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An OLTAM system for analysis of brown/beige fat thermogenic activity

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Abstract

BACKGROUND/OBJECTIVES—Thermogenic fat is present in humans and emerging evidence indicates that increasing the content and activity of these adipocytes may lead to weight loss and improved metabolic health. Multiple reporter systems have been developed to assay thermogenic fat activity based on the transcriptional and translational activation of *Ucp1*, the key molecule that mediates nonshivering thermogenesis. Our study aims to develop a much-needed tool to monitor thermogenic fat activity through a mechanism independent of *Ucp1* regulation, therefore effectively assaying not only canonical β-adrenergic activation but also various non-UCP1-mediated thermogenic pathways that have been increasingly appreciated.

METHODS—We detected increased luciferase activity upon thermogenic activation in interscapular brown and inguinal subcutaneous fat in ODD-Luc mice, a hypoxia reporter mouse model. We then developed an OLTAM (*O*DD-*L*uc based *T*hermogenic *A*ctivity *M*easurement) system to assay thermogenic fat cell activity.

RESULTS—In both primary murine and human adipocytes and an immortalized adipose cell line that were transduced with the OLTAM system, luciferase activity can be readily measured and visualized by bioluminescence imaging in response to a variety of stimuli, including UCP1-independent thermogenic signaling. This system can offer a convenient method to assay thermogenic activity for both basic and translational research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONCLUSIONS—The OLTAM system offers a convenient way to measure of the activation of thermogenic fat and presents opportunities to discover novel signaling pathways and unknown compounds targeting metabolically active adipocytes to counteract human obesity.

Keywords

OLTAM; brown fat; beige fat; thermogenesis

INTRODUCTION

Obesity, characterized by excessive fat accumulation in adipose tissues, is now considered to be an epidemic disease. In addition to white adipocytes, which store chemical energy as triglycerides, two types of thermogenic adipocytes have been identified to date, classical brown fat cells and inducible beige adipocytes.[1] Of note, thermogenic adipocytes have been detected in adult humans, and activation of these cells has been implicated in contributing to weight loss.[2, 3] Accurate and effective monitoring of the thermogenic activity of brown and beige adipocytes is one of the several key elements required in order to harness the therapeutic potential of these cells. Previously, a 'ThermoMouse' was generated with a transgene expressing luciferase under the control of the *Ucp1* transcriptional regulatory element[4] and the 'Ucp1-2A-luciferase knock-in mouse' was developed through introducing a luciferase reporter into the genetic locus of the *Ucp1* gene.[5] Both reporter models provide truthful recapitulation of *Ucp1* expression *in vivo* and *in vitro*. However these *Ucp1*-based designs render an inherent limitation of these systems, that they cannot be used to investigate thermogenic regulation through UCP1-independent mechanisms.[6–8]

It has been reported that hypoxia occurs in thermogenic fat tissues during cold exposure.[9, 10] A reporter mouse model, called the ODD-Luc mouse, has been previously developed to monitor hypoxia.[11] This transgenic mouse expresses luciferase fused with the oxygen-dependent degradation (ODD) domain of hypoxia-inducible factor 1 alpha (HIF1α). Proline residues within the ODD domain can be hydroxylated by prolyl hydroxylases (PHDs). This modification in turn induces ubiquitination-dependent degradation of HIF1α in oxygen-rich conditions. During hypoxia, non-hydroxylated prolines will prevent HIF1α protein degradation and activate downstream signaling. Here, we first evaluated the ODD-Luc mouse for its potential to be used as a reporter system to examine brown and beige fat thermogenic activity *in vivo* and *in vitro*. We then further developed an ODD-Luc based OLTAM system that can be widely used for a broad range of purposes in the thermogenic fat research field.

MATERIAL AND METHODS

Luciferase Assay

The luciferase activity assay was performed with the Dual-luciferase Reporter Assay kit (Promega, E1960) according to the manufacturer's instructions using a SpectraMax L plate reader (Molecular Devices). For *in vitro* bioluminescence imaging, cells were treated with luciferin to a final concentration of 100 μg/ml and then the luciferase activity was immediately measured using the Xenogen IVIS 200 bioluminescence system. For *in vivo*

bioluminescence imaging, 10–20 minutes before imaging mice were injected with 150 mg/kg of luciferin, then anesthetized or dissected to isolate interscapular brown fat and subcutaneous inguinal fat before luciferase activity was visualized using the Xenogen IVIS 200 bioluminescence system.

Construction of Adenoviral-OLTAM

ODD-Luc was cloned using cDNA from the liver of ODD-Luc mice with primers 5′-CTCGAGCCTAAGCTTGGATCCGAATTC-3′ and 5′-GGATATCTTATCTAGAATTACACGGCGATC-3′. The pAdTrack-CMV plasmid was amplified using primers 5′-GCCGTGTAATTCTAGATAAGATATCCGATC-3′ and 5′-ATTCGGATCCAAGCTTAGGCTCGAG-3′. pAdTrack-ODD-Luc was assembled using the Gibson Assembly method (New Engl Bio Labs; NEBE2611). Adenoviral-OLTAM was then generated and amplified.

