



Feedback regulation of Arid5a and Ppar- γ 2 maintains adipose tissue homeostasis

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Immune cells infiltrate adipose tissues and provide a framework to regulate energy homeostasis. However, the precise underlying mechanisms and signaling by which the immune system regulates energy homeostasis in metabolic tissues remain poorly understood. Here, we show that the AT-rich interactive domain 5A (Arid5a), a cytokine-induced nucleic acid binding protein, is important for the maintenance of adipose tissue homeostasis. Long-term deficiency of Arid5a in mice results in adult-onset severe obesity. In contrast, transgenic mice overexpressing Arid5a are highly resistant to high-fat diet-induced obesity. Inhibition of Arid5a facilitates the in vitro differentiation of 3T3-L1 cells and fibroblasts to adipocytes, whereas its induction substantially inhibits their differentiation. Molecular studies reveal that Arid5a represses the transcription of peroxisome proliferator activated receptor gamma 2 (Ppar- γ 2) due to which, in the absence of Arid5a, Ppar- γ 2 is persistently expressed in fibroblasts. This phenomenon is accompanied by enhanced fatty acid uptake in Arid5a-deficient cells, which shifts metabolic homeostasis toward prolipid metabolism. Furthermore, we show that Arid5a and Ppar- γ 2 are dynamically counterregulated by each other, hence maintaining adipogenic homeostasis. Thus, we show that Arid5a is an important negative regulator of energy metabolism and can be a potential target for metabolic disorders.

Arid5a | obesity | Ppar- γ | adipogenesis | cytokines

A significant population of metabolic tissues consists of immune cells, comprising mainly macrophages and lymphocytes. Although this evolutionarily conserved anatomical relationship was noticed long ago, the diverse roles of immune cells in metabolic tissue have remained poorly understood. A widely accepted traditional role of immune cells in metabolic tissue is to defend the tissue against pathogens. Apart from this role, emerging evidence indicates that immune cells dictate energy homeostasis (1). Particularly, cytokines secreted by immune cells have been shown to participate in energy regulation during physiological and pathological conditions (2). During exercise, interleukin 6 (IL-6) and transforming growth factor β are produced, improving glucose and fat metabolism (3). Moreover, during cancer cachexia and infectious conditions, excess production of cytokines drastically increases energy consumption, often resulting in weight loss (4). In contrast, cytokine deficiency or inhibition, particularly of IL-6, have been reported to lead to the development of obesity in mice and humans (5, 6). While several lines of evidence suggest that infiltrated immune cells induce chronic low-grade inflammation in adipose tissues, which might be the cause of metabolic disorders such as insulin resistance, recent evidence suggests that low-grade inflammation provided by cytokines in metabolic tissue is actually not bad but, rather, is a part of normal physiology that is necessary to maintain

adipogenic homeostasis (7). Although several in vitro and in vivo studies have provided a strong foundation demonstrating that cytokines regulate energy metabolism, the precise underlying mechanisms and signaling by which cytokines play a role in maintaining energy homeostasis are poorly understood.

AT-rich interactive domain 5a (Arid5a) is a member of the ARID protein family (8). It is rapidly expressed within a few hours upon activation of TLR4 signaling and upon direct stimulation by several cytokines, mainly IL-6 and IL-17 (9, 10). Arid5 proteins were initially discovered as a protein bound to the transcriptional modulator region of the human cytomegalovirus major immediate-early promoter (11). Recently, we discovered that Arid5a stabilizes messenger RNA of IL-6 by competing with IL-6 mRNA destabilizer Regnase1, resulting in stabilization of the IL-6 concentration in vivo over a longer period (12). Subsequently, we demonstrated its ability to stabilize several other mRNAs, including Stat3, Tbet, and Ox40, by binding to their 3' prime untranslated regions (3'UTRs) (13–15). Furthermore, we revealed its critical involvement in the inflammation milieu. Arid5a-deficient mice are resistant to experimental autoimmune

Significance

Obesity is now considered an epidemic problem worldwide. To develop effective interventions against obesity, we need to understand precisely the regulatory mechanism of adipogenesis, a process of forming adipocytes from fibroblast cells. In this study, we revealed that the Arid5a protein regulates the adipogenesis and the development of obesity in mice. Arid5a inhibits adipogenesis by suppressing the transcription of peroxisome proliferator activated receptor gamma 2 (Ppar- γ 2), which is the main facilitator of adipogenesis. Further, we showed adipose tissue homeostasis is maintained by the coordinated activity of Arid5a and Ppar- γ 2. Our study provides important insights into the novel mechanism of adipose tissue homeostasis.

