPAR Res. 2013) or protein level (Ringholm et al, PGC-1α Is Required for Exercise- and Exercise Training-Induced UCP1 Up-Regulation in Mouse White Adipose Tissue, PLoS One. 2013; 8(5): e64123. Published online 2013 May 22.). Each of these documents is incorporated herein by reference. Antibodies to UCP1 are commercially available e.g., ab10983 Abcam.

All publications cited in this specification, and US Provisional Patent Application No. 61/835,450, filed June 14, 2013, are specifically incorporated herein by reference. In addition, the publication Gerhart-Hines, et al, The nuclear receptor REV-erbα controls circadian thermogenic plasticity, Nature, 503(7476):410-3, Nature 2013 is hereby incorporated herein by reference in its entirety, including supplemental information. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

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What is claimed is:

1. A method of affecting calorie expenditure in a subject in need thereof, comprising modulating the expression of REV-ERBα in the subject.

2. The method of claim 1, comprising increasing the calorie expenditure in the subject, wherein the expression of REV-ERBα is decreased.

3. The method of claim 1, comprising decreasing the calorie expenditure in the subject, wherein the expression of REV-ERBα is increased.

4. A method of affecting the weight of a subject in need thereof, comprising modulating the expression of REV-ERBα in the subject.

5. The method of claim 4, comprising reducing weight in the subject, wherein the expression of REV-ERBα is decreased.

6. The method of claim 4, comprising increasing weight in the subject, wherein the expression of REV-ERBα is increased.

7. A method of regulating the function of brown adipose tissue (BAT) in a subject in need thereof, comprising modulating the expression of REV-ERBα.

8. The method of claim 7, comprising increasing the function of BAT in the subject, wherein the expression of REV-ERBα is decreased.

9. The method of claim 7, comprising decreasing the function of BAT in the subject, wherein the expression of REV-ERBα is increased.

10. A method of modulating the cold tolerance of a subject comprising modulating the expression of REV-ERBα.

11. The method of claim 10, comprising increasing the cold tolerance of the subject, wherein the expression of REV-ERBα is decreased.

12. The method of claim 10, comprising decreasing the cold tolerance of the subject, wherein the expression of REV-ERBα is increased.

13. A method of treating obesity in a subject, comprising decreasing the expression of REV-ERBα.

14. A method of affecting body temperature of a subject in need thereof, comprising modulating the expression of REV-ERBα.

15. The method of claim 14, comprising increasing the body temperature of the subject, wherein the expression of REV-ERBα is decreased.

16. The method of claim 14, comprising decreasing the body temperature of the subject, wherein the expression of REV-ERBα is increased.

17. A method of affecting glucose uptake in a subject in need thereof, comprising modulating the expression of REV-ERBα in the subject.

18. The method of claim 17, comprising increasing the glucose uptake, wherein the expression of REV-ERBα is decreased.

19. The method of claim 17, comprising decreasing the glucose uptake, wherein the expression of REV-ERBα is increased.

20. A method of modulating the expression of REV-ERBα comprising administering an agonist or antagonist of REV-ERBα.

21. The method according to any of claims 1-20, wherein said modulating comprises comprising administering an agent that modulates expression of REV-ERBα.

22. The method according to any of the preceding claims, wherein the modulation of REV-ERB occurs only at night.

23. The method according to claim 23, wherein the modulation occurs when the subject is sleeping or at rest.

24. The method according to any preceding claim, wherein the modulation occurs when the subject's body temperature is at a daily trough.

25. The method according to any of the preceding claims, wherein the subject has, or is at risk of developing obesity, diabetes, syndrome x, heart disease, obstructive sleep apnea, polycystic ovarian syndrome, hypertension, abnormal lipids, heart attack and stroke, cancers, fatty liver, anorexia, or bulimia.

26. A method of screening for a modulator of REV-ERBα expression or activity comprising:

analyzing the level of expression or activity of adipocytes cultured with a test compound, wherein an increase in expression or activity of UCP1 as compared to a control level corresponds with a decrease in expression or activity of REV-ERBα and wherein a decrease in expression or activity of UCP1 as compared to a control level corresponds with an increase in expression or activity of REV-ERBα.

