Full activation of thermogenesis in brown adipocytes requires Basigin action

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Full activation of thermogenesis in brown adipocytes requires Basigin action

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Keywords
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Exploring mechanisms responsible for brown adipose tissue’s (BAT) high metabolic activity is crucial to exploit its energy-dissipating ability for therapeutic purposes. Basigin (Bsg), a multifunctional highly glycosylated transmembrane protein, was recently proposed as one of the 98 critical markers allowing to distinguish ‘white’ and ‘brown’ adipocytes, yet its function in thermogenic brown adipocytes is unknown. Here, we report that Bsg is negatively associated with obesity in mice. By contrast, Bsg expression increased in the mature adipocyte fraction of BAT upon cold acclimation. Additionally, Bsg levels were highly induced during brown adipocyte maturation in vitro and were further increased upon β-adrenergic stimulation in a HIF-1α-dependent manner. siRNA-mediated Bsg gene silencing in cultured brown adipocytes did not impact adipogenesis nor mitochondrial function. However, a significant decrease in mitochondrial respiration, lipolysis and Ucp1 transcription was observed in adipocytes lacking Bsg, when activated by norepinephrine. Furthermore, using gas chromatography/mass spectrometry–time-of-flight analysis to assess the composition of cellular metabolites, we demonstrate that brown adipocytes lacking Bsg have lower levels of intracellular lactate and acetacetate. Bsg was additionally required to regulate intracellular AcAc and tricarboxylic acid cycle intermediate levels in NE-stimulated adipocytes. Our study highlights the critical role of Bsg in active brown adipocytes, possibly by controlling cellular metabolism.

Abbreviations
AcAc, acetoacetate; Ad, mature adipocyte fraction; AT, adipose tissue; AUC, area under the curve; BAT, brown adipose tissue; BSG, basigin; ECAR, extracellular acidification rate; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; FISH, fluorescent in situ hybridization; GC/MS-TOF, gas chromatography/mass spectrometry–time-of-flight; HFD, high-fat diet; Iso, isoproterenol; KD, knockdown; LC/MS-TOF, liquid chromatography/mass spectrometry–time-of-flight; MCT, monocarboxylate transporter; MMP, matrix metalloproteinase; NE, norepinephrine; OCR, oxygen consumption rate; Oligo, oligomycin; PPIA, peptidylprolyl isomerase A; qPCR, quantitative polymerase chain reaction; ROT/AA, rotenone/antimycin A; SVF, stromal vascular fraction; sWAT, subcutaneous white adipose tissue; TBP, TATA-box binding protein; TCA, tricarboxylic acid; UCP1, uncoupling protein 1; VEGF, vascular endothelial growth factor; vWAT, visceral white adipose tissue; WAT, white adipose tissue; βAR, β-adrenergic receptor.
Introduction

The ability of brown adipose tissue (BAT) to drive thermogenesis via the uncoupling protein 1 (UCP1) by burning fuels in response to physiological challenges such as cold temperature, presents a promising therapeutic opportunity for the treatment of obesity and its related comorbidities [1–3]. However, understanding the mechanisms that activate brown fat and promote energy dissipation is essential to utilize BAT for novel pharmacological intervention against metabolic disease.

Basigin (Bsg, CD147, EMMPRIN) was recently suggested as one of 98 marker genes allowing the distinction between ‘white’ and ‘brown’ adipocytes in mice and men [4]. Its abundance in mature brown, compared with white adipocytes, indicates it may play a pivotal role in sustaining brown adipose function. However, the role of Bsg has never been explored in the context of brown fat tissue. Bsg is a widely expressed multifunctional protein with an established role in a variety of physiological and pathophysiological processes such as spermatogenesis, the development of neural networks and retina, as well as cancer progression, inflammation and viral infections [5–8]. It promotes angiogenesis by controlling the expression of vascular endothelial growth factor (VEGF) and induces the degradation of basement membrane via matrix metalloproteinases (MMP), thus promoting tumour invasion [6,9]. Interestingly, these biological processes are also relevant for brown fat expansion during its activation [10]. Furthermore, Bsg interacts with a multitude of binding partners including integrins, cyclophilins and monocarboxylate transporters (MCTs) [11]. One of the better-described roles of Bsg is in trafficking MCT-1 and -4 to the plasma membrane in highly glycolytic tissues such as eye retina and cancer cells. This enables the transport of MCT substrate metabolites including lactate, pyruvate, succinate and ketone bodies acetoacetate (AcAc) and β-hydroxybutyrate [12–15]. Retina-specific Bsg knockout mice display a drastic decrease in the surface expression of MCT-1/4 in Müller cells and photoreceptors, compromising the shuttle of lactate between the two cell types, leading to blindness [12]. Similarly, Bsg aids the removal of lactate in cancer cells by scaffolding MCTs to the cell membrane, thus regulating glucose metabolism and promoting tumour progression [13]. Interestingly, MCT-1 and -4 are known to control lactate transport in white and brown adipocytes [16]. This raised speculations that Bsg might play a role in coordinating nutrient transport across the membrane and cellular metabolism in brown adipocytes.

Here we aimed to decipher whether Bsg is involved in brown adipocyte function, respiration and intracellular availability of metabolites in brown fat. In this study, we revealed the regulation of Bsg expression in BAT by temperature and obesity. Additionally, investigating its loss of function in brown adipocytes in culture, we demonstrated its critical role in sustaining the appropriate physiology of brown adipose cells.

Results

Bsg expression in BAT is regulated by temperature and obesity

To investigate whether Bsg plays a role in BAT, we first examined its expression during cold exposure, which robustly activates brown fat tissue [17]. Mice were initially housed at thermoneutrality for 3 weeks and subsequently exposed to cold for 3, 7 and 21 days. Quantitative PCR (qPCR) analysis of BAT revealed a 3- and 6-fold increase in Bsg gene expression upon 1 and 3 weeks of cold acclimation, respectively (Fig. 1A). Additionally, Bsg protein abundance in BAT significantly increased following 3 weeks of cold acclimation (Fig. 1B). Comparatively, its transcript levels remained unchanged in subcutaneous white adipose tissue (sWAT) and even decreased after 3 weeks of cold acclimation in visceral white adipose tissue (vWAT) (Fig. 1A). Consistent with the dynamic regulation of Bsg with temperature, we further uncovered that deactivating BAT, by transitioning mice from cold to thermoneutral temperatures for 3 or 7 days, resulted in an approximately 35% reduction of Bsg mRNA (Fig. 1C).