Generation of the Stable C3H/10T1/2 Cell Line Expressing Retroviral-OLTAM

ODD-Luc was cloned using cDNA from the liver of ODD-Luc mice with primers 5′-ATCTCTCGAGATGGAATTCAAGTTGGAATT-3′ and 5′-TTCGTTAACTTACACGGCGATCTTTC-3′. The pMSCV plasmid was amplified using primers 5′-CGCCGTGTAAGTTAACGAATTCTACCGGGT-3′ and 5′-TGAATTCCATCTCGAGAGATCTAATTCCGG-3′. pMSCV-ODD-Luc was assembled using the Gibson Assembly method. The stable C3H/10T1/2 cell line expressing retroviral-OLTAM was generated using the pMSCV puro retro system (Clontech; Cat. 634401).

Statistical analysis

The results are presented as mean ± SEM. Data are representative of two to four independent experiments. The statistical significance was determined using a two-tailed Student's *t* test for single comparisons. A value of $p < 0.05$ was considered statistically significant and was noted as *($p<0.05$), **($p<0.01$) and ***($p<0.001$).

RESULTS

ODD-Luc mice can be used for monitoring thermogenic fat activity mediated by cold exposure and β-adrenergic stimulation

To evaluate whether ODD-Luc mice could be used as an *in vivo* reporter model to monitor thermogenic fat activity, these mice were exposed to cold to activate nonshivering thermogenesis in fat cells. Luciferase activity in the interscapular brown fat (BAT), which contains classical brown adipocytes, was significantly increased in ODD-Luc mice after 8 hours of cold stimulation compared to those of control mice maintained at ambient temperature and remained elevated through 18 hour and 1 day time points. By day 2 luciferase activity in BAT returned to basal levels, likely due to an amelioration of hypoxia in response to cold-induced vascularization (Figure 1a). The canonical pathway by which the central nervous system regulates the adaptive thermogenic response to cold is through norepinephrine (NE)-mediated β-adrenergic receptor activation.[12] ODD-Luc mice were then injected with CL-316, 243 (CL), a specific agonist of the β3-adrenergic receptor which is the predominant form of β-adrenergic receptor in murine thermogenic fat.[12] As expected,

adaptive thermogenesis in ODD-Luc mice injected with CL was increased (Supplementary Figure S1) and mRNA and protein levels of the thermogenic gene UCP1 were induced in the brown fat of ODD-Luc mice injected with CL (Figure 1g, Supplementary Figure S2a). Similar to the response to cold exposure, luciferase activity in brown fat was significantly increased in CL injected ODD-Luc mice compared to those of control mice injected with vehicle (Figure 1c and e).

In addition to classical brown fat cells in the interscapular depot, inducible thermogenic beige adipocytes have been identified in white adipose depots, most prominently observed in subcutaneous fat.[13] Similar to the response observed in brown fat, luciferase activity was significantly increased in the inguinal (ING) fat tissue of ODD-Luc mice thermogenically stimulated by either cold exposure or CL injection (Figures 1b, d, f, and g; Supplementary Figure S2b). This increase of luciferase activity by CL injection was not observed in other tissues (Supplementary Figure S3), consistent with the notion that β3 adrenergic receptor-mediated increase of ODD-Luc activity is specific to thermogenic adipose tissues. Collectively, these results suggest that ODD-Luc mice can be used for analysis of brown and beige fat function *in vivo*, likely due to elevated respiration and reduced intracellular oxygen availability in activated thermogenic adipocytes.

To evaluate whether the ODD-Luc system works *in vitro*, the stromal vascular fraction (SVF) was isolated from the interscapular brown fat of ODD-Luc mice and induced to a full differentiation. When these primary brown fat cells were treated with NE or CL a substantial increase of luciferase activity was observed (Figure 1h and i), suggesting that ODD-Luc can be adapted to establish a cell-based system to monitor thermogenic fat activity *in vitro*.

The OLTAM system can be used to evaluate the activity of thermogenic adipocytes in response to canonical β-adrenergic signaling and various stimuli

To broadly apply this reporter system, we generated an adenoviral system with ODD-Luc, which contains a proline residue that can be hydroxylated by PHDs, thereby sensing intracellular oxygen levels and respiration rates (Supplementary Figure S4). When introduced into non-differentiated SVF cells, luciferase activity was dramatically increased by treatment of cobalt(II) chloride (CoCl2), a PHD protein inhibitor (Figure 2a). Moreover, the 75kDa ODD-Luc protein band was clearly detected by western blotting (Figure 2b). We named this system OLTAM (*O*DD-*L*uc based *T*hermogenic *A*ctivity *M*easurement). To test this system in cultured adipocytes, primary brown fat cells transduced with adenoviral-OLTAM were treated with CL for various amounts of time (Figure 2c). Luciferase activity and ODD-Luc protein levels were increased as the CL treatment persisted (Figure 2d, Supplementary Figure S5) but not in adenoviral-OLTAM transduced undifferentiated SVF cells, which express the β3 adrenergic receptor at a much lower level, indicating that the observed effects are specific (Figure 2a). In addition, significant increases in luciferase activity were observed in response to NE treatment in fully differentiated primary brown fat cells transduced with adenoviral-OLTAM (Figure 2e). Enhanced β adrenergic receptor signaling stimulates adenylyl cyclase and leads to elevated intracellular cAMP levels.[12] As expected, both luciferase activity and ODD-Luc protein levels were significantly increased by cAMP stimulation in adenoviral-OLTAM transduced primary brown fat cells (Figure 2e).