Figure 1

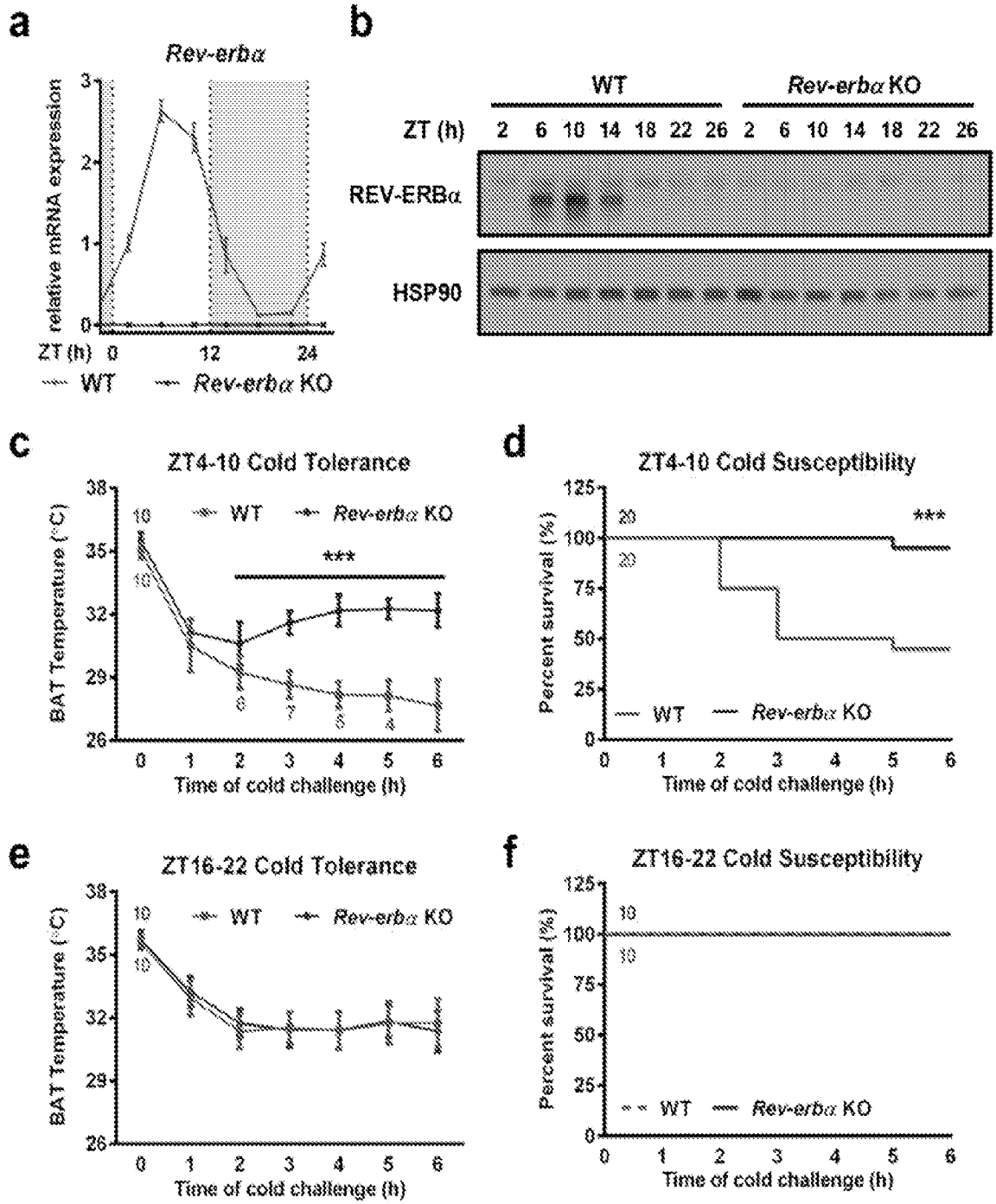

a
Rev-erbα
relative mRNA expression
ZT (h) 0 12 24
WT
Rev-erbα KO
b
WT
Rev-erbα KO
ZT (h) 2 6 10 14 18 22 26 2 6 10 14 18 22 26
REV-ERBα
HSP90
c
ZT4-10 Cold Tolerance
BAT Temperature (°C)
WT
Rev-erbα KO
***
Time of cold challenge (h)
d
ZT4-10 Cold Susceptibility
Percent survival (%)
***
WT
Rev-erbα KO
Time of cold challenge (h)
e
ZT16-22 Cold Tolerance
BAT Temperature (°C)
WT
Rev-erbα KO
Time of cold challenge (h)
f
ZT16-22 Cold Susceptibility
Percent survival (%)
WT
Rev-erbα KO
Time of cold challenge (h)

Figure 2

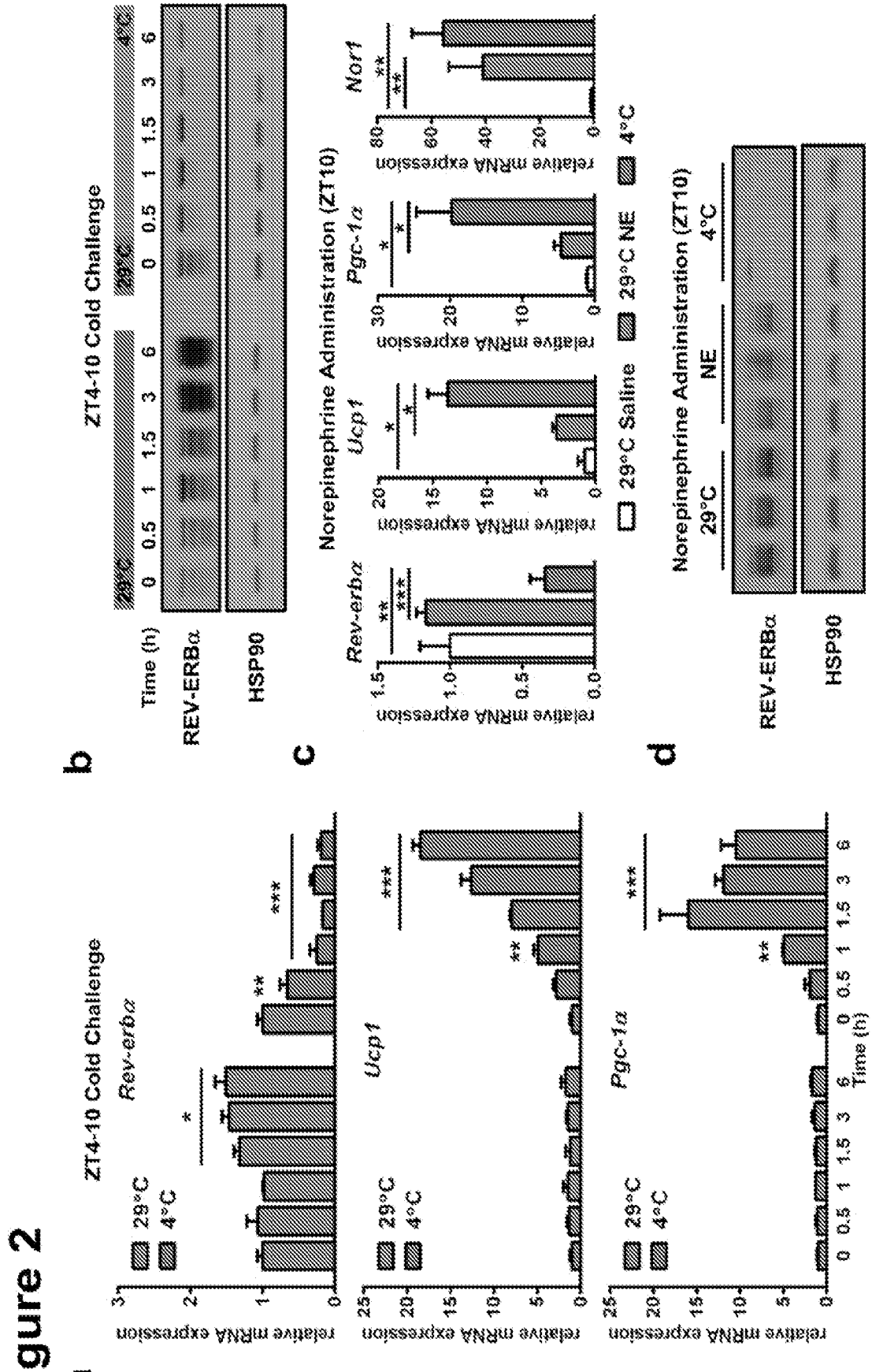

a
ZT4-10 Cold Challenge
29°C
4°C
Rev-erbα
Ucp1
Pgc-1α
relative mRNA expression
Time (h)
b
ZT4-10 Cold Challenge
29°C
4°C
Time (h)
REV-ERBα
HSP90
c
Norepinephrine Administration (ZT10)
Rev-erbα
Ucp1
Pgc-1α
Nor1
relative mRNA expression
29°C Saline
29°C NE
4°C
d
Norepinephrine Administration (ZT10)
29°C
NE
4°C
REV-ERBα
HSP90