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encephalomyelitis (EAE), bleomycin-induced lung injury, and septic shock (12, 15, 16).

In this article, we demonstrate a critical role for *Arid5a* in the regulation of energy homeostasis in adipose tissues. Modulation of *Arid5a* expression in vitro and in vivo severely affects adipogenesis and obesity, respectively, through the modulation of peroxisome proliferator activated receptor gamma 2 (*Ppar-γ2*) expression.

Results

Long-Term Genetic Deficiency of *Arid5a* in Mice Results in Adult-Onset Obesity. Utilizing the *Arid5a*^{-/-} mice, we investigated the possible role of *Arid5a* in metabolism (12). Under normal conditions and feeding with normal chow containing 4.5% crude fat, female *Arid5a*^{-/-} mice were phenotypically comparable in body size and weight to their wild-type (Wt) counterparts up to several weeks of age. However, after 15 to 20 wk, in comparison with Wt mice, female *Arid5a*^{-/-} mice slowly started to gain weight continuously over time (Fig. 1*A*). At ~30 to 35 wk of age, the difference became very clear in *Arid5a*^{-/-} mice, measuring ~30% higher than their Wt counterpart females (Fig. 1*A*). At 2 y of age, *Arid5a*^{-/-} mice became severely obese, weighing up to 70 g, which is more than twice the weight of average Wt mice that age (Fig. 1*B* and *C*). In addition to a maximum amount of accumulated fat in the visceral area, adipose tissue appeared to be increased all over the body in *Arid5a*^{-/-} mice (*SI Appendix*, Fig. S1*A*). Histochemical analysis revealed a similar size of adipocytes in *Arid5a*^{-/-} fat tissue, suggesting an increase in hyperplasia but not hypertrophy in *Arid5a*^{-/-} mice (*SI Appendix*, Fig. S1*B*). In addition, *Arid5a*^{-/-} mice developed severe fatty liver compared with Wt mice (*SI Appendix*, Fig. S1*C*). The serum levels of several adipokines, including leptin and total cortisol, were significantly increased in obese *Arid5a*^{-/-} mice (*SI Appendix*, Fig. S1*D* and *E*). This adult-onset obesity was also observed in male mice (*SI Appendix*, Fig. S2*A*).

Obesity is often associated with glucose metabolic disorders. While no significant differences in serum glucose were found between Wt and *Arid5a*^{-/-} mice at a young age (10 to 12 wk), old *Arid5a*^{-/-} mice (age 50 to 60 wk) had slightly increased serum

glucose levels compared with Wt mice the same age (*SI Appendix*, Fig. S2*B*). The glucose tolerance test (GTT) revealed that compared with Wt mice, *Arid5a*^{-/-} mice required more time to clear the serum glucose level (*SI Appendix*, Fig. S2*C*). Moreover, the insulin tolerance test (ITT) also suggested that old *Arid5a*^{-/-} mice had increased resistance to insulin (*SI Appendix*, Fig. S2*D*). These data for ITT and GTT revealed that obese *Arid5a*^{-/-} mice had some defects in glucose clearance from serum, suggesting some defects in glucose metabolism.

Since the development of obesity in *Arid5a*^{-/-} mice was observed at a late age on a normal low-fat diet, we next investigated whether *Arid5a*^{-/-} mice were more vulnerable to obesity at an early age by feeding a high-fat diet. We fed a high-fat diet (containing 32% crude fat, ~60% calories from fat) to young mice (8 wk old) for 12 wk. *Arid5a*^{-/-} mice gained weight more rapidly than Wt, but after a few weeks, no significant difference was observed between Wt and *Arid5a*^{-/-} (Fig. 1*D*). However, on a diet containing a moderately high amount of fat similar to the human diet (14% crude fat, ~25% calories from fat), both male and female *Arid5a*^{-/-} mice became obese within 10 to 15 wk compared with Wt mice (Fig. 1*E* and *SI Appendix*, Fig. S2*E*). These data clearly suggest *Arid5a* protects against obesity development during the low and moderately high fat fed condition.