Similar to what was observed in primary brown fat cells, treatments of CL, NE, or cAMP increased luciferase activity and ODD-Luc protein levels in differentiated SVF isolated from inguinal subcutaneous fat depots transduced with adenoviral-OLTAM (Figure 2f). Treatments with isoproterenol (ISO), a nonselective β-adrenergic receptor agonist, increased luciferase activity in both primary brown and inguinal fat cells (Supplementary Figure S6). The effectiveness of the adenoviral-OLTAM was further tested and confirmed in primary inguinal fat cells derived from various inbred mouse strains (Supplementary Figure S7), indicating the potential broad usage of this system. These results show that β-adrenergic receptor-mediated activation in both brown and beige adipocytes can be readily monitored via the OLTAM system *in vitro*.

In addition to canonical β-adrenergic signaling, various signaling molecules and pathways have been shown to regulate thermogenic fat activation. Intracellular cAMP levels are negatively regulated by phosphodiesterase (PDE) activity. As expected, treatment of IBMX (a non-selective PDE inhibitor) or cilostamide (a PDE3 inhibitor) increased luciferase activity in OLTAM transduced primary murine adipocytes (Figure 2g). Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α), as a transcriptional cofactor, plays an important role in mitochondrial biogenesis and oxidative metabolism.[14] The OLTAM system showed increased luciferase activity and ODD-Luc protein levels in primary brown fat cells overexpressing PGC1α compared to control brown fat cells expressing green fluorescence protein (GFP) (Figure 2h). Rosiglitazone, a thiazolidinedione (TZD), is a ligand of PPARγ that has been shown to cause “browning” and increase mitochondrial biogenesis both *in vitro* and *in vivo*.[15, 16] Rosiglitazone treatment in primary inguinal fat cells significantly increased luciferase activity and ODD-Luc protein levels (Figure 2h). It has recently been revealed that endogenous N-acyl amino acids regulated by peptidase M20 domain containing 1 (PM20D1) work as UCP1-independent endogenous uncouplers of mitochondrial respiration.[8] Indeed, treatments of N-arachidonyl glycine (NAGly), one of the endogenous N-acyl amino acids, effectively increase luciferase activity in primary inguinal fat cells with adenoviral-OLTAM (Figure 2i).These data collectively demonstrated that the OLTAM system provides a sensitive and convenient assay that directly measures functional changes in thermogenic fat under various conditions, regardless of the transcriptional or translational status of *Ucp1*.

Cellular respiration and oxidative phosphorylation (OXPHOS) play a pivotal role in thermogenic fat function. Cellular oxygen consumption rate (OCR) measurement is a commonly used functional assay when quantifying thermogenic adipocyte activity. During traditional OCR assays, treatment with carbonyl cyanide p-(trifluromethoxy) phenyl hydrazone (FCCP), a chemical mitochondrial uncoupler, reveals the maximal mitochondrial respiratory capacity, whereas treatment with rotenone, a mitochondrial complex I inhibitor, can be used to measure non-mitochondrial respiration. Primary inguinal fat cells with adenoviral OLTAM respond to FCCP treatments, showing increased luciferase activity by 220% and to rotenone, decreased by 70%, respectively (Figure 2k and l). These results were very similar to the results obtained by those determined using a Clark-type electrode which is a well-established tool for measurement of OCR (Figure 2j). Similar responses to FCCP and rotenone were also observed in primary brown fat cells transduced with adenoviral-OLTAM (Supplementary Figure S8).

OLTAM can be applied to various systems to specifically monitor thermogenic fat activity

As a complementary system to the adenoviral-OLTAM that can be conveniently applied in primary thermogenic fat cells, we established a retroviral-OLTAM system that can be readily incorporated into immortalized cell lines. We generated a stable cell line expressing retroviral-OLTAM based in C3H/10T1/2 cells, an immortalized line of mouse embryonic stem cells that can be robustly differentiated into adipocytes (Figure 3a). In response to $CoCl_2$ treatment, luciferase expression levels were significantly induced in these cells, indicating that the retroviral-ODD-Luc was stably expressed in these cells (Figure 3b). These fully differentiated cells were treated with the thermogenic stimuli Rosi or cAMP. Similar to primary fat cells with adenoviral-OLTAM, luciferase activity was significantly increased by Rosi or cAMP treatment (Figures 3c and d). Increased luciferase activity was further confirmed by visualization using the bioluminescence system (Figure 3e). Treatments with CL, ISO, or cilostamide increased luciferase activity in these cells (Supplementary Figure S9a–c). These results indicate that stable cell lines expressing retroviral-OLTAM could potentially be used for screening of genomic or small molecule libraries and reveal novel targets and signaling pathways that influence thermogenic fat activity.

We further investigated the specificity of OLTAM-mediated thermogenic activity measurement. Forced expression of CEBPβ and PRDM16 increases brown adipocyte specific thermogenic gene expression.[17] Adenoviral-mediated CEBPβ and PRDM16 transduction in these cells increased luciferase activity, whereas ectopic expression of PPARγ, which mediates adipogenesis rather than thermogenesis, only had a marginal effect (Supplementary Figure S9d), suggesting the specificity of the system for measurements of thermogenic activation. Next we injected WT and β-less mice, which lack all three isoforms of the β adrenergic receptor, with adenoviral-OLTAM and then assessed luciferase activity in these animals after CL stimulation. The luminescence signal was increased in the WT mice after CL injection whereas no changes were observed in the β-less mice (Figure 3f–i; Supplementary Figure S10). This loss of function mouse model provides genetic evidence to further validate that the signal measured using the OLTAM system is specific.