Figure 3

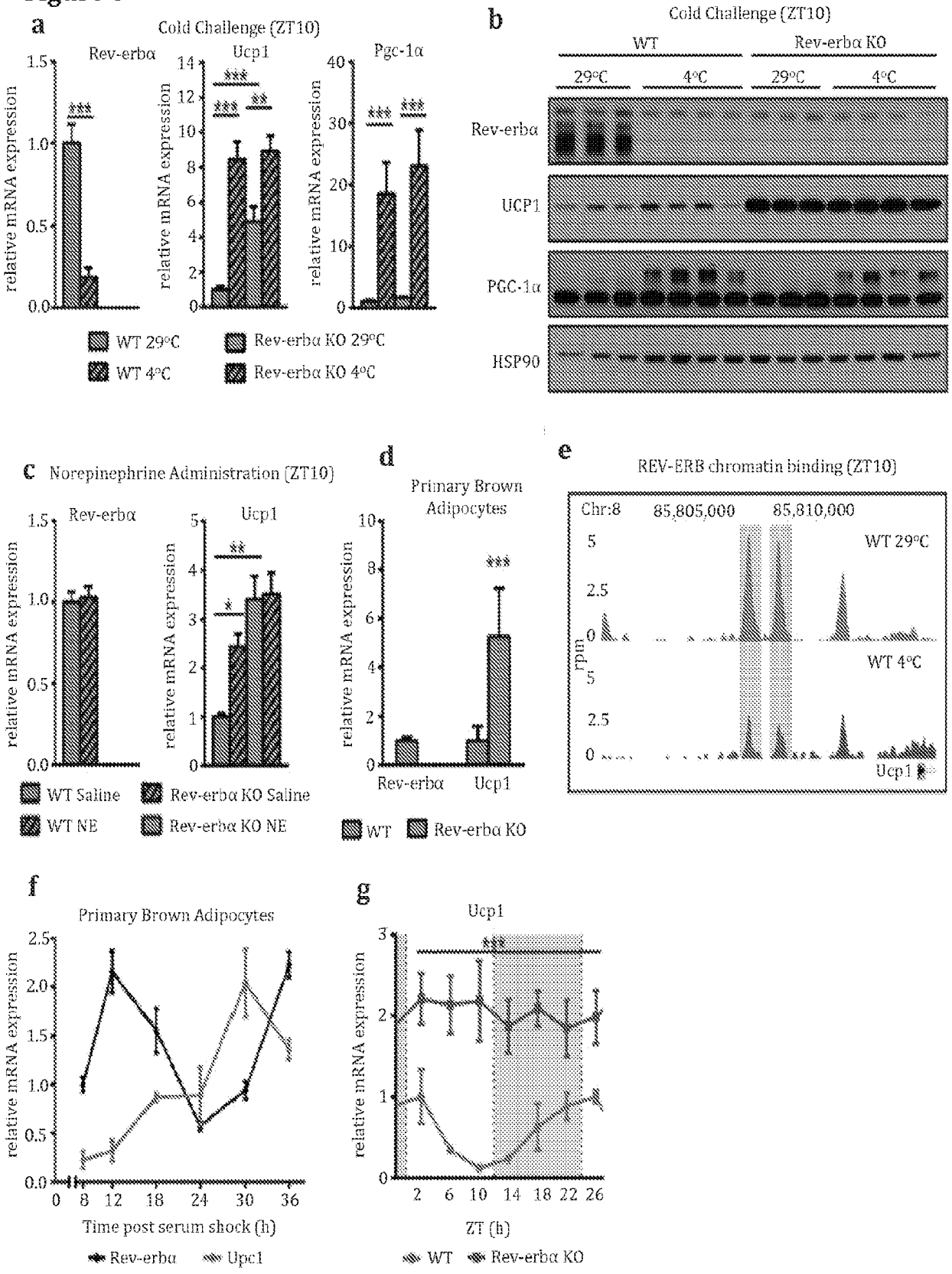

a
Cold Challenge (ZT10)
Rev-erbα
Ucp1
Pgc-1α
relative mRNA expression
WT 29°C
WT 4°C
Rev-erbα KO 29°C
Rev-erbα KO 4°C
b
Cold Challenge (ZT10)
WT
Rev-erbα KO
29°C
4°C
29°C
4°C
Rev-erbα
UCP1
PGC-1α
HSP90
c
Norepinephrine Administration (ZT10)
Rev-erbα
Ucp1
relative mRNA expression
WT Saline
WT NE
Rev-erbα KO Saline
Rev-erbα KO NE
d
Primary Brown Adipocytes
relative mRNA expression
Rev-erbα
Ucp1
WT
Rev-erbα KO
e
REV-ERB chromatin binding (ZT10)
Chr:8
85,805,000
85,810,000
WT 29°C
WT 4°C
rpm
Ucp1
f
Primary Brown Adipocytes
relative mRNA expression
Time post serum shock (h)
Rev-erbα
Upc1
g
Ucp1
relative mRNA expression
ZT (h)
WT
Rev-erbα KO

