A small molecule has recently been isolated that stimulated adipogenesis by acting as a gain-of-function ligand for PGRMC2, a poorly characterized single-pass transmembrane protein localized in the endoplasmic reticulum and the nuclear envelope. PGRMC2 belongs to the MAPR family, the members of which share a non-covalent haem-binding domain. Other MAPR proteins (such as PGRMC1, neudesin and neuferricin) bind haem reversibly. We found that PGRMC2 also reversibly bound haem. Of note, addition of haem boosts adipogenesis, whereas inhibition of biosynthesis blocks differentiation. The adipogenic effects of haem have been linked to the nuclear receptor Rev-Erbα. Haem is a ligand for Rev-Erbα, and its binding to Rev-Erbα leads to eventual Rev-Erbα degradation. Notably, the adipogenic effect of the PGRMC2 activator was dependent on binding of haem to Rev-Erbα incubated with wild-type PGRMC2, but not with a haem-binding domain. PGRMC2 traffics mitochondrial haem: PGRMC2 protein purified from *Escherichia coli* was noticeably reddish in colour (Fig. 1a). Its spectrum revealed the Soret peak of haemoproteins at 400–430 nm (Extended Data Fig. 1a), and liquid chromatography–mass spectrometry (LC–MS/MS) showed a 616.18-Da peak, corresponding to iron–protoporphyrin IX (Fig. 1b, Extended Data Fig. 1b, c), confirming that PGRMC2 co-purified with haem. To test the ability of PGRMC2 to transfer haem (a requirement for a haem-mobilizing chaperone), we incubated PGRMC2 with apo-horseradish peroxidase (apo-HRP), an inactive form of the enzyme lacking its prosthetic haem. Incubation of apo-HRP with haemin or PGRMC2 increased HRP activity, reflecting conversion of apo-HRP into active, haem-bound holohorserdsh peroxidase (apo-HRP) (Fig. 1c)—thus indicating that PGRMC2 can transfer haem to other proteins. To test the ability of PGRMC2 to transfer haem to Rev-Erbα itself, apo-Rev-Erbα was incubated with PGRMC2, the mixture was separated by native electrophoresis, and the gel was stained for haem and protein. In-gel staining revealed haem bound to PGRMC2, but not to apo-Rev-Erbα (Fig. 1d). By contrast, apo-Rev-Erbα incubated with wild-type PGRMC2, but not with a haem-binding domain.
Fig. 1 | PGRMC2 controls the intracellular distribution of labile haem. a, Purified PGRMC2 is similar in colour to haemoproteins. b, LC-MS/MS spectra of PGRMC2 and haemin standard. c, Peroxidase activity of apo-HRP with PGRMC2. PGRMC2, haemin, apo-HRP and apo-HRP plus protoporphyrin IX (PPIX) show no activity. Haemin served as positive control. Technical duplicates are shown. d, Native PAGE of wild-type and haem-binding mutant (3×M) PGRMC2 and apo-Rev-Erbα ligand-binding domain (LBD) alone or in combination stained in-gel for haem (top) or protein (bottom). Black arrows, PGRMC2; red arrows, Rev-Erbα. Haemin (20 μM) served as a positive control. PGRMC2 3×M and apo-Rev-Erbα LBD show no haem staining. e, Differential spectroscopy of PGRMC2 haem-binding domain with increasing amounts of ferric or ferrous (in presence of 10 mM dithionite) haemin. Titration curves represent differential absorbance at 405 (ferric) and 400 (ferrous) nm. $K_\text{d}$ is expressed as mean ± s.d. f, Peroxidase activity in HEK293T cells co-transfected with labile haem reporters and scrambled or Pgrmc2 siRNA, and exposed to succinylacetone (SA), haem-depleted FBS or both for 48 h. ER, endoplasmic reticulum. g, Endogenous PGRMC2 co-immunoprecipitates with endogenous PGRMC1 in primary brown adipocytes. h, Peroxidase activity in HEK293T cells co-transfected with labile haem reporters and scrambled, Pgrmc1, or Pgrmc1 and Pgrmc2 siRNA and treated as in f. The scrambled group is repeated from f. In f, h, n = 6 biologically independent samples. Representative results from two (b, g) or three (a, c–f, h) independent experiments. Data are presented as mean ± s.d.; *P < 0.05 and **P < 0.01 versus scrambled basal; determined by two-way analysis of variance (ANOVA) with multiple comparisons and Tukey’s post-test.

PGRMC2 is required for thermogenesis

To evaluate the importance of PGRMC2-mediated haem mobilization in vivo, we focused on adipose tissue. PGRMC2 is enriched in adipose depots, particularly brown adipose tissue (BAT) (Extended Data Fig. 2a, b). We generated adipose-specific PGRMC2-null mice—which we designated PGRMC2 adipose tissue knockout (PATKO)—that lack PGRMC2 only in mature adipocytes. To avoid compensation mechanisms, unless noted all procedures were conducted at thermoneutrality. PATKO mice adapted to 30 °C showed no difference in body weight or white adipose tissue (WAT) mass (Extended Data Fig. 2c) but had reduced BAT weight (Fig. 2a) relative to their wild-type littermates. Notably, the appearance of PGRMC2-deficient BAT was markedly altered, with loss of its distinctive reddish colour (Fig. 2b). There was, however, no difference in expression of brown adipocyte markers (Fig. 2c) and histological comparison failed to reveal any difference (Fig. 2d). These findings led us to test the functionality of PGRMC2-deficient BAT. Reflecting the minor role...
levels of 5-aminolevulinic acid, the product of ALAS1, tended to decrease (Extended Data Fig. 3a). We also noted decreased expression of Alas1 and Alas2 (Extended Data Fig. 3b), indicating that defects in biosynthesis contribute to decreased total haem in PATKO BAT. Iron content was the same as in wild-type BAT, indicating that the reduced haem levels were not caused by iron deficiency (Fig. 3b) and suggesting that tissue haem uptake was unaffected. Of note, labile haem levels were significantly decreased in nuclei purified from PATKO (Fig. 3c) and PGRMC1 and PGRMC2 double-knockout BAT, which had a similar discoloured appearance to PATKO BAT (Extended Data Fig. 3c). In the nucleus, haem regulates the activity of several transcription factors that, upon binding haem, are ultimately degraded. These include Rev-Erbα and the transcriptional repressor BACH123,24. Levels of Rev-Erbα and BACH1 proteins were higher in PATKO BAT (Fig. 3d), indicating that reduced nuclear labile haem resulted in stabilization of these factors. Accordingly, expression of Bmal1 (also known as Arnt1) and Fth1, targets of Rev-Erbα and BACH1, respectively, was reduced (Extended Data Fig. 3d). The circadian pattern of Rev-Erbα mRNA expression25 was not altered in PATKO mice (Extended Data Fig. 3e), suggesting that the increased Rev-Erbα protein levels in PATKO BAT are probably the result of reduced degradation. RNA-sequencing (RNA-seq) analysis showed that among differentially expressed genes (DEGs) between wild-type and PATKO BAT (adjusted P < 0.05; 312 DEGs upregulated and 236 DEGs downregulated) (Supplementary Table 1), haem and iron homeostasis genes were enriched (45 genes, 8.2% of DEGs versus 3.9% in the BAT transcriptome; P < 10−10) (Fig. 3e). Enhancer analysis of down-regulated DEGs in PATKO BAT revealed an enrichment (P < 10−10) of Rev-Erbα and BACH1 and BACH2 motifs (Fig. 3f, Supplementary Table 2), consistent with altered regulation of haem-sensitive transcription. The majority of haem and iron-linked DEGs were present in the three most downregulated pathways, which relate to metabolic processes and energy generation and contain many mitochondrial proteins. Expression of electron transport chain and tricarboxylic acid cycle genes (Extended Data Fig. 3f, g) was broadly decreased in PATKO BAT, and levels of all electron transport chain proteins analysed were notably lower (Fig. 3g, h). Further, PATKO BAT had substantially reduced levels of uncoupling protein 1 (UCP1) (Fig. 3i), a finding consistent with greater stability of Rev-Erbα, which directly represses Ucp126. Beyond its role in uncoupling mitochondrial electron transport, UCP1 regulates mitochondrial integrity27. PGRMC2-null brown adipocytes have large, swollen mitochondria with few, disorganized cristae (Fig. 3i), indicating mitochondrial dysfunction. Indeed, mitochondria isolated from PATKO BAT had reduced basal and markedly reduced uncoupled respiration (Fig. 3j). These findings demonstrate that in the absence of PGRMC2 there is a lower level of labile haem in the nucleus, leading to changes in the haem-responsive transcriptome that cause mitochondrial dysfunction.

