Brown and beige adipose tissue are emerging as distinct endocrine organs. These tissues are functionally associated with skeletal muscle, adipose tissue metabolism and systemic energy expenditure, suggesting an interorgan signaling network. Using metabolomics, we identify 3-methyl-2-oxovaleric acid, 5-oxoproline, and β-hydroxyisobutyric acid as small molecule metabolites synthesized in browning adipocytes and secreted via monocarboxylate transporters. 3-methyl-2-oxovaleric acid, 5-oxoproline and β-hydroxyisobutyric acid induce a brown adipocyte-specific phenotype in white adipocytes and mitochondrial oxidative energy metabolism in skeletal myocytes both in vitro and in vivo. 3-methyl-2-oxovaleric acid and 5-oxoproline signal through cAMP-PKA-p38 MAPK and β-hydroxyisobutyric acid via mTOR.

In humans, plasma and adipose tissue 3-methyl-2-oxovaleric acid, 5-oxoproline and β-hydroxyisobutyric acid concentrations correlate with markers of adipose browning and inversely associate with body mass index. These metabolites reduce adiposity, increase energy expenditure and improve glucose and insulin homeostasis in mouse models of obesity and diabetes. Our findings identify beige adipose-brown adipose-muscle physiological metabolite crosstalk.
Brown adipose tissue (BAT) functions to regulate body temperature through non-shivering thermogenesis; the dissipation of chemical energy to produce heat. Beige adipocytes are interspersed within the white adipose tissue (WAT) of rodents and humans, and can be induced to switch from a white-adipocyte-like phenotype to a brown-adipocyte-like phenotype; a process known as browning. Brown and activated beige cells are characterized by high levels of fatty acid β-oxidation, mitochondrial content, and thermogenesis. Thermogenesis occurs through the activity and increased expression of several specific gene products, including uncoupling protein 1 (UCP1), an inner mitochondrial membrane protein that uncouples substrate oxidation from ATP synthesis to generate heat. Activated brown and beige adipocytes alter systemic energy metabolism, increasing substrate oxidation and energy expenditure, with potential for therapeutic exploitation for metabolic diseases including Type 2 diabetes mellitus (T2DM) and obesity.

The effects of BAT and beige adipose tissue on energy balance may not solely depend on the action of UCP1. Activation of thermogenin in brown and beige adipose tissues may lead to propagation of thermogenesis in surrounding adipocytes and distal adipose depots. Transplantation studies of both beige and brown fat in mice suggest that these tissues can signal to activate endogenous beige and brown fat and improve glucose homeostasis in skeletal muscle. In murine models of both adipose tissue browning and increased BAT thermogenesis, fatty acid oxidation in skeletal muscle is increased. The anti-obesity and anti-diabetic effects of brown and beige adipose tissues are also not solely reliant on the thermogenic process. Mice lacking Ucp1 are resistant to diet-induced obesity at room temperature, yet mice lacking brown/beige fat are highly susceptible to an obese phenotype. Therefore, beige and brown fat may influence systemic metabolism through non-UCP1 thermogenic mechanisms, potentially mediated through the release of endocrine signals in the adipocyte secretome.

In this study, we aimed to identify and characterize signals released in the browning adipocyte secretome that may influence systemic metabolism. A discrete panel of small molecule metabolite paracrine and endocrine signals, secreted from both beige and brown adipocytes, is identified. These metabolites increase adipose tissue, skeletal muscle, and systemic energy metabolism. We propose these brown and beige adipokine-like small molecules function in an adipose–adipose and adipose–skeletal muscle interorgan signaling axis.

Results
Metabolite signals from browning adipocytes increase brown-adipocyte-associated gene expression in primary adipocytes. Adipocyte browning was induced in primary adipocytes differentiated from the stromal vascular fraction of subcutaneous (inguinal) WAT of mice using two distinct canonical signaling mechanisms, an adenylyl cyclase activator (forskolin), and peroxisome proliferator-activated receptor δ (Pparδ) agonist (GW0742). Cells were washed and fresh serum-free media was conditioned on the cells for 24 h. Conditioned media was transferred to naïve primary adipocytes (Fig. 1a) and induced expression of brown-adipocyte-associated genes (Fig. 1b, c).

Expression of brown-adipocyte-associated genes, including Ucp1, peroxisome proliferator-activated receptor γ co-activator1α (Pgc1α), cell death-inducing DFFA-like effector a (Cidea), carnitine palmitoyltransferase 1b, and acyl-CoA dehydrogenase very-long chain (Acadvl), and cytochrome C were further enhanced following media protein denaturation by boiling, implicating a nonprotein small molecule mediator(s). These data may also indicate the presence of a secreted protein inhibitor of browning. To define the physicochemical nature of the small molecule mediators, aqueous-soluble metabolites were extracted from media conditioned on activated beige adipocytes using solvent partition. The aqueous-soluble metabolites were reconstituted in fresh media and transferred to naïve primary adipocytes (Fig. 1d). Expression of brown-adipocyte-associated genes was induced by aqueous-soluble metabolites released from browning adipocytes.

To identify candidate metabolites that may induce browning, we applied both gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) metabolic profiling to media conditioned on browning adipocytes. GW0742 and forskolin were not detected in conditioned media. Multivariate statistical models of the metabolic profiling data were used to identify common metabolite species enriched in the media by both cyclic AMP (cAMP) and PPARδ-induced browning (Fig. 1e). The concentration of sugar species and the branched-chain amino acids (BCAAs) valine and isoleucine was decreased in the media of browning adipocytes (Fig. 1f). Concomitantly the concentration of 5-oxoproline (5OP) and the BCAA catabolites α-hydroxyisocaproic acid (HIC), α-ketoisovaleric acid (AKV), α-hydroxyisovaleric acid (AHI), 3-methyl-2-oxovaleric acid (MOVA), β-hydroxyisobutyric acid (BHIBA), and β-hydroxyisovaleric acid (BHVIA) was increased in the media. Glycerol, a marker of lipolysis, was also increased.

Next, we examined whether physiological plasma concentrations of the BCAA metabolites and 5OP increased the expression of brown-adipocyte-associated genes in primary adipocytes (Fig. 1g). Physiological plasma concentrations of the metabolites are given in Supplementary Table 1. MOVA, 5OP, BHIBA, and BHIVA significantly and robustly induced expression of brown-adipocyte-associated genes including Ucp1, Cidea, and Prgbt1. MOVA, 5OP, BHIBA, and BHIVA were also enriched in the media of primary mouse canonical brown adipocytes following cAMP (forskolin 1 μM) or Pparδ (GW0742 100 nM)-mediated induction of brown-adipocyte-associated gene expression (Fig. 1h, i).

Therefore, the metabolites MOVA, 5OP, BHIBA, and BHIVA are released from primary white and brown adipocytes in response to thermogenic stimuli and induce the expression of brown-adipocyte-associated genes in naïve adipocytes.