Figure 4

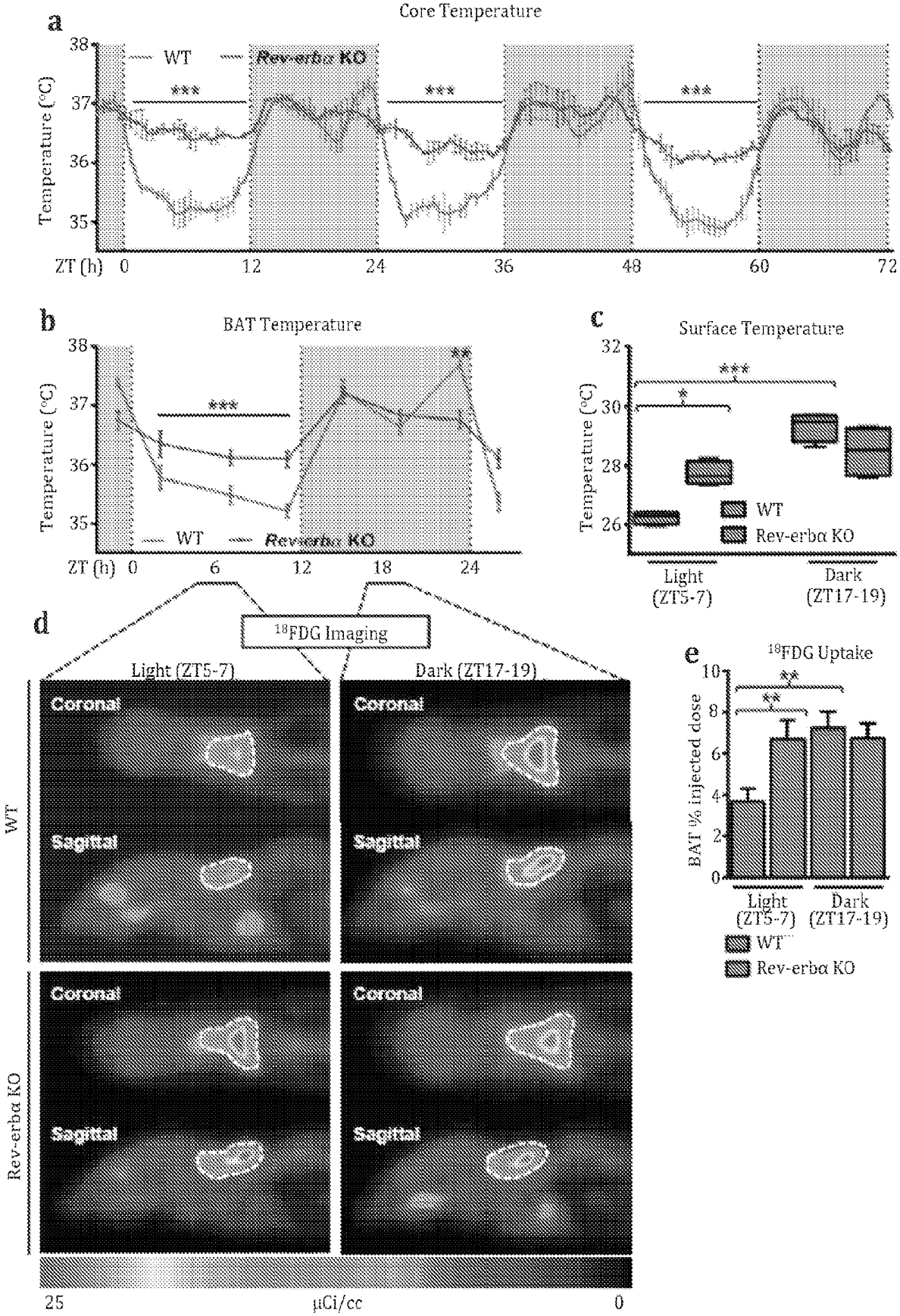

a
Core Temperature
WT
Rev-erbα KO
Temperature (°C)
ZT (h)
b
BAT Temperature
c
Surface Temperature
Light (ZT5-7)
Dark (ZT17-19)
d
18FDG Imaging
Light (ZT5-7)
Dark (ZT17-19)
Coronal
Sagittal
WT
Rev-erbα KO
25
µCi/cc
0
e
18FDG Uptake
BAT % injected dose