To assess whether Bsg is regulated by pathological metabolic conditions we examined its gene expression in fat depots of high-fat diet (HFD)-fed and leptin-deficient ob/ob mice. Compared with lean mice, the qPCR analysis revealed an approximately 2-fold reduction of Bsg in BAT of HFD and ob/ob animals. In contrast to brown fat, visceral depots from obese leptin-deficient mice had increased Bsg expression compared with lean controls. However, increased adiposity did not affect Bsg transcript levels in sWAT (Fig. 1D).

Collectively, these data demonstrate a dynamic regulation of Bsg expression in BAT, being increased during activation by chronic cold while a negative regulation of its expression occurred with obesity and warm temperature, suggesting Bsg is necessary to maintain a healthy BAT.

Dynamic regulation of Bsg occurs in brown adipocytes in vivo

Adipose tissue (AT) is highly heterogeneous and consists of various cell populations [18]. Additionally, Bsg is expressed in several cell types where it plays different
biological roles [8]. Given that chronic cold increased Bsg levels in BAT, we sought to elucidate whether a specific cellular population within AT is responsible for the observed induction of Bsg expression.

Mature adipocytes (Ad) and stromal vascular fractions (SVF) were obtained from AT of mice exposed to chronic cold. Gene expression data revealed that Bsg was uniquely induced with cold in the lipid-laden mature adipocyte population of BAT (Fig. 2A). Fluorescence-based in situ hybridization (FISH) of mouse BAT additionally confirmed cold-induced upregulation of Bsg. Moreover, we found that Bsg mRNA preferentially co-localized with mature adipocyte marker gene AdipoQ, demonstrating its enrichment in mature brown adipose cells compared with other cell types during cold exposure (Fig. 2B).

Collectively, our data revealed a dynamic regulation of Bsg expression primarily in mature brown adipocytes.

Brown adipocyte differentiation and β-adrenergic stimulation promote Bsg expression

To further investigate the regulation of Bsg in brown adipocytes we employed the WT-1 mouse brown pre-
Basigin regulates brown adipocyte activation

K. Rupar et al.

adipocyte cell line. WT-1 cells were differentiated into mature brown adipose cells, as denoted by the upregulation of adipogenic and thermogenic marker genes (AdipoQ, Fabp4, Ucp1). We found that the abundance of Bsg mRNA progressively increased to be significantly higher on day 4 of cell maturation and reached a plateau on day 6 post-induction of differentiation (Fig. 3A). Moreover, upregulation on Bsg protein level closely mirrored that of its gene expression (Fig. 3B).

Additionally, Bsg is known to be regulated by hypoxia in tumours via hypoxia-inducible factor-1α (HIF-1α), promoting glucose metabolism and tumour progression [19]. Since HIF-1α was previously demonstrated to contribute to the induction of glycolytic enzymes during hypoxic conditions and β-adrenergic stimulation in mouse brown adipocytes [20], we investigated the potential involvement of HIF-1α in the regulation of Bsg in these cells. Bsg gene expression was analysed in mature WT-1 cells, subjected to hypoxia and transfected with either control or HIF-1α siRNA. We detected a 3-fold increase in Bsg expression during the hypoxic condition, which was abolished upon Hif-1α depletion (Fig. 3D).

Altogether, Bsg abundance and upregulation upon cell differentiation suggest that Bsg might have a role in the function of mature brown adipocytes. Additionally, our data demonstrate that HIF-1α drives the expression of Bsg during hypoxia and β-adrenergic activation of brown adipocytes.

**Bsg depletion does not impact basal adipocyte and mitochondria function in brown adipocytes**

To assess Bsg function in mature brown adipocytes, we employed siRNA-mediated Bsg KD in mature mouse WT-1 cells. Compared with control cells, KD resulted in a 90% reduction of Bsg mRNA (Fig. 4A), resulting in 50% decrease of Bsg on protein level (Fig. 4B). Inhibition of Bsg expression did not affect the transcript levels of adipogenic markers (Acc1, AdipoQ, AldoA, Fabp4, Fasn), glucose transporters (Glut1, Glut4), pro-angiogenic factor Vegfa, MCTs (Mct-1, Mct-4), MMPs (Mmp-14, Mmp-19) or pro- (Cd38, Chemerin, iNOS) and anti- (IL-10) inflammatory markers during basal and thermogenic conditions (Fig. 4C). Accordingly, lipid accumulation and lipid droplet size were also comparable in control and Bsg KD cells (Fig. 4D).

Additionally, investigating mitochondrial function by measuring basal, maximal, ATP-linked and nonmitochondrial respiration as well as spare respiratory capacity and proton leak, we observed no differences between Bsg KD and control brown adipocytes (Fig. 5A). Furthermore, no significant changes were observed in basal or maximal extracellular acidification rate (ECAR) in Bsg KD cells (Fig. 5B), despite a trend for decreased abundance of MCT4 at the plasma membrane upon Bsg depletion (Fig. 5C), which is in line with the well described...
function of Bsg in targeting MCTs to the cell membrane. Consistent with a preserved mitochondrial function, decreased Bsg expression did not affect the levels of mitochondrial oxidative phosphorylation complexes (Fig. 5D).

Collectively, diminished Bsg function does not cause impairments in lipid accumulation and mitochondrial function in nonactivated mature brown adipocytes.

