BAF60a deficiency uncouples chromatin accessibility and cold sensitivity from white fat browning

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Brown and beige fat share a remarkably similar transcriptional program that supports fuel oxidation and thermogenesis. The chromatin-remodeling machinery that governs genome accessibility and renders adipocytes poised for thermogenic activation remains elusive. Here we show that BAF60a, a subunit of the SWI/SNF chromatin-remodeling complexes, serves an indispensable role in cold-induced thermogenesis in brown fat. BAF60a maintains chromatin accessibility at PPARγ and EBF2 binding sites for key thermogenic genes. Surprisingly, fat-specific BAF60a inactivation triggers more pronounced cold-induced browning of inguinal white adipose tissue that is linked to induction of MC2R, a receptor for the pituitary hormone ACTH. Elevated MC2R expression sensitizes adipocytes and BAF60a-deficient adipose tissue to thermogenic activation in response to ACTH stimulation. These observations reveal an unexpected dichotomous role of BAF60a-mediated chromatin remodeling in transcriptional control of brown and beige gene programs and illustrate a pituitary-adipose signaling axis in the control of thermogenesis.

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dipose tissue thermogenesis defends against cold stress and contributes to nutrient homeostasis and energy bal-
ance. Recent studies have identified beige (or brite) adi-
ocytes as an important thermogenic cell type in rodents and
humans. Brown and beige fat share a remarkably similar
transcriptional program underling fuel oxidation and engage
several biochemical processes to convert chemical energy into
heat, including uncoupled respiration via uncoupling protein 1
(UCP1) and UCP1-independent thermogenesis via futile sub-
strate cycling. Beyond thermogenesis, brown and beige fat also
release endocrine hormones such as Neuregulin 4, which act on
other tissues to influence metabolic physiology and disease
pathogenesis. While the clinical and therapeutic importance of
adipose tissue thermogenesis remains to be fully established,
brown and beige fat mass and activity have generally been asso-
ciated with desirable metabolic parameters. Importantly, cold-
induced and pharmacological activation of adipose thermogenesis
led to increased glucose utilization and improved insulin sensi-
tivity in humans. In rodents, genetic ablation of brown fat
exacerbates diet-induced weight gain and metabolic disorders,
whereas its activation results in increased energy expenditure,
reduced adiposity, improved insulin sensitivity, and plasma lipid
profiles. These observations illustrate the conserved nature of
the beneficial effects of thermogenic activation on systemic
metabolism and physiology.

A network of transcriptional regulators have been identified to
control brown and beige adipogenesis and thermogenesis, including
transcription factors (e.g., PPARγ), cofactors (PRDM16, PGC-1α, HDAC3),
histone-modifying epige-
etic regulators (EHMT1, JMJD1A, KDM4B, LSD1, HDAC), and
long noncoding RNAs (Blnc1, IncBATE). Many of these factors
form transcriptional complexes through physical interaction and
are recruited to the regulatory regions of the genes underlying
mitochondrial oxidation and thermogenesis in response to ther-
monic stimuli. The mammalian SWI/SNF chromatin-remodeling
complexes exist in three distinct assemblies: canonical BRG1-
associated factor (cBAF), polybromo-associated BAF, and non-
canonical BAF (ncBAF). These heterogeneous molecular complexes regulate diverse biological processes by mod-
ulating nucleosome structure, chromatin accessibility, and gene
transcription. Recent works have shown that brown adipocyte gene
expressions are regulated by EBF2 recruitment of BRG1- and
JMJD1A-associated SWI/SNF complexes. Despite these, the
role of SWI/SNF-mediated chromatin regulation in cold-induced
activation of the brown and beige gene programs remains largely
unknown. In this study, we generated mice harboring fat-specific
inactivation of BAF60a, a molecular link between the SWI/SNF
machinery and chromatin.

As BAF60a is abundantly expressed in brown fat, we postulated
that this factor may provide an important link between SWI/SNF-
mediated chromatin remodeling and thermogenesis. To test this
hypothesis, we inactivated BAF60a specifically in adipose tissue
and explored its role in gating chromatin accessibility during
thermogenic response.

Adipocyte-specific BAF60a knockout (AKO) mice exhibited
normal postnatal growth and appeared indistinguishable from
I/O mice. Following cold exposure, AKO mice exhibited heightened cold sensitivity and failed to maintain
core body temperature, this thermogenic defect was associated
with increased plasma levels of triglycerides, ketone body (β-
hydroxybutyrate), and non-esterified fatty acids (NEFAs) com-
pared to control (Fig. 1a, b). Histological staining revealed that,
while interscapular BAT appeared similar in two groups when
mice were kept at ambient temperature, cold-induced mobiliz-
alization of lipid storage failed to materialize, as indicated by the
presence of larger lipid droplets in BAF60a-deficient brown
adipocytes (Fig. 1c).