Metabolite signals are secreted from browning human adipocytes and induce a brown-adipocyte-like functional phenotype. We determined whether the secretion of the candidate metabolites from browning adipocytes was conserved in human cells. A brown-adipocyte-like phenotype was induced in human primary adipocytes using either forskolin (1 μM) or a PPARδ agonist (100 nM GW0742) (Supplementary Fig. 1a–h). MOVA, 5OP, BHIBA, and BHIVA were enriched in the media of forskolin and PPARδ-agonist-treated human adipocytes (Fig. 2a). Treatment of primary human adipocytes with physiological plasma concentrations (Supplementary Table 1) of MOVA (20 μM), 5OP (20 μM), BHIBA (20 μM), and BHIVA (10 μM) induced expression of a panel of brown-adipocyte-associated genes including UCP1, CIDEA, and PGC1α (Fig. 2b). Induction of UCP1 expression in primary human adipocytes treated with metabolites in the physiological micromolar range occurred in a dose-dependent manner (Supplementary Fig. 2a–d). The concentrations of UCP1 protein in metabolite-treated human primary adipocytes were also increased (Fig. 2c). We further investigated whether the metabolites induced functional effects consistent with browning on energy expenditure in human primary adipocytes. Both the basal and succinate-stimulated (complex II) oxygen consumption rates of adipocytes treated with MOVA
(20 μM), 5OP (20 μM), BHIBA (20 μM), and BHIVA (10 μM) were increased (Fig. 2d). Primary human adipocytes were treated with MOVA (20 μM), 5OP (20 μM), BHIBA (20 μM), and BHIVA (10 μM) and incubated in serum-free media containing U-13C-palmitate to monitor adipocyte fatty acid β-oxidation. The labeled palmitate is catabolized via β-oxidation, releasing labeled acetyl CoA, which enters the TCA cycle (Supplementary Fig. 2e). GC-MS analysis identified increased relative enrichment of downstream TCA cycle metabolites citrate, fumarate, and malate in MOVA, 5OP, and BHIBA-treated adipocytes (Fig. 2e–g), confirming that fatty acid β-oxidation is increased in these cells. The uptake of glucose and fatty acid into human primary adipocytes treated with the metabolites was measured with the fluorescent glucose analog 6-NBDG or the fluorescent fatty acid.
analog BODIPY-FA (Fig. 2h, i) (Supplementary Fig. 2f–m). Consistent with the browning response, the metabolites increased adipocyte glucose and fatty acid uptake.

We sought to further characterize the transcriptional program induced in adipocytes by the candidate metabolite signals, and to confirm that the effects on brown-adipocyte-associated gene expression are conserved in an independent in vitro model of human adipose tissue. A gene expression array of key adipocyte and brown-adipocyte-associated genes was used to probe immortalized human white preadipocytes isolated from neck fat and differentiated to mature adipocytes in the presence of MOV A (20 μM) (Supplementary Table 2), 5OP (20 μM) (Supplementary Table 3), BHIBA (20 μM) (Supplementary Table 4), or BHIVA (10 μM) (Supplementary Table 5). Confocal imaging of immortalized human adipocytes treated with the candidate metabolites identified MOV A, 5OP, and BHIBA significantly increased cellular UCP1 protein content (Fig. 2, k). Functionally, basal and leak respiration are both increased in immortalized human adipocytes treated with the metabolites, partially due to increased electron flux seen as chemically uncoupled maximal respiration, and partially due to increased proton conductance seen as decreased coupling efficiency (Fig. 2l–o) (Supplementary Fig. 2n–q).

These data indicate that MOV A, 5OP, BHIBA, and, to a lesser extent, BHIVA induce gene and protein expression and a functional phenotype consistent with browning in two human adipocyte models.

Transcriptional analysis and 13C-isotope substrate tracing reveal mechanisms of metabolite biosynthesis and secretion by browning adipocytes. Next we examined mechanisms by which adipocyte browning may increase the concentrations of MOV A, BHIBA, BHIVA, and 5OP. BCAs were depleted in the media of browning adipocytes (Fig. 1f). MOV A, BHIBA, and BHIVA are generated through the degradation of the BCAs isoleucine, valine, and leucine, respectively. These pathways share multiple enzymes. 5OP is synthesized from glutamate. U-13C-labeled isoleucine, valine, leucine, and glutamate were used to monitor stable isotope enrichment through the biosynthetic pathways and into extracellular accumulation of the candidate metabolites produced by human primary adipocytes treated with forskolin. Concomitantly, we performed RNA-Seq on human primary adipocytes treated with forskolin. Induction of the browning response increased both the intracellular and extracellular (culture media) 13C-enrichment of MOV A (Supplementary Fig. 3a–d), BHIBA (Supplementary Fig. 3e–j), BHIVA (Supplementary Fig. 3k–n), and 5OP (Supplementary Fig. 3o–u). The expression of the genes encoding BCAs catalytic enzymes, branched-chain amino acid transaminase 2 (BCAT2), branched-chain keto acid dehydrogenase E1 subunit beta (BCKDHB), acyl-CoA dehydrogenase short chain (ACADS), acyl-CoA dehydrogenase medium chain (ACADM), Enolyl-Coenzyme A Hydratase/3-Hydroxacyl Coenzyme A Dehydrogenase (EHHADH), hydroxacyl-CoA dehydrogenase (HADHA), and Enol-CoA Hydratase, Short Chain 1 (ECHS1) was increased in forskolin-treated adipocytes (Supplementary Fig. 3a–n). The expression of the genes encoding 5OP biosynthetic enzymes glutathione synthetase (GSS), γ-glutamyltransferase 7 (GGT7), and γ-glutamylcyclotransferase (GGCT) was also increased in browning adipocytes (Supplementary Fig. 3o–u).

These data identify that browning induces a transcriptional program upregulating expression of the metabolite biosynthetic enzymes and driving adipocyte synthesis and release of MOV A, 5OP, BHIBA, and BHIVA.
Metabolites are exported from browning adipocytes via monocarboxylate transporters. Next, we investigated the mechanisms through which the browning adipocytes export the metabolite signals. MOVA, 5OP, BHIBA, and BHIVA, structurally, share a common single carboxyl group. Our RNA-seq analysis identified that monocarboxylate transporter 1 (MCT1) expression was increased in human primary adipocytes treated with forskolin (SLC16A1, log fold-change = 0.37, P < 0.05, n = 3). MCT1 functions to both export and import monocarboxylates through the plasma membrane. 5OP, MOVA, and BHIVA are transported by MCT1. MCTs also transport the BHIBA, structurally related ketone body beta-hydroxybutyrate. We used a pharmacological MCT inhibitor (MCTi, α-cyano-4-hydroxycinnamate) to determine the involvement of MCTs in browning-mediated secretion of the metabolites. Inhibition of MCTs abrogated forskolin-induced secretion of the metabolite signals, decreasing MOVA, 5OP, BHIBA, and BHIVA extracellular concentration whilst increasing their intracellular concentration (Supplementary Fig. 4a–d). To confirm MCT1 contributed to the browning-mediated secretion of the metabolites from adipocytes, we decreased MCT1 expression in human adipocytes by 88% using siRNA (Fig. 3a). Knockdown of MCT1 inhibited forskolin-induced export of the metabolites from adipocytes, again decreasing the metabolite extracellular concentration whilst increasing their intracellular concentration (Fig. 3b–e). Although not assessed with transport assays, these data suggest that MCTs are required for metabolite export from browning adipocytes.

