Brown adipose tissue (BAT) dissipates chemical energy in the form of heat as a defence against hypothermia and obesity. Current evidence indicates that brown adipocytes arise from Mysf	extsuperscript{+} dermotomal precursors through the action of PR domain containing protein 16 (PRDM16) transcriptional complex	extsuperscript{1,2}. However, the enzymatic component of the molecular switch that determines lineage specification of brown adipocytes remains unknown. Here we show that euchromatic histone-lysine N-methyltransferase 1 (EHMT1) is an essential BAT-enriched lysine methyltransferase in the PRDM16 transcriptional complex and controls brown adipocyte fate. Loss of EHMT1 in brown adipocytes causes a severe loss of brown fat characteristics and induces muscle differentiation in vivo through demethylation of histone 3 lysine 9 (H3K9me2 and 3) of the muscle-selective gene promoters. Conversely, EHMT1 expression positively regulates the BAT-selective thermogenic program by stabilizing the PRDM16 protein. Notably, adipose-specific deletion of EHMT1 leads to a marked reduction of BAT-mediated adaptive thermogenesis, obesity and systemic insulin resistance. These data indicate that EHMT1 is an essential enzymatic switch that controls brown adipocyte fate and energy homeostasis.

Obesity develops when energy intake chronically exceeds total energy expenditure. All anti-obesity medications currently approved by the FDA act to repress energy intake, either by suppressing appetite or by inhibiting intestinal fat absorption. However, because of their side effects including depression, oily bowel movements and steatorrhoea, there is an urgent need for alternative approaches. BAT is specialized to dissipate energy through uncoupling protein 1 (UCP1). Recent studies using high-resolution liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), we found EHMT1 as the only methyltransferase that was co-purified preferentially with the differentiation-competent PRDM16 complexes	extsuperscript{2}. EHMT1 has enzymatic activity on H3K9 mono- or di-Me	extsuperscript{12}. Notably, haploinsufficiency of the EHMT1

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**Figure 1** Identification of EHMT1 in the PRDM16 transcriptional complex. **a,** Top: schematic illustration of PRDM16. Bottom: PRDM16 complex purified from brown adipocytes were subjected to in vitro histone methylation assay. **b,** Immunoprecipitation of EHMT1 protein followed by western blotting to detect PRDM16. Input is shown in lower panels. **c,** In vitro binding assay of [3H]-methyl EHMT1 or CtBP1 and purified PRDM16. **d,** Histone methylation assay of PRDM16 complex from brown adipocytes expressing wild type (WT) and ΔPR, but not ΔZF-1, had significant methyltransferase activities on H3 (Fig. 1a, bottom panel). Because this effect was independent of its SET domain, we searched for methyltransferases that were associated with differentiation-competent PRDM16 proteins (that is, WT and ΔPR), but not with differentiation-incompetent PRDM16 (ΔZF-1). By using high-resolution liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), we found EHMT1 as the only methyltransferase that was co-purified preferentially with the differentiation-competent PRDM16 complexes	extsuperscript{2}. EHMT1 has enzymatic activity on H3K9 mono- or di-Me	extsuperscript{2}. Notably, haploinsufficiency of the EHMT1

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gene, because of 9q34.3 microdeletions or point mutations in humans, is associated with clinical phenotypes including mental retardation. Importantly, 40–50% of patients with EHMT1 mutations develop obesity14,15; however, the underlying mechanism remains completely unknown. Given the essential role of the PRDM16 complex for BAT development, we considered that EHMT1 is a key enzymatic component that controls the lineage specification and thermogenic function of BAT.