Lipolytic function of epididymal white adipose tissue (eWAT) appeared to be enhanced by BAF60a
inactivation under basal and stimulated conditions (Supplementary
Fig. 1a). Quantitative PCR (qPCR) analysis indicated that
mRNA expression of genes involved in uncoupling and
thermogenesis (Ucp1, Dio2), fatty acid β-oxidation (Cpt1b, Acaca2,
Mead, Acadl, Elov3), and mitochondrial oxidative metabolism (Cox7a1, Cox8b) was significantly lower in AKO mouse brown
fat, whereas BAF60b and BAF60c mRNA levels remained largely
unaltered by BAF60a inactivation in BAT, inguinal WAT (iWAT), and eWAT from AKO mice (Fig. 1d and Supplementary
Fig. 1b). Accordingly, BAF60a inactivation markedly decreased
UCP1 protein levels and disrupted normal mitochondrial morphology in brown adipocytes lacking BAF60a, as revealed
by the appearance of dilated mitochondrial cristae (Fig. 1e, f).

We surveyed mRNA expression of several known regulators of
adipose tissue thermogenesis. While Ebf2, Zbtb7b, and Prdm16
mRNA levels remained comparable in two groups, mRNA
expression of PGC-1α, Blnc1, and PPARy2 was significantly
lower in AKO brown fat (Fig. 1d).

Cold exposure stimulates catecholamine release by the
sympathetic nerve fibers in adipose tissue, leading to adrenergic
receptor activation and thermogenesis. To directly assess whether
thermogenic response to adrenergic stimulation is impaired in
AKO mice, we performed thermal imaging on control and AKO
mice following a single intraperitoneal (i.p.) injection of CL-
316,243, a β3-selective adrenergic agonist and potent inducer of
brown fat thermogenesis. As expected, CL-316,243 treatment
increased surface body temperature in control flox mice (Fig. 1g,
h). In contrast, AKO mice failed to mount a robust thermogenic
response following CL-316,243 treatment. In fact, surface body
temperature remained similar to saline-treated mice. These
results illustrate an indispensable role for BAF60a-mediated
chromatin remodeling during cold-induced thermogenesis.

Results

The BAF60a transcriptional program is indispensable for cold-
induced thermogenesis. The mammalian SWI/SNF complexes
contain several core subunits including BAF60 that undergo

orderly assembly into functional molecular machines. Previous
studies have demonstrated that the BAF60 subunit plays a
uniquely important role in recruiting the SWI/SNF chromatin-
remodeling complexes to target chromatin, leading to cell type-
specific and context-dependent transcriptional response. BAF60a mediates induction of hepatic genes involved in fatty acid
β-oxidation and bile acid synthesis, whereas BAF60c serves as
a core component of myocyte glucose sensing and orchestrates
myogen gene expression and glycolytic muscle metabolism.

BAF60a governs chromatin accessibility in brown fat. BAF60a
mediates the recruitment of the SWI/SNF complexes to selective
genomic loci, thereby altering local chromatin structure and gene
transcription. We next performed assay for transposase-
accessible chromatin using sequencing (ATAC-seq) on nuclei
isolated from control and AKO mouse brown fat to delineate
the role of BAF60a in setting chromatin accessibility. ATAC-seq
provides a powerful tool to reveal the landscape of chromatin
accessibility across the entire genome. We identified a total of
47,250 ATAC-seq peaks (representing open chromatin) in brown
fat, of which 265 exhibiting significantly reduced accessibility in
response to BAF60a inactivation (Fig. 2a). Interestingly, 72 peaks

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exhibited increased accessibility in AKO mouse brown fat. We analyzed the effects of BAF60a deficiency on a set of brown fat-enriched genes and observed reduced accessibility at ATAC-seq peaks and mRNA expression for many of these genes, including Pparγ, Ifi27l2a, Cfd, Dgat2, and Pck1 (Fig. 2a and Supplementary Fig. 1c). Seven ATAC-seq peaks were detected across the Ucp1 locus, all of which exhibited reduced chromatin accessibility (Fig. 2b), consistent with significantly lower Ucp1 gene expression in AKO mouse brown fat.

To determine whether BAF60a preferentially affects chromatin accessibility at certain transcription factor binding sites, we performed transcription factor motif-enrichment analysis on...
ATAC-seq peaks that were decreased by BAF60a inactivation. We observed that the binding sites for several nuclear receptors, including PPAR, NUR77, RXR, and ERRγ, were highly enriched in BAF60a down-regulated peaks (Fig. 2c). We integrated our ATAC-seq data with previous chromatin immunoprecipitation sequencing (ChIP-seq) analyses of genome-wide PPARγ and EBF2 occupancy in brown fat21. As shown in Fig. 2d, 16,868 of 23,232 PPARγ and 6610 of 7584 EBF2 ChIP peaks were found within ATAC-seq peaks that we observed in brown fat. Interestingly, a large fraction of BAF60a-dependent ATAC peaks contained PPARγ (247/337) and EBF2 (92/337) binding sites. Co-immunoprecipitation (co-IP) studies indicated that BAF60a physically interacts with PPARγ (Supplementary Fig. 2a). Transcription factor footprint analysis of the ATAC-seq peaks indicated that chromatin regions near PPARγ binding sites exhibited reduced accessibility (Fig. 2e). Accordingly, ChIP analysis revealed diminished PPARγ binding at the Ucp1 locus (Supplementary Fig. 2b). In contrast, occupancy for CCCTC-binding factor (CTCF), a chromatin-binding factor that defines the boundary of chromatin domains, remained largely unaffected by inactivation of BAF60a in brown adipocytes. These results provide a mechanistic link between BAF60a-mediated chromatin remodeling and the global landscape of genome accessibility in brown fat.

