Thermal stress induces glycolytic beige fat formation via a myogenic state

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Environmental cues profoundly affect cellular plasticity in multicellular organisms. For instance, exercise promotes a glycolytic-to-oxidative fibre-type switch in skeletal muscle, and cold acclimation induces beige adipocyte biogenesis in adipose tissue. However, the molecular mechanisms by which physiological or pathological cues evoke developmental plasticity remain incompletely understood. Here we report a type of beige adipocyte that has a critical role in chronic cold adaptation in the absence of β-adrenergic receptor signalling. This beige fat is distinct from conventional beige fat with respect to developmental origin and regulation, and displays enhanced glucose oxidation. We therefore refer to it as glycolytic beige fat. Mechanistically, we identify GA-binding protein α as a regulator of glycolytic beige adipocyte differentiation through a myogenic intermediate. Our study reveals a non-canonical adaptive mechanism by which thermal stress induces progenitor cell plasticity and recruits a distinct form of thermogenic cell that is required for energy homeostasis and survival.

Cold acclimation stimulates non-shivering thermogenesis in adipose tissue, primarily through activation of the sympathetic nervous system, followed by increased β-adrenergic receptor (β-AR) signalling1. Beige fat, an inducible form of thermogenic adipocyte, has become the subject of intensive research because it has potent anti-obesity and anti-diabetic effects2-5, and the existence of beige adipocytes in adult humans has been verified by molecular analysis3-5. F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET/CT)-based imaging shows that cold acclimation promotes the recruitment of new thermogenic fat in adults6-11. Although extensive efforts have been made to activate adipose tissue thermogenesis using selective β3-adrenergic receptor (β3-AR) agonists as an anti-obesity medication, these attempts have been unsuccessful, partly because of poor bioavailability and concerns with respect to developmental origin and regulation, and displays enhanced glucose oxidation. We therefore refer to it as glycolytic beige fat. Mechanistically, we identify GA-binding protein α as a regulator of glycolytic beige adipocyte differentiation through a myogenic intermediate. Our study reveals a non-canonical adaptive mechanism by which thermal stress induces progenitor cell plasticity and recruits a distinct form of thermogenic cell that is required for energy homeostasis and survival.

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and that these cells underwent myogenes into GFP⁺ myotubes even when cultured under pro-adipogenic conditions (Fig. 1g).

**Identification of glycolytic beige fat**

Although cells in the Myod⁺ lineage do not typically give rise to either white or beige adipocytes, our results show that Myod⁺ cells emerge within inguinal WAT when β-AR signalling is inhibited. Accordingly, we performed lineage tracing using Myod⁻CreERT² reporter mice that were pre-treated with β-blocker or vehicle together with tamoxifen at ambient temperature for 5 days, and subsequently acclimated to 15 °C for an additional 5 days (Fig. 2a). By the end of a 5-day cold exposure, we observed a substantial number of UCP1⁺ beige adipocytes in the inguinal WAT, although no Myod⁺ beige adipocytes were found in vehicle-treated mice. On the other hand, we found that a subset of UCP1⁺ beige adipocytes were derived from Myod⁺ cells and localized near the lymph node where microvasculature (CD31⁺ endothelial cells) was enriched (Fig. 2b and Extended Data Fig. 2a, b). Because cold-induced GFP⁺ beige adipocytes do not express endogenous Myod despite their developmental origin from Myod⁺ progenitors, we refer to these cells as Myod⁺-derived beige fat. On average, Myod⁺-derived beige adipocytes accounted for 14.8% ± 2.5% of total UCP1⁺ beige adipocytes in the inguinal WAT of β-blocker-treated mice after 5 days at 15 °C (Fig. 2c). No detectable Myod⁺-derived adipocytes were found in iBAT and epididymal WAT (Extended Data Fig. 2c, d). Of note, Myod⁺-derived beige adipocytes were induced without β-blocker treatment when mice were maintained in a prolonged severe cold condition at 6 °C for 2 weeks (8.75% ± 1.59%) (Extended Data Fig. 2e). However, no Myod⁺-derived beige adipocytes were found after a 2-week treatment with a β-AR agonist (CL316,243) (Extended Data Fig. 2f, g), indicating that the effect of cold acclimation on Myod⁺-derived beige adipocyte biogenesis is mediated through a distinct mechanism from the activation of β-AR. Further, no beige adipocytes were derived from the Myog lineage (Extended Data Fig. 2h, i). These results suggest that stromal cells in inguinal WAT possess a unique progenitor population that expresses Myod1, but still retains cellular plasticity to give rise to beige adipocytes in cold conditions when β-AR signalling is inhibited.

To determine whether Myod⁺-derived beige adipocytes constitute a distinct adipose cell population, we used laser-capture microdissection to isolate Myod⁺-derived and non-Myod⁺-derived beige adipocytes in inguinal WAT for transcriptomics. As additional controls, transcriptome data from brown adipocytes, white adipocytes from inguinal WAT, and skeletal muscle were included. The transcriptome data suggests that Myod⁺-derived GFP⁺ adipocytes were classified as beige adipocytes, and not myocytes, because they abundantly expressed selective markers for brown or beige fat (Ucp1 and Kcnk3) and adipocyte-selective genes (Adipoq and Fabp4), whereas they did not express skeletal muscle-specific genes (Myh1 and Myog) (Extended Data Fig. 3a, b). Principal components analysis (PCA) showed that Myod⁺-derived beige fat exhibited a distinct molecular signature from that of conventional beige fat, which was also distinct from those of brown adipocytes, white adipocytes and skeletal muscle (Fig. 2d). Notably, many genes involved in glycolysis, glucose metabolism, carbohydrate metabolism, and fructose and mannose metabolism were enriched in Myod⁺-derived beige adipocytes relative to non-Myod⁺-derived beige fat (Fig. 2e). For example, expression of the glycolysis genes, including Enol1 and Pkm2 (also known as Pkm), was significantly higher in Myod⁺-derived beige adipocytes relative to GFP⁺ beige adipocytes (Fig. 2f and Extended Data Fig. 3c). These glycolytic genes were also highly enriched in the inguinal WAT of β-less mice following cold exposure (Fig. 2g). On the other hand, expression of β-AR was lower in Myod⁺-derived beige adipocytes than GFP⁺ beige adipocytes (Extended Data Fig. 3d).

The above results suggest that Myod⁺-derived beige fat displays enhanced glucose metabolism. Consistent with this notion, we found that eno1α (ENO1), a marker of glycolytic cells, was abundantly expressed in nearly all the Myod⁺-derived beige fat cells (Fig. 2h). Furthermore, Myod⁺-derived GFP⁺ beige fat exhibited significantly higher glucose oxidation than GFP⁺ beige fat (Fig. 2i), although no difference was found in fatty acid oxidation (Extended Data Fig. 3e). Similarly, extracellular acidification rate (ECAR) was significantly higher in Myod⁺-derived beige fat than in GFP⁺ beige fat in the presence of glucose (Fig. 2j), whereas both fat types exhibited similar oxygen consumption rate (OCR) (Fig. 2k). These results indicate that an adaptive thermogenic response promotes the formation of a distinct beige fat with enhanced glucose metabolism when β-AR signalling is blocked. We call this tissue glycolytic beige fat (g-beige fat).

