Adipocyte liver kinase b1 suppresses beige adipocyte renaissance through class IIa histone deacetylase 4

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Abstract

Uncoupling protein 1° (UCP1°) beige adipocytes are dynamically regulated by environment in rodents and humans; cold induces formation of beige adipocytes, while warm temperature and nutrient excess lead to their disappearance. Beige adipocytes can form through de novo adipogenesis, however how “beigeing” characteristics are maintained afterwards is largely unknown. Here we show that beige adipocytes formed postnatally in subcutaneous inguinal white adipose tissue (iWAT) lost thermogenic gene expression and multilocular morphology at adult stage, but cold restored their “beigeing” characteristics, a phenomenon termed as beige adipocyte renaissance. Ablation of these postnatal beige adipocytes inhibited cold-induced beige adipocyte formation in adult mice. Furthermore, we demonstrated that beige adipocyte renaissance was governed by Liver kinase b1 (Lkb1) and histone deacetylase 4 (Hdac4) in white adipocytes. Although neither presence nor thermogenic function of UCP1° beige adipocytes contributed to metabolic fitness in adipocyte Lkb1 deficient mice, our results reveal an unexpected role of white adipocytes in maintaining properties of preexisting beige adipocytes.

Introduction

Energy balance requires equivalent energy intake and energy expenditure, and when energy intake exceeds energy expenditure, animals store excess energy as fat in adipose and other metabolic tissues. Chronic energy excess can lead to obesity and further development into type II diabetes [1]. Adaptive thermogenesis, a major contributor of total energy expenditure, occurs mostly in brown fat [2], which contains specialized mitochondria-rich brown adipocytes whose thermogenic functionality is conferred by the uncoupling protein 1 (UCP1) [3, 4]. The presence of thermogenic, UCP1° fat depots in adult humans has been recognized recently using $^{18}$F-FDG.
PET scan [5-7]. In small rodents such as mice and rats, two types of UCP1\(^+\) adipocytes have been distinguished by their localization and developmental origin. Classical, also called constitutive, brown adipocytes are present in interscapular and perirenal adipose tissues, and they originate from Myf5\(^+\)/Pax7\(^+\) skeletal muscle progenitors [8]. In contrast, Ucp1\(^+\) multilocular adipocytes are also found scattered within white adipose tissue (WAT) in response to β-adrenergic (βAR) stimulation [9-13]. These Ucp1\(^+\) multilocular brown adipocytes in WAT are called beige adipocytes (or inducible brown adipocytes), although the multilocular morphology and Ucp1 expression are not always coupled [14, 15]. Notably, beige adipocytes in WAT are functionally identical as brown adipocytes in interscapular brown adipose tissue (iBAT) at single cell level. Indeed, the mitochondria from Ucp1\(^+\) multilocular adipocytes in iWAT and brown adipocytes in iBAT process similar thermogenic capacity in vitro [4].

The beige adipocyte formation is dominantly determined by the strength of βAR signaling in white adipose tissues, including amounts of catecholamines, βAR abundance and its intracellular signaling on target cells. Various sources can release catecholamines to induce browning. Sympathetic activation by cold and hormones has been recognized to be a dominant driver of beige adipocyte recruitment in both rodents and humans [16]. Myeloid cells can synthesize and secrete catecholamines to induce browning, especially under thermoneutral condition in mice [17]. Moreover, excess catecholamine secretion by adrenal chromaffin cells in pheochromocytoma patients also causes extensive beige adipocyte formation [18]. Additionally, increasing βAR signaling by overexpressing Adrb1 could promote beige adipocyte formation [19]. Human UCP1\(^+\) adipocytes are found in individuals exposed to cold environment, but their appearances are negatively correlated with aging and obesity; this feature resembles the plasticity
of beige adipocytes in mice [20-24]. Therefore, increasing beige adipocyte abundance has been considered as an attractive approach to promote metabolic health.

Besides the beige adipocyte population observed in adult mice, substantial amounts of beige adipocytes are present in 3-week-old pups during postnatal development [22]. However, the relationship between beige adipocytes formed postnatally and upon cold stimulation in adult stage is not determined. We have found that the beige adipocytes formed postnatally gradually lost their beigeing properties in adulthood, and cold stimulation can induce restoration of their beigeing properties, a process we termed as beige adipocyte “renaissance”. Liver kinase b1 (Lkb1) and Salt-inducible kinases (SIKs) suppressed βAR-induced histone deacetylase 4 (Hdac4) signaling in hepatocytes [25, 26] and fatbody in Drosophila [26, 27], although their roles in beige adipocyte homeostasis are unknown. Here we showed that specifically deletion of Lkb1 in adipocytes, not in brown and beige adipocytes themselves, led to a sustained beige adipocyte population regardless of βAR stimulation. Furthermore, loss of adipocyte Hdac4 in adipocyte Lkb1 knockout (Lkb1AKO) mice reversed the persistent beige adipocyte expansion phenotype. Lkb1AKO mice were protected against high-fat-diet induced obesity and insulin resistance. However, these metabolic benefits were not due to expanded beige adipocytes or Ucp1-dependent thermogenesis. Our results reveal that white adipocytes play an important role in beige adipocyte maintenance, and further studies are warranted to investigate whether targeting beige adipocyte renaissance could improve metabolic homeostasis.

**Research Design and Methods**

**Mice:** All animal experiments were approved by the UCSF IACUC Committee in adherence to NIH guidelines and policies. Adiponectin-Cre (JAX#010803), Rosa-iDTR (JAX#007900) and
Lkb1<sup>ff</sup> (JAX#014143) were obtained from JAX. Hdac4<sup>ff</sup> and Ucp1-Cre (JAX#024670) mice were provided by Drs. Eric Olson and Evan Rosen. Mice were in the C57Bl/6J background and both sexes were analyzed in this study. Additional mice were described in the Supplementary experimental procedures.

**Beige adipocyte ablation**: 3 week-old male or female mice were administrated with a single diphtheria toxin (DT) injection intraperitoneally at the dose of 10-25 ng/mouse (Biological Laboratories Inc.).

**Results**

**Beige adipocytes formed postnatally perdure but lose beigeing characteristics in adulthood**

In iWAT, *Ucp1* expression was absent in early postnatal period (less than 2-week of age). However, it reached a peak in 3-week-old pups and gradually reduced to baseline in 8-week-old adult mice. Cold stimulation could restore *Ucp1* expression (Fig.1A) [22, 28]. Immunoblots confirmed the gradual decline of Ucp1 protein levels from 3 to 8-week of age, and the reappearance after 7-day cold stimulation in 8-week-old mice (Fig.1B). *Ucp1* expression correlated with beige adipocyte abundance; H&E staining and Ucp1 immunostaining confirmed that ~30% of cells were the multilocular beige adipocytes in 3-week old pups and in cold-treated adult mice (Fig.1C, Supplementary Fig.1A-B), consistent with a previous report [24]. These beige adipocytes formed during postnatal development were referred as postnatal beige adipocytes. To trace the fate of postnatal beige adipocytes in iWAT, we performed Ucp1 immunofluorescence staining on Ucp1-Cre;Rosa-mT/mG mice, where past and current Ucp1<sup>+</sup> adipocytes were permanently labeled with membrane-targeted green fluorescent protein. At 1-week of age, Ucp1-Cre was not expressed in iWAT indicated by the absence of GFP<sup>+</sup> or Ucp1<sup>+</sup>
cells (Fig.1D). In contrast, all brown adipocytes in interscapular brown adipose tissue (iBAT) were GFP+ (meaning Ucp1+), while white adipocytes in epididymal WAT (eWAT) were GFP− (meaning Ucp1−) (Supplementary Fig.1C-D). At 3-week of age, about 30% of cells in iWAT were GFP+;Ucp1+ with multilocular morphology (Fig.1E and G). Although these GFP+ cells were still present in adults (at 8-week of age), they were no longer Ucp1+ anymore (Fig. 1F and G), suggesting that the postnatal beige adipocytes in iWAT lost their beigeing characteristics in adult stage. Although ambient temperature and nutrient status from birth to weaning can temporally affect the abundance of postnatal beige adipocytes, the responsiveness of cold-induced beige adipocyte expansion in adulthood remains intact [29, 30], suggesting that the plasticity of postnatal beige adipocytes is genetically controlled.

**Beige adipocyte renaissance: restoring beigeing characteristics in postnatal beige adipocytes in response to cold**

Beige adipocytes can reappear in iWAT of adult mice under β-adrenergic stimulation [16, 31]. For example, 8°C cold stimulation for one week drastically increased Ucp1 expression and induced the formation of multilocular beige adipocytes in iWAT (Fig.1A,C). Next, we employed an Ucp1-Cre inducible diphtheria toxin receptor (iDTR) mediated cell ablation system [32] (Fig.2A), to determine whether postnatal beige adipocytes and cold-induced beige adipocytes were the same cell population. In this system, current and past Ucp1+ adipocytes express iDTR and can be ablated by diphtheria toxin (DT) injection. The iDTR expression correlated with Ucp1 expression in iWAT of Ucp1-Cre; Rosa-iDTR (abbreviated as Cre+) pups until 3-week of age (Supplementary Fig.2A-B). However, iDTR retained while Ucp1 dropped to baseline in 8-week-old mice (Supplementary Fig.2A-B), which was consistent with the observations in Ucp1-
Cre;Rosa-mT/mG lineage tracing experiments (Fig.1D-F). In 3-week-old Cre+ pups, 3-day post-DT administration successfully ablated beige adipocytes, as Ucp1, Cidea and Cox8b mRNA levels were significantly reduced in iWAT (Fig.2B). Ucp1 protein levels in iWAT were also reduced after DT administration (Fig.2C). Indeed, the ratios of lipid droplets to nuclei correlated with Ucp1 mRNA levels in iWAT before or after DT administration (Fig.2B, Supplementary Fig.3B), confirming that multilocular Ucp1+ beige adipocytes were ablated in iWAT. The thermogenic gene expression in iWAT remained low at 8-week of age (Fig.2C), and the beige adipocytes were absent in iWAT by H&E staining and perilipin immunohistochemistry (Fig.2D, Supplementary Fig.3A). Moreover, increased macrophage markers Arg1, Cd68 and Emr1 in iWAT were only evident in 3-day post DT group (absent in 5-week post DT group) (Supplementary Fig.4A), suggesting there was transient macrophage infiltration after DTM induced beige adipocyte ablation iWAT.

Ucp1-Cre was also active in brown adipocytes in iBAT. Indeed, Cre+ mice had increased macrophage infiltration and reduced Ucp1 expression in iBAT 3-day post DT administration, and their core temperature dropped 8 Celsius degrees during the first hour of cold exposure (Supplementary Fig.4B, 5A-C). However, after one-week post DT injection, Ucp1 expression went back to normal levels in Cre+ mice (Supplementary Fig.5C-D), and they regained cold resistance (Supplementary Fig.5B-D). Histology analysis also revealed a progressive regeneration of iBAT (Supplementary Fig.E). Thus, iBAT exhibited rapid regeneration upon ablation. The differential responses of brown and beige adipocytes after ablation may be due to the fact that iBAT is the first defense mechanism for hypothermia. Once a functional iBAT has been regenerated, there is no need to regenerate beige adipocyte for the thermogenic purpose.
Therefore, the Ucp1-Cre;Rosa-iDTR ablation model offers us an opportunity to specifically manipulate postnatal beige adipocytes \textit{in vivo}.