Brown adipocyte β-adrenergic activation requires intact Bsg function

We next interrogated the functional consequences of Bsg KD upon activation of thermogenesis, by stimulating brown WT-1 cells with norepinephrine (NE). Assessment of mitochondrial respiration during
Fig. 4. Loss of Bsg does not affect brown adipocyte function in nonstimulated state. (A–D) Mature (day 4) WT-1 cells were reverse transfected with negative control or siRNA against Bsg. (A) Bsg transcript levels are normalized to Tbp and expressed as a percentage of control (n = 4). (B) Immunoblotting of Bsg protein levels. Graph represents quantification of Bsg band intensity normalized to Vinculin and expressed as a percentage of control. Western blot is representative of three independent experiments. (C) Relative expression of adipogenic marker genes, glucose transporters, Vegfa, MCTs, MMPs and inflammatory markers, normalized to Tbp mRNA during basal (upper panel) and stimulated conditions (1 μM NE, 6 h) (lower panel). Values are shown relative to basal control for each gene (n = 4). (D) Representative confocal microscopy images of cells stained using Nile red and DAPI. Graphs represent quantified Nile red intensity and area of lipid droplets. Data were normalized to cell number and expressed as a percentage of the control condition. Images are representative of three independent experiments. Data are shown as the mean ± SEM. *P < 0.05, ***P < 0.001 was measured by Student’s t-test and two-way ANOVA.
Fig. 5. Mitochondrial function in nonstimulated brown adipocytes remains intact in Bsg KD cells. (A–D) Mature (day 4) WT-1 cells were reverse transfected with negative control siRNA or siRNA targeting Bsg. (A) OCR and (B) ECAR were measured by performing Mito Stress Test in response to oligomycin (5 μM), FCCP (1 μM) and rotenone/antimycin A (1 μM) and are normalized to cell count (n = 5). Basal ECAR was determined prior and maximal ECAR level was determined after oligomycin injection. (C) Immunoblotting of MCT4 in the plasma membrane fraction of basal and NE-treated (1 μM, 1 h) cells. Graph represents quantified MCT4 band intensity normalized to insulin receptor β and expressed as relative to the control condition. Immunoblot is representative of three independent experiments. (D) Western blot of mitochondrial oxidative phosphorylation complex subunits I–V (CI–CIV) in cells stimulated with vehicle or NE (1 μM, 24 h). Western blots are representative of four independent experiments. Graphs demonstrate quantified band intensities normalized to Vinculin, expressed as fold change to control cells for each complex subunit. Data are shown as the mean ± SEM. Statistical significance was measured using one-way and two-way ANOVA.
thermogenesis showed a 35% decrease in oxygen respiration rate (OCR) (Fig. 6A) and a 37% reduction in ECAR (Fig. 6B) in Bsg KD cells. Compared with control cells, Bsg KD further reduced NE-stimulated lipolysis by 37% (Fig. 6C). Lack of Bsg profoundly downregulated Ucp1 mRNA in basal and NE-stimulated cells, without impairing the levels of Pgc-1α, Pparγ2, Prdm16 or Dio2 (Fig. 6D). These effects

Fig. 6. Loss of Bsg impedes NE-induced activation in brown adipocytes (A–E). Brown adipocytes were differentiated (day 4) and reverse transfected with control or anti-Bsg siRNA. (A) OCR upon oligomycin (5 μM) and NE (1 μM) injection, normalized to cell count. Graph represents the AUC of NE-stimulated OCR and is calculated from 3 independent experiments. (B) ECAR measured after oligomycin (5 μM) and NE (1 μM) injection. The AUC of NE-stimulated ECAR was calculated from three separate experiments and is shown in the graph. Data are normalized to cell count. (C) Glycerol concentration in vehicle or NE (1 μM, 6 h) treated cells (n = 7). (D) Gene expression of Ucp1, Pgc-1α, Pparγ2, Prdm16 and Dio2 in basal condition (left) and cells treated with NE (1 μM, 6 h) (right). mRNA levels are shown relative to control in basal condition for each gene and normalized to Tbp (n = 4). (E) Immunoblotting of β-adrenergic signalling in basal and NE-stimulated (1 μM, 15 min) conditions (n = 4). Graphs demonstrate quantified band intensities of phospho-proteins, normalized to the total amount of corresponding protein and are expressed as fold change to control cells for each phospho-protein. Data represent means ± SEM. Significance was determined by one-way ANOVA and two-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
were independent on β-adrenergic sensitivity since no signalling changes were observed between Bsg KD adipocytes and control cells (Fig. 6E).

Collectively, our data clearly demonstrate that Bsg is involved in the proper thermogenic activation of brown adipocytes.

**Bsg is necessary to maintain cellular metabolism in brown adipocytes**

Given the important role of Bsg regulating the traffic of MCT-1 and -4 as well as facilitating the transport of essential metabolites such as lactate, pyruvate, succinate and ketone bodies [14–16,21], we interrogated the involvement of Bsg in controlling the levels of intra- and extracellular metabolites in mature brown WT-1 cells, using a targeted metabolomics approach. In basal conditions, we observed a decrease in intracellular lactate and AcAc in cells lacking Bsg (Fig. 7A), while extracellular metabolite levels remained unchanged (Fig. 7B). In accordance with intact basal respiration, there was no change in tricarboxylic acid (TCA) cycle metabolites. Additionally, the levels of glucose and early glycolytic intermediates glucose-6-phosphate and fructose-6-phosphates were also comparable in control and Bsg KD adipocytes (Fig. 7A,B).

Since we found Bsg to be important for successful NE-induced mitochondrial respiration, we next investigated which metabolic dysfunction may occur during the stimulated state. NE treatment of brown adipocytes resulted in decreased intracellular AcAc levels in Bsg-depleted cells (Fig. 8A).

Furthermore, a discriminant analysis [22] of intracellular metabolites upon NE stimulation indicated that among all tested metabolites, AcAc and TCA intermediates were the main factors differentiating Bsg KD adipocytes from the control cells (Fig. 8B). 13C-isotopic glucose tracing for 1 h in NE-stimulated control and Bsg KD adipocytes showed comparable incorporation of glucose into TCA metabolites and glycolysis (Fig. 8C). No

![Fig. 7. Effect of Bsg KD on intracellular and extracellular metabolites in brown adipocytes.](image-url)
Basigin regulates brown adipocyte activation

K. Rupar et al.

(A) Intracellular metabolites upon NE-stimulation

Brown adipocytes

- MCT-1/4 substrates
- TCA intermediates
- Glucose metabolism

(B) Intracellular metabolites upon NE-stimulation

Brown adipocytes

Variable importance

(C) Intracellular glucose tracing upon NE-stimulation

Brown adipocytes

- Glucose
- Pyruvic acid
- Lactic acid
- Citric acid
- α-Ketoglutaric acid
- Succinic acid
- Malic acid
- Fumaric acid
- Acetoacetic acid
- β-Hydroxybutyric acid
significant differences in the specific isotopologue distributions of the tested metabolites were observed; however, the fractional labelling of the unlabeled (m + 0) citrate and α-ketoglutarate was significantly higher in Bsg KD cells (Fig. 8C).

