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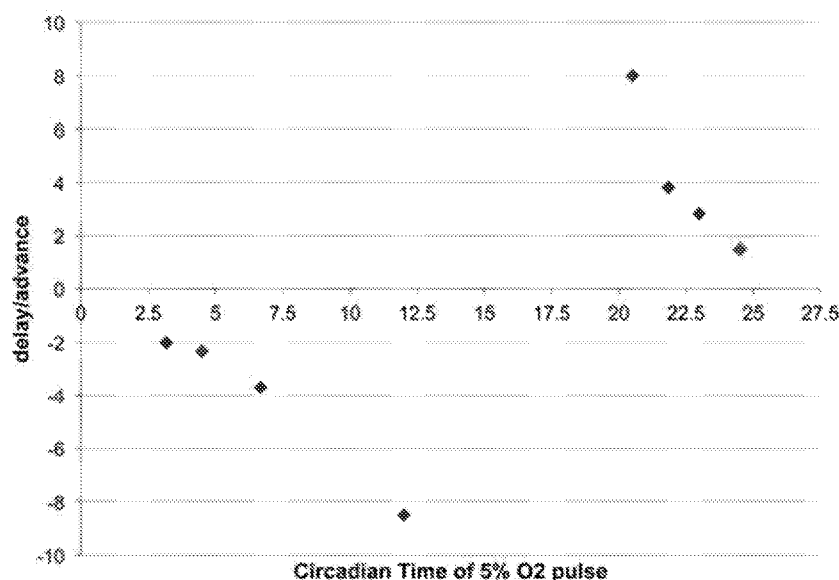


FIG. 49

(57) Abstract: Disclosed herein is a system is disclosed herein for use in the treatment of a circadian rhythm disorder, such as jet lag, and an aircraft or enclosed space in an airport terminal comprising such a system. The system is configured for exposing a subject to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 k Pa. Further disclosed is an agent that modulates an activity of HIF1 α , for use in the treatment of a circadian rhythm disorder, and pharmaceutical compositions and kits comprising such an agent.

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TREATMENT OF A CIRCADIAN RHYTHM DISORDER

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy, and
5 more particularly, but not exclusively, to systems, methods and compositions for treating
a circadian rhythm disorder.

Jet lag is a chronobiological problem, whereby upon travelling across time zones,
a person's body clock (circadian rhythm) becomes out of synchronization with the
daylight and darkness times at the destination. The body's rhythms that dictate times for
10 eating, sleeping, hormone regulation and body temperature variations no longer
correspond to the environment, and in some cases, may no longer correspond with each
other, until the body adjusts to the new schedule. Travelling east usually causes more
adjustment problems than travelling west. Shift work sleep disorder, in which work
hours overlap with a typical sleep period, involves problems similar to those of jet lag.

15 Control over the timing of exposure to and avoidance of light is a recommended
method for adjusting circadian rhythms. Administration of melatonin, a hormone
involved in synchronization of circadian rhythms, may also reduce the effects of jet lag,
although incorrect timing of melatonin administration may instead exacerbate effects of
jet lag.

20 In mammals, a master circadian clock in the brain synchronizes subsidiary
oscillators (a.k.a. cellular oscillators), which are present in peripheral tissues, in almost
every cell in the body. Because these cellular oscillators anticipate and function
together in a proactive manner to environmental changes, their temporal
synchronization is critical [Schibler et al., *Cold Spring Harbor Symposia on*
25 *Quantitative Biology* 2015, 80:223-232].

The molecular makeup for circadian rhythm generation is based on interlocked
negative transcription-translation feedback loops [Feng & Lazar, *Molecular Cell* 2012,
47:158-167; Partch et al., *Trends in Cell Biology* 2014, 24:90-99]. The basic helix-
loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins BMAL1 and CLOCK
30 heterodimerize and drive the expression of the *Period* (i.e., *Per1*, *Per2* and *Per3*) and
Cryptochrome (i.e., *Cry1* and *Cry2*) genes. Subsequently, PER and CRY proteins
accumulate and repress the transcription of their own genes. An auxiliary essential

feedback loop includes the orphan nuclear receptors of the REV-ERB and ROR families.

Both feeding rhythms and temperature cycles have been reported to synchronize peripheral clocks and even uncouple them from the master clock in the brain [Brown et al., *Current Biology* 2012, 12:1574-1583; Buhr et al., *Science* 2010, 330:379-385; 5 Damiola et al., *Genes & Development* 2000, 14:2950-2961; Saini et al., *Genes & Development* 2012, 26:567-580; Stokkan et al., *Science* 2001, 291:490-493; Vollmers et al., *PNAS* 2009, 106:21453-21458].

Hypoxia-inducible factor 1 α (HIF1 α) is a bHLH-PAS domain-containing 10 transcription factor that responds to and participates in oxygen homeostasis [Majmudar et al., *Molecular Cell* 2010, 40:294-309]. HIF1 α has been reported to heterodimerize with other bHLH-PAS domain-containing proteins such as BMAL1 [Hogenesch et al., *Genes & Development* 1998, 12:149-162], and to affect expression of *Per1* and *Clock* genes upon exposure to hypoxia [Chilov et al., *FASEB J* 2001, 15:2613-2622; Egg et al., 15 *Chronobiol Int* 2013, 30:510-529].

Coste et al. have reported that hypoxia depresses melatonin secretion [Coste et al., *J Pineal Res* 2004, 37:1-10], significantly alters blood chemistry, with a tendency towards phase delay [Coste et al., *Chronobiol Int* 2007, 24:1139-1157], and increases sleep onset latency [Coste et al., *J Sleep Int* 2009, 18:454-465]; and suggest that 20 exposure to hypoxia during air travel may contribute to jet lag. The partial pressure of oxygen in commercial aircraft has been reported to fall from 159 mm Hg (at sea level) to about 127 mm Hg [Cottrell, *Chest* 1988, 93:81-84].

U.S. Patent No. 5,799,652 (to Hypoxico Inc.) describes a hypoxic room system for hypoxic training or therapy at standard atmospheric pressure. The system employs 25 an oxygen content-reducing device that supplies oxygen-depleted air, such as 7 % to 15 % oxygen and 93 % to 85 % nitrogen, to a room having ventilating openings for equalizing atmospheric pressure inside the room. A variety of products for providing exposure to hypoxia, for applications such as enhancing athletic performance and preparing for high altitudes, are advertised by Hypoxico Inc. (e.g., at 30 [www\(dot\)hypoxico\(dot\)com](http://www.hypoxico.com)).

Additional background art includes Adamovich et al. [*Cell Metabolism* 2014, 19:319-330]; Asher et al. [*Cell* 2010, 142:943-953]; Asher & Sassone-Corsi [*Cell* 2015,

161:84-92]; Aviram et al. [*Molecular Cell* 2016, 62:636-648]; Chen et al. [*J Biol Chem* 2001, 276:9519-9525]; Dibner et al. [*Annu Rev Physiol* 2010, 72:517-549]; Dioum et al. [*Science* 2002, 298:2385-2387]; Koike et al. [*Science* 2012, 228:349-354]; Nagoshi et al. [*Cell* 2004, 119:693-705]; Rey et al. [*PLoS Biology* 2011, e1000595]; and Ripperger & Schibler [*Nature Genetics* 2006, 38:369-374].

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the invention, there is provided a system configured for exposing a subject to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa, the system being for use in the treatment of a circadian rhythm disorder.

According to an aspect of some embodiments of the invention, there is provided a system configured for exposing a subject in an aircraft to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa, the system being configured for use in an aircraft cabin, and comprising a breathing compartment configured for delivering the aforementioned atmosphere to a breathing orifice of a subject, and an apparatus configured for providing to the breathing compartment a gas selected to produce the atmosphere in the breathing compartment, the system being for use in the treatment of jet lag.

According to an aspect of some embodiments of the invention, there is provided an aircraft comprising a system configured for effecting a change in an oxygen partial pressure of an atmosphere within the aircraft cabin, wherein the change in an oxygen partial pressure comprises a change of at least 1 kPa during a flight of the aircraft at cruising altitude.

According to an aspect of some embodiments of the invention, there is provided an enclosed space in an airport terminal, the enclosed space comprising a system configured for effecting a daily change in an oxygen partial pressure of an atmosphere within the enclosed space, wherein the change in an oxygen partial pressure comprises a change of at least 1 kPa during a course of a day.

According to an aspect of some embodiments of the invention, there is provided an agent that modulates an activity of HIF1 α , for use in the treatment of a circadian rhythm disorder.

According to an aspect of some embodiments of the invention, there is provided a pharmaceutical composition comprising an agent that modulates an activity of HIF1 α , and a pharmaceutically acceptable carrier.

5 According to an aspect of some embodiments of the invention, there is provided a kit comprising the agent that modulates an activity of HIF1 α , according to any of the respective embodiments described herein, and instructions for enhancing the treatment by controlling exposure to a hypoxic atmosphere and/or to a controlled intensity of light.

According to some embodiments of the invention, the system described herein comprises a breathing compartment configured for exposing the subject to the atmosphere having an oxygen partial pressure which differs from a prevalent oxygen
10 partial pressure by at least 1 kPa, and an apparatus configured for providing to the breathing compartment a gas selected to produce the atmosphere in the breathing compartment.

According to some embodiments of the invention, the breathing compartment is
15 configured as a closed space.

According to some embodiments of the invention, the breathing compartment is configured to allow the subject to reside therein.

According to some embodiments of the invention, the breathing compartment and/or enclosed space described herein is in a form of a building, room and/or tent.

20 According to some embodiments of the invention, the breathing compartment is configured for delivering the atmosphere to a breathing orifice of a subject.

According to some embodiments of the invention, the breathing compartment is in a form of a mask, mouthpiece and/or helmet.

According to some embodiments of the invention, the system is a portable
25 system.

According to some embodiments of the invention, the atmosphere in the breathing compartment has an oxygen concentration of no more than 20 %.

According to some embodiments of the invention, the atmosphere in the breathing compartment has an oxygen concentration of at least 22.

30 According to some embodiments of the invention, the apparatus configured for providing the gas comprises a gas separation device configured for forming a gas having an oxygen concentration of no more than 20 %.

According to some embodiments of the invention, the apparatus configured for providing the gas comprises a gas separation device configured for forming a gas having an oxygen concentration of at least 22 %.

5 According to some embodiments of the invention, the apparatus configured for providing the gas comprises a reservoir of a gas having an oxygen concentration of at no more than 20 %.

According to some embodiments of the invention, the apparatus configured for providing the gas comprises a reservoir of a gas having an oxygen concentration of at least 22 %.

10 According to some embodiments of the invention, the prevalent oxygen partial pressure is an ambient oxygen partial pressure prior to a zeitgeber phase shift associated with the disorder.

According to some embodiments of the invention, the prevalent oxygen partial pressure is about 21 kPa.

15 According to some embodiments of the invention, the circadian rhythm disorder comprises jet lag, and the prevalent oxygen partial pressure is an ambient oxygen partial pressure during air travel associated with the jet lag.

According to some embodiments of the invention, the atmosphere has an oxygen partial pressure that differs from the prevalent oxygen partial pressure by at least 3 kPa.

20 According to some embodiments of the invention, the system is configured for exposing a subject to an atmosphere having an oxygen partial pressure of no more than 16 kPa.

According to some embodiments of the invention, the oxygen partial pressure of the atmosphere is in a range of from 10 kPa to 16 kPa.

25 According to some embodiments of the invention, a pressure of the atmosphere is about equal to an ambient atmospheric pressure.

According to some embodiments of the invention, the system is configured for exposing a subject to an atmosphere having an oxygen partial pressure in a range of from 33 to 100 kPa.

30 According to some embodiments of the invention, the system is further configured for controlling an intensity of light that reaches the eyes of the subject.

According to some embodiments of the invention, the system further comprises a control unit configured for controlling a parameter selected from the group consisting of an oxygen content of the atmosphere, a pressure of the atmosphere and an intensity of light that reaches the eyes of the subject.

5 According to some embodiments of the invention, the system further comprises instructions for controlling a parameter selected from the group consisting of an oxygen content of the atmosphere, a pressure of the atmosphere and an intensity of light which reaches the eyes of the subject, in accordance with a given time of day, the prevalent oxygen partial pressure, and/or a magnitude and/or direction of zeitgeber phase shift.

10 According to some embodiments of the invention, controlling the parameter described herein is determined in accordance with a given time of day, the prevalent oxygen partial pressure, and/or a magnitude and/or direction of zeitgeber phase shift.

According to some embodiments of the invention, controlling is adapted for treating jet lag by exposing a subject to the atmosphere during a pre-determined time of day according to local time at a point of departure of a journey associated with the jet lag, the pre-determined time of day being selected in accordance with a direction of the journey.

15 According to some embodiments of the invention, controlling is adapted for treating jet lag by exposing a subject to the atmosphere during a pre-determined time of day according to local time at a destination of a journey associated with the jet lag.

According to some embodiments of the invention, the pre-determined time of day is during the morning (from midnight to noon).

20 According to some embodiments of the invention, the pre-determined time of day is a time period of no more than 4 hours.

25 According to some embodiments of the invention, controlling is adapted for exposing a subject with a sleep disorder to the atmosphere during a pre-determined time of day selected in accordance with a direction of a desired circadian phase shift.

According to some embodiments of the invention, controlling is adapted for exposing a subject with a sleep disorder to the atmosphere during a pre-determined time period relative to a time of day during which the subject desires to sleep.

30 According to some embodiments of the invention, the treatment comprises exposure to the atmosphere for up to 4 hours.

According to some embodiments of the invention, the oxygen partial pressure of the atmosphere differs from the prevalent oxygen partial pressure by at least 5 kPa.

According to some embodiments of the invention, the oxygen partial pressure of the atmosphere is in a range of from about 10 kPa to about 14 kPa.

5 According to some embodiments of the invention, the treatment comprises exposure to the atmosphere for at least 6 hours.

According to some embodiments of the invention, the oxygen partial pressure of the atmosphere differs from the prevalent oxygen partial pressure by no more than 7 kPa.

10 According to some embodiments of the invention, the oxygen partial pressure of the atmosphere is about 16 kPa.

According to some embodiments of any of the embodiments of the invention relating to an aircraft, the prevalent oxygen partial pressure is an ambient oxygen partial pressure prior to flight of the aircraft.

15 According to some embodiments of any of the embodiments of the invention relating to an aircraft, the prevalent oxygen partial pressure is an ambient oxygen partial pressure in the aircraft cabin.

According to some embodiments of any of the embodiments of the invention relating to an aircraft, a pressure of the atmosphere described herein is about equal to an ambient pressure in the aircraft cabin.

20 According to some embodiments of any of the embodiments of the invention relating to an aircraft, controlling a parameter described herein is adapted for exposing the subject to the atmosphere during a pre-determined time of day according to local time at a point of departure of the aircraft, the pre-determined time of day being selected in accordance with a direction of the flight of the aircraft.

According to some embodiments of any of the embodiments of the invention relating to an aircraft, controlling a parameter described herein is adapted for exposing the subject to the atmosphere during a pre-determined time of day according to local time at a destination of the aircraft.

30 According to some embodiments of any of the embodiments of the invention relating to an aircraft, the change in an oxygen partial pressure described herein is

selected for use in the treatment of jet lag of subjects arriving at a destination of the aircraft.

According to some embodiments of any of the embodiments of the invention relating to an airport terminal, the daily change in an oxygen partial pressure described herein is selected for use in the treatment of jet lag of subjects arriving at the airport terminal following air travel.

According to some embodiments of the invention, the treatment further comprises exposure to a controlled intensity of light.

According to some embodiments of any of the embodiments of the invention relating to a system described herein, the treatment described herein further comprises administration of an effective amount of an agent that modulates an activity of HIF1 α .

According to some embodiments of the invention, the agent described herein is selected from the group consisting of an up-regulator of HIF1 α and a down-regulator of HIF1 α .

According some embodiments of the invention, the up-regulator of HIF1 α is selected from the group consisting of cobalt, dimethyloxalylglycine, desferrioxamine, deferiprone, deferasirox, IOX2, roxadustat (FG4592), and an inhibitor of von Hippel-Lindau tumor suppressor (VHL).

According to some embodiments of any of the embodiments of the invention relating to an agent that modulates an activity of HIF1 α , the agent is selected from the group consisting of cobalt, dimethyloxalylglycine, and an inhibitor of von Hippel-Lindau tumor suppressor (VHL).

According to some embodiments of the invention, the down-regulator of HIF1 α is selected from the group consisting of PX12, BAY87-2243 and KC7F2.

According to some embodiments of any of the embodiments of the invention relating to an agent that modulates an activity of HIF1 α , the treatment further comprises exposure to a hypoxic atmosphere and/or to a hyperoxic atmosphere.

According to some embodiments of the invention, the exposure to the hypoxic atmosphere and/or the hyperoxic atmosphere is for up to 4 hours, following the zeitgeber phase shift.

According to some embodiments of the invention, an oxygen partial pressure of the hypoxic atmosphere is no more than 16 kPa.

According to some embodiments of the invention, an oxygen partial pressure of the hyperoxic atmosphere is in a range of from 33 kPa to 100 kPa.

According to some embodiments of the invention, exposure to a hypoxic atmosphere is for at least 6 hours, prior to and/or during the zeitgeber phase shift.

5 According to some embodiments of the invention, the hypoxic atmosphere and/or the hyperoxic atmosphere is generated by a system described herein.

According to some embodiments of the invention, the circadian rhythm disorder is jet lag and the treatment is effected during a pre-determined time of day according to local time at a point of departure of a journey associated with the jet lag, the pre-determined time of day being selected in accordance with a direction of the journey.

10 According to some embodiments of the invention, the circadian rhythm disorder is jet lag and the treatment is effected during a pre-determined time of day according to local time at a destination of a journey associated with the jet lag.

According to some embodiments of the invention, the circadian rhythm disorder comprises a sleep disorder and the treatment is effected during a pre-determined time of day selected in accordance with a direction of a desired circadian phase shift.

According to some embodiments of the invention, the circadian rhythm disorder comprises a sleep disorder and the treatment is effected during a pre-determined time period relative to a time of day during which the subject desires to sleep.

20 According to some embodiments of the invention, controlling exposure is in accordance with a given time of day and/or a magnitude and/or direction of zeitgeber phase shift.

According to some embodiments of the invention, the composition described herein is formulated as a unit dosage form comprising an amount of the agent that is effective for treating a circadian rhythm disorder.

25 According to some embodiments of the invention, the circadian rhythm disorder is selected from the group consisting of jet lag and shift work sleep disorder.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent

specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

Implementation of the method and/or system of some embodiments of the invention can involve performing or completing selected tasks manually, automatically, or a combination thereof. Moreover, according to actual instrumentation and equipment of some embodiments of the method and/or system of the invention, several selected tasks could be implemented by hardware, by software or by firmware or by a combination thereof using an operating system.

For example, hardware for performing selected tasks according to some embodiments of the invention could be implemented as a chip or a circuit. As software, selected tasks according to some embodiments of the invention could be implemented as a plurality of software instructions being executed by a computer using any suitable operating system. In some embodiments of the invention, one or more tasks according to some embodiments of method and/or system as described herein are performed by a data processor, such as a computing platform for executing a plurality of instructions. Optionally, the data processor includes a volatile memory for storing instructions and/or data and/or a non-volatile storage, for example, a magnetic hard disk and/or removable media, for storing instructions and/or data. Optionally, a network connection is provided as well. A display and/or a user input device such as a keyboard or mouse are optionally provided as well.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A and 1B show oxygen consumption rate (OCR) of mice (in units of mm per hour per kg body weight) over the course of 3 days, presented as a graph showing OCR as a function of time (FIG. 1A) and as a bar graph showing mean $\pm$ standard

deviation OCR of mice during the light and dark phases (all data represent mean of results for 8 mice; light and dark phases depicted as light and shaded columns, respectively; *** indicates $p < 0.001$).

FIG. 2 is a schematic depiction of an oxygen telemetric measuring device used for continuous measurements of oxygen levels in kidney of freely moving animals.

FIG. 3 is a bar graph showing blood oxygen levels of mice during a light-dark cycle as a function of zeitgeber time (ZT), i.e., hours since initiation of light phase (data represent mean $\pm$ standard deviation of 4 mice; light and dark phase depicted as light and shaded column, respectively; oxygen levels shown in units of oxygen partial pressure (mm of mercury), and in units of oxygen percentage in air having said oxygen partial pressure at atmospheric pressure; * indicates $p < 0.05$).

FIG. 4 is a graph showing kidney oxygen levels of a rat over the course of 3 days (gray line represents raw data and black line represents moving average of raw data; light and dark phases depicted as light and shaded columns, respectively; oxygen levels shown in units of oxygen partial pressure, and in units of oxygen percentage in an atmospheric pressure air having said oxygen partial pressure).

FIG. 5 is a phase graph showing the peak time (mean $\pm$ standard deviation) of kidney oxygen levels in each of 5 rats over the course of several consecutive days (light and dark phase depicted as light and shaded column, respectively; zeitgeber time (ZT) represents hours since initiation of light phase).

FIG. 6 is a graph showing the difference between daily zenith and nadir (mean difference $\pm$ standard deviation) of kidney oxygen levels in each of 5 rats over the course of several consecutive days.

FIGs. 7A-7D presents an image of an immunoblot showing levels of HIF1 α , CLOCK, REV-ERB α and PER2 proteins in (wild-type) mouse kidney (FIGs. 7A and 7C) and brain (FIGs. 7B and 7D) at different zeitgeber times (ZT), i.e., hours since initiation of light phase (each time point represent a mix of 4 individual mice; U2AF levels serve as a control; light and dark phases indicated by light and dark bars, respectively).

FIG. 8 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Hif1a*, *Rora*, *Per1*, *Per2*, *Hif2a*, *Cry1*, *Cry2* and *Dbp*) in kidney of (wild-type) mice, as a function of

zeitgeber time (ZT), i.e., hours since initiation of light phase (mice were sacrificed at 4 hour time intervals throughout a daily cycle of light and dark phases, which are depicted as light and shaded column, respectively; data represent mean $\pm$ standard deviation of 3 individual experiments).

FIG. 9 presents a schematic depiction of a chamber with CO₂, O₂ and temperature control (left panel), and representative measurements of O₂ and temperature in the chamber during a cycle of 12 hours of 5 % O₂ and 12 hours of 8 % O₂ (right panel); CO₂ levels were maintained at 5 % throughout the experiment.

FIG. 10 is a schematic depiction of an experimental protocol subjecting cells to constant or rhythmic O₂ levels (arrow indicates sampling starting point, i.e., circadian time (CT) = 0).

FIG. 11 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) in Hepa-1c1c7 cells as a function of circadian time (CT), following exposure to constant or rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 12 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) in NIH3T3 cells as a function of circadian time (CT), following exposure to constant or rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; data represent mean $\pm$ standard deviation of 3 individual experiments).

FIG. 13 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Dbp* and *Hif1 α*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Hif1 α* siRNA (si*Hif1 α*) or control siRNA (siControl), following exposure to rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 14 presents an image of an immunoblot showing levels of CLOCK, REV-ERB α , PER2 and CRY2 proteins in Hepa-1c1c7 cells transfected with *Hif1 α* siRNA

(si*Hif1α*) or control siRNA (siControl), from 0 to 20 hours (in circadian time (CT)) after exposure to rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; tubulin levels serve as a control).

FIG. 15 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Dbp*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Clock* siRNA (si*Clock*) or control siRNA (siControl), and then exposed to a short pulse of dexamethasone (DEX) treatment (cells were cultured in 21 % O₂ and harvested at 4 hour time intervals from 24 to 48 hours after the dexamethasone treatment; data represent mean ± standard deviation of 3 individual experiments)

FIG. 16 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Dbp* and *Hif1α*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Hif1α* siRNA (si*Hif1α*) or control siRNA (siControl), exposed to 8 % O₂ for 2 days and then exposed to a short pulse of dexamethasone (DEX) treatment (cells were harvested at 4 hour time intervals from 24 to 48 hours after the dexamethasone treatment; data represent mean ± standard deviation of 3 experiments).

FIG. 17 is a schematic depiction of an experimental protocol subjecting cells to an oxygen cycle (Cue) (or constant oxygen levels as a control) after 3 days at constant 8 % O₂, in order to minimize the potential effects of cell seeding and/or media change (arrow indicates starting point of sampling and oxygen cycle, i.e., zeitgeber time (ZT) = 0).

FIG. 18 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) in Hepa-1c1c7 cells as a function of zeitgeber time (ZT), during exposure to constant or cyclic (Cue) O₂ levels (cells were harvested at 4 hour time intervals throughout the oxygen cycle, as depicted in FIG. 17; data represent mean ± standard deviation of 3 experiments).

FIG. 19 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Dbp*, *Hif2* and *Hif1α*) in Hepa-1c1c7 cells transfected with *Hif1α* siRNA (si*Hif1α*) or control siRNA (siControl), as a function of zeitgeber time (ZT), i.e., hours

since initiation of a cycle (Cue) in O₂ levels (cells were harvested at 4 hour time intervals throughout the oxygen cycle, as depicted in FIG. 17; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 20 presents bar graphs showing fold change in mRNA expression levels of *Hif1 α* , *Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Glut1*, *Pdk1* and *Ldha* genes in Hepa-1c1c7 cells transfected with *Hif1 α* siRNA (light bars) or control siRNA (dark bars), and harvested before (control) or after exposure to 5 %, 10 %, 15 % or 21 % O₂ for 4 hours (cells were cultured in 21 % O₂ prior to transfection; data are normalized to results for control siRNA and control oxygen exposure, and represent mean $\pm$ standard deviation of 3 experiments).

FIG. 21 presents bar graphs showing fold change in mRNA expression levels of *Rora*, *Cry2*, *Glut1*, *Pdk1* and *Ldha* genes in Hepa-1c1c7 cells cultured under 8 % O₂ for 3 days, upon changing the O₂ concentration from 8 % to 5 % (Δ 3 down, dark bars) or to 11 % (Δ 3 up, dark bars) for 4 hours, or with O₂ concentration remaining at 8 % (light bars) (data are normalized to results for constant 8 % oxygen exposure, and represent mean $\pm$ standard deviation of 3 experiments).

FIG. 22 is a schematic depiction of the *Cry2* gene, showing regions comprising an E-box (CACGTG) and/or hypoxia response element (HRE) (TACGTG), marked as blocks A to F, as well as exon 1 (E1) and exon 2 (E2) (block G is a region devoid of E-box of HRE, used as a negative control in some experiments described herein; bp values indicate location in base pairs relative to exon 1).

FIGs. 23A and 23B are bar graphs showing binding of BMAL1 (FIG. 23A) and HIF1 α (FIG. 23B) to each of chromatin blocks A-G (as identified in FIG. 22) of the *Cry2* gene, as determined by immunoprecipitation of liver chromatin prepared from mice sacrificed at 4 hour intervals (zeitgeber time (ZT) 0, 4, 8, 12, 16 and 20 hours) throughout the day (BMAL1 and HIF1 α binding levels presented as percentage of input; data represent mean $\pm$ standard deviation of 3 individual experiments per group; anti-HIF1 α antibody obtained from Novus Biologicals).

FIGs. 24A-24C are bar graphs showing binding of BMAL1 (FIG. 24A) and HIF1 α (FIGs. 24B and 24C) to each of chromatin blocks A-G (as identified in FIG. 22) of the *Cry2* gene, as determined by immunoprecipitation of kidney chromatin prepared from mice sacrificed at zeitgeber time (ZT) 4 hours (BMAL1 and HIF1 α binding levels

presented as percentage of input; data represent mean $\pm$ standard deviation of 3 individual experiments per group; anti-HIF1 α antibody obtained from R&D Systems (FIG. 24B) or Santa Cruz (FIG. 24C)).

FIGs. 25A and 25B presents a bar graph (FIG. 25A) showing BMAL1 binding to each of chromatin blocks A-G (as identified in FIG. 22) of the *Cry2* gene, as determined by chromatin immunoprecipitation, in Hepa-1c1c7 cells that were cultured for 4 hours either in 5 % O₂ or maintained at 21 % O₂; and a bar graph (FIG. 25) showing *Cry2* mRNA levels in the Hepa-1c1c7 cells (BMAL1 binding levels presented as percentage of input; data represent mean $\pm$ standard deviation of 3 individual experiments per group; N.S. indicates no statistically significant difference).

FIGs. 26A and 26B presents a bar graph (FIG. 26A) showing BMAL1 binding to each of chromatin blocks A-G (as identified in FIG. 22) of the *Cry2* gene, as determined by chromatin immunoprecipitation, in NIH3T3 cells that were cultured for 4 hours either in 1 % O₂ or maintained at 21 % O₂; and a bar graph (FIG. 26B) showing *Cry2* mRNA levels in the NIH3T3 cells (BMAL1 binding levels presented as percentage of input; data represent mean $\pm$ standard deviation of 3 individual experiments per group; N.S. indicates no statistically significant difference).

FIG. 27 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Cry2* siRNA (si*Cry2*) or control siRNA (siControl), following exposure to rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 28 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Dbp*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Cry2* siRNA (si*Cry2*) or control siRNA (siControl) and then cultured under 8 % O₂ for 2 days and then exposed to a short pulse of dexamethasone (DEX) treatment (cells were harvested at 4 hour time intervals from 24 to 48 hours after the dexamethasone treatment; data represent mean $\pm$ standard deviation of 3 individual experiments).

FIG. 29 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Rora* siRNA (si*Rora*) or control siRNA (siControl), following exposure to rhythmic O₂ levels (cells were harvested at 4 hour time intervals throughout the "free running" period, as depicted in FIG. 10; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 30 presents graphs showing fold change (relative to the lowest value) in mRNA expression levels of clock genes (*Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Dbp*) as a function of circadian time (CT) in Hepa-1c1c7 cells transfected with *Rora* siRNA (si*Rora*) or control siRNA (siControl), and then cultured under 8 % O₂ for 2 days and then exposed to a short pulse of dexamethasone (DEX) treatment (cells were harvested at 4 hour time intervals from 24 to 48 hours after the dexamethasone treatment; data represent mean $\pm$ standard deviation of 3 experiments).

FIG. 31 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of wild-type mice housed either under constant 21 % O₂ (left panel) or exposed to cycles of 12 hours of 21 % O₂ and 12 hours of 16 % O₂ (right panel), and subjected to cycles of 12 hours light and 12 hours dark, followed by constant dark (dark periods indicated by shaded area; 12 hour exposure of 16 % O₂ correspond to times of initial 12 hour light periods).

FIG. 32 is a bar graph showing the hours in a circadian period of wild-type mice housed either under constant 21 % O₂ (23.76 ± 0.03 hours) or exposed to cycles of 12 hours of 21 % O₂ and 12 hours of 16 % O₂ (23.73 ± 0.03 hours), under constant darkness (after an initial exposure to cycles of 12 hour light and 12 hour dark, as depicted in FIG. 31, wherein exposure of 16 % O₂ corresponded light periods; data represent mean $\pm$ standard deviation of 18 animals per group; $p = 0.42$ (N.S.)).

FIG. 33 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of wild-type (WT) mice housed either under constant 21 % O₂ (left panel) or exposed to 12 hours of 16 % O₂ (right panel; 16 % O₂ exposure indicated by "12hr"

bars) prior to a 6 hour advance in the lighting schedule (shown by shift in the shaded area, which indicates dark phase).

FIG. 34 is a graph showing the distribution of the number of days needed by individual wild-type mice to adapt to a 6 hour advance in the lighting schedule, when housed either under constant 21 % O₂ (WT) or exposed to 12 hours of 16 % O₂ (WT 12hr 16% O₂) prior to the advance in the lighting schedule (n = 28 for each test group; means ± standard deviation indicated by points with error bar: 7.4 ± 0.2 for constant 21 % O₂ and 5.5 ± 0.3 for 12 hours of 16 % O₂; *** indicates p = 1.82*10⁻⁶).

FIG. 35 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of wild-type mice housed either under constant 21 % O₂ (left panel) or exposed to 2 hours of 14 % O₂ (right panel; 14 % O₂ exposure indicated by "2hr" bars) following a 6 hour advance in the lighting schedule (shown by shift in the shaded area, which indicates dark phase).

FIG. 36 is a graph showing the distribution of the number of days needed by individual wild-type mice to adapt to a 6 hour advance in the lighting schedule, when housed either under constant 21 % O₂ (WT) or exposed to 2 hours of 14 % O₂ (WT 2hr 14% O₂) following the advance in the lighting schedule (n = 16 for each test group; means ± standard deviation indicated by points with error bar: 7.3 ± 0.3 for constant 21 % O₂ and 5.3 ± 0.2 for 2 hours of 14 % O₂; *** indicates p = 6.45*10⁻⁶).

FIG. 37 is a bar graph showing relative levels of *Hif1α* gene transcript in kidney of *Hif1α*^{+/+} and *Hif1α*^{+/-} mice (data represent mean ± standard deviation of 3 individual animals per group, normalized to levels in *Hif1α*^{+/+} mice).

FIG. 38 presents an image of an immunoblot showing relative levels of HIF1α protein in kidney of nuclear protein extracts of *Hif1α*^{+/+} and *Hif1α*^{+/-} mice (each sample represents a mix of 4 individual mice; U2AF levels serve as a control).

FIG. 39 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice subjected to cycles of 12 hours light and 12 hours dark, followed by constant dark (dark periods indicated by shaded area).

FIG. 40 is a bar graph showing the hours in a circadian period of *Hif1α*^{+/+} (23.49 ± 0.1 hours) and *Hif1α*^{+/-} (23.49 ± 0.09 hours) littermate mice under constant darkness (after an initial exposure to cycles of 12 hour light and 12 hour dark, as depicted in FIG. 39; data represent mean ± standard deviation of 16 animals per group; p = 0.51 (N.S.)).

FIG. 41 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice subjected to a 6 hour advance in the lighting schedule (shown by shift in the shaded area, which indicates dark phase).

FIG. 42 is a graph showing the distribution of the number of days needed by individual *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice to adapt to a 6 hour advance in the lighting schedule (n = 15 for each test group; means ± standard deviation indicated by points with error bar: 6.5 ± 0.4 for *Hif1α*^{+/+} and 6.4 ± 0.4 for *Hif1α*^{+/-}; p = 0.9 (N.S.)).

FIG. 43 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice exposed to 12 hours of 16 % O₂ (16 % O₂ exposure indicated by "12hr" bars) prior to a 6 hour advance in the lighting schedule (shown by shift in the shaded area, which indicates dark phase).

FIG. 44 is a graph showing the distribution of the number of days needed by individual *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice to adapt to a 6 hour advance in the lighting schedule, following exposure to 12 hours of 16 % O₂ (n = 15 for each test group; means ± standard deviation indicated by points with error bar: 5.1 ± 0.2 for *Hif1α*^{+/+} and 6.3 ± 0.3 for *Hif1α*^{+/-}; ** indicates p = 0.002).

FIG. 45 presents representative double-plot actograms showing the wheel-running activity over time (delineated by days (y-axis) and zeitgeber time (ZT) of each day (x-axis)) of *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice exposed to 2 hours of 14 % O₂ (14 % O₂ exposure indicated by "2hr" bars) following to a 6 hour advance in the lighting schedule (shown by shift in the shaded area, which indicates dark phase).

FIG. 46 is a graph showing the distribution of the number of days needed by individual *Hif1α*^{+/+} and *Hif1α*^{+/-} littermate mice to adapt to a 6 hour advance in the lighting schedule, prior to exposure to 12 hours of 14 % O₂ (n = 15 for each test group; means ± standard deviation indicated by points with error bar: 4.9 ± 0.2 for *Hif1α*^{+/+} and 6.3 ± 0.2 for *Hif1α*^{+/-}; *** indicates p = 0.0004).

FIG. 47 presents bar graphs showing fold change in mRNA expression levels of *Hif1 α* , *Clock*, *Bmal1*, *Rev-erba*, *Rora*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Ldha* genes in brain of *Hif1 α ^{+/+}* (+/+) and *Hif1 α ^{+/-}* (+/-) littermate mice housed either under constant 21 % O₂ (21%) or exposed to 2 hours of 14 % O₂ (14%), following a 6 hour advance in the lighting schedule (schedules are as depicted in FIGs. 35 and 45; data represent mean $\pm$ standard deviation of 4 individual animals per group, normalized to levels in *Hif1 α ^{+/+}* mice under constant 21 % O₂; * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, N.S. indicates no statistically significant difference).

FIG. 48 is a schematic depiction of a model for circadian clock resetting by oxygen through HIF1 α , wherein clock components that are especially responsive to oxygen levels in a HIF1 α -dependent manner (CRY, ROR) are emphasized.

FIG. 49 is a graph showing the phase shifts (in hours) of circadian rhythms of NIH3T3 cells following a 2 hour exposure to 5 % O₂, as a function of circadian time of the exposure to 5 % O₂ (circadian time determined as hours after replacement of cell medium; positive values of phase shift represent advance in circadian rhythm, negative values represent delay in circadian rhythm).

FIG. 50 is a graph showing the phase shifts (in hours) of circadian rhythms of NIH3T3 cells following a 2 hour exposure to 5 % O₂, as a function of circadian time of the exposure to 5 % O₂ (circadian time determined as hours after replacement of cell medium; positive values of phase shift represent advance in circadian rhythm, negative values represent delay in circadian rhythm; vertical bar at circadian time = 17.5 hours indicates that disruption of circadian rhythm prevented accurate characterization of phase shift).

FIG. 51 is a schematic depiction of a system according to some embodiments of the invention.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy, and more particularly, but not exclusively, to systems, methods and compositions for treating a circadian rhythm disorder.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set

forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

The present inventors have uncovered that changes in ambient oxygen levels surprisingly regulate circadian rhythms in a potent and controllable manner, and that this regulation is effected via HIF1 α activity. The inventors have further envisioned that such regulation of circadian rhythms can be useful for treating circadian rhythm disorders such as jet lag.

While reducing the present invention to practice, the inventors have accelerated the adaptation of mice to changes in lighting schedule in a model of jet lag, by temporarily exposing the mice to hypoxic atmospheres. The inventors have further shown that the accelerated adaptation is mediated by HIF1 α activity.

Referring now to the drawings, FIGs. 1A-6 show that oxygen levels in mice oscillate daily, with peak oxygen levels in blood and kidney coinciding with elevated oxygen consumption rates. As shown in FIGs. 7A-7D, HIF1 α nuclear protein levels also oscillate daily.