We next examined the effect of overexpressing *Arid5a* during obesity development. We developed transgenic mice that overexpress *Arid5a* (Tg-*Arid5a*) (17). Under normal conditions, these Tg-*Arid5a* mice exhibit size and weight comparable to those of Wt mice (*SI Appendix*, Fig. S2*F*). Interestingly, when subjected to a high-fat diet (containing 32% crude fat, ~60% calories from fat), they are highly resistant to weight gain. While Wt mice and *Arid5a*^{-/-} mice gain ~80 to 90% weight in 12 wk, Tg-*Arid5a* mice gain only ~10 to 15% weight (Fig. 1*F*). These data suggest that *Arid5a* protects against obesity development.

***Arid5a* Negatively Regulates the Adipogenic Program.** To explore the mechanism of obesity development in *Arid5a*^{-/-} mice, we examined whether the adipogenesis process was modulated by *Arid5a*. To achieve this goal, first, we developed *Arid5a*^{-/-} and *Arid5a*-overexpressing stable cell lines of 3T3-L1 origin. Then, we monitored the kinetics of adipogenesis in vitro (from day 0 to day 7) in these *Arid5a*-deleted or -overexpressing cells. The accumulation of fatty acids as lipid droplets was clearly visible within 3 d in 3T3-L1 *Arid5a*^{-/-} cells, while very few or no lipid droplets were observed in Wt and *Arid5a*-overexpressing cells (Fig. 2*A*, *a-f* and *SI Appendix*, Fig. S3*A*). At approximately day 5, lipid droplets were visible in both Wt and *Arid5a*^{-/-} cells, but the frequency and size of the lipid droplets in *Arid5a*^{-/-} cells were clearly larger than in Wt cells (Fig. 2*A*, *g-i*). Interestingly, at this time point in *Arid5a*-overexpressing cells, very little or no lipid droplets were formed (Fig. 2*A*, *i* and *SI Appendix*, Fig. S3*A*). At around day 7, both Wt 3T3-L1 and *Arid5a*^{-/-} cells developed into oval-shaped adipocytes with clear visible lipid droplets (stained red) (Fig. 2*A*, *j* and *k*). However, the size of the adipocytes and lipid droplets in *Arid5a*^{-/-} cells were significantly larger than in Wt cells. In comparison with Wt and *Arid5a*^{-/-} cells, very few lipid droplets were visible in *Arid5a*-overexpressing cells, and most importantly, the cell size had not expanded at all (Fig. 2*A*, *j-l* and *SI Appendix*, Fig. S3*A*). We also monitored the adipogenesis process in mouse embryonic fibroblasts (MEF) cells derived from Wt and *Arid5a*^{-/-} mice. In response to the same stimulants used for 3T3-L1 cells, MEF cells from Wt mice exhibited rare or no conversion of adipocytes up to day 10, but interestingly, a large number of *Arid5a*^{-/-} MEF cells had converted to adipocytes (Fig. 2*B* and *SI Appendix*, Fig. S3*B*). Moreover, several adipokines, mainly adiponectin and resistin, were highly increased in the lysates of *Arid5a*^{-/-} cells compared with Wt cells at day 10 of adipogenesis (*SI Appendix*, Fig. S3*C*). These data clearly indicate