BRD9 interacts with BAF60a and is required for thermogenic gene expression. To establish a cell-autonomous role of BAF60a in the control of thermogenic gene program, we immunolabeled brown preadipocytes from flox/flox neonatal pups and ablated BAF60a following transduction of a retroviral vector expressing Cre recombinase. As expected, expression of Ucp1, Cidea, and Fabp4 was markedly induced in control adipocytes during adipogenesis (Fig. 3a, b and Supplementary Fig. 3a). In contrast, the induction of these thermogenic genes was greatly diminished in adipocytes lacking BAF60a, which exhibited reduced lipid accumulation following differentiation (Supplementary Fig. 3b). Furthermore, adrenergic stimulation of thermogenic gene expression was greatly diminished in BAF60a-deficient adipocytes (Fig. 3c). MitoTracker staining indicated that mitochondrial content was lower in adipocytes lacking BAF60a (Fig. 3d). Consistently, basal oxygen consumption and total respiratory capacity of differentiated adipocytes were significantly lower following BAF60a inactivation (Fig. 3e). As such, BAF60a is required for transcriptional activation of thermogenic genes in a cell-autonomous manner.

BAF60a is a core subunit of the molecularly heterogeneous BAF chromatin-remodeling complexes. Recent studies have identified bromodomain-containing protein 9 (BRD9) as a subunit of ncBAF in mouse embryonic stem cells and cancer cells6,15,20,31,32. We performed co-IP assay to explore whether BAF60a is present in BRD9-containing ncBAF in brown adipocytes. As shown in Fig. 3f, BAF60a physically associated with BRD9 in addition to BRG1 and BAF47, suggesting that BAF60a is present in both cBAF and ncBAF complexes. These findings raised the possibility that BRD9-containing ncBAF may play an important role in the regulation of thermogenic gene expression. To explore this, we subjected brown preadipocytes to differentiation protocol without or with dBRD9, a BRD9-specific proteolysis targeting chimera compound33. As expected, dBRD9 markedly diminished endogenous BRD9 protein levels in treated adipocytes (Fig. 3g). While protein levels of FASN (fatty acid synthase) and PPARγ remained largely unaffected by dBRD9, UCP1 mRNA and protein expression was greatly reduced in differentiated brown adipocytes (Fig. 3g, h). We confirmed these results using a specific chemical inhibitor of BRD9 (t-BRD9)34. Both I-BRD9 and dBRD9 diminished the induction of Ucp1 mRNA and protein expression in response to adrenergic stimulation (Fig. 3i, j). These results demonstrate that BRD9 is required for induction of thermogenic gene expression during brown adipocyte differentiation and in response to adrenergic stimulation.

Inactivation of BAF60a enhances beige fat formation. Chronic cold exposure is known to stimulate browning of white fat, particularly the iWAT. Beige adipocytes within iWAT contribute to thermogenesis via UCP1-dependent and UCP1-independent mechanisms4. To determine whether BAF60a is required for beige fat formation, we subjected flox and AKO mice to cold acclimation. Unlike acute cold stress, chronic cold exposure reduced brown fat lipid content in both groups (Fig. 4a). Despite this, mRNA expression of Ucp1, Cpt1b, Elovl3, and Cox8b and UCP1 protein levels were significantly lower in BAF60a-deficient brown fat (Fig. 4b, c). To our surprise, we observed more pronounced cold-induced browning in iWAT from AKO mice, as illustrated by more abundant UCP1-positive beige adipocytes (Fig. 4c, d). Gene expression analysis indicated that BAF60a was efficiently deleted in adipocytes along with markedly reduced expression of PPARγ2 (Supplementary Fig. 4a). Accordingly, mRNA expression of a large number of genes involved in mitochondrial fuel oxidation and thermogenesis was more highly induced in AKO mouse iWAT than control, including Ucp1, Elovl3, Cidea, Cpt1b, and Ppara (Fig. 4e). Dio2 mRNA levels were comparable between two groups. Microarray transcriptional profiling of iWAT revealed a cluster of genes exhibiting more robust induction in AKO mice following cold acclimation, many of which are involved in mitochondrial fuel oxidation and thermogenesis (Fig. 4f). The expression of genes involved in creatine metabolism (Ckm1, Ckm2, Gatm, Giamt, Slc6a8) remained largely unchanged (Supplementary Fig. 4b). While Serca2a was upregulated in AKO mice during chronic cold, the expression of Serca2b and Ryr2, two major players in calcium cycling in response to cold challenge35, were not affected by BAF60a inactivation (Supplementary Fig. 4b).