**Characterizing progenitors of g-beige fat**

We next investigated the lineage relationship between Myod⁺ stromal cells and other cell types in the SVFs of inguinal WAT. The emergence of Myod⁺ cells was not a result of proliferation of pre-existing Myod⁺ progenitors, because we did not detect 5-bromo-2′-deoxyuridine (BrdU) incorporation in Myod⁺ cells following treatment with β-blocker (Extended Data Fig. 4a, b). Therefore, we next characterized the molecular signatures of Lin⁺ GFP⁺ stromal cells in the inguinal WAT of Myod⁻CreERT² reporter. The analysis also included satellite cell-derived myoblasts in skeletal muscle (Lin− Scgl− Vcam1⁺ integrin

**Fig. 1** β-AR blockade promotes myogenesis in inguinal WAT.

**a**, Transcriptomics in the inguinal WAT of wild-type (WT) and β-less mice at 23 °C or 15 °C. n = 3 biologically independent samples. P < 0.05 analysed by two-sided t-test. **b**, Gene ontology (GO) analysis of gene sets II and III. P values (−log₁₀) by delta-method-based test. **c**, Immunofluorescent staining of MyoD (arrows) in the inguinal WAT-derived SVFs from mice treated with β-blocker or vehicle. Scale bar, 100 μm. **d**, Immunofluorescent staining of MHC in differentiated SVF cells under pro-adipogenic conditions. Scale bar, 100 μm. **e**, Quantification of MHC⁺ myotubes in d. n = 4 biologically independent samples. Data are mean ± s.e.m., and analysed by unpaired Student’s two-sided t-test. **f**, Immunofluorescent staining of MyoD (arrows) in the inguinal WAT-derived SVFs from Myod⁻CreERT² reporter mice. Scale bar, 20 μm. **g**, Immunofluorescent staining of MHC in differentiated SVFs. Scale bar, 100 μm. **c**, **d**, **f**, **g**, DAPI as counter stain. Images represent four independent experiments.
α7) and fibro-adipogenic progenitors (FAPs) (Fig. 3a and Extended Data Fig. 4c). Transcriptome analysis suggested that GFP+ progenitors exhibited a distinct molecular signature from myoblasts and FAPs (Extended Data Fig. 4d, e). For instance, quantitative reverse transcription with PCR (qRT–PCR) analysis in independent samples verified that a myoblast-enriched marker Ncam1 (also known as Cd56) was not expressed in GFP+ progenitors, although GFP+ progenitors expressed Myod1 mRNA and Myod1 protein (Fig. 3b and Extended Data Fig. 4f). Moreover, Myod1 progenitors in inguinal WAT were not derived from the Pax7 lineage (Extended Data Fig. 4g, h). As GFP+ progenitors also expressed Pdgfra, Cd34, and Cd29 (also known as Igh1), markers for adipogenic progenitors38, we used Pdgfra-CreERT2 reporter mice to test the hypothesis that a subset of PDGFRα+ CD34+CD29+ adipocyte progenitors express Myod1 (Fig. 3c and Extended Data Fig. 5a, b). Following β-blocker treatment, we found that Myod1 protein was detected in a subset (10.4% ± 0.8%) of GFP+ (PDGFRα+) CD34+CD29+ progenitors in the inguinal WAT, whereas no Myod1 cell was observed in vehicle-treated mice (Fig. 3d, e and Extended Data Fig. 5c).

To elucidate the upstream signalling of Myod1 progenitors, we applied Ingenuity pathway analysis to the above transcriptome data. The analysis identified enhanced signalling pathways enriched in Myod1 progenitors, including but not limited to those induced by bone morphogenetic proteins (BMPs) (Fig. 3f). This is consistent with the observations that Myod1 progenitors abundantly expressed Smad5 and a BMP receptor Bmpr1b (Extended Data Fig. 6a). Because BMP7 is known to promote brown adipogenesis19, we probed whether BMP7 promotes beige adipogenesis in Myod1 progenitors using Myod1-CreERT2 reporter mice (Extended Data Fig. 6b). By 8 days of differentiation in pro-adipogenic conditions with recombinant BMP7 (rBMP7) pre-treatment, a large proportion of the GFP+ progenitors differentiated into adipocytes containing numerous lipid droplets, whereas all the GFP+ progenitors formed myotubes in the absence of rBMP7 pre-treatment (Fig. 3g). Known activators of α-adrenergic receptor (α-AR) or β-AR signalling did not promote adipocyte differentiation, indicating that adipogenesis in Myod1 progenitors was regulated independently from α-AR and β-AR signalling (Extended Data Fig. 6c).

**GABPα controls g-beige fat development**

To examine the transcriptional circuits that control g-beige adipocyte differentiation, we applied the transcriptome datasets of g-beige fat and β-less mice to hypergeometric optimization of motif enrichment (HOMER) analysis40. This analysis identified DNA-binding motifs for GA-binding protein (GABPα), oestrogen-related receptor α (ERRα) and oestrogen-related receptor γ (ERRγ), all of which were significantly enriched in Myod1-derived g-beige fat and cold-induced beige fat in β-less mice (Fig. 4a and Extended Data Fig. 7a–c). Previous reports show that GABPα, ERRα and ERRγ stimulate mitochondrial biogenesis and the OXPHOS program through the interaction with PGC1α21–23. Accordingly, we overexpressed each factor in C2C12 myoblasts and tested whether any of the transcription factors induced g-beige adipocyte differentiation in Myod1 progenitors (Extended Data Fig. 7d). Under pro-adipogenic conditions, C2C12 cells expressing an empty vector differentiated into myotubes; however, GABPα-expressing myoblasts efficiently differentiated into lipid-containing adipocytes (Fig. 4b). A small fraction of ERRα-expressing cells—but not ERRγ-expressing cells—differentiated into adipocytes, although their adipogenic differentiation potency was far lower than that of GABPα-expressing myoblasts. Notably, GABPα potently promoted beige adipogenesis in C2C12 myoblasts to levels comparable to PRDM16, with a 3,908-fold increase in Adipoq (Fig. 4c). Similar to thermogenic adipocytes, GABPα-expressing adipocytes expressed thermogenic genes such as Ucp1 (65.7-fold) and Pgc1a (also known as Ppara) (11.4-fold) in response to forskolin treatment (which increases...
in intracellular cAMP), at levels significantly higher than control cells (Fig. 4d). Furthermore, GABPα-expressing cells expressed ENO1, PPARγ and UCP1 under pro-adipogenic conditions, whereas GABPα inhibited myogenesis and Myod1 mRNA expression (Fig. 4e and Extended Data Fig. 7e, f). As C2C12 cells expressed undetectable levels of endogenous PRDM16, and GABPα did not induce Prdm16 expression (Extended Data Fig. 7g), GABPα appears to stimulate g-beige adipogenesis independently of PRDM16. At the functional level, GABPα-expressing adipocytes displayed higher ECAR than control cells and PRDM16-expressing adipocytes (Fig. 4f). Notably, GABPα-expressing adipocytes displayed high glucose uptake and oxidation (Fig. 4g and Extended Data Fig. 8a), whereas fatty acid oxidation and OCR were at comparable levels (Fig. 4h and Extended Data Fig. 8b). These data indicate that GABPα drives the g-beige fat differentiation program in Myod1+ progenitors.

To determine the requirement of GABPα for g-beige adipocyte development, two independent lentiviruses that expressed inhibiting short hairpin RNAs (shRNAs) targeting Galpa or a scrambled control (SCR) were introduced into primary Myod1+ progenitors from Myod1-CreERT2 reporter mice (Extended Data Fig. 8c). We found that adipogenesis in Myod1+ progenitors was severely impaired when Galpa was depleted by Galpa shRNA, whereas SCR control cells differentiated into adipocytes under pro-adipogenic conditions with rBMP7 pre-treatment (Extended Data Fig. 8d). Gene expression analysis shows that knockdown of Galpa significantly reduced the expression of Ucp1, Adipoq and Fabp4, whereas expression of the myogenic genes Myh1 and Myod1, was increased in Galpa-knockdown cells (Extended Data Fig. 8e). Furthermore, Galpa knockdown significantly reduced OCR and ECAR relative to SCR-control cells (Extended Data Fig. 8f, g). Accordingly, we examined whether GABPα was required for g-beige fat biogenesis in vivo. Because whole-body deletion of Galpa causes embryonic lethality24, we generated Myod1+ -cell-specific GABPα-knockout mice (GalpaMyod1lox/lox, Myod1-CreERT2;Gabaflox/fllox Rosa26-mTmG). Of note, these mice contained the Rosa26-mTmG reporter, which enabled us to trace and quantify the number of Myod1+ -derived g-beige adipocytes. Following β-blocker pretreatment and cold acclimation to 15 °C, control mice (Myod1-CreERT2, Gabpafllox/fllox Rosa26-mTmG) formed GFP+ UCP1+ g-beige adipocytes. By contrast, the formation of g-beige adipocytes was impaired in GalpaMyod1lox/lox mice, although GFP+ UCP1+ beige adipocytes were still observed (Fig. 4i, j). These results indicate that GABPα is required for g-beige fat development.