The OLTAM system revealed thermogenic activity in primary human subcutaneous adipocytes from multiple donors

Recent studies have shown that primary human adipose stem cells isolated from subcutaneous depots can fully differentiate *in vitro* and that these human primary subcutaneous fat cells respond to various thermogenic stimuli,[18, 19] suggesting that thermogenic adipocytes may exist in anatomic locations in addition to the supraclavicular region. To explore whether the OLTAM system can monitor thermogenic activity occurring in human adipocytes, we applied the adenoviral-OLTAM system to fully differentiated human subcutaneous fat cells from multiple donors of different ethnicities, ages and with a range of body mass indexes (BMIs) (Figures 4a and b; Supplementary Figure S11). Treatments of the PDE inhibitors IBMX and cilostamide increased luciferase activity in these differentiated primary human subcutaneous adipocytes (Figure 4c and d). cAMP treatments also significantly increased luciferase activity in these differentiated primary human subcutaneous adipocytes (Figure 4e). Moreover, bioluminescence imaging of these

cells suggests that increased thermogenic activity could be visualized via the adenoviral-OLTAM system in human subcutaneous adipocytes (Figure 4f), providing proof-of-principle evidence that future assays could be designed and developed based on this mechanism for *in vivo* imaging to monitor human thermogenic fat activity.

DISCUSSION

Here, we report that luciferase activity is increased in brown and inguinal fat of ODD-Luc mice upon thermogenic stimulation both *in vivo* and in cultured cells. These initial observations provided the rationale to develop a cell-based system using ODD-Luc to monitor thermogenic fat activity, which we named the OLTAM system.

Recent studies have revealed that UCP1-mediated thermogenesis can be significantly affected by splicing and post-transcriptional stabilization of *Ucp1* mRNA and posttranslational modification of the UCP1 protein, including sulfenylation at the 253th cysteine residue.[20–22] In addition, several UCP1 independent thermogenic mechanisms have been reported.[6–8] In contrast to previously developed systems, such as the 'ThermoMouse'[4] and the 'Ucp1-2A-luciferase knock-in mouse',[5] the OLTAM system does not depend on *Ucp1* regulation but instead directly assays the functional consequence of thermogenic fat activation through monitoring intracellular oxygen levels.[11] Indeed, activation of beige fat induced by NAGly, one of the endogenous uncouplers, was readily measured by the OLTAM system (Figure 2i). Luciferase activity in adenoviral-OLTAM transduced primary ING and BAT fat cells responds sensitively to FCCP or rotenone treatments (Figures 2k and l, Supplemental Figure S8). Additional applications of our OLTAM system were demonstrated with the C3H/10T1/2 stable cell line expressing retroviral-OLTAM. Immortalized cell line based systems not only permit gain- and loss- of function studies of individual molecules of interest, but also can potentially enable high throughput screens to identify novel signaling pathways.

The content and activity of thermogenic fat is heterogeneous among human populations.[3] Effective use of therapies targeting these cells to counteract human obesity requires identification of likely responders who have high thermogenic fat content. This challenge to personalize medical approaches demands improved diagnostic tools to accurately measure thermogenic fat in humans. Currently the most widely used approach is a combination of positron emission tomography (PET) using a ^{18}F-fluorodeoxyglucose ([^{18}F]FDG) tracer and computed tomography (CT) scanning.[3] On the other hand, an alternative tracer with ^{18}F-fluoro-thiaheptadecanoic acid ([^{18}F]FTHA, a fatty acid tracer) has also been used to detect adult human thermogenic fat activity during cold exposure.[23] These substrate-uptake based imaging assays do not comprehensively reveal the content of thermogenic fat since the preferred fuel under physiological conditions has not yet been illustrated. Furthermore, the oxygen consumption in [^{18}F]FDG PET defined "thermogenic fat" only accounted for a small fraction of the overall oxidative metabolism calculated with [^{15}O]O_2.[24] More interestingly, a significant portion of total energy expenditure was observed in white adipose tissue ([^{18}F]FDG-negative),[24] consistent with the hypothesis that thermogenic fat cells exist in these depots and may be too diffused to be detected through glucose-uptake based imaging. Hypoxia based imaging has been used in diagnosis of tumor progression in clinical