As shown in FIGs. 11-14 and 18-26B, expression and protein levels of clock genes were relatively constant in cells *in vitro* under constant oxygen levels, whereas exposure of the cells to physiological oxygen rhythms resulted in rhythmic expression of clock genes, characteristic of synchronized clocks in cells, and the rhythmic expression was HIF1 α -dependent.

The above results indicate that physiological oxygen rhythms reset the molecular clock in cultured cells in a HIF1 α -dependent manner.

As shown in FIGs. 31-32, exposure to rhythmic oxygen levels did not alter the circadian rhythm of mice *per se*. However, as shown in FIG. 33-36, exposure of mice to a hypoxic atmosphere before or after a change in lighting schedule (in an *in vivo* model of jet lag) accelerated the adaptation of mice to a new lighting schedule. As shown in FIGs. 37-46, HIF1 α -deficiency in mice did not alter circadian rhythms or normal adaptation to changes in the lighting schedule under normal conditions, but HIF1 α -deficiency inhibited hypoxia-induced acceleration of adaptation to changes in the lighting schedule. As shown in FIG. 47, HIF1 α -deficiency inhibited hypoxia-induced regulation of expression of the clock genes *Cry2*, *Per1* and *Cry1*.

The above results suggest a role of oxygen rhythms and HIF1 α on circadian clocks as depicted schematically in FIG. 48.

As shown in FIGs. 49 and 50, hypoxia induced advances and delays of various magnitudes in the circadian clock of cells, with a clear dependence on the phase of the circadian rhythm during which exposure to hypoxia occurs.

These results indicate that oxygen levels can modulate circadian rhythms in a controllable manner.

Embodiments of the present invention therefore relate to systems and methods usable in treating a circadian rhythm disorder, which are based on modulating the oxygen level to which a subject is exposed.

Embodiments of the present invention further relate to compositions, kits and methods usable in treating a circadian rhythm disorder, which are based on administering to a subject an agent that modulates HIF1 α activity.

Treatment by oxygen modulation:

According to an aspect of some embodiments of the invention, there is provided a method of treating a circadian rhythm disorder, the method comprising exposing a subject in need thereof to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa.

As used herein, the terms "treat", "treating" and "treatment" include inhibiting, preventing or arresting the development (e.g., appearance and/or progression) of a condition and/or clinical or aesthetical symptoms of a condition, enhancing a rate of recovery from a condition, and/or substantially ameliorating clinical or aesthetical symptoms of a condition. The terms "treat", "treating" and "treatment" should not be interpreted as implying that a treated condition (e.g., according to any of the respective embodiments described herein) is a pathological condition. For example, the condition may be a natural and/or transient result of external factors affecting a healthy individual.

In the context of some embodiments of the present invention, the treated condition is a circadian rhythm disorder, and the symptom of the condition which is treated (e.g., by inhibiting, preventing or arresting its development, and/or substantially ameliorating it) is a sleep disturbance (e.g., trouble falling asleep at a desired time, trouble remaining asleep during a desired time period, and/or trouble remaining awake

during a desired time period), poor performance on mental tasks and/or poor concentration, increased fatigue, headaches, irritability, abnormal digestion (e.g., indigestion, changes in frequency of defecation and/or consistency of feces), and/or reduced interest in and/or enjoyment of food.

5 As used herein, the term "preventing" refers to keeping a condition (e.g., disorder) from occurring in a subject who may be at risk for the condition, but has not yet been diagnosed as having the condition.

 In the context of some embodiments of the present invention, preventing a circadian rhythm disorder comprises inhibiting and/or preventing the development of a
10 symptom of the circadian rhythm disorder (e.g., any symptom of such a disorder described herein) in a subject who has not yet experienced such a symptom, for example, a traveling subject who has not yet experienced a symptom of jet lag, and optionally a subject who has not yet travelled sufficiently to develop jet lag, but who expects to do so in the near future (e.g., during a travel). In some embodiments,
15 preventing a circadian rhythm disorder comprises inhibiting and/or preventing the development of a jet lag in a travelling subject, before the subject experiences a jet lag.

 As used herein, the term "subject" includes mammals, preferably human beings at any age afflicted by a condition described herein. Preferably, this term encompasses individuals who are at risk to develop the condition, for example, travelers (e.g.,
20 humans and animals) at risk to develop jet lag, and night shift workers at risk to develop shift work sleep disorder.

 Herein, the term "prevalent oxygen partial pressure" refers to an oxygen partial pressure of an atmosphere (other than the atmosphere for effecting a treatment described herein) to which a subject using the system is known to be or expected to be exposed to,
25 for example, an ambient oxygen partial pressure in a vicinity of the system and/or an ambient oxygen partial pressure at a location in which the subject has been recently or is expected to be in the near future.

 In the context of some embodiments of the present invention, the prevalent oxygen partial pressure refers to an oxygen partial pressure of an atmosphere (other than
30 the atmosphere for effecting a treatment described herein) to which a traveling subject has been recently exposed to (e.g., at a departure location, in an aircraft, and/or at a

destination), or is expected to be exposed to (e.g., in an aircraft prior to travel on the aircraft, and/or at a destination prior to arrival thereat).

Herein, the term "partial pressure" refers to a hypothetical pressure of an indicated gas (e.g., oxygen) if that gas alone occupied an indicated volume (e.g., a volume occupied by a mixture of gases). A partial pressure may optionally be determined experimentally by removing all other gases from the volume. Alternatively, a partial pressure may optionally be determined as atmospheric pressure in an indicated volume multiplied by a mole fraction of the indicated gas (the mole fraction being less than 100 % in a mixture of gases, and 100 % in a pure state), the mole fraction being determined according to any suitable technique known in the art.

In the context of some embodiments of the present invention, an oxygen partial pressure refers to a mole fraction of oxygen in an atmosphere multiplied by the pressure of the atmosphere (e.g., a pressure of about 1 atmosphere at sea level).

In the context of some embodiments of the present invention, an oxygen partial pressure refers to a mole fraction of oxygen in air (about 21 %) multiplied by the pressure of the atmosphere.

Herein, the term "atmospheric pressure" refers to a pressure exerted by a gaseous environment, such as air or a similar gas. Atmospheric pressure may be determined by any suitable technique known in the art, such as by a barometer.

For example, in some embodiments of any of the embodiments described herein relating to treatment of jet lag, the prevalent oxygen partial pressure is an ambient oxygen partial pressure during air travel, such as an aircraft cabin pressure during a flight (e.g., at cruising altitude).

Alternatively, in some embodiments of any of the embodiments described herein, the prevalent oxygen partial pressure is an ambient oxygen partial pressure prior to a cause of a circadian rhythm disorder (e.g., prior to a phase shift, such as an aircraft flight to a different time zone, according to any of the respective embodiments described herein). For example, the prevalent oxygen partial pressure may optionally be an average ambient oxygen partial pressure to which a subject has been exposed in the prior 24 hours and/or the prior week or month. Alternatively or additionally, the prevalent oxygen partial pressure may optionally be an ambient oxygen partial pressure at a point

of departure of a subject travelling by air (e.g., in embodiments wherein the circadian rhythm disorder is jet lag).

An atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa (according to any of the respective
5 embodiments described herein) may be, for example, a hypoxic atmosphere, i.e., having an oxygen partial pressure which is at least 1 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere having an oxygen partial pressure of 20 kPa or less); or alternatively, a hyperoxic atmosphere, i.e., having an oxygen partial pressure which is at least 1 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic
10 atmosphere having an oxygen partial pressure of at least 22 kPa).

Herein throughout, the term "hypoxic" refers to an oxygen partial pressure lower than a prevalent oxygen partial pressure (e.g., by at least 1 kPa), and the term "hyperoxic" refers to an oxygen partial pressure higher than a prevalent oxygen partial pressure (e.g., by at least 1 kPa), according to any of the respective embodiments
15 described herein.

In some embodiments of any of the embodiments described herein, the atmosphere to which a subject is exposed has an oxygen partial pressure that differs from a prevalent oxygen partial pressure by at least 2 kPa. In some such embodiments, the atmosphere is a hypoxic atmosphere, i.e., having an oxygen partial pressure that is at
20 least 2 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere having an oxygen partial pressure of 19 kPa or less). In some embodiments, the atmosphere is a hyperoxic atmosphere, i.e., having an oxygen partial pressure that is at least 2 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 23 kPa).

25 In some embodiments of any of the embodiments described herein, the atmosphere to which a subject is exposed has an oxygen partial pressure that differs from a prevalent oxygen partial pressure by at least 3 kPa. In some such embodiments, the atmosphere is a hypoxic atmosphere, i.e., having an oxygen partial pressure that is at least 3 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere
30 having an oxygen partial pressure of 18 kPa or less). In some embodiments, the atmosphere is a hyperoxic atmosphere, i.e., having an oxygen partial pressure that is at

least 3 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 24 kPa).

In some embodiments of any of the embodiments described herein, the atmosphere to which a subject is exposed has an oxygen partial pressure that differs from a prevalent oxygen partial pressure by at least 5 kPa. In some such embodiments, the atmosphere is a hypoxic atmosphere, i.e., having an oxygen partial pressure that is at least 5 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere having an oxygen partial pressure of 16 kPa or less). In some embodiments, the atmosphere is a hyperoxic atmosphere, i.e., having an oxygen partial pressure that is at least 5 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 26 kPa).

In some embodiments of any of the embodiments described herein, the atmosphere to which a subject is exposed has an oxygen partial pressure that differs from a prevalent oxygen partial pressure by at least 7 kPa. In some such embodiments, the atmosphere is a hypoxic atmosphere, i.e., having an oxygen partial pressure that is at least 7 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere having an oxygen partial pressure of 14 kPa or less). In some embodiments, the atmosphere is a hyperoxic atmosphere, i.e., having an oxygen partial pressure that is at least 7 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 28 kPa).

In some embodiments of any of the embodiments described herein, the atmosphere to which a subject is exposed has an oxygen partial pressure that differs from a prevalent oxygen partial pressure by at least 9 kPa. In some such embodiments, the atmosphere is a hypoxic atmosphere, i.e., having an oxygen partial pressure that is at least 9 kPa less than a prevalent oxygen partial pressure (e.g., a hypoxic atmosphere having an oxygen partial pressure of 12 kPa or less). In some embodiments, the atmosphere is a hyperoxic atmosphere, i.e., having an oxygen partial pressure that is at least 9 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 30 kPa). In some embodiments, the hyperoxic atmosphere has an oxygen partial pressure that is at least 12 kPa more than a prevalent oxygen partial pressure (e.g., a hyperoxic atmosphere having an oxygen partial pressure of at least 33 kPa).

In some embodiments of any of the embodiments described herein, a prevalent oxygen partial pressure (according to any of the respective embodiments described herein) is about 21 kPa, e.g., such that an oxygen partial pressure differing from the prevalent oxygen partial pressure by at least 1 kPa (according to any of the respective
5 embodiments described herein) is at least about 22 kPa or no more than about 20 kPa.

In some embodiments of any of the embodiments described herein relating to an atmosphere having a low oxygen partial pressure relative to a prevalent oxygen partial pressure (e.g., wherein the prevalent oxygen partial pressure is about 21 kPa), the atmosphere has an oxygen partial pressure of no more than 20 kPa (e.g., in a range of
10 from 10 kPa to 20 kPa). In some embodiments, the oxygen partial pressure is no more than 19 kPa (e.g., in a range of from 10 kPa to 19 kPa). In some embodiments, the oxygen partial pressure is no more than 18 kPa (e.g., in a range of from 10 kPa to 18 kPa). In some embodiments, the oxygen partial pressure is no more than 16 kPa (e.g., in a range of from 10 kPa to 16 kPa). In some embodiments, the oxygen partial pressure is
15 no more than 14 kPa (e.g., in a range of from 10 kPa to 14 kPa). In some embodiments, the oxygen partial pressure is no more than 12 kPa (e.g., in a range of from 10 kPa to 12 kPa).

In some embodiments of any of the embodiments described herein relating to an atmosphere having a relatively low oxygen partial pressure (e.g., wherein the prevalent
20 oxygen partial pressure is about 21 kPa), the atmosphere has an oxygen partial pressure of at least 10 kPa, for example, in a range of from 10 kPa to 20 kPa, from 10 kPa to 18 kPa, from 10 kPa to 16 kPa, from 10 kPa to 14 kPa and/or from 10 kPa to 12 kPa. In some embodiments, the oxygen partial pressure is at least 12 kPa, for example, in a range of from 12 kPa to 20 kPa, from 12 kPa to 18 kPa, from 12 kPa to 16 kPa, and/or
25 from 12 kPa to 14 kPa. In some embodiments, the oxygen partial pressure is at least 14 kPa, for example, in a range of from 14 kPa to 20 kPa, from 14 kPa to 18 kPa, and/or from 14 kPa to 16 kPa.

In some embodiments of any of the embodiments described herein relating to an atmosphere having a relatively high oxygen partial pressure (e.g., wherein the prevalent
30 oxygen partial pressure is about 21 kPa), the atmosphere has an oxygen partial pressure of at least 22 kPa. In some embodiments, the oxygen partial pressure is at least 23 kPa. In some embodiments, the oxygen partial pressure is at least 24 kPa. In some

embodiments, the oxygen partial pressure is at least 26 kPa. In some embodiments, the oxygen partial pressure is at least 28 kPa. In some embodiments, the oxygen partial pressure is at least 30 %. In some embodiments, the oxygen partial pressure is at least 33 kPa. In some embodiments, the oxygen partial pressure is at least 40 kPa. In some
5 embodiments, the oxygen partial pressure is at least 50 kPa. In some embodiments, the oxygen partial pressure is at least 60 kPa. In some embodiments, the oxygen partial pressure is at least 70 kPa. In some embodiments, the oxygen partial pressure is at least 80 kPa. In some embodiments, the atmosphere is about 100 kPa oxygen.

In some embodiments of any of the embodiments described herein relating to an
10 atmosphere having a relatively high oxygen partial pressure (e.g., wherein the prevalent oxygen partial pressure is about 21 kPa), the atmosphere has an oxygen partial pressure of no more than 80 kPa, for example, in a range of from 22 kPa to 80 kPa, from 24 kPa to 80 kPa, from 26 kPa to 80 kPa, from 28 kPa to 80 kPa, from 30 kPa to 80 kPa, from 33 kPa to 80 kPa, from 40 kPa to 80 kPa, from 50 kPa to 80 kPa, and/or from 60 kPa to
15 80 kPa. In some embodiments, the oxygen partial pressure is no more than 60 kPa, for example, in a range of from 22 kPa to 60 kPa, from 24 kPa to 60 kPa, from 26 kPa to 60 kPa, from 28 kPa to 60 kPa, from 30 kPa to 60 kPa, from 33 kPa to 60 kPa, from 40 kPa to 60 kPa, and/or from 50 kPa to 60 kPa. In some embodiments, the oxygen partial pressure is no more than 50 kPa, for example, in a range of from 22 kPa to 50 kPa, from
20 24 kPa to 50 kPa, from 26 kPa to 50 kPa, from 28 kPa to 50 kPa, from 30 kPa to 50 kPa, from 33 kPa to 50 kPa, and/or from 40 kPa to 50 kPa. In some embodiments, the oxygen partial pressure is no more than 40 kPa, for example, in a range of from 22 kPa to 40 kPa, from 24 kPa to 40 kPa, from 26 kPa to 40 kPa, from 28 kPa to 40 kPa, from 30 kPa to 40 kPa, and/or from 33 kPa to 40 kPa. In some embodiments, the oxygen
25 partial pressure is no more than 30 kPa, for example, in a range of from 22 kPa to 30 kPa, from 24 kPa to 30 kPa, from 26 kPa to 30 kPa, and/or from 28 kPa to 30 kPa.

Although pressures are generally defined herein in Pa (pascal) units, the skilled person will appreciate that other units may be used to define equivalent pressures.

In some embodiments of any of the embodiments described herein, a pressure of
30 the atmosphere to which a subject is exposed is about equal to an ambient atmospheric pressure. Such an atmosphere may differ from an ambient atmosphere (e.g., of about 21 % O₂), for example, in a concentration of oxygen therein.

Without being bound by any particular theory, it is believed that exposure of a subject to an atmosphere having a pressure about equal to an ambient atmospheric pressure may in many cases be more convenient than exposure of the subject to an atmosphere at non-ambient pressure.

5 Herein (in accordance with common practice in the art), percentages of a gas (e.g., O₂) in the atmosphere refer to a mole fraction of the gas in the atmosphere.

Herein (in accordance with common practice in the art), compositions of atmospheres and percentages of a gas (e.g., O₂) in the atmosphere refer to a dry composition of the atmosphere, that is, water vapor therein is not considered as a portion
10 of the atmosphere.

In some embodiments of any of the embodiments described herein relating to an atmosphere having a low oxygen partial pressure relative to a prevalent oxygen partial pressure (e.g., at a pressure about equal to an ambient atmospheric pressure), the atmosphere has an oxygen concentration of no more than 20 % (e.g., in a range of from
15 10 % to 20 %). In some embodiments, the oxygen concentration is no more than 19 % (e.g., in a range of from 10 % to 19 %). In some embodiments, the oxygen concentration is no more than 18 % (e.g., in a range of from 10 % to 18 %). In some embodiments, the oxygen concentration is no more than 16 % (e.g., in a range of from 10 % to 16 %). In some embodiments, the oxygen concentration is no more than 14 %
20 (e.g., in a range of from 10 % to 14 %). In some embodiments, the oxygen concentration is no more than 12 % (e.g., in a range of from 10 % to 12 %).

In some embodiments of any of the embodiments described herein relating to an atmosphere having a relatively low oxygen partial pressure (e.g., at a pressure about equal to an ambient atmospheric pressure), the atmosphere has an oxygen concentration
25 of at least 10 %, for example, in a range of from 10 % to 20 %, from 10 % to 18 %, from 10 % to 16 %, from 10 % to 14 % and/or from 10 % to 12 %. In some embodiments, the atmosphere has an oxygen concentration of at least 12 %, for example, in a range of from 12 % to 20 %, from 12 % to 18 %, from 12 % to 16 %, and/or from 12 % to 14 %. In some embodiments, the atmosphere has an oxygen concentration of at least 14 %, for
30 example, in a range of from 14 % to 20 %, from 14 % to 18 %, and/or from 14 % to 16 %.

In some embodiments of any of the embodiments described herein relating to an atmosphere having a relatively high oxygen partial pressure (e.g., at a pressure about equal to an ambient atmospheric pressure), the atmosphere has an oxygen concentration of at least 22 %. In some embodiments, the oxygen concentration is at least 23 %. In some embodiments, the oxygen concentration is at least 24 %. In some embodiments, the oxygen concentration is at least 26 %. In some embodiments, the oxygen concentration is at least 28 %. In some embodiments, the oxygen concentration is at least 30 %. In some embodiments, the oxygen concentration is at least 33 %. In some embodiments, the oxygen concentration is at least 40 %. In some embodiments, the oxygen concentration is at least 50 %. In some embodiments, the oxygen concentration is at least 60 %. In some embodiments, the oxygen concentration is at least 70 %. In some embodiments, the oxygen concentration is at least 80 %. In some embodiments, the atmosphere is about 100 % oxygen.

In some embodiments of any of the embodiments described herein relating to an atmosphere having a relatively high oxygen partial pressure (e.g., at a pressure about equal to an ambient atmospheric pressure), the atmosphere has an oxygen concentration of no more than 80 %, for example, in a range of from 22 % to 80 %, from 24 % to 80 %, from 26 % to 80 %, from 28 % to 80 %, from 30 % to 80 %, from 33 % to 80 %, from 40 % to 80 %, from 50 % to 80 %, and/or from 60 % to 80 %. In some embodiments, the atmosphere has an oxygen concentration of no more than 50 %, for example, in a range of from 22 % to 50 %, from 24 % to 50 %, from 26 % to 50 %, from 28 % to 50 %, from 30 % to 50 %, from 33 % to 50 %, and/or from 40 % to 50 %. In some embodiments, the atmosphere has an oxygen concentration of no more than 40 %, for example, in a range of from 22 % to 40 %, from 24 % to 40 %, from 26 % to 40 %, from 28 % to 40 %, from 30 % to 40 %, and/or from 33 % to 40 %. In some embodiments, the atmosphere has an oxygen concentration of no more than 30 %, for example, in a range of from 22 % to 30 %, from 24 % to 30 %, from 26 % to 30 %, and/or from 28 % to 30 %.

In some embodiments of any of the embodiments described herein, the treatment further comprises controlling an intensity of light that reaches the eyes of the subject, for example, by increasing and/or decreasing an intensity of light. The modulation of circadian rhythms by light and/or darkness, and the treatment of a circadian rhythm

disorder by an appropriate time and/or degree of exposure to light and/or darkness has been extensively studied, and the skilled person will be readily capable of combining a suitable control over an intensity of light which reaches the eyes of a given subject with exposure to a suitable atmosphere according to any of the respective embodiments described herein.

In some embodiments of any of the embodiments described herein, the treatment further comprises administration of an effective amount of an agent capable of modulating an activity of HIF α , according to any of the respective embodiments described herein.

Circadian rhythm disorder:

Herein and in the art, the term "circadian rhythm" refers to any biological process that displays an endogenous oscillation of about 24 hours. Although a circadian rhythm is an endogenous process, it is to be appreciated that it may be affected by external cues.

Herein, the phrase "circadian rhythm disorder" refers to disorders wherein an afflicted subject's circadian rhythm (e.g., a rhythm of being inclined to sleep and be awake) does not accord with a desired daily schedule, for example, a typical daily schedule in which one sleeps at night and is awake during the day.

In some embodiment of any of the respective embodiments described herein, the circadian rhythm disorder comprises a sleep disorder, that is, it is characterized (at least in part) by a deleterious sleep pattern, for example, excessive sleepiness during the day and/or insomnia (e.g., at night).

Excessive sleepiness according to any of the respective embodiments described herein may be manifested, for example, as desire to nap, unintended dozing, impaired mental acuity, irritability, reduced performance, and/or accident proneness.

Circadian rhythm disorders may be non-pathological, e.g., transient conditions which may occur in healthy individuals due to extrinsic factors, such as travel and/or lifestyle factors (e.g., changes in sleep schedule).

Examples of non-pathological circadian rhythm disorders include, without limitation, jet lag and shift work sleep disorder.

Herein, the term "jet lag" refers to a disorder wherein a subject travels to a different time zone, and the subject's circadian rhythm does not accord with a local time in the destination.

Herein, the phrase "shift work sleep disorder" refers to a sleep disorder associated with adoption of a new sleep schedule; for example, wherein an abnormally high proportion of a subject's sleep is scheduled for daytime and/or abnormally high proportion of a subject's waking hours are scheduled for nighttime. A new sleep schedule may optionally be a normal sleep schedule adopted by a subject previously on an abnormal sleep schedule. Adoption of a new (e.g., abnormal) sleep schedule may optionally be due to work, for example, beginning or ceasing to work a night shift. However, the adoption of a new sleep schedule may be for any reason, and the term "shift work" in the phrase "shift work sleep disorder" is not intended to be limiting. Symptoms of shift works sleep disorder include, for example, excessive sleepiness during waking hours (according to the adopted sleep schedule), and decrease in sleep duration and/or quality (e.g., during daytime).

In some embodiment of any of the respective embodiments described herein, the circadian rhythm disorder is associated with a zeitgeber phase shift, as defined herein.

Herein and in the art, the term "zeitgeber" refers to an external cue, such as light, temperature, social interactions, exercise and/or eating/drinking patterns, which typically follow a daily cyclic pattern and affect a circadian rhythm, for example, by synchronizing an internal circadian rhythm to an external daily cycle (e.g., day/night).

Thus, a zeitgeber may be regarded as a part of a subject's environment, as opposed to a circadian rhythm, which refers to an internal biological process.

Herein, the phrase "zeitgeber phase shift" refers to an abrupt shift in schedule of one or more zeitgebers, as defined herein. For example, the difference between local time of different time zones (e.g., point of departure and destination associated with air travel) may be regarded as a zeitgeber phase shift (e.g., involving a shift in at least light/dark cycles) associated with jet lag; and a difference between a new sleep schedule and a previous sleep schedule may be regarded as a zeitgeber phase shift (e.g., involving a shift in social interactions, exercise and/or eating/drinking patterns) associated with, e.g., shift work sleep disorder.

A zeitgeber phase shift may induce a circadian rhythm disorder, for example, by resulting in a lack of synchronization between a subject's circadian rhythm and a zeitgeber cycle. Such a lack of synchronization can be undesirable *per se*, for example, when a subject becomes sleepy at times which are undesirable for social reasons (e.g.,

during the day, or during a night work shift). Additionally or alternatively, such a lack of synchronization may generate discomfort as the circadian rhythm undergoes a gradual phase shift in order to re-synchronize with zeitgebers. For example, different parts of the body may undergo circadian phase shift at different rates, resulting in an abnormal physiological state associated with desynchronization between different parts of the body.

A zeitgeber phase shift may be, for example, a delay of up to 12 hours or an advance of up to 12 hours. For example, an advance of 14 hours may optionally be considered equivalent to a delay of 10 hours, under the assumption that a circadian rhythm comprises a 24 hour cycle.

In some embodiment of any of the respective embodiments described herein, the circadian rhythm disorder is associated with an advance in zeitgeber phase, that is, a zeitgeber occurring earlier than usual, for example, jet lag associated with eastward travel.

Without being bound by any particular theory, it is believed that advances in zeitgeber phase (e.g., jet lag associated with eastward travel) are associated with more severe symptoms than are delays in zeitgeber phase, and thus especially advantageous to treat.

System:

A method as described herein in any of the respective embodiments can be executed by a system configured to expose a subject to a modulated oxygen level. In some embodiments, such a system is configured to provide a modulated oxygen level and optionally comprises means for exposing the subject to the modulated oxygen level. In some embodiments, the system is further configured for determining a desired modulation of the oxygen level (and optionally comprises means for determining a desired modulation of the oxygen level), e.g., based on a time of day according to any of the respective embodiments described herein. In some of any of the embodiments described herein, modulating oxygen level comprises modulating a partial oxygen pressure in an environment adjacent to a subject or to a breathing orifice of the subject, for example, within a breathing compartment, according to any of the respective embodiments described herein.

According to an aspect of some embodiments of the invention, there is provided a system configured for exposing a subject to an atmosphere having a modulated oxygen partial pressure.

Referring now to the drawings, FIG. 51 illustrates a system **10**, comprising a compartment **20** (also referred to herein as a “breathing compartment”) configured for exposing a subject to an atmosphere (e.g., an atmosphere having a modulated oxygen partial pressure) and an optional apparatus **30** configured for modulating an oxygen partial pressure of an atmosphere in compartment **20**.

According to an aspect of some embodiments of the invention, there is provided a system **10** configured for exposing a subject to an atmosphere (in breathing compartment **20**) having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa (according to any of the embodiments described herein relating to such an atmosphere), optionally provided by apparatus **30**. In some embodiments of any of the embodiments according to this aspect, the system **10** is for use in the treatment of a circadian rhythm disorder (e.g., a circadian rhythm disorder according to any of the respective embodiments described herein).

In some embodiments of any of the embodiments described herein, the system **10** comprises a breathing compartment **20** which is configured for exposing the subject to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa (according to any of the respective embodiments described herein), and an apparatus **30** configured for providing (to the breathing compartment) a gas selected to produce the atmosphere (having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa) in the breathing compartment **20**.

Herein, the phrase “breathing compartment” encompasses enclosed and semi-enclosed spaces of any shape or size configured to allow a subject to breathe an atmosphere within the compartment.

In some embodiments, apparatus **30** is in fluid communication with breathing compartment **20** via fluid connection **40**, for example, a pipe, valve and/or simple opening (e.g., in a wall of compartment **20** and/or apparatus **30**).

In some embodiments, breathing compartment **20** is configured such that a subject is exposed to a gas (suitable for forming a hypoxic and/or hyperoxic atmosphere)

generated by apparatus **30**. In such an embodiment, fluid connection **40** may optionally be configured as an inlet for compartment **20** (e.g., for inletting a gas for generating a hypoxic and/or hyperoxic atmosphere) and/or as an outlet for apparatus **30**.

Alternatively or additionally, breathing compartment **20** is configured such that a subject is exposed to an atmospheric pressure lower than that of a surrounding atmosphere. In such an embodiment, fluid connection **40** may optionally be configured as an outlet for compartment **20** (e.g., for removing gas from compartment **20**, thereby lowering an atmospheric pressure therein) and/or as an inlet for apparatus **30** (e.g., a pump).

Examples of breathing compartments (which may optionally serve as compartment **20**) include compartments sufficiently large to allow a subject to reside therein, such as a compartment **20** in a form of a building, a room, a tent, or the like; as well as smaller compartments such as a compartment **20** in a form of a mask (full or partial facial mask), a mouthpiece, a helmet, or the like.

A large breathing compartment **20** (according to any of the respective embodiments described herein) may optionally be adapted for home use (e.g., in one or more room of a subject's residence) and/or in a location at which afflicted subjects may be expected, for example, an airport terminal in embodiments relating to jet lag (e.g., according to any of the respective embodiments described herein), or at a facility (e.g., office or factory) with night shift workers in embodiments relating to shift work sleep disorder (e.g., according to any of the respective embodiments described herein). Such breathing compartments may optionally be identified for use (e.g., for "refreshment") shortly prior to and/or subsequent to an aircraft flight or night shift according to any of the respective embodiments described herein.

In some embodiments of any of the embodiments described herein, the breathing compartment **20** (e.g., a small compartment such as a mask, mouthpiece, and/or helmet described herein) is configured for delivering an atmosphere to a breathing orifice of a subject. In some such embodiments, the system **10** is portable.

In some embodiments of any of the embodiments described herein, the breathing compartment **20** is a closed space, for example, a large breathing compartment (e.g., a building, a room, and/or a tent) for which being closed facilitates control over the

voluminous atmosphere therein. In some such embodiments, the system **10** is portable, e.g., wherein a breathing compartment **20** is in a form of a foldable tent.

A system **10** (according to any of the respective embodiments described herein) may optionally comprise one or more breathing compartment **20** (according to any of the
5 respective embodiments described herein).

In addition, a system **10** (according to any of the respective embodiments described herein) may optionally comprise components outside of a breathing compartment(s) **20** (e.g., an apparatus **30** comprised by the system **10** according to any of the respective embodiments described herein), or alternatively, substantially all of the
10 components of the system **10** may be comprised within the space of the breathing compartment(s) **20**.

A gas provided by the abovementioned apparatus **30** may be, for example, substantially identical in content to the atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa according to any
15 of the respective embodiments described herein (e.g., wherein the breathing compartment **20** is sufficiently small and/or closed such that substantially all of the atmosphere therein consists of the provided gas); or alternatively, the gas may be selected such that mixture of the gas with another gas (e.g., air and/or a gas present in the breathing compartment **20**, especially wherein the breathing compartment **20** is
20 relatively large and/or open) results in the desired atmosphere in the breathing compartment **20** (e.g., at a given rate of provision of the gas to the breathing compartment **20**).

For example, a highly oxygen-enriched gas (e.g., 100 % O₂) may optionally be provided to a breathing compartment **20** at a rate which results in a moderately oxygen-enriched atmosphere according to any of the respective embodiments described herein
25 (e.g., 33 % O₂) in the breathing compartment **20**; and a very oxygen-poor gas (e.g., an oxygen-free gas, such as nitrogen) may optionally be provided to a breathing compartment **20** at a rate which results in a moderately oxygen-poor atmosphere according to any of the respective embodiments described herein (e.g., 10-16 % O₂) in
30 the breathing compartment **20**.

Any apparatus known in the art which is capable of providing a suitable gas according to any of the respective embodiments described herein, for example an

oxygen-enriched gas (e.g., having an oxygen concentration of at least 22 %) and/or an oxygen-poor gas (e.g., having an oxygen concentration of at least 22 %), may optionally be used as apparatus **30**. Examples of such apparatuses include, without limitation, apparatuses comprising a gas separation device (e.g., configured for effecting gas separation by pressure swing adsorption, vacuum swing adsorption, and/or membrane gas separation), a reservoir of a gas (e.g., a gas which differs from the ambient atmosphere), and/or a chemical oxygen generator (e.g., for providing an oxygen-enriched gas).

An oxygen-enriched gas according to any of the respective embodiments described herein may optionally be formed by adding oxygen to an ambient atmosphere (e.g., from a reservoir of oxygen gas or from a chemical oxygen generator) and/or by concentration of oxygen from the ambient atmosphere (e.g., by gas separation).

An oxygen-poor gas according to any of the respective embodiments described herein may optionally be formed by adding a gas such as nitrogen or argon to an ambient atmosphere (e.g., from a reservoir of nitrogen or argon), by concentration of nitrogen from the ambient atmosphere (e.g., by gas separation), and/or by removal of oxygen from the ambient atmosphere (e.g., by gas separation and/or by a chemical reaction, such as oxidation).

In some embodiments of any of the respective embodiments described herein, the system **10** includes, or is configured to be combined with, a gas reservoir (according to any of the respective embodiments described herein) in a form of a gas balloon (e.g., a portable gas balloon), which may optionally be replaced by a similar gas balloon as needed (e.g., upon depletion). The gas balloon may be one available from a commercial source for use in other types of systems and applications (e.g., for research, medical use, etc.).

In some embodiments of any of the embodiments described herein relating to a system (according to any of the aspects described herein), the system **10** is configured for controlling an intensity of light which reaches the eyes of a subject (e.g., according to any of the respective embodiments described herein). Control is optionally effected by a control unit according to any of the respective embodiments described herein.

In a system **10** with a large breathing compartment **20** (e.g., configured for allowing one or more subject to reside therein), control over intensity of light may

optionally be effected by a controllable lighting system for illuminating the breathing compartment **20**, and/or blocking of light from external sources (e.g., by opaque walls).

In a system **10** with a smaller breathing compartment **20** (e.g., a mask, mouthpiece and/or helmet), control over intensity of light may optionally be effected by a controllable light source configured to be positioned near a subject's eye (e.g., a relatively low-power light source), and/or by a device configured for blocking light from reaching the eyes (e.g., eye shades, a visor, and the like). Such controllable light sources and devices for blocking light may optionally be physically attached to the breathing compartment **20** and/or in a form of a physically separate module.

In some embodiments of any of the embodiments described herein, the system **10** further comprises a control unit configured for controlling one or more of any treatment parameter described herein, for example, an oxygen content of an atmosphere (percentage of oxygen in the atmosphere, e.g., in embodiments wherein oxygen partial pressure is modulated by modulating oxygen percentage), a pressure of the atmosphere (e.g., in embodiments oxygen partial pressure is modulated by modulating total pressure of the atmosphere), and an intensity of light which reaches the eyes of a subject. Control over such parameters may be effected in accordance with a treatment regimen according to any of the respective embodiments described herein.

The control unit may optionally be configured to operate in a single mode of operation (e.g., corresponding to a treatment regimen according to any of the respective embodiments described herein), for example, by presenting a binary "on"/"off" choice. Alternatively, the control unit may optionally be configured to operate in any one of a plurality of modes of operation (corresponding to different treatment regimens according to any of the respective embodiments described herein), optionally being configured to allow (e.g., by manual control and/or digital input) essentially any desired regimen (with parameters within the capabilities of the system), for example, wherein the system **10** includes instructions for selecting an appropriate mode of operation.

In some embodiments of any of the embodiments described herein, control over any one or more of the parameters described herein (e.g., by a control unit described herein) is determined (e.g., by a computer) in accordance with a given time of day (e.g., local time in the location of the system **10** or in a different location, according to any of the respective embodiments described herein), a prevalent oxygen partial pressure (e.g.,

determined by a sensor measuring ambient oxygen partial pressure and/or by a database listing prevalent oxygen partial pressures for different locations), and/or a magnitude and/or direction of zeitgeber phase shift (e.g., eastward or westward travel, and/or number of time zones traversed; or adoption of an earlier or later sleep schedule and/or number of hours in change of sleep schedule), for example, wherein the system **10** is configured for receiving the parameter(s) as input (e.g., via a user interface).

In some embodiments of any of the embodiments described herein, the system **10** comprises instructions for controlling any one or more of the parameters described herein (e.g., instructions for operating a control unit described herein) in accordance with a given time of day (e.g., instructions for determining a suitable time for treatment, according to any of the respective embodiments described herein), a prevalent oxygen partial pressure (e.g., instructions for determining a suitable oxygen partial pressure for treatment, according to any of the respective embodiments described herein), and/or a magnitude and/or direction of zeitgeber phase shift (e.g., instructions for determining a suitable treatment regimen, eastward or westward travel, and/or number of time zones traversed; or adoption of an earlier or later sleep schedule and/or number of hours in change of sleep schedule, according to any of the respective embodiments described herein). In some embodiments, the instructions may be adapted for instructing a user how to provide suitable input to the system **10** (e.g., via a user interface).

In some embodiments of any of the embodiments described herein, the control unit is in communication with a clock, e.g., for allowing the control unit effect exposure to different oxygen partial pressures and/or light intensities at different times (e.g., according to a treatment regimen according to any of the respective embodiments described herein). The clock may be for determining a local time where the system **10** is located (e.g., wherein a treatment time of a subject after an aircraft flight is according to local time at the destination) and/or for calculating a local time elsewhere (e.g., wherein a treatment time of a subject during or after an aircraft flight is according to local time at the point of departure), according to any of the respective embodiments described herein.

In some embodiments of any of the embodiments described herein relating to a treatment regimen based on a local time in a different location than the subject (e.g., wherein a treatment time of a subject during or after an aircraft flight is according to

local time at the point of departure), the system **10** further comprises a database and user interface configured for receiving said different location (e.g., point of departure) as an input, and calculating a local time at said location (e.g., based on local time of the location of the system **10** and time differences between the location of the system **10** and other locations).

In some embodiments, a system **10** according to any of the respective embodiments described herein is configured for use in an aircraft cabin, and for exposing a subject in the aircraft to a hypoxic and/or hyperoxic atmosphere according to any of the respective embodiments described herein, for example, for use in treating jet lag.

In some embodiments relating to a system **10** in an aircraft, the system **10** (optionally a portable system) comprises a breathing compartment **20** configured for delivering the atmosphere to a breathing orifice of a subject, such as a mask mouthpiece and/or helmet (e.g., according to any of the respective embodiments described herein). Such a system, for example, may be adapted to allow different passengers to use the system **10** if and as they so choose, without substantially affecting neighboring passengers.