To test this hypothesis, we first confirmed the PRDM16–EHMT1 interaction by immunoprecipitation followed by western blotting in brown adipocytes (Fig. 1b and Supplementary Fig. 1). The purified ZF-1 (224–454) and ZF-2 (881–1038) domains of glutathione S-transferase (GST)–PRDM16 protein bound to the in-vitro-translated EHMT1 protein, whereas the 680–1038 region of PRDM16 bound to CtBP1 as previously reported (Fig. 1c and Supplementary Fig. 2). These results indicate that EHMT1 directly interacts with PRDM16. EHMT1 is the main methyltransferase of the PRDM16 complex in brown adipocytes, because the histone methyltransferase activity of the PRDM16 complex was largely lost when EHMT1 was depleted using two short hairpin RNAs (shRNAs) targeted to EHMT1 (Fig. 1d and Supplementary Fig. 3). Furthermore, expression of EHMT1 protein was highly enriched in BAT and in cultured brown adipocytes, correlating well with PRDM16 (Fig. 1e and Supplementary Fig. 4). In contrast, amounts of EHMT2 protein were higher in white adipose tissue (WAT) than in BAT. To test if EHMT1 modulates the PRDM16 transcriptional activity, we performed luciferase assays using a luciferase reporter gene containing PPAR-γ binding sites. As shown in Fig. 1f, co-expression of EHMT1 and PRDM16 synergistically increased the reporter gene activity, whereas this induction was completely lost when the ZF-1 mutant was expressed. These data indicate that EHMT1 forms a transcriptional complex with PRDM16 and regulates its activity through direct interaction.

Next, we investigated the genetic requirement for EHMT1 in BAT development in vivo. Because a whole-body knockout of the Ehmt1 gene causes embryonic lethality before the emergence of brown adipocytes2, the Ehmt1 gene was deleted in brown adipocyte precursors by crossing Ehmt1flox/flox mice17 with Myf5-Cre mice. As shown in Fig. 2a–c, the interscapular BAT of Ehmt1flox/flox mice was substantially smaller than in WT mice at postnatal stage (P1). Haematoxylin and eosin staining showed that brown adipocytes in Ehmt1flox/flox knockout mice were significantly smaller and contained fewer lipids than in WT mice, whereas other tissues near the BAT including skin seemed normal (Fig. 2b and Supplementary Fig. 5). Similar results were observed in embryos at embryonic day (E)18.5 (Supplementary Fig. 6). Subsequently, we analysed the global gene expression of BAT from the WT and Ehmt1flox/flox knockout embryos by RNA-sequencing. The following gene ontology analysis found that the gene expression pattern in the Ehmt1flox/flox knockout BAT showed a skeletal-muscle phenotype: that is, a broad activation of the skeletal muscle-selective pattern in the

**Figure 2** | EHMT1 is required for BAT versus muscle lineage specification. a. Morphology of BAT from WT and Ehmt1flox/flox knockout embryos at P1. Scale bar, 2.5 mm. KO, knockout. b. Haematoxylin and eosin staining of WT and Ehmt1flox/flox knockout (KO) BAT. Scale bar, 600 μm. Bottom: high-magnification images. Scale bar, 30 μm. c. BAT weight from WT (n = 14) and knockout embryos (n = 8). d. Gene ontology analyses of RNA-sequencing data. The log2-fold changes in the expression of skeletal muscle (group M) and BAT (group B) genes are shown. e. Immunocytochemistry for MHC in C2C12 cells expressing indicated constructs under pro-myogenic culture conditions. Scale bar, 200 μm. f. Myogenin mRNA expression in e (n = 3). g. Chromatin immunoprecipitation assays using indicated antibodies (n = 3). Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
genes, and a broad reduction of the BAT-selective genes. Strikingly, 78.7% of the differentially expressed genes (118 out of 150 genes) between WT and knockout mice were stratified into categories of skeletal muscle development, BAT development and BAT function (glucose/fatty-acid metabolism). Specifically, 77.5% of the ectopically activated genes in the knockout BAT were related to skeletal muscle development, including myogenin and myosin heavy chains. On the other hand, 80.0% of the reduced genes in the knockout BAT were involved in BAT development and fatty-acid/glucose metabolism, including Ucp1, Pgc1a, Cebpβ, Cpt1a and Elov3 (Fig. 2d and Supplementary Fig. 7). These results indicate that EHMT1 is absolutely required for the cell-fate specification between BAT versus muscle.