The role of g-beige fat in metabolism

To examine the role of g-beige fat in vivo, we developed g-beige fat-deficient mice by crossing Myod1-CreERT2 mice with mice expressing a loxP-flanked stop cassette upstream of the diphtheria toxin receptor (DTR) (Extended Data Fig. 9a). This model enabled selective depletion of Myod1+ -derived adipocytes. Following diphtheria toxin administration, g-beige adipocytes were impaired in GalpaMyod1lox/lox mice, whereas control mice (Myod1-CreERT2 Rosa26-DTR) or littermate control mice (Myod1-CreERT2) were pre-treated with β-blocker and acclimated to 15 °C, leading to the formation of Myod1+ -derived glycolytic beige fat. Of note, β-blocker treatment reduced glucose uptake by iBAT and heart, and completely suppressed iBAT thermogenesis without affecting muscle shivering (Extended Data Fig. 9b–e). Following treatment with diphtheria toxin, glycolytic beige fat—as visualized by ENO1 and UCP1 immunostaining—was substantially reduced in Myod1-DTR+ mice relative to control mice without changing muscle shivering (Extended Data Fig. 9f–h). 18FDG-PET/CT imaging detected active glucose uptake in the inguinal WAT of control mice owing to g-beige formation; however, glucose uptake in the inguinal WAT of Myod1-DTR+ mice, but not in the muscle and liver, was significantly decreased (Fig. 5a, b). We also found that diphtheria-toxin-induced depletion of g-beige fat led to a significant reduction in OCR and ECAR in the inguinal WAT (Fig. 5c, d). Because of the possibility that muscle function is impaired in Myod1-DTR+ mice, we developed an alternative g-beige defective mouse model by deleting PPARγ in Myod1+ -derived cells (PparγMyod1lox/lox) (Extended Data Fig. 10a–c). Consistent with the results in Myod1-DTR+ mice, Myod1+ -cell-specific PPARγ deletion reduced OCR and ECAR in the inguinal WAT (Fig. 5e and Extended Data Fig. 10d, e).

CD34+CD29+ cells in the inguinal WAT of Pdgfra-CreERT2 reporter mice. n = 16 biologically independent samples. Data are mean ± s.e.m.
Data Fig. 10d). These data suggest that Myod1- derived g-beige fat contributes to cold-stimulated glucose uptake and thermogenesis in the inguinal WAT.

Lastly, we examined the role of g-beige fat in adaptive thermogenesis and systemic glucose homeostasis. Since no difference was seen in muscle shivering between PpargMyod1-KO mice and littermate controls (Extended Data Fig. 10e), the contribution of shivering thermogenesis appears to be negligible in this model. Upon acute β-blocker treatment followed by 10 °C cold exposure, wild-type mice without β-blocker pre-treatment quickly developed hypothermia because of the paucity of iBAT thermogenesis and g-beige fat biogenesis. On the other hand, mice with β-blocker pre-treatment were able to maintain their core body temperature for up to 4 h at 10 °C even after acute β-blocker treatment. PpargMyod1-KO mice exhibited slightly lower core-body temperature than control mice at 15 °C and developed severe hypothermia shortly after cold exposure at 10 °C (Fig. 5f). Consistent with these observations, PpargMyod1-KO mice exhibited modestly but significantly lower whole-body energy expenditure (VO2) than control mice following cold acclimation. Green arrows indicate 18F-FDG uptake in inguinal WAT. Fig. 5 | Requirement of g-beige fat for energy homeostasis. a, 18F-FDG PET/CT images of control mice and Myod1-DTR- mice following cold acclimation. Green arrows indicate 18F-FDG uptake in inguinal WAT. b, Quantification of 18F-FDG uptake in indicated tissues. Inguinal WAT (IngWAT): control, n = 10; Myod1-DTR-, n = 8. Skeletal (Sk.) muscle: control, n = 9; Myod1-DTR-, n = 7. Liver: control, n = 6; Myod1-DTR-, n = 5. Data are mean ± s.e.m. of biologically independent samples; two-sided unpaired Student's t-test. c, OCR in inguinal WAT. n = 5. d, ECAR in inguinal WAT. n = 5. e, Glucose stress test in inguinal WAT of PpargMyod1-KO and control mice. Glucose (25 mM) and 2-deoxyglucose (2-DG) were added at indicated points. n = 6. Data are mean ± s.e.m.; ANOVA followed by Tukey's test. f, Changes in rectal temperature during cold acclimation. Control and PpargMyod1-KO mice were pre-treated with β-blocker for 5 days and acclimated to 15 °C. Wild-type mice did not receive pre-β-blocker-treatment. Acute β-blocker was added at the indicated arrow. Data are mean ± s.d. of biologically independent mice. Control mice, n = 6; PpargMyod1-KO, n = 4; wild-type, n = 7. Inset, core body temperature at 15 °C. g, Glucose tolerance test (GTT) in mice treated with β-blocker and acclimated to 15 °C. Control, n = 7; PpargMyod1-KO, n = 5. Data are mean ± s.e.m. of biologically independent mice. h, A mechanism of g-beige adipocyte development, details in main text. a, Images represent three independent experiments. c, d, Data are mean ± s.e.m. of biologically independent samples; two-sided unpaired Student's t-test. f, g, Two-way ANOVA followed by Bonferroni's test.
alternative thermogenic program via g-beige fat biogenesis. In response to cold acclimation, progenitors expressing SMA (also known as Acta2), Pax3, Pdgfra or Pdgfrb in the SVF of WAT give rise to beige adipocytes through the β3-AR or β3-AR signalling pathways. However, when β3-AR signalling is impaired or in prolonged, severe cold conditions, a subset of Pdgfrα+CD34+CD29+ progenitors in subcutaneous WAT expresses MyoD, but retains the cellular plasticity to undergo beige fat adipogenesis following cold acclimation. MyoD1+ derived beige adipocytes are distinct from canonical beige adipocytes in their molecular signature, developmental origin and regulation, and cellular metabolism, exhibiting enhanced glucose utilization (Fig. 5h).

Two forms of thermogenic adipocytes, brown and beige adipocytes, have been described in mammals. As brown adipocytes in iBAT express substantially higher levels of UCP1 than beige adipocytes, beige fat was considered to have a marginal role in whole-body energy expenditure. However, recent studies demonstrated the crucial roles of beige fat in the regulation of whole-body energy homeostasis through UCP1-independent thermogenic mechanisms as well as non-thermogenic mechanisms (for example, anti-inflammatory and anti-fibrosis activity). These studies indicate the multifaced roles of beige adipocytes in the regulation of whole-body energy homeostasis in the absence of β3-AR signalling. It is conceivable that multiple subtypes of thermogenic adipocytes with distinct cellular origins exist, and that each subtype has unique biological roles, depending on the nature of external stimuli, such as cold acclimation, caloric restriction or manipulation, exercise, cachexia, bariatric surgery, and injury. A better understanding of adipose cell heterogeneity under such environmental changes will provide new insights into the molecular basis of metabolic adaptation in physiology and disease.