Figure 5

a

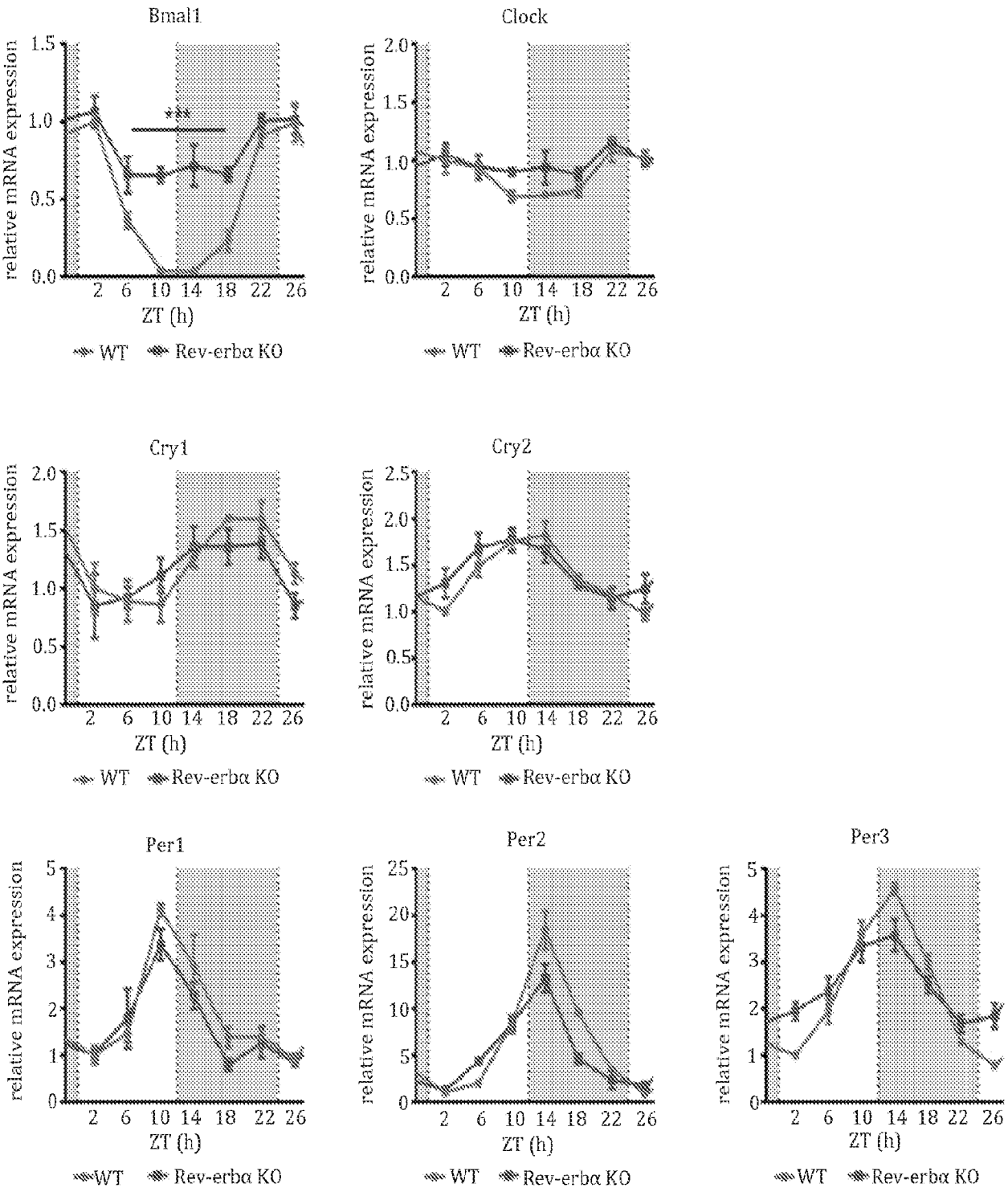
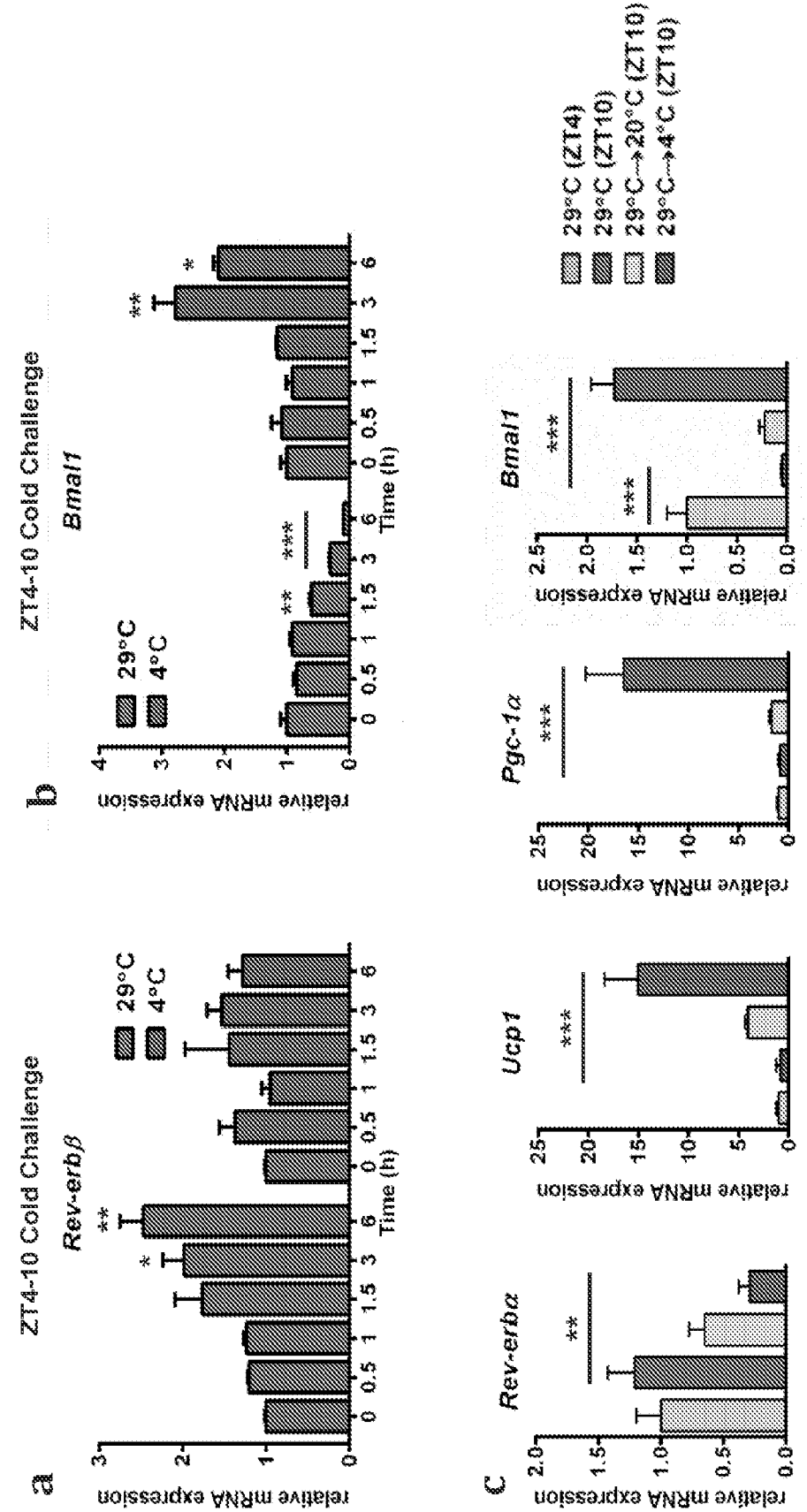
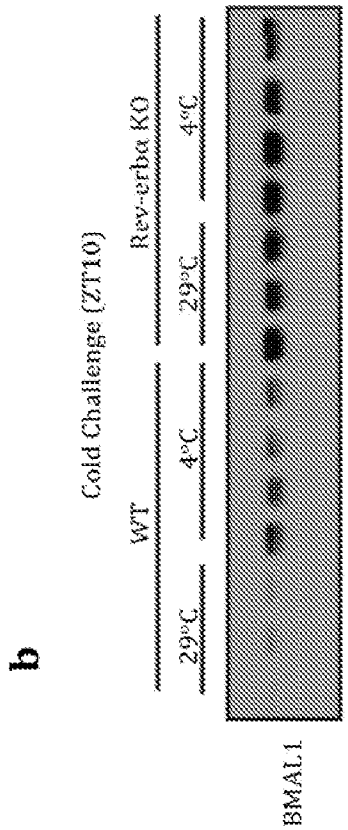

Bmal1
Clock
Cry1
Cry2
Per1
Per2
Per3
relative mRNA expression
ZT (h)
WT
Rev-erbα KO
***