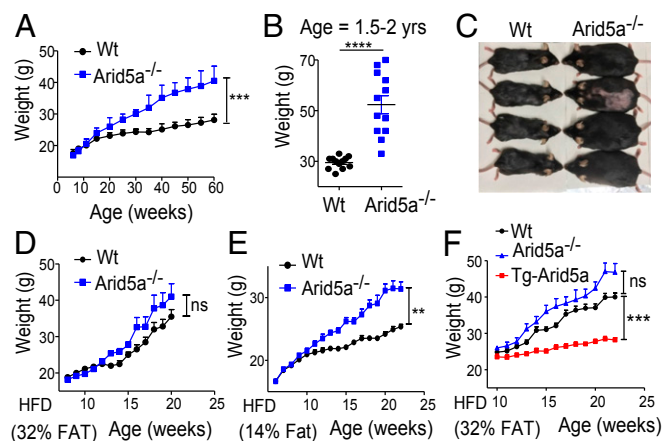


Fig. 1. *Arid5a* deficiency develops adult-onset obesity. (*A*) Monitoring of the total body weight of Wt and *Arid5a*^{-/-} mice with normal chow (4.5% crude fat amount), *n* = 8 to 10. This experiment was repeated 3 times. (*B*) Weight comparison of old female *Arid5a*^{-/-} and Wt mice (age: 18 to 24 mo, *n* = 12). (*C*) Representative images of female Wt and *Arid5a*^{-/-} mice (age 18 to 24 mo). (*D*) Total weight monitoring after mice were fed a high-fat diet (HFD) (32% crude fat), *n* = 8 to 10. (*E*) Total weight monitoring after mice were fed moderate fat (14% crude fat) *n* = 8 to 10. (*F*) Total weight monitoring in Wt, *Arid5a*^{-/-}, and Tg-*Arid5a* mice after they were fed a high-fat diet (32% crude fat; 2 independent experiments were performed). An unpaired Mann-Whitney *U* test was performed for statistical analysis. Error bars represent mean \pm SD (***P* < 0.01; ****P* < 0.001, *****P* < 0.0001; ns, not significant).

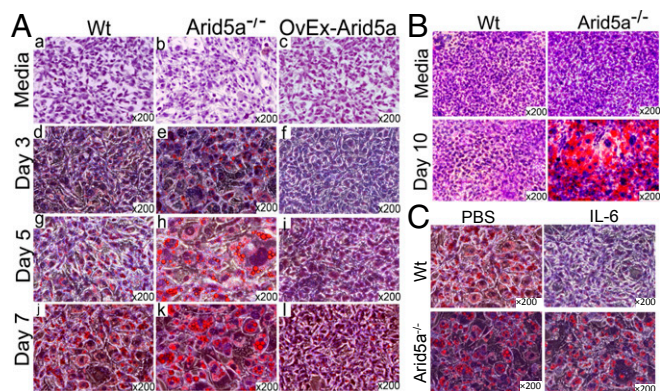


Fig. 2. Arid5a negatively regulates adipogenic differentiation. (A) Microscopic image of lipid-stained (red) cells during the monitoring of the adipogenesis process in Wt (empty vector), Arid5a^{-/-}, and Arid5a-overexpressing (OvEx) 3T3-L1 cells. Cells were subjected to adipogenesis as described in *Materials and Methods*. On days 0, 3, 5, and 7, the cells were fixed, stained with red oil O stain, and counterstained with hematoxylin, and phase contrast images were taken with a microscope. (B) Microscope images of Wt and Arid5a^{-/-} MEF cells on days 0 and 10 of adipogenesis. (C) Microscopic images of Wt and Arid5a^{-/-} 3T3-L1 cells on day 7 of adipogenesis in the presence of IL-6 (10 ng/mL) or phosphate buffered saline (PBS).

that Arid5a deletion enhances the adipogenesis process, while its overexpression inhibits adipogenesis.

IL-6 is known to inhibit the differentiation of adipogenesis. As expected, we also observed that IL-6 inhibited the differentiation of adipogenesis in Wt cells; however, IL-6 could not inhibit the differentiation in Arid5a^{-/-} cells, indicating that IL-6 utilized Arid5a to inhibit adipogenesis (Fig. 2C).

Arid5a Inhibition Enhances the Process of Lipid Anabolism. We next investigated the possible mechanisms of Arid5a-mediated molecular and biochemical effects in lipid metabolism. Physiologically, adipogenesis occurs in the stromal vascular environment. RNA sequencing analysis of the stromal vascular fraction (SVF) of Wt and Arid5a^{-/-} mice revealed that several genes related to lipid metabolism, including the master regulator of adipogenesis Ppar- γ , were enhanced by Arid5a deletion (*SI Appendix, Fig. S4A*). RT-PCR analysis confirmed the enhanced mRNA expression of Ppar- γ in the SVF of Arid5a^{-/-} mice (Fig. 3A). Consistent with the SVF data, Ppar- γ appeared to be greatly influenced by changes in Arid5a protein expression in 3T3-L1 and MEF cells during adipogenesis. The Ppar- γ protein has 2 distinct isoforms, Ppar- γ 1 and Ppar- γ 2, of which Ppar- γ 2 is important during the process of adipogenesis (18). In Arid5a^{-/-} cells, mRNA expression of Ppar- γ 2 was significantly elevated at early time points of adipogenesis in comparison with Wt cells of both 3T3-L1 and MEF origin (Fig. 3B and C). The increased mRNA level of Ppar- γ 2 in Arid5a^{-/-} cells was clearly reflected in the protein level at all of the time points of adipogenesis in MEF cells and on day 7 of adipogenesis in 3T3-L1 cells (Fig. 3D). However, in Arid5a-overexpressing 3T3-L1 cells, Ppar- γ 2 expression was significantly suppressed compared with Wt and Arid5a^{-/-} 3T3-L1 cells at all time points of adipogenesis (Fig. 3D). Instead, in Arid5a-overexpressing 3T3-L1 cells, Ppar- γ 1 appeared to be enhanced on day 7 of adipogenesis (Fig. 3D). These data show that Arid5a inhibits lipid metabolism, possibly through regulating the Ppar- γ 2 isoform. Ppar- γ is known to increase mitochondrial membrane potential and biogenesis (19). Consistently, we observed increased mitochondrial activity and numbers in Arid5a^{-/-} compared with Wt cells (*SI Appendix, Fig. S4B*). In addition to Ppar- γ 2, mRNA expression of the lipid uptake molecules Fabp4 and Cd36 was also significantly increased during adipogenesis