We then used this ablation model to address the contribution of postnatal beige adipocytes to cold-induced beige adipocyte expansion in adult mice. We administered DT in Cre- and Cre+ mice at 3-week of age to ablate postnatal beige adipocytes, followed by injection of CL316243 (a β3 specific agonist, abbreviated as CL) for 4- or 7-consecutive days at 8-week of age. Although CL strongly induced \textit{Ucp1}, \textit{Cidea} and \textit{Cox8b} mRNA levels in iWAT from Cre- mice, its effect in beige-ablated Cre+ mice was attenuated (Fig.2E). This observation indicated that CL restored \textit{Ucp1} and expression of other thermogenic genes in postnatal beige adipocytes. The similar result was obtained in 7-day 8°C cold stimulation (Fig.2E). However, longer cold stimulation (14-day at 8°C) did induce \textit{Ucp1} expression in beige-ablated Cre+ mice (Fig.2F). This could be due to induction of beige adipocyte adipogenesis from progenitors in adult mice [33-35], which might occur independently of beige adipocyte renaissance. The Pdgfra^+Sca1^+ progenitors were responsible for cold-induced beige adipocyte adipogenesis [31, 36]. These progenitors were present in iWAT and iBAT before and after beige adipocyte ablation (Supplementary Fig.6A-B), suggesting that they may contribute to the cold-induced beige adipocyte adipogenesis in iWAT and BAT regeneration after DT-mediated ablation. In contrast, both CL and cold induced eWAT \textit{Ucp1} expression similarly in postnatal beige adipocyte ablated and non-ablated mice (Supplementary Fig.7), suggesting the involvement of \textit{de novo} adipogenesis in cold-induced beige adipocyte formation in epididymal fat depot.

Collectively, there are two consecutive waves of beige adipocyte formation in iWAT; the first one is due to \textit{de novo} adipogenesis (genesis) during postnatal development and peaks at 3-week of age, and the other one is through restoring the beige characteristics in the postnatal beige
adipocytes by βAR stimulation at adult stage (renaissance) (Fig.2F). Notably, beige adipocyte renaissance and de novo beige adipogenesis are not mutually exclusive; thus new beige adipocytes did form in response to cold in beige-ablated mice, but to a lower degree.

**Salt-inducible kinases/SIKs and Liver kinase b1/Lkb1 suppress beige adipocyte renaissance**

βAR stimulation elevates secondary messenger cyclic adenosine monophosphate/cAMP levels and induces transcriptional program in target cells. Previously we have shown that Salt-inducible kinases/SIKs, a family of AMPK-related kinases, suppress cAMP-induced transcriptional program by phosphorylation and inhibition of two distinct classes of transcription cofactors: CREB regulated transcription coactivators (CRTC) and class IIa histone deacetylases (HDACs) [26, 37, 38]. This SIK-dependent regulation of cAMP transcriptional response is conserved in *C.elegans*, *Drosophila* and mammals, and all SIK members contain a conserved PKA phosphorylation site at their C-terminal [25, 39] (Supplementary Fig.8A-B). Mammalian SIK family contains three members: Sik1, 2 and 3. Sik2 was highly expressed in mature adipocytes compared to stromal-vascular fraction (SVF) cells from various depots, while Sik1 and Sik3 were expressed at lower levels in mature adipocytes (Supplementary Fig.8C). Although roles of SIKs in adipose have been explored [40, 41], whether SIKs regulate beige adipocyte formation remains unknown. We investigated thermogenic gene expression in iWAT from SIK deficient animals. Neither Sik1 or Sik2 global KO mice exhibited obvious changes in *Ucp1* expression in iWAT (data not shown), reflecting functional compensation between SIK members. However, we did observe elevated *Ucp1* expression in iWAT of 8-week-old Sik1;Sik2 KO mice without affecting PKA signaling (Supplementary Fig.8D-E), suggesting that SIKs were downstream components of βAR signaling and they suppressed *Ucp1* expression in iWAT. In
contrast, adipocyte AMPK deficient mice exhibited attenuated browning potential in response to CL stimulation in iWAT [42], suggesting that AMPK and SIKs differentially regulated beige adipocyte expansion in iWAT.

Because the Sik1;Sik2 KO mice employed here were global knockout models, we could not rule out the possibility that SIK deficiency in other tissues contributed to beige adipocyte renaissance in iWAT. We and others have shown that Liver kinase b1/Lkb1-mediated phosphorylation of a conserved Thr residue in the activating loop of SIK kinase domain was required for SIK activation [26, 27]. We used Adiponectin-Cre to generate Adiponectin-Cre;Lkb1^{f/f} (Lkb1^{AKO} mice), in which Lkb1 was specifically deleted in adipose tissues, such as iBAT, iWAT and eWAT (Supplementary Fig.9A). Lkb1 expression in other tissues (liver, spleen, kidney, lung, brain, gut and heart) was not affected (Supplementary Fig.9B). AMPK is another known Lkb1 substrate, and AMPK and its substrate ACC were hypophosphorylated in iWAT of Lkb1^{AKO} mice (Supplementary Fig.9B), confirming the absence of Lkb1 signaling in iWAT of Lkb1^{AKO} mice. We also use Ucp1-Cre to generate Ucp1-Cre;Lkb1^{f/f} (Lkb1^{BKO} mice) to delete Lkb1 in brown and beige adipocytes only. We did not observe a difference in Ucp1 expression or beige adipocyte abundance in 3-week-old Lkb1^{AKO} and Lkb1^{BKO} pups (Fig.3.A-C, Supplementary Fig.10B). We further investigated whether Lkb1 deletion in adipocytes affects beige adipocyte homeostasis in adult mice. At ~8-weeks of age, Lkb1^{AKO} mice exhibited an increase of Ucp1 mRNA and protein levels and a substantial population of beige adipocytes in iWAT compared with control mice (Fig.3A-C). Other thermogenic genes (Cox8b, Cidea and Dio2) were also elevated in iWAT (Supplementary Fig.10A). In contrast, increased Ucp1 expression and expanded beige population in iWAT were not observed in adult Lkb1^{BKO} mice (Fig.3A, Supplementary Fig.10B).
Constant β-adrenergic stimulation is necessary to induce and sustain beige adipocyte population in rodents and humans. As shown in Fig.3D, 4-day consecutively intraperitoneal injection of CL induced Ucp1 expression and beige adipocyte formation, and this effect was lost after 4-day recovery period (without CL). Surprisingly, the CL administration did not further increase iWAT Ucp1 expression in Lkb1^{AKO} mice, and Ucp1 expression was not diminished during the recovery phase (Fig.3D). Similarly, iWAT Ucp1 expression in Lkb1^{AKO} mice was largely unchanged upon either cold stimulation (8°C for 7 days) or withdrawn from the cold environment (7 and 14 days at room temperature after 8°C cold stimulation) (Fig.3D,F). Hence, beigeing characteristics of beige adipocytes, transient in nature, were persistently present in Lkb1^{AKO} mice regardless of cold stimulation. Furthermore, this persistent beige adipocyte expansion did not require Lkb1 signaling in beige adipocytes themselves, as Lkb1^{BKO} mice showed dynamic beige adipocyte profile similar to control mice (Fig.3E). Therefore, Lkb1 deficiency in white adipocytes led to persistent beige adipocyte renaissance in adult mice non-cell autonomously. In the same setting, Ucp1 expression in eWAT was upregulated by CL and dropped to baseline after recovery in both Lkb1^{AKO} and control mice, suggesting there are two different mechanisms for cold-induced beige adipocyte formation in iWAT and eWAT (Supplementary Fig.10C).

Notably, iWAT Ucp1 expression declined in Lkb1^{AKO} mice after two weeks on thermoneutrality (Fig.4A), suggesting that the absence of cold-induced SNS activity totally blocked beige adipocyte formation in Lkb1^{AKO} mice. However, there was no change of PKA activity in iWAT from Lkb1^{AKO} mice (also in Lkb1^{BKO} mice) at 8-week of age at room temperature (Fig.4B), suggesting that the appearance of beige adipocytes in Lkb1^{AKO} mice was not due to an increase of SNS activity in iWAT. This observation was also further confirmed by
no changes of tissue catecholamine levels (Fig.4C) or sympathetic nerve density (by tyrosine hydroxylase immunostaining) in iWAT of Lkb1\textsuperscript{AKO} mice (Fig.4D-E). Collectively, at thermoneutrality (with no thermal stress) there is no beige adipocyte in both control and Lkb1\textsuperscript{AKO} mice, due to lack of SNS activity and catecholamines; while at room temperature (with mild thermal stress for mice), Lkb1 deficiency in white adipocytes augments intracellular PKA-dependent transcriptional response and triggers beige adipocyte expansion in iWAT without increasing upstream PKA activity. Therefore, Lkb1 is functioning as a brake to suppress βAR-PKA signaling in white adipocyte during beige adipocyte renaissance.

**Lkb1 suppresses beige adipocyte renaissance through Hdac4 inhibition**

We and others have shown that deletion of class IIa HDAC4 fully rescued phenotypes of SIK3 and Lkb1 mutants in *Drosophila* [26]. Indeed, we observed that CL stimulation induced Sik2 phosphorylation at Ser587 and Hdac4 dephosphorylation at Ser245 in iWAT (Fig.5A). Hdac4 was also hypophosphorylated in adipocytes from iWAT of Lkb1\textsuperscript{AKO} mice (Fig.5B). We then examined whether adipocyte class IIa Hdac4 activation was required for beige adipocyte expansion in Lkb1\textsuperscript{AKO} mice. The adipocyte Lkb1;Hdac4 double KO (abbreviated as Lkb1;Hdac4\textsuperscript{AKO}) mice had no expanded beige adipocytes in iWAT, similar to wild-type and adipocyte Hdac4 KO (Adiponectin-Cre;Hdac4\textsuperscript{fl/fl}, abbreviated as Hdac4\textsuperscript{AKO}) mice (Fig.5C). In addition, Lkb1;Hdac4\textsuperscript{AKO} mice regained responsiveness to cold and cold plus recovery regarding *Ucp1* expression, similar to wild-type and Hdac4\textsuperscript{AKO} mice (Fig.5D). Furthermore, RNA-seq analysis revealed that 436 genes were upregulated in iWAT of 8-week-old Lkb1\textsuperscript{AKO} mice at normal chow, and roughly 40% of these genes were regulated in an Hdac4-dependent fashion (Fig.5E-F, and Supplementary table 2). The top five gene-ontology (GO) categories of Hdac4-
dependent transcripts that were upregulated in Lkb1^{AKO} mice included mitochondrial metabolic and muscle development processes (Fig.5G). We further confirmed that adipocyte Hdac4 deficiency rescued or partially rescued elevated expression levels of some thermogenic signature genes (including *Ucp1*, *Dio2* and *Cidea*) and metabolic genes (including *Cox7a1*, *Acaa2*, *Gyk*, *Ldhd* and *Scl27a1*) in Lkb1^{AKO} mice (Fig.5H). All data here suggests that Hdac4 is indeed a key downstream component of Lkb1 signaling in adipocytes regulating beige adipocyte renaissance in iWAT.