MOVA, 5OP, and BHIBA regulate metabolism in skeletal myocytes. In murine models of both adipose tissue browning and BAT activity, fatty acid oxidation in skeletal muscle is increased. We hypothesized that the metabolites secreted from browning adipocytes may contribute to the functional link between browning adipose tissue and muscle. We reconstituted this adipose tissue–muscle functional relationship in vitro. Conditioned serum-free media was collected from primary mouse adipocytes treated with forskolin (1 μM). Conditioned media was transferred to mouse C2C12 myotubes and induced expression of transcriptional regulators of metabolism (Pparα, Pgc1α), fatty acid β-oxidation genes including Cpt1b, and Acadvl, and mitochondrial genes Cys and respiratory chain complex 1 component NADH:Ubiquinone Oxidoreductase Core Subunit S1 (Ndufs1) (Fig. 4a). The metabolites MOVA and 5OP induced expression of the metabolic gene panel in mouse myotubes (Fig. 4b).

The adipose tissue–muscle in vitro signaling model was translated to human primary cells. Conditioned media from browning human adipocytes induced expression of key fatty acid metabolism genes in human myocytes (Fig. 4c). The effect of MOVA and 5OP on metabolic, mitochondrial, and fatty acid oxidation gene expression was conserved in human primary skeletal myocytes and was dose responsive in the physiological low micromolar range (Fig. 4d) (Supplementary Fig. 5a–d). BHIBA was also observed to increase expression of PPARα and CPT1b in human primary skeletal myocytes. To confirm that transcriptional changes in human myocytes are accompanied by a dose-dependent change in functional phenotype, the oxygen demand was measured using a cell respiration assay.
Fig. 2 Browning human adipocytes secrete metabolites, which induce a brown-adipocyte-like functional phenotype. a 3-methyl-2-oxovaleric acid (MOVA), 5-oxoproline (5OP), β-hydroxyisovaleric acid (BHIVA), and β-hydroxyisobutyric acid (BHIBA) are enriched in browning human adipocyte media (n = 3; One-way ANOVA Dunnett’s post hoc; Forskolin MOVA P = 0.0015, 5OP P = 0.034, BHIVA P = 0.012; PPARγ agonist MOVA P = 0.0017, 5OP P = 0.007, BHIVA P = 0.0003, BHIBA P = 0.0017). b MOVA, 5OP, BHIBA, and BHIVA induce brown-adipocyte-associated gene expression in human adipocytes. Forskolin treatment given as a positive control for browning (Control, MOVA, 5OP, BHIBA, and BHIVA n = 4; Forskolin n = 6; two-tailed t-test; MOVA UpcrP = 0.0006, Pgclu = 0.011, CptlbP = 0.005, AcadP = 0.003, CyscP = 0.008; 5OP UpcrP = 0.019, Pgclu = 0.0011, CideaP = 0.002, CptlbP = 0.025, CyscP = 0.0086; BHIBA UpcrP = 0.012, Pgclu = 0.015, CideaP = 0.009, AcadP = 0.0016, CyscP = 0.0038; BHIVA Pgclu = 0.002, CideaP = 0.014, CptlbP = 0.0024, AcadP = 0.01, CyscP = 0.012; Forskolin UpcrP = 0.0014, Pgclu = 0.009, CideaP = 0.0002, AcadP = 0.0001, CyscP = 0.0006). c UCP1 protein concentration in human primary adipocytes treated with MOVA, 5OP, and BHIVA determined by ELISA (n = 3; One-way ANOVA Dunnett’s post hoc; MOVA P = 0.025, 5OP P = 0.015, BHIVA P = 0.05). d Basal and stimulated (sucrose 20 mM/L) oxygen consumption increased in human adipocytes treated with MOVA, 5OP, BHIBA, and BHIVA, and Forskolin (provided for comparison) (Control n = 4, MOVA, 5OP, BHIBA, BHIVA n = 3, Forskolin n = 5; two-tailed t-test; Basal MOVA P = 0.023, 5OP P < 0.0001, BHIVA P = 0.006, Forskolin P < 0.0001; 20-mM Succinate MOVA P = 0.046, SOP P = 0.011, BHIVA P = 0.00017). e/g TCA cycle intermediates citrate, fumarate, and malate 13C-enrichment from 13C-palmitate metabolism in MOVA, 5OP, BHIBA, and BHIVA-treated human adipocytes. Data in bar charts are mean ± SEM with data points shown. Box and whisker plots show 25th to 75th percentile (box) min to max (whiskers), mean (°), and median (–). Source data are provided as a Source Data file.
fed a 60% fat diet for 17 weeks, had greater body weight and impaired glucose tolerance compared to matched chow-fed controls (Supplementary Fig. 6g, h). Obese mice exhibited markers of whitening within their intrascapular BAT, with decreased expression of thermogenic genes (Supplementary Fig. 6i) and a white-adipocyte-like morphology (Supplementary Fig. 6j). In agreement with these observations, the BAT concentrations of MOVA, 5OP, BHIBA, and BHIVA were decreased by diet-induced obesity (Supplementary Fig. 6k). Therefore the metabokine signals are modulated in adipose depots and systemically in in vivo physiological models of altered thermogenic function.
Concentrations of the adipokine-like metabolites in adipose tissue and plasma are inversely correlated with body mass index in humans. We investigated the association between genetic variants in the genes encoding the metabolokine biosynthetic enzymes and body mass index (BMI) in a large-scale Genome Wide Association Study (GWAS) database in Genetic Investigation of ANthropometric Traits and UK Biobank Meta-analysis27, included in the 795,640 subjects in the Type 2 Diabetes Knowledge Portal (http://www.type2diabetesgenetics.org/). We found that common noncoding variants in the MOVA, BHIBA, and BHIVA biosynthetic genes (BCAT2, BCKDHB, ACADS, and HADHA), the 5OP biosynthetic genes (GSS, GGCT1) and the gene for MCT1 were significantly associated with BMI (Supplementary Table 6). The most significant variants in each gene for BMI were: BCKDHB rs13220420, P = 0.00000750; BCAT2 rs73587808, P = 0.000488; ACADS rs12369156, P = 0.000131; HADHA rs559393527, P = 0.0000341; GSS rs2236270, P = 3.60e–8; GGCT1 rs549124813, P = 0.000875 and MCT1/SLC16A1 rs186286251, P = 0.000471.

We then examined the association of subcutaneous WAT MOVA, 5OP, BHIBA, and BHIVA concentrations with BMI in human volunteers (Supplementary Table 7). The WAT concentration of MOVA, 5OP, and BHIBA were significantly inversely correlated with BMI (Fig. 6a–d). Plasma concentrations of the metabolokine adipokine-like signals were also inversely correlated with BMI (Supplementary Fig. 7a–d). The association of metabolokine concentrations with beige adipose tissue in humans was also interrogated. RNA was isolated from the adipose tissue of volunteers and the expression of UCP1 and CPT1b measured using RT-qPCR. Associations between the adipose tissue metabolokine concentrations and the expression of UCP1 (Fig. 6e–h) and CPT1b (Fig. 6i–l) were analyzed. Concentrations of MOVA, 5OP, and BHIBA were significantly correlated with tissue expression of UCP1 and CPT1b.

These data suggest that the metabolokines are functionally associated with human physiology and may influence body mass phenotypes.