Combined, these results indicate that Bsg is important for maintaining cellular metabolic homeostasis in activated brown adipocytes.

Discussion

Brown adipose tissue has emerged as a promising candidate for addressing obesity and obesity-driven metabolic disturbances due to its extraordinarily efficient energy-dissipating function. However, molecular insights are still lacking to better understand the mechanisms allowing the unique metabolic properties of brown adipocytes and their fuel utilization adaptive capacity [23,24]. Albeit Bsg belonging to a specific set of 98 brown adipose marker genes, the role of this multifunctional protein in maintaining proper BAT function has never been explored [4].

Here we demonstrate that Bsg is highly enriched in mature thermogenic adipocytes of mouse BAT and is dynamically regulated by metabolic challenges such as temperature and obesity. Functional assessment revealed a requirement for intact Bsg function to preserve essential processes underpinning thermogenesis such as mitochondrial respiration, Ucp1 expression, lipolysis and cellular metabolism during brown adipocyte activation.

Active regulation of Bsg in mature brown adipose cells, positively by cold activation and on the contrary negatively by warm temperature and diet-induced or genetic obesity in brown fat, suggests that Bsg may be involved in key brown fat functions. Besides, the presence of BSG in human primary adipocytes from supraventricular BAT underlines the potentially important role of BSG in human brown fat. Notably, the opposite regulation of Bsg expression observed in BAT and vWAT of leptin-deficient obese mice may be linked to the remodelling activity and metabolic requirements of the tissues, respectively. It could also indicate a direct regulation of Bsg expression by leptin signalling in adipose depots.

Increased abundance of Bsg during brown adipogenesis further supports its role in sustaining brown adipocyte function. In mature brown adipocytes, Bsg expression was further increased upon β-adrenergic stimulation in a HIF-1α dependent manner, similar to its regulation in tumours [19]. This regulation, in concert with glycolytic factors via HIF-1α, suggests its involvement in glucose metabolism during high-energy demand in brown adipocytes, possibly for the regulation of lactate flux via MCT-1/4 trafficking in brown adipocytes. In favour of this is a dysregulation of intracellular lactate at basal state and significant reduction in NE-stimulated extracellular acid production upon loss of Bsg. However, in brown adipocytes, the ECAR is not only indicative of lactate secretion but also of FAs and reflecting CO2 production via oxidative phosphorylation [25], which is also dramatically impaired by the reduction in Bsg function.

Interestingly, while the presence of Bsg is dispensable for basal mitochondrial homeostasis, Bsg is required to sustain NE-stimulated respiration, a hallmark of brown fat metabolism [17]. This was accompanied by reduced NE-stimulated lipolysis, limiting the availability of FAs, which fuel β-oxidation as well as promote uncoupled respiration by binding to and activating UCP1 [17]. Combined with reduced Ucp1 expression and induction in cells lacking Bsg, our data suggest that Bsg is essential to promote several cellular processes boosting thermogenesis. Strikingly, this occurred while β-adrenergic signalling was preserved, indicating that Bsg acts via specific—yet to be identified—cellular components to target discrete cellular functions. Indeed, Bsg is known to have multiple
binding partners that mediate a multitude of physiological and pathophysiological roles [11]. Therefore, whether Bsg directly or indirectly regulates Ucp1 transcription, deciphering its interacting partners in brown adipocytes would aid to unravel the mechanisms by which Bsg controls Ucp1 expression and mitochondrial function and would constitute an interesting area of future research. Finally, our data point to an important role for Bsg in balancing substrate utilization and maintaining cellular metabolic homeostasis. This was evidenced by the different metabolic profiling of cells lacking Bsg as compared to controls, following NE stimulation. In particular, intracellular levels of the ketone body AcAc were severely impaired in brown adipocytes lacking Bsg, both at basal and after stimulation. Whether it is due to defective MCT function or metabolic rewiring remains to be determined. Since the downregulation of intracellular lactate and AcAc levels occurred in nonstimulated Bsg KD adipocytes, it is tempting to speculate that these disturbances prime the cells for further metabolic impairment once activated. However, additional experimentation would be required to test this hypothesis. Indeed, the metabolic perturbations observed in Bsg KD adipocytes may be causative of the altered mitochondrial function by limiting the necessary substrates to fuel the proper cellular metabolic requirements.

Collectively, our study provides new evidence that Bsg is required for full activation of thermogenesis in brown adipocytes. Further elucidation of the underlying mechanisms of Bsg action may uncover important metabolic tuning that sustains calorie burning in these cells and offer a deeper understanding of the metabolic adaptation of BAT in the face of chronic metabolic demand, to potentially exploit brown fat against obesity-associated metabolic disorders.

Materials and methods

Cell culture and pre-adipocyte differentiation

Mouse cell line

Mouse wild-type 1 brown pre-adipocyte cell line (WT-1) was kindly provided by C. Ronald Kahn [26]. WT-1 cells were propagated in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS (Invitrogen) and 1% penicillin-streptomycin (PS) (Invitrogen, Waltham, MA, USA) and maintained in humidified incubators at 37 °C and 5% CO2. Pre-adipocyte cells were differentiated into mature adipocytes by growing pre-adipocytes to confluence (day 0) in a culture medium supplemented with 20 nm insulin (Sigma) and 1 nm triiodothyronine (Sigma, St Louis, MO, USA) (differentiation medium). Once confluent, the cells were incubated in a differentiation medium, additionally supplemented with an induction cocktail [dexamethasone; 1 μM (Sigma), indomethacin; 0.125 mM (Sigma) and isobutylmethylxanthine; 0.5 mM (Sigma)] for 2 days. Subsequently, the cells were cultured in the differentiation medium, devoid of the induction cocktail, and the culture medium was changed every 2 days. All cell culture experiments were performed in independent replicates. For hypoxia study, cells were maintained in a humidified atmosphere at 37 °C with 1% O2 and 5% CO2 as described [20].

Human primary adipocytes

Human primary adipocytes were isolated from supraclavicular AT biopsies and differentiated for 12 days as described elsewhere [27]. The cells were obtained from a previously published cohort [27] and the study protocols of the original study were approved by the Scientific-Ethics Committee of the Capital Region journal number H-A-2009-020.

Laboratory animals

Animal experiments were performed in accordance with the Danish Animal Experiments Inspectorate (2015-15-0201-00728). All mice had ad libitum access to food and water. The animals were kept on a 12-h light/dark cycle at 22 °C, unless stated otherwise.