Figure 6


a
ZT4-10 Cold Challenge
Rev-erbβ
29°C
4°C
relative mRNA expression
Time (h)
b
ZT4-10 Cold Challenge
Bmal1
29°C
4°C
relative mRNA expression
Time (h)
c
Rev-erbα
Ucp1
Pgc-1α
Bmal1
29°C (ZT4)
29°C (ZT10)
29°C→20°C (ZT10)
29°C→4°C (ZT10)
relative mRNA expression


b
Cold Challenge (ZT10)
WT
Rev-erbα KO
29°C
4°C
29°C
4°C
BMAL1

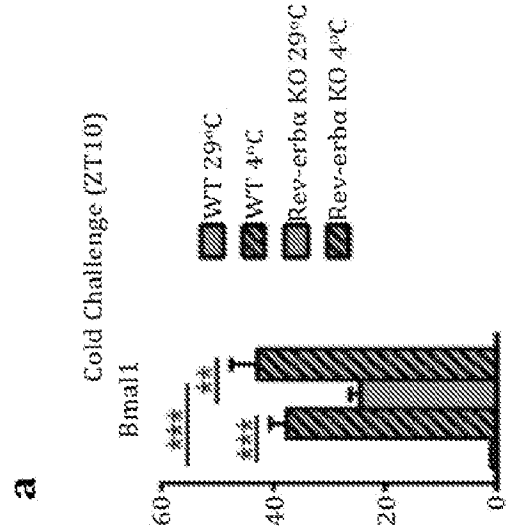

a
Cold Challenge (ZT10)
Bmal1
***
**
***
*
60
40
20
0
WT 29°C
WT 4°C
Rev-erbα KO 29°C
Rev-erbα KO 4°C

Figure 7

Figure 8

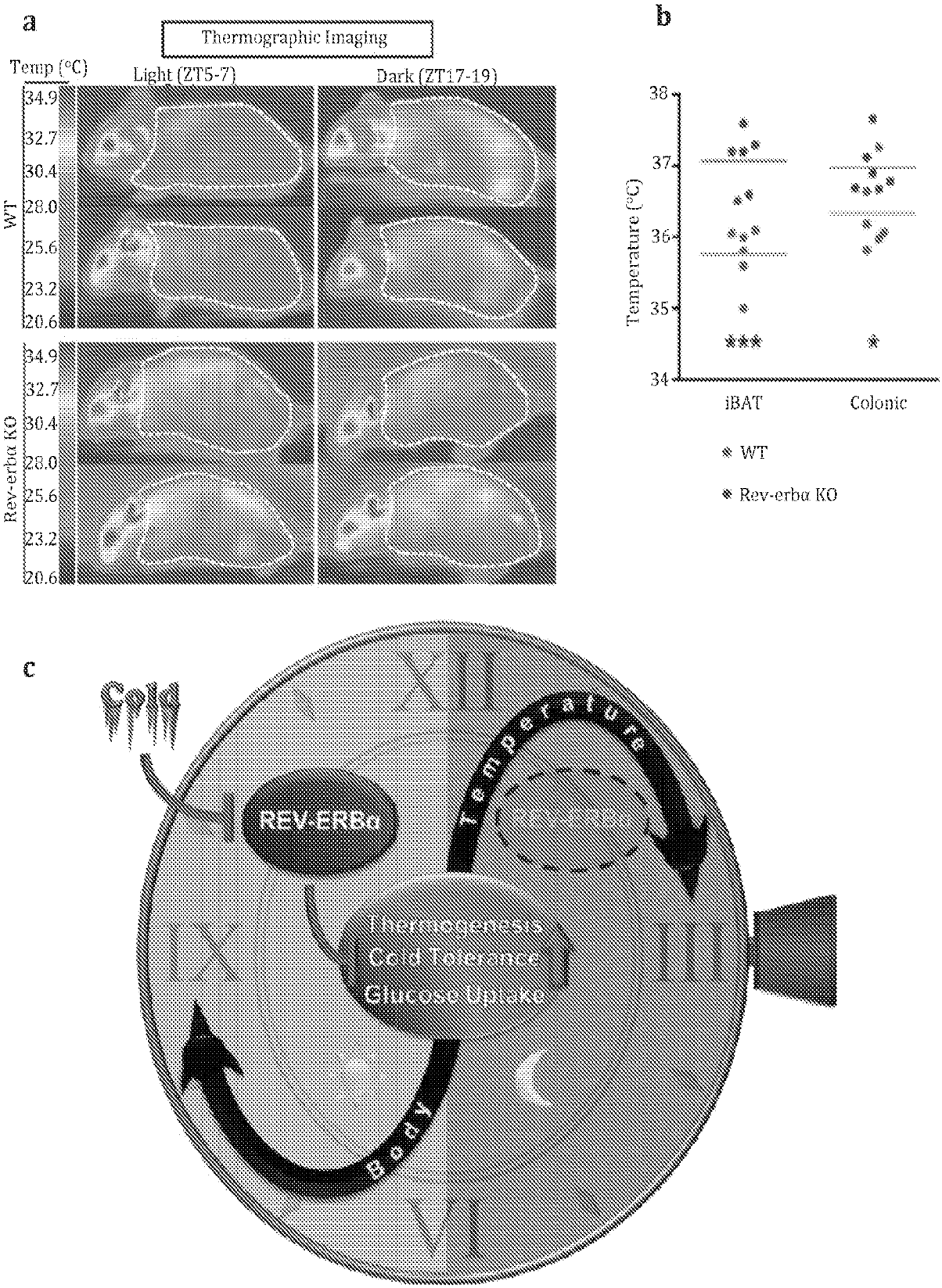

a
Thermographic Imaging
Temp (°C)
Light (ZT5-7)
Dark (ZT17-19)
34.9
32.7
30.4
28.0
25.6
23.2
20.6
WT
Rev-erbα KO
b
Temperature (°C)
38
37
36
35
34
iBAT
Colonic
WT
Rev-erbα KO
c
REV-ERBα
Temperature
Thermogenesis
Cold Tolerance
Glucose Uptake
Body