Recent work has uncovered a unique population of beige adipocytes characterized by elevated glycolytic activity56. Interestingly, mRNA expression of a number of glycolytic genes, including Hk2, Eno1, Fkp, Pkm2, and Ldhα, was significantly higher in iWAT from AKO mice following cold acclimation than...
We next performed ATAC-seq to examine how BAF60a inactivation alters chromatin accessibility in iWAT following cold acclimation. We identified a total of 39,558 peaks, 29,925 of which (75.7%) overlapped with peaks detected in BAT (Fig. 5a), indicating that brown and beige fat share remarkably similar features in their chromatin accessibility landscape. Motif-enrichment analysis revealed that the top 12 BAT-enriched motifs were also enriched among peaks detected in iWAT, including binding sites for C/EBP, CTCF, HLF, BORIS, EBF, PPAR, NF1, and SP1 (Fig. 5b). While ATAC-seq peaks for Ifi27l2a and Car3 were diminished by BAF60a inactivation in both BAT and iWAT, the peaks near Ucp1 and Cidea genes exhibited opposite regulation by BAF60a inactivation (Fig. 5c). Motif analysis indicated that the upregulated peaks in iWAT were enriched for EBF, C/EBP, and PPAR binding sites (Fig. 5d). Together, these observations illustrate a surprisingly dichotomous role of BAF60a in the control of thermogenic gene programs in brown and beige adipocytes. In this case, BAF60a is absolutely...
required for brown fat thermogenesis and defense against acute cold stress, yet its inactivation appears to sensitize beige fat formation in response to chronic cold stimulation.

BAF60a deficiency engages ACTH/MC2R signaling axis to induce browning. Secreted factors play an important role in driving beige fat formation in response to physiological and environmental stimuli. Adrenergic stimulation as a result of increased sympathetic outflow to adipose tissue provides a potent signal that promotes white fat browning. Further, a number of local and endocrine factors have been identified to mediate beige fat induction under different physiological conditions. To explore whether BAF60a inactivation may activate a dormant signaling pathway to enhance browning, we focused our analysis on membrane receptors that exhibit abundant expression in iWAT. Analysis of our microarray dataset revealed that mRNA expression of Mc2r, Oxtr, and Kiss1r, G-protein coupled receptors responding to pituitary hormones, was markedly elevated in iWAT from cold-acclimated AKO mice compared to control (Fig. 6a). In contrast, mRNA levels of other pituitary hormone receptors, including Avpr1a, Ghr, Tshr, and Prlr, remained largely unaltered by BAF60a inactivation. Similarly, mRNA expression of Adrb3 and Chrna2, which mediate the response to norepinephrine (NE) and acetylcholine, respectively, was comparable in two groups. We next examined the effects of ligand activation of Mc2r, Oxtr, and Kiss1r on thermogenic genes in adipocytes differentiated from primary stromal vascular cells isolated from iWAT. As expected, both isoproterenol (Iso) and CL-316,243 strongly induced mRNA expression of Ucp1 and PGC-1α (Fig. 6b). While KISS1 and OXT (oxytocin), physiological ligands for KISS1R and OXTR, respectively, had modest effects on Ucp1 and PGC-1α expression, activation of MC2R by its ligand adrenocorticotropic hormone (ACTH) elicited a strong induction of Ucp1 and PGC-1α. Cell fractionation studies indicated that Mc2r induction in iWAT occurred in BAF60α-deficient adipocytes, but not stromal vascular cells (Fig. 6c and Supplementary Fig. 4a). Further, this increase in Mc2r expression was observed in iWAT under thermoneutral condition and in BAT and eWAT from BAF60α AKO mice (Supplementary Fig. 5a, b). We noted the presence of two major ATAC-seq peaks at the proximal promoter and first intron of Mc2r that coincide with PPARY binding sites (Fig. 6d). Accordingly, rosiglitazone significantly increased mRNA expression of Mc2r in differentiated beige adipocytes (Fig. 6e). We next examined whether altered local release of catecholamines and ACTH levels in circulation may account for enhanced browning in AKO mice. To our surprise, iWAT epinephrine and norepinephrine (NE) and plasma ACTH concentrations were similar in control and AKO mice (Fig. 6f).