applications.[25] We demonstrated here that the OLTAM system could be used to visualize the thermogenic activation of human subcutaneous adipocytes *in vitro* (Figure 4), suggesting that imaging based on this mechanism can be adapted to enable a comprehensive measurement of thermogenic fat activity in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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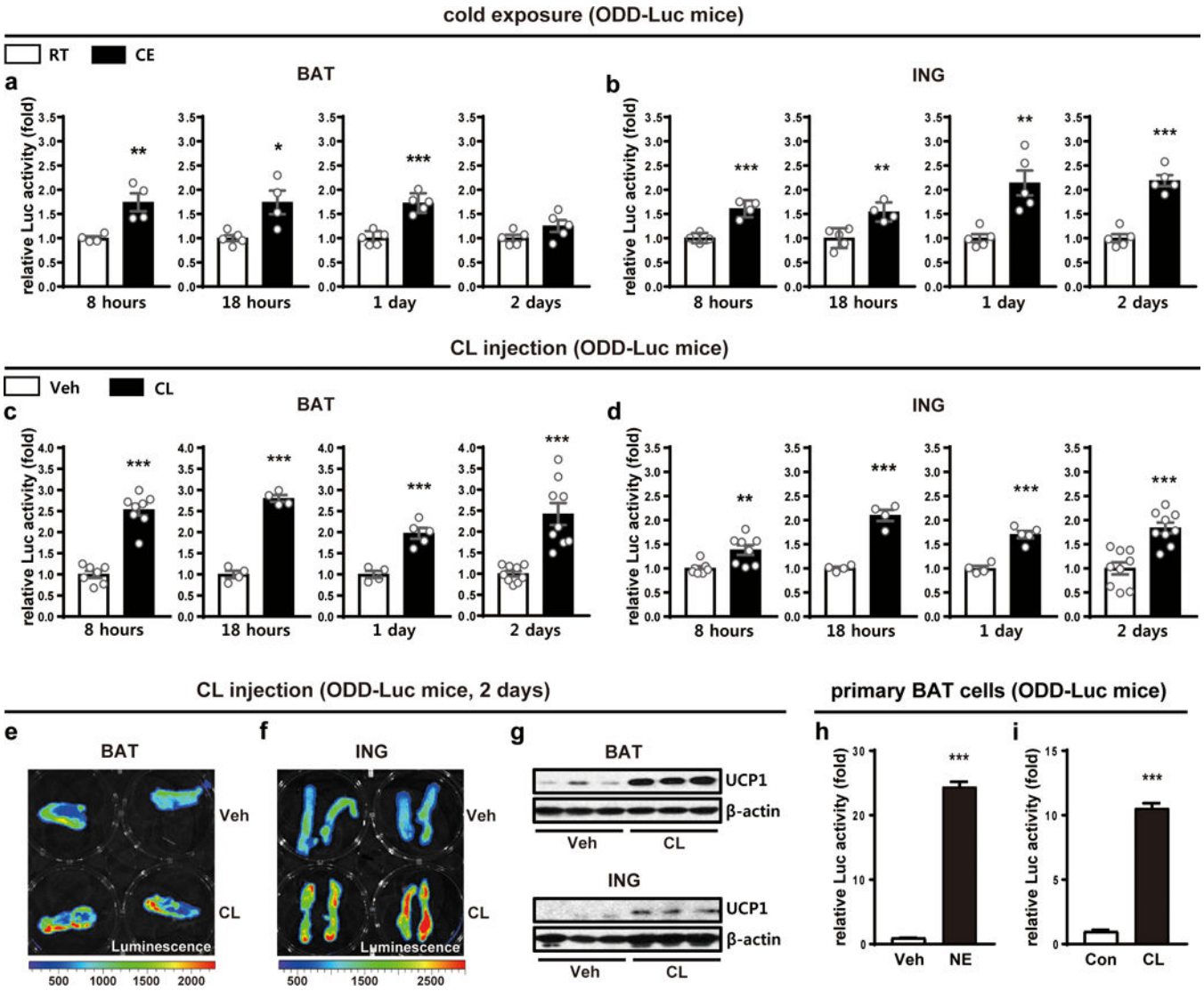

Figure 1.

ODD-Luc mice can be used for monitoring thermogenic fat activity mediated by cold exposure and β-adrenergic stimulation. (a and b) ODD-Luc mice were housed at ambient temperature (RT) or cold environment (CE) for the indicated amount of time. (a) Brown and (b) inguinal fat were isolated and luciferase activity was measured. (c-g) ODD-Luc mice were intraperitoneally injected with PBS or 1 mg/kg/day of CL-316, 243 for the indicated amount of time. Luciferase activity was measured from homogenized (c) brown and (d) inguinal fat. Each animal used in these experiments is represented on the graph as a dot. (e) Brown and (f) inguinal fat tissues were isolated and bioluminescence imaging was performed. The colored bar code indicates the intensity of the luciferase signal. (g) Brown and inguinal fat tissue were isolated and UCP1 protein expression was measured by Western blotting. Representative immunoblots are shown. β-actin was used as a loading control. (h and i) Primary BAT fat cells derived from ODD-Luc mice were treated with 200 nM norepinephrine (NE), vehicle (Veh), 100 nM CL-316, 243 (CL) or control (Con) for 24

hours and luciferase activity was measured (n=3). Data are presented as mean ± SEM *(p<0.05), **(p<0.01) and ***(p<0.001).