For cold exposure experiments, 10 weeks old C57BL/6 male mice (Taconic, Germantown, NY, USA) were used and given a chow diet (1310; Altromin, Lage, Germany), as previously described [28]. Ob/ob male mice (Jackson Laboratories, Bar Harbor, ME, USA, Cat.000632) were fed the control diet (D12450B, Research Diets, Inc., New Brunswick, NJ, USA). For the HFD challenge previously described [29], 6 weeks old male mice (Scanbur, Denmark) were used and given either an HFD (D12492, Research Diets, Inc., USA) or a control diet (D12450B, Research Diets, Inc., USA), respectively, for 8 weeks.

Mouse temperature studies

Mice were first acclimated to 29 °C in climate chambers (Mammert HPP750Life) for 21 days and subsequently transitioned to 5 °C for 3, 7 or 21 days. The mice used are previously described [28]. For BAT deactivation experiment, mice were housed at 5 °C for 21 days and then transitioned to 29 °C for either 3 or 7 days.

Isolation of mouse AT fractions

Interscapular BAT, epidydimal and inguinal WAT were excised from mice. Adipose depots were washed with PBS supplemented with 10 mM CaCl2 and 0.5% BSA (Sigma),
minced and collagenized in digestion buffer (2 mg·mL⁻¹ Collagenase type II (Sigma Aldrich) in PBS with 10 mM CaCl₂ and 0.5% BSA) for 30 min at 37 °C. Floating mature adipocytes (Ad) and SVF were separated by centrifugation (800 rcf, 15 min). The two adipose fractions were resuspended and lysed in TRIzol (Thermo Fisher Scientific), followed by RNA isolation.

### Gene silencing

Transient knockdown of Bsg was achieved with small interfering RNA (siRNA). On day 4 of cell differentiation, for protein and gene expression analysis 4 × 10⁵ cells/well were seeded in a 24-well culture dish (Fisher Scientific); for gas chromatography/mass spectrometry/time-of-flight (GC/MS-TOF) and liquid chromatography/mass spectrometry (LC/MS-TOF) and gene expression analysis 4 × 10⁴ cells/well were seeded in a ViewPlate-96 black (Perkin Elmer), for subcellular fractionation, 3 × 10⁶ cells/well were seeded in a 6-well culture dish (Fisher Scientific). Once seeded, cells were reverse transfected as described in [30] with a 50 nM pool of four siRNAs targeting mouse Bsg (L-042995-00-0005, Dharmacon, Lafayette, CO, USA) or a 9 protein and gene expression analysis 4 × 10⁴ cells/well in a ViewPlate-96 black (Perkin Elmer); for Seahorse experiments, 10⁴ cells/well were seeded in a 96-well XF Cell Culture Microplates (Agilent Technologies, Santa Clara, CA, USA); for lipid staining, 10⁵ cells/well in 12-well culture dish (Fisher Scientific); for subcellular fractionation, 3 × 10⁶ cells/well were seeded in a ViewPlate-96 black (Perkin Elmer); for subcellular fractionation, 3 × 10⁶ cells/well were seeded in a 6-well culture dish (Fisher Scientific). Once seeded, cells were reverse transfected as described in [30] with a 50 nM pool of four siRNAs targeting mouse Bsg (L-042995-00-0005, Dharmacon, Lafayette, CO, USA) or a pool of four nontargeting control siRNAs (D-001810-10-05, Dharmacon) using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific) at a final concentration of 5 µL·mL⁻¹ and Opti-MEM Reduced Serum Medium (Thermo Fisher Scientific) according to the manufacturer’s protocol. The medium was changed 2 days post-transfection, and cells were further incubated in cell-culture media for 24 h. siRNA transfection protocol for Hif-1α KD has been described [20].

### Real-time quantitative PCR

For total RNA isolation from cell culture, RNeasy spin columns (Qiagen) were used and for RNA extraction from AT, a combined method of TRIzol-CHCl₃ (Invitrogen) and RNeasy spin column (Qiagen, Hilden, Germany) was employed. RNA (1 µg from cell culture and 250 ng from mouse adipose depot samples) was reverse transcribed to cDNA using the iScript kit (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR was carried out using Brilliant III Ultrafast SYBR Green QPCR Master Mix (AH Diagnostics, Tilst, Denmark) in a CFX384 Real-Time PCR Detection System according to the manufacturer’s manual (Bio-Rad). For relative quantification of gene expression, the 2⁻ΔΔCt method was used with normalization to Ppia (for mouse AT samples) or Tbp mRNA (for cell culture samples). Gene expression analysis was performed using CFX-Maestro software (Bio-Rad). qPCR primer (TAG Copenhagen) sequences used are listed in Table 1.

### Table 1. List of mouse qPCR primers sequences.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<td>Acc1</td>
<td>TGACTACAGGGTTCTTTTGTTGTTG</td>
<td>GTCGTTCCCTTCCTCATTTTGT</td>
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<td>AdipoQ</td>
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<td>AldoA</td>
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<td>Bsg</td>
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Fluorescent in situ hybridization

Mouse BAT

5 µm formalin-fixed and paraffin-embedded brown fat was sectioned and stained with RNAscope Multiplex Fluorescent V2 Assay (Advanced Cell Diagnostics, Newark, CA, USA) and a HybEZ™ II Hybridization System (Advanced Cell Diagnostics), following the manufacturer’s protocol to visualize Bsg (probe: mm-Bsg-01 #491721, Advanced Cell Diagnostics) and AdipoQ (probe: mm-Adipoq-01-C3 #508121-C3, Advanced Cell Diagnostics) mRNA. Opal 520 (Akoya Biosciences, FP1487001KT), Opal 570 (FP1488001KT; Akoya Biosciences, Marlborough, MA, USA) and Opal 690 (Akoya Biosciences, FP1497001KT) at 1 : 1000 dilution were used to visualize signals. Images were obtained using Zeiss Axio Observer microscope (Zeiss, Oberkochen, Germany) with LIGHTNING super-resolution was used.