To investigate the mechanisms by which EHMT1 determines BAT lineage, retroviruses expressing a scrambled control RNA (scr) or shRNAs targeting EHMT1 (shEHMT1-1 and -2) were transduced into C2C12 myoblasts together with PRDM16 (Supplementary Fig. 8a). As shown in Fig. 2e (upper panels), PRDM16 expression powerfully blocked myogenic differentiation in a dose-dependent fashion, as shown by immunohistochemistry using a pan-skeletal myosin heavy chain (MHC) antibody. In contrast, EHMT1 depletion significantly impaired the PRDM16-mediated repression on myogenesis (Fig. 2e, lower panels). Gene expression analysis showed that the repression on muscle-selective genes such as myogenin was near completely abolished when EHMT1 was depleted (Fig. 2f and Supplementary Fig. 8b). This repressive effect was mediated through the methyltransferase activity of EHMT1, because ectopic expression of the EHMT1 mutant (N1198L/H1199E) that lacks methyltransferase activity18 significantly blunted the PRDM16-mediated repression on myogenesis (Supplementary Fig. 9a). Additionally, two chemical inhibitors of EHMT1/2, BIX-01294 and UNC0638, blocked the repressive effects of PRDM16 (Supplementary Fig. 9b, c). BIX-01294 treatment in brown adipocytes also significantly reduced the expression of BAT-selective genes (Supplementary Fig. 9d). Consistent with these data, chromatin immunoprecipitation assays found that EHMT1 depletion robustly reduced amounts of H3K9me2 and me3 at the proximal region of the myogenin gene promoter on which EHMT1 depletion robustly reduced amounts of H3K9me2 and me3 (Fig. 3b, and Supplementary Fig. 10). Conversely, under pro-adipogenic culture conditions, knockdown of EHMT1 largely blocked the PRDM16-induced brown adipogenesis in C2C12 cells (Supplementary Fig. 11). Together, these results indicate that EHMT1 determines BAT versus muscle cell lineage through PRDM16 by controlling H3K9 methylation status of the muscle-selective gene promoters.

To investigate the role of EHMT1 in BAT thermogenesis, EHMT1 was depleted in immortalized brown adipocytes by retrovirus-mediated shRNA knockdown (Fig. 3a) and Supplementary Fig. 12a, b). Total and uncoupled (oligomycin-insensitive) oxygen consumption rate in the EHMT1-depleted brown adipocytes was significantly reduced both at the basal and cyclic AMP (cAMP)-stimulated states (Fig. 3a). Conversely, EHMT1 overexpression significantly increased messenger RNA (mRNA) amounts of BAT-selective thermogenic genes, including Ucp1, Pgc1a and Dio2 (Fig. 3b), and oxygen consumption rate (Supplementary Fig. 12c). To test further if this EHMT1 action requires PRDM16, EHMT1 was ectopically introduced into mouse embryonic fibroblasts that did not express endogenous PRDM16. As shown in Fig. 3c, mouse embryonic fibroblasts expressing PRDM16 and C/EBP-β uniformly differentiated into lipid-containing adipocytes as previously reported19. Although EHMT1 alone did not stimulate brown adipogenesis, the combination of EHMT1 with PRDM16 and C/EBP-β synergistically increased mRNA amounts of the BAT-selective genes, including Ucp1, Cidea, Cox7a and Cox8b (Fig. 3d). These data indicate that EHMT1 positively regulates the BAT-selective thermogenic gene program through PRDM16.

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expression of EHMT1 robustly increased the amount of PRDM16 protein, independently of its mRNA expression (Fig. 3f and Supplementary Fig. 16). This effect was due to changes in the rate of protein degradation, because cycloheximide chase experiments showed that expression of EHMT1 extended the half-life of PRDM16 protein from 8.5 to 16.5 h. The N1198L;H1199E mutant also extended the half-life of PRDM16 protein as potently as the WT form (Fig. 3g). PRDM16 protein stability was not affected by PRDM16 (Supplementary Fig. 17).

These results collectively suggest that EHMT1 has dual functions: that is, repressive effects on the muscle-selective gene program through its methyltransferase activity, and activation of the BAT-selective gene program through stabilization of PRDM16 protein through direct association.