The metabolokines MOVA, 5OP, and BHIBA increase systemic energy expenditure and regulate the adipose tissue and skeletal muscle metabolic phenotype in vivo. Next, we investigated the effect of MOVA, 5OP, BHIBA, and BHIVA on the in vivo metabolic phenotype of mice. Six-week-old mice fed standard chow were either treated with MOVA (100 mg/kg/day), 5OP (100 mg/kg/day), BHIBA (150 mg/kg/day), or BHIVA (125 mg/kg/day) in drinking water for 17 weeks (based on preliminary dose escalation studies) or remained untreated (control mice). Treatment increased the plasma concentrations of the metabolites in the mice within the low micromolar physiological range. (Supplementary Fig. 8a–d). Water intake was not different between groups (Supplementary Fig. 8e). Weight gain of SOP- and MOVA-treated mice was decreased compared with controls (Supplementary Fig. 8f). Analysis with metabolic cages indicated BHIBA, MOVA, and 5OP increased energy expenditure (Supplementary Fig. 8g–j) and oxygen consumption (Supplementary Fig. 8k–n) independent of body mass (as determined by ANCOVA). Metabolite treatment did not affect the activity of the mice (Supplementary Fig. 8o). Food intake was increased in the 5OP and BHIBA-treated groups, which likely underpin the lack of difference in weight between BHIBA-treated mice and control (Supplementary Fig. 8p). BHIVA had no effect on the metabolic parameters independent of body mass.

MOVA, 5OP, and BHIBA increased systemic energy expenditure in mice. We examined the expression of thermogenic and mitochondrial metabolism genes in BAT, subcutaneous inguinal WAT, and skeletal muscle of the metabolokine-treated mice (Supplementary Fig. 8q–t). Metabolite treatment also increased citrate synthase activity, a marker of mitochondrial density and TCA cycle flux, in the BAT, inguinal WAT, and muscle of metabolokine-treated mice (Supplementary Fig. 8u–x).
glucose tolerance in the mice. MOVA treatment demonstrated a mild but significant improvement in insulin sensitivity.

We then examined markers of thermogenesis and mitochondrial metabolism in BAT and subcutaneous WAT of the MOVA-, 5OP-, and BHIBA-treated mice. The activity of citrate synthase was significantly increased in the BAT of mice following MOVA, 5OP, and BHIBA treatment, suggesting increased mitochondrial biogenesis (Fig. 7k). Consistent with these data, IHC analysis of the BAT of MOVA-, 5OP-, and BHIBA-treated mice indicated increased concentrations of Ucp1 (Supplementary Fig. 9o), which were confirmed by ELISA (Fig. 7l). Pgc1α protein concentration was also increased in the BAT of BHIBA-treated mice (Fig. 7l). Citrate synthase activity was increased in subcutaneous WAT of mice following 5OP and BHIBA treatment (Fig. 7m).
ICHC analysis of inguinal subcutaneous WAT from these mice indicated increased Ucp1 concentrations following SOP and BHIBA treatment (Supplementary Fig. 9o), which were again confirmed by ELISA (Fig. 7n). SOP and BHIBA also increased the concentration of Pgc1α protein in inguinal WAT (Fig. 7n), with SOP, BHIBA, and MOVA all increasing Cpt1 concentrations (Fig. 7n). MOVA and SOP decreased adipocyte hypertrophy, significantly reducing adipocyte size within the inguinal WAT depot, consistent with effects of the metabolites on weight gain (Supplementary Fig. 9p).

MOVA, SOP, and BHIBA increased expression of mitochondrial and metabolic genes in skeletal muscle in vitro and in vivo. Consequently we investigated markers of mitochondrial metabolism in the soleus muscle of the MOVA-, 5OP-, and BHIBA-treated murine model of obesity. Mitochondrial density was increased in skeletal muscle by all three metabolite signals (Fig. 7o). Protein concentrations of Pgc1α and Ndufs1 were significantly increased in the muscle of metabolite-treated mice (Fig. 7p).

Positron emission tomography/computed tomography (PET/CT) using the glucose analogue 18F-fluorodeoxyglucose (18F-FDG) was used to determine the tissue-specific metabolic effects of MOVA (100 mg/kg/day), SOP (100 mg/kg/day), and BHIBA (150 mg/kg/day) treatment in vivo in the mouse model of obesity and T2DM28 (Fig. 7q). The metabolic activity of BAT was significantly increased in BHIBA- and MOVA-treated mice (Fig. 7r). Hind limb skeletal muscle metabolic activity was increased in BHIBA-, MOVA-, and SOP-treated mice (Fig. 7s), with forelimb muscle metabolic activity significantly increased in MOVA- and SOP-treated mice (Fig. 7t).

The candidate metabolites are concomitantly increased in the plasma by stimulation of thermogenesis. MOVA and SOP produced the most robust and significant reduction in weight gain and adiposity in high-fat-fed mice. We examined whether these metabolites would have combinatorial anti-obesity and anti-diabetic effects on systemic metabolism. Six-week-old mice were treated with a combination of MOVA (100 mg/kg/day) and SOP (100 mg/kg/day) in drinking water for 17 weeks and fed a 60% fat diet. The combination of metabolites additively reduced weight gain when compared to either SOP or MOVA treatments alone (Supplementary Fig. 10a). CT analysis identified that a combination of MOVA and 5OP reduced body fat by 24.6% in treated mice compared with controls (Supplementary Fig. 10b). Glucose tolerance was further improved by a combination of 5OP and MOVA treatment (Supplementary Fig. 10c). PET/CT analysis using 18F-FDG indicated that mice treated with both MOVA and 5OP had enhanced glucose uptake into the hind limb skeletal muscle when compared to the singly administered treatments (Supplementary Fig. 10d, e).

Together, these data show that the metabolites increase energy expenditure, reduce weight gain, improve glucose homeostasis, and increase glucose and fatty acid catabolism in BAT, WAT, and skeletal muscle. The results of MOVA and 5OP combinatorial studies also suggest MOVA and 5OP function through disparate mechanisms and that the small molecule adipokine-like signals function in concert to mediate systemic metabolism and anti-obesity effects on release from brown/beige adipose tissue.

MOVA and 5OP signal through cAMP–PKA–p38 MAPK and BHIBA via mTOR to regulate adipocyte and myocyte metabolic gene expression. We determined whether the metabolites function extracellularly or intracellularly at the human adipocyte to induce expression of UCP1. MCTs function to both import and export monocarboxylate species.29 Treatment of human adipocytes with MOVA, SOP, or BHIBA increased the intracellular concentration of the metabolites; this effect was abrogated by co-treatment with the MCTi (a-cyano-4-hydroxycinnamate) (Fig. 8a–c). Concomitant treatment of primary adipocytes with the MCTi and 5OP impaired 5OP-induced UCP1 expression. (Fig. 8d). Conversely, inhibition of MCT activity did not impair MOVA or BHIBA-mediated UCP1 expression, with dual metabolokine and MCTi treatment trending toward increased UCP1 expression compared to metabolokine treatment alone (Fig. 8e, f). We then examined whether the metabolokines signaled via similar mechanisms in human primary skeletal myocytes. Treatment of human skeletal myocytes with the metabolokines increased their intracellular concentration; this effect was impaired by co-treatment with the MCTi (Fig. 8g–i). Combined MCTi and SOP treatment impaired 5OP-mediated CPT1b expression in
myocytes (Fig. 8j). The MOVA and BHIBA-induced expression of CPT1b was not impaired by MCTi (Fig. 8k, l). These data suggest that 5OP requires import into the cells to induce molecular signals leading to increased metabolic gene expression. Conversely, these results indicate MOVA and BHIBA function through extracellular signal transduction and may require a receptor in the adipocyte and myocyte membrane.