Human primary adipocytes

Pre-adipocytes were seeded at a density of 1 × 10⁵ cells per well in a 24-well tissue culture dish. Once confluent, cells were differentiated as described [27]. Fully differentiated cells were starved for 2 h in DMEM/F12 supplemented with 1% PS. Cells were then stimulated with control solution or 10 µM NE (Sigma) for 4 h. Cells were then washed once with PBS and then fixed at room temperature for 15 min with 10% Neutral Buffered Formalin and subsequently dehydrated using increasing ethanol concentrations. Cells were stored in 100% ethanol at −20 °C until used for FISH. RNAscope Multiplex Fluorescent V2 Assay (Advanced Cell Diagnostics) following the manufacturer’s protocol was used to visualize human BSG (probe: Hs-BSG #472721) and UCP1 (Hs-UCP1 #475821) mRNA. For imaging, Zeiss Axiovert 200 M inverted microscope or a Leica TCS SP8 Axiovert 200 M inverted microscope (Zeiss, Oberkochen, Germany) with LIGHTNING super-resolution was used.

Lipid droplet staining

Cells were washed with PBS and incubated in 4% paraformaldehyde in PBS for 20 min at room temperature. Cells were then washed with PBS for the second time and incubated with a solution containing DAPI (0.5 µg·mL⁻¹) and Nile Red (0.5 µg·mL⁻¹) in PBS for 20 min at room temperature protected from light. Cells were washed again with PBS and stored in PBS at 4 °C until further analysis.

Image analysis and quantification of lipid droplets

Images were acquired with high-content screening spinning disc confocal microscope, Opera QEHS, (PerkinElmer). Light sources used were the UV 365 nm channel for the nucleus (DAPI) and the 640 nm channel for Nile red. 20× and 60× objectives were used for visualizing the nuclei and the lipid droplets. All the plates were scanned with the same microscope settings. Thirty different fields of view per well (at least 20 wells per condition) were acquired in a sequential mode. The number of adipocytes, intensity of Nile red per cell and area of the lipid droplets were analyzed using the Columbus™ software following this guide [31]. In brief, the nuclei were detected analyzing the images obtained with the 20× objective, using the intensity of the DAPI channel, employing algorithm B with common threshold 0.4, nucleus area > 30 µm², split factor 7.0, individual threshold 0.4 and contrast 0.1. Once the nuclei were identified, using the cell segmentation function, the cytoplasm was selected using the Nile red channel, selecting method D with an individual threshold of 0.15. Proper identification of the nuclei and cytoplasm was confirmed by manually checking 10–20 images. Cells located outside the border of the image were excluded from the analysis. Once identified, the cytoplasm of each adipocyte and the intensity of Nile red were automatically quantified. Finally, analyzing the images taken with the higher magnification (60×), the lipid droplets were detected using the spot detection function. Selecting the Nile red channel, Method C was employed with radius ≤5.0, contrast <0.31, uncorrected spot to region intensity >1.3 and distance between the spot 2.0 pixels. Correct identification of the spots was manually checked in 10–20 figures. Once the spots were identified, the mean area of them was automatically calculated.

Subcellular fractionation of WT-1 adipocytes

Cells were rinsed and harvested in ice-cold TES buffer (20 mM Tris–pH 7.4, 5 mM EDTA, 250 mM sucrose) containing a protease inhibitor cocktail (SigmaFast protease inhibitor tablets, cat. no. S8820-20TAB). The suspension was homogenized by three passages through a 25G needle, followed by centrifugation at 4 °C and 13 000 g for 20 min, resulting in a pellet and a supernatant fraction. The pellet fraction was washed and resuspended in the TES buffer. The pelleted was then layered onto a 1.12M sucrose cushion and ultracentrifuged at 4 °C and 77 000 g for 60 min to obtain the plasma membrane pellet, which was resuspended in TES buffer.

Lipolysis measurements

Lipolysis was determined by measuring glycerol (Free Glycerol Reagent, F6428, Sigma Aldrich) concentration in media of basal and NE-stimulated (1 µM, 6 h) (Sigma, St Louis, MO, USA) cells according to the manufacturer’s protocol.

Analysis of β-adrenergic signalling pathway

Brown adipocytes were incubated in DMEM (Thermo Fisher Scientific) containing 0.1% fatty acid-free BSA
(Sigma) for 2 h. Cells were subsequently treated with 1 μM NE (Sigma) for 15 min, washed with cold PBS and subjected to protein immunoblotting as described below.

Protein immunoblotting

Upon washing with cold PBS, cells were lysed in RIPA buffer (Thermo Fisher Scientific) supplemented with a protease inhibitor cocktail (SigmaFast protease inhibitor tablets, cat. no. S8820-20TAB). The lysates were centrifuged at 4 °C and 16 000 g for 20 min. Protein concentration was determined with bicinchoninic acid assay (BCA) according to the manufacturer’s protocol. For plasma membrane immunoblotting, the plasma membrane pellet was first resuspended in TES buffer, followed by protein concentration analysis using BCA assay. Laemmli buffer was added and samples were incubated at 95 °C for 5 min. Between 15 and 20 μg of protein was loaded on 4%–12% Criterion™ TGX Stain-Free™ Protein Gels (Bio-Rad). For immunoblotting of mitochondrial oxphos subunits, 15 μg of protein was loaded onto a 4%–12% Criterion XT Bis-Tris Protein Gels (Bio-Rad) and proteins were separated using MOPS running buffer (Bio-Rad). Proteins were then transferred onto a 0.45 μm PVDF membrane. Membranes were blocked using 5% milk in TBS-T and first incubated in either primary mouse anti-mouse anti-Vinculin [(E1E9V) XP Rabbit mAb #13901, Cell Signaling Technology, Danvers, MA, USA], mouse anti-BSG antibody [EMMPRIN Antibody (B-5), sc-47600, Santa Cruz Biotechnology], Total Oxyphos Rodent WB antibody cocktail (ab-110413, Abcam), Phospho-PKA Substrate (RRXS-T*) [(100G7E) Rabbit mAb #9624, Cell Signaling Technology], Phospho-HSL (Ser660) (Antibody #45804, Cell Signaling Technology), HSL (Antibody #4107, Cell Signaling Technology), Phospho-CREB (Ser133) [(87G3) Rabbit mAb #9198, Cell Signaling Technology], CREB (48H2) Rabbit mAb #9197, Cell Signaling Technology), Phospho-ATF-2 (Thr71/ATF-7 (Thr53) (Antibody #24329, Cell Signaling Technology), ATF-2/ATF-7 (A9G1M) Rabbit mAb #82870, Cell Signaling Technology), p38 MAPK (Antibody #9212, Cell Signaling Technology), Phospho-p38 MAPK (Thr180/Tyr182) [(D3F9) XP® Rabbit mAb #4511, Cell Signaling Technology], Insulin Receptor β [(4B8) Rabbit mAb #3025, Cell Signaling Technology] or Anti-Monocarboxylate Transporter 4 Antibody (AB3314P, Sigma Aldrich). This was followed by incubation in HRP-conjugated secondary antibodies. For protein visualization, Chemidoc XRS was used (Bio-Rad). Band intensity was quantified using Image Lab software (Bio-Rad).