In some embodiments relating to a system **10** in an aircraft, the system **10** is configured for effecting a change in an oxygen partial pressure of an atmosphere within the aircraft cabin.

According to an aspect of some embodiments of the invention, there is provided an aircraft comprising a system **10** configured for effecting a change in an oxygen partial pressure of an atmosphere within the aircraft cabin (e.g., a system according to any of the respective embodiments described herein).

In some embodiments of any of the embodiments relating to a system **10** in an aircraft, the change in an oxygen partial pressure effected by the system **10** is a pre-determined change (e.g., not associated with malfunction of a pressurization system or failure of structural integrity of a pressurized cabin) of at least 1 kPa during a flight of the aircraft at a normal cruising altitude of the aircraft (e.g., in the absence of any change in altitude of the aircraft).

In some embodiments of any of the respective embodiments, the time and/or degree (e.g., in kPa) of change in the oxygen partial pressure in an aircraft cabin is in

accordance with a pre-determined regimen, e.g., a treatment regimen according to any of the respective embodiments described herein. The treatment regimen may optionally be selected in accordance with a local time of a destination of the aircraft and/or a point of departure of the aircraft, according to any of the respective embodiments described
5 herein, for use in treatment (e.g., prophylactic treatment) of jet lag of subjects (including passengers and/or crew members) upon arrival at a destination of the aircraft.

In some embodiments, a system **10** according to any of the respective embodiments described herein is installed in an airport terminal.

According to an aspect of some embodiments of the invention, there is provided
10 an enclosed space in an airport terminal, the enclosed space comprising a system **10** configured for effecting a change in an oxygen partial pressure of an atmosphere within the enclosed space (e.g., a system according to any of the respective embodiments described herein). The enclosed space may be, for example, a building, a room (e.g., a departure lounge or an adjacent room) and/or a tent in the airport terminal. Optionally,
15 the enclosed space comprises a non-permanent barrier (e.g., a curtain) as part of the enclosure (e.g., the area may be open upon movement of the non-permanent barrier).

In some embodiments of any of the embodiments relating to a system **10** in an airport terminal, the change in an oxygen partial pressure effected by the system **10** comprises a change of at least 1 kPa during a course of a day.

20 In some embodiments of any of the respective embodiments, the time and/or degree (e.g., in kPa) of change in the oxygen partial pressure effected by a system **10** in an airport terminal is in accordance with a pre-determined regimen, e.g., a treatment regimen according to any of the respective embodiments described herein.

The treatment regimen may optionally be selected in accordance with a local
25 time of the airport (e.g., being in a form of a daily cycle of oxygen levels) according to any of the respective embodiments described herein, for use in treatment of jet lag of subjects in need thereof upon arrival at the airport. Such a treatment regimen may optionally be independent of a circadian rhythm of a subject and/or a local time at a point of departure of a subject arriving at the airport (according to any of the respective
30 embodiments described herein), such that a large number of people arriving at the airport may receive a beneficial treatment without necessitating the tailoring of different treatments to different individuals. For example, a large enclosed space (e.g., a building,

or a room or tent configured to hold a plurality of subjects) may follow a treatment regimen independent of specific status of an individual subject. Alternatively, a smaller enclosed space, for example, a room or tent configured to hold a small number of subjects, optionally one subject, may optionally be configured to effect a treatment regimen based on a circadian rhythm of a subject and/or a local time at a point of departure of a subject arriving at the airport, according to any of the respective embodiments described herein.

In some embodiments of any of the embodiments described herein (according to any of the aspects described herein), control over one or more parameters described herein according to any of the respective embodiments (e.g., by a control unit described herein and/or according to instructions described herein) is adapted for treating jet lag by exposing a subject to a suitable atmosphere during a pre-determined time of day (e.g., according to any of the respective embodiments described herein).

In some embodiments, the pre-determined time of day (according to any of the respective embodiments described herein) is according to local time at a destination associated with the jet lag. For example, a system **10** in an aircraft (according to any of the respective embodiments described herein) may optionally be configured according to local time at the aircraft's destination, and/or a system **10** in an airport terminal (according to any of the respective embodiments described herein) may optionally be configured according to local time at the airport for treatment or subjects arriving thereat.

Alternatively, in some embodiments, the pre-determined time of day (according to any of the respective embodiments described herein) is according to local time at a point of departure associated with the jet lag. For example, a system **10** in an aircraft (according to any of the respective embodiments described herein) may optionally be configured according to local time at the aircraft's point of departure, and/or a system **10** in an airport terminal (according to any of the respective embodiments described herein) may optionally be configured according to local time at the airport for treatment or subjects arriving therefrom. Alternatively or additionally, a system **10** in an airport terminal (according to any of the respective embodiments described herein) configured for treatment or subjects arriving thereat may optionally be configured for effecting treatment according to local time at a point of departure of a subject, for example, upon

receiving an input indicating a point of departure and/or local time at a point of departure.

As exemplified herein, exposure to modulated oxygen levels did not alter the circadian rhythm of individuals *per se* (in a mouse model), but rather accelerated
5 adaptation to a new schedule.

Thus, in some embodiments relating to concomitant treatment of multiple people (e.g., in a large enclosure such as in an airport), the treatment selectively affects individuals in need thereof (e.g., travelers at risk of jet lag) with little or no effect on others who do not need to adapt to a different schedule, such as workers, visitors, and
10 travelers not at risk of jet lag (e.g., travelers who have not crossed many time zones) in an airport.

In some embodiments of any of the embodiments described herein relating to treatment of jet lag according to a pre-determined time of day according to local time at a point of departure, control of the system **10** described herein comprises different pre-
15 determined times of day (e.g., as options according to instructions and/or settings of a system **10** described herein) for different directions of journeys, for example, at least one pre-determined time of day suitable for eastward travel and at least one other pre-determined time of day suitable for westward travel. Two or more pre-determined times may optionally be included for the same direction (eastward and/or westward), for
20 example, a pre-determined time for traversing a relatively large number (e.g., at least 4, 5, 6, 7, 8 or 9) of time zones (e.g., suitable for a effecting a large circadian rhythm phase shift), and another pre-determined time for traversing a lesser number of time zones (e.g., suitable for a effecting a moderate circadian rhythm phase shift).

Treatment regimen:

25 The treatment regimens according to any of the embodiments described in this section may utilize a hypoxic and/or hyperoxic atmosphere according to any or the respective embodiments described elsewhere herein (unless indicated otherwise). For convenience, it is noted that in general, the embodiments of this section relate to times of treatment, whereas atmospheres used in treatment according to various embodiments are
30 generally described hereinabove. However, the aforementioned division of topics is not absolute and is not to be considered limiting.

In some embodiments of any of the embodiments described herein, treatment comprises exposure to a hypoxic and/or hyperoxic atmosphere according to any of the respective embodiments described herein for no more than about 4 hours, for example, for a period in a range of from 10 minutes to 4 hours, optionally from 30 minutes to 4 hours, and optionally from 1 hour to 4 hours. In some such embodiments, the exposure to the hypoxic and/or hyperoxic atmosphere is for no more than about 3 hours (e.g., from 10 minutes to 3 hours, from 30 minutes to 3 hours, and/or from 1 hour to 3 hours). In some such embodiments, the exposure to the hypoxic and/or hyperoxic atmosphere is for no more than about 2 hours (e.g., from 10 minutes to 2 hours, from 30 minutes to 2 hours, and/or from 1 hour to 2 hours). In exemplary embodiments, the exposure is for about 2 hours.

An exposure time may optionally be limited in duration, e.g., to no more than 4 hours (according to any of the respective embodiments described herein), in order to minimize inconvenience of the treatment. Alternatively or additionally, exposure time may optionally be limited in duration, e.g., to no more than 4 hours (according to any of the respective embodiments described herein), in order to avoid possible safety issues associated with longer exposure times, for example, when the oxygen partial pressure differs considerably from a prevalent oxygen partial pressure (e.g., about 21 kPa), for example, by at least 5 kPa (e.g., according to any of the respective embodiments described herein).

In some embodiments of any of the embodiments described herein relating to exposure to a hypoxic and/or hyperoxic atmosphere for no more than 4 hours, the oxygen partial pressure differs considerably from a prevalent oxygen partial pressure (e.g., about 21 kPa), for example, by at least 5 kPa (e.g., according to any of the respective embodiments described herein). In some such embodiments, the oxygen partial pressure of a hypoxic atmosphere is in a range of from about 10 kPa to about 14 kPa, according to any of the respective embodiments described herein. An exemplary oxygen partial pressure in the context of exposure times of hours (e.g., about 2 hours) is about 14 kPa.

In some embodiments of any of the embodiments described herein, treatment comprises exposure to a hypoxic and/or hyperoxic atmosphere according to any of the

respective embodiments described herein for at least about 6 hours, for example, for a period in a range of from 6 hours to 24 hours, and optionally from 6 hours to 12 hours.

An exposure time may optionally be extended in duration, e.g., to at least 6 hours (according to any of the respective embodiments described herein), in order to enhance efficacy of the treatment, e.g., a treatment comprising exposure to a hypoxic and/or hyperoxic atmosphere wherein the oxygen partial pressure differs only moderately from a prevalent oxygen partial pressure (e.g., about 21 kPa), for example, by no more than 7 kPa (e.g., according to any of the respective embodiments described herein). An exemplary oxygen partial pressure in the context of exposure times of at least 6 hours (e.g., about 12 hours) is about 16 kPa.

For example, an inconvenience of a longer treatment time may be outweighed by reduction in discomfort and/or safety risks associated with a more extreme hypoxic and/or hyperoxic atmosphere.

In some embodiments of any of the embodiments described herein relating to exposure for at least 6 hours, the exposure is effected prior to and/or during a zeitgeber phase shift (e.g., as a prophylactic treatment of a disorder associated with the zeitgeber phase shift) according to any of the respective embodiments described herein.

As exemplified herein, oxygen level modulation (according to any of the respective embodiments described herein) may result in phase shifts of different directions and/or magnitudes at different times in a circadian rhythm. A treatment (according to any of the respective embodiments described herein) may thus be optionally effected at a suitable time, such as a time suitable for generating a circadian rhythm phase shift which is similar in magnitude and direction to a zeitgeber phase shift associated with the circadian rhythm disorder.

In some embodiments of any of the embodiments described herein, treatment is effected during a suitable phase in a subject's circadian rhythm.

In some embodiments, a subject's circadian rhythm is assumed to be in accordance with a local time (i.e., treatment is effected during a suitable local time), for example, a traveler's circadian rhythm may be represented by local time at a point of departure, and a circadian rhythm of a subject afflicted by shift work sleep disorder associated with beginning night shift work may be represented by local time. Alternatively, for example, a circadian rhythm of a subject afflicted by shift work sleep

disorder associated with ceasing night shift work may be unsynchronized with local time, and may be calculated based on the subject's previous lifestyle, e.g., sleep patterns.

In some embodiments of any of the embodiments described herein, treatment is effected during a time period (e.g., relative to a subject's circadian rhythm, according to
5 any of the respective embodiments described herein) during which an oxygen level modulation (according to any of the respective embodiments described herein) typically results in a maximal or near-maximal phase shift. Such a time period may optionally be determined, for example, as a time point at which the expected phase shift is maximal $\pm$ 2 hours, or $\pm$ 1 hour. Alternatively, such a time period may optionally be determined,
10 for example, as a time point at which the expected phase shift is no less than a threshold value, for example, 4 hours, 6 hours or 8 hours.

Expected phase shifts may be determined quantitatively, for example, based on studies on humans and/or on animals.

In some embodiments of any of the embodiments described herein, treatment is
15 effected (according to any of the respective embodiments described herein) during a time period (e.g., time of day) which results in a maximal or near-maximal positive phase shift (e.g., advance) in circadian rhythm, or a time period which results in a maximal or near-maximal negative phase shift (e.g., delay), depending on a desired direction in phase shift. Such a treatment is suitable, for example, for treating jet leg
20 associated with crossing a large number (e.g., at least 4, 5, 6, 7, 8 and even 9) of time zones.

In some embodiments of any of the embodiments described herein, treatment is effected (according to any of the respective embodiments described herein) during a time period (e.g., time of day) which results in a moderate positive phase shift in
25 circadian rhythm (e.g., a positive phase shift smaller than a maximal or near-maximal positive phase shift described herein) and/or a time period which results in a moderate negative phase shift (e.g., a negative phase shift smaller than a maximal or near-maximal negative phase shift described herein). Such a treatment is suitable, for example, for treating jet leg associated with crossing a small number (e.g., less than 9, 8, 7, 6, 5 and
30 even 4) of time zones.

In some embodiments of any of the embodiments described herein, treatment is effected during a time period (e.g., time of day) selected in accordance with a

mathematical function and/or algorithm based on a desired phase shift magnitude and direction. For example, a graph may optionally display pre-determined times of treatment corresponding to various phase shifts, and/or a computerized module (e.g., in a system according to any of the respective embodiments described herein) may optionally
5 be configured to provide (as output) a pre-determined time for any inputted phase shift.

In some embodiments of any of the embodiments described herein, treatment is effected during a pre-determined time of day.

In some embodiments of any of the embodiments described herein relating to treatment during a pre-determined time of day, the pre-determined time of day is
10 selected to result in a maximal or near-maximal positive or negative phase shift, in a maximal or near-maximal negative phase shift, or in a moderate positive or negative phase shift, according to any of the respective embodiments described herein.

Without being bound by any particular theory, it is believed that a treatment regimen (e.g., included in instructions or settings of a system according to any of the
15 respective embodiments described herein) adapted to offer a relatively small number of options, for example, an option of a large positive phase shift and/or a large negative phase shift (e.g., a positive and/or negative phase shift of at least 4 hours, optionally at least 6 hours, and optionally at least 8 hours) may have the advantage of simplicity (e.g., by avoiding a large and potentially confusing number of options) and/or flexibility (e.g.,
20 by providing a relatively broad time period during which the treatment may optionally be effected), even though the magnitude of the phase shift expected upon treatment may not precisely correspond to a desired circadian phase shift. For example, subjects for whom a smaller circadian phase shift is desirable (e.g., less than 4 hours) may be less motivated to undergo treatment, such that it may not be necessary to devote treatment
25 options (e.g., included in instructions or settings of a system according to any of the respective embodiments described herein) for such subjects. Furthermore, subjects for whom an optimal circadian phase shift is slightly different (e.g., by no more than 4 hours, or by no more than 2 hours) from the actual circadian phase shift associated with the pre-determined time of treatment may not suffer any significant disadvantages upon
30 receiving a slightly sub-optimal treatment.

For example, instructions or settings of a system according to any of the respective embodiments described herein may optionally provide a choice between two

or more of the pre-determined times of day described herein, optionally with an explanation of how to select an appropriate time period based on needs of a subject (e.g., based on a direction and/or magnitude of a change in time zones associated with travel by a subject).

5 Herein, the phrase "during a pre-determined time of day" encompasses pre-determined time periods during which exposure to a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein) is to end, pre-determined time periods during which exposure to a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein) is to
10 begin, and pre-determined time periods during which an entire exposure to a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein) is to occur.

 In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the pre-determined time of day is a time during which
15 exposure to a hypoxic and/or hyperoxic atmosphere ends (e.g., upon exposure for a duration according to any of the respective embodiments described herein).

 It is to be appreciated that the phrase "during a pre-determined time of day" may encompass a range of different (albeit optionally similar) regimens. For example, in embodiments wherein a pre-determined time of day is a 4 hour time period, exposure to
20 an atmosphere a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein) may optionally begin and/or end at various points within the 4 hour time period.

 Such a variable exposure time may be suitable, for example, for embodiments configured for treating individual subjects (e.g., in a small room, or tent, or using a
25 portable system), according to any of the respective embodiments described herein, as it allows the subject to select a convenient exposure time from among a range of possible exposure times.

 In some embodiments of any of the embodiments described herein, exposure to an atmosphere a hypoxic and/or hyperoxic atmosphere according to any of the respective
30 embodiments described herein) is for a time period consisting of a pre-determined time of day described herein (i.e., exposure begins at the beginning of the pre-determined time of day and continues for the duration of the pre-determined time of day). Such a

fixed exposure time may be suitable, for example, for embodiments in which a captive population is treated (e.g., in an aircraft or in an airport terminal building), according to any of the respective embodiments described herein.

In some embodiments, the pre-determined time of day (e.g., for treating jet lag according to any of the respective embodiments described herein) is according to a local time according to a point of departure of a journey, such as an aircraft flight, (e.g., in embodiments wherein the system is in an aircraft or airport). In some such embodiments, the pre-determined time of day may depend on the particular subject being treated.

For example, a pre-determined time of day (e.g., daytime or nighttime) for treatment of jet lag according to such embodiments may optionally depend on the direction (e.g., eastward or westward) of the journey, and optionally also on the number of time zones to be crossed during the journey. Optionally, a pre-determined time of day for treatment of jet lag (according to any of the respective embodiments described herein) is in the afternoon (e.g., daytime afternoon) for one direction of travel (e.g., westward) and/or is at night (e.g., midnight $\pm$ 2 hours, and/or or later) for another direction of travel (e.g., eastward).

In another example, a pre-determined time of day for treatment of a sleep disorder (e.g., shift work sleep disorder) according to such embodiments may be optionally depend on the direction of a desired circadian phase shift (e.g., desiring to awake and/or sleep sleeping earlier or later), and optionally also on the magnitude (e.g., in hours) of the desired circadian phase shift.

In some embodiments, the pre-determined time of day (e.g., for treating jet lag according to any of the respective embodiments described herein) is according to a local time in a location of the system and/or a local time according to a destination of an aircraft flight (e.g., in embodiments wherein the system is in an aircraft or airport). In such embodiments, the pre-determined time of day may be independent of the particular subject being treated (e.g., independent of the initial phase of a circadian rhythm of the subject).

As exemplified herein, oxygen level modulation (according to any of the respective embodiments described herein) at any of a variety of different phases of a circadian rhythm (i.e., a circadian rhythm phase prior to treatment) may each result in a

phase of a circadian rhythm following treatment being in a certain range. In other words, some ranges of circadian rhythm phase (which correspond to a time period during the day, e.g., morning) in a subject following treatment can be readily obtained by resetting a circadian phase at a variety of treatment times.

5 Without being bound by any particular theory, it is believed that performing treatment at a pre-determined time of day which corresponds to such a readily obtained range of circadian rhythm phases may have the advantage of simplicity (e.g., by providing a uniform treatment to different subjects), for example, in embodiments wherein a plurality of subjects whose circadian rhythms are not necessarily in
10 synchronization are treated.

For example, in some embodiments of any of the embodiments described herein relating to treatment of subjects in an airport terminal (e.g., a system **10** in an airport terminal configured for allowing more than one subject to reside in a breathing compartment thereof), exposure to a hypoxic and/or hyperoxic atmosphere according to
15 any of the respective embodiments described herein is effected during a pre-determined time of day (according to local time).

In some embodiments of any of the embodiments described herein relating to treatment of subjects in an aircraft (e.g., a system in an aircraft), exposure to a hypoxic and/or hyperoxic atmosphere according to any of the respective embodiments described
20 herein is effected during a pre-determined time of day according to local time at a destination of the aircraft. For example, an aircraft may have substantial numbers of passengers and/or crewmembers who made a previous connecting flight, such that their circadian rhythms are not necessarily synchronized with local time at the aircraft's point of departure or with each other.

25 Times according to any of the respective embodiments described herein (e.g., pre-determined time of treatment, a time period in a sleep schedule) may optionally be defined in accordance with clock time (e.g., in accordance with a standard time used in a given location) and/or in accordance with an apparent motion of the sun (e.g., based on time relative to local sunrise, apparent noon and/or sunset). In many situations,
30 differences between clock time (e.g., wherein daytime is defined as being from 6:00 to 18:00 and/or nighttime is defined as being from 18:00 to 6:00) and apparent motion of the sun (e.g., wherein daytime as being from sunrise to sunset and/or nighttime is

defined as being from sunset to sunrise) will be insignificant, such that determination of time may optionally be selected, for example, based on convenience.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is during the morning, i.e., from midnight to noon (e.g., wherein midnight and noon are each a midpoint between sunrise and sunset, and/or from 12:00 midnight to 12:00 noon). In some such embodiments, the time of day is in a range of from sunrise to noon and/or from 6:00 AM to 12:00 noon. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is during the afternoon, i.e., from noon to midnight (e.g., wherein midnight and noon are each a midpoint between sunrise and sunset, and/or from 12:00 noon to 12:00 midnight). In some such embodiments, the time of day is in a range of from sunset to midnight and/or from 6:00 PM to 12:00 midnight. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is a time period of no more than 4 hours.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 0 to 4 hours after beginning of daytime, as defined herein (e.g., from 0 to 4 hours after sunrise and/or from 6:00 to 10:00 AM). In some such embodiments, the time of day is in a range of from 0 to 2 hours after beginning of daytime. In some such embodiments, the time of day is in a range of from 2 to 4 hours after beginning of daytime. In some embodiments, the time

of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 2 to 6 hours after beginning of daytime, as defined herein (e.g., from 2 to 6 hours after sunrise and/or from 8:00 AM to 12:00 noon). In some such embodiments, the time of day is in a range of from 4 to 6 hours after beginning of daytime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 4 to 8 hours after beginning of daytime, as defined herein (e.g., from 4 to 8 hours after sunrise and/or from 10:00 AM to 14:00). In some such embodiments, the time of day is in a range of from 6 to 8 hours after beginning of daytime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 6 to 10 hours after beginning of daytime and/or from 6 to 2 hours before beginning of nighttime, as defined herein (e.g., from 6 to 10 hours after sunrise, from 6 to 2 hours before sunset, and/or from 12:00 noon to 16:00). In some such embodiments, the time of day is in a range of from 8 to 10 hours after beginning of daytime and/or from 4 to 2 hours before beginning of nighttime. In some such embodiments, the time of day is in a range of from 6 to 4 hours before beginning of nighttime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the

time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 8 to 12 hours after beginning of daytime and/or from 4 to 0 hours before beginning of nighttime, as defined herein (e.g., from 8 to 12 hours after sunrise, from 4 to 0 hours before sunset, and/or from 14:00 to 18:00). In some such embodiments, the time of day is in a range of from 10 to 12 hours after beginning of daytime and/or from 2 to 0 before beginning of nighttime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 10 to 14 hours after beginning of daytime and/or from 2 hours before to 2 hours after beginning of nighttime, as defined herein (e.g., from 10 to 14 hours after sunrise, from 2 hours before to 2 hours after sunset, and/or from 16:00 to 20:00). In some such embodiments, the time of day is in a range of from 12 to 14 hours after beginning of daytime and/or from 0 to 2 hours after beginning of nighttime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 12 to 8 hours before beginning of daytime and/or from 0 to 4 hours after beginning of nighttime, as defined herein (e.g., from 12 to 8 hours before sunrise, from 0 to 4 hours after sunset, and/or from 18:00 to 22:00). In some such embodiments, the time of day is in a range of from 10 to 8 hours before beginning of daytime and/or from 2 to 4 hours after beginning of nighttime. In some embodiments, the time of day is a local time at a destination of a

subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

5 In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 10 to 6 hours before beginning of daytime and/or from 2 to 6 hours after beginning of nighttime, as defined herein (e.g., from 10 to 6 hours before sunrise, from 2 to 6 hours after sunset, and/or from 20:00 to 24:00). In some such embodiments, the time of day is in a range of from
10 8 to 6 hours before beginning of daytime and/or from 4 to 6 hours after beginning of nighttime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject
15 being treated for jet lag associated with westward travel.

 In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 8 to 4 hours before beginning of daytime, as defined herein (e.g., from 8 to 4 hours before sunrise and/or from 22:00 to 2:00 AM). In some such embodiments, the time of day is in a range of
20 from 6 to 4 hours before beginning of daytime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

25 In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 6 to 2 hours before beginning of daytime, as defined herein (e.g., from 6 to 2 hours before sunrise and/or from 24:00 to 4:00 AM). In some such embodiments, the time of day is in a range of from 4 to 2 hours before beginning of daytime. In some embodiments, the time of day is
30 a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag

associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 4 to 0 hours before beginning of daytime, as defined herein (e.g., from 4 to 0 hours before sunrise and/or from 2:00 to 6:00 AM). In some such embodiments, the time of day is in a range of from 2 to 0 hours before beginning of daytime. In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein relating to a pre-determined time of day, the time of day is in a range of from 2 hours before beginning of daytime to 2 hours after beginning of daytime, as defined herein (e.g., from 2 before sunrise to 2 hours after sunrise and/or from 4:00 to 8:00 AM). In some embodiments, the time of day is a local time at a destination of a subject being treated for jet lag. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with eastward travel. In some embodiments, the time of day is a local time at a point of departure of a subject being treated for jet lag associated with westward travel.

In some embodiments of any of the embodiments described herein, the treatment comprises exposing a subject with a sleep disorder to a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein) at a pre-determined time relative to a time of day during which the subject desires to sleep (e.g., desires to begin to sleep). That is, there is a pre-determined time difference (e.g., in hours) between treatment and a selected time for sleep. For example, the sleep disorder is optionally shift work sleep disorder and the selected time for sleep is optionally an abnormal time for sleep necessitated by a subject's work schedule (e.g., when the subject can be at home to sleep).

In some embodiments of any of the embodiments described herein, a pre-determined time of day relative to a time of day during which the subject desires to sleep

(according to any of the respective embodiments described herein) is no more than 12 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described
5 herein) is from 12 to 8 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 12 to 10 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described
10 herein) is from 10 to 6 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 10 to 8 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described
15 herein) is from 8 to 4 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 8 to 6 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described
20 herein) is from 6 to 2 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 6 to 4 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described
25 herein) is from 4 to 0 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 4 to 2 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 2 to 0 hours prior to a time of day during which the subject desires to sleep.

30 In some embodiments of any of the embodiments described herein, a pre-determined time of day relative to a time of day during which the subject desires to sleep

(according to any of the respective embodiments described herein) is at least 12 hours prior to a time of day during which the subject desires to sleep.

It is to be appreciated that periods of time which are considerably more than 12 hours prior to a time of day during which the subject desires to sleep may correspond to periods of time during which the subject desires to sleep. In such embodiments, the subject may be awake (e.g., insomniac) or undergoing treatment in sleep, for example, by sleeping in a compartment (e.g., a room or tent) comprising a suitable atmosphere.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is from 16 to 12 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 14 to 12 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is from 18 to 14 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 16 to 14 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is from 20 to 16 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 18 to 16 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is from 22 to 18 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 20 to 18 hours prior to a time of day during which the subject desires to sleep.

In some embodiments of any of the embodiments described herein, a pre-determined time of day (according to any of the respective embodiments described herein) is from 24 to 20 hours prior to a time of day during which the subject desires to sleep. In some embodiments, the pre-determined time of day is from 22 to 20 hours prior to a time of day during which the subject desires to sleep. In some embodiments,

the pre-determined time of day is from 24 to 22 hours prior to a time of day during which the subject desires to sleep.

A treatment regimen according to any of the respective embodiments described herein, and any combination thereof, may optionally utilize (unless indicated otherwise) an oxygen partial pressure which is at least 1 kPa less, at least 2 kPa, at least 3 kPa, at least 5 kPa, at least 7 kPa, and even at least 9 kPa less than a prevalent oxygen partial pressure (according to any of the respective embodiments described herein), and/or an oxygen partial pressure which is at least 1 kPa less, at least 2 kPa, at least 3 kPa, at least 5 kPa, at least 7 kPa, and even at least 9 kPa more than a prevalent oxygen partial pressure (according to any of the respective embodiments described herein). For example, a hypoxic atmosphere utilized in a treatment regimen may optionally have an oxygen partial pressure of 20 kPa or less, 19 kPa or less, 18 kPa or less, 16 kPa or less, 14 kPa or less, 12 kPa or less (according to any of the respective embodiments described herein); and a hyperoxic atmosphere utilized in a treatment regimen may optionally have an oxygen partial pressure of at least 22 kPa, at least 23 kPa, at least 24 kPa, at least 26 kPa, at least 28 kPa, at least 30 kPa, at least 33 kPa, at least 40 kPa, at least 50 kPa, at least 60 kPa, at least 70 kPa, at least 80 kPa, and optionally about 100 kPa (according to any of the respective embodiments described herein).

It is to be appreciated that although many embodiments herein are described in terms of treating jet lag, the corresponding treatment of circadian rhythm disorders other than jet lag will be readily apparent to the skilled person based on the descriptions relating to jet lag, by replacing a local time of a point of departure described herein with any suitable indication of a present phase of a circadian rhythm of a subject (e.g., based on a sleep pattern), and/or by replacing a local time of a destination described herein with any suitable indication of a desired phase of a circadian rhythm of a subject (e.g., based on a desired sleep pattern).

According to some embodiments of any of the embodiments described herein relating to a method of treating a circadian rhythm disorder, the method comprises exposing a subject in need thereof to a hypoxic and/or hyperoxic atmosphere according a regimen described herein (according to any of the respective embodiments).

HIF1 α modulation:

As exemplified herein, HIF1 α activity plays an important role in modulating circadian rhythms. HIF1 α activity may optionally be modulated by techniques other than oxygen level modulation, either in addition to or as an alternative to oxygen level modulation.

According to an aspect of some embodiments of the invention, there is provided an agent capable of modulating an activity of HIF1 α , for use in the treatment of a circadian rhythm disorder (e.g., according to any of the respective embodiments described herein). The treatment optionally further comprises exposure to a controlled intensity of light (according to any of the respective embodiments described herein) and/or to a hypoxic and/or hyperoxic atmosphere (according to any of the respective embodiments described herein), optionally using a system according to any of the respective embodiments described herein.

The agent may optionally be an up-regulator of HIF1 α or a down-regulator of HIF1 α .

Up-regulation of HIF1 α can be effected at the genomic level (e.g., activation of transcription via promoters, enhancers, regulatory elements), at the transcript level (e.g., correct splicing, polyadenylation, activation of translation) or at the protein level (e.g., post-translational modifications, interaction with substrates, inhibition of protein degradation, and the like).

Downregulation of HIF1 α can be effected on the genomic and/or the transcript level using a variety of molecules which interfere with transcription and/or translation (e.g., RNA silencing agents (e.g., antisense, siRNA, shRNA, micro-RNA), ribozyme and DNzyme), or on the protein level, e.g., by antagonistic binding to HIF1 α , and or by enhancing degradation of HIF1 α .

In some embodiments, treatment with an up-regulator of HIF1 α exhibits an effect corresponding to that of exposure a hypoxic atmosphere and is optionally combined with an exposure a hypoxic atmosphere (according to any of the respective embodiments described herein).

In some embodiments, treatment with a down-regulator of HIF1 α exhibits an effect corresponding to that of exposure a hyperoxic atmosphere and is optionally

combined with an exposure a hyperoxic atmosphere (according to any of the respective embodiments described herein).

Examples of up-regulators of HIF1 α include, without limitation, cobalt, iron chelators (e.g., desferrioxamine, deferiprone, deferasirox), dimethyloxalylglycine, HIF
5 prolyl hydroxylase inhibitors (HIF-PHIs, e.g., IOX2, roxadustat (FG4592)) and inhibitors of von Hippel-Lindau tumor suppressor (VHL).

Herein, "cobalt" which is a HIF1 α up-regulator refers to cobalt ion (preferably Co²⁺) and any compound (e.g., cobalt salt) comprising cobalt ion. CoCl₂ is an example of a suitable cobalt salt.

10 Without being bound by any particular theory, it is believed that inhibition of VHL (which is associated with degradation of HIF1 α) and/or of one or more hydroxylase (e.g., prolyl hydroxylase) which promotes degradation of HIF1 α (e.g., by cobalt, iron chelator, HIF-PHI, and/or dimethyloxalylglycine) is an effective route for up-regulating HIF1 α . It is further believed that inhibition of a hydroxylase may be
15 effected by inhibition of binding of iron (e.g., by iron chelator and/or cobalt) or α -ketoglutarate (e.g., by dimethyloxalylglycine) to the hydroxylase (e.g., as hydroxylase cofactors).

Examples of down-regulators of HIF1 α include, without limitation, PX12, BAY87-2243 and KC7F2.

20 It is expected that during the life of a patent maturing from this application many relevant agent capable of modulating an activity of HIF1 α will be developed and the scope of the terms "agent", "up-regulator" and "down-regulator" is intended to include all such new technologies *a priori*.

The HIF1 α -modulating agents of some embodiments of the invention may
25 optionally be administered to a subject *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

Thus, according to an aspect of some embodiments of the invention, there is provided a HIF1 α -modulating agent (according to any of the respective embodiments described herein) and a pharmaceutically acceptable carrier.

30 As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein (e.g., a HIF1 α -modulating agent) with other chemical components such as physiologically suitable carriers and excipients.

The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier", which may be interchangeably used, refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such

as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions that can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-
5 tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a
10 powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be
15 suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection
20 suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which
25 increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may
30 also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, an effective amount means an amount of active ingredients (e.g., HIF1 α -modulating agents) effective to treat a disorder (e.g., a circadian rhythm disorder) according to any of the respective embodiments described herein.

Determination of an effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount (e.g., included in a single unit dosage form) and interval may be adjusted individually to provide blood levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations. Dosage amount and interval may optionally be lower than an MEC for treating a condition (e.g., pathological condition) other than a circadian rhythm disorder (e.g., an MEC for treating cancer with a HIF1 α downregulator).

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment

lasting from a single administration to several days or several weeks, or until cure is effected or diminution of the disorder and/or a symptom thereof is achieved.

The amount of a composition to be administered will of course be dependent on the subject being treated, the severity and nature of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in kit such as a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient (e.g., a HIF1 α -modulating agent described herein). A pack may, for example, comprise metal or plastic foil, such as a blister pack. The kit may further comprise instructions for administration of the active ingredient, and/or for controlling exposure to a hypoxic atmosphere and/or to a controlled intensity of light (according to any of the respective embodiments described herein) in order to treat a circadian rhythm disorder (e.g., by enhancing an effect of a HIF1 α -modulating agent described herein). The kit may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutically acceptable carrier may also be prepared, placed in an appropriate container, and optionally labeled for treatment of an indicated condition, as is further detailed above.

As used herein the term "about" refers to $\pm 10\%$.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

The word "exemplary" is used herein to mean "serving as an example, instance or illustration". Any embodiment described as "exemplary" is not necessarily to be construed as preferred or advantageous over other embodiments and/or to exclude the incorporation of features from other embodiments.

5 The word "optionally" is used herein to mean "is provided in some embodiments and not provided in other embodiments". Any particular embodiment of the invention may include a plurality of "optional" features unless such features conflict.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or
10 "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible
15 limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well
20 as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges
25 from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners,
30 means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available

immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

Materials:

Anti-HIF1 α antibody (mouse anti-HIF1 α ; 3C144) was obtained from Santa Cruz (unless indicated otherwise).

Anti-tubulin and anti-U2AF antibodies were obtained from Sigma.

Dexamethasone was obtained from Sigma.

Protease inhibitors (N-(α -aminoethyl)benzene-sulfonyl fluoride, bestatin, E65, leupeptin, and pepstatin) were obtained from Sigma.

siRNAs (SMARTpool ON-TARGETplus™ mouse *Hif1 α* siRNA (L-040638), *Cry2* siRNA (L-040486), *Rora* siRNA (L-040430) and control siRNA (D-001810) (D-001810)) were obtained from Dharmacon.

Silver wire (0.2 mm diameter) was obtained from Advent Research Materials.

Animals:

For biochemical and behavioral experiments, three months old male mice were used. For telemetric oxygen monitoring, three month old male Wistar rats were used. Animals were housed under 12-hour light/dark regimen unless indicated differently;

ZT0 corresponded to the time lights were turned on and ZT12 to the time lights were turned off in the animal facility.

Activity measurement:

Circadian voluntary locomotor activity was determined by monitoring wheel-running activity. The circadian phase and period were analyzed with the ClockLab™ software (Actimetrics) under 12 hour light/dark and constant dark regimens.

Oxygen measurement:

The oxygen consumption rate of mice was monitored using PhenoMaster™ metabolic cages (TSE Systems). Blood oxygen levels were measured with FireStingO₂™ oxygen meter (Pyroscience).

Telemetry-based recordings of renal pO₂ were performed according to procedures described previously [Koeners et al., *Renal Physiology* 2013, 304:F1471-F1480; Koeners et al., *Methods Mol Biol* 2016, 1397:93-111]. Briefly, a TR57Y tissue oxygen telemeter (Millar) was equipped with a carbon paste electrode (CPE, 0.27 mm in diameter) for electrochemical detection of tissue O₂ levels. This CPE electrode was implanted in the rat kidney, so that the tip of the electrode was approximately 2 mm below the cortical surface. Reference and auxiliary electrodes, made of silver wire (0.2 mm diameter), were also implanted in the kidney. This maintained a potentiostat circuit with a potential of -650 mV on the CPE. The telemeter was placed in the abdomen of the rat and attached to the inner abdominal muscle layer. After a recovery, the rat's cage was placed on a SmartPad™ TR181 receiver-charging unit (Millar), which received the data from, and recharged the battery of, the telemeter. This setup allowed renal tissue O₂ levels to be measured continuously at a frequency of 5 Hz. The CPE measures the molar concentration of oxygen (the amount of oxygen) by electrochemical reduction of the oxygen present in the fluid surrounding the tip of the electrode. To facilitate the comparison with the gases used for the cell culture experiments, percentage O₂ in kidney tissue was described using the following conversion: 1 μM = 0.631 mmHg = 0.08 %.

In order to implant the telemeters, the telemeters were sterilized in a 2 % w/v glutaraldehyde solution for at least 4 hours or in a Cidex® OPA solution of 0.55 % w/v ortho-phthalaldehyde (Advanced Sterilization Products) for 30 minutes and rinsed thoroughly with sterile 0.9 % w/v NaCl solution before implantation. Rats were

anesthetized with 5 % v/v isoflurane in an induction box and maintained at 2 - 2.5 % v/v isoflurane on a heated operating table. Rats were pre-medicated with 30 µg/kg subcutaneous buprenorphine (Temgesic® injection, Reckitt Benckiser). Under sterile conditions, the left kidney and aorta were exposed by laparotomy. The cables
5 connecting the electrodes and telemeter were secured by suturing them on the adventitia of the abdominal aorta or dorsal muscles adjacent to the spine near the left kidney. After pre-puncturing the kidney with a 30-gauge needle, the reference electrode and CPE were inserted in the kidney and secured in place with Histoacryl® tissue glue (B. Braun) approximately 1 mm apart from each other, while the auxiliary electrode was
10 affixed onto the kidney surface. With the telemeter placed and secured in the abdomen, the abdomen was closed with sutures and the rat was placed on a heated pad for at least 12 hours to recover. Post-operative analgesia was administered (buprenorphine, 3 µg/100 gram rat every 8–14 hours for up to 3 days or as required). All rats were allowed to recover for 1 week before experiments started.