**Lkb1^{AKO} mice exhibit improved metabolic performance under high-fat diet regardless of beige adipocyte expansion and Ucp1-mediated thermogenesis**

We next investigated whether deletion of Lkb1 in adipocytes affected metabolic performance. We did not observe noticeable metabolic changes in Lkb1^{AKO} mice under normal chow (NC) condition. Compared with controls, Lkb1^{AKO} mice had identical growth rates until ~6-month of age, similar fasting glucose and insulin sensitivity (Supplementary Fig.11A-C). *In vitro* lipolysis rate and CL-induced free fatty acid release *in vivo* were not affected in Lkb1^{AKO} mice (Supplementary Fig.11D-E), which was consistent with previous observation that PKA activity was not altered in Lkb1^{AKO} mice (Fig.4B). CLAMS studies indicated that Lkb1^{AKO} mice had similar energy expenditure, RER, food intake, physical activity, and other parameters compared to their control littermates (Supplementary Fig.12A-L).

HFD feeding can whiten the beige adipocyte. Interestingly, beige adipocyte population in Lkb1^{AKO} mice even persisted after 4-5 week of HFD feeding (Fig.6A-B). In fact, enhanced expression of thermogenic genes *Ucp1*, *Dio2* and *Pgc1α* in both NC and HFD conditions were also observed through screening the expression levels of ~200 key genes involved in
adipogenesis and lipid metabolism pathways (Supplementary Fig.13A). Furthermore, the thermogenic gene signature identified by RNA-seq from Lkb1\textsuperscript{AKO} mice at normal chow was also present after HFD (Supplementary Fig.13B). Since substantial beige adipocyte population was absent in Lkb1\textsuperscript{BKO} mice (Fig.6A-B), we then compared metabolic performance of Lkb1\textsuperscript{AKO} and Lkb1\textsuperscript{BKO} mice under short-term HFD. Surprisingly, both Lkb1\textsuperscript{AKO} and Lkb1\textsuperscript{BKO} mice showed reduced weight gain under HFD and similar insulin resistance (Fig. 6C-E). CLAMS studies of Lkb1\textsuperscript{AKO} mice after HFD showed no differences in organismal oxygen consumption (when normalized per lean body weight or per animal), RER and food intake (Supplementary Fig.14A-C), although there was a slight increase of physical activities (Supplementary Fig.14D-F). There were no differences in fasting glucose, serum insulin and FFA levels in Lkb1\textsuperscript{AKO} mice under short-term HFD (Supplementary Fig.15.A-C).

We have shown previously that Hdac4 was required for beige adipocyte expansion in Lkb1\textsuperscript{AKO} mice. Lkb1;Hdac4\textsuperscript{AKO} mice did not have expanded beige adipocytes (Fig.5C-D), however, they still showed reduced adiposity and improved insulin sensitivity (Fig.6F-H). No differences were observed in Hdac4\textsuperscript{AKO} mice compared to control mice. Therefore, beige adipocytes do not contribute to metabolic benefits in Lkb1\textsuperscript{AKO} mice.

In order to further determine whether Ucp1-mediated adaptive thermogenesis was required for adiposity phenotype of Lkb1\textsuperscript{AKO} mice under HFD, we then generated Lkb1\textsuperscript{AKO} mice in Ucp1 KO background (Lkb1\textsuperscript{AKO};Ucp1 KO mice). Lkb1\textsuperscript{AKO};Ucp1 KO mice still maintained multilocular beige adipocytes in iWAT under HFD, consistent with elevated thermogenic gene expression (\textit{Dio2}, \textit{Cox8b}, \textit{Cidea}, \textit{Pgc1a}) (Fig.7A-B). They also showed reduced adiposity under HFD (Fig.7C-D). Therefore, we conclude that Ucp1-dependent thermogenesis do not contribute to leanness in Lkb1\textsuperscript{AKO} mice under HFD. Taken together, comparison of the metabolic
performance of Lkb1\(^{\text{AKO}}\) mice (with beige expansion) with Lkb1\(^{\text{BKO}}\) (without beige expansion), Lkb1;Hdac4\(^{\text{AKO}}\) (without beige expansion) and Lkb1\(^{\text{AKO}}\);Ucp1 KO mice (without Ucp1-mediated adaptive thermogenesis) further suggests that neither beige adipocytes nor Ucp1-dependent adaptive thermogenesis contributes to metabolic benefits in Lkb1\(^{\text{AKO}}\) mice.

**Lkb1 regulates thermogenic capacity in iBAT independently of Hdac4**

Since Lkb1\(^{\text{BKO}}\), Lkb1\(^{\text{AKO}}\), and Lkb1;Hdac4\(^{\text{AKO}}\) mice had Lkb1 deficiency in classical brown adipocytes, we also investigated whether adaptive thermogenesis was affected in these three mouse models. Their brown adipocyte morphology in iBAT was not significantly altered, although \(Ucp1\) expression in iBAT exhibited a trend of upregulation in Lkb1\(^{\text{AKO}}\) and Lkb1\(^{\text{BKO}}\) mice compared with control (Supplementary Fig.16A-B). However, Lkb1\(^{\text{AKO}}\) mice were cold sensitive; they failed to maintain their core temperature in a 4\(^\circ\)C cold tolerance test, although they could maintain their core temperature when we group-housed mice during 4\(^\circ\)C cold challenge (Supplementary Fig.16C). Hdac4 was also hypophosphorylated in iBAT of Lkb1\(^{\text{AKO}}\) mice (Supplementary Fig.16D). However, Hdac4 activation in BAT of Lkb1\(^{\text{AKO}}\) mice did not affect Ucp1 expression (Supplementary Fig.16E). And the thermogenic defects in Lkb1\(^{\text{AKO}}\) mice was Hdac4 independent; Lkb1;Hdac4\(^{\text{AKO}}\) mice remained cold-sensitive as Lkb1\(^{\text{AKO}}\) mice (Supplementary Fig.16F).

Recently, Shan et al showed that Lkb1\(^{\text{AKO}}\) mice exhibited enhanced adaptive thermogenesis and improved metabolic performance under HFD [43]. The discrepancy of the role of Lkb1’s deficiency on adaptive thermogenesis could originate from the use of different methodologies. For example, we used single-housed animals for cold-tolerance assay, because
group-housed Lkb1\textsuperscript{AKO} mice remained cold resistant as littermate controls (Supplementary Fig.15C). Also, we normalized O\textsubscript{2} consumption per lean body weight or per mouse in CLAMS experiments, especially after HFD, as previously suggested in the literature [44, 45]. Collectively, our results suggest that Lkb1 regulates two different pathways in iWAT and iBAT; in iWAT, Lkb1 regulates beige adipocyte renaissance in an Hdac4M-dependent manner (Fig.8); while in iBAT, it controls thermogenic capacity independently of Hdac4. How Lkb1 signaling in iBAT regulates adaptive thermogenesis and energy homeostasis requires further studies.

**Discussion**

Multiple lines of investigations have established that beige adipocytes arose from either 	extit{de novo} adipogenesis from progenitors or transdifferentiation from white adipocytes under various conditions [23, 24, 31, 33, 35, 46]. Granneman’s laboratory previously reported that iWAT denervation at 3-week of age impaired CL’s ability to reinstate beige adipocyte characteristics at adult stage [24, 28]. However, the underlying cellular and molecular mechanisms are not addressed. Our studies indicate that cold induces beige adipocyte renaissance in adult mice; it restores the “beigeing” properties in the postnatal beige adipocytes, which have lost their beige properties in iWAT in adulthood. At morphological level, our renaissance model is similar to the transdifferentiation model; both suggest a drastic white-to-beige morphology change without new adipocyte formation. However, the distinct feature of beige adipocyte renaissance is that white-like adipocyte converted to beige adipocyte by cold was once Ucp1\textsuperscript{+} beige adipocyte during postnatal development. Thus, not every white adipocyte in iWAT can be transdifferentiated to beige adipocyte in response to cold. Wolfrum’s laboratory had also reported that more than 70\% of cold-induced beige adipocytes came from white adipocytes with
Ucp1-expressing memory [23]. Based on lineage tracing experiments using Ucp1-Cre;RosamT/mG mice (Fig.1D-G), we expected that at adult stage, about 30% of cells in iWAT were Ucp1+ at 3-week of age, and they could regain Ucp1 expression and other beiging properties after cold stimulation. This number is in agreement with previous studies; both Granneman’s and Graff’s laboratories showed 30-40 % of cold-induced beige adipocytes were from preexisting white adipocytes labeled by adiponectin-CreERT2 in adult mice [24, 35].

Beige adipocyte renaissance may be the default mechanism of beige adipocyte formation in iWAT, which can provide a rapid adaptive response to cold (restoring thermogenic machinery rather than generating new thermogenic adipocytes). The de novo beige adipocyte adipogenesis program can be activated under persistent cold stimulation [33] or in the absence of beige adipocyte renaissance. Interestingly, postnatal beige adipocytes may potentially stimulate recruitment of progenitors upon cold stimulation, since postnatal beige adipocyte ablation suppressed cold-induced beige adipocyte formation from progenitors (Fig.2F). Notably, beige adipocyte renaissance is a depot specific phenomenon; it does not occur in epididymal WAT.

We further delineate an Lkb1-Hdac4 dependent mechanism in white adipocytes that governs the beige adipocyte renaissance non-cell autonomously (Fig.8). Lkb1AKO mice exhibited persistent beige adipocyte population regardless of cold stimulation, while removing Hdac4 in Lkb1AKO mice restored responsiveness to cold (Fig. 5D), suggesting that Hdac4 activation in adipocytes determines dynamic behavior of beige adipocytes. Further studies are warranted to investigate how beige adipocytes reprogram during their transitions (beige to white and to beige again by cold), and what are the Hdac4-dependent and white adipocyte derived signals that maintain characteristics of preexisting beige adipocytes in iWAT.
Classic brown adipocytes and beige adipocytes are capable of Ucp1-dependent thermogenesis. The classic brown adipocytes may be the major heat-producing Ucp1$^+$ adipocytes in the setting of hypothermia. In our Ucp1-Cre;Rosa-iDTR mice, iBAT regeneration occurred rapidly after DT-mediated ablation of all Ucp1$^+$ brown and beige adipocytes. The regenerated iBAT itself sufficiently rendered cold resistance regardless of beige adipocyte numbers. However, beige adipocytes could contribute to thermogenesis significantly in the absence of functional iBAT. Mice lacking Bmpr1a in Myf5$^+$ lineage cells exhibited iBAT defect and compensatory increase in beige adipocytes [47]. However, a compensatory increase of sympathetic inputs to iWAT at room temperature was not observed in Lkb1$^{AKO}$ mice. One of possible explanations is that metabolic reprogramming in mitochondria caused by Lkb1 deficiency [48] might be sufficient to cope with mild cold stress (room temperature housing), but not with strong cold stress (4°C cold tolerance test).