Next, we examined the requirement for EHMT1 in adaptive thermogenesis in vivo. To exclude potential defects in the skeletal muscle of Ehmt1adipo knockout mice, we generated adipose tissue-specific Adiponectin-Cre mice. To exclude potential defects in the skeletal muscle of Ehmt1adipo knockout mice, we generated adipose tissue-specific Adiponectin-Cre mice. Hence, the Ehmt1adipo knockout mice allow us to examine the role of EHMT1 in BAT/beige fat-mediated thermogenesis in vivo. As shown in Fig. 3h, rectal temperature of Ehmt1adipo knockout mice strikingly dropped within 1 h after a cold challenge to 4 °C, whereas that of control mice remained constant. Expression of BAT-selective genes in skeletal muscle was not altered in Ehmt1adipo knockout mice (Supplementary Fig. 19). We subsequently measured oxygen consumption rate at thermoneutrality (29–30 °C) in response to an activation of the β3-adrenergic pathway. As shown in Fig. 4a, the oxygen consumption rate of WT mice was significantly increased after administering CL316,243 whereas this induction was completely lost in knockout mice. The impaired thermogenesis in knockout mice was accompanied by higher serum amounts of free fatty acids (FFAs) (Fig. 4b). This is consistent with previous findings that BAT serves as a major sink of FFAs for heat generation, and that reduced fatty-acid oxidation in BAT leads to an increase in amounts of serum FFA. Indeed, fatty-acid oxidation capacity in the knockout BAT was significantly lower than in WT mice at the basal rate of WT and Ehmt1adipo knockout mice treated with CL316,243 (0.5 mg kg⁻¹) at thermoneutrality (n = 6). Amounts of serum FFA in mice treated with saline or CL316,243. c. Fatty-acid oxidation in BAT (n = 6–10). d. Body mass change under a high-fat diet at thermoneutrality (n = 16). e. Adipose tissue mass after 4-week high-fat diet (n = 16). f. Haematoxylin and eosin staining of adipose tissues. Scale bar, 100 μm. g. Glucose tolerance test in 9-week high-fat diet-fed mice (n = 9). h. Insulin tolerance test in 10-week high-fat diet-fed mice (n = 9). i. Amounts of serum insulin at the fasted and glucose-stimulated states (n = 9). j, Haematoxylin and eosin staining of liver in d. Scale bar, 50 μm. k. Amounts of liver triglyceride in j (n = 9). l. Hepatic insulin signalling as assessed by phosphorylated (S473) and total Akt amounts. Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
mice was observed only at thermoneutrality. As shown in Fig. 4d and Supplementary Fig. 21, EHMT1<sup>−/−</sup> knockout mice gained significantly more body mass than WT mice without any change in food intake (Supplementary Table 1). Knockout mice had higher amounts of epididymal VAT and interscapular BAT that contained substantially larger lipid droplets than WT mice (Fig. 4e, f). A glucose tolerance test found that knockout mice showed significantly higher blood glucose concentrations than WT mice (Fig. 4g). Similarly, knockout mice showed impaired responses to insulin during an insulin tolerance test (Fig. 4h) and higher amounts of serum insulin at the fastest and glucose-stimulated states (Fig. 4i). Knockout mice showed an insulin-resistance phenotype even at ambient temperature, whereas no statistically significant difference was observed in body mass (Supplementary Fig. 22). Notably, the liver from knockout mice contained higher amounts of lipids and triglyceride (Fig. 4j, k) and showed impaired insulin signalling as assessed by phosphorylation of Akt in response to insulin (Fig. 4l and Supplementary Fig. 23). Together, these results indicate that EHMT1 deficiency in BAT leads to obesity, systemic insulin resistance and hepatic steatosis under a high-fat diet.

In conclusion, we have identified EHMT1 as an essential BAT-enriched methyltransferase that controls brown adipose cell fate, adaptive thermogenesis and glucose homeostasis in vivo. Although presence of BAT in adult humans is now widely appreciated, no mutation that causes defects in human BAT development and thermogenesis had been described except polymorphisms in UCP1 and β3-adrenergic receptor genes. Delineating the causal link between EHMT1 mutations and BAT thermogenesis will provide a new perspective in understanding the molecular control of energy homeostasis through the epigenetic pathways, which may lead to effective therapeutic interventions for obesity and metabolic diseases.

**METHODS SUMMARY**

**Animals.** All animal experiments were performed according to procedures approved by University of California, San Francisco’s Institutional Animal Care and Use Committee. EHMT1<sup>−/−</sup> and Adiponectin-Cre mice were provided by A. Tarakhovsky and E. D. Rosen. For metabolic studies, male mice in Bl6 background were fed with a high-fat diet for 4 weeks at thermoneutrality and ambient temperature.