Figure 9

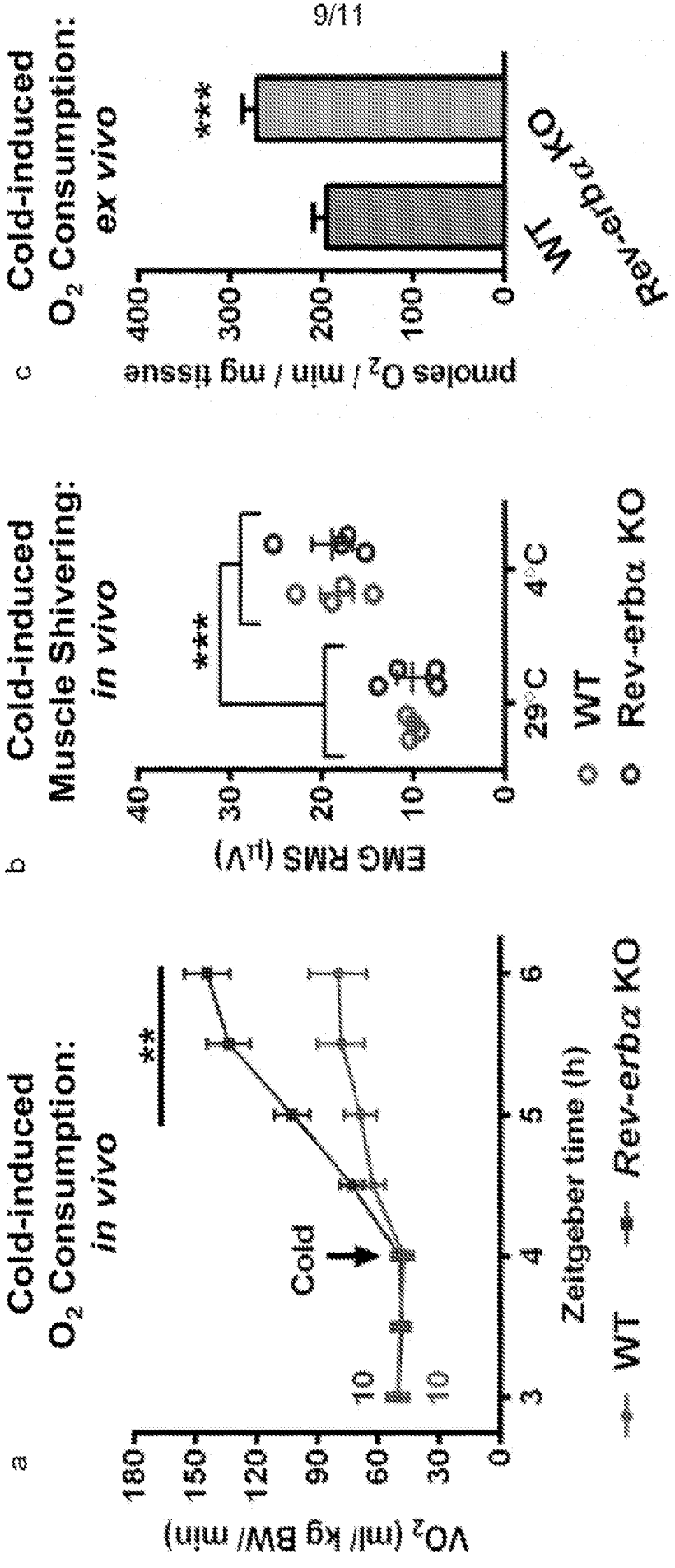

a
Cold-induced
O_2 Consumption:
in vivo
**
Cold
10
10
VO_2 (ml/ kg BW/ min)
180
150
120
90
60
30
0
3
4
5
6
Zeitgeber time (h)
WT
Rev-erbα KO
b
Cold-induced
Muscle Shivering:
in vivo
***
EMG RMS (μV)
40
30
20
10
0
29°C
4°C
WT
Rev-erbα KO
c
Cold-induced
O_2 Consumption:
ex vivo
***
pmoles O_2 / min / mg tissue
400
300
200
100
0
WT
Rev-erbα KO