Improved metabolic performance accompanied by beige adipocyte expansion was also observed in Ucp1 knockout mice alone or together with the inactivation of glycerol phosphate cycle [49, 50], however, it has not been demonstrated whether expanded beige adipocytes did functionally contribute to their elevated energy expenditure and/or decreased adiposity under HFD in vivo. Our studies suggest that thermogenic capacity and metabolic homeostasis are uncoupled in Lkb1$^{AKO}$ mice. For example, mice with Ucp1-Cre mediated deletion of Lkb1 specifically in brown and beige adipocytes, the Lkb1$^{BKO}$ mice, showed similar metabolic improvements under HFD without beige adipocyte expansion. This suggests that Lkb1 deficiency in iBAT alone may improve metabolic performances in Lkb1$^{AKO}$ mice, although we cannot absolutely rule out possible contributions of Lkb1 deletion in other adipose depots. Beige adipocyte expansion is dependent on Hdac4 activation in white adipocytes. Removing Hdac4
demolishes beige adipocyte population in iWAT of Lkb1\textsuperscript{AKO} mice in adult mice without thermogenic or metabolic consequences. Therefore, beige adipocytes do not contribute to metabolic benefits observed in Lkb1\textsuperscript{AKO} mice. However, beige adipocytes may influence energy homeostasis through thermogenic and non-thermogenic functions in other settings. Since Lkb1 regulates SIKs-Hdac4 pathway specifically in white adipocytes to promote beige adipocyte renaissance in adult mice, additional animal models that only target the Lkb1-dependent beige adipocyte renaissance program and spontaneously preserve iBAT thermogenesis may reveal specific functions of beige adipocytes and novel therapeutic approaches to tackle metabolic disorders.

Accession numbers

The accession number for RNA-seq data reported in the manuscript is GEO: GSE85335.

Supplementary Information

Supplementary information includes Supplementary Experimental Procedures, 9 supplementary figures, and 2 supplementary tables.

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**Figure 1. Beige adipocyte dynamics in iWAT.**

A, q-PCR analysis of Ucp1 mRNA levels in iWAT from wild-type B6 male and female mice at different ages and under 8°C cold treatment. n=3-10. B, Immunoblots showing protein amounts of Ucp1, and Hsp90 in iWAT from B6 male and female mice at different age. C, Representative H&E staining of iWAT from 1, 3, 8 week-old, and after 7-day 8°C cold treatment. Insert: Arrows showing multilocular beige adipocytes. Scale bar: 50µm. Confocal images of membrane GFP (mG), membrane Tomato (mT), and Ucp1 (by immunostaining) in iWAT from 1w (D), 3w- (E) and 8w- (F) old Ucp1-Cre;Rosa-mT/mG mice. Scale bar: 100µm (20µm for inserts). G. Statistics of GFP+ and Ucp1+ positive adipocytes at 3w- and 8w-old of age.
Figure 2. Beige adipocyte renaissance in response to cold in adult mice. A, Schematic of the beige adipocyte ablation mouse model. Ucp1-Cre mice were crossed with Rosa-iDTR mice to generate Ucp1-Cre;Rosa-iDTR mice. B, q-PCR analysis of mRNA levels of thermogenic genes Ucp1, Cidea and Cox8b in iWAT from Rosa-iDTR (Cre-) and Ucp1-Cre;Rosa-iDTR (Cre+) mice before and post DT administration. No DT: No DT injection; 3d post DT: 3 days after DT injection; 5w post DT: 5 weeks after DT injection. DT was injected at 3-week of age. Sample sizes: No DT Cre- (n=5), No DT Cre+ (n=4), 3d post DT Cre- (n=13), 3d post DT Cre+ (n=8), 5w post DT Cre- (n=6) and 5w post DT Cre+ (n=6). Both male and female mice were used. C, Immunoblots of Ucp1 and Hsp90 in iWAT from Cre- and Cre+ mice at indicated time points. D, Representative H&E staining of iWAT from Cre- and Cre+ mice at indicated time points. Scale bar: 200µm. Arrows: multilocular beige adipocytes. E, q-PCR analysis of Ucp1, Cidea and Cox8b mRNA levels in iWAT from ~8-week-old male and female Cre- and Cre+ mice treated with PBS or CL (0.2 mg/kg for 4-consectutive days, or 1 mg/kg for 7-consectutive days), 7 or 14-day 8°C cold. DT was injected at 3-week of age. Sample sizes: PBS Cre- (n=6), PBS Cre+ (n=4), 4d_CL Cre- (n=5), 4d_CL Cre+ (n=5), 7d_CL Cre- (n=6), 7d_CL Cre+ (n=4), 7d_Cold Cre- (n=9), 7d_Cold Cre+ (n=9), 14d_Cold Cre- (n=6) and 14d_Cold Cre+ (n=6). F, Diagram showing the life of beige adipocyte. “Genesis” refers to de novo beige adipocyte formation from progenitors during postnatal phase, while “renaissance” refers to the process that once Ucp1+ white adipocytes regain beiging properties (Ucp1 and other thermogenic gene expression and multilocular morphology) upon cold in adult mice.
Figure 3. Lkb1 in white adipocyte suppresses beige adipocyte renaissance.  

A, q-PCR analysis of *Ucp1* mRNA levels in iWAT from male Lkb1^Ef^ (CON), Adiponectin-Cre;Lkb1^Ef^ (Lkb1^AKO^) and Ucp1-Cre;Lkb1^Ef^ (Lkb1^BKO^) mice at 1, 3 and 8-week of age. Sample sizes: 1w CON (n=17), 1w Lkb1^AKO^ (n=4), 1w Lkb1^BKO^ (n=6), 3w CON (n=14), 3w Lkb1^AKO^ (n=13), 3w Lkb1^BKO^ (n=2), 8w CON (n=12), 8w Lkb1^AKO^ (n=9) and 8w Lkb1^BKO^ (n=3).  

B, Immunoblots showing protein amounts of Ucp1 and Hsp90 in iWAT from 3 and 8-week-old male CON and Lkb1^AKO^ mice.  

C, Representative H&E staining of iWAT from 3 and 8-week-old CON and Lkb1^AKO^ male mice. Scale bar: 50 µm.  

D, q-PCR analysis of *Ucp1* mRNA levels in iWAT from 8-week-old male and female CON and Lkb1^AKO^ mice at different conditions. CL: 0.2 mg/kg for 4 days; CL+4d recovery: CL injections followed by 4 days without CL; Cold: 8°C for 7 days; Cold+7d (or14d) recovery: 8°C cold followed by 7 (or 14) days on room temperature. Sample sizes: PBS CON (n=16), PBS Lkb1^AKO^ (n=13), CL CON (n=5), CL Lkb1^AKO^ (n=3), CL+4d recovery CON (n=4), CL+4d recovery Lkb1^AKO^ (n=5), Cold CON (n=14), Cold Lkb1^AKO^ (n=10), Cold+7d recovery CON (n=3), cold+7d recovery Lkb1^AKO^ (n=5), Cold+14d recovery CON (n=3) and Cold+14d recovery Lkb1^AKO^ (n=5).  

E, q-PCR analysis of *Ucp1* mRNA levels in iWAT from 8-week-old male and female CON and Lkb1^BKO^ mice after CL and CL plus 4-day recovery conditions. Sample sizes: CL CON (n=14), CL Lkb1^BKO^ (n=6), CL+4d recovery CON (n=6) and CL+4d recovery Lkb1^BKO^ (n=9).  

F, Representative H&E staining of iWAT from 8-week-old male CON and Lkb1^AKO^ mice at cold and cold plus 7 days recovery conditions. Scale bar: 50 µm.
Figure 4. Sympathetic activities in iWAT of Lkb1\textsuperscript{AKO} mice. A. q-PCR analysis of \textit{Ucp1} mRNA levels in iWAT from 8-weeks old male CON and Lkb1\textsuperscript{AKO} mice housed at room temperature and thermoneutral conditions. Sample sizes: CON/RT (n=12), Lkb1\textsuperscript{AKO}/RT (n=9), CON/30 (n=6) and Lkb1\textsuperscript{AKO}/30 (n=4). B. Immunoblots of p-PKA substrates, Ucp1 and Hsp90 in iWAT from ~8-weeks old male CON and Lkb1\textsuperscript{AKO} and Lkb1\textsuperscript{BKO} mice housed at RT or 30°C. C. Relative iWAT tissue catecholamine levels in ~8-weeks old male CON and Lkb1\textsuperscript{AKO} mice. Sample sizes: CON (n=5) and Lkb1\textsuperscript{AKO} (n=5). Representative Tyrosine hydroxylase (Th) immunostaining (D) and counts of Th+ fibers (E) in iWAT from ~8-weeks old male CON and Lkb1\textsuperscript{AKO} mice.
Figure 5. Adipocyte Hdac4 activation is required for beige adipocyte renaissance. A, Immunoblots showing pS245 and total Hdac4, pS587 and total Sik2, p-PKA substrates and Hsp90 in iWAT from 8-week-old male C57Bl/6J mice, before and 30 minutes after 1 mg kg$^{-1}$ CL injection. B, Immunoblots showing pS245 and total Hdac4 and Hsp90 in isolated iWAT adipocytes from 8-week-old male CON and Lkb1$^{AKO}$ mice. C, Representative H&E staining of iWAT of 8-week-old male CON, Hdac4$^{AKO}$ and Lkb1;Hdac4$^{AKO}$ mice at normal chow. Scale bar: 100µm. D, q-PCR analysis of Ucp1 mRNA levels in iWAT from 8-week-old male and female CON and Lkb1;Hdac4$^{AKO}$ mice. Sample sizes: RT CON (n=13), RT Hdac4$^{AKO}$ (n=4), RT Lkb1;Hdac4$^{AKO}$ (n=14), Cold CON (n=7), Cold Hdac4$^{AKO}$ (n=7), Cold Lkb1;Hdac4$^{AKO}$ (n=10), Cold+recovery CON (n=18), Cold+recovery Hdac4$^{AKO}$ (n=6) and Cold+recovery Lkb1;Hdac4$^{AKO}$ (n=13). E, Clustering analysis and heatmap of differentially expressed genes (1.5 fold up or down) of iWAT from 8-weeks old male CON, Lkb1$^{AKO}$ and Lkb1;Hdac4$^{AKO}$ mice at normal chow housed at room temperature. F, Pie chart analysis of Hdac4 dependency of upregulated genes. G, List of gene ontology (GO) number, description, count, percentage, and Log10(p) value of the top five processes enriched in Hdac4-dependent genes that were upregulated in Lkb1$^{AKO}$ mice. H, q-PCR analysis of a panel of beige adipocyte selected genes in iWAT from 8-week-old male and female CON, Lkb1$^{AKO}$, Lkb1;Hdac4$^{AKO}$ and Hdac4$^{AKO}$ mice. Sample sizes: CON (n=10), Lkb1$^{AKO}$ (n=8), Lkb1;Hdac4$^{AKO}$ (n=10) and Hdac4$^{AKO}$ (n=4).
Figure 6. Adipocyte Lkb1 deficient mice exhibit improved metabolic performances under high-fat diet independently of beige adipocyte expansion. A, q-PCR analysis of Ucp1 mRNA levels in iWAT from 10-12 week-old male CON, Lkb1\(^{AKO}\) and Lkb1\(^{BKO}\) mice after 4-5-week HFD. Sample sizes: CON (n=18), Lkb1\(^{AKO}\) (n=10) and Lkb1\(^{BKO}\) (n=6). B, Representative H&E staining of iWAT from male CON, Lkb1\(^{AKO}\) and Lkb1\(^{BKO}\) mice after 4-5-week HFD. Scale bar: 50\(\mu\)m. Body weight (C) and body weight gain (D) upon HFD of male CON, Lkb1\(^{AKO}\) and Lkb1\(^{BKO}\) mice. Sample sizes: CON (n=71), Lkb1\(^{AKO}\) (n=34) and Lkb1\(^{AKO}\) (n=30). E, Insulin tolerance tests of male CON, Lkb1\(^{BKO}\) and Lkb1\(^{AKO}\) mice after HFD. Sample sizes: CON (n=30), Lkb1\(^{AKO}\) (n=28) and Lkb1\(^{BKO}\) (n=9). Body weight (F) and body weight gain (G) upon HFD of male CON, Hdac4\(^{AKO}\) and Lkb1;Hdac4\(^{AKO}\) mice. Sample sizes: CON (n=13), Hdac4\(^{AKO}\) (n=5) and Lkb1;Hdac4\(^{AKO}\) (n=11). H, Insulin tolerance test of male CON and Lkb1;Hdac4\(^{AKO}\) mice after 4-5-week HFD. Sample sizes: CON (n=7), Hdac4\(^{AKO}\) (n=5) and Lkb1;Hdac4\(^{AKO}\) (n=9).
Figure 7. Ucp1-mediated adaptive thermogenesis does not contribute to metabolic benefits in Lkb1\(^{AKO}\) mice under HFD. 