**Bioinformatics.** RNA-sequencing libraries were constructed at the University of California, San Francisco Genomic Core Facility. Gene ontology enrichment analyses were performed on the differentially expressed genes (P < 0.05, the delta-method-based hypothesis test) using RefSeq as the background data set. The accession number for the data is E-MTAB-1704.

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Fatty-acid oxidation assay. To analyse embryonic BAT development, lysate. After centrifugation at 735. Subsequently, equal amounts (0.4 ml) of chloroform and water were added to the strips (Abbott). To measure liver triglyceride contents, the liver tissue (25 mg) was minced to small pieces and incubated with DMEM supplemented with 10% FBS for 15 min. 

In vivo insulin stimulation assay. Mice were anaesthetized with Tribromoethanol (Avertin). Insulin (5 U) was injected into the inferior venae cavae. Livers were removed 2 min after the injection and lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 1 mM xanthine, 125 mM NaCl, 12 mM HEPES, containing 0.5% Tween 20, 1 mM EDTA, 10% FBS). Once the liver was minced, samples were homogenized in 1.25 ml of lysis buffer (chloroform/methanol, 2:1, v/v). Subsequently, equal amounts (0.4 ml) of chloroform and water were added to the lysate. After centrifugation at 735 g for 3 min, the chloroform phase was collected and dried. The pellet was dissolved in isopropanol. Amounts of triglycerides were determined by an Infinity Triglycerides kit (Thermo).

Fat lipolysis assay. WT and Ehnmt1<sup>fl/fl</sup> knockout mice at 11 weeks old were intraperitoneally injected with saline or CL316,243 at a dose of 0.5 mg kg<sup>−1</sup>. After the injection, the interscapular BAT depots were isolated. Fatty-acid oxidation assay was performed according to the protocol described by Mao et al.2 Briefly, the adipose tissues were minced to small pieces and incubated with DMEM supplemented with 1 mM pyruvate, 1% FFA-free BSA and 0.5 mM oleate. [14C]oleic acid at 1 μCi ml<sup>−1</sup> was added for 2 h at 37°C. After adding 70% perchloric acid into each well, CO2 was captured by Whatman paper soaked in 3 M NaOH solution for 1 h. 14C radioactivity was measured by liquid scintillation counter and normalized to the total protein content.

RNA-sequencing and gene ontology analysis. Total RNA was isolated from tissues using Trizol (Invitrogen) or Ribozol reagents (AMRESCO) following the manufacturers’ protocols. Quality of RNA from all the samples was checked by spectrophotometer. Reverse transcription reactions were performed using an iScript complementary DNA (cDNA) synthesis kit (Bio-Rad). The sequences of primers used in this study can be found in Supplementary Table 2. Quantitative reverse transcriptase PCR (qRT–PCR) was performed with SYBR green fluorescent dye using an ABI ViiA7 PCR machine. Relative mRNA expression was determined by the ΔΔCT method using TATA-binding protein as an endogenous control to normalize samples.

Protein interaction analyses. Immortalized brown fat cells stably expressing Flag-tagged WT, PR-domain deletion mutant and ZF-1 deletion mutant of PRDM16 or by 10 μg total RNA from the Ehnmt1<sup>fl/fl</sup> knockout and Ehnmt1<sup>myf5</sup> knockout BAT using an Ovation RNA-sequencing system version 2 (NuGEN). mRNA was reverse transcribed to cDNAs using a combination of random hexameric and a poly-T chimaeric primer. The cDNA libraries were subsequently amplified by single primer isoamplification<sup>36</sup> using an UltraLo DR library kit (NuGEN) according to the manufacturer’s instructions. The qualities of the libraries were determined by Bioanalyzer (Agilent Technologies). Subsequently, high-throughput sequencing was performed using a HiSeq 2500 instrument (Illumina) at the University of California, San Francisco Genomics Core Facility. RNA-sequencing reads for each library were mapped independently using TopHat version 2.0.8 against the University of California, Santa Cruz (UCSC) mouse genome build mm9 indexes, downloaded from the TopHat website (http://tophat.cbcb.umd.edu/igenomes.shtml). The mapped reads were converted to fragments per kilobase of exon per million fragments mapped (FPKM) using cufflinks<sup>2</sup>. (red: 31.5% were unique alignments to TopHat and the UCSC coding genes to estimate amounts of gene and isoform expression. Based on the list of genes that showed significant difference (P<0.05, the delta-method-based hypothesis test) from the RNA-sequencing data, enrichment of the Gene Ontology biological process terms (GO FAT category) was analysed using the Gene Set Enrichment Analysis (GSEA) program, according to the method described by the previous paper<sup>33</sup>. RNA-sequencing reads have been deposited in ArrayExpress (www.ebi.ac.uk) under accession number E-MTAB-1704.