Canonical activation of adipocyte thermogenesis through β3-adrenergic signaling requires intracellular signal transduction by cAMP and downstream activation of protein kinase A (PKA)\(^1\). Using LC-MS, we measured the intracellular cAMP content in human adipocytes and myocytes treated with 5OP, MOVA, and BHIBA (Fig. 8m). The concentration of cAMP was unchanged in BHIBA-treated cells but increased in 5OP- and MOVA-treated
cells. We analyzed the AMP content of BAT (Supplementary Fig. 11a), subcutaneous WAT (Supplementary Fig. 11b), and soleus skeletal muscle (Supplementary Fig. 11c) of SOP-, MOVA-, and BHIBA-treated mice. The AMP content was increased in BAT, subcutaneous WAT, and skeletal muscle of SOP- and MOVA-treated mice. We then co-treated primary adipocytes with either MOVA or 5OP and the selective PKA inhibitor H89. Inhibition of PKA impaired MOVA and 5OP-induced expression of 

**Discussion**

The canonical role of BAT, and to some extent beige adipose tissue, has long been regarded as to generate heat through uncoupled respiration. However, BAT and beige adipose tissue may have a more varied capacity to regulate systemic metabolism. The ability of WAT to function as an endocrine organ, releasing messengers known as adipokines that coordinate a systemic response to energetic status, feeding behaviors and inflammatory responses, amongst other physiological processes, is well established\(^\text{33}\). A similar endocrine function of BAT and beige adipose tissue remains poorly understood and characterized. However, evidence suggests the presence of endocrine and paracrine signals emanating from BAT and beige adipose tissue. Transplantation studies of both beige and brown fat in mice have demonstrated the capacity to induce weight loss in mouse models of obesity. Surprisingly, these studies have identified direct improvements in glucose homeostasis in skeletal muscle and activation of endogenous beige and brown fat\(^\text{8,9,32}\). In addition, the total loss of BAT has a more profound effect on metabolic status than the tissue-specific ablation of Ucp1. The ability of BAT to influence systemic energy balance is not solely reliant on non-shivering thermogenesis\(^\text{11-14}\).

Recent discoveries have highlighted a number of peptidic, lipid, and miRNA brown adipokines\(^\text{33}\), including the discovery by Kong and colleagues of a BAT-to-muscle signaling axis mediated through the protein myostatin\(^\text{10}\). We have previously highlighted a small molecule metabolite-mediated skeletal muscle to beige adipose tissue signaling axis\(^\text{34}\). Here we identify MOVA, SOP, and BHIBA as a discrete set of brown and beige adipose metabolites. The effects of MOVA, 5OP, and BHIBA in vitro and in vivo have been summarized and compared in Supplementary
Table 8. These metabolites function in concert to mediate crosstalk between BAT, beige adipose tissue, and skeletal muscle by inducing expression of key mitochondrial genes and an oxidative phenotype in adipose and muscle, increasing whole-body energy expenditure, complementary to BATokine proteins and lipokines.

MOVA, BHIBA, and BHIVA are monocarboxylic acids generated via the catabolism of BCAAs. Catabolites of BCAAs are emerging as bioactive metabolites and have been implicated in endocrine signaling. Notably, the valine catabolite β-aminoisobutyric acid acts as an exercise-stimulated myokine, signaling to induce browning of WAT and hepatic fatty acid oxidation. BHIBA is also a product of valine metabolism and signals between skeletal muscle and the endothelium in a PGC1α-dependent manner to increase fatty acid uptake. Increased circulating plasma concentrations of BCAAs are a distinguishing feature of obesity and may predict T2DM onset. BHIBA, MOVA, and BHIVA are monocarboxylic acids generated via the catabolism of BCAAs.

5OP uncoupling protein 1 palmitoyltransferase 1b gene expression in human adipose tissue. Dark green = MOVA, light green = SOP, purple = BHIBA, dark blue = BHIVA. Analysis by Pearson correlation. Source data are provided as a Source Data file.
metabolic diseases including obesity. Metabolic risk associated with increased circulating BCAAs may, in part, be mediated by decreased biosynthesis and secretion of these brown and beige adipocyte metabolines and perturbation of the interorgan signaling axes they mediate.

5OP is a neglected amino acid, which links glutamine metabolism to glutathione biosynthesis. Glutathione is a potent antioxidant and protects against adverse cellular redox states. Browning of adipose tissue has been postulated as an adaptive mechanism to alleviate redox pressure, with recent studies demonstrating that the expression of UCP1 is regulated by redox status including oxidative stress and antioxidants. Thus, our identification of 5OP as a brown and beige metabokine may represent a mechanism through which beige/brown adipose can
communicate redox state and, amongst other effects, recruit additional beige adipocytes to rescue systemic redox stress.

We acknowledge limitations to our study. Our work does not preclude the presence of other metabolite factors, protein signals, or bioactive lipids that may contribute to the functional signaling between adipose–adipose and adipose–skeletal muscle. Indeed, our data suggests the presence of an, as yet unidentified, secreted peptidic inhibitor of browning. Due to the importance of the biosynthetic enzymes involved in the generation of BHIBA, BHIVA, 5OP, and MOVA, shared across multiple metabolic pathways including BCAA catabolism, fatty acid oxidation, TCA cycle, glutamine/glutamate, and redox glutathione metabolism, directly targeting these in loss-of-function experiments would be incapable of unambiguously distinguishing the effect of the metabolites from perturbations of multiple pathways. However, this observation supports our finding that these metabolites function as key brown and beige adipokines, as the need to closely associate tissues during metabolic processes responding to cellular energy and redox metabolism would be essential. Our analyses suggest that MOVA and 5OP function through cAMP–PKA–p38 MAPK signaling, and BHIBA functions through mTOR, to induce adipocyte and myocyte metabolic gene expression. Both cAMP–PKA–p38 MAPK and mTOR signaling regulate the activation of BAT thermogenesis, WAT browning, and mitochondrial biogenesis.33,34,35 Our study suggests that BHIBA and MOVA function through extracellular receptors and that 5OP induces effects through a direct metabolic mechanism to induce adipocyte browning and myocyte β-oxidation. Future work may also uncover the effects of BHIBA, SOP, and MOVA on other tissues, and the identity of the discreet receptors through which the metabolites exert their effects.

In addition, we ascertained that the metabolite BHIVA demonstrated some capacity to regulate adipose tissue metabolism in vitro, although no effect on energy or glucose homeostasis was observed in vivo. BHIVA has been shown to have bioactive metabolic properties in other settings, including skeletal muscle protein synthesis and exercise,36 and our work does not preclude an alternative signaling role for this metabolite linking beige adipose tissue and BAT to the regulation of systemic physiology.

We identify MOVA, SOP, and BHIBA as a discrete set of small molecule brown and beige adipokines. The identification of these metabolites as interorgan signals has significant implications, not only for our understanding of the integration and regulation of physiological energy metabolism and its protective role against the development of metabolic diseases, but also for understanding the pathophysiology of, and potential therapeutics for obesity, T2DM, and the metabolic syndrome.