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1. Collins, S. β3-adrenoceptor signaling networks in adipocytes for recruiting stored fat and energy expenditure. Front. Endocrinol. 2, 102 (2011).
2. Seale, P. et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin. Invest. 121, 96–105 (2011).
3. Ikeda, K. et al. UCPI-independent signalling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis. Nat. Med. 23, 1454–1465 (2017).
4. Cohen, P. et al. Ablation of PRDM16 and beige adipocytes cause metabolic dysfunction and a subcutaneous to visceral fat switch. Cell 156, 304–316 (2014).
5. Sharp, L. Z. et al. Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS ONE 7, e49452 (2012).
6. Wu, J. et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376 (2012).
7. Lidell, M. E. et al. Evidence for two types of brown adipose tissue in humans. Nat. Med. 19, 631–634 (2013).
8. Shinoda, K. et al. Genetic and functional characterization of clonally derived adult human brown adipocytes. Nat. Med. 21, 389–394 (2015).
9. Yonesshiro, T. et al. Recruited brown adipose tissue as an antiobesity agent in humans. J. Clin. Invest. 123, 3404–3408 (2013).
10. van der Lans, A. A. et al. Cold acclimation recruits human brown fat and increases nonsivering thermogenesis. J. Clin. Invest. 123, 3395–3403 (2013).
11. Hanssen, M. J. et al. Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. Nat. Med. 21, 863–865 (2015).
12. Arch, J. R. Challenges in β3-adrenoceptor agonist drug development. Ther. Adv. Endocrinol. Metab. 2, 59–64 (2011).
13. Bachman, E. S. et al. β3AR signaling required for diet-induced thermogenesis and obesity resistance. Science 297, 843–845 (2002).
14. Ye, L. et al. Fat cells directly sense temperature to activate thermogenesis. Proc. Natl Acad. Sci. USA 110, 12480–12485 (2013).
15. Razoni, M. et al. Stress-induced activation of brown adipose tissue prevents obesity in conditions of low adaptive thermogenesis. Mol. Metab. 5, 19–33 (2016).
16. Sanchez-Gurmaches, J. & Guertin, D. A. Adipocytes arise from multiple lineages that are heterogeneous and dynamically distributed. Nat. Commun. 5, 4099 (2014).
17. Berry, D. C., Jiang, Y. & Graf, J. M. Mouse strains to study cold-inducible beige progenitors and beige adipocyte formation and function. Nat. Commun. 7, 10184 (2016).
18. Rodeheffer, M. S., Birsoy, K. & Friedman, J. M. Identification of white adipocyte progenitor cells in vivo. Cell 135, 240–249 (2008).
19. Tseng, Y. H. et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454, 1000–1004 (2008).
20. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38, 576–589 (2010).
21. Yang, Z. F., Drumea, K., Mott, S., Wang, J. & Rosmarin, A. G. GABP transcription factor (nuclear respiratory factor 2) is required for mitochondrial biogenesis. Mol. Cell. Biol. 34, 3194–3201 (2014).
22. Mootha, V. K. et al. Envo and Gabpa/b specify PGCG1α-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. Proc. Natl Acad. Sci. USA 101, 6570–6575 (2004).
23. Gantner, M. L., Hazen, B. C., Eury, E., Brown, E. L. & Krailli, A. Complementary roles of estrogen-related receptors in brown adipose thermogenic function. Endocrinology 157, 4770–4781 (2016).
24. Jaworski, A., Smith, C. L. & Burden, S. J. GA-binding protein is dispensable for neuromuscular synapse formation and synapse-specific gene expression. Mol. Cell. Biol. 27, 5040–5046 (2007).
25. Lee, Y. H., Petkova, A. P., Mottillo, E. P. & Granemann, J. G. In vivo identification of bipotential adipocyte progenitors recruited by β3-adrenoceptor activation and high-fat feeding. Cell Metab. 18, 480–491 (2013).
26. Vishwanath, L. et al. Pdgfra+ mural progenitors contribute to adipocyte hyperplasia induced by high-fat-diet feeding and prolonged cold exposure in adult mice. Cell Metab. 23, 350–359 (2016).
27. Long, J. Z. et al. A smooth muscle-like origin for beige adipocytes. Cell Metab. 19, 810–820 (2014).
28. Kajimura, S., Spiegelman, B. M. & Seale, P. Brown and beige fat: physiological roles beyond heat generation. Cell Metab. 22, 546–559 (2015).
29. Nedergaard, J. & Cannon, B. UCP1 mRNA does not produce heat. Biochem. Biophys. Acta 1831, 943–949 (2013).
30. Kazak, L. et al. A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. Cell 163, 643–655 (2015).
31. Hasegawa, Y. et al. Repression of adipose tissue fibrosis through a PRDM16–GTF2IRD1 complex improves systemic glucose homeostasis. Cell Metab. 27, 180–194 (2018).

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Author contributions Y.C. and S.K. conceived the study and designed experiments. Y.C., KI, TY, A.S., K.T., Q.W., K.K., C.H.S. and Z.B. performed experiments. K.S. performed bioinformatics analyses. Y.C., KI, TY, A.S., K.T., Q.W., K.K., C.H.S., and S.K. analysed and interpreted the data. Y.C. and S.K. wrote the manuscript. Y.C., Z.B., A.B. and S.K. edited the manuscript.

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METHODS

Animals. All animal experiments were performed following the guidelines established by the UCSF Institutional Animal Care and Use Committee. All the mice were 12–16-week-old males, had free access to food and water, 12-h light cycles, and were caged at 23 °C. Rosa26-mTmG mice (Stock No. 007576), Myod1-CREERT2 mice (Stock No. 025667), Pdgfra-CreERT2 mice (Stock No. 018280), Rosa26-IDTR mice (Stock No. 007900), Fpf2a-Cre (Stock No. 004384), and Rosa26-tdTomato (Stock No. 007997) were obtained from the Jackson Laboratory. Primary: Table 1. Supplementary: Papers Table 1. Mice. Gfp^{+}\text{mice} were gifts from C. Keller and S. Burden, respectively. Myogenin-Cre mice were obtained from S. Haldar. Mice were randomly assigned at the time of purchase or weaning to minimize any potential bias. For cold-exposure experiments, mice were singly caged and exposed to mild cold temperature at 15 °C, 10 °C or 6 °C. Mice were treated intraperitoneally with β-blocker (propranolol hydrochloride) at a dose of 25 mg per kg (body weight) per day, with saline as a vehicle control at room temperature for 5 days. Subsequently, the treated mice were acclimated to a mild cold condition for an additional 5 days. Metabolic cage analyses were performed by a staff scientist who was blinded to the experimental groups. Other experiments were not blinded.

Chemicals and antibodies. All chemicals were obtained from Sigma-Aldrich unless otherwise specified. The following antibodies were used in this study: GFP antibody (GFP-1020, Aves), UCP1 antibody (ab-10983, Abcam), ENO1 antibody (ab-151502, Abcam), myosin (skeletal) antibody (M7523-2ML, Sigma-Aldrich), myoD antibody (sc-780, Santa Cruz Biotech); Alexa Fluor 488 goat anti-chicken (A-11039, Life Technologies), Alexa Fluor 546 goat anti-rabbit (A-11035, Life Technologies), Alexa Fluor 647 goat anti-rabbit (A-21244, Life Technologies), and biotinylated secondary antibody. CD31-PE/Cy7 antibody (BD Biosciences), CD45-PE/Cy7 antibody (BD Biosciences), CD45-PE/Cy7 antibody (BD Biosciences), CD34-APC/Cy7 antibody (Biolegend), and CD29-APC/Cy7 antibody (Biolegend).

Genotyping, tamoxifen and diphertheria toxin injection. Genotyping of all Cre-transgenic mice was performed by PCR using primers designing the Cre sequence, according to the protocol provided by the Jackson Laboratory. Genotyping of the Rosa locus was performed according to the Jackson laboratory protocol. To induce Cre expression in Myod1-CREERT2 mice, Pax7-CREERT2 mice, or Pdgfra-CREERT2 mice, tamoxifen at 3 mg in 100 μl/100 g of body weight was injected intraperitoneally for 5 days. To ablate systemic myod1<sup>−</sup>-derived cells in Myod1-CREERT2/Rosa26-IDTR mice, diphertheria toxin was injected intraperitoneally for two to three consecutive days at a dose of 4 ng per g (body weight) per injection<sup>3,34</sup>. All mice controls were sex- and age-matched.