ACTH stimulates cAMP signaling and PKA activation and has been shown to regulate thermogenic gene expression. To directly assess whether increased MC2R expression may sensitize adipocyte response to ACTH, we performed treatments in differentiated beige adipocytes expressing GFP or MC2R with different concentrations of ACTH. As shown in Fig. 7a, MC2R overexpression markedly augments the induction of Ucp1 and Elolv3 mRNA expression in response to ACTH. Consistently, ACTH-induced PKA activation, as revealed by phosphorylation of PKA substrates, was more pronounced in adipocytes over-expressing MC2R (Fig. 7b). RNA interference knockdown of MC2R in beige adipocytes impaired activation of PKA signaling by ACTH, but not Iso (Fig. 7d). Induction of Ucp1 and Elolv3 mRNA expression in response to ACTH was diminished by knockdown of MC2R (Fig. 7c). These results illustrate that MC2R expression levels modulate hormonal responsiveness of beige adipocytes to ACTH. To further assess whether the induction of Mc2r expression sensitizes ACTH response in vivo, we cultured adipose tissue explants from control and AKO mouse iWAT and stimulated them with ACTH or Iso. As expected, Iso stimulated phosphorylation of PKA substrates in control and AKO iWAT explants (Fig. 7e). In contrast, ACTH-induced PKA activation was notably stronger in AKO mouse iWAT compared to control, indicating that BAF60α deficiency sensitizes response of iWAT to ACTH stimulation. In support of this, we found that induction of iWAT Ucp1 mRNA expression was significantly higher in AKO mice than control following ACTH treatment (Fig. 7f). Together, these studies demonstrate that BAF60α per se is not essential for cold-induced white fat browning; instead, its deficiency activates the ACTH-MC2R pituitary-adipose signaling axis to promote beige fat formation.

Discussion

The brown and beige thermogenic programs are regulated by an overlapping set of transcription factors and cofactors. In this study, we uncover a surprisingly dichotomous role of BAF60α-mediated chromatin remodeling in thermogenic regulation (Fig. 7g). BAF60α is absolutely required for stimulation of brown fat thermogenesis in response to cold exposure and adrenergic activation; its deficiency impaired cold-induced thermogenesis and rendered mice hypersensitive to cold stress. At the molecular level, BAF60α physically associates with both cBAF- and BRD9-containing ncBAF complexes; the latter is required for thermogenic gene expression during brown adipogenesis and in response to adrenergic stimulation. BAF60α inactivation results in restricted chromatin accessibility near thermogenic genes such as Ucp1 in brown adipose tissue. Many of the ATAC-seq peaks regulated by BAF60α coincide with PPARY and EBF2 binding sites, suggesting that these two transcription factors may direct BAF60α-mediated chromatin remodeling. PPARY and EBF2 have been shown to recruit SWI/SNF complexes through interaction with the histone demethylase JMJD1A and the ATPase BRG1, respectively. Our results reveal a critical role of BAF60α in gating chromatin accessibility at these regulatory regions.

A surprising observation here is that BAF60α is essentially dispensable for induction of beige fat formation; its deficiency
**BAF60a deficiency promotes cold-induced beige fat formation.**

a. H&E staining of BAT from mice kept at ambient room temperature (RT) or after cold acclimation (Cold) (scale bar = 200 μm; n = 4 per group).

b. qPCR analysis of BAT gene expression (floxed, n = 7 for RT, n = 6 for Cold; AKO, n = 6 for RT, n = 7 for Cold).

c. Immunoblots of brown fat and inguinal fat lysates.

d. H&E and UCP1 immunostaining of iWAT sections (scale bar = 200 μm) (floxed, n = 6 for RT, n = 7 for Cold; AKO, n = 8 for Cold).

e. qPCR analyses of iWAT gene expression (floxed, n = 6 for RT, n = 6 for Cold; AKO, n = 6 for RT, n = 7 for Cold).

f. Heatmap representation of microarray data showing a cluster of genes exhibiting elevated expression in AKO iWAT following cold acclimation.

g. qPCR analysis of genes enriched in glycolytic beige fat (floxed, n = 6, filled; AKO, n = 9, open). Data in b, e, g represent mean ± s.e.m. two-tailed unpaired Student’s t-test, flox vs. AKO. Source data are provided as a Source Data file.
instead augmented white fat browning following chronic cold acclimation. ATAC-seq studies indicate that BAF60a deficiency differentially affected chromatin accessibility in brown and beige fat. These results illustrate that, despite the similarity between the brown and beige thermogenic gene programs, BAF60a are differentially required for transcriptional activation of these genes. These findings are in contrast to other thermogenic regulators such as PRDM16 and EBF2, which are required for thermogenic gene activation in both brown and beige adipocytes\(^\text{40-43}\). Given that BAF60a is largely dispensable for cold-induced browning of white fat, it is possible that BAF60b and/or BAF60c may compensate for the loss of BAF60a in beige adipocytes. Similar redundancy has been observed in a recent study on skeletal myocyte gene regulation by BAF60 family members\(^\text{44}\). Defects in brown fat thermogenesis have been shown to trigger compensatory browning response\(^\text{45}\). Our work here reveals a mechanism mediated by activation of the ACTH/MC2R pituitary-adipose endocrine pathway to promote iWAT browning. In this case, elevated MC2R expression sensitizes BAF60a-deficient adipocytes to PKA activation and thermogenic gene expression in response to ACTH. Recent studies have implicated ACTH and MRAP in thermogenic gene induction\(^\text{38,46}\). Our work illustrates that, in

Fig. 5 ATAC-seq analysis of iWAT from cold-acclimated mice. a Overlap of ATAC-seq peaks detected in BAT and cold-acclimated iWAT. b Top 12 motifs enriched in BAT ATAC-seq peaks and their ranks in beige fat. c UCSC genome browser traces of ATAC-seq peaks at the Ifi27l2a, Car3, Ucp1, and Cidea loci. d Motif-enrichment analysis of the peaks upregulated in AKO iWAT.
addition to the regulation of ACTH levels, the expression of MC2R modulates the sensitivity of adipocyte response to ACTH.