upon Arid5a deletion (Fig. 3E and F). In contrast, Arid5a-overexpressing 3T3-L1 cells had significantly lower expression of these genes (Fig. 3E and F). The increased expression of CD36 and FABP4 by Arid5a deletion was confirmed at the protein level in both 3T3-L1 and MEF cells by fluorescence-activated cell sorting (FACS) and Western blot analysis, respectively (Fig. 3G and H). Moreover, the lipid uptake function was also increased in Arid5a-deleted 3T3-L1 cells under normal conditions and early adipogenesis (Fig. 3I). Overall, we show that the absence of Arid5a enhances the process of lipid anabolism.

Arid5a Represses the Transcription of Ppar- γ 2. As we showed that Ppar- γ 2, a master regulator of lipid metabolism, is largely enhanced by Arid5a deletion during adipogenesis, we next investigated the relationship between Ppar- γ 2 and Arid5a at the molecular level. Arid5a is a nucleic acid binding protein with the ability to modulate both transcriptional and posttranscriptional processes. While Arid5a localizes in the nucleus under normal conditions, after receiving an inflammatory signal, it is transported to the cytoplasm and then, upon resolution of the inflammation, back to the nucleus via the importin- α /B pathway (17). In 3T3-L1 cells, we found that Arid5a was localized mostly in the nucleus under normal conditions and in the early phase (day 3) of adipogenesis (*SI Appendix, Fig. S5A*). We analyzed the effects of Arid5a on the promoter activity of Ppar- γ 2 by the luciferase assay during adipogenesis. Ppar- γ 2 promoter activity increased significantly during early adipogenesis compared with normal conditions, but the addition of an Arid5a-expressing plasmid significantly decreased the promoter activity of Ppar- γ 2, suggesting that Arid5a repressed the transcription of Ppar- γ 2 (Fig. 4A). We next performed the promoter deletion experiment to identify the Arid5a regulatory region in the Ppar- γ 2 promoter. We prepared Ppar- γ 2 promoter vectors of several lengths starting from 1,000 to 150 base pair (bp) upstream from the transcription initiation site. The promoter activity of Ppar- γ continued to decrease with shortening of the promoter, but the promoter activity was still significantly decreased in the presence of Arid5a up to 350 kb of the Ppar- γ 2 promoter (Fig. 4B). These data suggest that Arid5a inhibits the transcription of Ppar- γ 2 during the early adipogenesis process by modulating the proximal Ppar- γ 2 promoter.

We next analyzed the promoter sequence of Ppar- γ 2 in detail. Arid5a binds to the AATATT or AATACT region of nucleic acid (*SI Appendix, Fig. S5B*). We found these Arid5 binding sites (highlighted in yellow) in several regions of the Ppar- γ 2 promoter of mice and humans within 500 bp upstream of the transcription initiation site (*SI Appendix, Fig. S5C and D*). Of note, both in mice and humans, the Ppar- γ 2 gene Arid5a binding site is placed immediately after the CEBP site, a transcription factor that is important for the activation of Ppar- γ 2 transcription (*SI Appendix, Fig. S5C and D*; indicated in bold letters). To confirm the binding of Arid5a in Ppar- γ 2 during adipogenesis, we performed qPCR analysis to identify the Arid5a-bound promoter fragments of Ppar- γ 2 prepared by chromatin immunoprecipitation analysis. During early adipogenesis, the promoter region of 270 to 430 bp upstream of the transcription initiation site was highly amplified in comparison with the immunoglobulin G (IgG) control (Fig. 4C). All these experiments clearly show that Arid5a can directly modulate the promoter of Ppar- γ 2 and repress the transcription of Ppar- γ during adipogenesis.