Cell culture. SVF cells were isolated from the inguinal WAT of reporter mice according to our protocol<sup>35</sup>. MyoD<sup>+</sup> progenitors isolated from Myod1-CREERT2 reporter mice were immortalized by using the SV40 Large T antigen according to the cell immortalization protocol<sup>36</sup>. HEK293T and C2C12 cell lines were purchased from ATCC. No commonly missidentified cell line was used. All cell lines used in this study were regularly tested negative for mycoplasma contamination. The cells were pre-treated with 3.3 nM human recombinant BMP7 (PCH9544, Thermo Fisher Scientific) for two days before inducing adipogenesis in Myod1<sup>+</sup> progenitors and C2C12 cells. Adipogenesis was induced by culturing 100% confluent cells in DMEM containing 10% FBS, 0.5 mM isobutylmethylxanthine, 125 nM indomethacin, 2 μg ml<sup>−1</sup> dexamethasone, 850 nM insulin, 1 nM T3, and 0.5 μM rosiglitazone. Three days after adipogenic induction, cell medium was replaced with the maintenance medium containing 10% FBS, 850 nM insulin, 1 nM T3 and 0.5 μM rosiglitazone<sup>37</sup>. Differentiated cells were treated with or without 10 μM forskolin (cAMP) for 4 h before collecting cells for analyses. To stimulate α-ARs and β-ARs, we used agonists for α1-AR (phenoxyphrine, 10 μM), α2-AR (clonidine, 10 μM), α3-AR (denopamine, 10 μM), α2-AR agonist (formoterol, 2.5 μM), and α3-AR (CSL16243, 0.1 μM). The myoblast cell line C2C12 cells were purchased from ATCC. Lipid droplets were visualized by Oil-red-O staining or Nile red staining (N1142, Thermo Fisher Scientific). The cells have been correctly authenticated by RNA-seq and tested negative for mycoplasma contamination.

Fluorescence-activated cell sorting. To obtain highly purified GFP<sup>+</sup>, tdTomato<sup>+</sup> cells, the SVF cells were isolated from the inguinal WAT of tamoxifen-treated Myod1-CREERT2/Rosa26-mTmG reporter mice, and subsequently sorted into sorting medium (PBS containing 2% FBS, 7-aminocaproic acid D (7-AAA) was used for viable cell gating, CD31-PE/Cy7 (BD Biosciences), CD45-PE/Cy7 (BD Biosciences), CD34-APC (Biolegend), and CD29-APC/Cy7 antibody (Biolegend) antibodies or MACS MicroBeads (Miltenyi Biotec) were used to remove lineage<sup>−</sup> (Lin<sup>−</sup>) cells from tissue samples. Flow cytometric analysis and sorting were performed using FACS-Aria II (BD Biosciences).

Isolation of myogenic cells. To obtain highly purified myogenic cells, mononucleated cells were isolated from unjured muscles. Cells in sorting medium (10% horse serum, 2% FBS, 2% L-glutamine) were incubated with V2C4-SP for quantification of glycolytic beige adipocytes, we randomly chose 10 slides from each mouse and counted the number of GFP<sup>+</sup> and UCP1<sup>+</sup> or ENO1<sup>+</sup> adipocytes. Because glycolytic beige adipocytes were enriched in the central part of the inguinal WAT near lymph node, all the image-based
analyses were performed using histology sections in the central part of inguinal WAT containing the lymph node.

**Oxygen consumption and glucose stress assays.** OCR and ECAR were measured in isolated tissues or cultured adipocytes using the Seahorse XFe Extracellular Flux Analyzer (Agilent). For tissue respiration assays, 1.0 mg adipose tissue was dissected from inguinal WAT depots by using a surgical biopsy instrument (Integra Millex Standard Biopsy Punches, Thermo Fisher) and placed into XF24 Islet Capture Microplates and pre-incubated with XF assay medium with pH value at 7.4. XF assay medium supplemented with 1 mM sodium pyruvate, 2 mM GlutaMaxTM-I, and 25 mM glucose. Tissue or cells were subjected to a mitochondrial stress test by adding oligomycin (5 μM) followed by carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 5 μM), and 2-DG (50 mM). For glucose stress assay and ECAR measurement, XF assay medium supplemented only with GlutaMaxTM-I. Tissue or cells were subjected to a glucose stress test by adding highly concentrated glucose (for tissue, 25 mM; for cells, 10 mM), followed by adding oligomycin (5 μM), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 5 μM), and 2-DG (50 mM).

**Temperature recording.** Tissue temperature in the iBAT and the skeletal muscle was recorded using the type T thermocouple probes and recorded by using the TC-2000 Meter (Sable Systems International), according to our protocol1. Core body temperature was monitored using a TH-5 thermometer (Physitemp) by recording the rectal temperature of the mice. For core-body temperature recording experiment in Fig. 5f, both control (recording the rectal temperature of the mice). For core-body temperature recording experiment in Fig. 5f, both control (recording the rectal temperature of the mice) and MyoD1-Cre ERT2; Ppargc1a KO (MyoD1-Cre ERT2; Ppargc1a KO) mice were pre-treated with β-blocker and tamoxifen at room temperature for 5 days and acclimated to 15 °C for an additional 5 days. Wild-type mice did not receive pre-β-blocker-treatment. Subsequently, all the mice were acutely treated with β-blocker and exposed to 10 °C.

**Glucose and fatty acid oxidation assays.** Differentiated cells cultured in six-well plates were incubated with DMEM containing 2% FBS for 2 h and 4 h for glucose oxidation assays and fatty acid oxidation assays, respectively. Cells were subsequently incubated with KRB–HEPES buffer that contained 2% BSA and 5 mM glucose in the presence of 0.5 μCi/ml [1-14C]glucose or [1-14C]oleic acid and 1 mM carnitine. After 1 h incubation at 37 °C, 30% hydrogen peroxide (350 μl) was added into each well; then the plates were sealed with wipe smears supplemented with 300 μl 1 M benzenthonium hydroxide solution. Radioactivity trapped in the wipe smears was determined by a liquid scintillation counter (PerkinElmer).

**Glycogen uptake assay.** Differentiated immortalized beige adipocytes or cells expressing an empty vector or GABPα were plated in a six-well plate and incubated in the medium containing 2% FBS for 2 h. Subsequently, the cells were incubated with PBS containing 100 nM insulin, washed in PBS and incubated with 0.1 mM 2-deoxyglucose and 1 μCi/ml 2-deoxy-o-[1-14H]glucose for 10 min. After washing in cold PBS, cells were collected in 1% SDS. [14H]glucose uptake was detected by scintillation counter. Values were normalized by total protein concentrations measured by the bicinchoninic acid (BCA) method.

**Metabolic studies.** Twelve-week-old mice (control, MyoD1-Cre ERT2; Ppargc1a KO and MyoD1-Cre ERT2; Ppargc1a KO) were chronically pre-treated with β-blocker for 5 days, and then the mice were transferred from room temperature to mild cold at 15 °C for an additional 5 days. Whole-body energy expenditure (VO2, VCO2), food intake and locomotor activity (beam break counts) were monitored using the Comprehensive Laboratory Animal Monitoring System (CLAMS, Columbus Instruments) at 15 °C. For glucose tolerance test, mice were pre-treated with β-blocker and mild cold at 15 °C, and then the mice were acutely treated with β-blocker. After 6 h of fasting, the mice were injected intraperitoneally with glucose (2.0 g/kg). Blood samples were collected at several time points, and glucose levels were measured using blood glucose test strips (Abbott).

**Electromyography.** To perform electromyography (EMG), 29-gauge needle electrodes were placed close to the back muscles near the back leg under anaesthesia. The recorded signal was processed (low-pass filter, 3 kHz; high-pass filter, 10 Hz; notch filter, 60 Hz) and amplified 1,000× with Bio Amp (ADInstruments). EMG data were collected at a sampling rate of 2 kHz using LabChart 8 Pro Software (ADInstruments). Before the data analysis, the raw signal was converted to root mean square (RMS) activity, which was ultimately analysed for shivering bursts in 5-s windows.

**18F-FDG-PET/CT scan.** Following treatment with β-blocker or vehicle, mice were administered 100 μCi of 18F-FDG via a tail vein injection under 2% isoflurane anaesthesia. The micro-PET/CT imaging system was applied to scan the whole mouse at the UCSF PET/CT Imaging Core Facility. Subsequently, mice were euthanized, and their iBAT, inguinal WAT, liver and skeletal muscle were collected. The radioactivity in the tissues was measured against known activity standards using a gamma counter (Wizard 3; PerkinElmer) at the UCSF Imaging Facility.