**Endogenous haem controls mitochondrial function**

Primary brown PATKO adipocytes recapitulated these defects: they exhibited severely reduced respiratory capacity, a markedly blunted response to adrenergic stimuli without alterations in the transcriptional response to noradrenaline, and decreased levels of UCP1 and electron transport chain proteins (Extended Data Fig. 4a–j). Similar, and perhaps greater, defects were noted in adipocytes deficient in both PGRMC1 and PGRMC2 (Extended Data Fig. 4k). The introduction of human PGRMC2 into mouse PGRMC2-null brown adipocytes restored mitochondrial bioenergetics and UCP1 levels, whereas expression of a PGRMC2 haem-binding mutant did not, indicating that these defects are related to the ability of PGRMC2 to mobilize haem (Extended Data Fig. 4l–o). Notably, mirroring the effect of PGRMC2 deletion, inhibition of haem synthesis was sufficient to impair mitochondrial function and deplete UCP1 in wild-type cells (Extended Data Fig. 5a–d). Neither depletion of exogenous haem nor addition of haemin affected mitochondrial function of BAT at thermoneutrality, PATKO mice were indistinguishable from wild-type mice in energy balance studies (Extended Data Fig. 2d). However, in contrast to wild-type mice, which activated thermogenesis and preserved body temperature when exposed to cold (4°C), PATKO mice (Extended Data Fig. 2f) were minimally reduced (Extended Data Fig. 2e). To confirm that the thermogenic defect was independent of noradrenaline levels, we used the β2-adrenergic receptor agonist CL316,243. Injection of CL316,243 elicited an immediate and sustained increase in oxygen consumption in wild-type mice; this response was significantly blunted in PATKO mice (Extended Data Fig. 2f). Further, consistent with our model of mitochondrial haem mobilization, adiopose-specific PGRMC1 and PGRMC2 double-knockout mice were also cold-sensitive, perhaps more so than PATKO mice (Extended Data Fig. 2g). These findings stress the importance of the PGRMC1–PGRMC2 haem-trafficking pathway for adaptive thermogenesis.

**Loss of PGRMC2 causes mitochondrial dysfunction**

To determine the basis of the defects of PGRMC2-null BAT, we measured total haem content and found it considerably reduced (about 60%) (Fig. 3a). To probe the origins of this difference, we quantified haem precursors and found reduced levels of succinyl-CoA and glycine, the substrates of 5′-aminolevulinate synthase 1 (ALAS1), the rate-limiting enzyme of haem biosynthesis (Extended Data Fig. 3a). Accordingly, of BAT at thermoneutrality, PATKO mice were indistinguishable from wild-type mice in energy balance studies (Extended Data Fig. 2d). However, in contrast to wild-type mice, which activated thermogenesis and preserved body temperature when exposed to cold (4°C), PATKO mice rapidly became hypothermic and perished if not rescued (Fig. 2e, f). This total impairment of adaptive thermogenesis was not a result of changed mitochondrial biogenesis and function (Fig. 2c). Expression of thermogenic genes is decreased in PATKO BAT (wild type n = 5; PATKO n = 6). Haematoxylin and eosin (H&E) staining of BAT. Representative images from two independent experiments (n = 5). Scale bar, 100 μm. e, PATKO mice are cold intolerant (n = 12). Challenge started at Zeitgeber time (ZT) 5. f, Survival curves at 4°C (homeothermia is at 31°C). g, PATKO BAT responds normally to adrenergic signalling (wild type, n = 4; PATKO, n = 5). Pgc-1α is also known as Ppargc1a. In a, g, n represents biologically independent samples. Data are mean ± s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001; PATKO versus wild type. ###P < 0.001; 30°C versus 4°C, determined by two-tailed Student’s t-test (a, c) or two-way ANOVA with multiple comparisons and Bonferroni’s post-test (e, g).
Fig. 3 | PGRMC2 regulates haem-sensitive transcription and mitochondrial function in BAT. a, b, Total haem (a; wild type, n = 6; PATKO, n = 8) and iron (b; n = 8) levels in BAT. c, Labile haem in mitochondrial and nuclear fractions of BAT (wild type, n = 5; PATKO, n = 8). d, Rev-ERBα and BACH1 levels in BAT. e, Genes related to haem and iron metabolism (red portions) are enriched in genes downregulated in PATKO BAT. g, Heat map of haem- and iron-related genes shows a global decrease of electron transport chain (ETC) and tricarboxylic acid cycle (TCA) gene expression. h, UCPI and oxidative phosphorylation (OXPHOS) proteins are reduced in PATKO BAT. i, Electron microscopy shows altered mitochondrial morphology in PATKO BAT. Representative images from four independently samples. Representative results from two (a–c, j) or three (d, h) independent experiments. Data are mean ± s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001 versus wild type; by two-tailed Student’s t-test.

Adipose PGRMC2 regulates systemic metabolism

We next gauged the importance of adipose PGRMC2 for glucose homeostasis. PATKO mice housed at room temperature and fed a high-fat diet

Fig. 4 | PGRMC2 controls systemic glucose homeostasis. a, Blood glucose (wild type, n = 9; PATKO, n = 10) and insulin (n = 6) in wild-type and PATKO mice on HFD. b, c, Glucose tolerance test (GTT) (b) and insulin tolerance test (ITT) (c) after 10 (GTT) and 12 (ITT) weeks of HFD. d, Glucose and insulin levels in DI0 mice treated with vehicle or CPAG-1 for 30 days (n = 7). e, f, GGT (f) and ITT (g) in DI0 mice after 14 (GTT) and 20 (ITT) days of treatment (n = 7). h, H&E staining of BAT. Representative images from four independently samples. i, Ucp1 mRNA levels in BAT of treated DI0 mice (n = 7). j, UCPI and Rev-ERBα levels in BAT of treated DI0 mice. k, Nuclear labile haem levels in BAT of DI0 mice treated with CPAG-1 for four days (n = 4). l, Nuclear labile haem levels in BAT of DI0 mice treated with vehicle or CPAG-1 for 30 days (n = 7).
(HFD) showed no differences in body weight or composition, except for decreased BAT mass (Extended Data Fig. 6a, b). However, they had higher fasting glycaemia (Fig. 4a) and decreased glucose tolerance and insulin sensitivity (Fig. 4b, c). They also exhibited hyperlipidaemia and exacerbated liver steatosis (about 70% more triglycerides) (Extended Data Fig. 6c–e), factors that probably increased insulin resistance. The BAT of HFD-fed PATKO mice showed no histological abnormalities (Extended Data Fig. 7a) but had substantially reduced Ucp1 expression (approximately 40% less) (Fig. 4d). Expression of Bmal1 and Pth1r was also decreased (Extended Data Fig. 7b). Analysis of VAT depots did not show extensive differences in adipocyte size, immune cell infiltration or gene expression in inguinal or epididymal VAT (Extended Data Fig. 7c–e). Notably, Bmal1 expression was reduced in PATKO inguinal VAT (Extended Data Fig. 7e). We propose that hastened metabolic deterioration in HFD-fed PATKO mice probably reflects the aggregate of defects in BAT and VAT.

**PGRMC2 activation mitigates metabolic disease**

The deleterious effects on metabolism of adipose PGRMC2 deletion suggest that activation of PGRMC2 function might reverse features of metabolic syndrome. Thus, we treated diet-induced-obese (DIO) mice at room temperature with a small-molecule PGRMC2 activator (compound 27 in ref. 1; hereafter referred to as CPAG-1). CPAG-1 treatment had no effect on weight or food intake (Extended Data Fig. 8a), but treated mice had reduced fasting glycaemia and insulin levels (Fig. 4e) and improved glucose tolerance and insulin sensitivity (Fig. 4f, g). BAT histology showed decreased lipid content and an increase in multilocular adipocytes (Fig. 4h), features indicative of improved function. Expression of Ucp1 and Bmal1 was also upregulated (Fig. 4i, Extended Data Fig. 8b), changes suggestive of reduced levels of Rev-Erba. Indeed, Rev-Erba protein was decreased and UCPI protein was increased in BAT of CPAG-1-treated mice (Fig. 4j). Labile haem in the nucleus of brown adipocytes from CPAG-1-treated mice was significantly increased within four days of treatment (Fig. 4k), suggesting that decreased Rev-Erba protein was probably the result of haem-induced degradation. No histological differences were found in inguinal VAT (Extended Data Fig. 8c), but expression of Ucp1 and Pgc-1a was increased (Extended Data Fig. 8d). Histology revealed a marked improvement in epididymal VAT, with fibrosis and inflammation noticeably decreased (Extended Data Fig. 8e, f). The liver of CPAG-1-treated mice appeared slightly less steatotic and expression of gluconeogenic genes and Tfα was reduced (Extended Data Fig. 8g, h). CPAG-1 treatment also increased hepatic nuclear labile haem levels (Extended Data Fig. 8i). Given that CPAG-1 interacts very weakly with PGRMC1 (Extended Data Fig. 9), we suggest it may act primarily through PGRMC2 to increase haem flux to the nucleus.