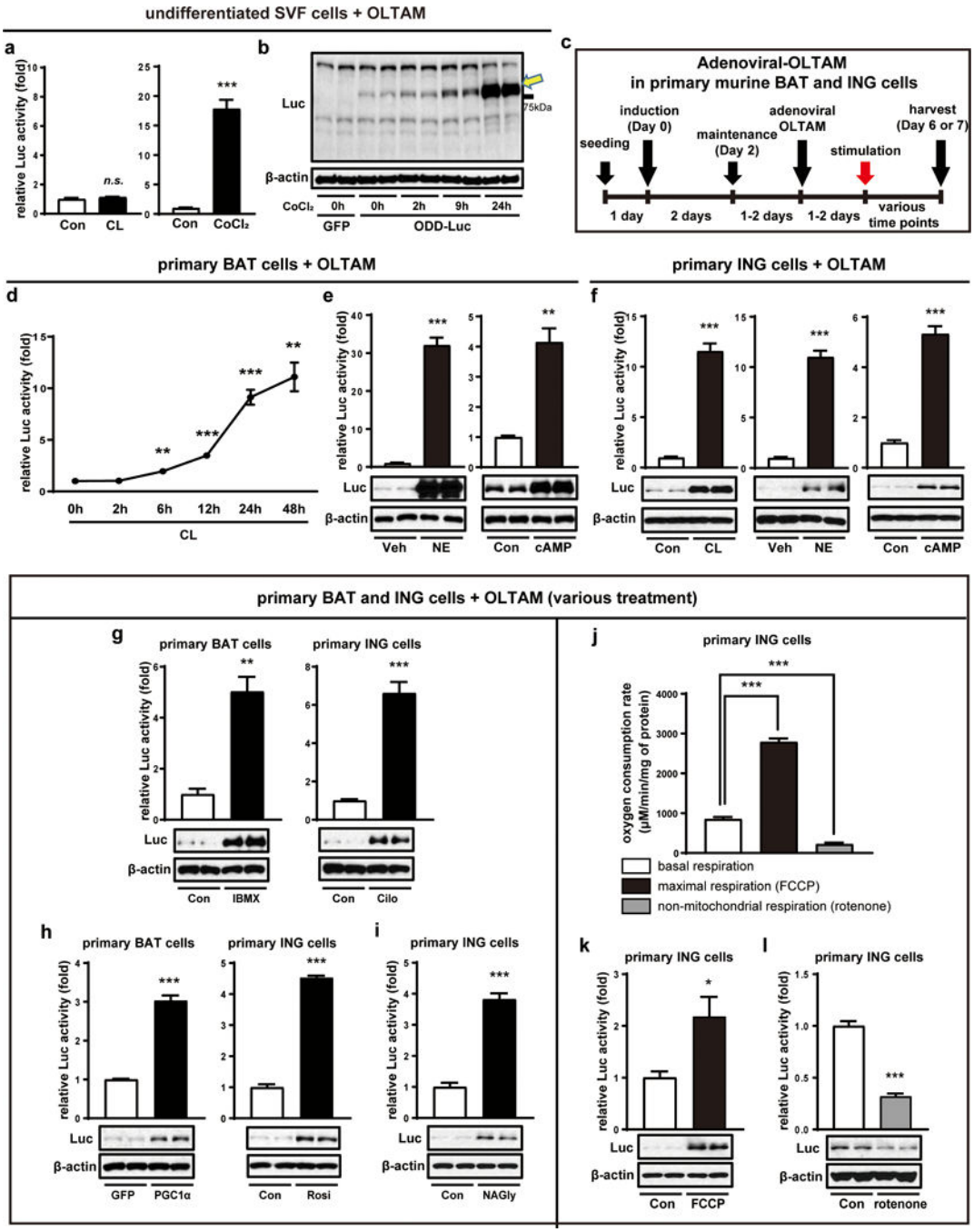

Figure 2.

The OLTAM system can be used to evaluate the activity of thermogenic adipocytes in response to canonical β-adrenergic signaling and various stimuli. (a) Adenoviral-OLTAM transduced primary inguinal SVF cells were treated with 100 nM CL, 500 μM $CoCl_2$ or control for 24 hours and then luciferase activity was measured (n=3). (b) Adenoviral-OLTAM transduced primary inguinal SVF cells were treated with 500 μM $CoCl_2$ for various time intervals. ODD-Luc protein expression was measured by Western blotting. A yellow arrow indicates the 75 kDa ODD-Luc band. Representative immunoblots are shown.

Experiments using an adenovirus expressing GFP was included as a negative control. (c) Diagram of the experimental timetable of the adenoviral-OLTAM system in primary murine adipocytes. (d) Primary BAT cells were transduced with adenoviral-OLTAM and then were treated with 100 nM CL for the indicated amount of time. Luciferace activity was measured (n=3). (e and f) Primary (e) BAT and (f) ING fat cells were transduced with adenoviral-OLTAM and then were treated with the indicated drugs (200 nM NE, 100 nM CL or 100 μM cAMP) for 24 hours. Luciferase activity was measured (n=3) and ODD-Luc expression was assayed by Western blot analysis. (g) Primary BAT and ING fat cells were transduced with adenoviral-OLTAM and then were treated with 100 μM IBMX, 10 μM cilostamide (Clio) or control for 24 hours. Luciferase activity was measured (n=3) and ODD-Luc expression was assayed by Western blot analysis. (h) Primary BAT and ING fat cells were transduced with adenoviral-OLTAM. 24 hours later, BAT cells were transduced with adenovirus expressing PGC1α or GFP as a control and primary ING cells were treated with 5 μM rosiglitazone (Rosi). Two days later, cells were harvested and luciferase activity (n=3) and ODD-Luc expression were measured. (i) Primary ING fat cells were transduced with adenoviral-OLTAM and were treated with 50 μM N-arachidonyl glycine (NAGly) for 24 hours. Cells were harvested and luciferase activity (n=3) and ODD-Luc expression were measured. (j) Oxygen consumption rate in primary ING cells was measured with a Clark-type electrode. Respiration was determined in the absence of drugs (basal, n=4) or the presence of 1μM FCCP (maximal, n=4) or 2 μM rotenone (non-mitochondrial, n=4). (k and l) Primary ING fat cells were transduced with adenoviral-OLTAM and were treated with (k) 1 μM FCCP for 24 hours or (l) 500 nM rotenone for 5 hours. Cells were harvested and luciferase activity (n=3) and ODD-Luc expression were measured. β-actin was used as a loading control. Data are presented as mean ± SEM *n.s.*(not significant), *($p<0.05$), **($p<0.01$), and ***($p<0.001$).