**Statistics.** Statistical analyses were performed using GraphPad Prism 5.0 or 7.0 (GraphPad Software), and Excel (Microsoft). All data were represented as mean ± s.e.m, except where noted. A two-sample unpaired Student’s t-test was used for two-group comparisons. One-way ANOVA followed by Tukey’s test was used for multiple group comparisons, two-way ANOVA followed by Bonferroni’s test was used for Seahorse measurement from multiple groups or core body temperature measurement of animals from two groups. No statistical methods were used to predetermine sample size. P-values below 0.05 were considered significant throughout the study.

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

**Data availability**

RNA-seq datasets generated in this study are available at ArrayExpress under the following accession codes: E-MTAB-4526 (adipose tissues in β-less mice), E-MTAB-4528 (skeletal muscle in β-less mice), E-MTAB-6392 (MyoD1+ derived beige fat), E-MTAB-7175 (BAT, WAT and skeletal muscle), E-MTAB-6441 (MyoD1+ derived progenitors), and E-MTAB-7164 (myoblasts). The datasets in the present study are available from the corresponding author upon request.

32. Nishijo, K. et al. Biomarker system for studying muscle, stem cells, and cancer in vivo. *FASEB J.* 23, 2681–2690 (2009).
33. Jung, S. et al. In vivo depletion of CD11c<sup>+</sup> dendritic cells abrogates priming of CD8<sup>+</sup> T cells by exogenous cell-associated antigens. *Immunity* 17, 211–220 (2002).
34. Buch, T. et al. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat. Methods* 2, 419–426 (2005).
35. Lipsberg Aune, U., Ruiz, L. & Kajimura, S. Isolation and differentiation of stromal vascular cells to beige/brite cells. *J. Vis. Exp.* (2013).
36. Ohno, H., Shinoda, K., Spiegelman, B. M. & Kajimura, S. PPARγ agonists induce a white-to-brown fat conversion through stabilization of PPARδ protein. *Cell Metab* 15, 395–404 (2012).
37. Trapnell, C. et al. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat. Biotechnol.* 31, 46–53 (2013).
38. Tripathi, S. et al. Meta- and orthogonal integration of inflammation "OMICS" data defines a role for UBR4 in virus budding. *Cell Host Microbe* 18, 723–735 (2015).
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Mouse models with defects in β-AR signalling.

a, Schematic of the experiment. The inguinal WAT from wild-type and β-less mice at 23 °C or 15 °C was collected and analysed by RNA-seq. 
b, mRNA expression of indicated genes in interscapular BAT (iBAT) of mice in Fig. 1a. n.s., not significant. n = 5. c, GO analysis of gene set I in Fig. 1a. P values (−log10) by delta-method–based test. d, mRNA expression of gene set I: Ucp1, n = 10; Elovl3, n = 5; Cidea, n = 5; Pgc1a, n = 5; Cox8b, n = 5. e, mRNA expression of skeletal muscle-related genes in inguinal WAT. Myh1, n = 9; Myl1, n = 8; Mylpf, n = 8; Mybpc1, n = 9; Acta1, n = 8. f, mRNA expression of myogenesis-related genes in the iBAT of mice in a. n = 5. g, mRNA expression of indicated genes in the skeletal muscle of mice in a. n = 5. h, Schematic of the experiment. Wild-type mice were treated with β-blocker or vehicle (saline) at 23 °C for 5 days. i, mRNA expression of indicated genes in inguinal WAT (left), iBAT (middle) and skeletal muscle (right) of mice treated with β-blocker or vehicle. *P < 0.05. n = 4. Data are mean ± s.e.m. of biologically independent samples; unpaired two-sided Student’s t-test. j, Immunofluorescent staining of MHC in differentiated SVFs from the iBAT of mice in h. k, Immunofluorescent staining of MHC in differentiated SVF cells from the epididymal WAT of mice in h. l, Schematic of the experiment. SVFs from β-blocker-treated Myod1-CreERT2 reporter mice were cultured. b–g, Data are mean ± s.e.m. of biologically independent samples; one-way ANOVA followed by Tukey’s test. j, k, DAPI for counter staining. Images represent three independent replicates. Scale bar, 50 μm.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2  |  Myod1<sup>+</sup>-derived beige adipocytes in adipose tissue. a, Immunofluorescent staining of GFP and UCP1 in the anterior, middle and posterior regions of inguinal WAT of Myod1-Cre<sup>E<sup>E</sup>RT2</sup> reporter mice. Mice were pre-treated with β-blocker and acclimated to 15 °C. Scale bar, 100 μm. Note that GFP<sup>+</sup> adipocytes were enriched in the middle part of inguinal WAT near the lymph node (LN). The ratio (%) of UCP1<sup>+</sup> cells among total GFP<sup>+</sup> cells is shown on the top. N.D., not detected. b, Immunofluorescent staining of GFP and CD31 in the middle part of inguinal WAT. Myod1-Cre<sup>E<sup>E</sup>RT2</sup> reporter mice treated with β-blocker. Scale bar, 100 μm. Note that GFP<sup>+</sup> adipocytes are localized near the CD31<sup>+</sup> microvasculature. c, Immunofluorescent staining of GFP and UCP1 in the iBAT of Myod1-Cre<sup>E<sup>E</sup>RT2</sup> reporter mice treated with β-blocker or vehicle. Scale bar, 100 μm. d, Immunofluorescent staining of GFP and UCP1 in the epididymal WAT of mice in c. Scale bar, 100 μm. e, Immunofluorescent staining of GFP and UCP1 in the inguinal WAT of Myod1-Cre<sup>E<sup>E</sup>RT2</sup> reporter mice that were acclimated to 6 °C for 2 weeks without β-blocker treatment. Scale bar, 100 μm. f, Immunofluorescent staining of GFP and UCP1 in the inguinal WAT of Myod1-Cre<sup>E<sup>E</sup>RT2</sup> reporter mice treated with CL316,243 (1 mg per kg (body weight) per day) for 2 weeks. Scale bar, 100 μm. g, Quantification of GFP<sup>+</sup> beige adipocytes among total UCP1<sup>+</sup> beige adipocytes in e and f. n = 8. Data are mean ± s.e.m. of biologically independent experiments. h, Schematic of the experiment. Myog-Cre;Rosa26-mTmG reporter mice were treated with β-blocker at room temperature for 5 days and subsequently acclimated at 15 °C for 5 days. i, Immunofluorescent staining of GFP and UCP1 in the inguinal WAT of mice in h. Scale bar, 100 μm. a–f, i, tdTomato or DAPI for counter stain. The images represent three independent replicates.
Extended Data Fig. 3 | Molecular analyses of Myod1+ derived beige adipocytes. a, mRNA expression (in fragments per kilobase of transcript per million mapped reads (FPKM)) of Myh1, Myog, Fabp4, Adipoq, Ucp1 and Kcnk3 in indicated tissues. n = 3 biologically independent experiments. Data are mean ± s.e.m.; ANOVA followed by Tukey’s test. b, mRNA expression of beige fat markers in GFP+ and GFP− beige fat from Myod1-CreERT2 reporter mice. n = 3 biologically independent experiments. Data are mean ± s.e.m.; unpaired Student’s t-test. c, mRNA expression of glucose metabolism genes in indicated tissues. n = 3 biologically independent samples. Data are mean ± s.e.m., and analysed by ANOVA followed by Tukey’s test. d, mRNA expression of adrenergic receptors in GFP+ and GFP− beige fat. n = 3 biologically independent samples. Data are mean ± s.e.m.; unpaired two-sided Student’s t-test. e, Fatty acid oxidation in GFP+ and GFP− beige fat. n = 6 biologically independent samples. Data are mean ± s.e.m.; unpaired two-sided Student’s t-test.
Extended Data Fig. 4  |  MyoD+ progenitors in inguinal WAT.