Arid5a and Ppar- γ 2 Counterregulate Each Other to Maintain Metabolic Homeostasis. Since expressing Arid5a in 3T3-L1 cells inhibited adipogenesis and Arid5a^{-/-} cells have enhanced adipogenesis, we hypothesized that the intrinsic Arid5a in fibroblasts or preadipocytes should be inhibited for effective adipogenesis. We observed a gradual decrease in the mRNA expression of Arid5a during the adipogenesis process (Fig. 5A). However, Ppar- γ 2 expression was simultaneously observed to increase (Fig. 5B),

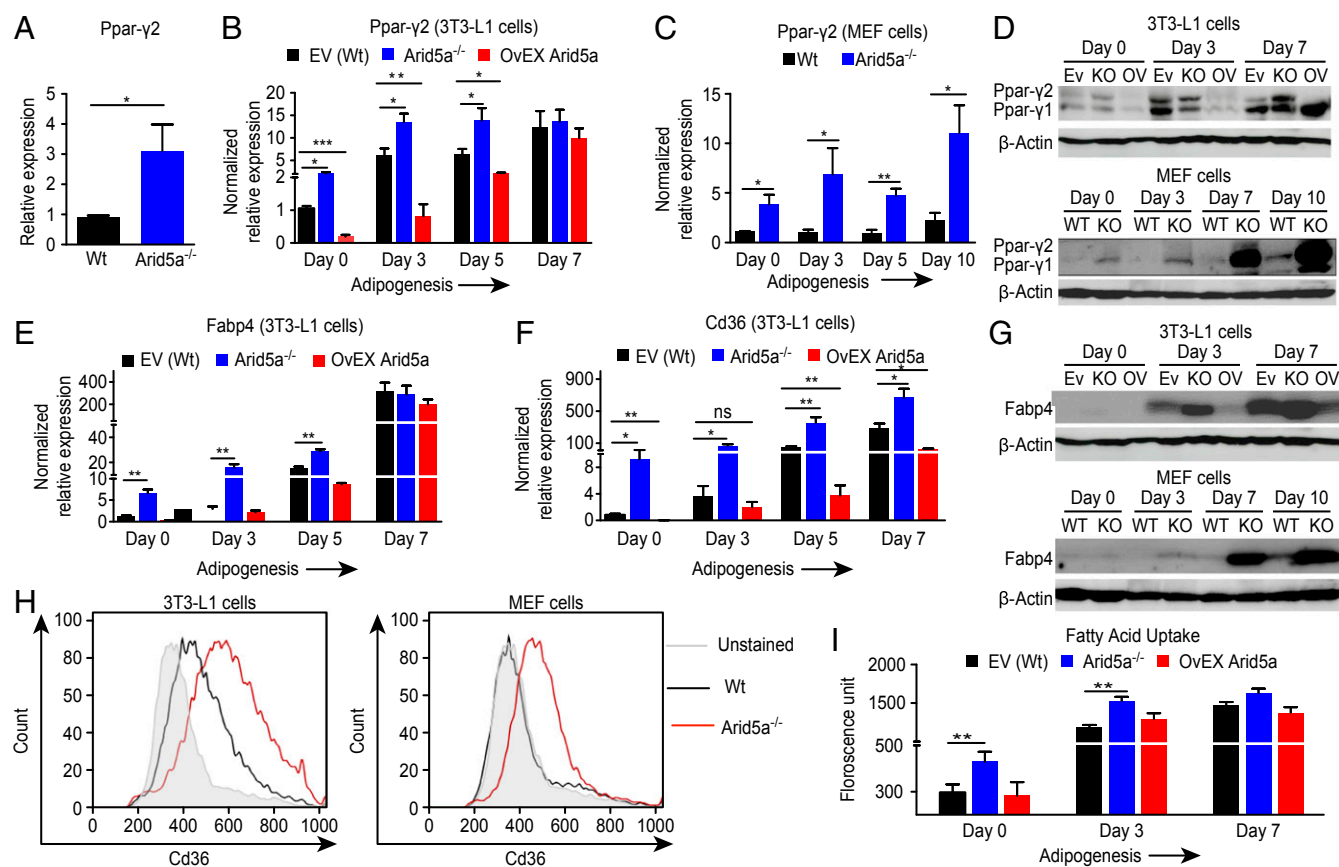


Fig. 3. Arid5a deletion enhances the process of lipid anabolism. (A) Ppar-γ expression (mRNA) in SVF collected from 24 wk old female Wt and Arid5a^{-/-} mice. (B) Ppar-γ expression (mRNA) in Wt (empty vector, EV), Arid5a^{-/-}, and Arid5a-overexpressing 3T3-L1 cells during adipogenesis. (C) Ppar-γ expression (mRNA) in MEF cells prepared from Wt and Arid5a^{-/-} mice during adipogenesis. (D) Ppar-γ protein expression levels in 3T3-L1 (Top) and MEF cells (Bottom) during adipogenesis. (E and F) Fabp4 and Cd36 expression (mRNA) in Wt, Arid5a^{-/-}, and Arid5a-overexpressing cells during adipogenesis. (G) Fabp4 protein expression levels in 3T3-L1 (Top) and MEF cells (Bottom) during adipogenesis. (H) Surface expression of Cd36 in 3T3-L1 and MEF cells as analyzed by FACS on day 3 of adipogenesis. (I) Fatty acid uptake analysis performed in Wt, Arid5a^{-/-}, and Arid5a-overexpressing 3T3-L1 cells during adipogenesis. *n* = 4 to 5 for all mRNA analyses and fatty acid