15 Renal O₂ levels were continuously measured in Wistar rats for several days. After the experimental period, rats were killed by intraperitoneal injection of an overdose (>200 mg/ml) of sodium pentobarbitone (Euthatal® solution, Merial Animal Health), and post-mortem O₂ values were determined for offset correction of individual O₂ recordings.

20 For statistical analyses of kidney mean daily O₂ levels and the range of O₂ oscillations (i.e., the difference between the measured O₂ zenith and nadir levels during the day) only considered animals that showed continuous and steady measurements for at least 3 consecutive days were considered. Data were smoothed with a moving average of a six-hour time window. Baseline was calculated with a moving average of
25 a 48-hour time window and subtracted from the smoothed data. Values and times of peaks and troughs were calculated using MATLAB® findpeaks function (MathWorks) for each 24-hour cycle.

Chromatin immunoprecipitation:

30 Chromatin from mouse liver and kidney was prepared and chromatin immunoprecipitations were performed according to procedures previously described with anti-BMAL1 antibody [Ripperger & Schibler, *Nature Genetics* 2006, 38:369-374] and anti-HIF1α antibodies obtained from Novus Biologicals (NB100-134), R&D

Systems (AF1935) and Santa Cruz (3C144). Chromatin from cultured cells was prepared according to standard protocols, such as described by Schmidt et al. [*Methods* 2009, 48:240-248]. The immunoprecipitated DNA was reversed cross-linked and purified with QIAquick® PCR purification kit (Qiagen). Samples were analyzed by quantitative real-time PCR, employing a SYBR™ green assay, using primers listed in Table 1 below for blocks A-G in the vicinity of the *Cry2* gene (as depicted in FIG. 22).

Table 1: Primers used in chromatin immunoprecipitation analysis

Blocks	Primer sequences
A	Forward primer 5'-CTGCCTGTCTGCTTCCATTT-3' (SEQ ID NO: 1) Reverse primer 5'-CATGGTAAACCACCCTGACC-3' (SEQ ID NO: 2)
B	Forward primer 5'-GCCAATGCAAGCCTCTTATG-3' (SEQ ID NO: 3) Reverse primer 5'-GGCTCACCTCATTTGGTGTC-3' (SEQ ID NO: 4)
C	Forward primer 5'-CAACCCACGTCCTCTGAAAC-3' (SEQ ID NO: 5) Reverse primer 5'-TCCACTTCCTCGCAGAGAAT -3' (SEQ ID NO: 6)
D	Forward Primer 5'-GTAAGGCAATCACCGCTCAG-3' (SEQ ID NO: 7) Reverse Primer 5'-TCGGATCTCCTACGCATTTC-3' (SEQ ID NO: 8)
E	Forward Primer 5'-CTTCCCTAAGCCTCGGATTT-3' (SEQ ID NO: 9) Reverse Primer 5'-TCACCACCACGTTTGTCTGT-3' (SEQ ID NO: 10)
F	Forward Primer 5'-GCTTCAGTGCATTGACCAGA-3' (SEQ ID NO: 11) Reverse Primer 5'-GGCTCAGAAGCAGCTACCAT-3' (SEQ ID NO: 12)
G	Forward Primer 5'-TCCCTGGCTAAGACAGCCTA-3' (SEQ ID NO: 13) Reverse Primer 5'-AAAGCACCGAAAGAACTGGA-3' (SEQ ID NO: 14)

Cell culture and reagents:

Hepa-1c1c7 and NIH3T3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % FBS (fetal bovine serum), 100 units/ml penicillin, and 100 mg/ml streptomycin, and cultured at 37 °C in a humidified incubator with 5 % CO₂. SMARTpool ON-TARGETplus™ mouse *Hif1α*, *Cry2*, *Rora* and control siRNA were delivered into the cells with Lipofectamine® RNAi reagent 48 hours prior to the experimental procedure. Cells were synchronized with 100 nM dexamethasone

treatment for 20 minutes. Experiments with different oxygen levels were conducted in special chambers with O₂, CO₂ and temperature control (Coy Laboratory).

RNA preparation and real-time PCR analysis:

RNA extraction and transcript quantification by real-time PCR were carried according to procedures described by Adamovich et al. [Cell metabolism 2014, 19:319-330]. Synthesis of cDNA was done using qScript™ cDNA SuperMix (Quanta Biosciences). Quantitative real-time PCR measurements were performed using SYBR™ green or TaqMan® probes with a LightCycler® II machine (Roche) and normalized to the geometrical mean of 3 housekeeping genes: *Tbp*, *Hprt*, and *Rplp0*.

Primers and probes used for real-time PCR measurements are listed in Table 2 below.

Table 2: Primers and probes used in quantitative real-time PCR

Gene	Primer/Probe sequences
<i>Tbp</i>	Forward primer 5'-CCCTATCACTCCTGCCACACCAGC-3' (SEQ ID NO: 15) Reverse primer 5'-GTGCAATGGTCTTTAGGTCAAGTTTACAGCC-3' (SEQ ID NO: 16)
<i>Hprt</i>	Forward primer 5'-GGCCAGACTTTGTTGGATTTG-3' (SEQ ID NO: 17) Reverse primer 5'-TGCGCTCATCTTAGGCTTTGT-3' (SEQ ID NO: 18)
<i>Rplp0</i>	Forward primer 5'- AGATTCGGGATATGCTGTTGGC-3' (SEQ ID NO: 19) Reverse primer 5'- TCGGGTCCTAGACCAGTGTTTC-3' (SEQ ID NO: 20)
<i>Clock</i>	Forward Primer 5'- AGAACTTGGCATTGAAGAGTCTC-3' (SEQ ID NO: 21) Reverse Primer 5'- GTCAGACCCAGAATCTTGGCT-3' (SEQ ID NO: 22)

Gene	Primer/Probe sequences
<i>Bmal1</i>	Forward Primer 5'-CCAAGAAAGTATGGACACAGACAAA-3' (SEQ ID NO: 23) Reverse Primer 5'-GCATTCTTGATCCTTCCTTGGT-3' (SEQ ID NO: 24) Probe 5'-FAM-TGACCCTCATGGAAGGTTAGAATATGCAGAA-TAMRA-3' (SEQ ID NO: 25)
<i>Rev-erba</i>	Forward Primer 5'-TGCAGGCTGATTCTTCACACA-3' (SEQ ID NO: 26) Reverse Primer 5'-AGCCCTCCAGAAGGGTAGGA-3' (SEQ ID NO: 27) Probe 5'-FAM-ACACTCTCTGCTCTTCCCATGCAAATCAG-TAMRA-3' (SEQ ID NO: 28)
<i>Rora</i>	Forward primer 5'- GTGGAGACAAATCGTCAGGAAT-3' (SEQ ID NO: 29) Reverse primer 5'- TGGTCCGATCAATCAAACAGTTC-3' (SEQ ID NO: 30)
<i>Per1</i>	Forward primer 5'-ACCAGCCATTCCGCCTAAC-3' (SEQ ID NO: 31) Reverse primer 5'-CGGGGAGCTTCATAACCAGA-3' (SEQ ID NO: 32)
<i>Per2</i>	Forward Primer 5'-ATGCTCGCCATCCACAAGA-3' (SEQ ID NO: 33) Reverse Primer 5'-GCGGAATCGAATGGGAGAAT-3' (SEQ ID NO: 34) Probe 5'-FAM-ATCCTACAGGCCGGTGGACAGCC-TAMRA-3' (SEQ ID NO: 35)
<i>Cry1</i>	Forward Primer 5'-CTGGCGTGGAAGTCATCGT-3' (SEQ ID NO: 36) Reverse Primer 5'-CTGTCCGCCATTGAGTTCTATG-3' (SEQ ID NO: 37) Probe 5'-FAM-CGCATTTACATACACTGTATGACCTGGACA-TAMRA-3' (SEQ ID NO: 38)

Gene	Primer/Probe sequences
<i>Cry2</i>	Forward Primer 5'- TGTCCCTTCCTGTGTGGAAGA-3' (SEQ ID NO: 39) Reverse Primer 5'- GCTCCCAGCTTGGCTTGA-3' (SEQ ID NO: 40) Probe 5'-FAM- CAGTCACCCTGTGGCAGAGCCTGG-TAMRA-3' (SEQ ID NO: 41)
<i>Dbp</i>	Forward Primer 5'- TGGCCCGAGTCTTTTTGC-3' (SEQ ID NO: 42) Reverse Primer 5'- GCGTCCAGGTCCACGTATTC-3' (SEQ ID NO: 43) Probe 5'-FAM- CCGCTGCTGTGGGAACGCACT-TAMRA-3' (SEQ ID NO: 44)
<i>Hif1a</i>	Forward primer 5'- AGATCTCGGCGAAGCAAAGAGT-3' (SEQ ID NO: 45) Reverse primer 5'- CGGCATCCAGAAGTTTTCTCACAC-3' (SEQ ID NO: 46)
<i>Hif2a</i>	Forward primer 5'- GTGACATGATCTTTCTGTCGGAA-3' (SEQ ID NO: 47) Reverse primer 5'- CGCAAGGATGAGTGAAGTCAAA-3' (SEQ ID NO: 48)
<i>Glut1</i>	Forward primer 5'- CAGTTCGGCTATAACACTGGTG-3' (SEQ ID NO: 49) Reverse primer 5'- GCCCCCGACAGAGAAGATG-3' (SEQ ID NO: 50)
<i>Pdk1</i>	Forward primer 5'- GGACTTCGGGTCAGTGAATGC-3' (SEQ ID NO: 51) Reverse primer 5'- TCCTGAGAAGATTGTCGGGGA-3' (SEQ ID NO: 52)
<i>Ldha</i>	Forward primer 5'- TGTCTCCAGCAAAGACTACTGT-3' (SEQ ID NO: 53) Reverse primer 5'- GACTGTACTTGACAATGTTGGGA-3' (SEQ ID NO: 54)

FAM = 6-carboxyfluorescein; TAMRA = tetramethylrhodamine

Protein assays:

Mouse kidney nuclei and brain were isolated according to procedures described by Aviram et al. [*Molecular Cell* 2016, 62:636-648]. Nuclei and cultured cells were homogenized in RIPA buffer (150 mM NaCl, 1 % NP-40, 0.5 % Na-deoxycholate, 0.1 % SDS (sodium dodecyl sulfate), 50 mM Tris-HCl pH 8, 1 mM dithiothreitol) supplemented with protease inhibitors (1 mM N-(α -aminoethyl) benzene-sulfonyl fluoride, 40 μ M bestatin, 15 μ M E65, 20 μ M leupeptin, 15 μ M pepstatin). The extracts were centrifuged to remove cell debris at 13,000 rotations per minute for 10 minutes at 4 °C. Samples were heated at 95 °C for 5 minutes in Laemmli sample buffer and analyzed by SDS-PAGE and immunoblotting, according to standard procedures. Antibodies used were rabbit anti-CLOCK, anti-CRY2, anti-PER2 and anti-REV-ERB α antibodies [Asher et al., *Cell* 2010, 142:943-953]; and mouse anti-HIF1 α , anti-tubulin and anti-U2AF antibodies.

EXAMPLE 1***Daily rhythms in blood and tissue oxygen levels***

In order to determine whether daily variations in oxygen levels occur, the oxygen consumption rate of mice was monitored using metabolic cages.

As shown in FIGs. 1A and 1B, the oxygen consumption rate was higher in the dark phase than in the light phase, suggesting that oxygen consumption increases during the night in mice, coinciding with their activity onset and food ingestion, both of which consume oxygen.

In view of the above result, it was examined whether daily changes in blood and tissue oxygen levels occur as well. Oxygen levels in blood of mice were measured using an oxygen optical fiber, and a telemetric oxygen electrode device was used to continuously monitor oxygen levels in kidney of freely moving rats, as depicted schematically in FIG. 2.

As shown in FIG. 3, oxygen levels in the blood oscillated with zenith levels during the dark phase.

As shown in FIGs. 4 and 5, kidney oxygenation was rhythmic, and reached peak levels during the dark phase.

Mean daily O₂ levels were calculated by averaging the mean daily maximal O₂ level with the mean daily minimal O₂ levels, among 5 animals. The mean daily O₂ levels in kidney were approximately 7 % (6.94 ± 1.34 %, mean $\pm$ SEM), which is in accordance with Carreau et al. [J Cell Mol Med 2011, 15:1239-1253].

As shown in FIG. 6, the range of the rhythmic daily changes in kidney oxygenation was approximately 3 % O₂.

Taken together, the above results indicate that daily oscillations in oxygen consumption and oxygen levels occur in various parts of the body throughout the day, with peak levels occurring during the night in mice.

Under normoxia, HIF1 α is rapidly degraded via the Von Hippel-Lindau (VHL)-mediated ubiquitin-proteasome degradation pathway. However, once oxygen levels decrease, HIF1 α degradation is inhibited and HIF1 α accumulates. Hence, HIF1 α protein levels are tightly regulated post-transcriptionally and inversely correspond to oxygen levels. The effect of the above-described rhythms in oxygen levels on HIF1 α protein levels throughout the day was therefore determined.

As shown in FIGs. 7A-7D, HIF1 α nuclear protein levels exhibited daily rhythms with peak levels at Zeitgeber Time (ZT) ~8 and ~12 in mouse kidney (FIGs. 7A and 7C) and brain (FIGs. 7B and 7D), respectively, indicating that accumulation of HIF1 α in brain is delayed by about 4 hours relative to kidney. As further shown therein, the peak in HIF1 α nuclear protein coincided with the peak in REF-ERB α protein levels in both kidney and brain tissue.

Overall, the above results indicate that daily rhythms in blood and tissue oxygenation occur in association with daily oscillations in HIF1 α protein levels.

EXAMPLE 2

Effect of physiological oxygen and HIF1 α rhythms on circadian clock synchronization in cultured cells

The effect of physiological rhythms in oxygen levels, with a 5 % and 8 % O₂ nadir and zenith respectively, on synchronization of circadian clocks was examined in a population of cultured cells. Special efforts were made to minimize any potential confounding effects by: (i) refraining from using firefly luciferase based circadian reporters, as the luciferase enzymatic activity is reported to be sensitive to oxygen levels

[Doran et al. *Biochimie* 2011, 93:361-368], and (ii) maintaining the incubator gas composition and temperature as constant as possible, by using special chambers with CO₂, O₂ and temperature controls (as detailed in the Materials and Methods section, and depicted schematically in FIG. 9). Temperature, CO₂, and O₂ levels were continuously
5 monitored throughout the experiment with constant temperature of 37 °C, 5 % CO₂ and O₂ levels as indicated. In order to generate rhythms in oxygen levels, O₂ was replaced with the inert gas nitrogen.

24 hours after cells (i.e., Hepa-1c1c7 or NIH3T3 cells) were seeded, they were exposed to 3 consecutive cycles of 12 hours 5 % O₂, and 12 hours 8 % O₂ (mimicking
10 physiological O₂ rhythms), and subsequently released to constant 8 % O₂ (free running period). The control cells were maintained under constant 8 % O₂ throughout the entire experiment. Samples were collected at 4-hour intervals in the course of the free running period (Day 4) and the expression level of clock genes was determined. The experimental protocol is depicted schematically in FIG. 10

15 As shown in FIGs. 11 and 12, the transcript levels of the clock genes *Clock*, *Bmal1*, *Cry1*, *Cry2*, *Per1*, *Per2*, *Rora*, *Rev-erba* and *Dbp* were relatively constant throughout the circadian cycle in a population of Hepa-1c1c7 (FIG. 11) or NIH3T3 (FIG. 12) cells cultured under constant 8% O₂ for 4 successive days. This result accords with the reported failure of clocks in individual cells in culture to maintain phase
20 coherence with their neighboring cells after several days, due to the weak coupling between cells in culture [Nagoshi et al., *Cell* 2004, 119:693-705].

In contrast, as further shown in FIGs. 11 and 12, exposure of Hepa-1c1c7 (FIG. 11) and NIH3T3 (FIG. 12) cells to physiological oxygen rhythms was associated with rhythmic expression of clock genes. As further shown therein, the phase relation (the
25 relative peak time expression of the different clock genes) closely resembled the one obtained with a dexamethasone pulse, which is widely used to synchronize clocks in cells, both in culture (as shown, for example, in FIG. 15) and *in vivo* (as shown, for example, in FIG. 8).

The above results indicate that physiological oxygen rhythms reset the
30 molecular clock in cultured cells.

HIF1 α not only responds to changes in oxygen levels, but has also been reported to play a role in oxygen homeostasis through gene expression regulation [Majmundar et

al., *Molecular Cell* 2010, 40:294-309]. The role of HIF1 α in resetting the molecular clock upon oxygen rhythms was therefore investigated. To this end, *Hif1 α* siRNA was employed in order to specifically knockdown *Hif1 α* .

As shown in FIG. 13, oxygen rhythms failed to elicit cyclic expression of clock genes in *Hif1 α* -deficient cells, and the expression levels of *Cry1*, *Cry2*, *Per1*, *Per2*, *Rora*, *Rev-erba* and *Dbp* were constantly low compared to those in control cells. *Hif1 α* knockdown exhibited a prominent effect on *Cry2* and *Rora* expression in particular, as their transcript levels were substantially lower in *Hif1 α* -deficient cells already at CT0.

As further shown in FIG. 13, *Hif2 α* transcript levels were not affected by knockdown of *Hif1 α* , indicating the specificity of the knockdown process.

As shown in FIG. 14, the protein levels of several clock genes (*Clock*, *Rev-erba*, *Per2*, and *Cry2*) were in accordance with the gene expression data presented in FIG. 13. As further shown therein, CLOCK protein levels were elevated in *Hif1 α* -deficient cells.

Thus, rhythmic expression of clock gene was not observed in *Hif1 α* -deficient cells. The above results raised the possibility that HIF1 α is either absolutely required for circadian rhythmicity (namely, a core clock component), or specifically essential for clock resetting by oxygen rhythms. In order to distinguish between these two scenarios, the requirement of HIF1 α for resetting the clock by dexamethasone was examined.

As shown in FIG. 15, knockdown of the *Clock* gene, a principal component of the core clock circuitry, completely abolished the circadian rhythmicity in cells synchronized by dexamethasone.

In contrast, as shown in FIG. 16, *Hif1 α* knockdown had little effect on the rhythmic expression of clock genes in cells synchronized by dexamethasone. Specifically, the expression profiles of *Clock*, *Bmal1*, *Cry1*, *Rev-erba* and *Dbp* were very similar irrespectively of *Hif1 α* levels, and the rhythmicity of *Cry2*, *Per1*, *Per2*, and *Rora*, was preserved although their expression levels were lower.

Taken together, the above results indicate that HIF1 α is specifically required for resetting the molecular clock by oxygen rhythms, but not for resetting the molecular clock by dexamethasone, and that HIF1 α is not an integral component of the core clock circuitry but rather functions upstream to the clock in response to changes in oxygen levels. Thus, the above results indicate that HIF1 α is the molecular link between oxygen and the circadian clock.

EXAMPLE 3***Effect of oxygen levels on core clock gene expression***

In order to identify potential downstream effectors within the core clock circuitry that connect oxygen rhythms and HIF1 α with circadian clock resetting, expression levels of clock genes in cultured cells were determined throughout an oxygen cycle, upon exposure to 12 hours of 5 % O₂ followed by 12 hours of 8% O₂, as depicted schematically in FIG. 17.

As shown in FIG. 18, transcript levels of *Cry2*, *Rora*, *Per1*, *Per2*, and *Rev-erba* were upregulated in response to a decrease in oxygen levels to 5 %, and *Cry1* levels were only increased when oxygen levels were restored to 8 %, whereas expression levels of *Clock* and *Bmal1* were mostly unaffected.

The role of HIF1 α in the induction of clock genes in the course of an oxygen cycle was then evaluated.

As shown in FIG. 19, induction of *Cry2*, *Rora*, *Per2*, *Cry1*, as well as *Glut1* (a known target of HIF1 α [Chen et al., *J Biol Chem* 2001, 276:9519-9525]), was blunted upon knockdown of *Hif1 α* . As further shown therein, the transcript levels of both *Cry2* and *Rora* were also downregulated in the absence of HIF1 α under 8 % O₂ (i.e., ZT0).

As shown in FIG. 20, *Cry2* and *Rora* responded to decrease in oxygen levels in a dose-dependent and HIF1 α -dependent manner.

Moreover, as shown in FIG. 21, the transcript levels of *Cry2* and *Rora* were highly sensitive to variations in oxygen levels within the physiological range, namely $\pm 3\%$ O₂.

These results indicate that in comparison to different known HIF1 α target genes, such as *Glut1*, *Pdk1* and *Ldha*, both *Cry2* and *Rora* are highly sensitive to small changes in O₂ levels, similarly to *Glut1*.

Bioinformatics analysis of the *Cry2* gene motifs was performed by mapping the gene using the MatInspectorTM software tool (Genomatix Software).

As shown in FIG. 22, several hypoxia response elements (HREs) and E-box motifs were found in the vicinity of the *Cry2* gene.

As HREs and E-box motifs are pertinent for HIF1 α and BMAL1 binding respectively, the binding of HIF1 α and BMAL1 in the vicinity of the *Cry2* gene was investigated by chromatin immunoprecipitation studies of the regions comprising HREs

and/or E-box motifs, as identified in FIG. 22, according to procedures described in the Materials and Methods section hereinabove. A region lacking an HRE or E-box motif served as a negative control (block G shown in FIG. 22). Real time PCR was performed with primers specifically designed for each of the indicated regions (blocks A-G shown in FIG. 22).

As shown in FIG. 23A, BMAL1 specifically exhibited rhythmic binding to a region within the *Cry2* promoter that contains both E-box and HRE motifs (i.e., block D, as shown in FIG. 22), with peak binding at ZT8 (zeitgeber time 8 hours), as determined by immunoprecipitation of chromatin from mouse liver.

As shown in FIG. 23B, specific binding of HIF1 α to the *Cry2* gene was not detected by immunoprecipitation of chromatin from mouse liver.

Similarly to the abovementioned results with mouse liver, as shown in FIGs. 24A-26A, BMAL1 specifically bound the same region within the *Cry2* promoter (i.e., block D, as shown in FIG. 22) in chromatin from mouse kidney (FIG. 24A) and cultured Hepa-1c1c7 cells (FIG. 25A) and NIH3T3 cells (FIG. 26A); whereas specific binding of HIF1 α to the *Cry2* gene was not detected by immunoprecipitation of chromatin (using anti-HIF1 α antibodies from different sources) from mouse kidney (FIGs. 24B and 24C).

As shown in FIGs. 25A-26B, hypoxia induced the expression of *Cry2* (FIG. 25B and FIG. 26B), but did not affect the binding of BMAL1 to the *Cry2* promoter (FIG. 25A and FIG. 26A), in both Hepa-1c1c7 cells (FIGs. 25A and 25B) and NIH3T3 cells (FIGs. 26A and 26B). These results indicate that the sensitivity of *Cry2* expression to oxygen levels does not reflect any effect of oxygen levels on BMAL1 levels.

The above results indicate that *Cry2* and *Rora* may connect the oxygen-HIF1 α axis and the circadian clock, as they readily responded to changes in oxygen levels in a HIF1 α -dependent manner.

In order to further examine the role of *Cry2* and *Rora* as a molecular link between the oxygen-HIF1 α axis and the circadian clock, their ability to phenocopy the effect of HIF1 α knockdown on circadian clock resetting by oxygen rhythms was evaluated.

As shown in FIGs. 27 and 28, knockdown of *Cry2* resulted in phase delay in the expression of clock genes upon exposure to oxygen rhythms (FIG. 27) or dexamethasone (FIG. 28).

As shown in FIGs. 29 and 30, knockdown of *Rora* resulted in reduction of the daily expression levels of several clock genes following oxygen rhythms (FIG. 29), but had little effect upon exposure to dexamethasone (FIG. 30).

Although both *Cry2* and *Rora* responded to changes in oxygen levels in HIF1 α -dependent manner, neither knockdown of *Cry2* nor of *Rora* mimicked the effect of *Hif1 α* knockdown, suggesting that resetting the circadian clock by the oxygen-HIF1 α axis is mediated by the concerted action of several clock genes and potentially by other factors.

EXAMPLE 4

Effect of low oxygen on adaptation of mice in an in vivo jet lag model

In order to assess the role of the above-described mechanisms *in vivo*, the effect of moderate reduction in oxygen levels on the daily voluntary locomotor activity of mice was evaluated under different light-dark regimens. Upon 12-hour light-dark cycles, mice exhibit robust rest-activity cycles, with activity onset every 24 hours once light is turned off. This activity pattern is preserved in mice under constant dark, albeit with a slightly shorter period than 24 hours [Partch et al., *Trends in Cell Biology* 2014, 24:90-99].

The effect of cycles of 12 hours of 21 % and 12 hours of 16 % O₂ (in the subjective dark and light phase, respectively) on 24 hour schedules of mice in constant dark.

As shown in FIGs. 31 and 32, the circadian period of mice housed under rhythmic oxygen levels was similar to those of mice housed under constant oxygen levels.

One of the key properties of the circadian clock is its phase resetting to a new lighting schedule. The effect of ambient oxygen levels was therefore determined in an experimental jet lag protocol, with a 6-hour phase advance in the lighting schedule, corresponding to a phase shift associated with travelling eastward.

As shown in FIGs. 33 and 34, a 12-hour exposure to 16 % O₂ prior to the shift in lighting schedule considerably accelerated the adaptation of mice to the new lighting schedule.

Furthermore, as shown in FIGs. 35 and 36, even a short pulse of 2 hours of 14 % O₂ following the shift in lighting schedule shortened the adaptation time of mice.

These results indicate that low oxygen levels, before or after a shift in schedule (e.g., as in jet lag), enhance *in vivo* adaptation to a new schedule.

The role of HIF1 α in the above-described *in vivo* effect was then determined. As *Hif1 α* -null homozygous mice are reported to be nonviable [Iyer et al., *Genes & Development* 1998, 12:149-162], *Hif1 α* heterozygous mice were tested.

As shown in FIGs. 37 and 38, *Hif1 α* transcript (FIG. 37) and protein levels (FIG. 38) were decreased by approximately 50 % in *Hif1 α ^{+/-}* mice, as compared to their wild-type littermates.

As shown in FIGs. 39 and 40, *Hif1 α* -deficient mice did not differ from their wild-type littermates in their circadian period.

Similarly, as shown in FIGs. 41 and 42, *Hif1 α* -deficient mice did not differ from their wild-type littermates in their adjustment to 6-hour shifts in the lighting schedule.

However, as shown in FIGs. 43-46, neither 12 hours of 16 % O₂ (FIGs. 43 and 44) nor 2 hours of 14 % O₂ (FIG. 45 and 46) accelerated the adaptation of HIF1 α -deficient mice to the new schedule in a jet lag protocol, in contrast to the results in wild-type mice (e.g., as shown in FIGs. 33-36).

The expression levels of clock genes in whole brain samples of wild-type mice and *Hif1 α* -deficient littermates were then evaluated, upon exposure for 2 hours to 14 % O₂.

As shown in FIG. 47, 2-hour exposure to 14 % O₂ induced a statistically significant increase in *Cry2* expression (as well as a similar increase in expression of *Ldha*, a known HIF1 α target) in wild-type mice, and this increase was abrogated in *Hif1 α* -deficient mice.

As further shown in FIG. 47, both *Per1* and *Cry1* transcript levels were down-regulated in *Hif1 α* -deficient mice irrespectively of oxygen levels.

The above results indicate that HIF1 α is required for resetting of circadian clocks by oxygen levels in both cultured cells and *in vivo*.

The role of oxygen rhythms and HIF1 α on circadian clocks is depicted schematically in FIG. 48.

EXAMPLE 5

Effect of low oxygen pulse on circadian phase shifting

In order to further characterize the effect of oxygen levels on circadian phase shifting, NIH3T3-Bmal-Luc1 cells were used in order to monitor circadian gene expression. These cells express a short-lived luciferase transcript from Bmal1 promoter [Nagoshi et al., *Cell* 2004, 119:693-705].

Cells were grown under 21 % O₂ and were synchronized by exposure to fresh medium at time 0. The phase of the circadian rhythm of cell samples was determined by real-time bioluminescence recording using a LumiCycle™ 32 luminometer (Actimetrics). During the second luminescence cycle (circadian time 0 = 24 hours after synchronization by fresh medium, corresponding to a minimum of luminescence), cells were exposed to 5 % O₂ for 2 hours at the indicated circadian times (3, 4.5, 7, 12, 20.5, 22, 23, 24.5 hours). The phase shift was quantified by recording the time during the next luminescence cycle at which luminescence was at a maximum (corresponding to 1.5 circadian periods after CT = 0), and plotted against circadian time. In untreated cells, the maximum was at about CT = 43 hours.

As shown in FIG. 49, phase shifts of at least 8 hours, as either an advance or as a delay of the circadian rhythm, were induced by a 2 hour exposure to 5 % O₂ at particular circadian times (~12 and ~20 hours), whereas at other circadian times (~0 and ~24 hours) the magnitude of the phase shift was much smaller.

These results indicate that a magnitude and direction of a circadian rhythm phase shift can be selected by modulating oxygen levels at an appropriate time of day.

As further shown in FIG. 49, the magnitude and direction of phase shifts induced by low oxygen treatment at various times were such that the cells after treatment were in similar stages of the circadian rhythm, corresponding to untreated cells at a CT in a range of about 1 to 4 hours (following phase delay upon treatment at CT = 3-12 hours) or about 26 to 29 hours (following phase advance upon treatment at CT = 20.5-24.5 hours). As the circadian period the cultured cells was slightly more

than 24 hours, the CT time periods of about 1-4 hours and about 26-29 hours are nearly equivalent.

The above experiment was repeated with measurements at a greater number of time points.

5 As shown in FIG. 50, maximal phase shifts induced by a 2 hour exposure to 5 % O₂ were observed at circadian times of about 13 hours (about a 5 hour phase shift) and about 20 hours (about an 8 hour phase shift), similarly to the results shown in FIG. 49. As further shown therein, the magnitude and direction of phase shifts induced by low oxygen treatment at various times were such that the cells after treatment were in
10 similar stages of the circadian rhythm (about 0-4 hours or about 24-28 hours, for most treatment times).

As further shown in FIG. 50, low oxygen treatment at a circadian time of 17.5 hours, disrupted the circadian rhythms of the cells. This result is consistent with the circadian time being between a circadian time range (~13 hours) associated with a
15 marked phase delay in the circadian rhythm and a circadian time range (~20 hours) associated with a marked phase advance.

These results indicate that cells can be "reset" to a particular range of the circadian period by modulating oxygen levels.

Furthermore, based on the expression of the reporter gene in cells and in mice, it
20 was estimated that the circadian time of 20 hours in cells (at which a maximal phase advance was observed) corresponded to the middle of the sleeping period of mice (e.g., about noon, as mice are nocturnal).

EXAMPLE 6

Effect of low oxygen on adaptation of mice in an in vivo jet lag model with a delay in lighting schedule

In order to measure the efficacy of transient low oxygen levels in a jet lag model with a delay in lighting schedule, corresponding to a phase shift associated with travelling westward, the effect of moderate reduction in oxygen levels on the daily
30 voluntary locomotor activity of mice is evaluated according to procedures such as described in Example 4, except that the protocol comprises a delay (e.g., a 6 hour delay) in the lighting schedule instead of an advance in the lighting schedule. Adaptation of

animals exposed to transient low oxygen levels to the change in lighting schedule is compared to adaptation of animals maintained under ambient oxygen levels.

EXAMPLE 7

Effect of high oxygen on circadian rhythms

The effect of transient high oxygen levels on circadian rhythms is evaluated *in vitro* and/or *in vivo*.

After cells (e.g., Hepa-1c1c7 or NIH3T3 cells) are seeded (e.g., 24 hours after seeding), they are exposed to cycles of more than 8 % O₂ (e.g., about 12 % O₂, about 30 % O₂, and/or about 100 % O₂) and 8 % O₂, (optionally followed by a running period of constant O₂ concentration), according to procedures such as described in Example 2, except that high O₂ levels, i.e., more than 8 % O₂ (e.g., about 12 % O₂, about 30 % O₂, and/or about 100 % O₂) are used rather than the low levels (5 % O₂) described in Example 2 and/or Example 3. Control cells may optionally be maintained under constant O₂ concentration (e.g., about 12 % O₂, about 30 % O₂, and/or about 100 % O₂) throughout the entire experiment. Expression of clock genes is then evaluated, according to procedures described hereinabove. The ability of high O₂ levels to enhance rhythmicity of clock gene expression represents an efficacy at synchronizing/resetting circadian clocks in cells.

The effect of transient high oxygen levels is optionally determined in *Hif1α*-deficient cells, according to procedures such as described in Example 2 and/or Example 3, in order to assess whether effects of transient high oxygen levels are mediated by modulation (e.g., reduction) of HIF1α levels.

In order to measure the efficacy of transient high oxygen levels in an *in vivo* jet lag model, the effect of increased oxygen levels (e.g., in a range of from 30 % to 100 % O₂, optionally from 30 % to 60 % O₂) on the daily voluntary locomotor activity of mice is evaluated in a model corresponding to eastward travel, according to procedures such as described in Example 4, and/or in a model corresponding to eastward travel, according to procedures such as described in Example 6, except that transient exposure to high oxygen levels (e.g., 12 hours of 30-100 % (optionally 30-60 %) O₂ prior to the shift in lighting schedule, and/or a 1-2 hour pulse prior to and/or following the shift in lighting schedule) is performed instead of transient exposure to low oxygen levels.

Adaptation of animals exposed to transient high oxygen levels to the change in lighting schedule is compared to adaptation of animals maintained under ambient oxygen levels.

The effect of transient high oxygen levels is optionally determined in *Hif1 α* -deficient mice, according to procedures such as described in Example 4, in order to assess whether effects of transient high oxygen levels are mediated by modulation (e.g., reduction) of HIF1 α levels.

EXAMPLE 8

Effect of HIF activators and repressors on adaptation to circadian rhythm phase shifts

The effect of HIF-modulating agents on adaptation to circadian rhythm phase shifts is evaluated *in vivo* in an *in vivo* jet lag model. The effect of HIF-modulating agents on the daily voluntary locomotor activity of mice is evaluated in a model corresponding to eastward travel, according to procedures such as described in Example 4, and/or in a model corresponding to eastward travel, according to procedures such as described in Example 6, except that administration of a HIF-modulating agent is performed instead of exposure of mice to low oxygen levels. In addition, the effect of HIF-modulating agents on circadian period length is optionally evaluated *in vivo* (e.g., as described in Example 4).

The HIF-modulating agents may optionally be a lentivirus expressing HIF (in a non-inducible or inducible form), dimethyloxallylglycine, IOX2, roxadustat (FG4592), cobalt (e.g., cobalt chloride) or an iron chelator (e.g., desferrioxamine, deferiprone, deferasirox), for activating HIF; or a lentivirus expressing shRNA for HIF (in a non-inducible or inducible form), PX12, BAY87-2243 or KC7F2, for repressing HIF.

Adaptation of animals treated with a HIF activator may optionally be compared to adaptation of animals exposed to HIF-activating low oxygen levels (e.g., as described in Example 4 and/or 6). Adaptation of animals treated with a HIF repressor may optionally be compared to adaptation of animals exposed to high oxygen levels (e.g., as described in Example 7).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all

such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

5 All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A system configured for exposing a subject to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa, the system being for use in the treatment of a circadian rhythm disorder.
2. The system of claim 1, comprising a breathing compartment configured for exposing the subject to said atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa, and an apparatus configured for providing to said breathing compartment a gas selected to produce said atmosphere in said breathing compartment.
3. The system of claim 2, wherein said breathing compartment is configured as a closed space.
4. The system of any one of claims 2 to 3, wherein said breathing compartment is configured to allow said subject to reside therein.
5. The system of any one of claims 2 to 3, wherein said breathing compartment is configured for delivering said atmosphere to a breathing orifice of a subject.
6. The system of any one of claims 1 to 5, being a portable system.
7. The system of any one of claims 2 to 6, wherein said atmosphere in said breathing compartment has an oxygen concentration of no more than 20 %.
8. The system of any one of claims 2 to 6, wherein said atmosphere in said breathing compartment has an oxygen concentration of at least 22 %.
9. The system of any one of claims 1 to 8, wherein said circadian rhythm disorder is selected from the group consisting of jet lag and shift work sleep disorder.

10. The system of any one of claims 1 to 9, wherein said prevalent oxygen partial pressure is an ambient oxygen partial pressure prior to a zeitgeber phase shift associated with said disorder.

11. The system of any one of claims 1 to 9, wherein said prevalent oxygen partial pressure is about 21 kPa.

12. The system of claim 9, wherein said circadian rhythm disorder comprises jet lag, and said prevalent oxygen partial pressure is an ambient oxygen partial pressure during air travel associated with said jet lag.

13. The system of any one of claims 1 to 12, wherein said atmosphere has an oxygen partial pressure which differs from said prevalent oxygen partial pressure by at least 3 kPa.

14. The system of any one of claims 1 to 13, being configured for exposing a subject to an atmosphere having an oxygen partial pressure of no more than 16 kPa.

15. The system of any one of claims 1 to 14, wherein a pressure of said atmosphere is about equal to an ambient atmospheric pressure.

16. The system of any one of claims 1 to 13, being configured for exposing a subject to an atmosphere having an oxygen partial pressure in a range of from 33 to 100 kPa.

17. The system of any one of claims 1 to 16, being further configured for controlling an intensity of light which reaches the eyes of said subject.

18. The system of any one of claims 1 to 17, further comprising a control unit configured for controlling a parameter selected from the group consisting of an oxygen content of said atmosphere, a pressure of said atmosphere and an intensity of light which reaches the eyes of said subject.