A, q-PCR analysis of Dio2, Pgc1α, Cidea and Cox8b mRNA levels in iWAT from male CON, Lkb1\(^{AKO}\), Ucp1 KO and double Lkb1\(^{AKO}\);Ucp1 KO mice after 4-week HFD. Sample sizes: CON (n=6, genotype: Lkb1\(^{ff}\)), Lkb1\(^{AKO}\) (n=4), Ucp1 KO (n=4, genotype: Lkb1\(^{ff}\);Ucp1 KO) and Lkb1\(^{AKO}\);Ucp1 KO (n=9). 

B, Representative H&E staining of iWAT from male Ucp1 KO and Lkb1\(^{AKO}\);Ucp1 KO mice after 4-5-week HFD. Scale bar: 50µm.

Body weight (C) and body weight gain (D) upon HFD of male CON, Lkb1\(^{AKO}\);Ucp1 het and Lkb1\(^{AKO}\);Ucp1 KO mice. Sample sizes: CON (n=12, including 4 Lkb1\(^{ff}\);Ucp1 heterozygotes and 8 Lkb1\(^{ff}\);Ucp1 KO), Lkb1\(^{AKO}\);Ucp1 het (n=5) and Lkb1\(^{AKO}\);Ucp1 KO (n=7).
Figure 8. Diagram showing cellular and molecular mechanisms of cold-induced beige adipocyte renaissance. Under cold stimulation, adipocyte Hdac4 activation could promote beige adipocyte renaissance non-cell autonomously. Lkb1 activates SIKs to suppress Hdac4 activation, while cold stimulates Hdac4 activation through βAR-mediated inhibition of SIKs. Additional mechanisms of cold-induced beige adipocyte de novo formation demonstrated in other studies not shown here.
Beige adipocyte dynamics in iWAT

502x322mm (300 x 300 DPI)
Beige adipocyte renaissance in response to cold in adult mice

400x260mm (300 x 300 DPI)
Lkb1 in white adipocyte suppresses beige adipocyte renaissance

371x198mm (300 x 300 DPI)
Sympathetic activities in iWAT of Lkb1AKO mice

Fig. 4
Adipocyte Hdac4 activation is required for beige adipocyte renaissance

Diabetes
Adipocyte Lkb1 deficient mice exhibit improved metabolic performances under high-fat diet independently of beige adipocyte expansion.
Ucp1-mediated adaptive thermogenesis does not contribute to metabolic benefits in Lkb1AKO mice under HFD

361x207mm (300 x 300 DPI)
Fig. 8

Diagram showing cellular and molecular mechanisms of cold-induced beige adipocyte renaissance

158x151mm (300 x 300 DPI)
Supplementary information

Supplementary experimental procedures

**Mice:** Rosa-mT/mG (JAX#007576) mice were obtained from JAX. Two independent ES cells of Sik3 conditional allele from EUCOMM (Sik3<sup>tm1a(EUCOMM)Hmguy</sup>) were injected into C57Bl/6 mice, and the founders were initially crossed with ACTB-FLPe to remove lacZ and neo cassettes to get Sik3 flox allele.

**Browning of white adipose tissue:** For cold stimulation experiments, mice were placed in an 8°C rodent chamber (Power Scientific RIS52SD Rodent Incubator) for 7 or 14 days with or without recovery phase at room temperature. For CL 316,243 treatment, mice were injected intraperitoneally with 0.2 mg g<sup>-1</sup> daily for 4 days or 1 mg g<sup>-1</sup> daily for 7 days. To identify beige adipocytes, subcutaneous inguinal fat (iWAT) was isolated and Ucp1 RNA and protein levels were analyzed. Paraffin sections and hematoxylin & eosin (H&E) staining were performed at AML Labs using standard protocols.

**Cold tolerance test (CTT):** 6-8-week-old male mouse was single-housed with free-access to food and water during CTT. The core temperatures prior to and during 4°C cold exposure (at one hour interval) were measured using BAT-12 Microprobe Thermometer with probe RET-3 (Physitemp). Cold challenge was started at 11am (4 hours after light on).

**Metabolic measurements:** Oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>) and respiratory exchange ratio (RER) were measured using CLAMS (Columbus Instruments), installed under a constant temperature-controlled environment, under a 12 hour light: dark cycle with free access to food and water. The raw data were normalized per lean body weight or per mouse [1, 2]. Magnetic resonance imaging scans for fat and lean mass were performed using an
Echo MRI-100 instrument according to manufacturer’s instructions. Investigators were blinded to mice genotypes for CLAMS, which were performed by the UCSF Diabetes and Endocrinology Research Center Metabolic Research Unit. For high fat diet (HFD) studies, 6-week-old mice were transferred to a 60% fat diet (Research Diets, D12492) for additional 4 weeks at room temperature. Two times per week, body weight and food intake were monitored. For GTT, mice were fasted overnight and then injected with glucose (1.5 g kg⁻¹, intraperitoneally). For ITT, mice were fasted 4-6h before intraperitoneal administration of insulin (Humulin; 0.75U kg⁻¹). For both tests glucose was measured from tail vein at indicated time points with a glucometer (Contour, Bayer). Free fatty acid (WAKO Chemicals) levels were determined by ELISA. For in vitro lipolysis mice were fasted for 6 hours and 20 mg of fat tissues were collected and incubated at 37°C in modified Krebs-Ringer buffer (121 mM NaCl, 5 mM KCl, 0.5 mM MgCl, 0.4 mM NaH₂PO₄, and 1 mM CaCl₂) supplemented with 1% free fatty acid BSA, 0.1% glucose, and 20 mM HEPES. Glycerol release before or after 20 µM Forskolin (FSK) was determined by Free Glycerol Reagent (Sigma, F6428). Investigators were not blinded to genotypes.

**Tissue catecholamine measurement**: Catecholamines levels were measured by ELISA (Rocky Mountain Diagnostics, # BA E-6600) according to the manufacturer’s protocol. Tissues were homogenized in homogenization buffer (0.01N HCl, 1 mM EDTA, 4 mM Na₂S₂O₅), and centrifuged at 13,000 rpm for 15 min at 4°C. The supernatants were collected and stored at −80°C prior to quantification. All measurements were normalized to total tissue protein concentration.

**RNA-seq**: iWAT RNAs of ~8-week-old CON, Lkb1AKO and Lkb1;Hdac4AKO male mice (n=5 each genotype) were isolated using RNeasy Mini Kit (Qiagen). RNA-seq was performed at the
UCSF Next Generation Sequencing Service (NGS Core), analyzed at the Gladstone Bioinformatics Core and deposited at GEO [3]. Briefly, cDNA libraries were prepared using Ovation RNA-seq Systems V2 and Ovation Ultralow Library Systems V2 (Nugen) according to manufacturer’s instructions. cDNA libraries were run on 100bp single-end reads on the HiSeq 2500 System (Illumina) in UCSF Genomics Core. Data analysis was performed by Gladstone Bioinformatics Core. Briefly, known adapters and low-quality regions of reads were trimmed using Fastq-mcf, and sequence quality control was assessed using the program FastQC and RSeQC. Reads were aligned to the mm9 reference assembly using "featureCounts", part of the Subread suite (http://subread.sourceforge.net/) [4, 5]. We calculated differential expression P-values using edgeR, an R package available through Bioconductor [6]. We used the built-in R function "p.adjust" to calculate the FDR (false discovery rate) for each P-value, using the Benjamini-Hochberg method. Heat map was generated by ClustVis and GO analysis was performed by Metascape [7].

Q-PCR: RNA from cells and tissues was extracted using RNeasy Mini Kit (QIAGEN). Total RNA (1 µg) was reverse-transcribed by iScript™ cDNA synthesis kit (Bio-Rad) and cDNA was used for real time PT-PCR (CFX384, Bio-Rad), using 2 ng of cDNA template and a primer concentration of 400 nM. Values were normalized to 36b4 housekeeping gene. Primer sequences were listed in Supplementary table 1. Obesity and Adipogenesis Plates with predesigned primers (Bio-Rad) were used.

Western blot: Tissues were homogenized on ice in lysis-buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 6 mM EGTA, 20 mM NaF, 1% Triton X-100, and protease inhibitors) for 15–20 min. After centrifugation at 13000 rpm for 15 min, supernatants were reserved for protein quantification followed by SDS-PAGE analysis. The following antibodies were used: anti-
**Immunostaining:** Frozen sections of iWAT were washed in PBS for 2x10 min, and treated with 0.1% Triton X-100 in PBS for 5 min. Wheat germ agglutinin (WGA Alex FluroTM 488, ThermoFisher, #W11261) was applied to stain membrane. Slides were blocked by 5% goat serum and incubated in TH (1:400, Millipore #AB152) or Ucp1 (1:500, Abcam #ab10983) antibodies at 4 °C overnight. After 3 times washing, slides were incubated in with fluorescent secondary antibody (1:200, Thermo). Then the slides were washed again, stained with DAPI and mounted. Fluorescence was observed using Leica SPE confocal microscope. TH positive nerves were counted in each field, and normalized to the counts in control sections.

Immunohistochemistry was performed using standard protocol. Briefly, iWAT tissues were dissected, fixed in 10% formalin and subsequently stored in 70% ethanol prior to paraffin embedding. For Perilipin immunohistochemistry, 4 µM sections of iWAT were incubated with anti-perilipin (1:1000, D1D8, Cell signaling) overnight, followed by detection with ABC detection kit (004303, Invitrogen), and counterstaining with hematoxylin (VWR, 95057-844). Lipid droplet and nuclei were counted in ImageJ software in a double-blind fashion.