Figure 10

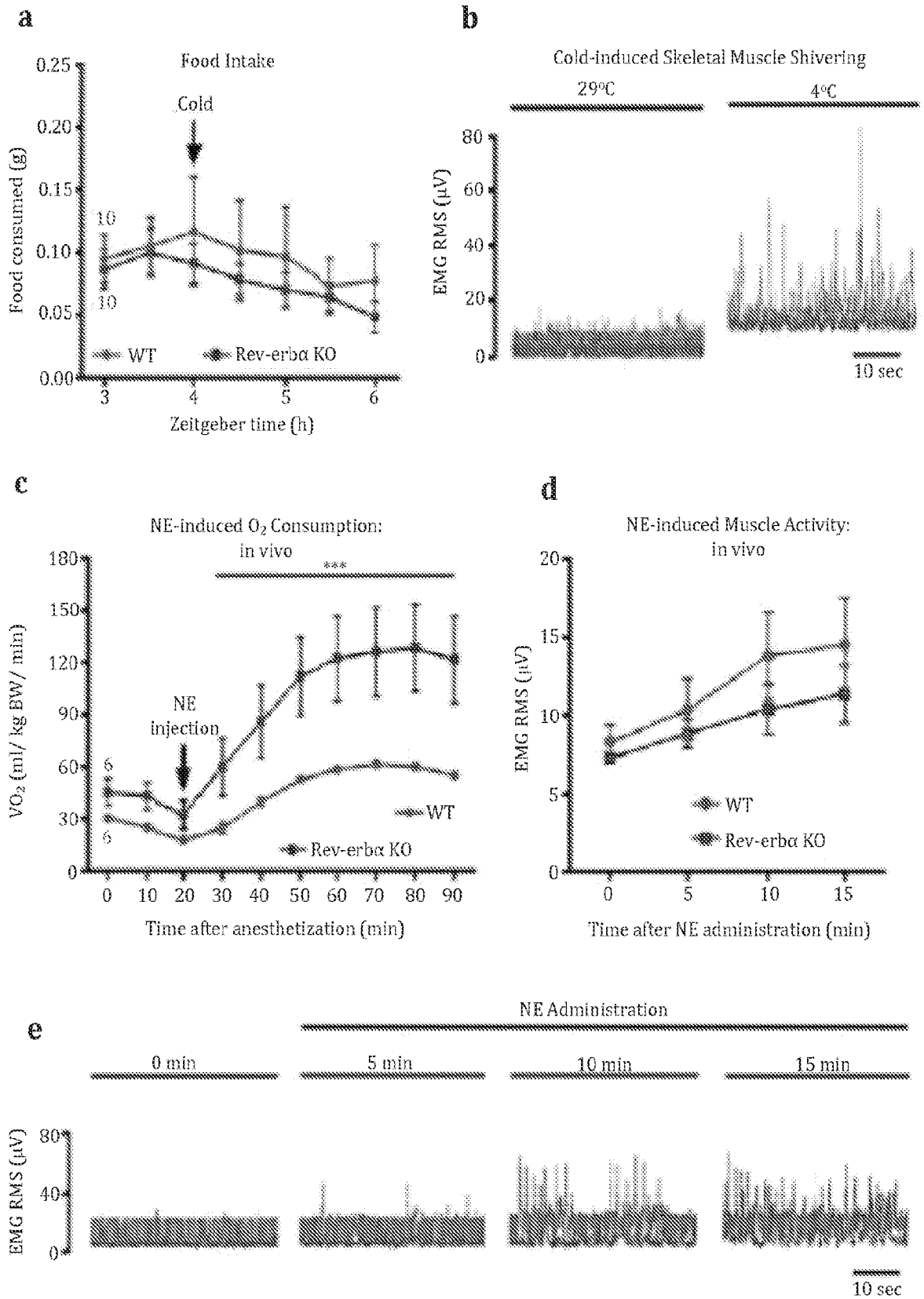
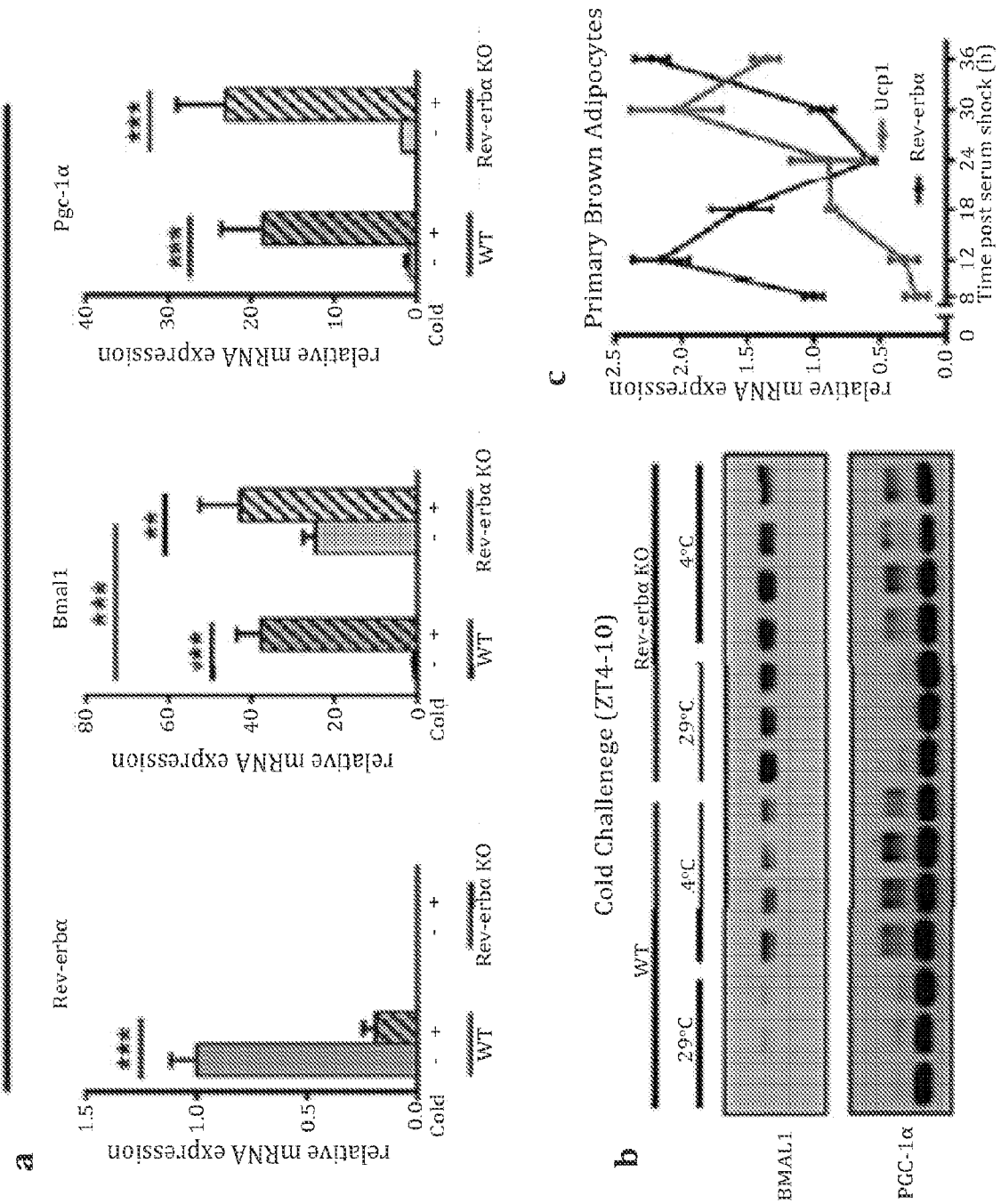

a
Food Intake
Cold
Food consumed (g)
0.25
0.20
0.15
0.10
0.05
0.00
10
10
WT
Rev-erbα KO
3
4
5
6
Zeitgeber time (h)
b
Cold-induced Skeletal Muscle Shivering
29°C
4°C
EMG RMS (μV)
80
60
40
20
0
10 sec
c
NE-induced O2 Consumption:
in vivo
***
VO2 (ml/ kg BW/ min)
180
150
120
90
60
30
0
NE
injection
6
6
WT
Rev-erbα KO
0 10 20 30 40 50 60 70 80 90
Time after anesthetization (min)
d
NE-induced Muscle Activity:
in vivo
EMG RMS (μV)
20
15
10
5
0
WT
Rev-erbα KO
0 5 10 15
Time after NE administration (min)
e
NE Administration
0 min
5 min
10 min
15 min
EMG RMS (μV)
80
40
0
10 sec

Figure 11


Cold Challenge (ZT4-10)
a
Rev-erbα
Bmal1
Pgc-1α
relative mRNA expression
Cold
WT
Rev-erbα KO
b
Cold Challenege (ZT4-10)
WT
Rev-erbα KO
29°C
4°C
BMAL1
PGC-1α
c
Primary Brown Adipocytes
Ucp1
Rev-erbα
Time post serum shock (h)