19. The system of any one of claims 1 to 18, further comprising instructions for controlling a parameter selected from the group consisting of an oxygen content of said atmosphere, a pressure of said atmosphere and an intensity of light which reaches the eyes of said subject, in accordance with a given time of day, said prevalent oxygen partial pressure, and/or a magnitude and/or direction of zeitgeber phase shift.

20. The system of any one of claims 18 and 19, wherein said controlling of said parameter is determined in accordance with a given time of day, said prevalent oxygen partial pressure, and/or a magnitude and/or direction of zeitgeber phase shift.

21. The system of any one of claims 18 to 20, wherein controlling is adapted for treating jet lag by exposing a subject to said atmosphere during a pre-determined time of day according to local time at a destination of a journey associated with said jet lag.

22. The system of any one of claims 1 to 21, wherein said treatment comprises exposure to said atmosphere for up to 4 hours.

23. The system of claim 22, wherein said oxygen partial pressure of said atmosphere differs from said prevalent oxygen partial pressure by at least 5 kPa.

24. The system of any one of claims 1 to 21, wherein said treatment comprises exposure to said atmosphere for at least 6 hours.

25. A system configured for exposing a subject in an aircraft to an atmosphere having an oxygen partial pressure which differs from a prevalent oxygen partial pressure by at least 1 kPa, the system being configured for use in an aircraft cabin, and comprising a breathing compartment configured for delivering said atmosphere to a breathing orifice of a subject, and an apparatus configured for providing to said breathing compartment a gas selected to produce said atmosphere in said breathing compartment, the system being for use in the treatment of jet lag.

26. The system of claim 25, wherein said prevalent oxygen partial pressure is an ambient oxygen partial pressure prior to flight of said aircraft.

27. The system of claim 25, wherein said prevalent oxygen partial pressure is about 21 kPa.

28. The system of claim 25, wherein said prevalent oxygen partial pressure is an ambient oxygen partial pressure in said aircraft cabin.

29. The system of any one of claims 25 to 28, wherein a pressure of said atmosphere is about equal to an ambient pressure in said aircraft cabin.

30. The system of any one of claims 25 to 29, wherein said atmosphere in said breathing compartment has an oxygen concentration of no more than 20 %.

31. The system of any one of claims 25 to 29, wherein said atmosphere in said breathing compartment has an oxygen concentration of at least 22 %.

32. The system of any one of claims 25 to 31, further comprising a control unit configured for controlling a parameter selected from the group consisting of an oxygen content of said atmosphere, a pressure of said atmosphere and an intensity of light which reaches the eyes of said subject.

33. The system of claim 32, wherein said controlling of said parameter is determined in accordance with a given time of day, said prevalent oxygen partial pressure, and/or a magnitude and/or direction of zeitgeber phase shift.

34. The system of claim 32, wherein controlling is adapted for exposing said subject to said atmosphere during a pre-determined time of day according to local time at a destination of said aircraft.

35. The system of any one of claims 1 to 34, wherein the treatment further comprises administration of an effective amount of an agent which modulates an activity of HIF1 α .

36. An aircraft comprising a system configured for effecting a change in an oxygen partial pressure of an atmosphere within the aircraft cabin, wherein said change in an oxygen partial pressure comprises a change of at least 1 kPa during a flight of the aircraft at cruising altitude.

37. The aircraft of claim 36, wherein said change in an oxygen partial pressure is selected for use in the treatment of jet lag of subjects arriving at a destination of said aircraft.

38. An enclosed space in an airport terminal, the enclosed space comprising a system configured for effecting a daily change in an oxygen partial pressure of an atmosphere within the enclosed space, wherein said change in an oxygen partial pressure comprises a change of at least 1 kPa during a course of a day.

39. The enclosed space of claim 38, wherein said daily change in an oxygen partial pressure is selected for use in the treatment of jet lag of subjects arriving at said airport terminal following air travel.

40. An agent which modulates an activity of HIF1 α , for use in the treatment of a circadian rhythm disorder.

41. The agent of claim 40, being selected from the group consisting of an up-regulator of HIF1 α and a down-regulator of HIF1 α .

42. The agent of claim 41, wherein said up-regulator of HIF1 α is selected from the group consisting of cobalt, dimethyloxalylglycine, desferrioxamine, deferiprone, deferasirox, IOX2, roxadustat (FG4592), and an inhibitor of von Hippel-Lindau tumor suppressor (VHL).

43. The agent of claim 41, wherein said down-regulator of HIF1 α is selected from the group consisting of PX12, BAY87-2243 and KC7F2.

44. The agent of any one of claims 40 to 42, wherein said circadian rhythm disorder is selected from the group consisting of jet lag and shift work sleep disorder.

45. The agent of any one of claims 40 to 44, wherein said treatment further comprises exposure to a hypoxic atmosphere and/or to a hyperoxic atmosphere.

46. The agent of claim 45, wherein said hypoxic atmosphere and/or said hyperoxic atmosphere is generated by the system of any one of claims 1 to 20.

47. The agent of any one of claims 40 to 46, wherein said circadian rhythm disorder is jet lag and said treatment is effected during a pre-determined time of day according to local time at a destination of a journey associated with said jet lag.

48. A kit comprising the agent of any one of claims 40 to 46, and instructions for enhancing said treatment by controlling exposure to a hypoxic atmosphere and/or to a controlled intensity of light.

49. A pharmaceutical composition comprising an agent which modulates an activity of HIF1 α , and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49, being formulated as a unit dosage form comprising an amount of said agent which is effective for treating a circadian rhythm disorder.