Methods
Animal studies. All relevant ethical regulations for animal testing and research have been complied. All animal procedures were approved by the University of Michigan Institutional Animal Care and Use Committee. The protocol number is PRO00007676. Wild-type C57BL/6J mice (JAX stock #000664) were purchased from the Jackson Laboratory. BAF60a<sup>fl</sup>ox/ox mice were generated as previously described<sup>25</sup>. To inactivate BAF60a in adipocytes, BAF60a<sup>fl</sup>ox/ox mice were crossed with Adiponectin-Cre transgenic mice. Mice were fed ad lib with standard rodent chow and maintained under 12/12 h light/dark cycles. For cold exposure, 4-month-old mice were individually housed in cages pre-chilled at 4 °C. Mice had
free access to food and water throughout the experiment. A rectal thermometer was used to measure core body temperature. For cold acclimation, mice at 6–8 months of age were kept in a temperature-controlled chamber. Temperature in the chamber was lowered 3 °C per day until it reached 10 °C. Mice were then maintained at 10 °C for 7 more days before analysis. For ACTH treatment, ACTH (Phoenix Biologicals) was dissolved in phosphate buffered saline and injected into inguinal locations at a dose of 5.6 μg/kg body weight per side.

### Primary cell culture and differentiation
Primary brown preadipocytes from B6F06a mice were isolated and immortalized as previously described21. Interscapular brown adipose tissues were removed from postnatal day 1 pups. After the tissues were minced, cell suspensions were filtered through 70 μm strainers, followed by centrifugation. The pellets were resuspended in primary cell culture medium containing 20% fetal bovine serum/DMEM/F12 GlutaMAX supplemented with 15% FBS. To induce differentiation, various hormones at increasing doses, ACTH (5 and 50 nM), KISS1 (112 nM), and FSK (1 μM) were added every 2 days. For NE stimulation, differentiated adipocytes were treated for 5 h with vehicle or 1 μM NE before harvest for RNA analyses.

The subsequent immortalized brown preadipocytes were maintained in 10% FBS/DMEM/F12 GlutaMAX containing BSA, followed by 15-min treatments of saline, ACTH, Iso, and FSK.

### Co-immunoprecipitation
Nuclear extracts were prepared from differentiated brown adipocytes. After incubating in a hypotonic buffer (10 mM Tris (pH 7.5), 10 mM KCl, 1.5 mM MgCl2, 20% glycerol, 0.2 mM EDTA, and the protease inhibitors), nuclear proteins were subsequently extracted by end-to-end rocking at 4 °C for 45 min before centrifugation. The pellets were then resuspended in high salt buffer containing 20 mM Tris, pH 7.5, 0.42 M NaCl, 1.5 mM MgCl2, 20% glycerol, 0.2 mM EDTA, and the protease inhibitors. Nuclear proteins were then immunoprecipitated using antibodies against BAF60a or BAF47. After washing, the immunoprecipitated proteins were eluted by boiling the beads in 1× sodium dodecyl sulfate (SDS) sample buffer.

### Histology and immunohistochemistry
Adipose tissues were dissected and directly fixed overnight in 10% formalin before paraffin embedding and H&E (hematoxylin and eosin) staining. Immunohistochemical analysis of Ucp1 protein was performed as described before28. Briefly, unstained tissue sections were heated at 60 °C for 30 min before rehydration. After antigen retrieval by boiling the slides in 10 mM sodium citrate-citric acid solution (pH = 6.2) for 20 min, the tissue sections were incubated overnight at 4 °C with anti-Ucp1 antibody (UCP1-1-A, Abclonal). Tissue sections were then incubated with mouse anti-BAF60a antibody before the addition of 20 μl Protein G agarose (THERMO FISHER) to capture antibody-bound protein complexes. After three washes, the immunoprecipitated proteins were eluted by boiling the beads in 1× sodium dodecyl sulfate (SDS) sample buffer.

### qPCR and immunoblotting analyses
qPCR analysis of gene expression and immunoblotting was performed as previously described29,30. Total RNA was isolated from tissues and cultured adipocytes using PureLink RNA Isolation Kit (Thermo Fisher). qPCR primer information can be found in Supplementary Table 1. Total cell lysates were prepared in a buffer containing 50 mM Tris-HCl (pH = 7.8), 137 mM NaCl, 10 mM NaF, 1 mM EDTA, 1% Triton X-100, 10% glycerol, and the protease inhibitor cocktail (Roche), followed by three freeze/thaw cycles. The crude lysates were prepared by homogenizing in a buffer containing 50 mM Tris (pH = 7.6), 130 mM NaCl, 5 mM NaF, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 10% glycerol, 1% Triton X-100, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (PMSF), and the protease inhibitor cocktail. After centrifugation, tissue lysates were separated on SDS-polyacrylamide gel (SDS-PAGE) and analyzed by the antibodies below. Primary antibodies used were as follows: mouse anti-Baf60a (617228, BD Biosciences), rabbit anti-Baf60a (109982-AP, Proteintech), rabbit anti-UCP1 (UCP11-A, Alpha Diagnostic), rabbit anti-HSP90 (sc-7947, Santa Cruz), mouse anti-HSP90 (sc-13119, Santa Cruz), rabbit anti-phospho-PKA substrate (#9624, Cell Signaling), rabbit anti-BRD9 (619368, Active Motif), rabbit anti-BRG1 (H-88, Santa Cruz), and rabbit anti-BAF47 (A301-087A, Bethyl). Detailed information can be found in Supplementary Table 2.