Oxygen consumption and extracellular acid production

For mitochondrial stress test, cells were cultured in Seahorse XF DMEM medium (Agilent Technologies) supplemented with 5 mM glucose (Agilent Technologies) and 2 mM glutamine (Agilent Technologies) 1 h prior to the assay. Oxygen consumption rate (OCR) and ECAR were measured under basal conditions and upon oligomycin (5 μM), FCCP (1 μM) and rotenone/antimycin A (1 μM) (all from Agilent Technologies) injection. Data for oxygen consumption were generated using Agilent Seahorse XF Cell Mito Stress Test report generator (Agilent Technologies). Basal ECAR levels were measured prior to oligomycin injection and maximal ECAR was measured after the injection of FCCP. For measurements of cellular respiration in response to NE, cells were incubated for 1 h prior to the assay in Seahorse XF DMEM medium (Agilent Technologies) supplemented with 5 mM glucose (Agilent Technologies) and 2 mM glutamine (Agilent Technologies). OCR and ECAR were measured under basal conditions and upon NE (1 μM) injection. The area under the curve (AUC) for NE-stimulated OCR and ECAR was calculated using GraphPad Prism 9 software (GraphPad). All OCR and ECAR measurements were performed using Seahorse XFe96 Extracellular Flux Analyser (Agilent Technologies). For normalization of OCR and ECAR measurements, cells were stained using Hoechst stain and counted with Biotek Cytation 5 Cell Imaging Multimode Reader (Agilent).

Extraction of intracellular metabolites

Cells from each sample were washed with PBS (Thermo Fisher Scientific) and the sample was harvested with 300 μL of ice-cold working solution with labelled internal standards (90% methanol containing succinic acid-2,2,3,3-d4, 0.22 mg L−1; l-valine-d8, 4.41 mg L−1; l-glutamic acid-13C5, 22.06 mg L−1; D-3-hydroxybutyrate-13C4, 0.22 mg L−1; palmitic acid-d31, 22.06 mg L−1; and decanoic acid-d19, 4.41 mg L−1). The suspension was snap frozen in liquid nitrogen, thawed at 4 °C and vortexed. This process was repeated three times. For protein precipitation, the suspension was incubated at 4 °C for 1 h. Sample was further centrifuged at 16 000 g for 15 min at 4 °C and 50 μL of supernatant extract was aliquoted into GC vials. Additionally, to generate pooled samples dedicated for quality control, 10 μL of extract from each sample was taken. Pellet was used for protein content determination using the BCA method. Extracts were dried using a nitrogen stream with 6 L min−1 flow for 1 h at room temperature. Samples were then subjected to targeted gas chromatography–mass spectrometry (GC–MS) analysis as described below. For blank control, deionized water was used. Normalized peak areas to internal standards were normalized again to protein content to correct for biological variation.

Extraction of extracellular metabolites

Conditioned cell medium was collected, and 100 μL of sample was aliquoted. To the aliquot, 400 μL of ice-cold working solution with labelled internal standards (90% methanol containing succinic acid-2,2,3,3-d4, 0.22 mg L−1; l-valine-d8, 4.41 mg L−1; l-glutamic acid-13C5, 22.06 mg L−1; D-3-hydroxybutyrate-13C4, 0.22 mg L−1; palmitic acid-d31, 22.06 mg L−1; and decanoic acid-d19, 4.41 mg L−1). The suspension was snap frozen in liquid nitrogen, thawed at 4 °C and vortexed. This process was repeated three times. For protein precipitation, the suspension was incubated at 4 °C for 1 h. Sample was further centrifuged at 16 000 g for 15 min at 4 °C and 50 μL of supernatant extract was aliquoted into GC vials. Additionally, to generate pooled samples dedicated for quality control, 10 μL of extract from each sample was taken. Pellet was used for protein content determination using the BCA method. Extracts were dried using a nitrogen stream with 6 L min−1 flow for 1 h at room temperature. Samples were then subjected to targeted gas chromatography–mass spectrometry (GC–MS) analysis as described below. For blank control, deionized water was used. Normalized peak areas to internal standards were normalized again to protein content to correct for biological variation.
methanol containing succinic acid-2,2,3,3-d₄, 0.28 mg L⁻¹; L-valine-d₆, 5.51 mg L⁻¹; L-Glutamic acid-¹⁵C₁, 27.58 mg L⁻¹; D-3-hydroxybutyrate-¹³C₄, 0.28 mg L⁻¹; Palmitic acid-d₃₁, 27.58 mg L⁻¹; and decanoic acid-d₁₉, 5.51 mg L⁻¹) was added. After 1 h incubation at 4 °C for protein precipitation, the suspension was centrifuged at 16,000 g for 15 min at 4 °C and 50 µL of supernatant extract was aliquoted into GC vials. For quality control, 10 µL of extract per sample was taken. Using a nitrogen stream with 6 L min⁻¹ flow, the extracts were dried for 1 h at room temperature. Samples were further analysed with targeted GC–MS as described below. Deionized water was used as blank control.

**Stable isotope labelling of intracellular metabolites**

Cells were washed with 37 °C warm PBS (Thermo Fisher Scientific) and incubated for 1 h with 1 µM NE and no-glucose DMEM (Thermo Fisher Scientific) supplemented with 10% FBS (Invitrogen), 1% penicillin–streptomycin (Invitrogen) and 25 mM of U-¹³C labelled glucose (Sigma). After the incubation, cells were harvested in 90% methanol as described under the Extraction of intracellular metabolites section. The resulting supernatant extract was analysed using targeted GC–MS (for AcAc analysis) and targeted liquid chromatography–mass spectrometry (LC–MS) (for analysis of the remaining metabolites) as described in the respective sections. The resulting isotopeologue abundances were corrected for naturally occurring C₁₃ using IsoCor [32] and further analysed with Python.