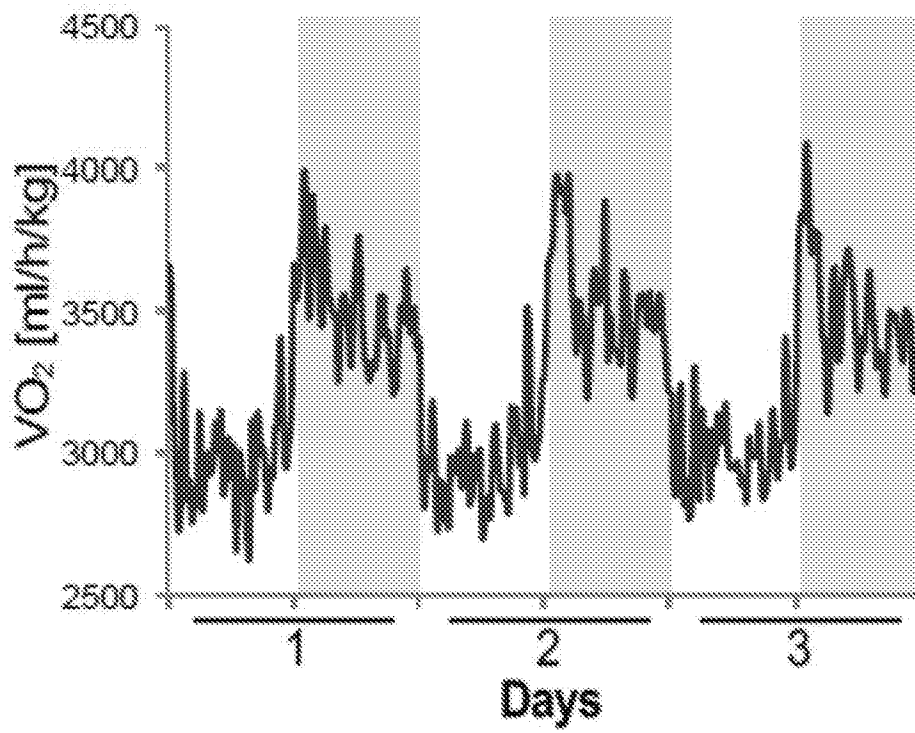


FIG. 1A

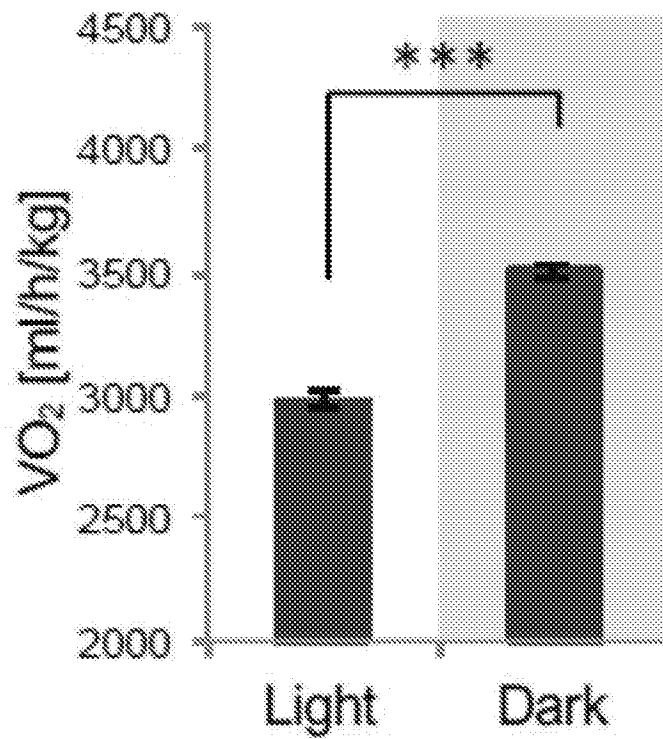


FIG. 1B

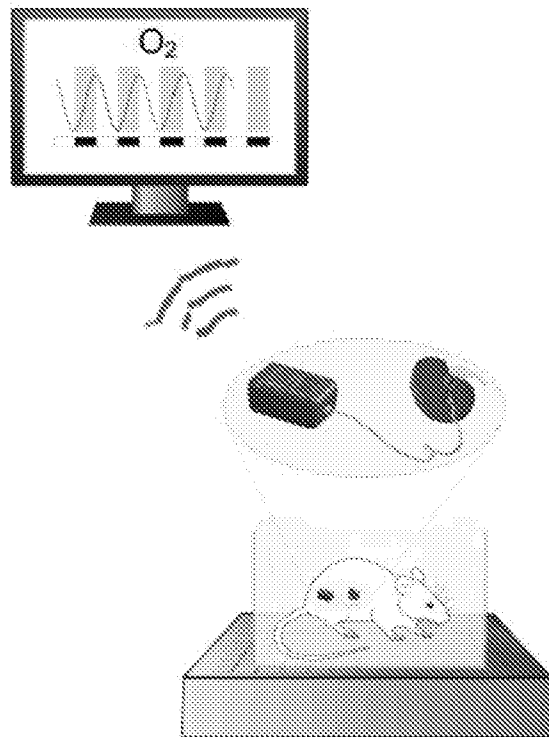


FIG. 2

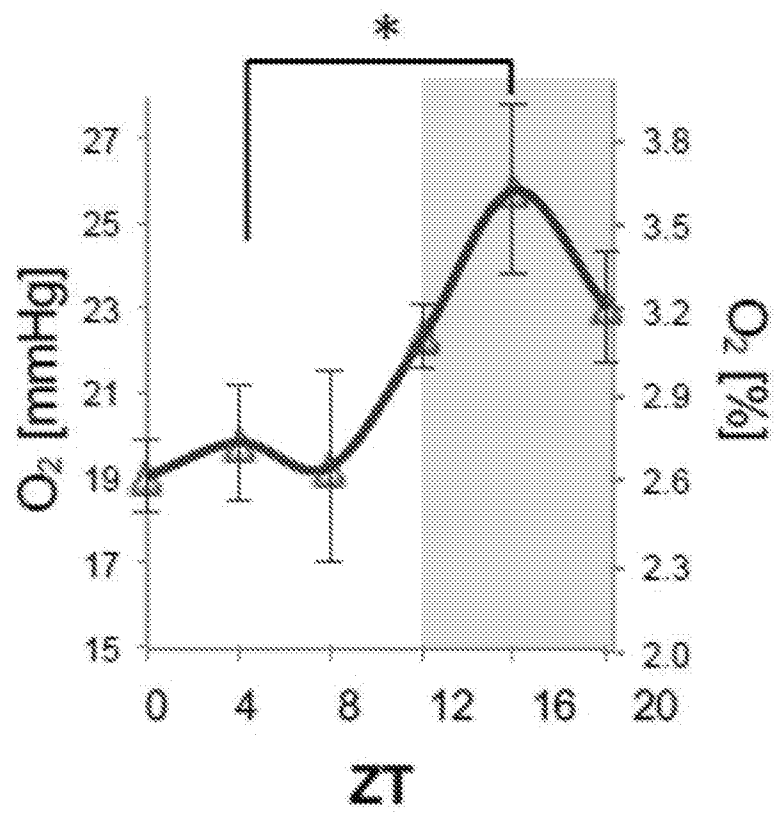


FIG. 3

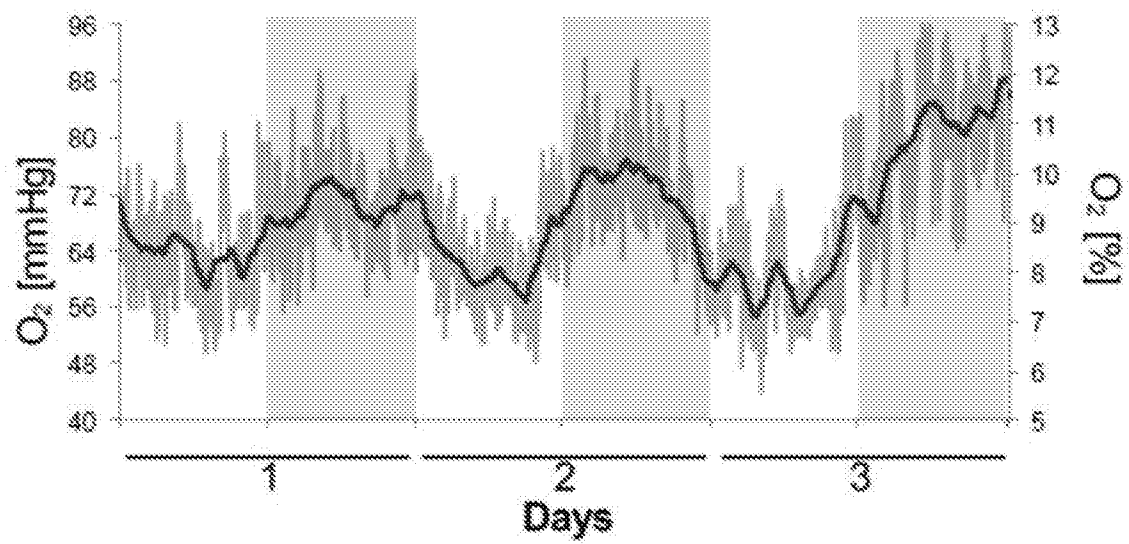


FIG. 4

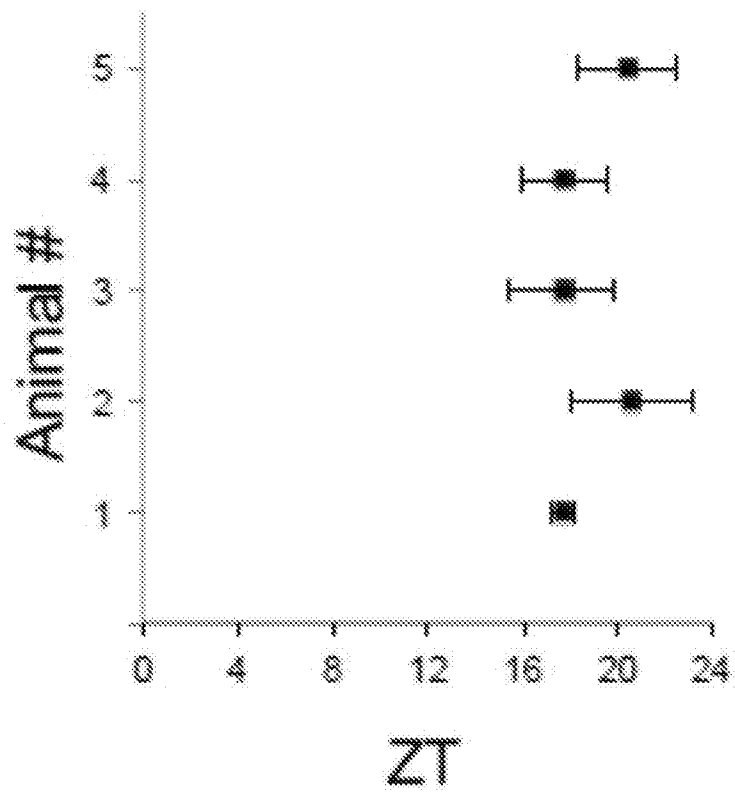


FIG. 5

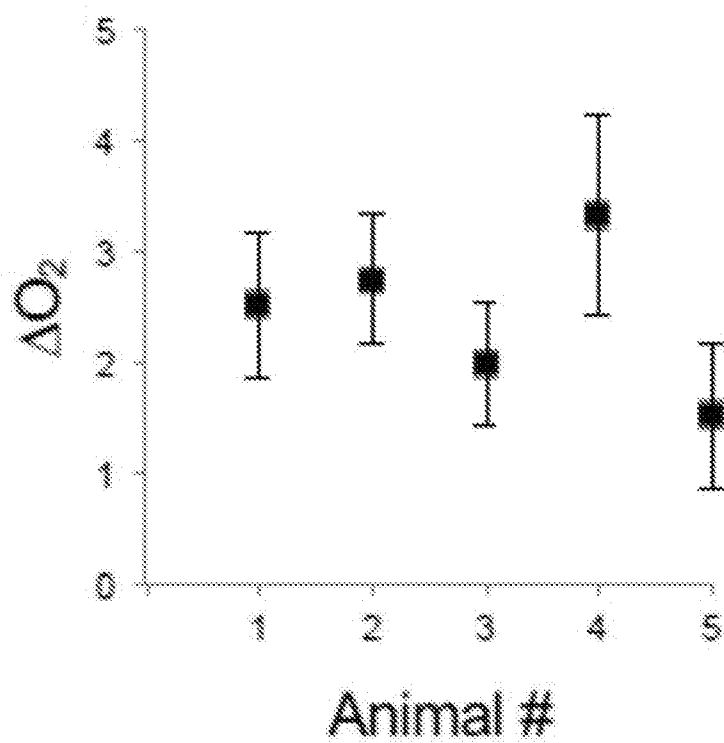


FIG. 6

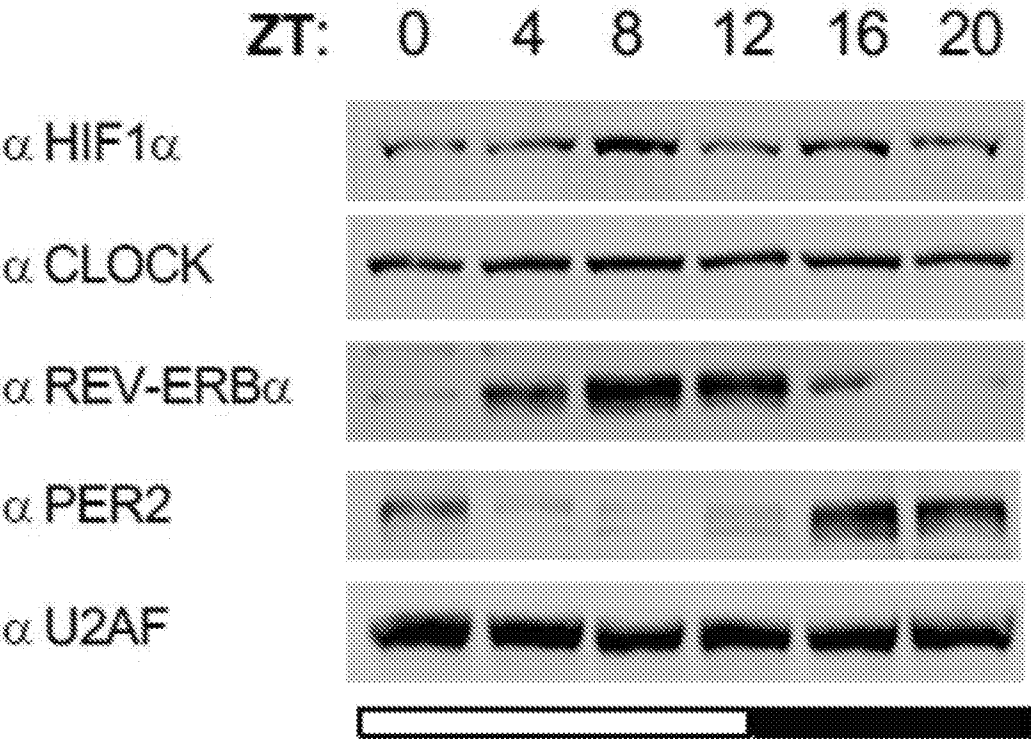


FIG. 7A

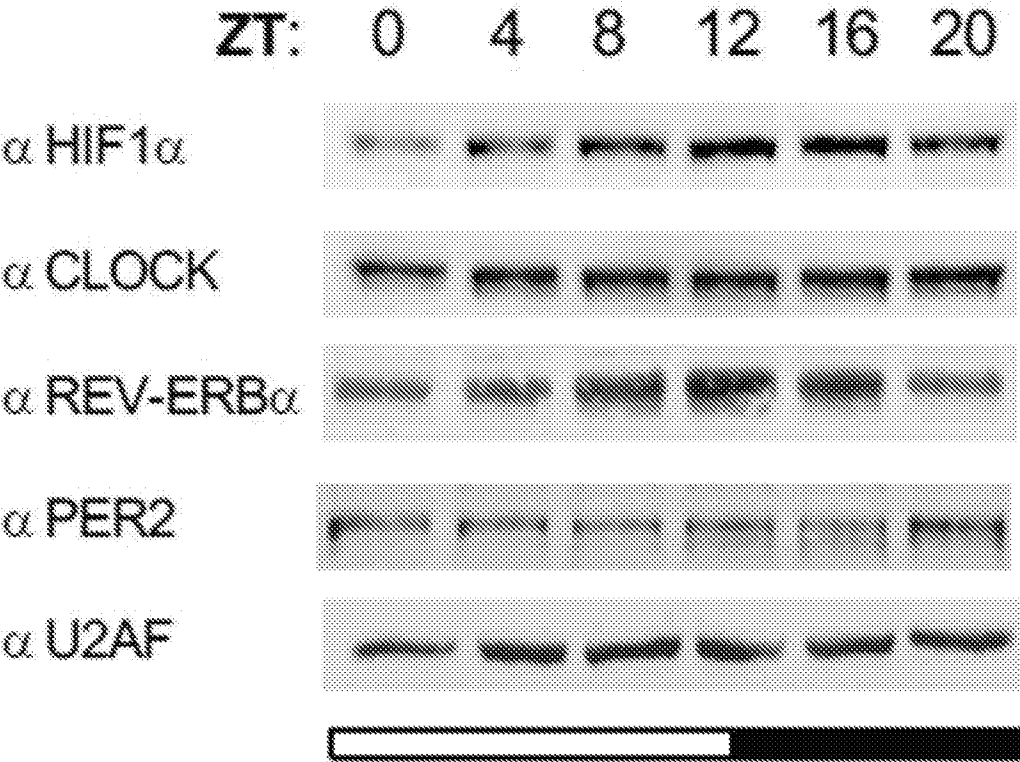


FIG. 7B

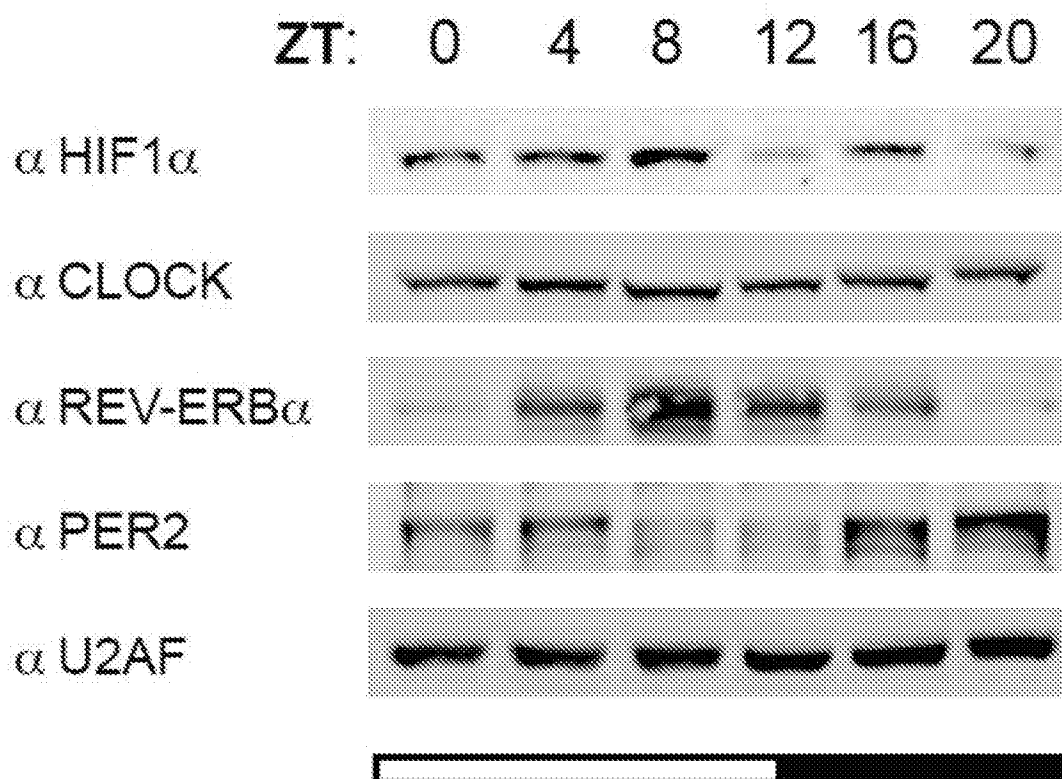


FIG. 7C

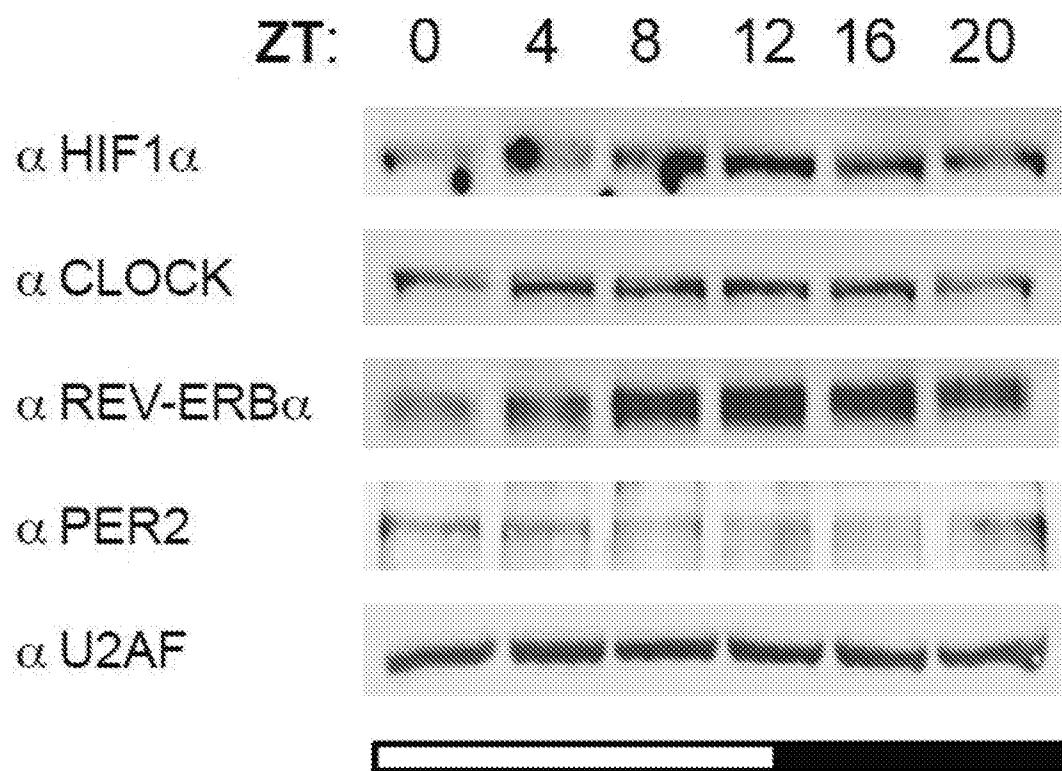


FIG. 7D

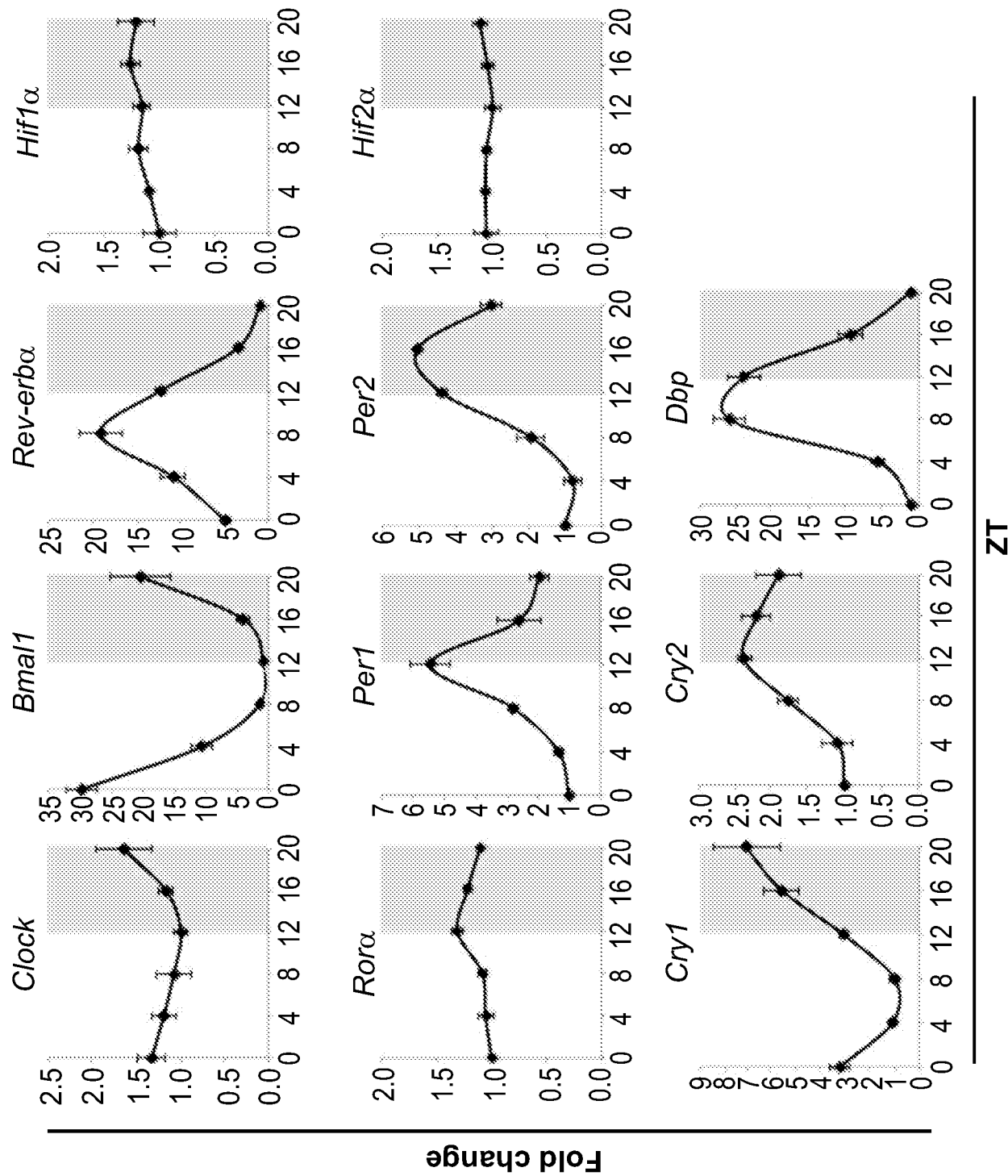


FIG. 8

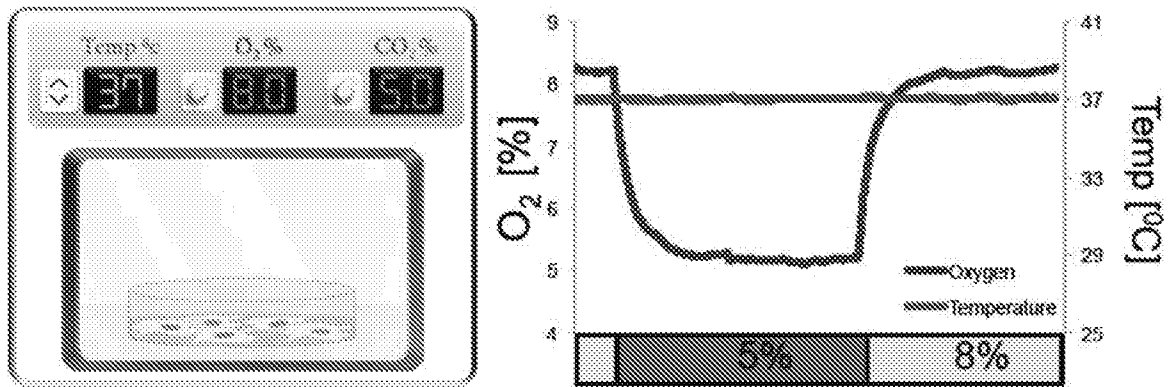


FIG. 9

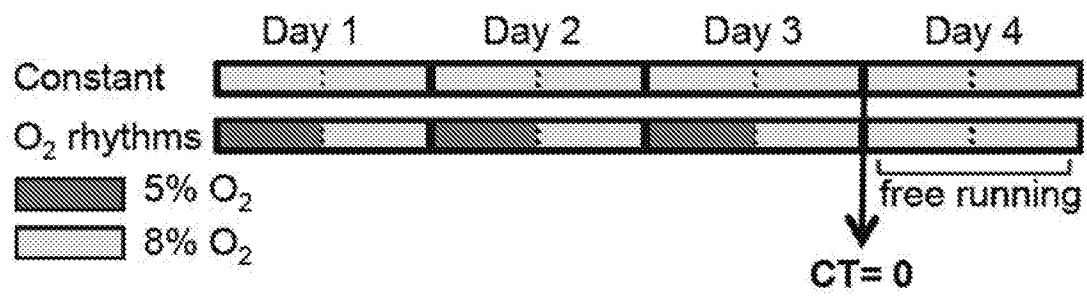


FIG. 10

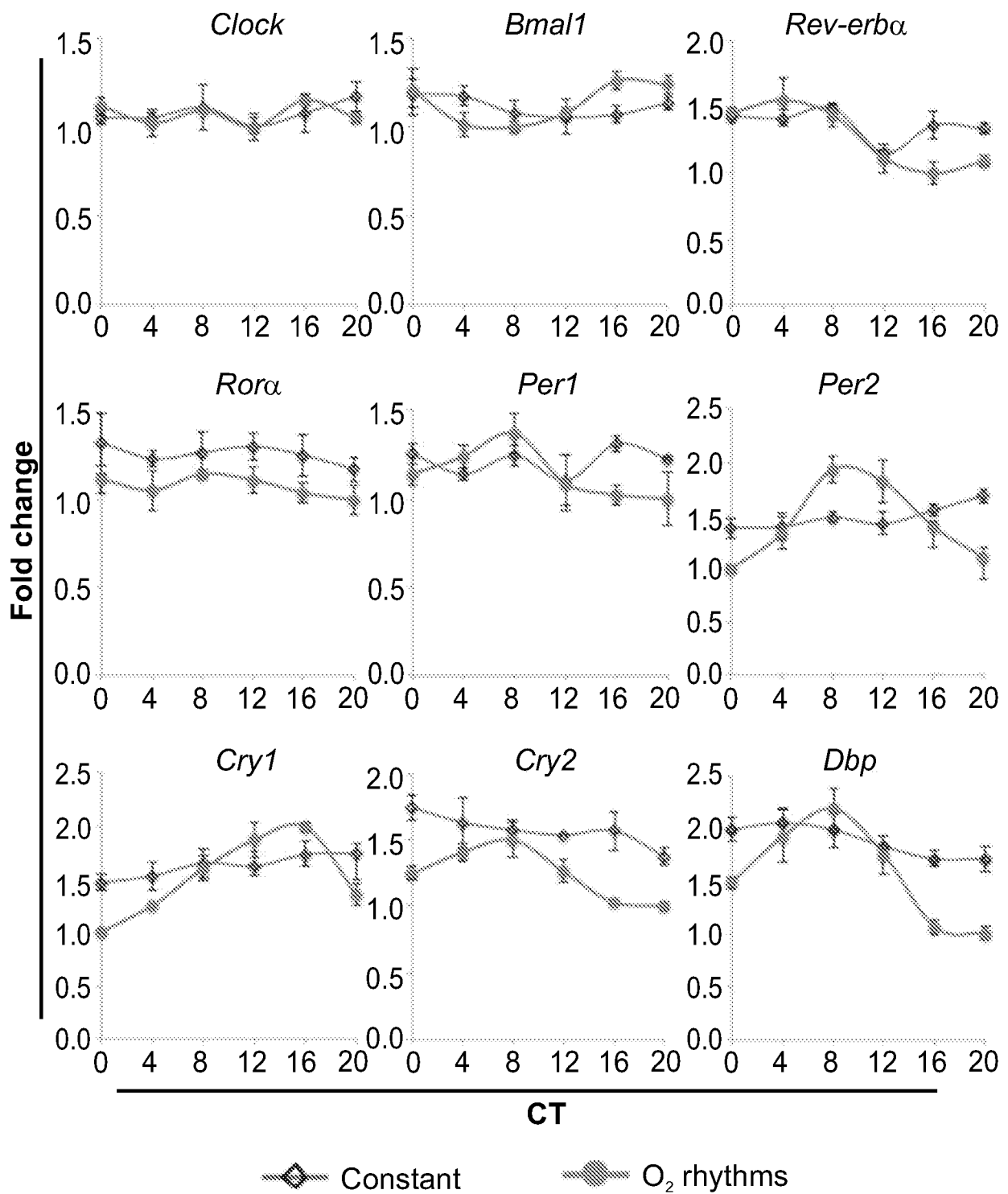


FIG. 11

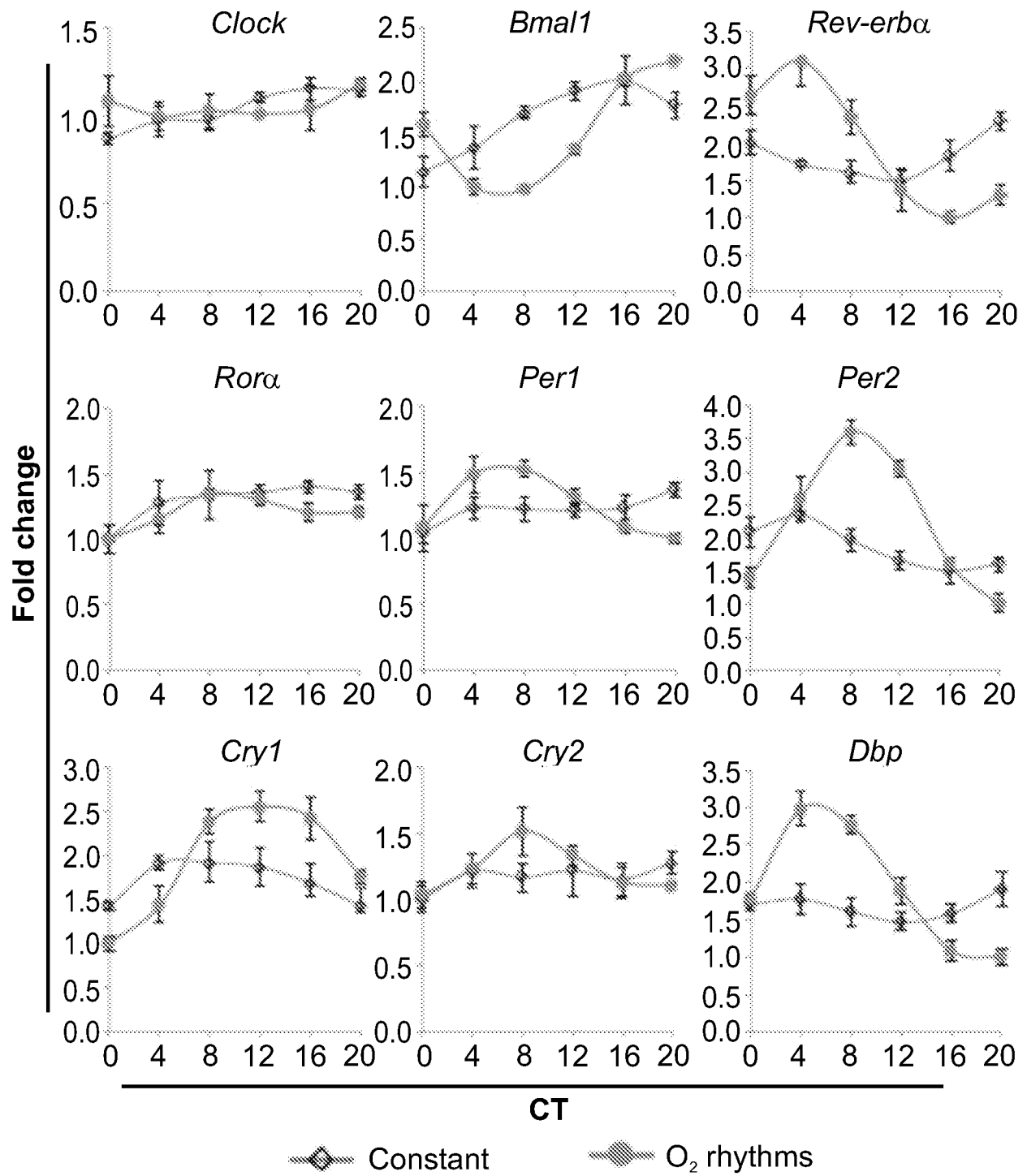


FIG. 12

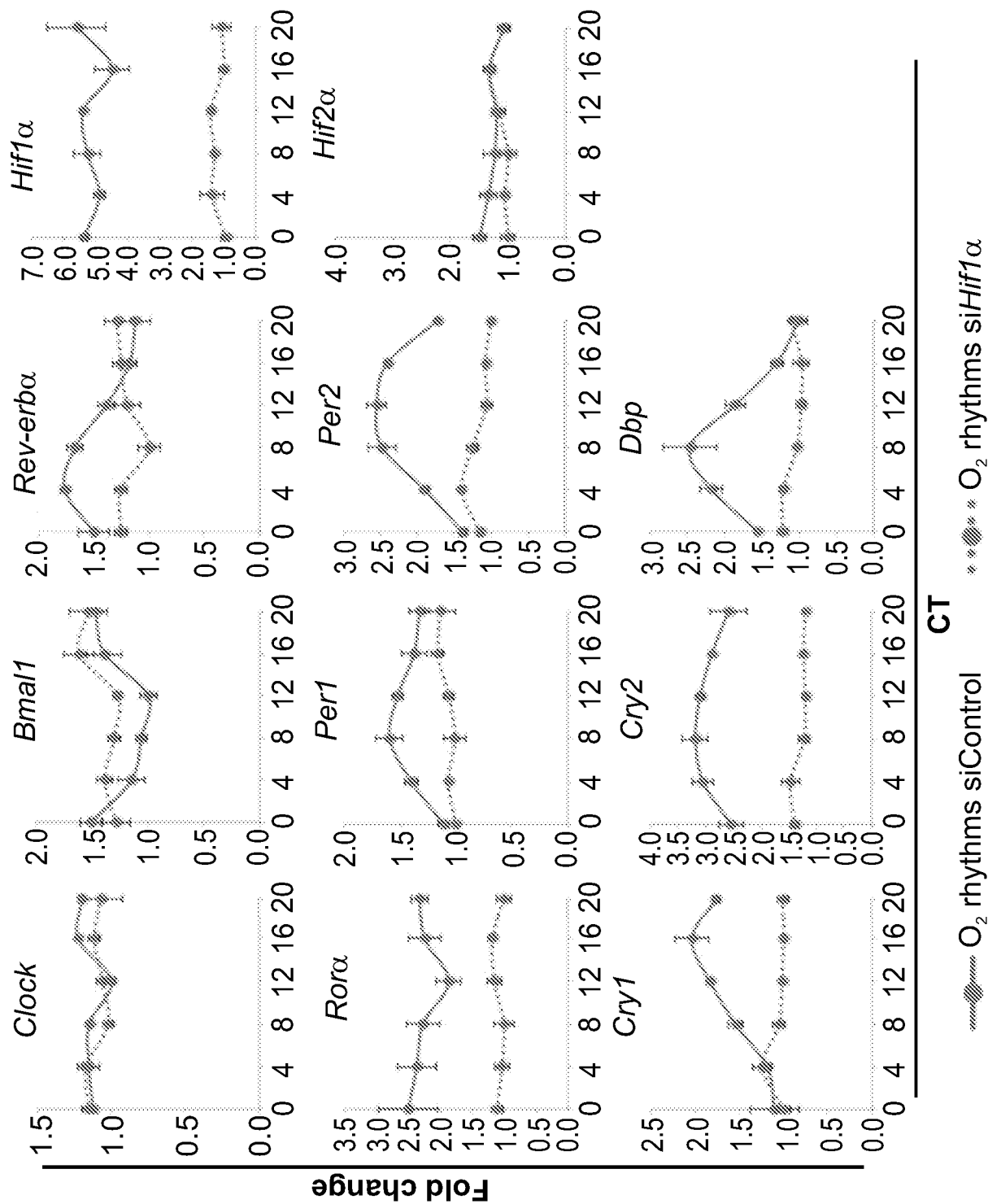


FIG. 13

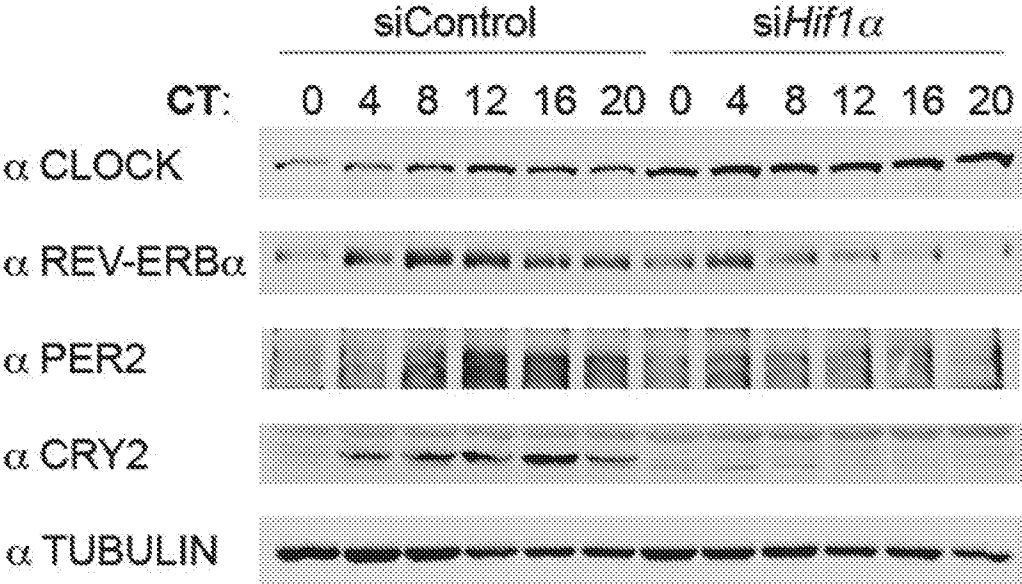


FIG. 14

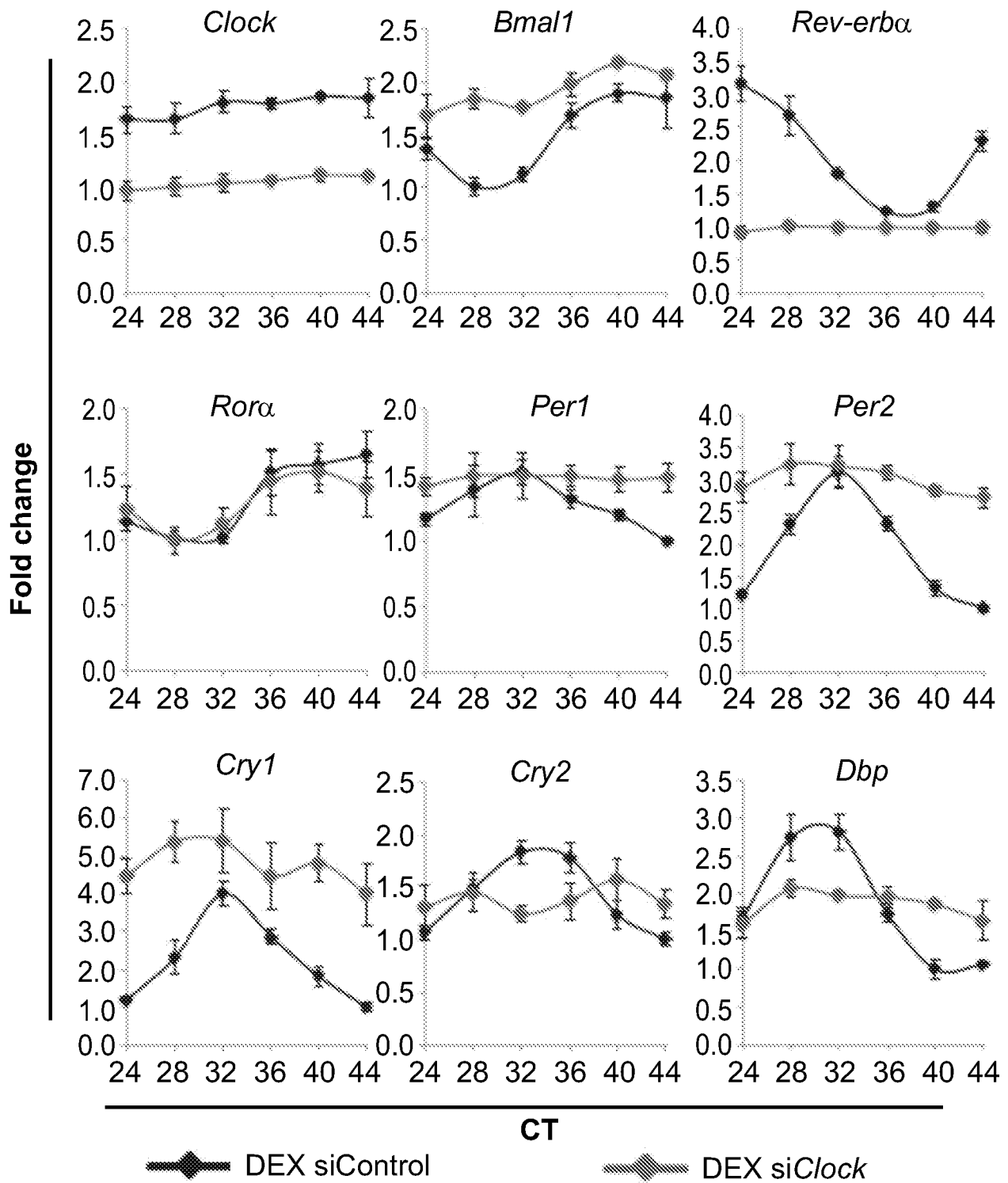


FIG. 15

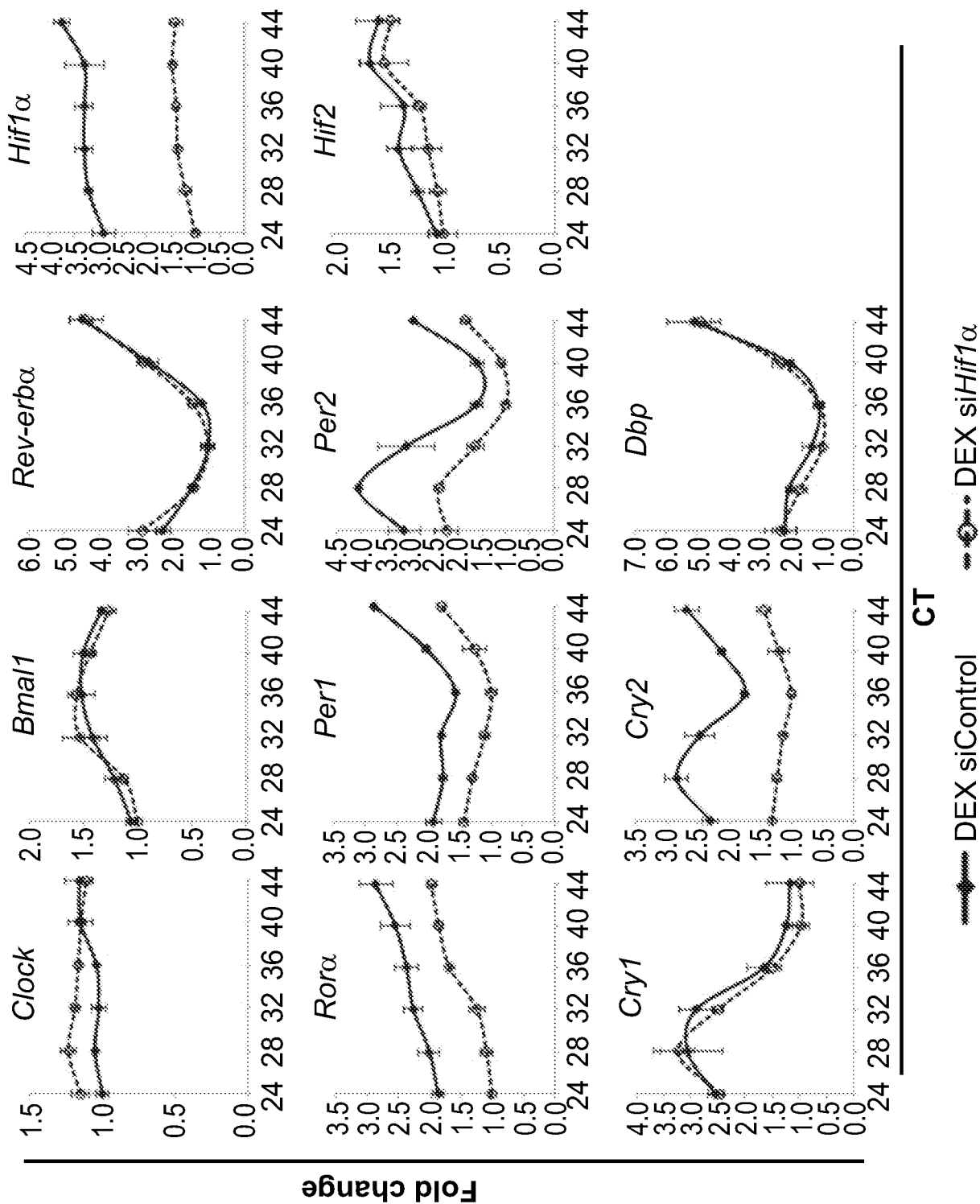
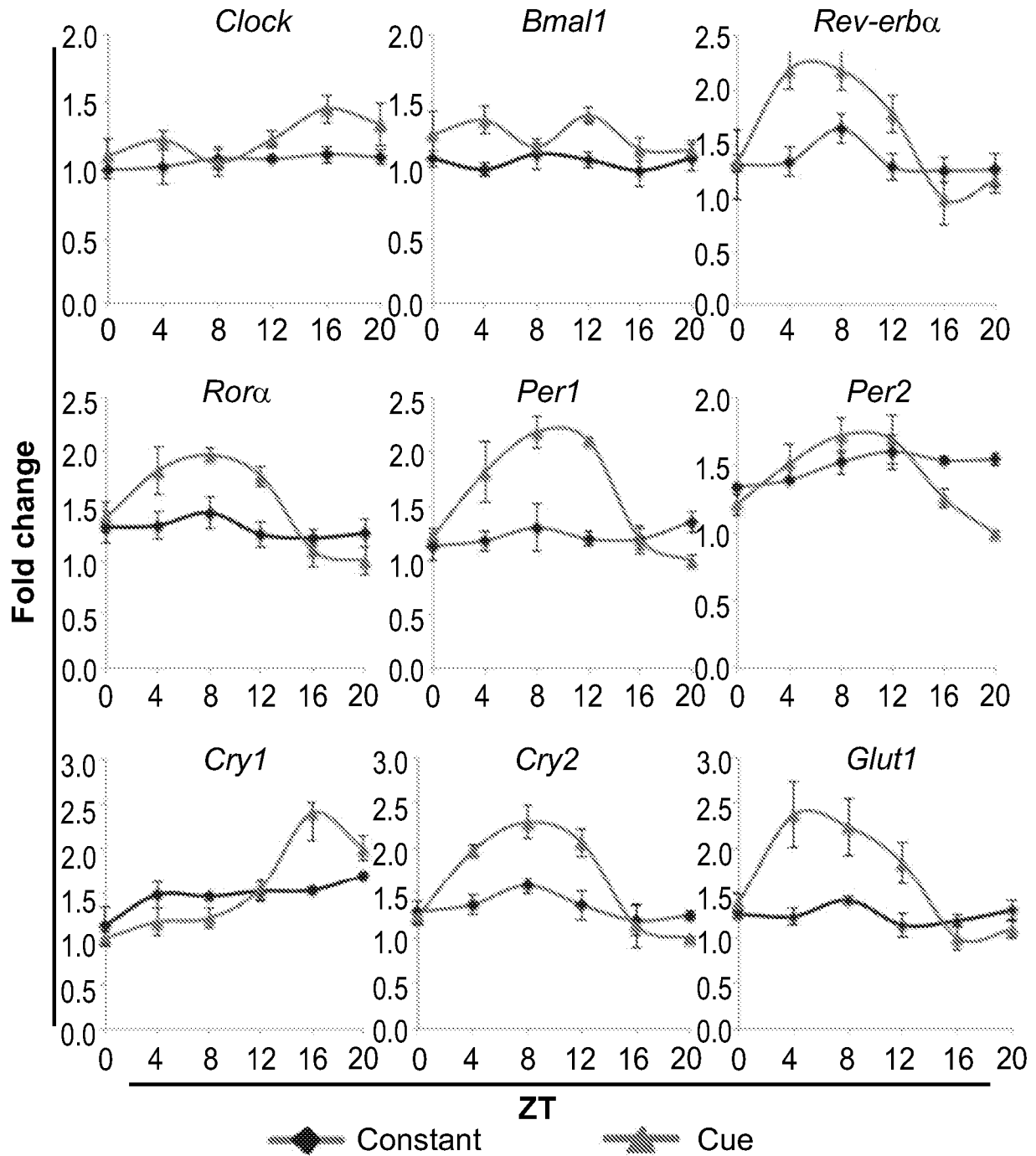
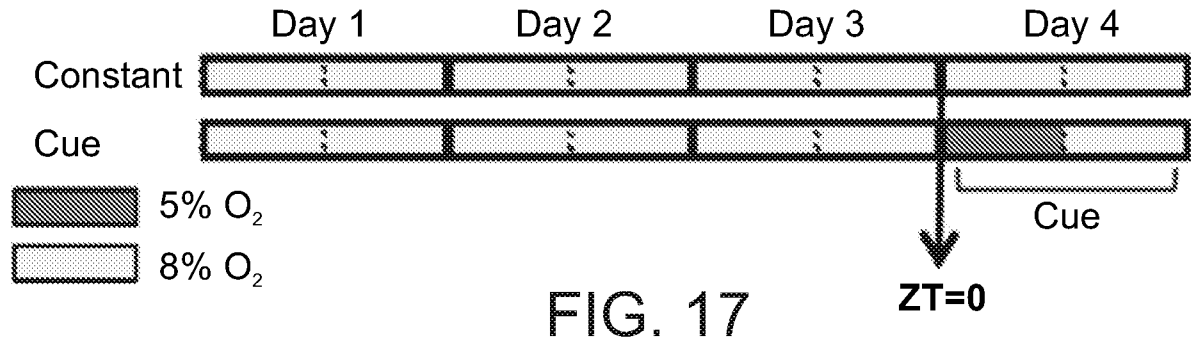


FIG. 16

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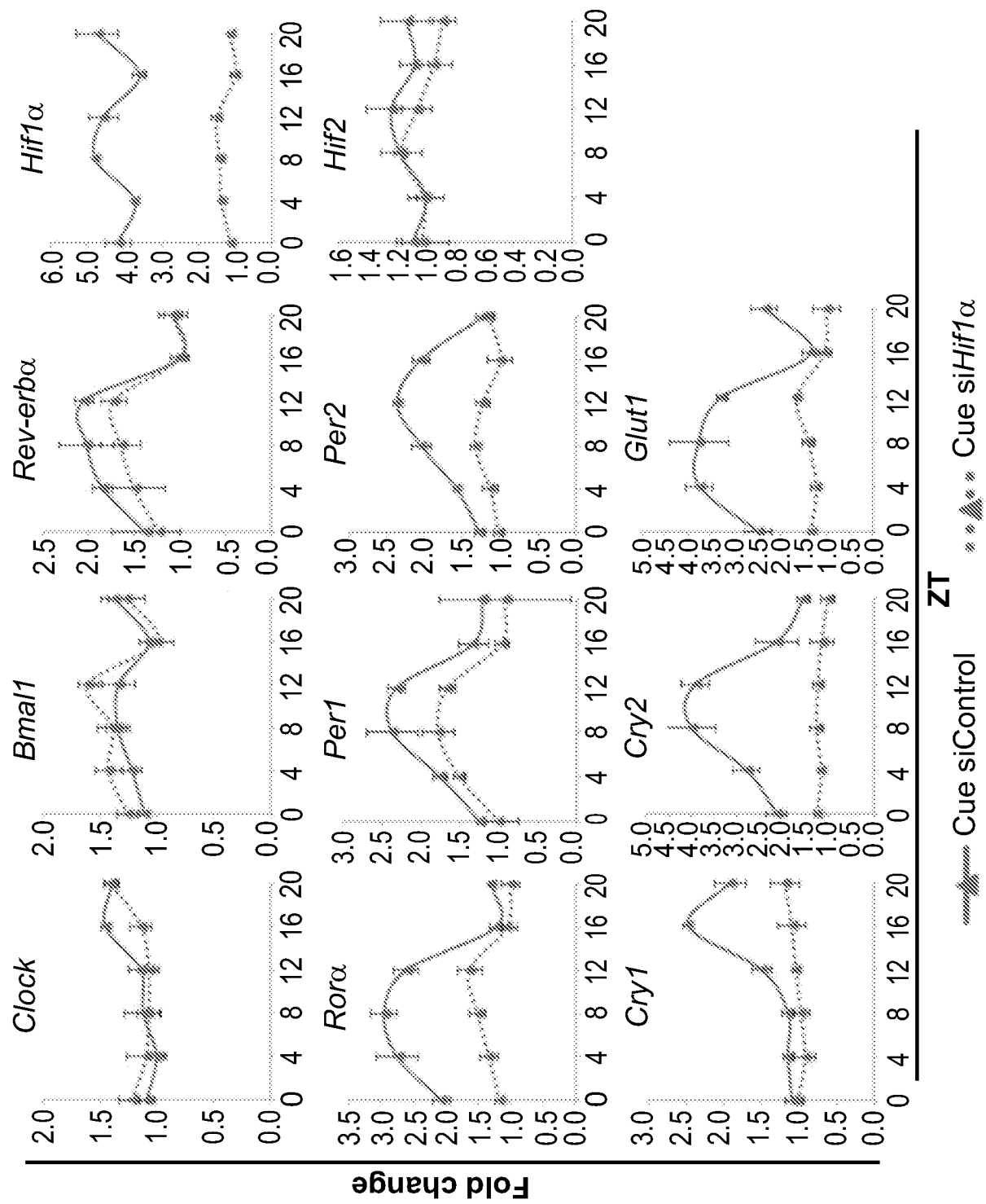