**Discussion**

In this study we have described a role for PGRMC2 in transport of mitochondrial haem. In the absence of PGRMC2, less labile haem reaches the nucleus, resulting in alterations in haem-sensitive transcription that cause mitochondrial dysfunction in brown adipocytes (Extended Data Fig. 10). These defects compromise not only the primary function of BAT (preservation of normal body temperature), but also its contribution to systemic glucose homeostasis. Given its high expression across white fat depots, further studies will be needed to determine whether PGRMC2 performs a similar role in VAT. Nevertheless, our findings provide a view of how haem dynamics in adipocytes can affect physiology. Haem levels and expression of biosynthetic enzymes are reduced in visceral fat of obese humans15, stressing the link between adipocyte haem homeostasis and metabolic disease. Because PGRMC2 is restricted in its tissue distribution, additional haem chaperones probably remain to be discovered. Finally, we have shown that pharmacological activation of PGRMC2 may be of use in treating metabolic disease. Given the interest in identifying signalling pathways that enhance adipocyte function and correct obesity-linked adipose tissue defects9, our findings suggest that modulation of intracellular haem dynamics could be a potentially innovative therapeutic strategy.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1774-2.
Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Reagents

Haemin, protoporphyrin IX, oligomycin A, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), rotenone, antimycin A, 3-isobutyl-1-methylxanthine (IBMX), BSA, mannitol, noradrenaline, isotroperenol, β-Br-CAMP and succinylacetone were purchased from Sigma-Aldrich. CL 316, 243 was obtained from Cayman Chemical. Forskolin was obtained from Chem Impex International. Insulin (Novolin) was purchased from Novo-Nordisk. Complete EDTA-free protease inhibitor cocktail was obtained from Roche. DMEM and other Gibco-branded cell culture products were purchased from Thermo Fisher. Haem-depleted FBS was prepared by treating FBS with 20 mM ascorbic acid for 16 h, followed by 24 h dialysis against PBS. Haem depletion was verified by measuring optical absorbance at 405 nm. CAPG-1 was synthesized as previously reported. ON-TARGET siRNA SMARTpools against human PGRMC2 (L-051721-00-0005), and mouse NR1D1 (L-051721-00-0005), and BACH1 (L-042956-01-0005), as well as a Non-Targeting Pool (D-00810-10-05) were purchased from Dharmacon. HEK293T cells were obtained from ATCC (CRL-3216) having undergone short-tandem repeat verification. Cells were routinely tested for mycoplasma and were never positive.

Protein production

Full-length PGRMC2 in a bacterial expression vector (GenBank accession number NM_027558; Genecopoeia Ex-Mm25103-B01) was transformed into chemically competent BL21(DE3) cells (Thermo Fisher) and grown at 37 °C to an OD600 of 0.8. Cells were induced with 1 mM IPTG for 5 h, collected and resuspended in 50 mM Tris-HCl, 150 mM NaCl, pH 7.4 containing 1% Triton X-100, 100 μg/ml lysozyme, 100 μg/ml DNase I, 10 mM β-mercaptoethanol and 1% Triton X-100. Complete EDTA-free protease inhibitor cocktail (Roche) for 1 h. After sonication, the lysate was centrifuged at 6,000 g for 30 min and the supernatant purified using nickel affinity chromatography. After elution, the protein was dialysed into 50 mM Tris-HCl, 150 mM NaCl, pH 7.4, and purified by HiLoad 16/600 Superdex 75 size exclusion chromatography (GE Healthcare). A mouse PGRMC2 haem-binding mutant was created by mutating 3 amino acids (Y131F, K187A and Y188F) using a Quikchange II XL Site-Directed Mutagenesis Kit (Agilent), verified by DNA sequencing, and expressed and purified for the haem Soret peak (ε415) of 101.85 = 1 mM, and 6×His–REV-ERα LBD bound to haem was assessed using the extinction coefficient for the haem Soret peak (ε405 nm) was plotted in relation to haemin concentration per addition. Spectra were recorded 3 min after each addition of haemin. The difference in absorbance at 420 nm and absorbance at 405 nm measured immediately for 15 min. As a positive control, apo-HRP was incubated with 0.3 nM haemin for 5 min and washed extensively with 50 mM Tris-HCl, 1 mM EDTA, 0.01% NaN3, 1 mM DTT, 25% sucrose, 100 mM sodium chloride, 1% sodium deoxycholate and 1% Triton X-100. Inclusion bodies were subjected to a final wash in the same buffer without Triton X-100. To denature inclusion bodies, about 200 mg of inclusion bodies was resuspended in 100 mM Tris-HCl, 6 M guanidinium chloride and 20 mM β-mercaptoethanol for 1 h at room temperature (RT). Denatured inclusion bodies were refolded overnight at 4 °C in an oxidative refolding buffer containing 400 mM L-arginine, 100 mM Tris-HCl, 5 mM reduced glutathione, 0.5 mM oxidized glutathione, 10 mM EDTA and 200 mM phenylmethylsulphonyl fluoride. Refolded protein was concentrated and purified using HiLoad 16/600 Superdex 75 size exclusion chromatography (GE Healthcare). Purity of PGRMC2 proteins was confirmed using SDS–PAGE. Soret and a, β absorption spectra were measured on a SpectraMax 250 reader (Molecular Devices) at room temperature. Purified PGRMC2 protein was incubated for 15 min with 10 mM dithionite to reduce the haem group. Human REV-ERβ LBD (residues 281–614) with an N-terminal hexahistidine tag and a tobacco etch virus (TEV) protease cleavage site was inserted into a pET46 vector and expressed in E. coli BL21(DE3) cells. Cells were grown in 37 °C overnight and induced in autoinduction medium at 37 °C for 5 h, 30 °C for 1 h, and 22 °C for 16 h. Cells were collected and pellets stored at −80 °C. Pellets were thawed on ice and resuspended in lysis buffer without imidazole (40 mM NaHPO4, pH 7.4, 500 mM NaCl, 10% glycerol, 2.5 mM DTT and 0.1% Tween-20) at 40 ml buffer per 5 g pellet. The cell slurry was sonicated on ice in 15 s on/30 s off intervals (75% amplitude) for 5 min total. Lysed cells were centrifuged at 14,000 rpm for 30 min at 4 °C. The supernatant was filtered through a 0.4-μm PES membrane Nalgene Rapid-Flow bottle-top filter and affinity purification using 2 × 5 ml HisTrap IMAC columns (GE Healthcare) affixed to an Akta Start. After loading, columns were washed with 100 ml wash buffer (40 mM NaHPO4, pH 7.4, 500 mM NaCl, 10% glycerol, 15 mM imidazole and 1 mM DTT). The protein was eluted using a 10 column-volume elution gradient with elution buffer (40 mM NaHPO4, pH 7.4, 500 mM NaCl, 10% glycerol, 500 mM imidazole and 1 mM DTT). The protein was eluted after >50% elution buffer then pooled and dialysed in 10-kDa MWCO SnakeSkin dialysis tubing (Thermo Fisher) for 24 h at 4 °C to remove imidazole and bound haem in 2 dialysis buffer (40 mM NaHPO4, pH 7.4, 500 mM NaCl, 10% glycerol, 10 mM DTT, 0.1% Tween-20 and 0.5 mM EDTA). After dialysis, the protein was concentrated using a 30 kDa MWCO Amicon Ultra centrifugal concentrator (EMD Millipore). The protein was further purified by size exclusion chromatography (Akta Pure) using a Superdex 75/10/300 GL column in gel filtration buffer (20 mM NaHPO4, pH 7.4, 50 mM NaCl, 50 mM L-arginine, 50 mM L-glutamate and 0.5 mM EDTA). The protein was pooled and confirmed to be >95% pure by LC–MS and SDS–PAGE. The fraction of final purified 6×His–REV-ERβ LBD bound to haem was assessed using the extinction coefficient for the haem Soret peak (ε405 nm) was plotted in relation to haemin concentration per addition. Spectra were recorded 3 min after each addition of haemin. The difference in absorbance at 420 nm was plotted in relation to haem concentration, and dissociation constants (Kd) calculated with GraphPad Prism 6 using a quadratic binding equation.

Haem titration assay

The affinity of PGRMC2 cytochrome b5 haem-binding domain for ferric and ferrous haem was measured by spectrophotometry of the UV-visible spectrum in the Soret region using a SpectraMax 250 reader. Sequential aliquots of haemin in DMSO were added to the sample well containing 10 μM apo-PGRMC2 and the reference well to obtain a 2 μM increment of haemin concentration per addition. Spectra were recorded 3 min after each addition of haemin. The difference in absorbance at 420 nm was plotted in relation to haem concentration, and dissociation constants (Kd) calculated with GraphPad Prism 6 using a quadratic binding equation.

Haem transfer assay

Twenty-five microlitres of 200 mM apo-HRP (Calzyme Laboratories) was incubated with 25 μl of 5 μM purified PGRMC2 protein. After 5 min at room temperature, 150 μl of BioFX TMB One Component HRP microwell Substrate (Surmodics) was added to wells and absorbance at 405 nm measured immediately for 15 min. As a positive control, apo-HRP was incubated with 0.3 mM haemin for 5 min and absorbance measured as described.