Immunocytostaining. Differentiated C2C12 myotubes or COS7 cells expressing green fluorescent protein (GFP)–PRDM16 and EHMT1 constructs were fixed with 4% paraformaldehyde for 10 min, permeabilized with PBS containing 0.1% Triton X-100, rinsed with PBS, and then exposed to 0.2% Triton X-100 in PBS for 5 min. The cells were subsequently incubated with anti-MF20 mouse antibody (DSHB, 1:50) for MHC and then with Flag antibody (M2, 1:200) for EHMT1. After washing with PBS, Alexa 594-labelled anti-mouse IgG (1:800) was added as a secondary antibody.

Protein interaction analyses. Immortalized brown fat cells stably expressing Flag-tagged WT, PR-domain deletion mutant and ZF-1 deletion mutant of PRDM16 or an empty vector were grown to confluence<sup>36</sup>. Nuclear extracts were isolated from these cells and incubated with Flag M2 agarose beads, washed in a binding buffer (180 mM KCl) and subsequently eluted either by 3× or by 1× Flag peptides from agarose beads on a conjugated to the beads to assay or to reverse-phase LC–MS/MS for peptide sequencing using a high-resolution hybrid mass spectrometer (LTQ-Orbitrap, Thermo Scientific) with TOP10 method. Data obtained was annotated using the IPI mouse database<sup>37</sup>. Proteins were considered significantly identified with at least two unique valid peptides, and the false
discovery rate was estimated to be 0% using the target-decoy approach. To confirm the interaction between PRDM16 and EHMT1 in brown adipocytes, the immunopurified complex was purified using anti-EHMT1 (R&D Systems) or Flag antibody (M2) and subjected to 4–12% SDS–PAGE. Rabbit polyclonal PRDM16 antibody or EHMT1 antibody (R&D Systems) was used for western blotting. COS7 cells expressing haemagglutinin (HA)-tagged PRDM16 or deletion fragments of Flag-tagged EHMT1 were collected 48 h after transfection. Total cell lysates were incubated overnight at 4 °C with Flag M2 agarose beads, washed and eluted by boiling. The immunoprecipitants were analysed by western blot analysis using HA antibody (Roche). For in vitro binding assays, various fragments of the GST–fusion PRDM16 fragments were purified as previously described.

**Protein stability assay.** COS7 cells expressing haemagglutinin (HA)-tagged PRDM16 or deletion fragments of Flag-tagged EHMT1 were collected 48 h after transfection. Total cell lysates were incubated overnight at 4 °C with Flag M2 agarose beads, washed and eluted by boiling. The immunoprecipitants were analysed by western blot analysis using HA antibody (Roche). For in vitro binding assays, various fragments of the GST–fusion PRDM16 fragments were purified as previously described.

**Chromatin immunoprecipitation assay.** After cross-linking with 1% formaldehyde at room temperature (24 °C) for 10 min, total cell lysates from brown adipocytes were sonicated to shear the chromatin, and immunoprecipitated overnight at 4 °C using antibodies for H3 di-methyl and tri-methyl K9 (Abcam), acetyl-H3K9/K14 (Millipore), pan-H3 (Cell Signaling), EHMT1 (R&D Systems) or IgG (Santa Cruz). After extensive washing, the immunoprecipitants were eluted with 2% SDS in 0.1 M NaH2CO3. Cross-linking was reversed by heating at 65 °C with in vitro translated proteins in a binding buffer containing 20 mM HEPES pH 7.7, 300 mM KCl, 2.5 mM MgCl2, 0.05% NP40, 1 mM DTT and 10% glycerol. The sepharose beads were then washed five times with the binding buffer. Bound proteins were separated by SDS–PAGE and analysed by autoradiography.