FIG. 19

18/46

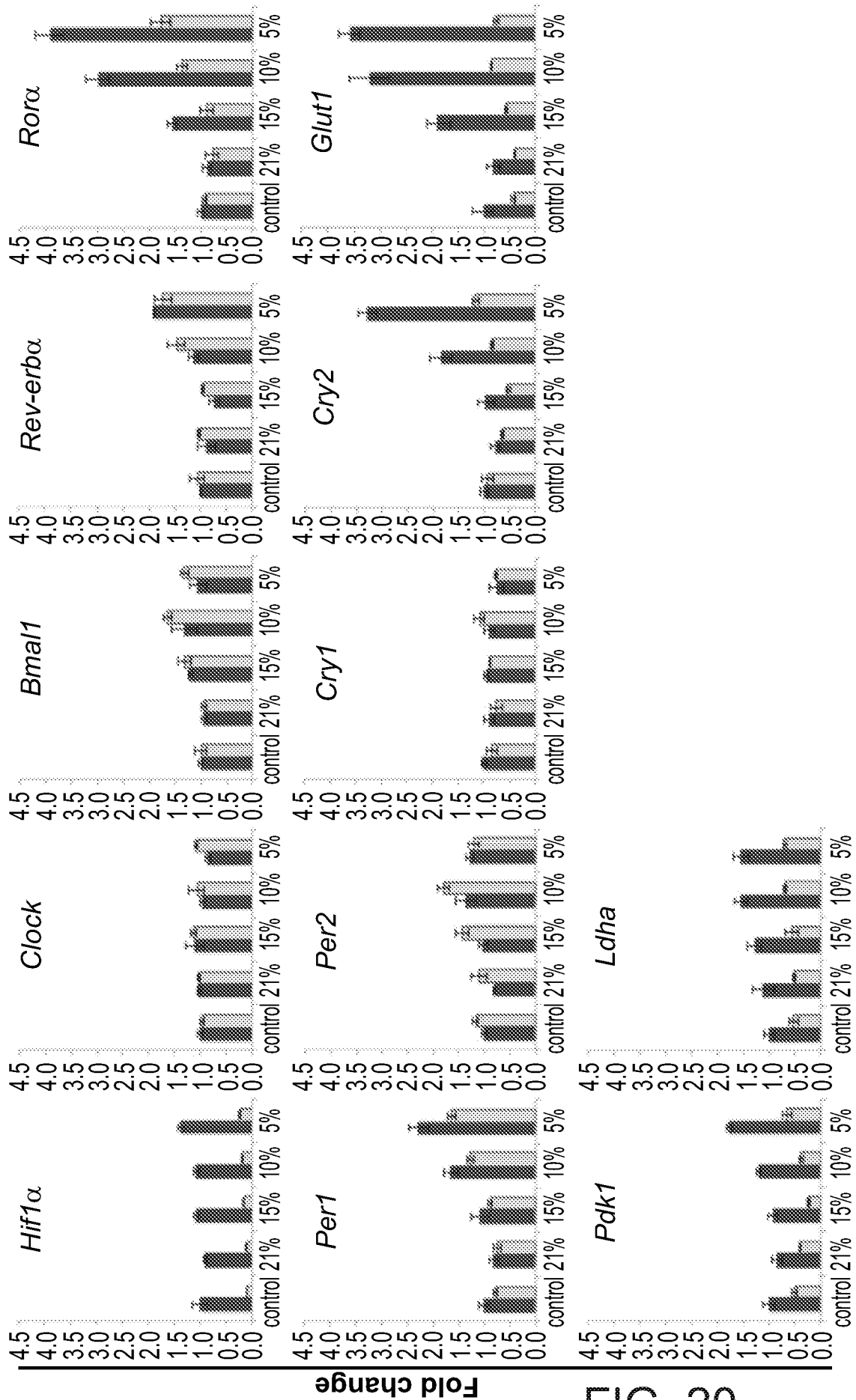


FIG. 20

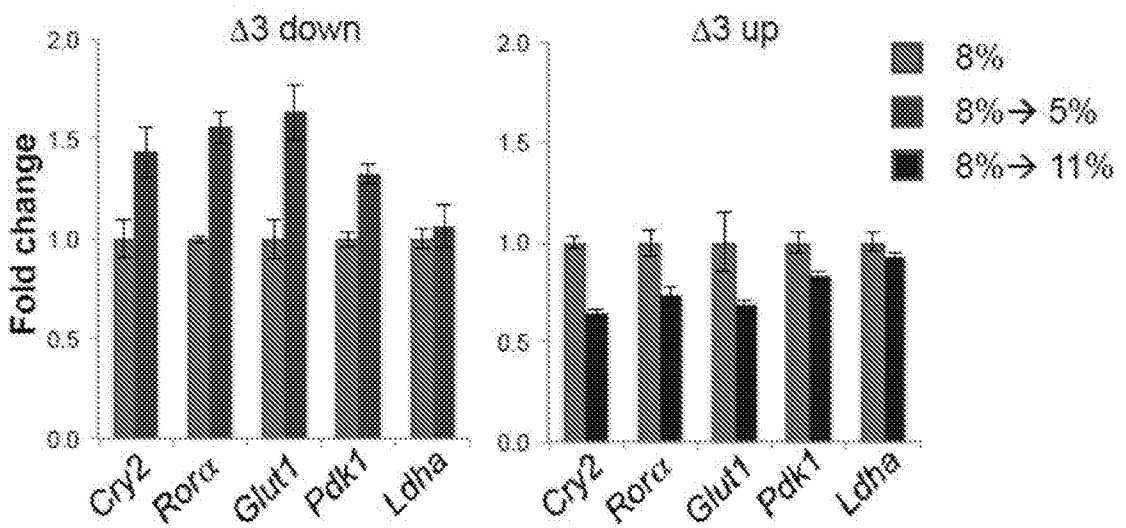


FIG. 21

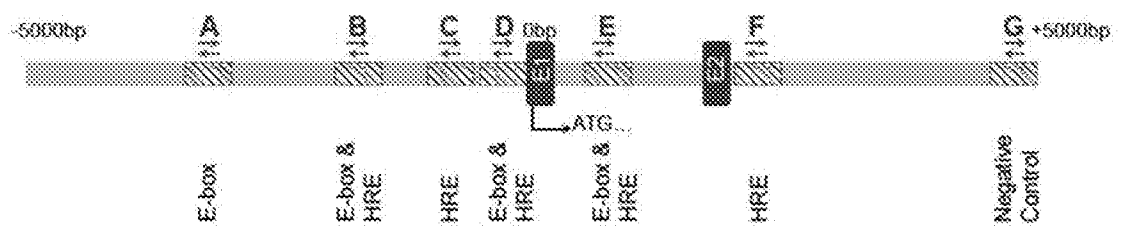


FIG. 22

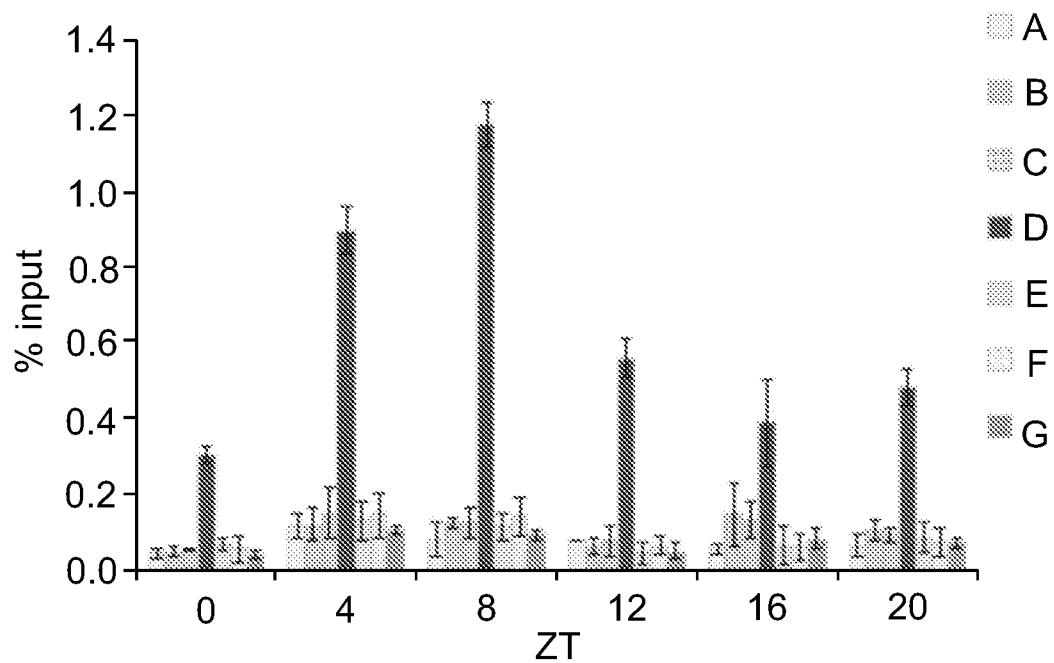


FIG. 23A

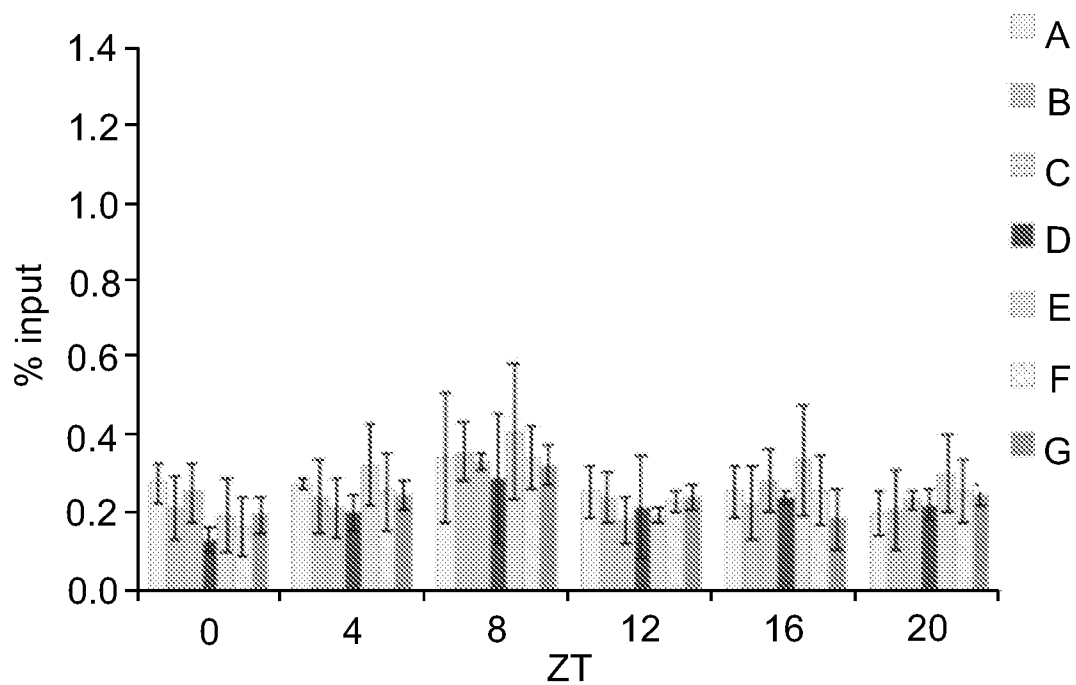


FIG. 23B

21/46

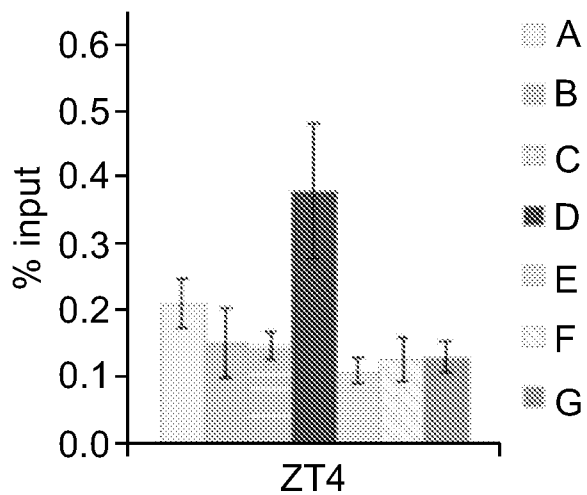


FIG. 24A

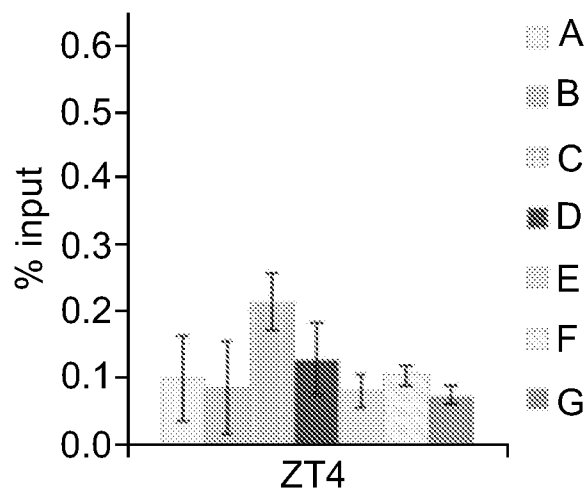


FIG. 24B

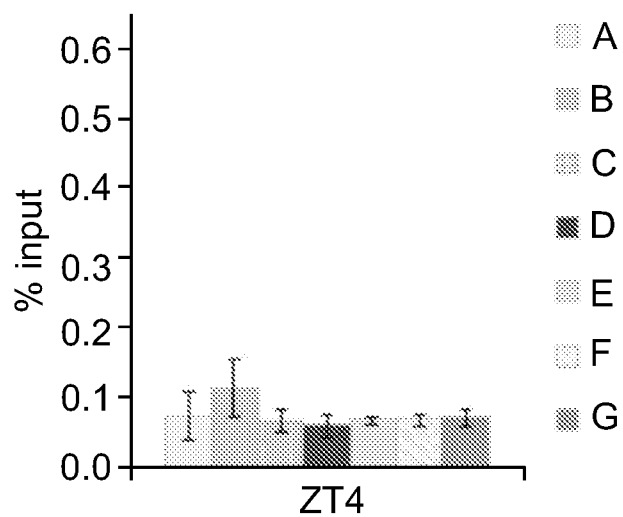


FIG. 24C

22/46

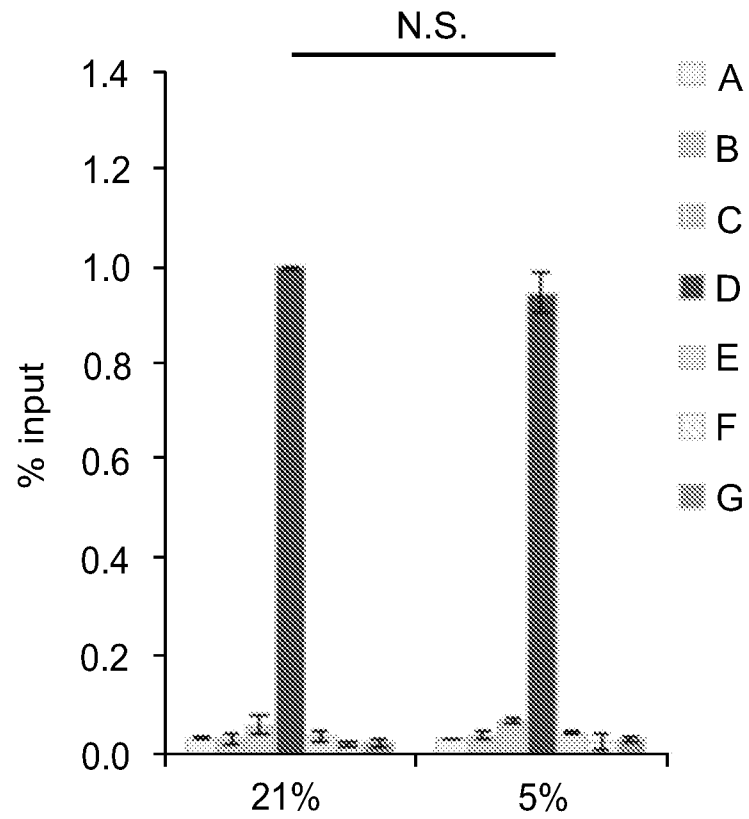


FIG. 25A

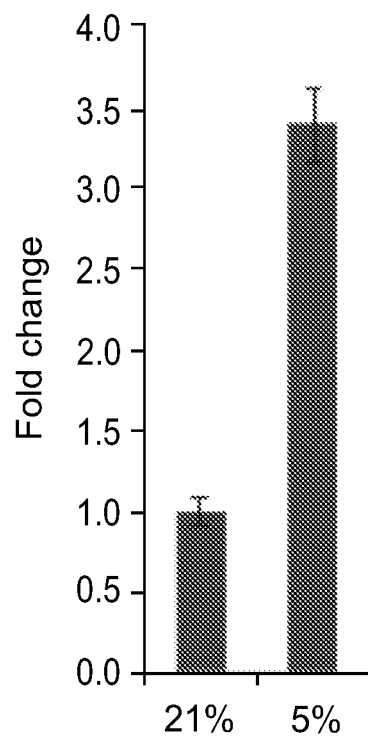


FIG. 25B

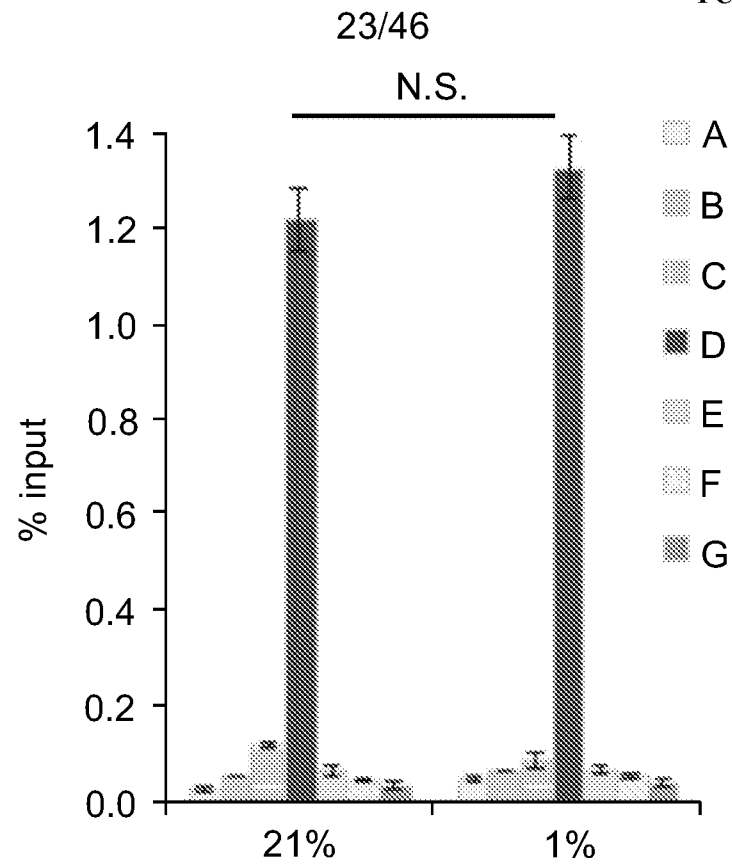


FIG. 26A

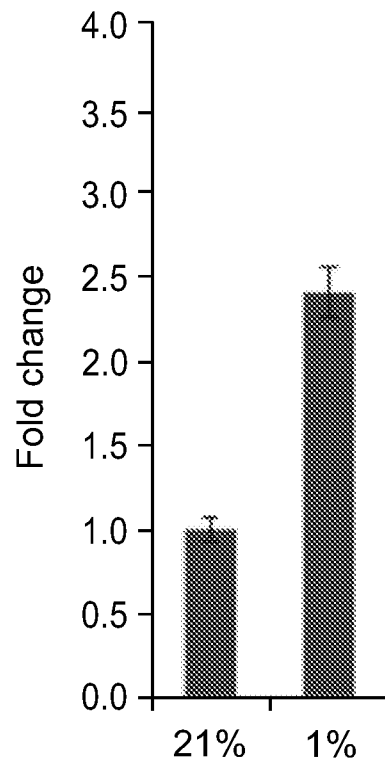


FIG. 26B

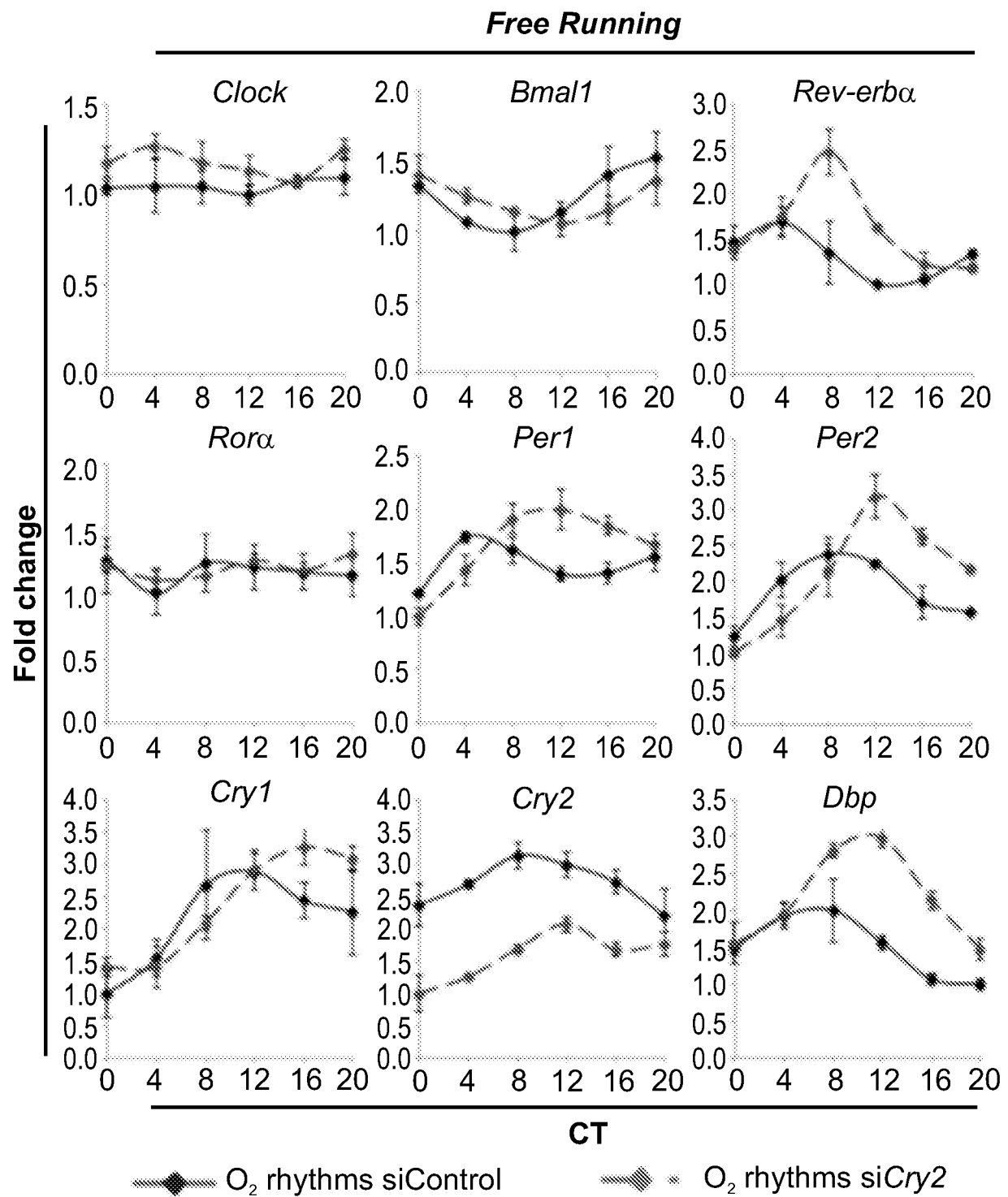


FIG. 27

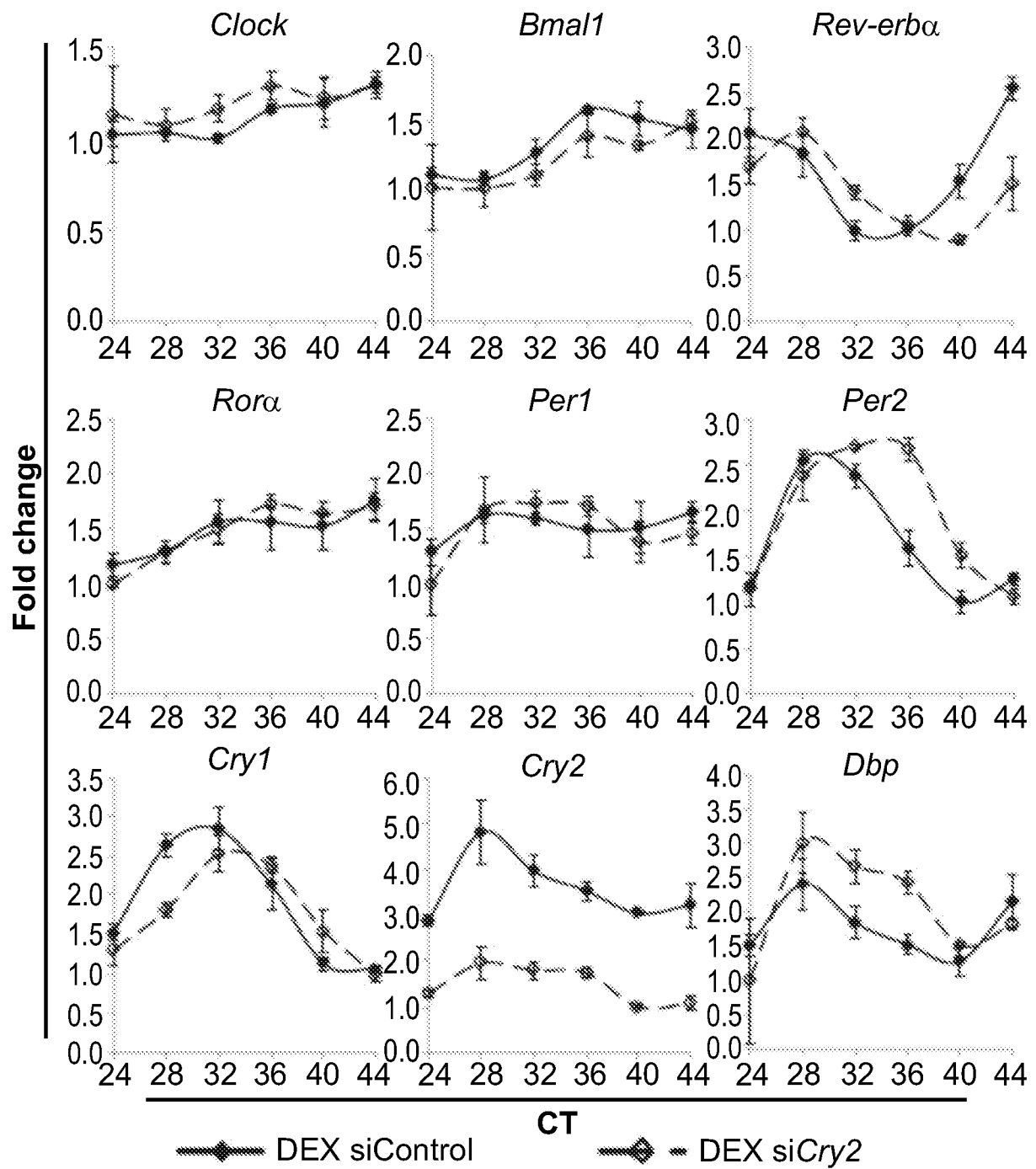


FIG. 28

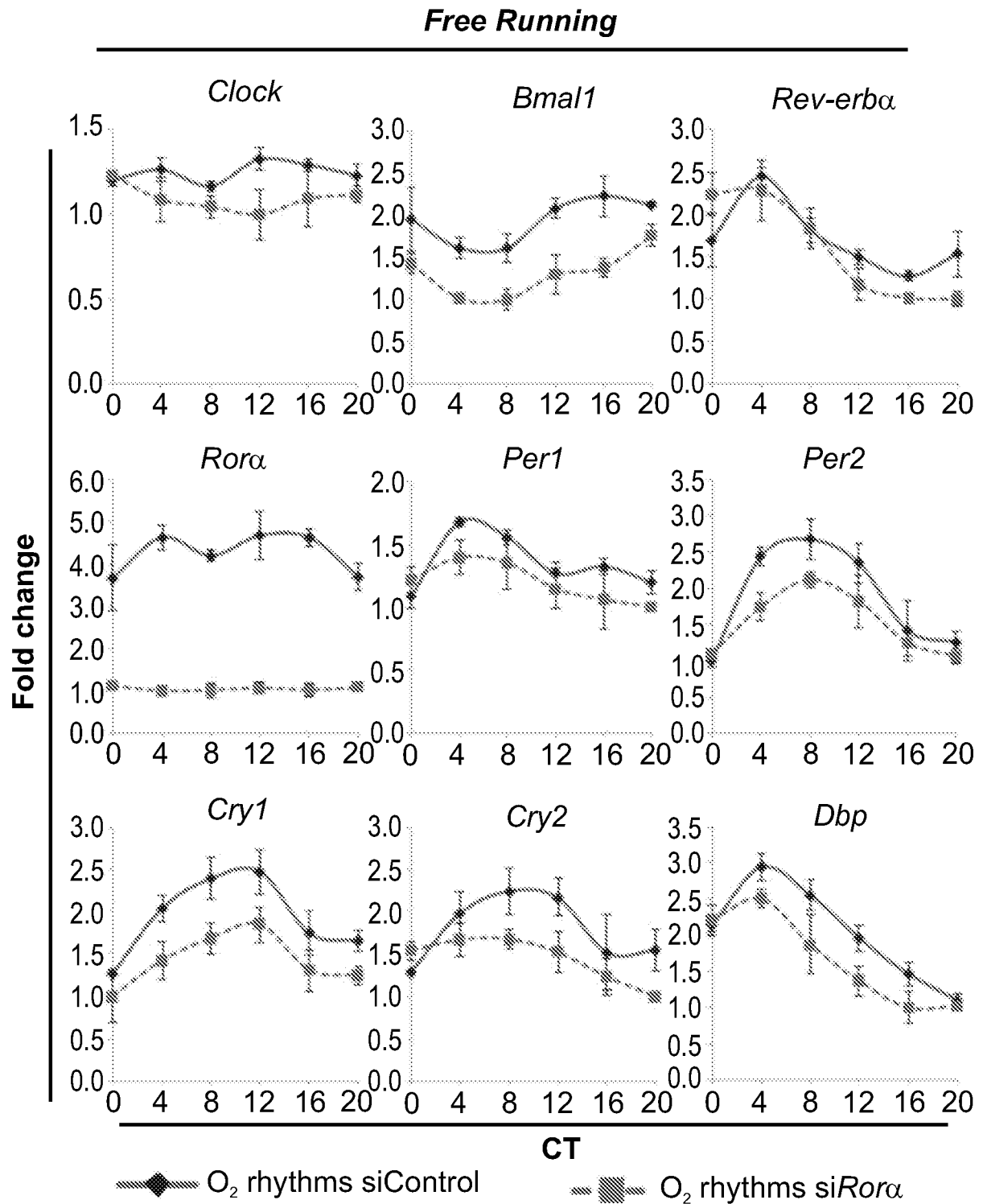


FIG. 29

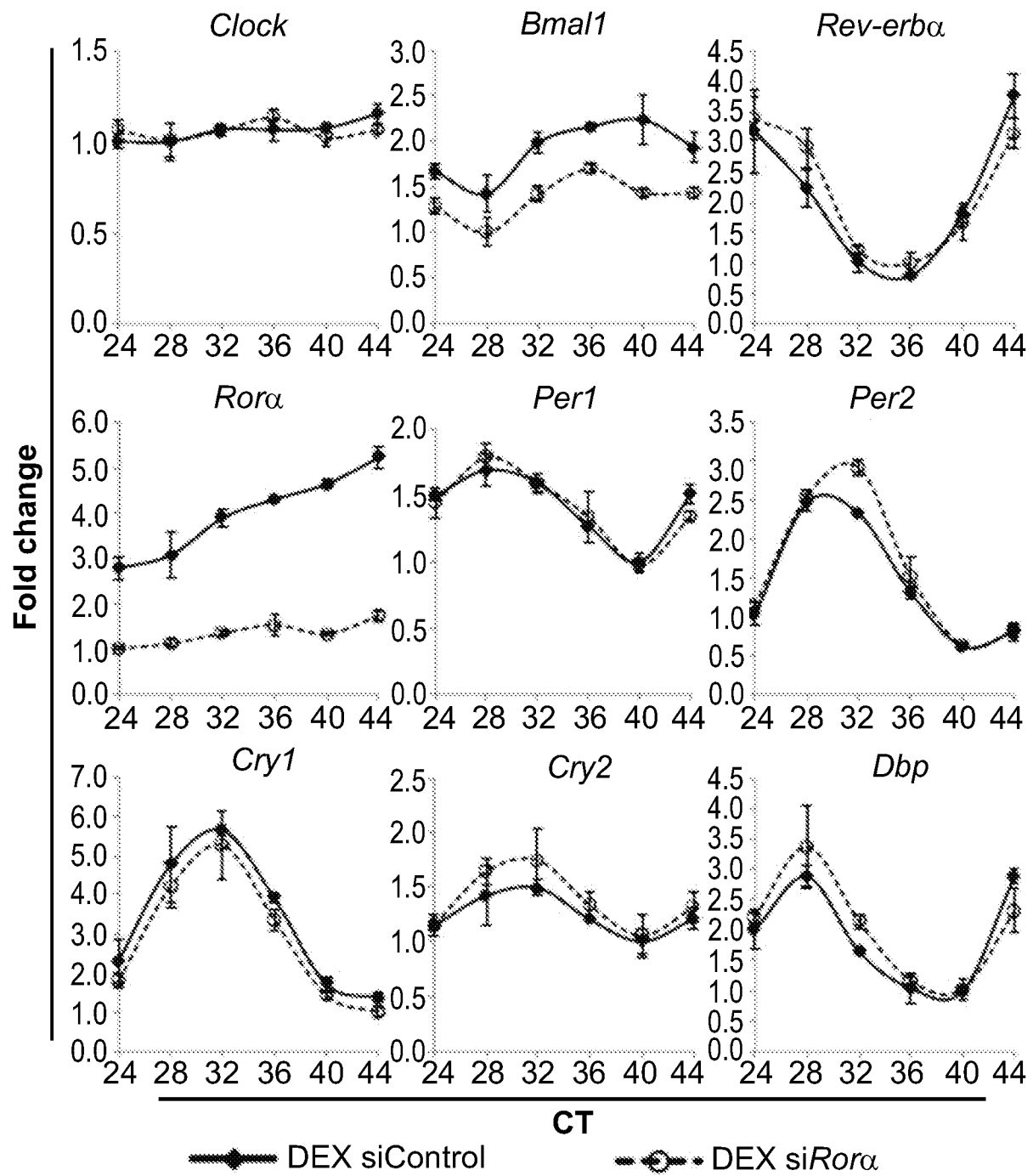


FIG. 30

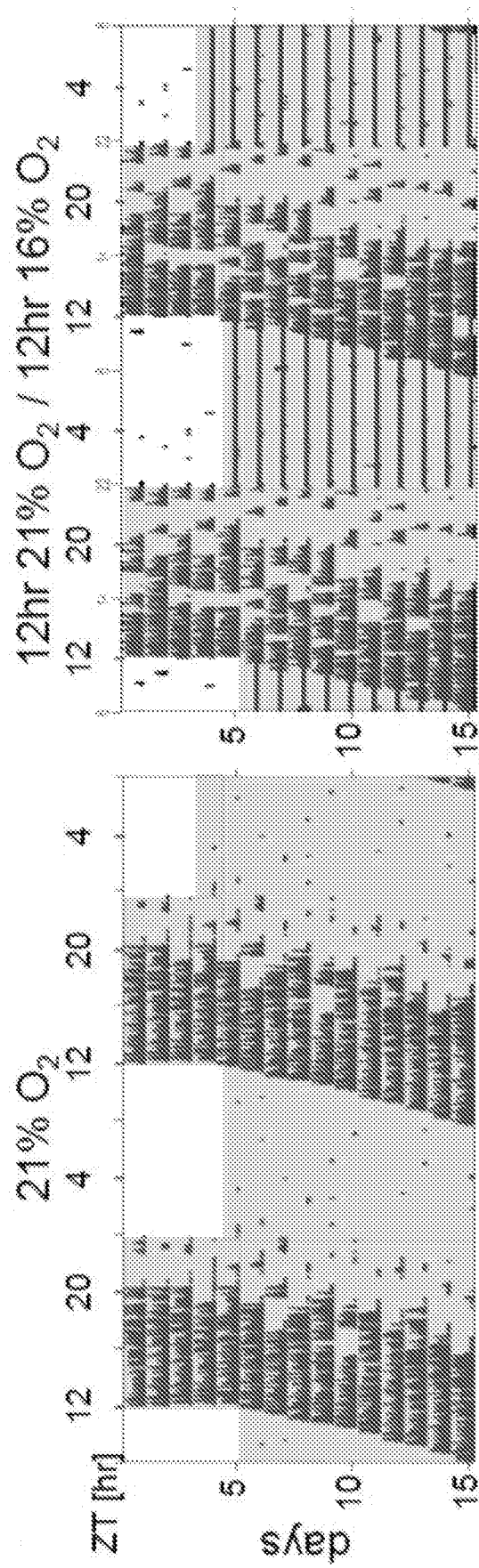


FIG. 31

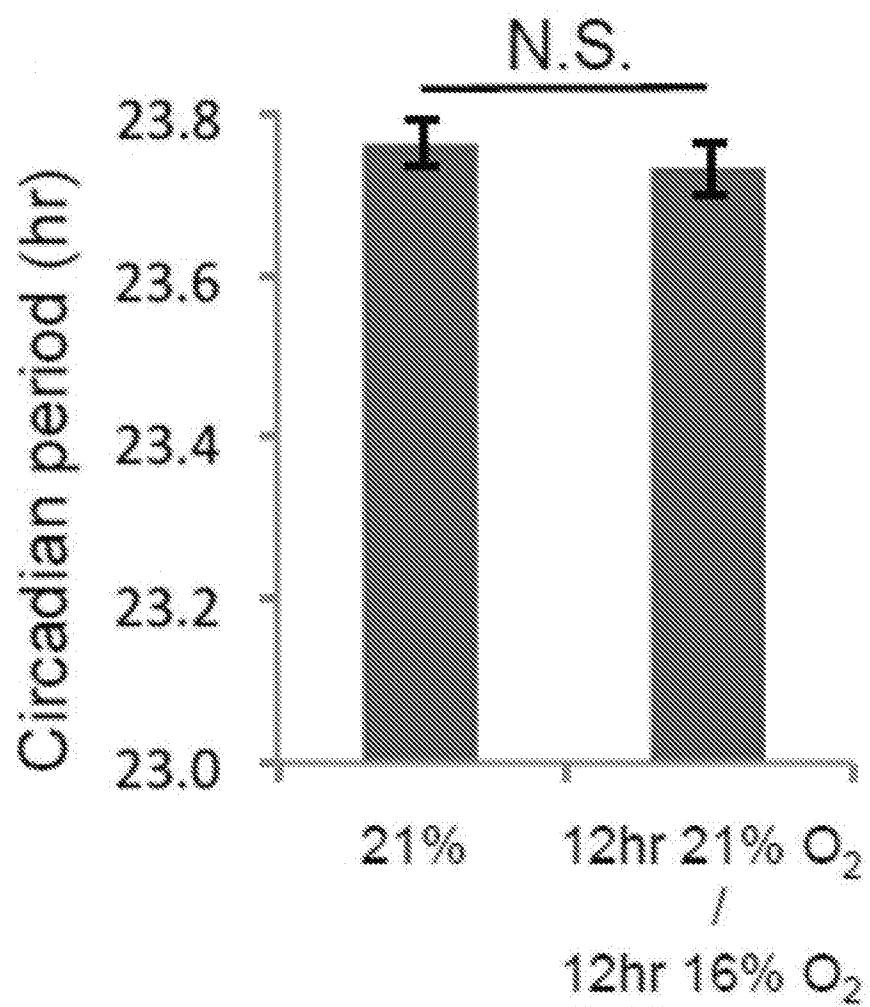


FIG. 32