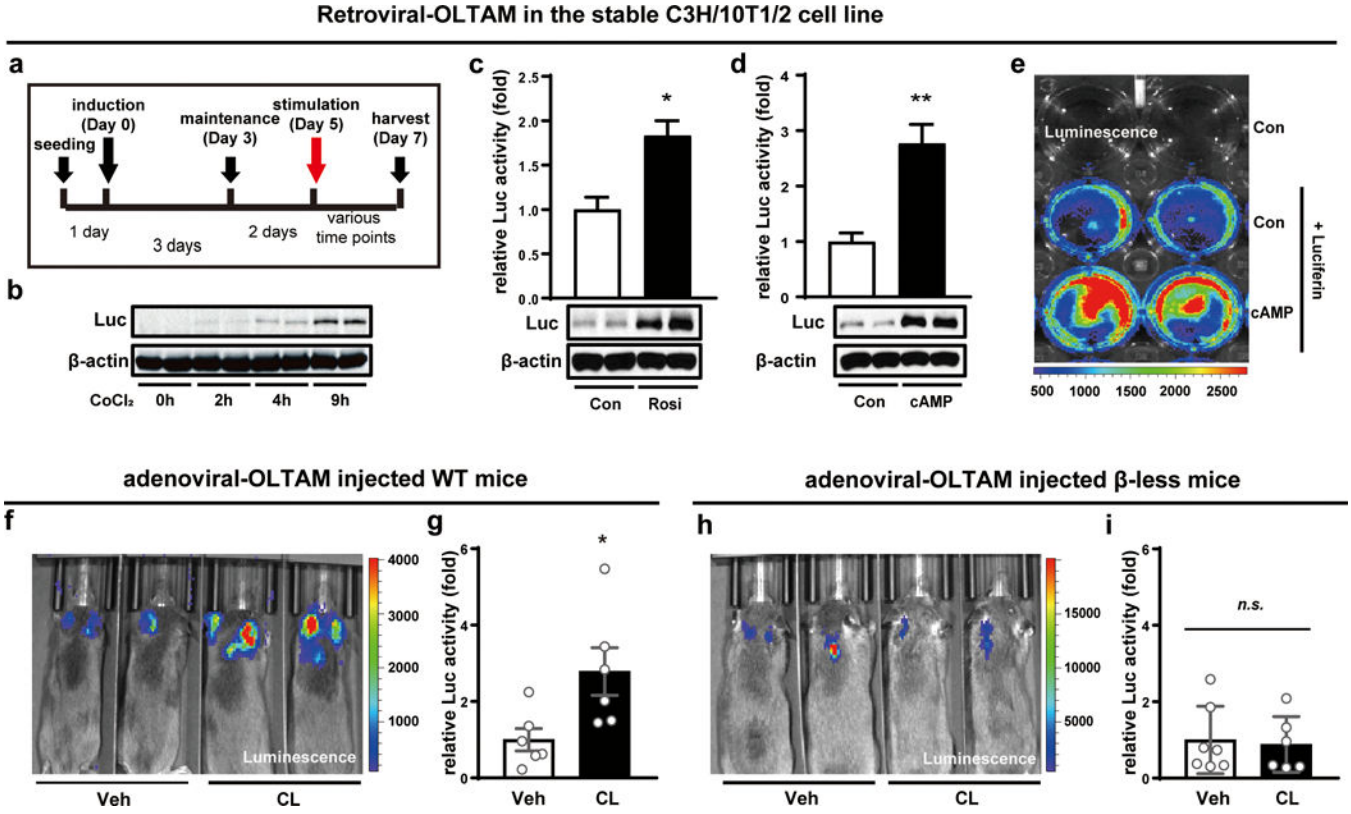

Figure 3.

OLTAM can be applied to various systems to specifically monitor thermogenic fat activity. (a) Diagram of the experimental timetable of the retroviral-OLTAM system in the stable cell line. (b) The C3H/10T1/2 stable cell line expressing retroviral-OLTAM was fully differentiated and treated with 500 μM $CoCl_2$ for various time intervals. Cells were harvested and ODD-Luc expression was assayed by Western blot analysis. β-actin was used as a loading control. (c-e) The C3H/10T1/2 stable cell line expressing retroviral-OLTAM was differentiated and treated with (c) 1 μM Rosi or (d and e) 100 μM cAMP for 48 hours. (c and d) Luciferase activity (n=3) and ODD-Luc expression were measured. (e) Cells were incubated with luciferin and bioluminescence imaging was performed. The colored bar code indicates the intensity of the luciferase signal. (f-i) Wild-type (WT) and β-less mice were injected with adenoviral-OLTAM in the BAT depot. Two days later, the mice were intraperitoneally injected with PBS or 1 mg/kg of CL-316, 243. (f and h) 24 hours later mice were injected with luciferin and bioluminescence imaging was performed. The colored bar code indicates the intensity of the luciferase signal. (g and i) Brown fat was isolated and luciferase activity was measured. Each animal used in these experiments is represented on the graph as a dot. Data are presented as mean ± SEM *n.s.*(not significant), *(p<0.05) and **(p<0.01).