Native PAGE and in-gel haem staining

Haem transfer was assessed by mixing 10 μg of wild-type or mouse PGRMC2 haem-binding mutant (3×M) with 10 μg of apo-REV-ERβ protein and incubating for 30 min at 37 °C. After incubation, 2 × Native Tris-Glycine sample buffer (Life Technologies) was added and samples separated by electrophoresis using Novex Tris-Glycine 4–20% gels and
Tris-Glycine Native Running Buffer (Life Technologies) for 6 h. The gel was washed for 10 min with water and haem staining was performed using the BioFX TMB One Component HRP Microwell Substrate (Surmodics). After imaging the haem stain, the gel was washed overnight with water and counterstained with Coomassie for protein detection.

**Mass spectrometry**

To detect haem in purified PGRMC2 protein, 5 μl of 20 mg/ml PGRMC2 was extracted with 1 ml of Folch solution (2:1 chloroform:methanol) and washed with 200 μl of water. The extraction solution was then vortexed and centrifuged at 1,000g, 4°C for 10 min and the lower phase extracted and dried down. Before LC–MS analysis, the sample was reconstituted in methanol. A haemin standard solution was prepared at 10 μM. LC–MS analysis was performed on an I-class UPLC system coupled with a Synapt G2-Si mass spectrometer via an electrospray ionization (ESI) source from Waters. The positive-mode (+) ESI conditions were as follows: capillary, +3.00 kV; sampling cone, 40 V; source temperature, 100°C; desolvation temperature, 250°C; desolvation gas flow, 600 l/h; and cone gas flow, 50 l/h, respectively. Leucine–enkephalin (m/z 556.2771) was used for lock mass correction. Liquid chromatography was performed with A = 40:60 water:acetonitrile + 1 mM ammonium formate, B = 90:10 2-propanol:acetonitrile. A Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm) was used at a flow rate of 250 μl/min. Initially, the mobile phase composition consisted of 32% B and held for 1 min after injection and its composition was increased over the length of the gradient (15 min, B = 97%) in short increments adapted from a previous study. The injection volume was 2 μl. For haem quantification in tissue, BAF was isolated from wild-type and PATKO mice housed at 30°C after 10 min of perfusion with cold PBS. Ten to twenty-five milligrams of frozen tissue was homogenized in 300 μl of 1% formic acid in dH₂O and an internal standard added. Haem was extracted in Folch solution (2:1 chloroform:methanol). After centrifugation at 4,000g for 10 min at 4°C, haem was re-extracted from the organic phase with 1 volume of 1.4 N NaOH. Samples were centrifuged at 4,000g for 10 min at 4°C and the aqueous phase collected for mass spectrometry analysis. Haemin was quantified on an Agilent 6495 triple quadrupole with a jet stream source coupled to an Agilent 1290 UPLC. As internal standard, deuteroporphyrin (Frontier Scientific) was used and the monitored transitions were m/z 616.1 → 557.1 (quantitative), m/z 616.1 → 498.2 (qualitative) for haemin, and m/z 564.0 → 505.0 for deuteroporphyrin. Jet stream was set at gas temperature 200°C, gas flow 12 l/min, nebulizer pressure 30 psi, sheath gas temperature 325°C, sheath gas flow 10 l/min, cap V = 400 V, nozzle V = 2,000 V. Liquid chromatography was performed with A = 90:10 water:methanol + 0.1% ammonium hydroxide and + 10 mM ammonium formate, B = 65:35:10 2-propanol:methanol:water + 0.1% ammonium hydroxide and + 10 mM ammonium formate. All solvents were LC–MS grade. An Agilent extend-C18 column (1.8 μm, 2 × 50 mm) was used at a flow rate of 0.2 ml/min. Initially, the mobile phase consisted of 5% B and, after injection, its composition increased linearly to 95% B in 6 min and held at 95% for 3 min. The injection volume was 5 μl. Haem content was normalized per milligram of tissue. Quantitative analysis of glycine, aminolevulinic acid and succinyl-CoA was performed using a QQQ mass spectrometer operated in positive-ion mode (Xevo TQ-XS from Waters). In brief, samples were extracted and dried down. Before LC–MS analysis, an aliquot was reconstituted in 1:1 acetonitrile:water and injected into a Waters ACQUITY UPLC BEH Amide column (1.7 μm, 2.1 mm × 100 mm) at a flow rate of 400 μl/min. The mobile phases consisted of A = water + 0.1% formic acid and B = acetonitrile + 0.1% formic acid. Initially, the mobile phase composition consisted of 95% B and held for 1 min after injection and its composition was decreased to 65% over 6 min and then to 40% over 3 min and held for an additional 1 min. The following quantifier and qualifier transitions (collision energy in eV) were used for each metabolite: glycine: 76.0 → 30.3 (6 eV) and 48.2 (4 eV); glutamine: 78.0 → 31.0 (6 eV) and 49.0 (4 eV); aminolevulinic acid: 132.2 → 55.1 (18 eV), 68.3 (18 eV), 86.0 (10 eV), 114.0 (6 eV). For succinyl-CoA, an aliquot was reconstituted in 50 mM ammonium acetate (pH 6.8 adjusted with ammonium hydroxide) and analysed as soon as possible once samples had been reconstituted to avoid degradation. Liquid chromatography was performed with A = 50 mM ammonium acetate (pH 6.8) and B = 80% methanol. A Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm) was used at a flow rate of 250 μl/min. Initially, the mobile phase composition consisted of 2% B and held for 1.5 min after injection and its composition was increased to 15% over 1.5 min and then to 95% over 1.5 min and held for 9 min. The following quantifier and qualifier transitions (collision energy in eV) were used for succinyl-CoA: 868.1 → 99.0 (54 eV), 136.3 (54 eV), 259.1 (54 eV) and 361.3 (54 eV).

**Labile haem reporters targeted to subcellular compartments**

HEK293T cells grown in DMEM with 10% FBS were transiently transfected in OptiMEM for 8 h using Dharmafect Duo transfection reagent (Dharmafect) in 96-well plate format. Peroxidase reporters (peGFPMitoAPX, peGFPP-APX, peGFPP-LS-APX, and pm-Cherry-ER-HRP) were co-transfected with 50 nM siRNA against Pgrmc2, Pgrmc1, the combination or a scramble control. After transfection, cells were switched to basal medium (DMEM with 10% FBS), basal medium plus 0.5 mM succinylacetone, haem-depleted medium (DMEM with 10% haem-depleted FBS) or haem-depleted medium plus 0.5 mM succinylacetone. Cells were lysed 72 h later in 100 μl haem lysis buffer (150 mM NaCl, 20 mM HEPES, 0.5% Triton X-100, with Protease Inhibitor Cocktail Set III). Fifty microlitres of lysate was incubated with the BioFX TMB One Component HIRP microwell Substrate (Surmodics). Absorbance at 620 nm was measured after 5 min for the ER-HRP reporter, and after 30 min for mitochondrial, nuclear, and cytosolic APX reporters.

**Co-immunoprecipitation**

Endogenous PGRMC2 and PGRMC1 were immunoprecipitated from primary brown adipocytes differentiated in vitro using anti-PGRMC2 and anti-PGRMC1 antibodies. Cells were lysed in IP lysis buffer (150 mM NaCl, 20 mM Tris-Cl, 10% glycerol, 1% Triton X-100 and complete EDTA-free protease inhibitor cocktail) and protein quantified using the DC assay (BioRad). One milligram of total proteome was incubated with 4 μg of anti-PGRMC2, anti-PGRMC1 or rabbit IgG control antibody pre-bound to 0.75 mg of Dynabeads Protein G (Thermo Fisher). After overnight incubation at 4°C, beads–antibody–protein complexes were washed three times with PBS –0.02% Tween 20 for 5 min at RT, eluted in 50 mM glycine buffer pH = 2.8 for 10 min at 60°C and separated by SDS–PAGE for immunodetection.