**Gas chromatography/mass spectrometry–time-of-flight analysis**

The method was previously developed by [33], and it was adopted for specific matrix and experimental design. The GC/MS-TOF analysis was performed with a Leco Pegasus BT GC/TOFMS instrument (Leco Corp., Saint Joseph, Michigan) equipped with a Gerstel MSP multisampler (Mülheim an der Ruhr, Germany). Preceding the analysis, dried extracts were silylated by an automatic two-step derivatization reaction with methoxamine (MOX) reagent (2% in pyridine) and N,O-Bis(trimethylsilyl) trifluoroacetamidamide (BSTFA) with 1% trimethylchlorosilane (TMCS). In the first step of the reaction, 12.5 µL of MOX reagent was added to the dried extracts, and the mix was incubated at 45 °C for 60 min. Next, 12.5 µL of BSTFA in 1% TMCS was added, and the mixture was incubated again at 45 °C for 60 min. Lastly, 50 µL of 10 mg L⁻¹ 4,4’-dibromoocotfluorobiphenyl in hexane was added to the mix as an injection standard. The trimethylsilil derivatives were then separated on a Restek Rxi 5 ms column (pn:13423–6850) with 1.2 mL min⁻¹ of helium flow and an inlet temperature of 270 °C. The temperature gradient started at 40 °C and it was kept constant for 1 min, until acceleration at 20 °C min⁻¹ to 340 °C, where it remained stable for 3 min. Mass fragmentation occurred at 70 eV with an electron ionization source. The detector acquired mass spectra in scan mode from 50 to 750 Da at 10 Hz speed.

For data processing, raw files were converted into centroid mode and exported as netCDF files. Data were extracted using a targeted approach using the in-house Swedish Metabolome Center (SMC; www.swedishmetabolomicscentre.se) GC/MS software. A predefined list of target compounds based on mass spectra and retention index was applied to obtain a list of metabolites and their corresponding areas. Internal standards were used to normalize peak areas of the target metabolites.

MOX reagent and BSTFA with 1% TMCS were from Thermo Fisher Scientific (Waltham, Massachusetts). Hexane, L-valine-d₆, succinic acid-2,2,3,3-d₄, L-Glutamic acid-¹⁵C₁,¹⁵N and 4,4-dibromoocotfluorobiphenyl were purchased at Sigma Aldrich (Saint Louis, Missouri). D-3-hydroxybutyrate-¹³C₄ was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, Massachusetts). Palmitic acid-d₃₁ and decanoic acid-d₁₉ were from Cayman chemicals (Ann Arbor, Michigan).

**Liquid chromatography/mass spectrometry–time-of-flight analysis**

To increase the sensitivity and improve the separation of carboxylic acids, samples were derivatized with 3-Nitrophenylhydrazine (3NP), N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) and Butyldihydroxytoluene (BHT). To derivatize the samples, 20 µL of supernatant extracts was combined with 20 µL of a 3NP solution (200 mm in 50% methanol) and 20 µL of an EDC solution (120 mm in 50% methanol and 6% pyridine) and briefly vortexed. After 1 h incubation at room temperature, 40 µL of BHT solution (0.05 mg mL⁻¹ in methanol) was added and briefly vortexed. Samples were kept at 4 °C until analysis on the same day.

The samples were analysed by LC/MS-TOF using an Agilent 1290 II Liquid chromatograph (Agilent) for the chromatographic separation, a Tims TOF Pro mass spectrometer (Bruker) equipped with a VIP-ion source for detection and TASQ 2021b (Bruker) for data extraction. A Waters HSS T3 10 cm × 2.1 × 1.8 µm column (Waters #186003539) was used for the chromatographic separation. Water with added 0.1% formic acid (Thermo Fisher Scientific #A117-50) was used for mobile phase A, while a mix (V/V = 3:1) of acetonitrile (Honeywell #14261) and isopropanol (Honeywell #34965) 0.1% formic acid was used for mobile phase B. The mobile phase gradient started with 3% of mobile phase B and increased to 100% of mobile phase B over the course of 9 min. Then, it was left stable for 3 min and subsequently decreased again to initial
conditions over the course of 30 s where it remained for 2 min 30 s. The column oven temperature was kept at 40 °C, the multisampler cooled to 4 °C, and the injection volume was 2 µL. The acquisition was performed in full scan negative mode in the mass range from 50 to 1000 m/z at 2 Hz. Sodium formate was used as an internal calibrant. The TASQ method for data extraction was previously set up based on retention times and m/z values of derivatized standards.

**Statistical data analysis**

Data are represented as mean ± SEM with individual data points. All experiments were repeated in three or more independent biological replicates. All statistical tests were performed using GraphPad Prism 9 software (GraphPad), except for discriminant analysis and variable importance visualization, which were performed as described in [22] using orthogonal projections to latent structures discriminant analysis in SIMCA-P v.17 (Sartorius, Umeå Sweden). The Student’s (unpaired, two-tailed) t-test was performed for comparisons between two groups. Multiple comparison testing was performed using one- or two-way ANOVA, followed by post-hoc tests as recommended by GRAPHPAD PRISM 9 software (Sidak’s, Dunnett’s or Tukey’s multiple comparison tests were applied after a two-way ANOVA, and the Dunnett’s post-hoc test was used after one-way ANOVA). P < 0.05 was considered significant. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

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**Conflict of interest**

Z.G.-H. works or has worked, in some capacity, for Embark Biotech ApS, a company developing therapeutics for the treatment of diabetes and obesity. All other authors declare no conflict of interest.

**Author contributions**

BE and KR performed the conceptual design of the study. KR, MSI, ZGH, SN and BE planned experiments and interpreted the data. KR, MSI, LAM, MAO, KP, EB, MM, SB, MT, DT, PSSP, TIH and KT performed experiments and the data analysis. JBH, SN and TM contributed the essential material. KR and BE wrote the manuscript. All authors contributed to the study and approved the final version of the manuscript.

**Peer review**

The peer review history for this article is available at https://publons.com/publon/10.1111/febs.16716.

**Data availability statement**

All the data are presented in the manuscript.

**References**


Basigin regulates brown adipocyte activation

K. Rupar et al.