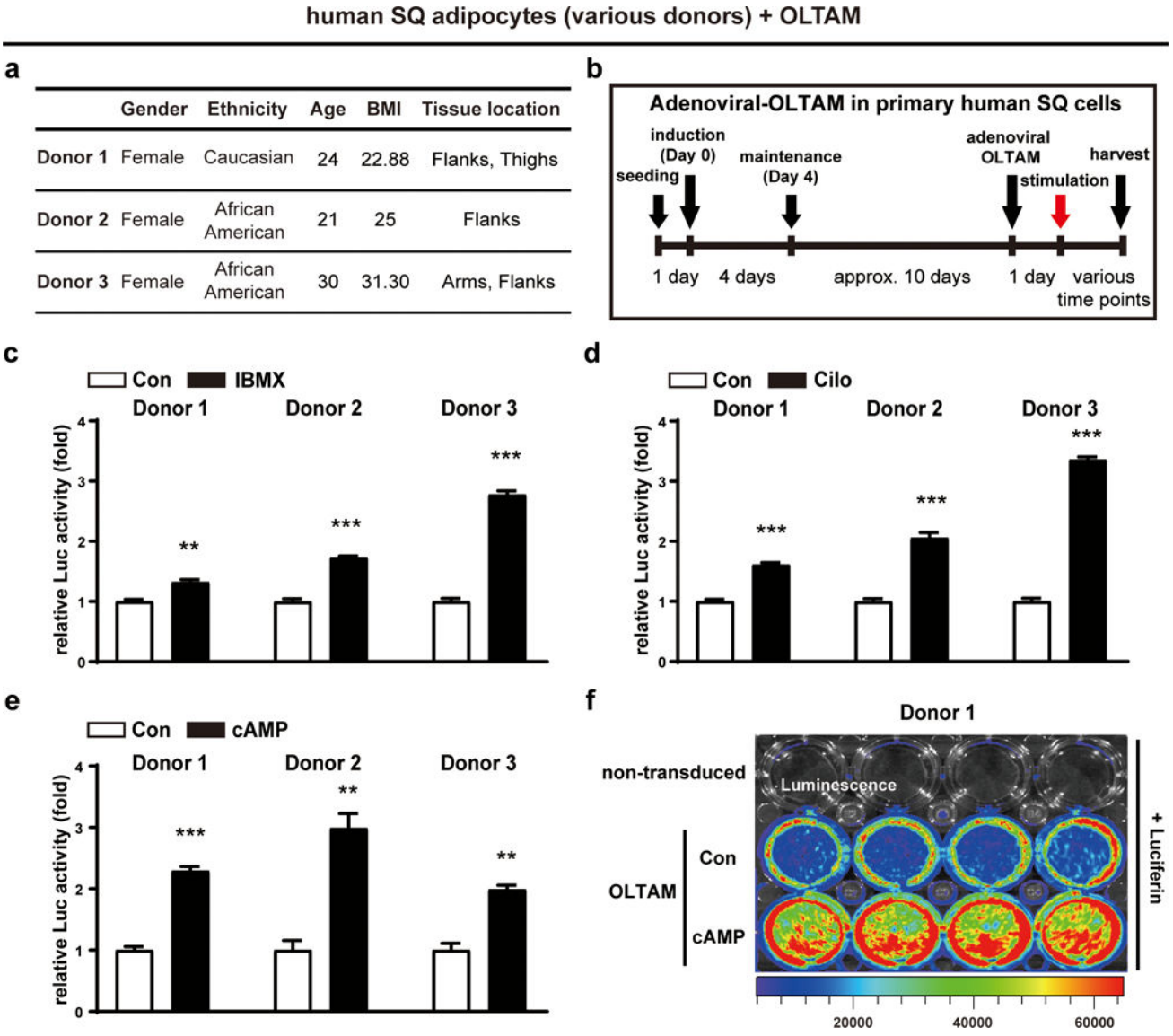

Figure 4.

The adenoviral-OLTAM system revealed thermogenic activity in primary human subcutaneous adipocytes from multiple donors. (a) Subject information of human donors in this study. (b) Diagrams of the experimental timetable of the adenoviral-OLTAM system in primary human subcutaneous adipocytes. (c-f) Primary human SQ adipocytes were transduced with adenoviral-OLTAM and then were treated with (c) 100 μM IBMX for 24 hours, (d) 10 μM cilostamide (Clio) for 24 hours (e and f) 200 μM cAMP or control (Con) for 48 hours. (c-e) Cells were harvested and luciferase activity was measured (n=3). (f) Cells were incubated with luciferin and bioluminescence imaging was performed. The colored bar code indicates the intensity of the luciferase signal. Data are presented as mean ± SEM **(p<0.01) and ***(p<0.001).