**Flow Cytometry:** iBAT and iWAT were minced and then digested in 2 ml of Collagenase I buffer (2 mg ml⁻¹ at 250U/mg, Worthington, and 30 mg ml⁻¹ bovine serum albumin in Hams F-10 medium) at 37°C for 45-60 min. The homogenates were washed with PBS and filtered (70 µm) prior to immunostaining for flow cytometric analysis. Cell suspensions were stained with ZombieAqua (1:1000), anti-CD45 (30-F11, 1:200), anti-CD31 (390, 1:200), anti-Sca1 (D7,
1:200), anti-Pdgfrα (APA5, 1:200) for 30 mins in FACS buffer (PBS, 5 mM EDTA, and 2.5% FBS). All antibodies were from Biolegend. They were then spin down, resuspended in FACS buffer, and analyzed on a BD FACSVerse flow cytometer.

**Statistical analysis:** We used GraphPad Prism 6.0 to assess data for normal distribution and similar variance between groups. Data were presented as the mean ± s.d. Statistical significance was determined using an unpaired two-tailed Student’s *t* test with unequal variance between groups: ns: not significant, *: p<0.05 and **: p<0.01. We selected sample size for animal experiments based on numbers typically used in similar published studies. We did not perform randomization of animals or predetermine sample size by a statistical method. *In vivo* metabolic measurements such as body weight and ITT were repeated 2-3 times, and *in vitro* measurements of glycerol and FFA were done with 3 technical replicates.
Supplementary Fig.1. Ucp1 immunostaining in wild-type and Ucp1-Cre;Rosa-mT/mG mice. A, Confocal images of Ucp1, wheat germ agglutinin (WGA), and DAPI in iWAT from wild-type mice at 3-week-old (3w), 8-week-old (8w), or with 7-days 8°C stimulation (Cold). Scale bar: 100 µm. B. Statistics of Ucp1+ positive adipocytes at 3w-, 8w-old of age and with Cold stimulation. Confocal images of membrane GFP (mG), membrane Tomato (mT), and DAPI in iBAT (C) and eWAT (D) from 1-week-old Ucp1-Cre;Rosa-mT/mG pups. Scale bar: 25 µm.
Supplementary Fig.2. *Ucp1* and *iDTR* expression in Ucp1-Cre;Rosa-iDTR mice in iWAT.

t-PCR analysis of *Ucp1* (A) and *iDTR* (B) mRNA levels in iWAT from 1, 3 and 8-week-old male and female Rosa-iDTR (Cre-) and Ucp1-Cre;Rosa-iDTR (Cre+) mice. Sample sizes: 1w Cre- (n=4), 1w Cre+ (n=6), 3w Cre- (n=4), 3w Cre+ (n=4), 8w Cre- (n=2) and 8w Cre+ (n=2).
Supplementary Fig. 3. Perilipin immunohistochemical staining in iWAT before and after DT. 

A, Representative IHC staining of perilipin in iWAT of Rosa-iDTR (Cre-) and Ucp1-Cre;Rosa-iDTR (Cre+) mice before (No DT), 3-day (3d post DT) and 5-week (5w post DT) after DT administration. 

B, Ratios of lipid droplets to nuclei (LD/NUC) in iWAT of Cre- and Cre+ mice before, 3-day and 5-week after DT administration.
Supplementary Fig. 4. Inflammatory gene expression in iWAT and iBAT before or after DT. q-PCR analysis of mRNA levels of macrophage markers *Arg1*, *Cd68* and *Emr1* in iWAT (A) and iBAT (B) of male and female Rosa-iDTR (Cre-) and Ucp1-Cre;Rosa-iDTR (Cre+) mice before and post DT administration. No DT: No DT injection; 3d post DT: 3 days after DT injection; 5w post DT: 5 weeks after DT injection. DT was injected at 3-week of age. Sample sizes: No DT Cre- (n=5), No DT Cre+ (n=4), 3d post DT Cre- (n=13), 3d post DT Cre+ (n=8), 5w post DT Cre- (n=6) and 5w post DT Cre+ (n=6).
Supplementary Fig.5. iBAT shows an active regeneration after ablation.  A, CTT of male and female mice 3 days post DT injection. Sample size: Cre- (n=5) and Cre+ (n=4).  B, CTT of male and female mice 7 days post DT injection. Sample size: Cre- (n=5) and Cre+ (n=5).  C, qPCR analysis of mRNA levels of Ucp1 in iBAT from male and female Rosa-iDTR (Cre-) and Ucp1-Cre;Rosa-iDTR (Cre+) mice before and post DT administration. No DT: no DT injection; 3d post DT: 3 days after DT injection; 5w post DT: 5 weeks after DT injection. DT was injected at 3-week of age. Sample sizes: No DT Cre- (n=5), No DT Cre+ (n=4), 3d post DT Cre- (n=13), 3d post DT Cre+ (n=8), 5w post DT Cre- (n=6) and 5w post DT Cre+ (n=6).  D, Immunoblots of Ucp1 and Hsp90 in iBAT from Cre- and Cre+ mice at indicated time points.  E, Representative H&E staining of iBAT from Cre+ mice prior, 3 days and 5 weeks post DT injection. Scale bar: 100 µm.
Supplementary Fig. 6. Abundance of Pdgfra$^+$Sca1$^+$ progenitors in iWAT and iBAT before or after DT. Numbers of Pdgfra$^+$Sca1$^+$ progenitors in iWAT (A) and iBAT (B) of male and female Rosa-iDTR (Cre-) and Ucp1-Cre; Rosa-iDTR (Cre+) mice before and post DT administration. No DT: No DT injection; 3d post DT: 3 days after DT injection; 5w post DT: 5 weeks after DT injection. DT was injected at 3-week of age. Sample sizes: No DT Cre- (n=4), No DT Cre+ (n=4), 3d post DT Cre- (n=8), 3d post DT Cre+ (n=8), 5w post DT Cre- (n=8) and 5w post DT Cre+ (n=10).
Supplementary Fig. 7. Ablation of beige adipocytes does not affect cold-induced Ucp1 expression in eWAT. q-PCR analysis of Ucp1 mRNA levels in eWAT from ~10-week-old male and female Cre- and Cre+ mice treated with PBS or CL or cold. Sample sizes: PBS Cre- (n=5), PBS Cre+ (n=5), 4d_CL Cre- (n=4), 4d_CL Cre+ (n=4), 7d_CL Cre- (n=5), 7d_CL Cre+ (n=5), 4d_Cold Cre- (n=3), 4d_Cold Cre+ (n=4), 14d_Cold Cre- (n=6) and 14d_Cold Cre+ (n=4).
Supplementary Fig. 8. Effects of SIK deficiency on Ucp1 expression in iWAT. A, Schematic showing SIK family members in different species using HYPERTREE view. ce: Caenorhabditis elegans; dm: Drosophila melanogaster; sp: Strongylocentrotus purpuratus; mm: Mus musculus; hs: Homo sapiens. B, Up: Schematic of SIKs with catalytic and ubiquitin association (UBA) domain shown. *: Regulatory phosphorylation site by protein kinase A (PKA). Bottom: Alignment of PKA phosphorylation sites in SIK members. C, q-PCR analysis of Sik1, Sik2, and Sik3 mRNA levels of mature adipocytes and SVF cells in interscapular BAT (iBAT), inguinal WAT (iWAT) and epididymal WAT (eWAT) from ~8-week-old C57Bl/6 male mice. n=3-4. D, E, q-PCR analysis for Ucp1 mRNA and immunoblots for Ucp1, phosphor-PKA substrates and Hsp90 in iWAT from 8-10 week-old male and female control (CON) and Sik1;Sik2 double knockout (Sik1/Sik2 KO) mice. Sample sizes: n=4 for both genotypes.
Supplementary Fig. 9. Lkb1 is specifically deleted in adipose tissues in Lkb1^{AKO} mice. A, Immunoblots showing Lkb1 and Hsp90 in iBAT, iWAT and eWAT of ~8-week-old male CON and Lkb1^{AKO} mice. B, Immunoblots showing Lkb1 and Hsp90 in liver, spleen, kidney, lung, brain and heart of ~8-week-old male CON and Lkb1^{AKO} mice. B, Immunoblots showing pS79 and total Acc, pT172 and total AMPK and Hsp90 in iWAT of 8-week-old male CON and Lkb1^{AKO} mice.
Supplementary Fig.10. Thermogenic program in Lkb1^{AKO} mice. A, q-PCR analysis of Ucp1, Cox8b, Cidea and Dio2 mRNA levels in iWAT from male CON and Lkb1^{AKO} mice at 3- and 8-week of age. Samples sizes: 3w CON (n=11), 3w Lkb1^{AKO} (n=10), 8w CON (n=10) and 8w Lkb1^{AKO} (n=6). B, Immunoblots showing protein amounts of Ucp1 and Hsp90 in iWAT from 3 and 8-week-old male CON and Lkb1^{BKO} mice. C, q-PCR analysis of Ucp1 mRNA levels in eWAT from 8-week-old male CON and Lkb1^{AKO} mice at different conditions. CL: 0.2 mg/kg for 4 days; CL+4d recovery: CL injections followed by 4 days without CL. Sample sizes: PBS CON (n=5), PBS Lkb1^{AKO} (n=2), CL CON (n=3), CL Lkb1^{AKO} (n=3), CL+4d recovery CON (n=3) and CL+4d recovery Lkb1^{AKO} (n=3).
Supplementary Fig.11. Metabolic performance of Lkb1\textsuperscript{AKO} mice under normal chow. A, Body weight of male CON and Lkb1\textsuperscript{AKO} mice at different ages. Sample sizes: CON (n=43) and Lkb1\textsuperscript{AKO} (n=21). B,C, Insulin and glucose tolerance tests of 8-week-old male CON and Lkb1\textsuperscript{AKO} mice. Sample sizes: CON (n=7) and Lkb1\textsuperscript{AKO} (n=4). D, Glycerol release from eWAT isolated from ~8-week-old male CON and Lkb1\textsuperscript{AKO} mice before and after 20mM Forskolin (FSK) stimulation. Sample sizes: n=5 for both CON and Lkb1\textsuperscript{AKO}. E, Serum free-fatty acid levels in ~8-week-old male CON and Lkb1\textsuperscript{AKO} mice before and after 2 mg kg\textsuperscript{-1} CL injection. Sample sizes: CON (n=6) and Lkb1\textsuperscript{AKO} (n=4).
Supplementary Fig.12. CLAMS measurements of Lkb1\textsuperscript{AKO} mice under normal chow.  