Methods
Culture and differentiation of mouse primary adipocytes. Primary white adipose stromal vascular cells were fractionated from 6- to 10-week-old C57BL/6J male mice as previously described.42,47 Stromal vascular cells were then cultured and differentiated into adipocytes according to published methods.42,48,49 During the 6-day differentiation, cells were cultured with 100 nM GW0742 (Sigma Aldrich), 250 nM HIC (Sigma Aldrich), 10 μM AKV (Sigma Aldrich), 5 μM AHI (Sigma Aldrich), 20 μM MOVA (Sigma Aldrich), 20 μM SOP (Sigma Aldrich), 20 μM BHIBA (Sigma Aldrich), and 10 μM BHIVA (Sigma Aldrich) or with 1 μM forskolin (Sigma Aldrich) on days 5–6 of differentiation.

For media transfer experiments, treatment media was removed, cells were washed three times with phosphate-buffered saline (PBS), and 1:1 serum-free media was conditioned on fully differentiated primary white adipocytes that had remained untreated (control) or had received either 100 nM GW0742 throughout differentiation or 1 μM forskolin on days 5–6 of differentiation within each well of a 12-well plate. Media was removed after 24 hrs. Media was boiled at 100 °C for 5 mins for denaturation experiments. Conditioned media (1 ml) was added to naively differentiated primary adipocytes in each well of a 12-well plate.

For assessment of aqueous fraction bioactivity and metabolomic analysis, after 24 hrs conditioning on cells, serum-free media was removed from the cells and spun at 1000 g for 2 min to remove debris. Aqueous soluble metabolites were extracted from 1 ml of conditioned media as previously described.49. For aqueous fraction bioactivity assessment, dried aqueous fractions were then reconstituted in 1 ml of fresh serum-free media by vortex-mixing (5 min) followed by sonication (15 min) and then added to naive primary adipocytes in a 12-well plate for 24 hrs. Human primary adipocyte culture. Human primary preadipocytes (PromoCell, Heidelberg, Germany, Cat no. C-12735) were seeded (10,000–15,000 cells/cm²) and grown to confluence (37 °C, 5% CO₂) in preadipocyte growth medium (0.05 ml/ml fetal calf serum, 0.004 ml/ml endothelial cell growth supplement).
10 ng/ml epidermal growth factor, 1 µg/ml hydrocortisone, 90 µg/ml heparin). Preadipocytes were differentiated according to supplier’s instructions. Briefly, growth medium was replaced by differentiation media (8 µg/ml d-biotin, 0.5 µg/ml insulin, 440 ng/ml dexamethasone, 44 µg/ml isobutylmethylxanthine, 9 ng/ml L-thyroxine, 3 µg/ml ciglitazone) for 48 h. Differentiation medium was replaced with adipocyte nutrition media (0.03-ml/ml fetal calf serum, 8-µg/ml d-biotin, 0.5-µg/ml insulin, 400-ng/ml dexamethasone) for 12 days until cells were fully differentiated. Media was changed every 48 h.

Human white primary adipocytes were treated throughout differentiation with either 100 nM GW0742, MOVA (10, 20, 40 µM), SOP (10, 20, 40 µM), BHIBA (10, 20, 40 µM), and BHIVA (10, 20, 40 µM) with 1 µM forskolin on days 10–12 of differentiation and with either 2 mM α-cyano-4-hydroxycinnamate for 24 h on day 11 of differentiation, or 500 nM of the p38 MAPK inhibitor Birb 796, 500 nM of the mTOR inhibitor temsirolimus, or 10 µM of the PKA inhibitor H89 for 12 h on day 12 of differentiation. All experiments were performed using adipocytes (day 12) at passages 3–5.
Informed consent was obtained from donors. The cells were approved and complied with ethics according to:

- Collection, generation, research purpose, and sale in compliance with the Declaration of Helsinki: PromoCell GmbH Sickingenstr. 63/65 69126 Heidelberg Germany.

- Use in compliance with Human Tissue Act (UK) by Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, LS2 9JT UK in 2017.

siRNA MCT1 knockdown. FlexiTube siRNA against MCT1 (SI04330396), AllStars negative control siRNA, and HiPerFect Transfection Reagent were purchased from Qiagen. Adipocyte transfection was performed according to the manufacturer’s instructions (75 ng siRNA, 3 μl transfection reagent per well, 60 nmol/L final siRNA concentration) on days 0, 5, and 10 of differentiation.

C2C12 myocytes. C2C12 myocytes were cultured as previously described41. C2C12 cells (Sigma Aldrich Cat no. 90313101) were cultured in 1 ml Dulbecco’s Modified Eagle’s Medium (DMEM; 45 g/l glucose, L-glutamine, NaHCO3, and pyridoxine-HCl) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) in 12-well plates (Millipore, USA) at 37°C in 5% CO2. Once confluent, cells were maintained in differentiation medium for 6 days (DMEM supplemented with 2% horse serum and 1% penicillin/streptomycin). Medium was changed daily.

Human primary skeletal myocyte culture. Adult human skeletal myoblasts (Cell applications Inc. Cat no. 150-05a) were grown in human skeletal muscle cell growth medium (Cell applications Inc.) at 37°C within a humidified atmosphere at 5% CO2. Subculture of human skeletal myoblasts occurred once 85–95% confluency was reached. Experiments were limited to the 5th passage. Human skeletal myoblasts were seeded at 9500 cells per cm2. Once confluent, myoblasts were cultured for 6–8 days in skeletal muscle differentiation media (Cell applications Inc.) to induce myoblast differentiation to myotubes. Myoblasts were treated with human skeletal muscle cell differentiation medium (Cell applications Inc.) containing either BHIBA (5 and 20 μM), MOVA (5 and 20 μM), BHIBA (2.5 and 10 μM), α-cyano-4-hydroxyphenylacetate (24 h on day 8, 2 mM), p38 MAPK inhibitor Birb 796 (12 h on day 8, 500 nM), or BHIBA (20 μM) (Two-tailed t-test; adipoctyes n = 4; MOVA P = 0.02, 5OP P = 0.039; myoblasts n = 3, MOVA P = 0.012, 5OP P = 0.05). Human primary adipoctyes treated with the selective protein kinase A inhibitor H89 (10 μM) (PKAi) with and without n 20 μM MOVA UCP1 C2C12 vs. Control P = 0.004, MOVA vs. MOVA + PKAi P < 0.0001; CIDEA MOVA vs. MOVA + PKAi P = 0.0001; CPT1b MOVA vs. Control P = 0.0003, MOVA vs. MOVA + PKAi P = 0.029) and o 20 μM UCP1 (Control vs. 5OP P = 0.0019, 5OP vs. PKAi P = 0.036; CIDEA 5OP vs. Control P = 0.03, CYCS 5OP vs. Control P = 0.05) (n = 4; Two-way ANOVA with Tukey’s post hoc).

Immortalized human white preadipocyte culture. Immortalized human white preadipocytes, isolated from human neck fat, were obtained from Yu-Hsa Tseng (Joslin Diabetes Center, Harvard Medical School, USA)32. Cells were cultured according to a previously published protocol42. Cells were treated throughout induction and maintenance phases with either 20 μM MOVA, 20 μM BHIBA, or 10 μM BHIBA or for the final 4 days of differentiation with 1 μM forskolin.