**Western blot analysis**

Samples separated by SDS–PAGE were transferred onto nitrocellulose membranes. Membranes were incubated in blocking buffer (TBS-Tween 0.1%, BSA 5% w/v) for 1 h at room temperature. Membranes were incubated overnight at 4°C with primary antibodies diluted in blocking buffer, washed three times for 15 min with TBS-Tween 0.1%, and incubated for 1 h at room temperature with HRP-conjugated secondary antibodies diluted in blocking buffer (1:20,000 dilution). The antibodies and dilutions used in this work were: PGRMC2 (1:1,000, Bethyl Laboratories, A302-954A and A302-955A), PGRMC1 (1:1,000, Bethyl Laboratories, A304-561A), PPARy, EV-ERBa (1:200, Santa Cruz Biotechnology, sc-7273 and sc-100910), BACH1 (1:500, R&D Systems, AF5777), UCP1 and OxPhoS (1:5,000 and 1:300, Thermo Fisher Scientific, PA124894 and 458099), GAPDH, TUBULIN, and HSP90 (1:5,000, GeneTex, GTX627408, GTX27291, and GTX101423), and CEBPβ (1:1,000, Abgent, AP20492c).
Primary adipocyte culture
Primary brown adipocytes were isolated from the interscapular BAT depot of wild-type and PATKO newborn mice. BAT depots were minced and digested by shaking for 40 min at 37 °C in isolation buffer containing 6.15 mM NaCl, 2.5 mM KCl, 0.65 mM CaCl2, 2.5 mM glucose, 50 mM HEPES, 50 U/ml, 50 μg/ml Pen/Strep, BSA 2% (w/v) and 1.5 mg/ml collagenase type I (Worthington). Cells were filtered through a 70-μm strainer and plated in DMEM with 25 mM glucose, 20 mM HEPES, 20% FBS and Pen/Strep. Differentiation was induced when cells reached confluence by switching to the medium to DMEM, 10% FBS, 20 mM insulin, 1 mM triiodothyronine (T3), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) and 2 μg/ml dexamethasone (Dex). Two days later, medium was replaced with DMEM, 10% FBS, 20 mM insulin and 1 mM T3. On day 4 of differentiation, cells were treated with 0.5 mM succinylacetone, or switched to haem-depleted FBS, for bioenergetics and gene/protein expression studies and analysed at day 7. Exogenous haemin at a final concentration of 20 μM was added 48 h before bioenergetics studies were performed. On day 7 of differentiation, adipocytes were treated with vehicle or 100 nM noradrenaline for 2 h for gene expression studies. For complementation experiments, cells were infected with lentiviruses expressing mCherry, wild-type human PGRMC2, a human PGRMC2 haem-binding mutant (3×M; Y137F, K193A and Y194F) at day 0 of differentiation in the presence of 5 μg/ml polybrene. Revertase and BAC1I knockdown in mature adipocytes was performed as previously described.

Mitochondrial bioenergetics measurements
The oxygen consumption rate of adipocytes was measured on a Seahorse XFe96 instrument. Primary brown adipocytes differentiated in vitro were re-plated at day 5 of differentiation on gelatin-coated XFe96 plates at a density of 8,000 cells per well. Two days after plating, cells were equilibrated in serum-free DMEM (Sigma Aldrich D5030) containing 25 mM glucose, 10 mM sodium pyruvate, 2 mM glutamine and 5 mM HEPES pH 7.4 for 1 h before a mitochondrial stress test was performed at day 7 consisting of 3 min cycles of mixing and 2 min cycles of measurements. Basal respiration rates were measured, followed by sequential injections of oligomycin (2 μM), FCCP (1 μM) and rotenone (2 μM) plus antimycin A (RAA, 2 μM). To measure the acute response to adrenergic signalling stimulators, compounds were injected using one of the ports after measurements of basal respiratory rates were complete. Freshly isolated BAT mitochondria (4 μg per well) were transferred onto XFe96 plates containing isolation buffer 2 (IB2 = 220 mM mannitol, 70 mM sucrose, 10 mM KH2PO4, 5 mM MgCl2, 1 mM EGTA, 0.5 mM ADP, 2 μM rotenone, 10 mM succinate, 0.2% BSA and 2 mM HEPES pH 7.4), and plates centrifugated at 2,000 g for 20 min at 4 °C. Oxygen consumption rate was measured after sequential injections at final concentrations of 4 μM oligomycin, 4 μM FCCP and 4 μM antimycin A. Each cycle consisted of 30 s of mixing followed by 2.5 min of measurements.

Quantitative PCR and RNA-seq
Total RNA was isolated from cells and tissues using the Direct-zol RNA MiniPrep Plus kit (Zymo Research). Taqman-based quantitative real-time PCR was performed using the SuperScript III Platinum One-Step qRT–PCR reagent (Thermo Fisher Scientific). Samples were run in triplicate as multiplexed reactions normalized to an internal control (36B4; acidic ribosomal phosphoprotein P0 mRNA). Sequences of primers and probes used are included in Supplementary Information.

For RNA-seq, total RNA was extracted from BAT of wild-type and PATKO mice at 30 °C using the Direct-zol RNA extraction kit (Zymo Research). PolyA+ RNA was fragmented and prepared into strand-specific libraries using the Illumina True-seq stranded RNA kit (Illumina) and analysed on an Illumina HiSeq 2500 sequencer. Libraries were sequenced using single-end 50-bp reads at a depth of 10–15 million reads per library. Single-end sequencing reads were mapped to the mouse reference genome (mm9, NCBI37) using STAR (version 2.3.0.c, default parameters). Only reads that aligned uniquely to a single genomic location were used for downstream analysis (MAPQ >10).

Gene expression values were calculated for read counts on exons of annotated RefSeq genes using HOMER. DEGs were calculated with four replicates per condition using EdgeR, and a threshold of adjusted P value <0.05 was used to call DEGs. DEGs were used for pathway and gene ontology functional enrichment analysis using Ingenuity Pathway Analysis (Qiagen and Metascape (http://metascape.org)). Heat maps were generated using RStudio software (package ‘ggplot2’). Pie charts and Circos plots were generated with Metascape and Adobe Illustrator. Data are available in GEO (GSE124621). Cell type-specific regulatory elements were download from the ENCODE SCREEN portal, using biosequence ‘C57BL/6 brown adipose tissue male adult 24 weeks’. BAT-specific enhancers as annotated by ENCODE (typically high DNase2 and H3K27ac signal but no H3K4me3 signal) were lifted over to mm9 using UCSC LiftOver and associated to genes by proximity (20 kb from TSS). HomER 4.9.1 was used to find enriched known and de novo motifs in enhancers associated to genes of interest.

Mouse studies
All procedures were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute and conducted in accordance with relevant ethical regulations. To generate mice with adipo-specific deletion of Pgrmc2, mice with floxed Pgrmc2 alleles37 and backcrossed to the C57BL/6j background (NNT mutant) were crossed with an Adipoq-Cre strain38 (JAX stock 010803). Similarly, mice with dual deletion of Pgrmc139 and Pgrmc2 in adipose tissue were generated by crossing mice with floxed Pgrmc1 and Pgrmc2 alleles to the Adipoq-Cre strain. Floxed littersmates without the cre transgene were used as controls and are referred to as wild type. Mice were born at room temperature and moved to 30 °C two weeks after weaning. Experiments were performed after a minimum of 4 weeks of acclimatization to 30 °C. Mice were kept on a 12 h light–dark cycle and fed standard chow breeder diet (5058, Picolab) or 60% HFD (D12492, Research Diets) as specified. Male and female mice were used in separate gender-matched experiments. No gender-specific differences were observed. For molecular characterization, mice were euthanized at around ZTS, extensively perfused with ice-cold PBS, and tissues collected and immediately frozen in liquid nitrogen. For circadian time-course analysis, wild-type and PATKO mice (n = 3 per group, per time point) were euthanized every 4 h over a period of 24 h and tissues harvested as described above.

Energy balance studies
Energy balance parameters were determined in a computer-controlled open-circuit system (Oxymax) that is part of an integrated Comprehensive Laboratory Animal Monitoring System (Columbus Instruments), as previously described40. Body temperature was monitored using a rectal probe (RET-3 probe, TH-5 Thermalet Monitoring Thermometer, Physitemp) in cold exposure experiments, and by radiotelemetry in all other experiments. Radiotelemetry was enabled by surgically implanting a transmitter (TA10TA-F10; Data Sciences) into the peritoneal cavity, as previously described41. Male mice (20 weeks old) were allowed to recover for 14 d post-surgery and were then acclimated for 3 d to the experimental environment before measurements were taken. Data were recorded by placing a cage containing a mouse implanted with a transmitter on a receiver plate (RPC-1; DataScience). Data collection and offline analysis were performed using the DATAQUEST A.R.T. software (DataScience). To test the response to the β3-adrenergic receptor agonist CL316,243 (1 mg/kg) or an equivalent volume of PBS, was administered via intraperitoneal injection to 20-week-old male mice housed at thermoneutrality at ZT4.5. Oxygen consumption rate and activity levels were monitored using the CLAMS system.
Exposure to cold
Experiments were performed on male and female mice 12–14 weeks of age. Mice were individually caged with minimal bedding and free access to food and water. To start the cold challenge, they were transferred to 4 °C, with controls remaining at 30 °C, and body temperature was monitored every 30 min for a total of 2.5 h. In that amount of time, all PATKO mice became severely hypothermic and all cold-exposed mice were euthanized. Cold challenge experiments started at or around ZT5 (11:00).