A, Average of oxygen consumption during day and night of 8-week-old male CON and Lkb1\textsuperscript{AKO} mice. B, Oxygen consumption recordings of 8-week-old male CON and Lkb1\textsuperscript{AKO} mice over 3-day period in a CLAMS experiment. C, Transient changes of oxygen consumption after 0.2 mg g\textsuperscript{-1} CL injection. Red arrow: CL injection. All values are calculated per lean body mass. D, E, F, Similar to F, G, H, but the values are calculated per mouse. G, H, RER and food intake recordings of CON and Lkb1\textsuperscript{AKO} mice. I, Average food intakes from day and night of CON and Lkb1\textsuperscript{AKO} mice. J, K, Recordings of ambulatory and total activities of CON and Lkb1\textsuperscript{AKO} mice. L, Average ambulatory and total activities from day and night of CON and Lkb1\textsuperscript{AKO} mice. Sample sizes: CON (n=6) and Lkb1\textsuperscript{AKO} (n=4).
Supplementary Fig.13. iWAT thermogenic gene expression in Lkb1\textsuperscript{AKO} mice under HFD.

A, Volcano plot showing differentially expressed genes in iWAT of male CON and Lkb1\textsuperscript{AKO} mice under NC and HFD conditions. Thermogenic genes \textit{Ucp1}, \textit{Dio2} and \textit{Pgc1\alpha} circled. B, q-PCR analysis of beige adipocyte selective genes in iWAT from male CON and Lkb1\textsuperscript{AKO} mice under 4-5-week HFD. Sample sizes: CON (n=12) and Lkb1\textsuperscript{AKO} (n=8).
Supplementary Fig.14. CLAMS measurements of Lkb1\textsuperscript{AKO} mice under HFD. A, Oxygen consumption recordings of male CON and Lkb1\textsuperscript{AKO} mice after 4-5-week HFD in a CLAMS experiment. All values are calculated as per lean body mass. B, RER recording of CON and Lkb1\textsuperscript{AKO} mice after HFD. C, Average food intakes from day and night of CON and Lkb1\textsuperscript{AKO} mice after HFD. D, E, Recordings of ambulatory and total activities of CON and Lkb1\textsuperscript{AKO} mice after HFD. F, Average ambulatory and total activities from day and night of CON and Lkb1\textsuperscript{AKO} mice after HFD. Sample sizes: CON (n=6) and Lkb1\textsuperscript{AKO} (n=4).
Supplementary Fig.15. Measurements of serum metabolites in Lkb1\textsuperscript{AKO} mice under HFD.

A, Fasting glucose levels in ~10-12-week-old male CON and Lkb1\textsuperscript{AKO} mice at normal chow and after 4-week-HFD. Sample size: CON/NC (n=19), Lkb1\textsuperscript{AKO}/NC (n=7), CON/HFD (n=8), Lkb1\textsuperscript{AKO}/HFD (n=7).

B, Insulin levels in ~10-12-week-old male CON and Lkb1\textsuperscript{AKO} mice at normal chow and after 4-week-HFD. Sample size: CON/NC (n=6), Lkb1\textsuperscript{AKO}/NC (n=4), CON/HFD (n=9), Lkb1\textsuperscript{AKO}/HFD (n=8).

C, Serum FFA levels in ~10-12-week-old male CON and Lkb1\textsuperscript{AKO} mice at normal chow and after 4-week-HFD. Sample size: CON/NC (n=5), Lkb1\textsuperscript{AKO}/NC (n=3), CON/HFD (n=6), Lkb1\textsuperscript{AKO}/HFD (n=4).
Supplementary Fig.16. Defective adaptive thermogenesis in adipose Lkb1 deficient mice.

A. Representative H&E staining of iBAT from 6-8 week-old male CON, Lkb1\textsuperscript{AKO} and Lkb1\textsuperscript{BKO} mice. B, q-PCR analysis of Ucp1 mRNA levels in iBAT from 8-week-old male CON, Lkb1\textsuperscript{AKO}, and Lkb1\textsuperscript{BKO} mice. Sample sizes: CON (n=12), Lkb1\textsuperscript{BKO} (n=4) and Lkb1\textsuperscript{AKO} (n=8). C, Core temperature of ~8-week-old male and female CON and Lkb1\textsuperscript{AKO} mice upon 4°C cold challenge in both single- and group-housed conditions. Sample sizes: CON/single-housed (n=12), Lkb1\textsuperscript{AKO}/single-housed (n=20), CON/group-housed (n=5) and Lkb1\textsuperscript{AKO}/group-housed (n=5). D, Immunoblots showing pS245, total Hdac4 and Hsp90 in iBAT from 8-week-old male CON and Lkb1\textsuperscript{AKO} mice. E, q-PCR analysis of Ucp1 mRNA levels in iBAT from 8-week-old male CON, Lkb1\textsuperscript{AKO}, Hdac4\textsuperscript{AKO} and Lkb1;Hdac4\textsuperscript{AKO} mice. Sample sizes: CON (n=5), Hdac4\textsuperscript{AKO} (n=5), Lkb1\textsuperscript{AKO} (n=5), and Lkb1;Hdac4\textsuperscript{AKO} (n=5). F, Core temperature of 8-week-old male and female CON, Hdac4\textsuperscript{AKO} and Lkb1;Hdac4\textsuperscript{AKO} mice upon 4°C cold challenge. Sample sizes: CON (n=6), Hdac4\textsuperscript{AKO} (n=3) and Lkb1;Hdac4\textsuperscript{AKO} (n=5).
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Supplementary Fig. 1
Supplementary Fig. 2
Supplementary Fig.4
Supplementary Fig. 5
Supplementary Fig. 6
Supplementary Fig. 7
Supplementary Fig. 8
Supplementary Fig.9
Supplementary Fig. 10
Supplementary Fig. 14
Supplementary Fig. 15

A

B

C

362x106mm (300 x 300 DPI)
Supplementary Fig. 16
Supplementary table 1: List of primer sequences for q-PCR

| Primer | Sequence            |
|--------|---------------------|
| 36B4-F | TTTGGGCATCACCACGAAA |
| 36B4-R | GGACACCCCTCCAGAAAGCG |
| iDTR1-F | CTTGCCACTGGATCTACGGAC |
| iDTR1-R | TGCTTTGTCCTGGAGGATA |
| iDTR2-F | GCTGCAATTCTTTCGGCAC |
| iDTR2-R | CGTATAGTCTCGGAAGGT |
| Ucp1-F | ACTGCCACACCTCAGTCAT |
| Ucp1-R | CGTATAGTCTCGGAAGGT |
| Lkb1-F | TGGAGGACAGGCTCCTCCAG |
| Lkb1-R | TGCTTTGTCCTGGAGGATA |
| Sik1-F | TGGACGTCTGGAGCCTCGGT |
| Sik1-R | CGTATAGTCTCGGAAGGT |
| Sik2-F | TGGAGGACAGGCTCCTCCAG |
| Sik2-R | TGCTTTGTCCTGGAGGATA |
| Sik3-F | TGGAGGACAGGCTCCTCCAG |
| Sik3-R | TGCTTTGTCCTGGAGGATA |
| Cox8b-F | TGGAGGACAGGCTCCTCCAG |
| Cox8b-R | TGCTTTGTCCTGGAGGATA |
| Cidea-F | TGGAGGACAGGCTCCTCCAG |
| Cidea-R | TGCTTTGTCCTGGAGGATA |
| Dio2-F | TGGAGGACAGGCTCCTCCAG |
| Dio2-R | TGCTTTGTCCTGGAGGATA |
| Pgc1a-F | TGGAGGACAGGCTCCTCCAG |
| Pgc1a-R | TGCTTTGTCCTGGAGGATA |
| Plin5-F | TGGAGGACAGGCTCCTCCAG |
| Plin5-R | TGCTTTGTCCTGGAGGATA |
| Cox7a1-F | TGGAGGACAGGCTCCTCCAG |
| Cox7a1-R | TGCTTTGTCCTGGAGGATA |
| Acaa2-F | TGGAGGACAGGCTCCTCCAG |
| Acaa2-R | TGCTTTGTCCTGGAGGATA |
| Gyk-F | TGGAGGACAGGCTCCTCCAG |
| Gyk-R | TGCTTTGTCCTGGAGGATA |
| Ldhb-F | TGGAGGACAGGCTCCTCCAG |
| Ldhb-R | TGCTTTGTCCTGGAGGATA |
| Slec27a2-F | TGGAGGACAGGCTCCTCCAG |
| Slec27a2-R | TGGAGGACAGGCTCCTCCAG |
| Elov13-F | TGGAGGACAGGCTCCTCCAG |
| Elov13-R | TGGAGGACAGGCTCCTCCAG |
| Letmd1-F | TGGAGGACAGGCTCCTCCAG |
| Letmd1-R | TGGAGGACAGGCTCCTCCAG |
| Arg1-F | TGGAGGACAGGCTCCTCCAG |
| Arg1-R | TGGAGGACAGGCTCCTCCAG |
| Cd68-F | TGGAGGACAGGCTCCTCCAG |
| Cd68-R | TGGAGGACAGGCTCCTCCAG |
| Emr1-F | TGGAGGACAGGCTCCTCCAG |
| Emr1-R | TGGAGGACAGGCTCCTCCAG |
Supplemental table 2: List of genes upregulated in Lkb1AKO mice in an Hdac4-dependent manner.