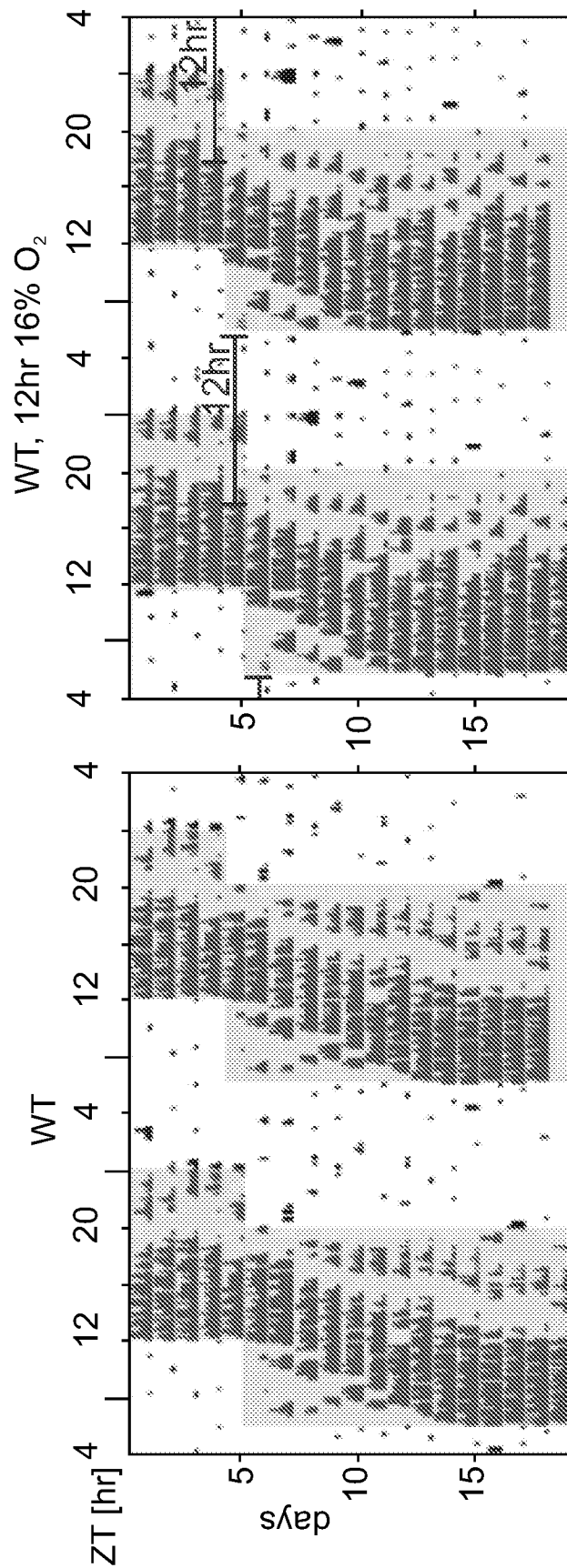


FIG. 33

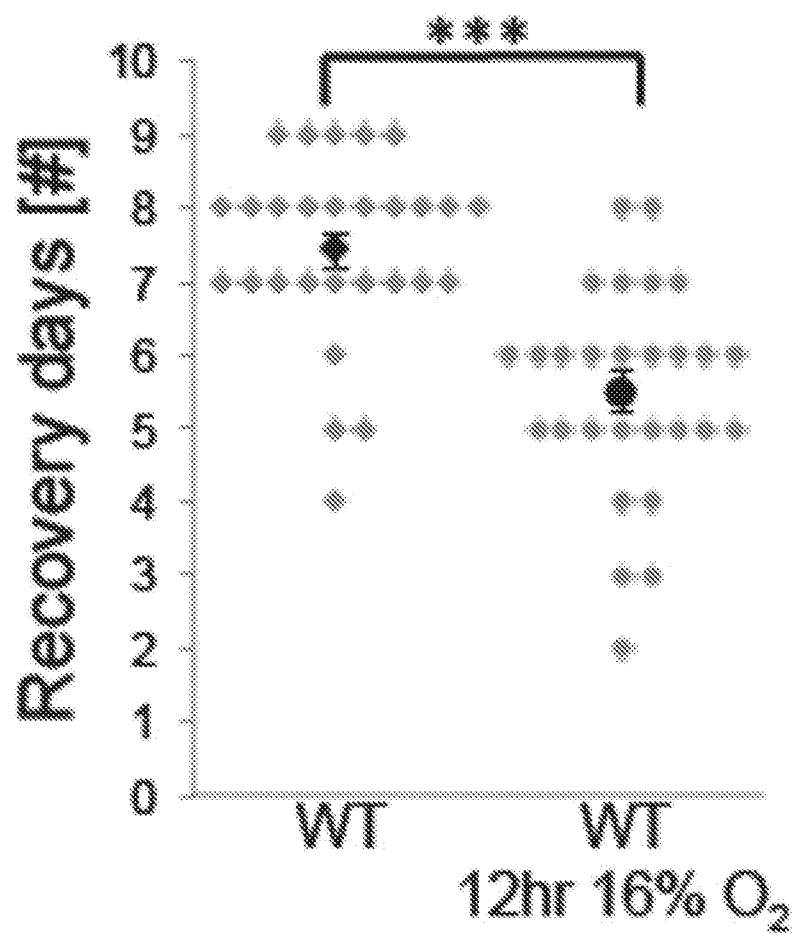


FIG. 34

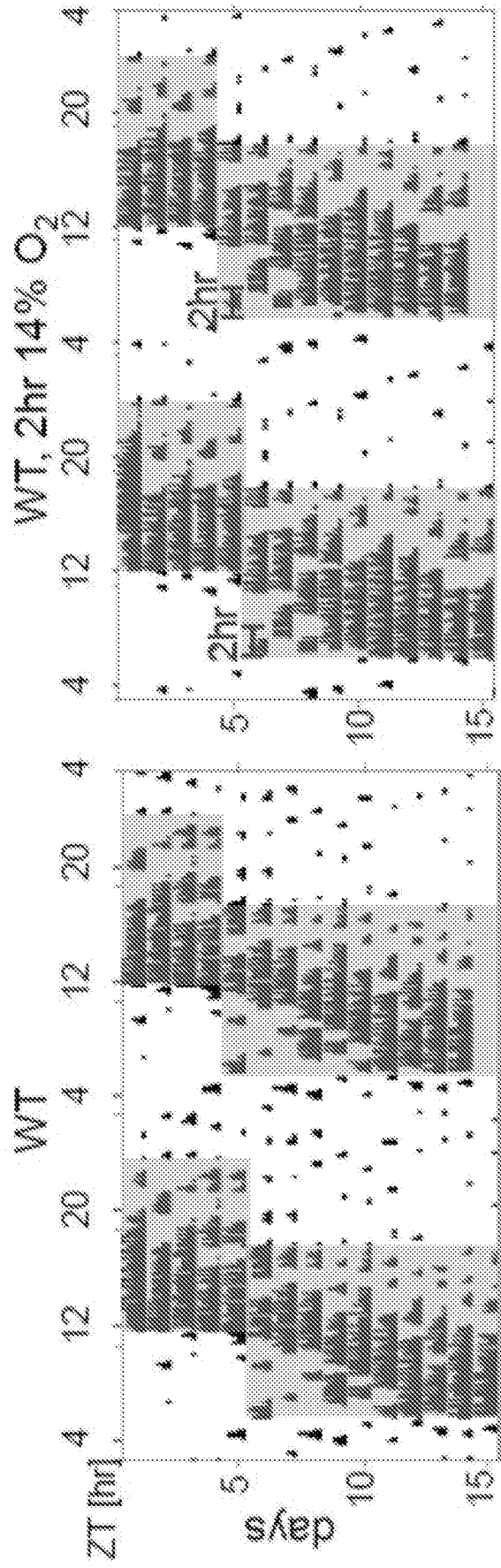


FIG. 35

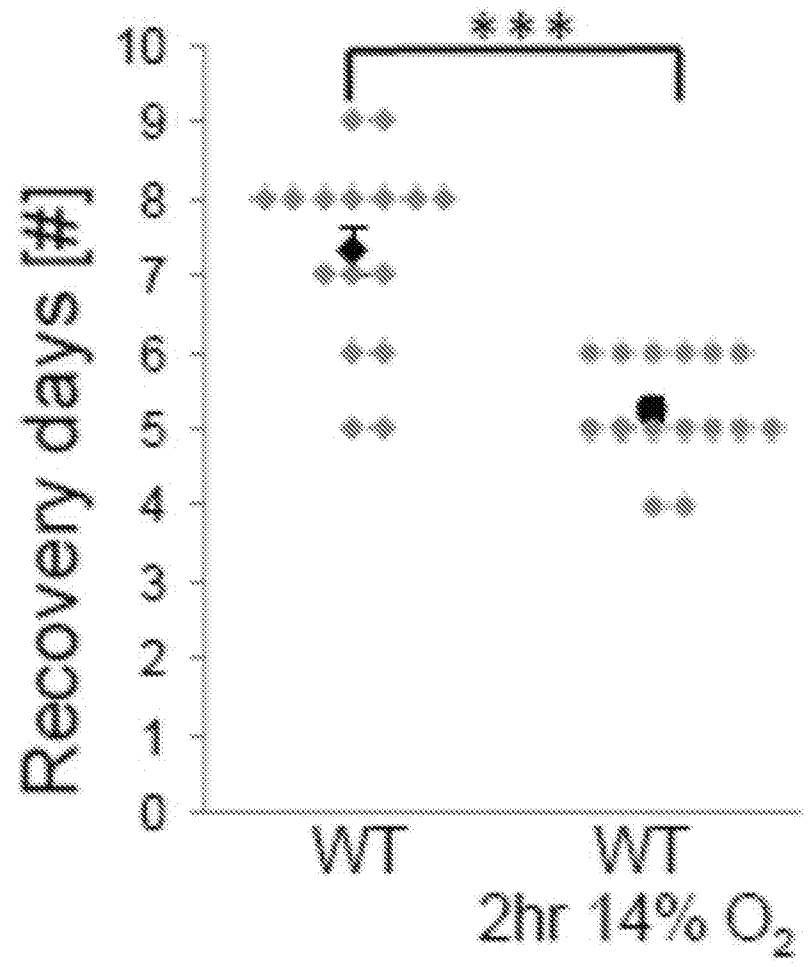


FIG. 36

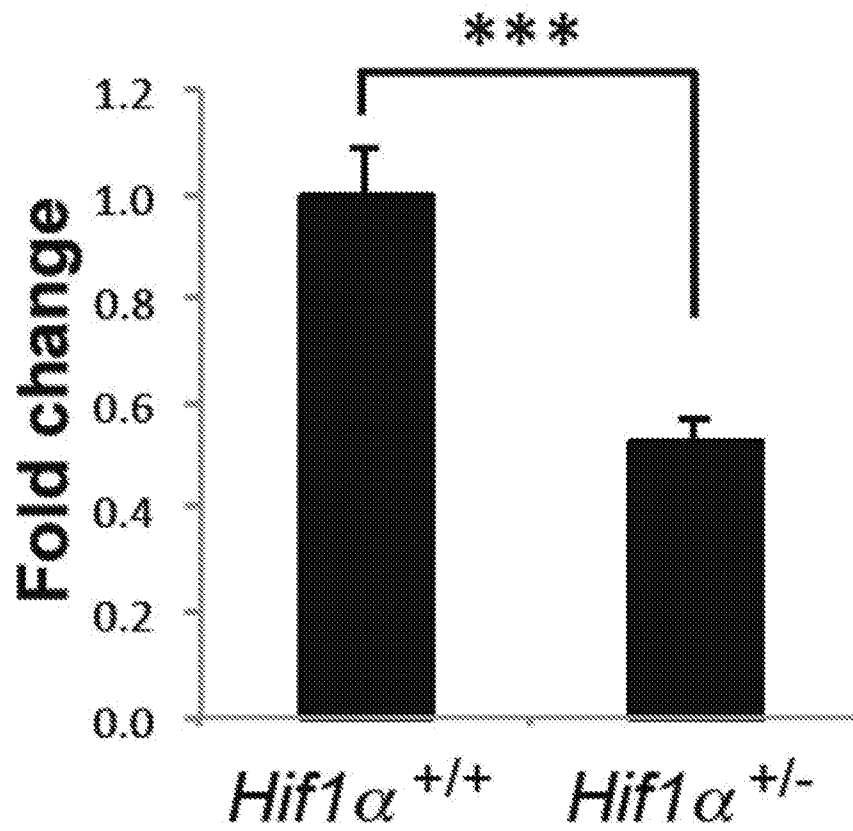


FIG. 37

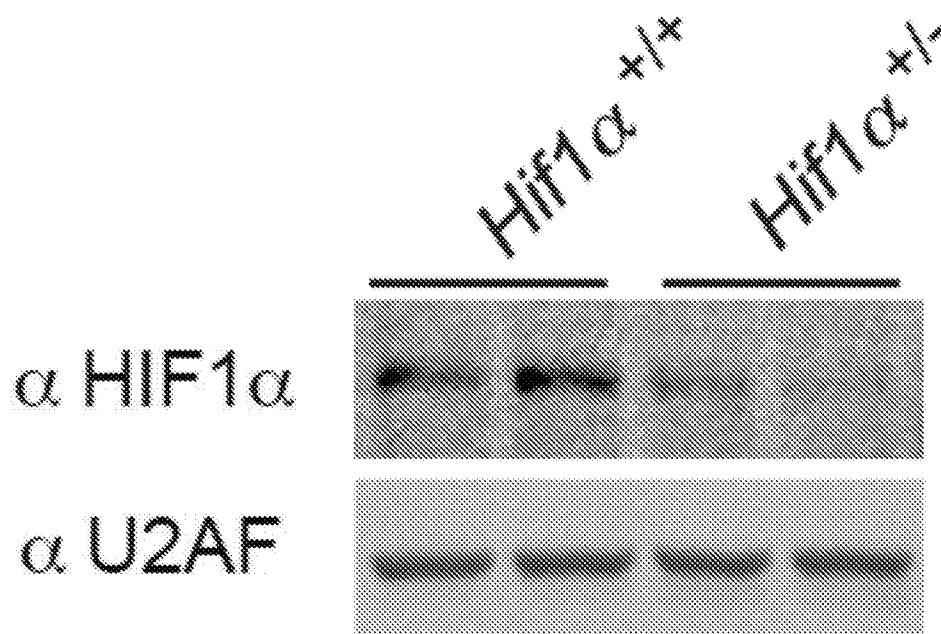


FIG. 38

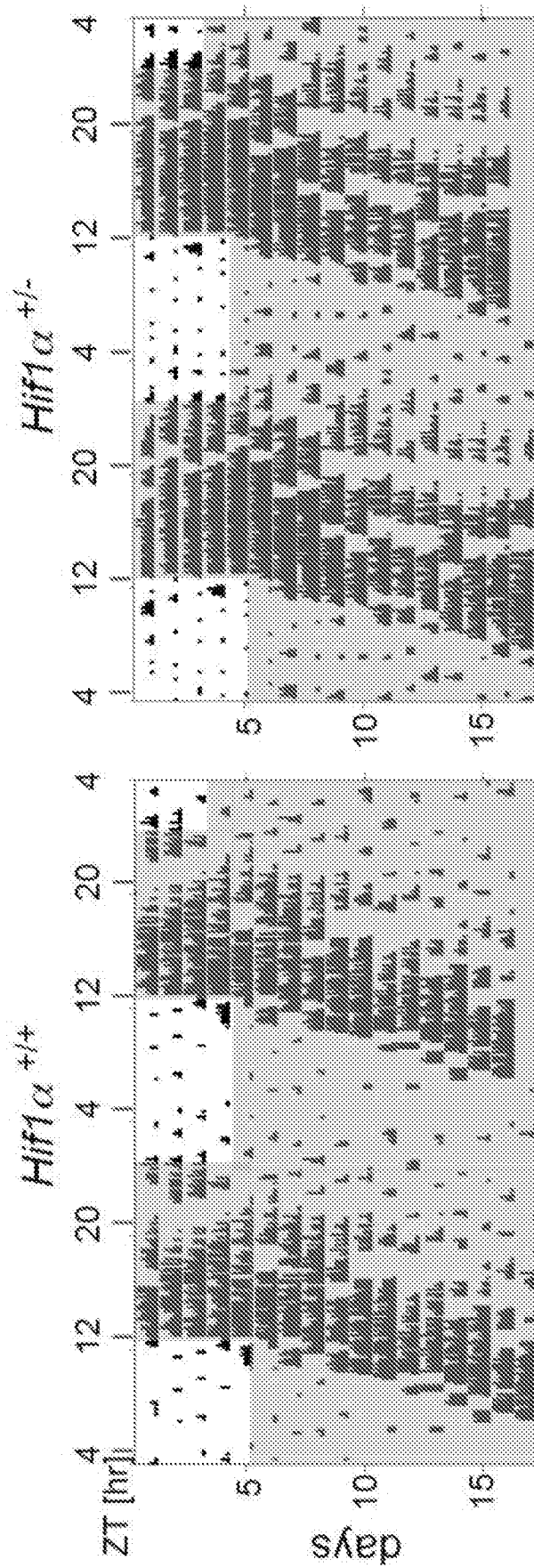


FIG. 39

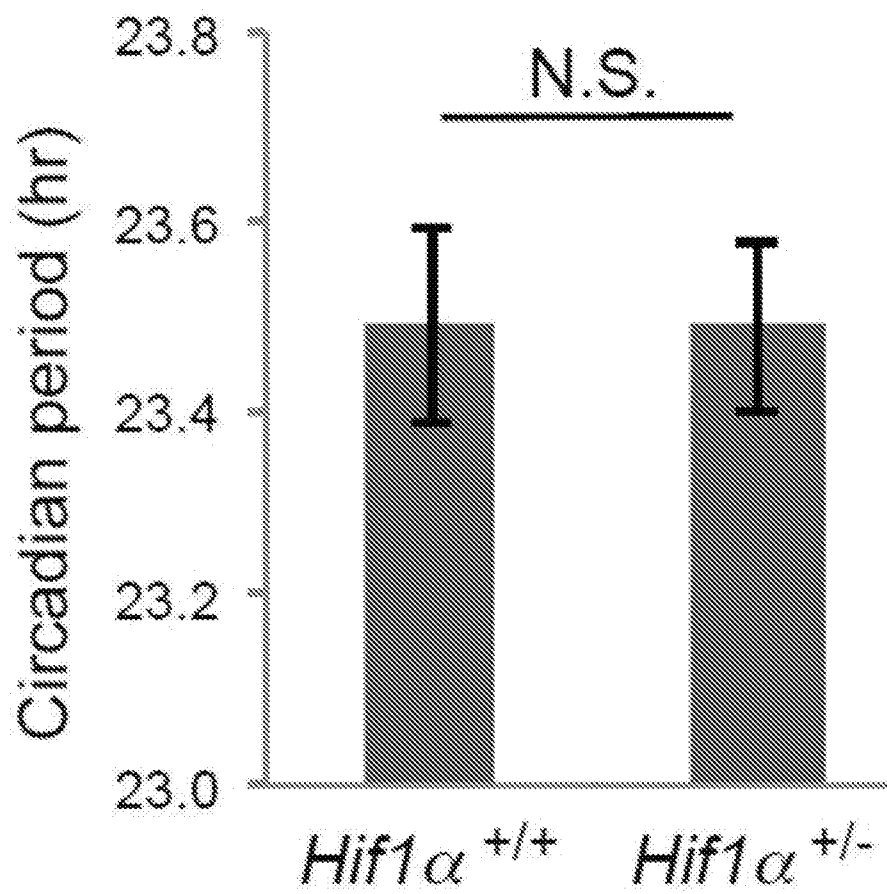


FIG. 40

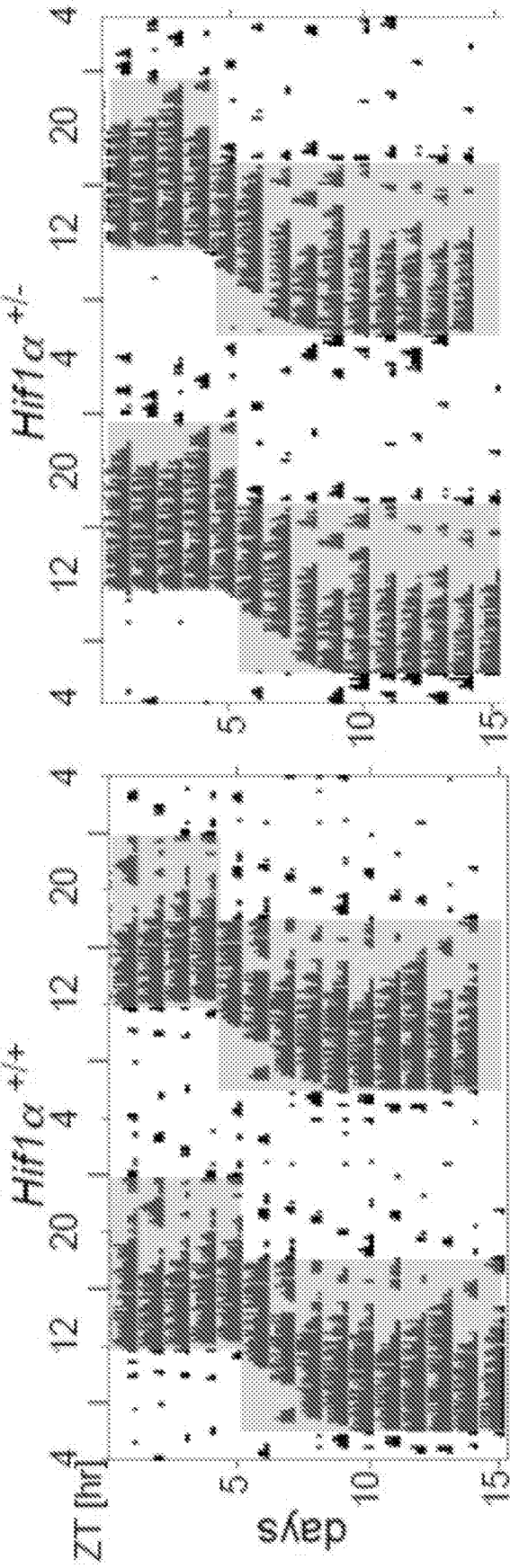


FIG. 41

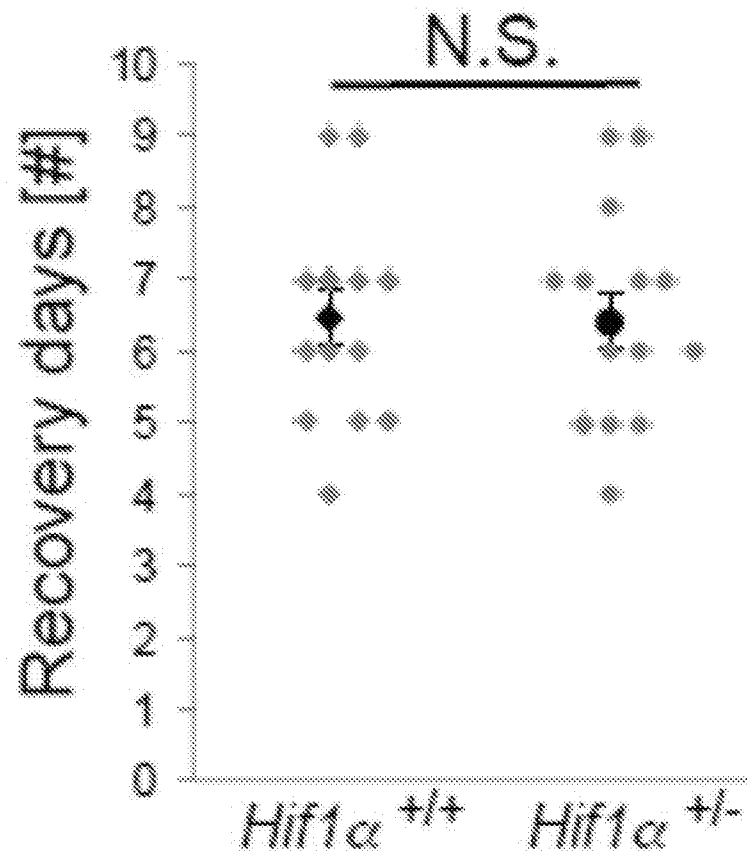


FIG. 42

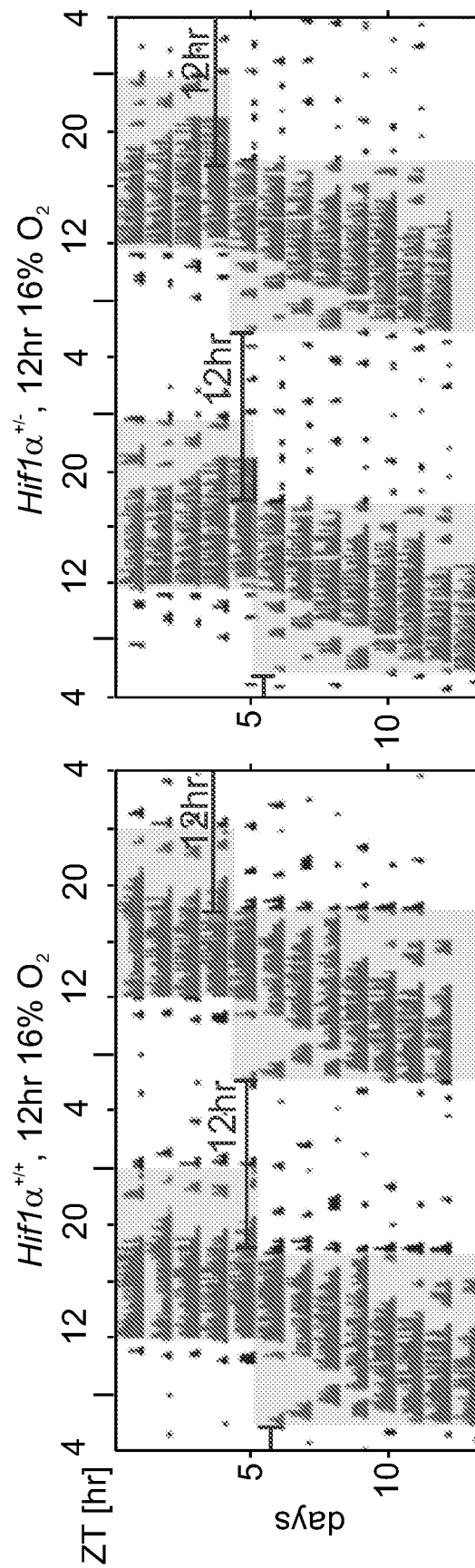


FIG. 43

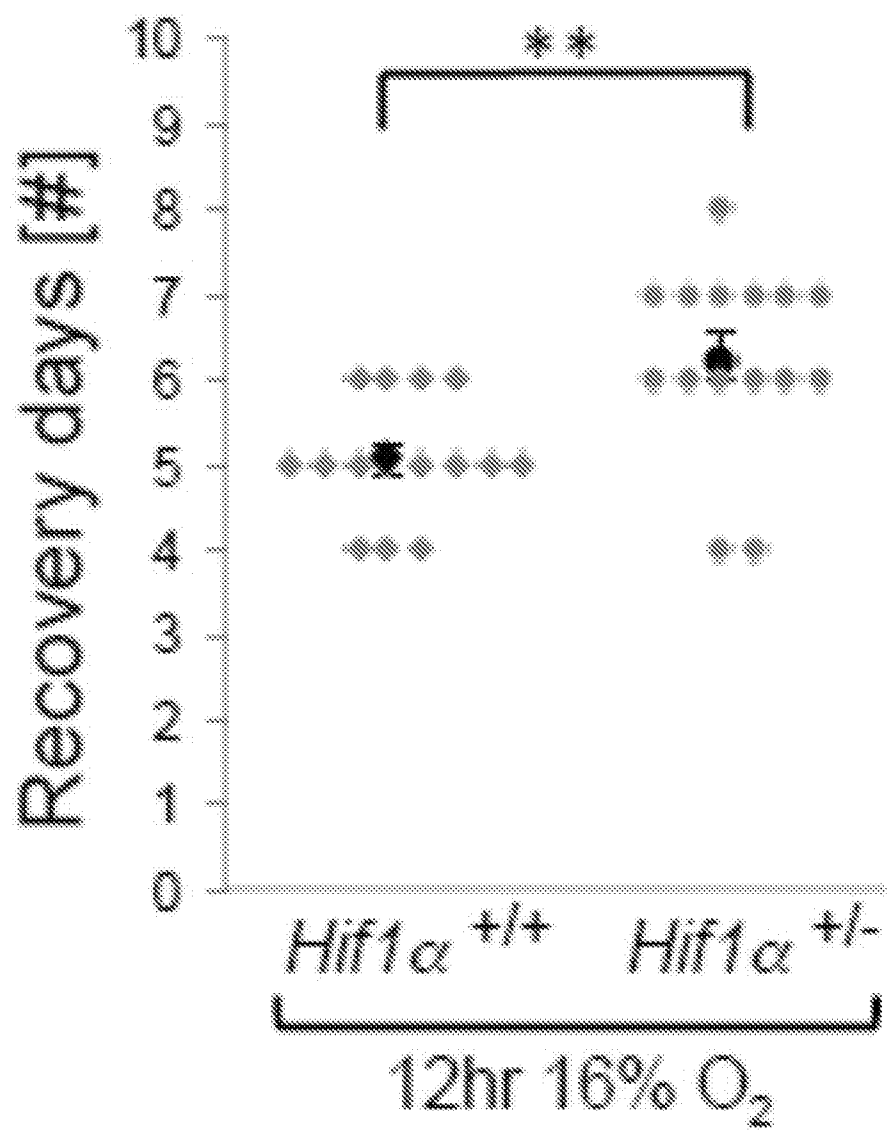


FIG. 44

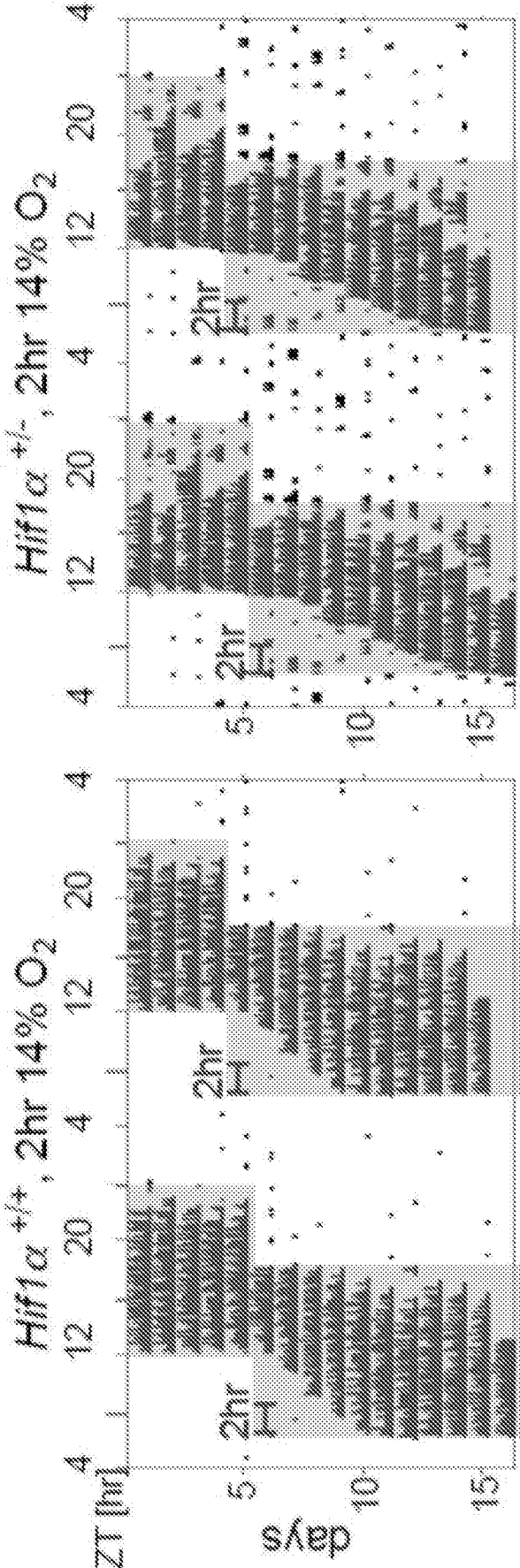


FIG. 45

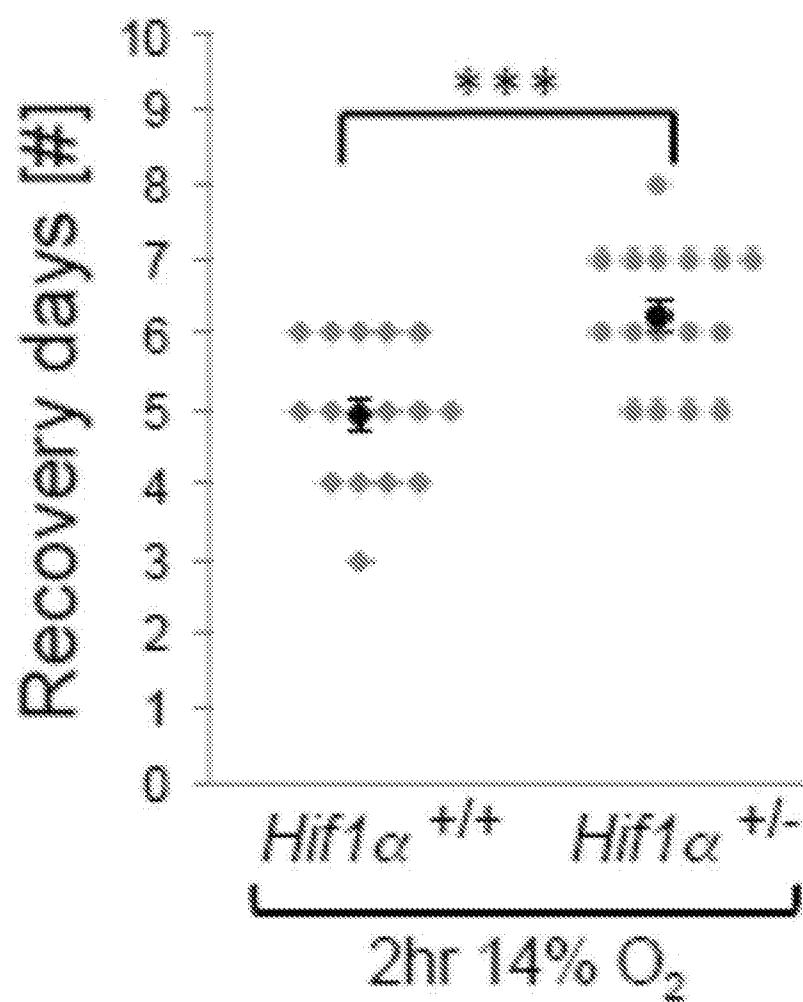


FIG. 46

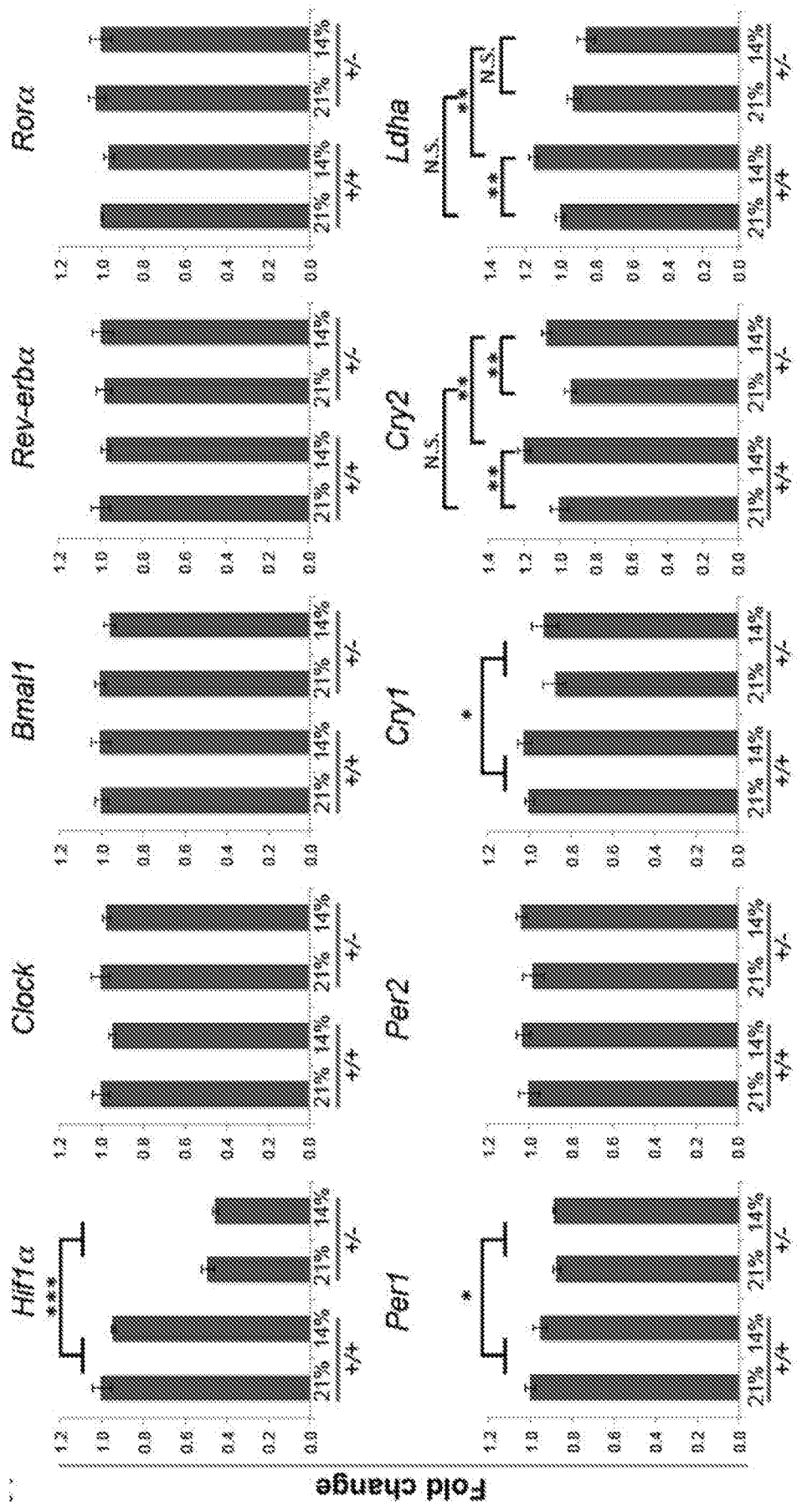


FIG. 47

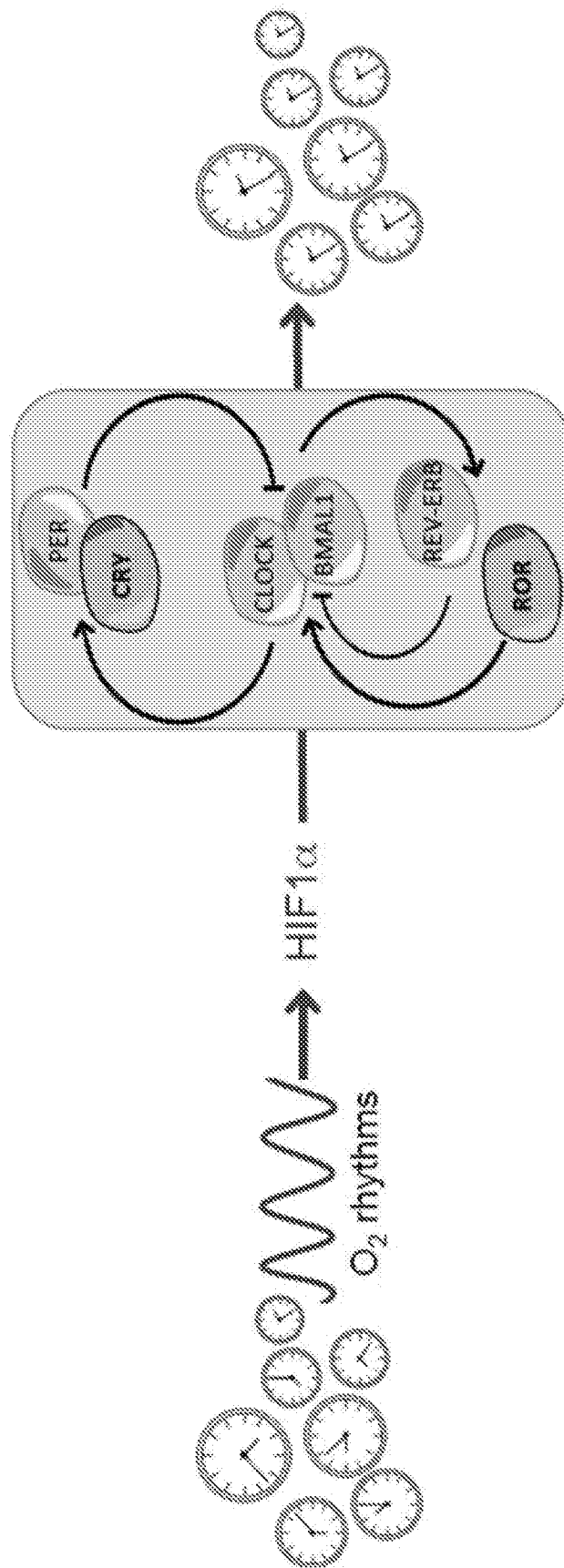


FIG. 48

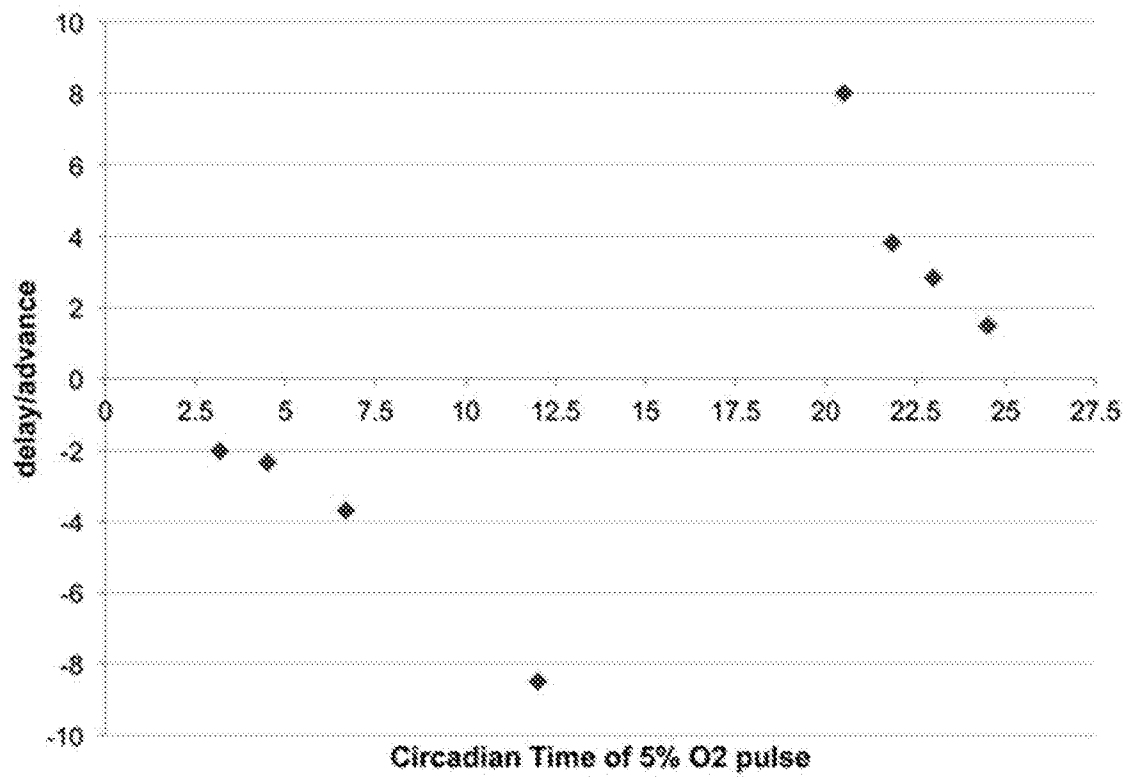


FIG. 49

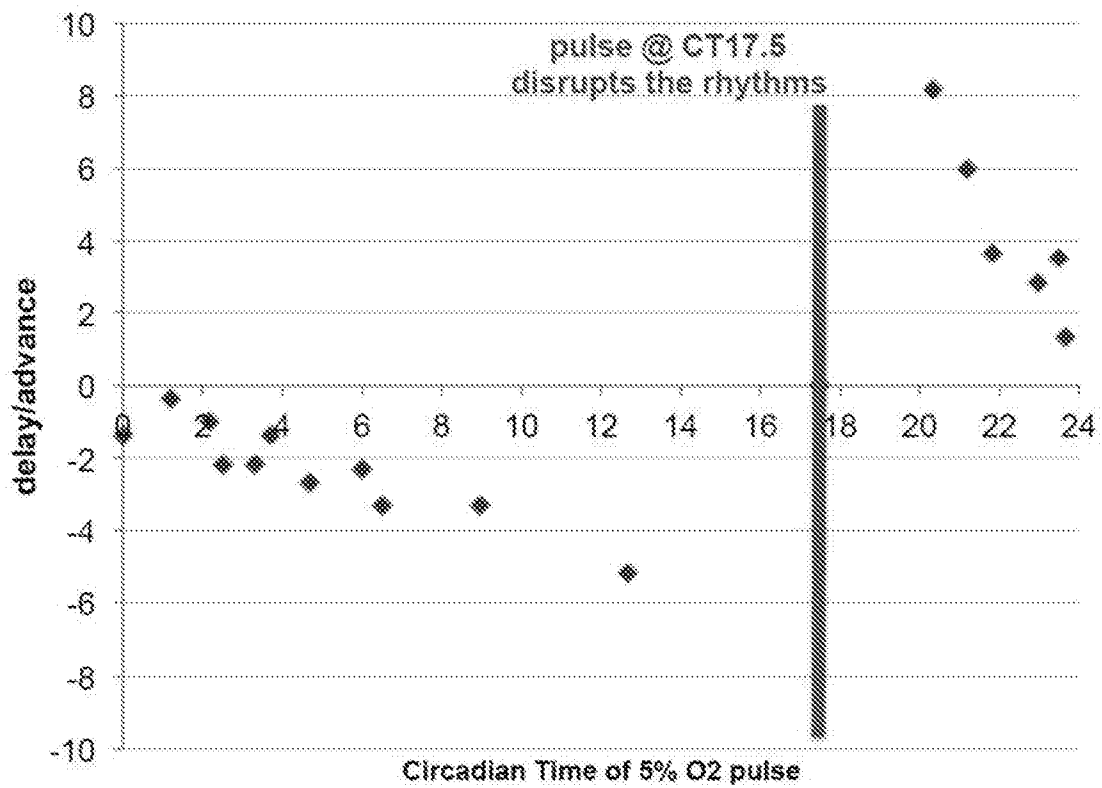


FIG. 50

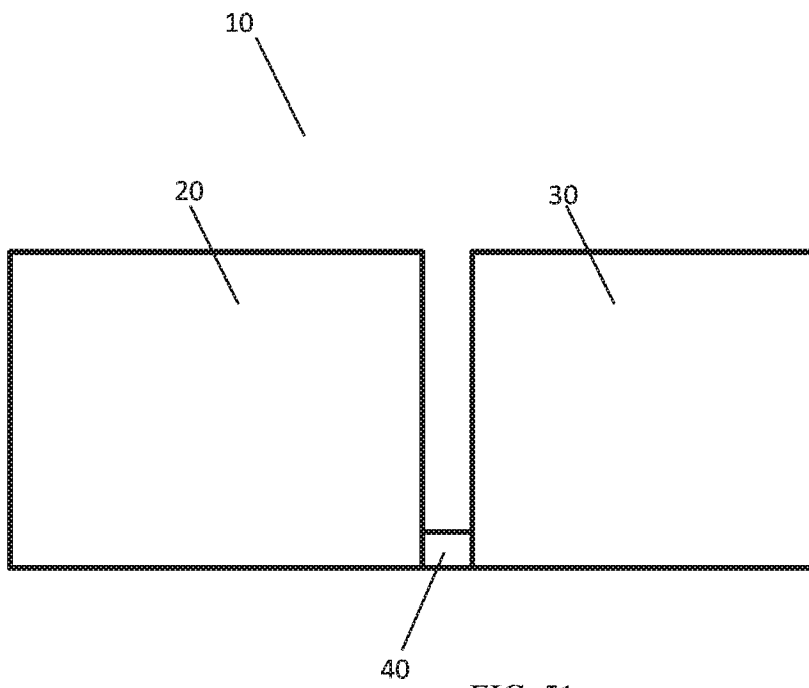


FIG. 51