### Transmission electron microscopy
Brown adipose tissues were quickly removed and placed in a Petri dish containing a fixative buffer (2.5% glutaraldehyde, 0.1 M phosphate buffer, pH 7.4). The tissues were sliced into small pieces and fixed for 24 h. Transmission electron microscopy (TEM) images were acquired using Philips CM-100 TEM at the University of Michigan Microscopy and Image Analysis Laboratory.

### ChIP assay
ChIP analyses on brown adipose tissues were performed as previously described27. Briefly, brown fat tissue was minced and fixed in 1% formaldehyde for 15 min at room temperature, followed by quenching with 125 mM glycine for 5 min. The cross-linked tissue samples were further ground using PowerGen 125 Homogenizer (Fisher Scientific). The pellets from crude lysates were prepared as described above. After washing, the cross-linked tissue sections were resuspended and further washed by filtering again through 40 μm strainer, followed by centrifugation. The cell pellets were resuspended in primary cell culture medium containing 20% fetal bovine serum/DMEM/F12 GlutaMAX supplemented with 0.5% bovine serum albumin (BSA) at 37 °C with constant rocking. The isolated precursor cells were filtered through 70 μm strainer, followed by centrifugation. The cell pellets were resuspended in primary cell culture medium containing 20% fetal bovine serum/Dulbecco’s modified Eagle’s medium (F/125 mM NaHCO3) and incubated at 37 °C until reaching about 5% confluency. To immortalize the cells, retroviruses expressing SV40 large T antigen and Cre recombinase were filtered through 40 μm strainer, followed by centrifugation. The cell pellets were resuspended and further washed by centrifugation. The subsequent immortalized brown preadipocytes were maintained in 10% FBS/DMEM. To delete Baf60a, preadipocytes were transduced with control or a retroviral vector expressing Cre recombinase. The resulting stable cell lines were maintained in DMEM supplemented with 10% FBS. To induce differentiation, confluent cultures were exposed to induction medium containing 10% FBS/DMEM supplemented with 0.15% (w/v) IBMX, 125 μM indomethacin, 1 μM dexamethasone, 1 mM T3, and 20 nM insulin. After 48 h, the induction medium was replaced with maintenance medium (10% FBS/DMEM supplemented with 1 nM T3 and 20 nM insulin). Fresh maintenance medium was added every 2 days until day 7, when the cells were treated with 5 μg of vehicle or 1 μM NE before harvest for RNA analyses.

MitoTracker Red (Thermo Fisher) was added to the cells (cells with mitochondrial staining) to a final concentration of 1 μM and stained for 20 min. After centrifugation, cell pellets were resuspended and further washed by centrifugation. Nuclear proteins were subsequently extracted by end-to-end rocking at 4 °C for 45 min before centrifugation. The pellets were then resuspended in high salt buffer containing 10 mM Tris, pH 7.5, 0.42 M NaCl, 6 μg/ml polybrene. At 4–8 h after AAV infection, differentiated cells were treated with saline, ACTH, Iso, or forskolin (FSK) for 3 h before harvesting for RNA. For Western blot analyses, differentiated cells were starved in DMEM/F12 GlutaMAX containing BSA, followed by 15-min treatments of saline, ACTH, Iso, and FSK.
Cell Disrupter) for six times (10 s on, 30 s off) at set 6. After clearing by centrifugation, the chromatin lysates were diluted with ChIP dilution buffer (1% Triton X-100, 0.15 M NaCl, 1 mM EDTA, 20 mM Tris at pH 8) to a final concentration of 0.1% SDS and incubated overnight with antibodies at 4 °C with rotation. One microgram of antibodies against PPARγ (sc-7196, Santa Cruz) or control IgG was used for each binding assay. To recover the antibody-bound chromatin, Protein A agarose beads (Thermo Fisher Scientific, #20334) were added and incubated for 2 h at 4°C. After washing with a series of wash buffers (A—0.1% SDS, 0.1% NaDOC, 1% Triton X-100, 0.15 M NaCl, 1 mM EDTA, 20 mM Tris at pH 8; B—0.1% SDS, 0.1% NaDOC, 1% Triton X-100, 0.5 M NaCl, 1 mM EDTA, 20 mM Tris at pH 8; C—0.25 M LiCl, 0.5% NaDOC, 0.5% NP-40, 1 mM EDTA, 20 mM Tris at pH 8; and D—1 mM EDTA, 20 mM Tris at pH 8), bound chromatin DNA was eluted in 1 mM EDTA/1% SDS buffer. Samples were reverse cross-linked overnight at 65 °C, followed by the treatment of RNase A and proteinase K.
DNA was further purified using PCR Purification Kit (Thermo Fisher). Quantitative real-time PCR using SYBR Green (Thermo Fisher) was performed using the primers located on Ucp1 promoters and 18s gene. ChIP enrichment was normalized to input and IgG control.