Informed consent was obtained from donors. The cells were approved and complied with ethics according to:

- Generation and use including drug discovery purposes: Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA in 2015.

Gene expression array. Total RNA was extracted and reverse transcribed into cDNA using amion TagMan Gene Expression Cells-to-Ct kits (AM1729). cDNA was preamplified using TaqMan PreAmp MasterMix Kits (Applied Biosystems 4384676), prior to loading on TaqMan OpenArray Real-Time PCR Plates (Applied Biosystems 4406947) and analysis by RT-PCR on a QuantStudio 12K Flex RT-PCR System using QuantStudio software version 1.2.10 (ThermoFisher).
Data analysis was carried out in the Thermofisher Cloud Relative Quantification App (3.4.1-PCR-build5) 2017-09-26. Data were normalized to PPIA, RPLP0, and R2M. Primer details are given in Supplementary Table 9.

Confocal images and image quantification. Cells were washed with PBS and labeled with 1:500 HCS Lipidtox Green (ThermoFisher, H34475) and 1:10,000 Hoechst 33342 (ThermoFisher, H3750) at 37 °C for 30 min. Afterwards, cells were washed three times with PBS, fixed in 3.7% formaldehyde for 15 min, and washed four times with PBS. Cells were resuspended in donkey blocking buffer (1xPBS, 5% donkey serum, 0.3% Triton X-100) and incubated at 4 °C overnight. Blocking buffer was removed prior to addition of 10-μg/ml mouse anti-human/anti-mouse UCP1 monoclonal antibody (R&D Systems MBA6158, Minneapolis, USA) in antibody buffer (1xPBS, 1% BSA, 0.3% Triton X-100) for 1 h at 21 °C. Cells were washed three times with PBS, 3-μg/ml Alexa455 AffiniPure donkey anti-mouse IgG (H + L) antibody (Jackson ImmunoResearch 715-545-150, West Grove, USA) in antibody buffer (1xPBS, 1% BSA, 0.3% Triton X-100) was added for 1 h at 21 °C and washed three times with PBS. Samples were afterwards kept in PBS. Imaging was performed on an OPERA QHS spinning disc confocal microscope (PerkinElmer) using an ×20-magnification water immersion lens (numerical aperture 0.7), and 405, 488, and 561-nm lasers with appropriate filter sets. Image analysis was performed using Columbus software version 2.9.1 (PerkinElmer). To determine UCP1 protein levels, first, areas with differentiated adipocytes were defined using LipidTox labeling of lipid droplets followed by measurement of mean UCP1 intensity levels within this region.

Fatty acid and glucose uptake assays. The cellular fatty acid uptake assay was performed as described in the literature. The cellular glucose uptake assay was performed as follows. Cells were grown and differentiated in 12-well plates. Cells were cultured with Dulbecco’s phosphate-buffered saline (DPBS) and placed in low glucose (1 g/L) serum-free DMEM for 24 h. Media was aspirated and replaced with fresh low glucose serum-free DMEM for 1 h. Following media aspiration DPBS containing 6-deoxy-6-[7-nitro-2,1,3-benzoazadil-4-yl]-(amin)-D-glucose (200 μM) was added for 1 h and cells were kept at 37 °C, 5% CO2. Cells were washed three times with DPBS and fluorescence measured using a microscope reader (excitation 485 nm, emission 528 nm).

13C-Palmitate substrate labeling study. Palmitate was solubilized using a dia-lyzed albumin solution. 13C-Palmitate labeling studies were performed as previously described. Briefly, fully differentiated human primary white adipocytes were conditioned with serum-free medium containing insulin 850 nM/L, triiodothyronine 1 nM/L, and rosiglitazone 1- and 140-μM/L. U-13C-labeled palmitate (Cambridge Isotope Laboratories). After 24 h, cells were collected and metabolites were extracted as previously described. During differentiation, cells were cultured with either 25 μM MOA, 20 μM 50P, 20 μM BHIBA, or 10 μM BHIVA.

13C-labeled amino acid substrate analysis. Fully differentiated human primary white adipocytes were conditioned with serum-free medium containing insulin 850 nM/L, triiodothyronine 1 nM/L, and rosiglitazone 1 μM/L and either 200 μM U-13C-labeled leucine, 200 μM U-13C-labeled isoleucine, 200 μM U-13C-labeled valine or 100 μM U-13C-labeled glutamate (Cambridge Isotope Laboratories). After 24 h, cells were collected and metabolites were extracted as previously described. During differentiation, cells were cultured with 1 μM forskolin on days 10–12 of differentiation. Extracted metabolites were analyzed by GC-MS as described below and previously. Animal experimentation. Six-week-old C57BL6/J mice (Charles River) were weight-matched and assigned to groups for treatment. Mice were treated with either 100 mg/kg/day 50P, 100 mg/kg/day MOA, 150 mg/kg/day BHIBA, or 125 mg/kg/day BHIVA in their drinking water for 17 weeks and fed either standard chow or a 60% fat diet ad libitum (Bio Serv F3282). Animals were housed in conventional cages at room temperature with humidity maintained at 40–60% and a 12 h light/dark photoperiod. The cold exposure study was conducted at the University of Cambridge. Animals were housed in a specific pathogen-free facility with 12 h-light and dark cycles and humidity maintained at 60%. Four groups of eight mice (C57BL6/J mice (Charles River) underwent thermal adaptation at 12 weeks of age. One group was placed at 8 °C for 4 weeks, a second group was maintained at room temperature for 3 weeks then placed at 8 °C for 1 week, a further group was placed at 28 °C for 4 weeks and a final group was maintained at room temperature for 4 weeks (21–23 °C). All mice were killed at 16 weeks of age. This study was regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body. All studies complied with national and local ethical regulations for animal research. All procedures were carried out in accordance with U.K. Home Office protocols under a U.K. Home Office Project License by a U.K. Home Office Personal License Holder.

Indirect calorimetry. All experiments were performed according to previously published protocols. Briefly Combined Laboratory Animal Monitoring System (CLAMS, Columbus Instruments) was used to monitor oxygen consumption, carbon dioxide production, food intake, and locomotory activity using Oxymax software (version 5.37.05, Columbus Instruments). The CLAMS was calibrated before each experiment. Animals were subjected to a 3-day acclimation period in a training cage to habituate to the environment of the metabolic cages. Animals were kept on a normal bedding in 22 °C throughout the monitoring period. Ten minute interval measurements for each animal were obtained for oxygen and carbon dioxide with ad libitum access to food and water (or water plus metabolites) on a controlled 12 h light/dark cycle. Cages contained one mass sensor to monitor food intake. Data were analyzed using CalR (version 1.1) (https://calrapp.org)53.

Intraperitoneal glucose tolerance tests (IPGTTs). IPGTTs were performed as previously described. Mice were fasted for 8 h with free access to water prior to baseline glucose measurements. Administration of glucose (Sigma Aldrich) was performed by intraperitoneal injection (glucose 1.5 mg/g of body weight; glucose solution 150 mg/ml). Blood was obtained from the tail vein immediately prior to glucose injection and then at 50, 60, 90, and 120 min post injection. Glucose levels were measured using a Bayer Contour Glucose Meter (Bayer Healthcare).