**a**, Schematic of the experiment. BrdU was administered in Myod1-CreERT2 reporter mice during β-blocker treatment. **b**, Immunofluorescent staining of BrdU and GFP in the inguinal WAT-derived SVFs from Myod1-CreERT2 reporter mice. Scale bar, 50 µm. Enlarged image, scale bar, 10 µm.

**c**, Schematic of the experiment. GFP+ and GFP− progenitors were isolated from lineage-negative (Lin−) stromal cells in the inguinal WAT of Myod1-CreERT2 reporter mice by fluorescence-activated cell sorting.

**d**, Transcriptome analysis in c. Each type of progenitor was pooled from 3 mice. Cut-off values was $P < 0.05$ by the delta-method-based hypothesis test. **e**, PCA of transcriptome dataset from indicated cells. Transcriptome of FAPs (GSE86073) is included in the analysis. **f**, Immunofluorescent staining of MyoD in isolated GFP+ progenitors in c. Note that all the GFP+ cells express MyoD protein. Scale bar, 100 µm. **g**, Immunofluorescent staining of MyoD and PAX7 in the SVFs from the inguinal WAT of Pax7-CreERT2; Rosa26-tomato reporter mice. **h**, Immunofluorescent staining of MyoD and PAX7 in the SVFs from the iBAT of Pax7-CreERT2; Rosa26-tomato reporter mice in g. Scale bar, 10 µm. **b–h**, DAPI was used as counter stain. Images represent three independent experiments.
Extended Data Fig. 5 | Isolation of MyoD+ progenitors from inguinal WAT. a, Expression of CD29 and CD34 in the SVFs from Pdgfra-CreERT reporter mice. b, Sequential gating to isolate GFP^+ (PDGFRα^+) CD34^+CD29^+ cells in the SVFs from the inguinal WAT of Pdgfra-CreERT reporter mice treated with β-blocker. c, Immunofluorescent staining of MyoD in GFP^+ CD34^+CD29^+ cells isolated from the SVFs in b. DAPI was used as counter-stain. Scale bar, 25 μm. Data represent three independent experiments.
Extended Data Fig. 6 | Developmental regulation of MyoD+ progenitors in the inguinal WAT. a, mRNA expression of indicated genes in isolated GFP+ and GFP− progenitors from the inguinal WAT of Myod1-CreERT2 reporter mice treated with β-blocker. Smad5 (GFP+, n = 6; GFP−, n = 8), Myod1, Bmpr1a, Bmpr1b and Bmpr2 (n = 4). Data are mean ± s.e.m. of biologically independent samples; unpaired two-sided Student's t-test. b, Schematic of the experiment. The inguinal WAT-derived SVF cells from Myod1-CreERT2 reporter mice were pre-treated with rBMP7 or vehicle for 2 days. Cells were subsequently differentiated under pro-adipogenic conditions for 8 days. c, Immunofluorescent staining of GFP and lipid droplets stained with Nile red in differentiated MyoD+ cells prepared as in b. Cells were treated with indicated compounds, including Br-cAMP (200 µM), 3-isobutyl-1-methylxanthine (IBMX, 0.5 µM), adenosine (100 nM), agonists for α1-AR (phenylephrine, 10 µM), α2-AR (clonidine, 10 µM), β1-AR (denopamine, 10 µM), β2-AR agonist (formoterol, 2.5 µM), β3-AR (CL316,243, 0.1 µM), noradrenaline (1 µM), and recombinant human BMP7 (rBMP7, 3.3 nM), or vehicle control. DAPI was used as counter stain. The images represent three independent experiments. Scale bar, 100 µm.
Extended Data Fig. 7 | Transcriptional regulation of beige adipocyte differentiation.  

**a**, mRNA expression of Gabpa, Errα, and Errγ in GFP⁺ and GFP⁻ progenitors in the inguinal WAT of Myod1-CreER⁺ reporter mice treated with β-blocker. *n = 4.*  

**b**, mRNA expression of indicated genes in GFP⁺ and GFP⁻ beige fat from Myod1-CreER⁺ reporter mice. *n = 3.*  

**c**, Protein expression of PRDM16, EHMT1 and CKIα in GFP⁺ and GFP⁻ progenitors in the inguinal WAT of Myod1-CreER⁺ reporter mice. β-actin was used as a loading control. Molecular mass (kDa) is shown on the right.  

**d**, mRNA expression of indicated genes in C2C12 myoblasts expressing empty vector, GABPα, ERRα or ERRγ using lentivirus. *n = 3.*  

**e**, mRNA expression of *Myod1* in C2C12 myoblasts expressing an empty vector, GABPα, ERRα or ERRγ by lentivirus. *n = 4.*  

**f**, Protein expression of PPARγ and UCP1 in differentiated C2C12 cells expressing an empty vector or GABPα in pro-adipogenic conditions. β-actin was used as a loading control. Blots represent five independent samples.  

**g**, mRNA expression of *Prdm16* in differentiated beige adipocytes expressing empty vector, GABPα, ERRα or ERRγ using lentivirus. Differentiated immortalized beige adipocytes are included as a reference. *n = 4.*  

**a**, **b**, Data are mean ± s.e.m. of biologically independent replicates; unpaired two-sided Student’s *t*-test.  

**d**, **e**, **g**, Data are mean ± s.e.m. of biologically independent replicates; ANOVA followed by Tukey’s test.
Extended Data Fig. 8 | GABPα controls g-beige fat development.

a, Glucose uptake in differentiated cells expressing an empty vector or GABPα, and in immortalized beige adipocytes. The values were normalized to total protein concentration. n = 5. b, OCR of differentiated C2C12 cells expressing empty vector, GABPα or PRDM16. n = 3. c, mRNA expression of Gabpa in differentiated cells expressing a scramble control (sh-SCR) or shRNAs targeting Gabpa (shGabpa#1 and shGabpa#2). n = 4. d, Immunofluorescent staining of lipid droplets, MHC and GFP in differentiated C2C12 cells expressing sh-SCR or shGabpa.

Scale bar, 50 µm. DAPI was used as counter stain. Images represent three independent experiments. e, mRNA expression of indicated genes in differentiated cells expressing sh-SCR or shGabpa. Ucp1, n = 5; Adipoq, Fabp4, Myhl, Myod1, n = 6. f, OCR in differentiated cells in c. n = 14. g, ECAR in differentiated cells in c. n = 14. a–c, e, Data are mean ± s.e.m. of biologically independent samples; one-way ANOVA followed by Tukey’s test. f, g, Data are mean ± s.e.m. of biologically independent samples; two-way ANOVA followed by Bonferroni’s test.

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Extended Data Fig. 9 | A mouse model of g-beige fat depletion.

a, Schematic of the experiment. Myod1-DTR+ and control mice were pre-treated with β-blocker and tamoxifen, and subsequently acclimated to 15 °C for 5 days. Diphtheria toxin (DT) was administered to deplete cold-induced Myod1+ derived g-beige fat. 

b, 18F-FDG PET/CT images of vehicle- or β-blocker-treated mice at 15 °C.

c, Schematic of tissue temperature recording in iBAT and skeletal muscle of mice.

d, Changes in tissue temperature (ΔT) in iBAT and skeletal muscle. Mice were treated with saline (control) or β-blocker for 5 days (chronic β-blocker). A subset of the saline-treated mice was acutely treated with β-blocker (acute β-blocker). To stimulate thermogenesis, noradrenaline (NE) was administered at the indicated time point (black arrow). n = 4 for control, n = 6 for acute β-blocker treatment and n = 6 for chronic β-blocker treatment. Data are mean ± s.e.m. of biologically independent mice; two-way ANOVA followed by Bonferroni’s test.

e, EMG measurement of skeletal muscle shivering in wild-type mice treated with β-blocker or vehicle (saline) at 30 °C or 15 °C. The shivering data were converted to the root mean square (RMS, µV). n = 4 biologically independent mice. Data are mean ± s.e.m.; ANOVA followed by Tukey’s test.

f, H&E staining (left) and immunostaining of UCP1 (middle) or ENO1 (right) in the inguinal WAT of control and Myod1-DTR+ mice. Scale bar, 100 µm; enlarged image scale bar, 20 µm. Images represent five independent animals.