Labile haem quantification
To purify nuclear and mitochondrial fractions from BAT and liver, one lobe of BAT or 100 mg of liver were dounce-homogenized in isolation buffer 1 (IB1 = 220 mM mannitol, 70 mM sucrose, 5 mM EGTA, and 50 mM MOPS pH 7.4). After centrifugation at 1,000g for 10 min at 4 °C, the nuclear pellet was passed through a 100-μm strainer and washed 5 times in IB1. Mitochondria in the supernatant were isolated from the cytosolic fraction after a second centrifugation at 9,500 g for 10 min at 4 °C and washed twice with IB1. The nuclear and mitochondrial pellets were resuspended in 50 μl dH2O, sonicated and protein content quantified using the DC assay (Bio-Rad). Five microliters of 25 mM apo-HRP was incubated in 384-well format with 10 μg in 5 μl of purified mitochondrial or nuclear protein lysates. After 5 min at room temperature, 40 μl of haem assay buffer (30 μM Amplex UltraRed, 0.02% H2O2 in 1 M NaH2PO4/Na2HPO4 buffer, pH 6) was added and fluorescence (ex–em: 490/585) measured immediately for 15 min.

Iron quantification
Frozen tissue (BAT, about 50 mg) was pulverized and lysed in 50 mM NaOH. Non-haem iron content was quantified using 200 μg of protein lysate and the ferrozine method as previously described.

Blood chemistry measurements
Blood samples were collected either from the retro-orbital plexus of anaesthetized mice, or by cardiac puncture after euthanasia. Plasma was separated using BD Microtainer PST tubes with lithium heparin. Triglycerides and non-esterified free fatty acids were measured using the Serum Triglyceride Determination Kit (Sigma) and the HR Series NEFA-HR(2) kit (Wako). Norepinephrine levels were quantified using an ELISA kit (Abnova). Insulin levels were determined using an Ultra-Sensitive Rat ELISA Kit (Crystal Chem).

Tissue lipid content
Frozen tissue (liver, about 30 mg) was pulverized and lysed in RIPA buffer. Triglycerides were quantified in 10 μl of tissue lysate using the EnzyChrome Triglyceride Assay kit (EGTA-200, Bioassay Systems). Triglyceride content was normalized to tissue weight.

Treatment with CPAG-1
C57BL/6 male mice fed a 60 kcal% fat diet (D12492, Research Diets) were purchased from The Jackson Laboratory (D1O, JAX stock 380050) and kept in the same diet throughout the studies. D1O mice (>12 weeks of HFD, 20 weeks of age), randomized based on weight and fasting glycaemia, were dosed intraperitoneally with CPAG-1 every other day (45 mg/kg in a 2:3:1:4 DMSO:PEG40:ethanol:PBS vehicle solution). Weight and fasted glucose levels were monitored weekly. Mice were fasted for 16 h before analysis of basal blood chemistry parameters. At the conclusion of treatment, tissues were collected and snap-frozen for RNA extraction and western blot analysis or fixed for histological examination.

Glucose and insulin tolerance tests
For glucose tolerance tests, mice were fasted for 6 h, and blood was collected from the tail vein before and at timed intervals after oral gavage of glucose (1 g/kg). Plasma glucose was measured with a One-touch Ultra glucometer (Johnson & Johnson). For insulin tolerance tests, mice fasted for 4 h were injected intraperitoneally with insulin (0.4 U/kg; Novolin, Novo Nordisk). Glucose levels were determined before and at timed intervals after injection of insulin.

Histology
Liver, and brown (BAT) and white (WAT) adipose tissue were fixed in 2-Fix (Anatech), dehydrated, embedded in paraffin, and 3-μm- (liver and BAT) or 10-μm- (WAT) thick sections stained with haematoxylin and eosin. Cell size was analysed using ImageJ software.

Electron microscopy
BAT depots were collected and immediately placed in fixative buffer (2.5% paraformaldehyde, 3% glutaraldehyde, 0.02% picric acid in cacodylate buffer, pH 7.3) and stored at 4 °C for 72 h. Fixative buffer was refreshed after 48 h. Tissues were extensively washed in 0.1 M sodium cacodylate buffer (pH 7.3) prior to post-fix incubation in 2% OSO4 in 0.1 M sodium cacodylate buffer for 4 h (buffer was refreshed after 2 h). Tissues were then washed in 0.1 M sodium cacodylate buffer (pH 7.3) followed by water. Tissues were dehydrated in a graded ethanol series and infiltrated and embedded in Spurr resin (Sigma-Aldrich). Thin sections were post-stained with 2% uranyl acetate followed by lead citrate and examined in a FEI Philips CM100 electron microscope at 80 kV. Images were taken using Radius 1.3 software with a Megaview G2 CCD Camera (EMGIS GmbH).

Statistics
Results from in vitro assays and cell culture data are presented as mean ± s.d. Data generated in mouse studies are presented as mean ± s.e.m. The number of mice used in each experiment is indicated in the figure legends. Statistical analysis was performed on Prism software (GraphPad) using Student’s t-test for comparisons between two groups, one-way ANOVA with multiple comparisons for assessment of more than two groups, and two-way ANOVA with multiple comparisons for repeated measurements. Comparisons among specific groups were done using post-tests as indicated in the figure legends.

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

Data availability
Source data tables are provided for Figs. 1–4 and Extended Data Figs. 1–8. Full scans of all western blots are shown in the Supplementary Information. RNA-seq data are available in the Gene Expression Omnibus under accession number GSE124621. All other data supporting the findings in this study are available from the corresponding author upon request.

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**Author contributions** A.G. and E.S. conceived the project, designed research and analysed data. A.G. and B.P.K. performed in vivo experiments. A.G., C.G., V.A. and B.P.K. carried out cell-based assays. A.G. and J.Y.L. performed gene-expression and biochemical analyses. A.S.K. prepared PGRMC2 proteins. S.M. prepared apo-Rev-Erbα protein. J.R.M.-B. and W.R.W. carried out mass spectrometry experiments. A.G. and R.S. performed bioinformatic analysis. C.G.P. synthesized CPAG-1. J.J.P. and J. K.P. provided Pgrmc2 and Pgrmc1 floxed mice. R.C.-C. and B.C. contributed to energy balance studies. L.A.S., D.K., C.G.P., G.S. and B.F.C. provided advice and reagents. A.G. and E.S. wrote the manuscript and integrated comments from the other authors.