**Infrared imaging analysis.** Infrared thermography was performed as previously described. Briefly, mice were injected i.p. saline or 0.1 mg/kg body weight of CL-316,243 (Sigma-Aldrich) dissolved in saline. Three hours later, an infrared camera (T650sc, emissivity of 0.98, FLIR Systems) was used to capture the thermal pictures at a distance of ~30 cm above the cage. Multiple images were taken while mice could move freely. To analyze the thermal images, a region of interest (ROI) for each mouse was drawn for whole body (whole body) or the area above interscapular brown fat. The image files were then converted to CSV file using the FBR Research IR program. Mean body surface temperature was calculated from top 10% pixels for each ROI.

**Cellular respiration assay.** Oxygen consumption rate was measured in fully differentiated brown adipocytes using Oxygen Meter (Strathkelvin Instruments) with a Mitocell (MT200) mixing chamber. Cells suspended in 400 μl maintenance medium (10% FBS/DMEM supplemented with 1 nM T3 and 20 nM insulin) were loaded into the mixing chamber. The change of oxygen concentration was recorded for 5 min, following injections of FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone, 3 μM, Cayman) to the chamber. Oxygen consumption rate was calculated using software (782 Oxygen System version 4.0) and its sample was normalized to its protein content.

**Ex vivo culture and lipolysis assay.** After removing lymph nodes, inguinal adipose tissues collected from several mice were pooled and kept in 100 mm dishes containing 20 ml warm DMEM. Tissues were cut into small pieces (2–3 mm) using the scissors, followed by three PBS washes. Tissue pieces were then evenly distributed into 6-well tissue culture plates and maintained in 2 ml Medium 199 containing 10% FBS. Three hours later, T0CH (Phoenix Pharma), 30 nM ISO, or PBS were added and incubated for 15 min before harvest for protein analysis. For lipolysis assay, epididymal fat explants were obtained similarly as above. After 1 h incubation at 37 °C in HBSS containing 2% BSA, the tissues were stimulated with saline or 100 nM ISO for 3 h. NEFA concentrations in media were measured at different time points (Wako).

**Adipose ATAC-seq.** Brown adipose tissues were dissected from three mice for each genotype and snap-frozen individually in liquid nitrogen. For iWAT ATAC-seq, inguinal adipose tissue was isolated as previously described. Nuclei from brown and inguinal adipose tissue were isolated from three mice for each genotype and snap-frozen individually in liquid nitrogen. For iWAT ATAC-seq, inguinal adipose tissue was isolated as previously described. For downstream analysis, the peaks were filtered against an mm10 blacklist (downloaded from http://mitra.stanford.edu/kundaje/akundaje/release/blacklists/mm10-mouse/mm10.blacklist.bed.gz). The treat_pileup.bedgraph files (generated by macs2) from each individual sample were normalized to its own library size. We took the average of treat_pileup.bedgraph files across samples within a biological condition to visualize ATAC-seq coverage in the UCSC genome browser. The final track files were displayed in the bigwig format, which was converted from normalized and averaged treat_pileup.bedgraph file by bedGraphToBigWig (v.4). Atavy (v. 1.0.6; https://github.com/ParkerLab/atavy) was used to perform the ATAC-seq data quality control analysis.

**Statistical analysis.** Experiments of similar conditions were independently performed two or more times. Data were analyzed using Prism8 (GraphPad Software Inc.). Two-tailed unpaired Student’s t test and two-way ANOVA (analysis of variance) were used for statistical analysis as indicated in the figure legends. Statistical significance between groups was called when P value is smaller than 0.05. Motif enrichment was statistically analyzed using cumulative binomial distribution (HOMER, findMotifs.pl).

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

**Data availability**

The data that support the findings of this study are available from the corresponding authors upon reasonable request. ATAC-seq and microarray data have been deposited in GEO database (GSE128747 and GSE145498, respectively). The source data underlying Figs. 1a, b, d, e, 2d, 3a, 4b, 5b, 6a, –j, 7a–d, Supplementary Figs. 1–7, 3a, 4, and 5 are provided as a Source Data file. PPARy and EB2 ChIP-seq of brown fat bigwig files were downloaded from GEO database (GSE97116); PPARy inguinal ChIP-seq bigwig file was downloaded from GEO sample GSM2433426.

**Code availability**

Code for processing and analyzing ATAC-seq data is available on GitHub (https://github.com/ltonguyu/ATAC-seq-pipeline).

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Competing interests
The authors declare no competing interests.

Additional information
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