Positron emission tomography/computed tomography (PET/CT). PET/CT scans were performed on an Albira Si (Bruker). Mice were anesthetized under 2–3% isoflurane, weighed and injected intravenously with 8.9 ± 2.3-MBq 18F-FDG in 200 μL via the lateral tail vein and flushed with saline, followed by a 1 h uptake period. Mice were scanned for 20-min static PET, followed by a 10-min CT protocol for anatomical registration. The CT scans were performed at a 3.5-kV tube voltage and 200 μA over 250 projections. Animal temperature was maintained and monitored throughout the procedure alongside the respiratory rate. PET/CT data were reconstructed using the Albira Reconstructor Software in PMOD (version 3.807, Bruker). PET data were reconstructed using a maximum likelihood expectation maximization iterative method at 25 iterations with scatter, random event, and radiotracer decay corrections. The PET data were fused with the CT data, which was reconstructed with filtered back projection. All PET and CT image data were analyzed in PMOD (version 3.807, Bruker). The methods used to calculate adiposity have been described previously.

Blood and tissue collection. Mice were killed by cervical dislocation. Blood was obtained by cardiac puncture, collected in tubes containing EDTA (2.5 mM/L), and immediately centrifuged to obtain plasma. WAT, interscapular BAT, soleus, and gastrocnemius muscle were removed and flash-frozen in liquid nitrogen.

Gene expression analysis. Total RNA extraction from WAT, adipocytes, and myocytes; cDNA conversion; and quantitative RT-PCR were performed according to published protocols. All data were normalized to 18S rRNA (human WAT, myocytes; cDNA conversion; and quantitative RT-PCR were performed according to published protocols58. Cell and tissue samples were homogenized in 100 mM K2HPO4/ KH2PO4, 5 mM EDTA, 0.1-mM fructose-2,6-bisphosphate, 0.1% Triton X-100, 0.2 mM acetyl CoA, 0.2 mM di-thiobis(2-nitrobenzoic acid) (DTNB). Reaction buffer composition was 100 mM Tris · HCl, 0.2 mM acetyl CoA, 0.1 mM DTT, 1 μM sample, and radiotracer decay corrections. The PET data were fused with the CT data, which was reconstructed with filtered back projection. All PET and CT image data were analyzed in PMOD (version 3.807, Bruker). The methods used to calculate adiposity have been described previously.

RNA-Seq. Next-generation RNA sequencing was performed by Cambridge Genomic Services (Cambridge, UK). The Lexogen mRNA-seq with integrated Chromium (10X Genomic Services) was used for library preparation. Samples were analyzed using a NextSeq300 (illumina) with 75 bp per read and 10 million reads per sample. Data analysis was performed using R package edgeR v3.8.6.

Protein analysis. Analysis of UCP1, PGC-1α, CPT1, and NDUF51 was performed using ELISA per the manufacturer’s instructions (UCP1 Kit SEF557Ra, PGC-1α Kit SEH373Ra; CPT1 SE16050; NDUF51 SE15979; Cloud-Clone Corp., Houston, TX). Kinase profiling was performed using the Proteome Prover Phospho-MAPK Array Kit (Bio-techne Ltd; ARY020) according to the manufacturer’s instructions.

Citrates synthase assay. Citrate synthase was assayed according to published protocols. Cell and tissue samples were homogenized in 100 mM K HPO4/ KH2PO4, 5 mM EDTA, 0.1-mM fructose-2,6-bisphosphate, 0.1% Triton X-100, and radiotracer decay corrections. pH 7.2, 2 M L-malate, and radiotracer decay corrections. pH 7.2 (DTNB). Reaction buffer composition was 100 mM Tris · HCl, 0.2 mM acetyl CoA, 0.1 mM DTT, and 1 mM oxaloacetate (omitted for control), pH 8.0. The reaction rates were linear for ≥4 min. All assays were run in duplicate, and means were analyzed. Specific activities were expressed in international units (μmol substrate transformed to product/min) normalized to tissue weight.

Histology and immunohistochemistry. Tissue was fixed in 4% paraformaldehyde, processed in paraffin, and sectioned into 4-μm sections for staining. Sections were
dehydration in xylene, rehydrated through a 95–50% ethanol series, then placed in water before staining. The VECTASTAIN Elite ABC HRP Kit (Vector Labs, cat no: PK 6100) was used per manufacturer’s instructions to retrieve antigens. Anti-UCP1 primary antibody (abcam ab23841) was used at a 1:20 dilution and incubated on sections in a humidity chamber at room temperature for 60 min. Visualization of VECTASTAIN peroxidases was achieved using VECTOR NovaRED Peroxidase (HRP) Substrate Kit (Vector Labs, cat no: SK-4800). Sections were rinsed in tap water and counterstained with hematoxylin (VECTOR Hema- toxylin Q5, Vector Labs, Cat no: H-3404). Sections were rinsed in tap water, dehydrated through a 50–95% ethanol series, cleared in xylene, and mounted onto coverslips using DPX. Pictures were captured using a standard light microscope and Zen 2 pro software (Version 2.0, ZEISS).

**Respirometry.** Basal respiration was measured in adipocytes and skeletal myocytes maintained in Krebs-Henseleit buffer at 37°C using Clark-type oxygen electrodes (Strathkelvin, U.K.). and full-scan spectra were collected using 3 scans/s over a range of 50–650 m/z. GC-MS chromatograms were processed using Xcalibur (version 2.2, Thermo Scientific). Each individual peak was integrated and then normalized. Overlapping signals were deconvoluted with the Type 2 Diabetes Knowledge Portal (version 1.1, https://calib.org). Post hoc test as indicated. Univariate statistics were performed using Student’s t-test. Error bars depict SEM. A two-tailed Student’s t test, Benjamini Hochberg-adjusted t-test, Exact test, One or Two-way ANOVA were used to determine P value set as a nominal significance of P <0.05 with multiple comparisons correction using a Dunn’s post hoc test as indicated. Univariate statistics was conducted using Prism (version 6.02, Graphpad).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

RNA-Seq data associated with this study are available from Gene Expression Omnibus (GSE129153) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE129153). Mass spectrometry metabolomics data associated with this study have been deposited to EBI Metabolomics Database (MTBLS2426). In addition, all identified metabolites with identifying information are presented in the Source data provided with this paper. Human GWAS data are available from The Type 2 Diabetes Knowledge Portal (http://www.type2diabetogenetics.org/).
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**Author contributions**

A.W. and A.M. were directly involved in the majority of experiments. F.N.K. and C.C. performed and analyzed immortalized human adipocyte studies. F.N.K. performed mass spectrometry cAMP assays in mouse tissues. J.L.S., A.M., and E.B. assisted with experiments throughout. S.A.M., B.D.M., L.D.R. and J.L.G. assisted with metabolomic screens and 13C isotope studies. G.R.D. and J.D. assisted with quantitative microscopy and open array analysis. A.J.M. and A.D.V.M. assisted with design and performance of respirometry. A.D.V.M. assisted with myocyte culture studies. S.V. and A.Y.-P. led and designed the mouse cold acclimatization studies. J.W. and J.E.S. ran the PET/CT imaging studies. J.G. and K.K.W. isolated the human adipose biopsies and plasma samples. L.D.R. designed and led the studies, interpreted the results, and wrote the paper with input from all co-authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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