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**Extended Data Fig. 1** PGRMC2 binds haem and, with PGRMC1, coordinates its intracellular distribution. a, Absorbance spectra of mouse PGRMC2 protein shows peaks of haem–protein complexes in the 390–450-nm range. Dotted spectra indicate haem–protein complexes after 10 mM dithionite reduction of the iron moiety. b, LC–MS/MS spectra of haemin standard (left) and PGRMC2 protein (right) with collision energy of 40 V. c, Isotope envelope of haemin calculated on the basis of isotope natural abundance for C₃₄H₃₂ClFeN₄O₄ (left), PGRMC2 protein (centre) and haemin standard (right). d, Purified mouse PGRMC2(3×M) mutant (Y131F/K187A/Y188F) does not bind haem. e, The Soret peak typical of haemoproteins is absent in PGRMC2(3×M). f, Representative fluorescence imaging of cells expressing targeted HRP or APX labile haem reporters, showing their localization to mitochondria, endoplasmic reticulum, nucleus and cytosol. g, Levels of Pgrmc2 and Pgrmc1 mRNA in siRNA-transfected HEK293T cells (n = 3 biologically independent samples). h, Interaction of PGRMC1 with PGRMC2 is not observed when PGRMC2 is immunoprecipitated using an antibody that recognizes the haem-binding domain at the C terminus of PGRMC2. Representative results from two (a–e, h) or three (f, g) independent experiments. Data presented as mean ± s.d., ***P < 0.001 versus scrambled basal; two-way ANOVA with multiple comparisons and a Tukey’s post-test.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Pgrmc2 is enriched in adipose tissue and regulates BAT function. a, PGRMC2 protein levels increase during adipocyte differentiation. 3T3-L1 preadipocytes were induced to differentiate and protein extracts prepared at the indicated time points. PPARγ and CEBPδ are markers of mature adipocytes and preadipocytes, respectively. Representative results from three independent experiments. b, Profile of Pgrmc2 mRNA expression across mouse tissues (n = 5 biologically independent samples). c, Whole-body and inguinal subcutaneous fat weight of chow-fed wild-type and PATKO mice housed at 30 °C (WT, n = 8; PATKO, n = 9). d, OCR, core body temperature, CO₂ production rate, respiratory exchange ratio (RER), and activity oscillations of PATKO mice housed at 30 °C (WT, n = 5; PATKO, n = 6). e, Levels of plasma noradrenaline, glucose and non-esterified fatty acids (NEFA) in wild-type and PATKO mice on cold challenge (WT, n = 5; PATKO, n = 7). f, Increased oxygen consumption upon acute injection of the β₃-agonist CL316,243 (1 mg kg⁻¹) is reduced in PATKO mice housed at 30 °C, despite comparable motor activity (n = 5 biologically independent samples). g, Adipose-specific PGRMC1 and PGRMC2 double-knockout mice (DKO) housed at 30 °C are cold-intolerant (WT, n = 13; DKO, n = 8 biologically independent samples). Survival curves of wild-type and DKO mice exposed to 4 °C (homeothermia is at 31 °C). Mice were exposed to 4 °C at ZT5. Data presented as mean ± s.e.m. *P < 0.05 and ***P < 0.001 versus wild type; two-tailed Student’s t-test (e, f) or two-way ANOVA with multiple comparisons and a Tukey’s post-test (g).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Effect of Pyrmc2 deletion in BAT. BAT from chow-fed wild-type and mutant mice housed at 30 °C was analysed. a, Levels of succinyl-CoA, glycine and aminolevulinic acid (ALA) in BAT quantified using targeted metabolomics (n = 5 biologically independent samples per group). b, PATKO mice show reduced expression of Alas1 and Alas2 in BAT (n = 3 biologically independent samples per group). c, Nuclear labile haem is significantly lower in BAT of fat-specific PGRMC1 and PGRMC2 DKO mice housed at 30 °C (n = 4 biologically independent samples per group). Similar to PATKO mice, BAT of DKO mice is discoloured. Representative results from two independent experiments. d, Expression of Rev-Erbα and BACH1 targets (Bmal1 and Fth1, respectively) in BAT of PATKO mice housed at 30 °C (WT, n = 5; PATKO, n = 6). e, Circadian oscillation of clock components is not altered in PATKO BAT (n = 3 biologically independent samples per group per time point). f, Gene ontology (GO) category analysis (biological process) of significantly downregulated genes in RNA-seq analysis of BAT from wild-type and PATKO mice housed at 30 °C (n = 4 biologically independent samples per group). P values determined by standard accumulative hypergeometric statistical test. g, Circos plot of haem-related DEGs showing that the majority (28 out of 45) of them belong to the top 3 downregulated biological processes. Number in parentheses below each biological process represents the total number of DEGs in PATKO BAT in that category. Blue lines refer to downregulated DEGs and red lines to upregulated DEGs. Data presented as mean ± s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001 versus wild type determined by two-tailed Student’s t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Primary brown adipocytes recapitulate the mitochondrial defects of PATKO BAT. a, Wild-type and PGRMC2-null primary brown adipocytes differentiated in vitro imaged on day eight. Lipid stained with Nile red (red) and nuclei stained with Hoechst (blue). Scale bar, 100 μm. b, Protein levels of adipocyte markers during the course of differentiation. c, PGRMC2-null brown adipocytes have impaired mitochondrial respiration (n = 3). d–h, Lack of PGRMC2 in brown adipocytes results in a defective mitochondrial response to endogenous (d), synthetic pan β-adrenergic agonists (e) and pan β3-adrenergic agonists (f), and to downstream activators of adrenergic signalling (g, h) (n = 5). i, Induction of noradrenaline-responsive genes is similar in wild-type and PGRMC2-null brown adipocytes (n = 3) exposed to 100 nM noradrenaline for 2 h. j, OXPHOS proteins and UCP1 are reduced in primary brown PATKO adipocytes. k, PGRMC1 and PGRMC2 DKO primary brown adipocytes differentiated in vitro show severe mitochondrial dysfunction, an inability to increase oxygen consumption on noradrenaline exposure (n = 3), and reduced UCP1 and OXPHOS proteins. l, m, Overexpression of human wild-type PGRMC2, but not of a haem-binding mutant (3×M (Y137F/K193A/Y194F)), can rescue mitochondrial function and the response to noradrenaline in PATKO adipocytes (l, n = 4; m, WT–mCherry, WT–WT, PATKO–WT, n = 8; WT–3×M, PATKO–3×M, n = 7; PATKO–mCherry, n = 6). n, Ucp1 mRNA expression is restored when human wild-type PGRMC2, but not the haem-binding mutant 3×M, is expressed in PATKO cells (n = 3). o, Levels of mouse and human Pgrmc2 mRNA in primary adipocytes used in I–n (n = 3). In a–o, n represents biologically independent samples. Representative results from two (j–o) or three (a–i) independent experiments. Data presented as mean ± s.d. *P < 0.05, **P < 0.01 and ***P < 0.001 versus wild type; ###P < 0.001 versus vehicle; two-way ANOVA with multiple comparisons and a Bonferroni’s post-test.
Extended Data Fig. 5 | PGRMC2-mediated transport of endogenous labile haem regulates mitochondrial function in primary brown adipocytes. 

**a, b,** Inhibition for 48 h of endogenous haem synthesis with 0.5 mM succinylacetone (FBS + SA), but not exogenous haem depletion (haem-depleted FBS), in wild-type primary brown adipocytes phenocopies the mitochondrial defects of PATKO cells (a, n = 8; b, n = 4). 

**c, d,** Treatment with succinylacetone (0.5 mM) markedly reduces *Ucp1* mRNA and protein levels (n = 3). 

**e,** Exogenous haemin (20 μM) does not correct mitochondrial dysfunction in PATKO cells (n = 3). 

**f,** PATKO brown adipocytes show higher levels of Rev-Erbα and BACH1 protein. 

**g,** Dual knockdown of Rev-Erbα and BACH1 in mature PATKO adipocytes restores mitochondrial respiration (n = 5). 

**h,** Pgrmc2, Rev-Erbα (also known as Nr1d1) and Bach1 mRNA in control and knockdown cells. In a–h, n represents biologically independent samples. Representative results from two independent experiments. Data presented as mean ± s.d. **P < 0.01 and ***P < 0.001 versus wild type; ###P < 0.001 versus scrambled; two-way ANOVA with multiple comparisons and a Bonferroni’s post-test.
Extended Data Fig. 6 | Body composition of PATKO mice fed a HFD. Wild-type and PATKO mice were fed HFD for 20 weeks. a, Body weight progression (WT, n = 7; PATKO, n = 9). b, BAT of PATKO mice fed HFD is smaller compared to BAT of HFD-fed wild-type mice. No difference was seen in inguinal WAT (iWAT), epididymal WAT (eWAT) or liver weight (WT, n = 7; PATKO, n = 9). c, PATKO mice fed HFD had higher levels of plasma triglycerides and NEFA (WT, n = 7; PATKO, n = 8). d, H&E staining of liver shows increased steatosis in PATKO mice. Scale bar, 100 μm. Representative images of seven biologically independent samples. e, PATKO mice fed HFD had more lipid accumulation in liver (n = 8). In a–e, n represents biologically independent samples. Data presented as mean ± s.e.m.; *P < 0.05 versus wild type; two-tailed Student’s t-test.
Extended Data Fig. 7 | Analysis of adipose depots of PATKO mice fed a HFD. Wild-type and PATKO mice were fed a HFD for 20 weeks. a, H&E stain images of BAT from wild-type and PATKO mice on a HFD show similar morphology. Insets are magnified on the right. Scale bar, 100 μm. Representative images of seven biologically independent samples. b, Gene expression analysis in BAT shows reduced levels of *Fth1* and *Bmal1*, targets of BACH1 and Rev-Erbα respectively, in PATKO BAT (WT, *n* = 7; PATKO, *n* = 8). c, H&E staining of iWAT and eWAT from wild-type and PATKO mice fed HFD do not show clear differences. Scale bar, 100 μm. Representative images of seven biologically independent samples. d, Size analysis of iWAT and eWAT adipocytes from HFD-fed wild-type and PATKO mice. The x axis indicates area in μm² (*n* = 5 images of biologically independent samples). e, Gene expression analysis in iWAT reveals a modest increase in expression of genes involved in lipid handling. Similar to BAT, *Bmal1* expression is significantly reduced in iWAT of PATKO mice (WT, *n* = 7; PATKO, *n* = 9). In a–e, *n* represents biologically independent samples. Data presented as mean ± s.e.m.; *P* < 0.05, **P < 0.01 and ***P < 0.001 versus wild type; two-way ANOVA with multiple comparisons and a Bonferroni's post-test.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Effect of pharmacological activation of PGRMC2 in DIO mice. DIO mice were treated with CPAG-1 for 30 days. **a**, Body weight (left) and food intake (right) progression (n = 8). **b**, Expression of Pgc-1α and Bmal1 is increased in BAT of treated DIO mice (n = 8). **c**, H&E staining of iWAT shows no difference between vehicle- and CPAG-1-treated DIO mice. Scale bar, 100 μm. **d**, Gene expression analysis reveals increased expression of Pgc-1α and Ucp1 in iWAT of CPAG-1-treated DIO mice (n = 8). **e**, H&E staining shows reduced fibrosis and immune cell infiltration in eWAT of DIO mice treated with CPAG-1. Scale bar, 100 μm. **f**, Gene expression analysis shows decreased expression of markers of inflammation in eWAT of treated mice (n = 8). **g**, H&E staining of liver shows that CPAG-1 treatment modestly reduces lipid deposition. Scale bar, 100 μm. **h**, Hepatic gene expression analysis shows decreased levels of gluconeogenic genes and inflammation markers in liver of treated mice (n = 8). **i**, Treatment with CPAG-1 for four days significantly increases nuclear labile haem levels in the liver of DIO mice (n = 4). In **a**–**i**, n represents biologically independent samples. Representative images of eight biologically independent samples per group (**d, e, g**). Data presented as mean ± s.e.m.; *P < 0.05, **P < 0.01 and ***P < 0.001 versus vehicle; two-way ANOVA with multiple comparisons and a Bonferroni’s post-test.
Extended Data Fig. 9 | Evaluation of interaction of CPAG-1 with PGRMC1 and PGRMC2 in live cells. a, HEK293T cells transfected with expression vectors for either PGRMC1 or PGRMC2 were treated with 10 μM probe 25 (the photoreactive form of CPAG-1) and DMSO, 100 μM haemin or 100 μM CPAG-1 for 30 min followed by UV photocross-linking, lysis and conjugation of labelled proteomes to a tetramethylrhodamine (TAMRA)-azide tag. Labelled proteomes were separated by SDS–PAGE and visualized by in-gel fluorescence scanning. The intensity of the signals indicates the affinity of probe 25 for the overexpressed proteins. The black asterisk marks PGRMC1 protein and the red asterisk marks PGRMC2 protein. Although detectable, PGRMC1 shows very poor labelling with probe 25 relative to PGRMC2. Both interactions can be competed by haemin or CPAG-1. Western blot analysis confirms expression of PGRMC1 and PGRMC2 in transfected cells. Representative results from two independent experiments.
Extended Data Fig. 10 | PGRMC2 is an intracellular haem chaperone critical for adipocyte function. Model of the proposed role for PGRMC2 in haem dynamics in brown adipocytes. PGRMC2 acquires haem from PGRMC1, which forms a complex with FECH, the last enzyme in haem synthesis. PGRMC2, located in the endoplasmic reticulum and the nuclear envelope, facilitates delivery of labile haem to the nucleus. Nuclear labile haem alters expression of genes regulated by haem-responsive transcriptional repressors such as Rev-Erbα and BACH1, which influence mitochondrial bioenergetics. FVLCR1b, a mitochondrial haem exporter identified in erythrocytes, and HRG-1, a plasma membrane haem importer characterized in macrophages, are also shown. FVLCR1b and HRG-1 are both expressed in brown adipocytes, but their role in haem dynamics in this cell type remains to be defined.
# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| -   |           |