**Histone methylation assay.** The PRDM16 transcriptional complex was immunopurified from nuclear extracts of brown adipocytes using Flag M2 agarose or IgG (negative control). The immunoprecipitants were incubated with 2 μg of core histone (Millipore) with [3H]-adenosyl-methionine at 30 °C for 1 h. Subsequently, the reaction was stopped by addition of sample buffer. Core histone was resolved by 4–12% SDS–PAGE and detected by autoradiography or by scintillation counter.

**Cellular respiration assay.** Uncoupled respiration was measured as previously described.

**Statistical analyses.** Statistical analysis used JMP version 9.0 (SAS Institute). Two-way repeated-measures analysis of variance was applied to determine the statistical difference in glucose tolerance test, insulin tolerance test, body mass gain and rectal temperatures between genotypes. Effect size and power analysis were done by the pwr.t.test function of the R statistics package. Other statistical comparisons were assessed by an unpaired Student’s t-test. P < 0.05 was considered significant throughout the study.
Extended Data Figure 1 | EHMT1 regulates endogenous PRDM16 protein expression in vivo. a, The putative BAT was micro-dissected from WT and Ehmt1<sup>myf5</sup> knockout embryos. mRNA expression of Prdm16 was measured by qRT–PCR. Data are presented as mean and s.e.m. (n = 8–10). b, Western blotting to detect endogenous EHMT1, PRDM16, UCP1 and MHC in BAT from WT and Ehmt1<sup>myf5</sup> knockout embryos. α-Tublin protein was shown as a loading control.
Extended Data Figure 2 | Ectopic activation of skeletal-muscle-selective genes and reduction of BAT-selective genes in the BAT from Ehmt1<sup>adipo</sup> knockout mice. a, Western blotting for endogenous EHMT1 in BAT and liver from WT and Ehmt1<sup>adipo</sup> knockout mice. β-Actin protein was shown as a loading control. b, Amounts of mRNA expression of BAT, skeletal muscle, white fat and beige-fat selective genes in BAT from Ehmt1<sup>adipo</sup> knockout mice. Values were normalized to those in WT mice. The amounts of mRNA were visualized by a heat-map using Multi Experiment Viewer. c, Venn diagram showing the overlapped genes between Ehmt1<sup>myf5</sup> knockout and Ehmt1<sup>adipo</sup> knockout mice. RNA-sequencing and gene ontology analyses identified 33 genes that were similarly dysregulated both in the Ehmt1<sup>myf5</sup> knockout BAT and the Ehmt1<sup>adipo</sup> knockout BAT. The mRNA expression values were normalized to WT mice for each knockout model and visualized by a heat-map using Multi Experiment Viewer. The colour scale shows the amounts of mRNA of the genes in a blue (low)–white (no change)–red (high) scheme.
Extended Data Figure 3 | EHMT1 is required for beige/brite cell development. a, The β3-AR agonist CL316,243 at a dose of 0.5 mg kg⁻¹ or saline were administered to WT or Ehmt1adipo knockout mice for 7 days. Inguinal WAT was collected for gene expression analysis. Amounts of mRNA expression of BAT and beige-fat selective genes (as indicated) were measured by qRT–PCR (n = 3–6). †Significant between saline and CL316,243 in WT mice. b, Immunohistochemistry for UCP1 in a. Scale bar, 100 μm. Nuclei were stained with DAPI. c, To test a cell-autonomous requirement for EHMT1 in beige/brite cell development, the stromal vascular (SV) fractions were isolated from the inguinal WAT of Ehmt1flox/flox mice. Cells were infected with adenovirus expressing GFP or Cre. The SV cells were differentiated in the presence or absence of rosiglitazone (Rosi) at 0.5 μM. Amounts of mRNA expression of BAT-selective genes (as indicated) were measured by qRT–PCR. Deletion of Ehmt1 was confirmed by qRT–PCR (right graph) (n = 3); data are presented as mean and s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.