Extended Data Fig. 10 | Cold acclimation and β-blocker treatment increase skeletal muscle shivering.

a-h, Quantification of data in Extended Data Fig. 9. Data are mean ± s.e.m.; ANOVA followed by Tukey’s test.

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Extended Data Fig. 10 | Requirement of g-beige fat for adaptive thermogenesis in the absence of β-AR signalling. 

**a.** Schematic of the experiment. *Pparg<sup>Myod1-KO</sup>* mice (*Myod1-Cre<sup>ERT2</sup>;*Pparg<sup>flx/flx</sup>) and littermate control mice (*Pparg<sup>flx/flx</sup>*) were pre-treated with β-blocker and tamoxifen. Subsequently, these mice were acclimated to 15 °C for 5 days.

**b.** Immunofluorescent staining of UCP1 and ENO1 in the inguinal WAT of *Pparg<sup>Myod1-KO</sup>* mice and controls. Scale bar, 100 µm. Images represent three independent experiments.

**c.** Quantification of glycolytic beige fat in **b.** *n* = 10.

**d.** OCR in the inguinal WAT of *Pparg<sup>Myod1-KO</sup>* mice and controls. Scale bar, 100 µm. Images represent three independent experiments.

**e.** EMG measurement of skeletal muscle shivering in *Pparg<sup>Myod1-KO</sup>* (*n* = 6) and littermate control mice (*n* = 5) at 30 °C or 15 °C. The shivering data were converted to RMS (µV). Data are mean ± s.e.m. of biologically independent mice; ANOVA followed by Tukey’s test.

**f.** Whole-body oxygen consumption (VO₂) in *Pparg<sup>Myod1-KO</sup>* mice and littermate controls. Mice were treated with β-blocker at indicated time points at 15 °C. *n* = 5 for *Pparg<sup>Myod1-KO</sup>* mice, *n* = 6 for control. Data are expressed as mean ± s.e.m. of biologically independent mice; ANOVA followed by Bonferroni’s test.

**g.** Total food intake in **f.**

**h.** Locomotor activity in **f.**

**i.** Body weight of *Pparg<sup>Myod1-KO</sup>* mice and littermate controls on a regular chow diet. Mice were treated with β-blocker and acclimated to 15 °C for 5 days. *Pparg<sup>Myod1-KO</sup>* mice, *n* = 5; littermate control mice, *n* = 7. Data are mean ± s.e.m. of biologically independent samples; unpaired two-sided Student’s *t*-test.
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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).

| Item                                                                 | 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  |           |
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| A description of all covariates tested                              |           |
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| 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

RNAseq analysis: TopHat version 2.0.8; Cuffdiff 2.1.1; Metascape; Ingenuity Pathway Analysis
Histology: Leica Sp 8; Volocity 6; ImageJ 1.49v; Adobe Photoshop CS2 9.0
QPCR: QuantStudio Real-time PCR system 1.2v
Seahorse: Wave 2.4
EMG: LabChart 8
PET-CT image: Amide1.0.4

Data analysis

Microsoft office Excel 2016; GraphPad Prism 5; GraphPad Prism 7

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

The sequencing data were uploaded to ArrayExpress (https://www.ebi.ac.uk/arrayexpress) and open to public with the following accession numbers: E-MTAB-4526 (adipose tissue in $\beta$-less mice), E-MTAB-4528 (skeletal muscle in $\beta$-less mice), E-MTAB-6392 (MyoD-derived beige fat), E-MTAB-7175 (BAT, WAT, and skeletal muscle), and E-MTAB-6441 (MyoD-derived progenitors), and E-MTAB-7164 (Myoblasts). RNA-seq dataset in FAPs is available in GSE86073.

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
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

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.

Sample size
The sample size was determined by the power analysis with $\alpha = 0.05$ and power of 0.8, developed by Cohen (1988), and based on our experience with experimental models, anticipated biological variables, and previous literatures. This is described in the Method (statistics). Sample numbers were described in the Figure legends.

Data exclusions
No data were excluded in the study.

Replication
All the biological experiments were repeated, at least, twice and reproduced. RNA-sequencing and metabolomics were performed once but three independent samples were analyzed and further validated by alternative approaches, such as qRT-PCR. Western blotting data were confirmed by two or three independent samples.

Randomization
Mice were randomly assigned at the time of purchase or weaning to minimize any potential bias. This is described in the Method (animals).

Blinding
RNA sequencing and library constructions were performed by technical staffs at the UCLA genome core who were blinded to the experimental groups. RNA sequencing alignment and analyses were performed by the authors who were blinded to the experimental groups. CLAMS was performed by a staff scientist who was blinded to the experimental groups. Other experiments were not blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

- n/a Involved in the study
- ✗ Unique biological materials
- ✗ Antibodies
- ✗ Eukaryotic cell lines
- ✗ Palaeontology
- ✗ Animals and other organisms
- ✗ Human research participants

Methods

- 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 the unique materials used are available from the authors upon request.
Antibodies

**Antibodies used**
Following antibodies were used in this study: GFP antibody (GFP-1020, Aves, Chicken antibody 1:500), UCP1 antibody (ab-10983, Abcam, Rabbit antibody 1:200), ENO1 antibody (ab-155102, Abcam, Rabbit antibody 1:200), Myosin (Skeletal) antibody (M7523-.2ML, Sigma-Aldrich, Rabbit antibody 1:500), MyoD antibody (sc-760, Santa Cruz Biotech, Rabbit antibody 1:75); Alexa Fluor 488 (A-11039, Life Technologies, goat anti-chicken 1:500), Alexa Fluor 546 (A-11035, Life Technologies, goat anti-rabbit 1:500), Alexa Fluor 647 (A-21244, Life Technologies, goat anti-rabbit 1:500), and Biotinylated (BA-1000, Vector, goat anti-rabbit 1:200), CD31-PE/Cy7 antibody (#561410, BD Biosciences, rat anti-mouse, 1:100), CD45-PE/Cy7 antibody (#552848, BD Biosciences, rat anti-mouse, 1:100), CD34-APC antibody (#119309, Biolegend, rat anti-mouse, 1:20), and CD29-APC/Cy7 antibody (#102225, Biolegend, hamster anti-mouse/rat, 1:100). All antibodies were validated for the application and species used in this study by their manufacturers.

**Validation**
Antibodies were validated based on the size of band in western blotting (molecular weight), specificity/selectivity assessed by using samples from knockout mouse/knockdown mouse/knockdown cells/over expression cells, and reproducibility of the results.

Eukaryotic cell lines

Policy information about [cell lines](#)

**Cell line source(s)**
HEK293T, C2C12 cell lines used in this study were purchased from ATCC. Primary progenitor cells used in this study were isolated from inguinal WAT or IBAT of experimental housed mice.

**Authentication**
RNA-sequencing of the cell lines provide authentification.

**Mycoplasma contamination**
All the cell lines used in this study were routinely tested negative for mycoplasma contamination.

**Commonly misidentified lines**
No commonly misidentified cell line was used.

Animals and other organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research

**Laboratory animals**
Mice of both sexes aged 12-16 weeks were used for all the experiments. Littermate controls with same sex were used whenever it is possible. All the mice had free access to food and water, 12 hr light cycles, and were caged at 23 °C. Rosa26-mTmG mice (Stock No. 007576), Myod-CreERT2 mice (Stock No. 025667), Pdgfra-CreERT (Stock No. 018280), Rosa26-iDTR mice (Stock No. 007900), Ppargflox/flox (Stock No. 004584), and Rosa26-tdTomato (Stock No. 007905) were obtained from the Jackson Laboratory. Pax7-CreERT mice and Gapbaflox/flox mice were generous gifts from Dr. Keller and Dr. Burden, respectively. Myogenin-Cre mice were obtained from Dr. Haldar’s lab at Gladstone Institutes.

**Wild animals**
This study did not involve wild animals

**Field-collected samples**
This study did not involve samples collected from the field