- The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. \( F \), \( t \), \( r \)) with confidence intervals, effect sizes, degrees of freedom and \( P \) value noted
  - Give \( P \) values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's \( d \), Pearson's \( r \)), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

## Software and code

Policy information about availability of computer code

| Data collection | Datasets in this study were collected using the following software: |
|-----------------|------------------------------------------------------------------|
|                 | - SDS v2.4.1                                                     |
|                 | - Wave v2.4.1.1                                                  |
|                 | - SoftMax Pro v5.4.1                                             |
|                 | - STAR v2.3.0.c                                                  |
|                 | - NIS-Elements AR v3.22.15                                        |
|                 | - ImageJ v1.48                                                   |
|                 | - VitalView v5.1                                                 |
|                 | - DATAQUEST A.R.T v4.0                                            |
|                 | - Radius v1.3                                                    |
|                 | - MassLynx v4.1                                                  |
|                 | - UCSC LiftOver [https://genome.ucsc.edu/cgi-bin/hgLiftOver?hgsid=754627323_iu7bnkhsbYlo3wLsyVlbM1844A](https://genome.ucsc.edu/cgi-bin/hgLiftOver?hgsid=754627323_iu7bnkhsbYlo3wLsyVlbM1844A) |
Data analysis

RNAseq reads were mapped to the mouse genome mm9 NCBI37 using STAR 2.3.0.c (default parameters). Gene expression values were calculated using HOMER 4.9.1. DEGs were calculated with four replicates per group using EdgeR v3.5. DEGs were analyzed with Ingenuity Pathway Analysis (version 01-07, QIAGEN) Metascape (http://metascape.org), and HOMER (version 4.9.1, http://homer.ucsd.edu/homer/motif/). Additional software used in data analysis: GraphPad Prism v6.0h, RStudio v1.1.383, Image Lab v5.2.1, Microsoft Excel v16.28.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source Data for Figs. 1-4 and Extended Data Figs. 1-8 are provided. Full scans for all western blots are provided in Supplementary Information. RNAseq data have been deposited in GEO under accession number GSE124621. All other data present in this study are available from the corresponding author upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Sample size Sample sizes were determined on the basis of previous experiments and account for biological/technical variability.
Data exclusions No data were excluded from the analyses.
Replication All data reported in this study were reproduced as biological replicates as stated in figure legends. All in vitro experiments were replicated at least twice. All in vivo experiments were replicated at least two independent cohorts.
Randomization MS samples were processed in random order.
Blinding For MS analyses, experimenters were blinded to experimental conditions. Blinding was not possible in mouse studies due to the need to genotype or treat mice accordingly.

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Reporting for specific materials, systems and methods

Materials & experimental systems

Methods

n/a Involved in the study

n/a Involved in the study

☑️ ChIP-seq
☑️ Flow cytometry
☑️ MRI-based neuroimaging
Unique biological materials

Policy information about availability of materials

Obtaining unique materials  All unique materials are available from the corresponding author upon reasonable request.

Antibodies

Antibodies used

The antibodies and dilutions used in this work were:
- PGRMC2 (1:1,000, Bethyl Laboratories, A302-954A and A302-955A)
- PGRMC1 (1:1,000, Bethyl Laboratories, A304-561A)
- PPARg (1:200, Santa Cruz Biotechnology, sc-7273)
- REV-ERBa (1:200, Santa Cruz Biotechnology, sc-100910)
- BACH1 (1:500, R&D Systems, AF5777)
- UCP1 (1:5,000, Thermo Fisher Scientific, PA124894)
- OxPhoS (1:300, Thermo Fisher Scientific, 458099)
- GAPDH (1:5,000, GeneTex, GTX627408)
- TUBULIN (1:5,000, GeneTex, GTX27291)
- HSP90 (1:5,000, GeneTex, GTX101423)
- CEBPd (1:1,000, Abgent, AP20492c)
- Rabbit IgG (Abcam, 37415)
- Anti-rabbit IgG HRP-conjugated (1:10,000, Jackson immunoresearch, 211-035-109)
- Anti-mouse IgG HRP-conjugated (1:20,000, Jackson immunoresearch, 315-035-045)
- Anti-goat IgG HRP-conjugated (1:10,000, Jackson immunoresearch, 705-035-003)

Validation

Validation data for the antibodies used can be found as follows:
- PGRMC2 https://www.bethyl.com/product/A302-954A/PGRMC2+Antibody
- PGRMC1 https://www.bethyl.com/product/A304-561A/PGRMC1+Antibody
- PPARg https://www.scbt.com/scbt/product/ppargamma-antibody-e-8
- REV-ERBa https://www.scbt.com/scbt/product/rev-erbalpha-antibody-14?requestFrom=search
- BACH1 https://www.rdsystems.com/products/mouse-bach1-antibody_a5777
- UCP1 https://www.thermofisher.com/antibody/product/UCP1-Antibody-Polyclonal/PA1-24894
- OxPhoS https://www.thermofisher.com/antibody/product/OxPhos-Rodent-Clone-Cocktail/45-8099
- GAPDH https://www.genetex.com/Product/Detail/GAPDH-antibody-GT239/GTX627408
- TUBULIN https://www.genetex.com/Product?category=0&keyword=GTX27291&page=1
- HSP90 https://www.genetex.com/Product?category=0&keyword=GTX101423&page=1
- CEBPd https://www.abgent.com/products/AW5199-R-Cebpd-Antibody-Center
- Rabbit IgG https://www.abcam.com/rabbit-igg-polyclonal-isotype-control-ab37415.html
- Anti-rabbit IgG HRP-conjugated https://www.jacksonimmuno.com/catalog/products/211-035-109
- Anti-mouse IgG HRP-conjugated https://www.jacksonimmuno.com/catalog/products/315-035-045
- Anti-goat IgG HRP-conjugated https://www.jacksonimmuno.com/catalog/products/705-035-003

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  HEK293T cells were obtained from ATCC (CRL-3216).

Authentication  Short-tandem repeat (STR) profiling.

Mycoplasma contamination  Cells were routinely tested for mycoplasma (at least once every two months) and were always negative.

Commonly misidentified lines

(See ICLAC register)  No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals  Mice with floxed Pgrmc2 alleles and backcrossed to the C57BL/6j background (NNT mutant) were crossed with an Adipoq-CRE strain (JAX stock 010803) to generate mice with adipose-specific deletion of Pgrmc2. Floxed littermates without the CRE transgene were used as controls and are referred to as WT. Similarly, mice with dual-deletion of Pgrmc1 and Pgrmc2 in adipose tissue were generated by crossing mice with floxed Pgrmc1 and Pgrmc2 alleles to the Adipoq-CRE strain. Mice were born and weaned at room temperature and moved to 30°C two weeks after weaning. Studies were performed in male and female mice. Primary brown preadipocytes were isolated from male and female pups (0-2 days old). C57BL/6 DIO mice were purchased from Taconic at 18 weeks of age.

Wild animals  This study did not involve wild animals.
Field-collected samples

This study did not involve samples collected from the field.