| Gene           | CON   | Lkb1<sup>AKO</sup> | Lkb1;Hdac4<sup>AKO</sup> |
|----------------|-------|---------------------|---------------------------|
| Tbx3           | 16.942| 24.146              | 21.35                     |
| Gm9949         | 0.102 | 0.852               | 0.542                     |
| Uxt            | 8.88  | 12.59               | 11.148                    |
| Gm9978         | 0.142 | 1.104               | 0.698                     |
| 2210411K11Rik  | 1.08  | 2.828               | 2.09                      |
| Gm5806         | 0.78  | 2.08                | 1.524                     |
| Dapk2          | 3.074 | 5.036               | 4.25                      |
| Trmt5          | 13.39 | 21.79               | 18.342                    |
| Gm17357        | 0.288 | 1.444               | 0.944                     |
| Pcdh12         | 7.708 | 11.496              | 9.98                      |
| 4933400A11Rik  | 0.758 | 2.332               | 1.586                     |
| Aqp4           | 0.588 | 1.914               | 1.298                     |
| Tmem147        | 10.942| 17.782              | 14.784                    |
| Mrps33         | 9.004 | 16.308              | 13.062                    |
| Arxes1         | 6.034 | 15.004              | 10.696                    |
| Gm14966        | 2.296 | 4.254               | 3.398                     |
| Myo5b          | 2.658 | 4.834               | 3.884                     |
| Gm11839        | 0.194 | 1.088               | 0.688                     |
| Yars2          | 14.756| 21.366              | 18.574                    |
| Peg3           | 232.814| 471.466             | 361.83                    |
| Ldhb           | 48.916| 89.77               | 71.552                    |
| Tmem181a       | 2.132 | 4.24                | 3.328                     |
| Acaa2          | 106.96| 233.714             | 174.754                   |
| Cacng5         | 0     | 4.048               | 1.76                      |
| Tmem45b        | 105.562| 242.024             | 177.676                   |
| Gmpr           | 1.554 | 5.398               | 3.554                     |
| Tmed5          | 155.47| 298.92              | 234.818                   |
| Khdrbs3        | 3.436 | 9.998               | 6.882                     |
| Gtf2ird1       | 9.818 | 17.44               | 14.156                    |
| Gm10822        | 0.302 | 1.314               | 0.954                     |
| 9530034E10Rik  | 0.21  | 1.342               | 0.924                     |
| Gm15247        | 0.348 | 1.162               | 0.876                     |
| 1700084J12Rik  | 0.698 | 2.26                | 1.682                     |
| Ces4a          | 0     | 0.352               | 0.23                      |
| Suclg1         | 51.108| 106.704             | 85.032                    |
| Ogdh           | 271.322| 396.908             | 353.408                   |
| Ankrd40        | 92.516| 135.354             | 120.508                   |
| Chchd7         | 14.244| 21.148              | 18.542                    |
| Gm15793        | 3.702 | 8.568               | 6.55                      |
| Msrb2          | 7.02  | 11.54               | 9.802                     |
| Sirt3          | 6.146 | 10.658              | 8.874                     |
| Otop1          | 20.578| 112.1               | 63.728                    |
| Gene     | Value1  | Value2  | Value3  |
|----------|---------|---------|---------|
| Zfp770   | 20.624  | 34.402  | 28.976  |
| Cox7a1   | 6.974   | 37.942  | 22.426  |
| Idh3g    | 138.004 | 221.492 | 189.996 |
| Gnao1    | 9.784   | 17.674  | 14.608  |
| Gpx1     | 264.43  | 401.47  | 350.278 |
| Hspe1    | 59.008  | 89.858  | 74.09   |
| 4930432B10Rik | 0.754 | 2.06    | 1.382   |
| Nr4a1    | 8.958   | 20.096  | 14.042  |
| Ucp1     | 24.508  | 790.618 | 166.778 |
| Dtx4     | 27.456  | 43.94   | 35.548  |
| Cxadr    | 16.166  | 25.274  | 20.674  |
| BC034090 | 4.444   | 9.392   | 6.792   |
| Aifm2    | 69.172  | 102.364 | 86.002  |
| Hspb6    | 27.824  | 47.03   | 37.22   |
| Atf5     | 43.052  | 69.862  | 56.248  |
| Sucl2    | 100.642 | 253.918 | 171.122 |
| Cbs      | 1.292   | 4.026   | 2.582   |
| Pde4d    | 31.742  | 64.318  | 57.432  |
| Ak1      | 2.514   | 7.744   | 4.908   |
| Tiparp   | 48.298  | 75.122  | 62.15   |
| Scp2     | 365.726 | 504.594 | 438.044 |
| Gypa     | 0       | 0.316   | 0.168   |
| Lrrc48   | 0.578   | 2.108   | 1.314   |
| Gm12141  | 2.306   | 5.78    | 4.034   |
| Aspg     | 3.412   | 6.756   | 5.14    |
| Krt79    | 0.664   | 6.016   | 2.878   |
| B230369F24Rik | 3.606 | 6.134   | 4.96    |
| Adra1a   | 23.952  | 58.502  | 40.174  |
| Agk      | 16.37   | 23.55   | 20.222  |
| Gm12346  | 0.992   | 2.144   | 1.596   |
| mt-Nd1   | 5728.496| 10676.274| 8224.25 |
| Tuba8    | 4.552   | 9.444   | 6.62    |
| Gm13341  | 3.718   | 6.062   | 4.78    |
| Rbak     | 8.908   | 13.862  | 11.176  |
| Slc16a1  | 41.164  | 119.288 | 71.168  |
| Gyk      | 10.33   | 50.908  | 23.658  |
| Car2     | 5.116   | 9.248   | 6.96    |
| Cav2     | 140.162 | 192.19  | 164.938 |
| Pank1    | 23.174  | 55.122  | 36.474  |
| Pde4a    | 18.542  | 27.964  | 22.96   |
| Adrbk2   | 39.712  | 57.536  | 48.148  |
| Rgs7     | 20.794  | 43.234  | 29.05   |
| Gm10032  | 0.924   | 4.078   | 2.012   |
| Xylt1    | 11.782  | 17.548  | 14.162  |
| Slc7a6   | 22.902  | 40.122  | 30.046  |
| Cald1    | 243.166 | 422.84  | 318.832 |
| Gene          | Value1 | Value2 | Value3 |
|--------------|--------|--------|--------|
| Hrsp12       | 7.45   | 12.89  | 9.774  |
| Jun          | 21.294 | 40.48  | 28.788 |
| Hr           | 16.038 | 29.592 | 21.476 |
| Adam3        | 0.308  | 2.362  | 1.05   |
| Pdk4         | 130.554| 446.918| 234.348|
| Cdkn1a       | 22.792 | 43.152 | 30.906 |
| Azgp1        | 0      | 0.5    | 0.168  |
| Gm13574      | 0.526  | 1.744  | 0.896  |
| Acadvl       | 10.928 | 16.974 | 12.832 |
| Ifrd1        | 92.602 | 140.804| 107.592|
| Srl          | 11.956 | 30.914 | 16.84  |
| Mospd1       | 37.446 | 73.26  | 47.524 |
| Tomm5        | 16.296 | 28.402 | 19.812 |
| 9530008L14Rik| 7.844  | 15.834 | 10.018 |
| Ncoa7        | 30.008 | 46.762 | 34.95  |
| C030037D09Rik| 3.142  | 6.638  | 4.436  |
| Kng2         | 0      | 5.104  | 1.168  |
| Lipt1        | 6.622  | 10.196 | 7.982  |
| Snx24        | 7.552  | 13.398 | 9.706  |
| Mmachc       | 22.24  | 31.436 | 25.822 |
| Fer1l5       | 1.944  | 3.724  | 2.622  |
| Bend3        | 12.892 | 19.138 | 15.324 |
| Gm14421      | 3.492  | 7.842  | 4.866  |
| SLEC25A42    | 37.06  | 58.788 | 44.424 |
| Hspb1        | 10.242 | 21.98  | 13.986 |
| 9430025C20Rik| 2.518  | 5.56   | 3.512  |
| S100b        | 5.646  | 40.192 | 12.744 |
| Chpt1        | 473.694| 757.668| 571.238|
| SLEC25A17    | 31.936 | 63.838 | 42.652 |
| BC0397771    | 9.55   | 14.88  | 11.532 |
| Vnn1         | 11.016 | 44.97  | 20.062 |
| Hadhb        | 402.212| 596.258| 474.378|
| Palld        | 22.864 | 35.352 | 27.378 |
| Letmd1       | 46.712 | 111.004| 66.416 |
| Gstk1        | 23.466 | 33.028 | 26.99  |
| Fbp2         | 0.474  | 1.62   | 0.74   |
| Sall4        | 0      | 0.748  | 0.174  |
| mt-Nd3       | 357.762| 760.746| 444.434|
| Gm13814      | 0.206  | 1.554  | 0.482  |
| Arxes2       | 33.708 | 72.398 | 41.644 |
| Hpcal4       | 1.412  | 3.364  | 1.846  |
| Gm7707       | 1.32   | 3.384  | 1.774  |
| Cyp4v3       | 83.942 | 123.872| 93.652 |
| Hsp90aa1     | 151.37 | 256.63 | 179.57 |
| Mcee         | 12.804 | 19.676 | 14.77  |
| 1700012A16Rik| 1.6    | 3.598  | 2.096  |
| Gene   | Value1 | Value2  | Value3  |
|--------|--------|---------|---------|
| Lox    | 73.07  | 115.538 | 83.972  |
| BC024139 | 1.066  | 4.752   | 1.838   |
| Scoc   | 44.054 | 61.612  | 48.976  |
| Gm13141 | 1.268  | 3.74    | 1.676   |
| Shisa4 | 2.242  | 4.94    | 2.704   |
| Cd59a  | 97.76  | 140.424 | 105.856 |
| Gm17082 | 4.476  | 7.656   | 5.144   |
| 2900053O20Rik | 0.4  | 1.636   | 0.624   |
| U6     | 0.372  | 2.164   | 0.684   |
| Myl1   | 73.296 | 140.236 | 85.866  |
| Esrrg  | 9.684  | 24.01   | 12.116  |
| Clic5  | 30.582 | 69.952  | 37.312  |
| Zfand6 | 38.726 | 77.786  | 44.078  |
| Clic4  | 338.372| 477.854 | 360.648 |
| Pkp2   | 23.666 | 51.698  | 27.48   |
| Cxcr7  | 33.13  | 53.194  | 36.288  |
| Gm13152| 3.512  | 6.86    | 4.05    |
| Grhl3  | 0.184  | 1.764   | 0.396   |
| Trib1  | 47.078 | 76.266  | 51.712  |
| 6330403K07Rik | 0.188 | 1.24    | 0.346   |
| B230216N24Rik | 8.862 | 13.242  | 9.608   |
| Tacr2  | 0      | 0.61    | 0.078   |
| Pla2g5 | 0.94   | 3.564   | 1.242   |
| Tbx5   | 7.076  | 14.388  | 8.05    |
| mt-Co2 | 3826.792 | 5440.592 | 4077.188 |
| Cidea  | 18.18  | 33.146  | 12.524  |
| Ttl7   | 6.17   | 10.98   | 4.48    |
| Dusp6  | 15.164 | 21.278  | 15.888  |
| Elovli3| 0.186  | 3.428   | 0.382   |
| Acot11 | 8.692  | 25.368  | 9.858   |
| mt-Nd2 | 3181.696 | 6985.448 | 3518.822 |
| Gm17067| 0      | 1.54    | 0.122   |
| 2700089E24Rik | 66.552 | 134.65  | 72.732  |
| Gm13641| 0      | 0.782   | 0.036   |
| Fabp3  | 1.132  | 6.96    | 1.292   |
| Arhgef19 | 0.504 | 2.138   | 0.564   |
| Gm15197| 0.184  | 1.058   | 0.24    |
| Jph1   | 4.354  | 9.352   | 4.604   |
| Ubc    | 87.182 | 136.838 | 90.066  |
| 4930538K18Rik | 0.21 | 1.4     | 0.232   |
| Smyd1  | 4.644  | 11.122  | 4.774   |
| Lgr6   | 0.094  | 2.128   | 0.138   |
| Gm11772| 0.28   | 1.126   | 0.308   |
| mt-Atp8| 175.5  | 246.534 | 176.096 |
| Klk9   | 0.302  | 1.688   | 0.318   |
| Grb14  | 21.516 | 33.888  | 21.682  |
| Gene       | Mean 1 | Mean 2 | Mean 3 |
|------------|--------|--------|--------|
| Tpm1       | 163.58 | 244.814| 162.988|
| Slc47a1    | 0.234  | 2.102  | 0.234  |
| Pkia       | 13.88  | 24.338 | 13.902 |
| Adamtsl3   | 5.958  | 11.074 | 4.724  |
| Gm10800    | 0.756  | 7.036  | 0.186  |
| Slc40a1    | 12.696 | 20.802 | 11.028 |
| Gm10925    | 301.874| 422.332| 285.104|
| Slc27a2    | 3.878  | 13.562 | 3.244  |
| Gm13111    | 9.608  | 13.98  | 9.05   |
| 2310042D19Rik | 2.196 | 4.616  | 1.948  |
| Tceanc     | 8.618  | 13.978 | 8.026  |
| Rbfox1     | 1.194  | 4.252  | 1.086  |
| Ampd1      | 5.798  | 17.342 | 5.194  |

Note: The average of normalized CPM values are shown.