uptake analyses. mRNA expressions of target gene at all time points were normalized with the day 0 Wt cell value. All mRNA experiments and Western blot analyses were repeated at least twice. An unpaired Student's *t* test was performed for statistical analysis. Error bars show the means ± SEM (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, not significant).

indicating that Arid5a and Ppar-γ2 acted reciprocally during the adipogenesis process. Hence, we hypothesized that some molecules that are involved in adipogenesis should be inhibiting the intrinsic expression of Arid5a. We analyzed the direct effect of dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), insulin,

and rosiglitazone, which are used to induce adipogenesis in vitro. Of these compounds, rosiglitazone, a strong inducer of Ppar-γ, inhibited the expression of Arid5a (Fig. 5C). This effect was confirmed by troglitazone, another inducer of Ppar-γ, which also showed an inhibitory effect on Arid5a expression (Fig. 5D). As

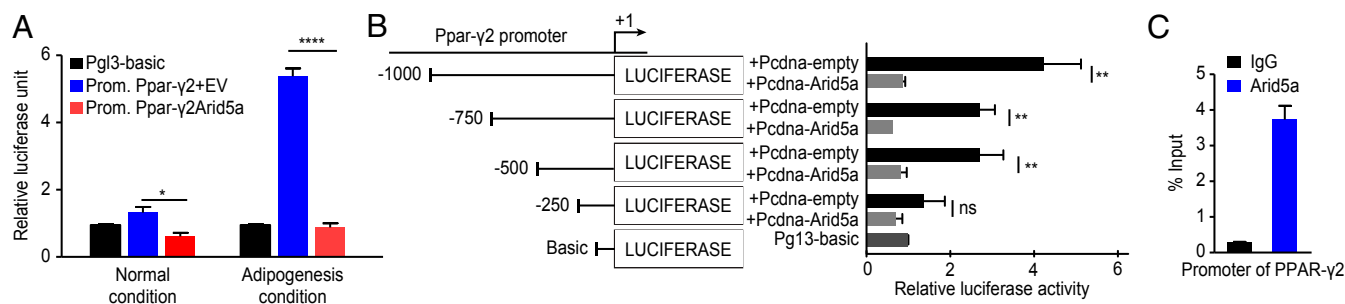


Fig. 4. Arid5a represses the transcription of Ppar-γ. (A) Relative promoter luciferase assay of Ppar-γ2 during normal and adipogenic condition. (B) Relative luciferase activity of Ppar-γ2 promoter deletion constructs transfected into 3T3-L1 cells and subjected to adipogenesis. A series of 5'-deleted Ppar-γ2 promoter fragments were generated. A schematic representation of the 5'-flanking region and the reporter constructs used is shown on the Left. The data shown are the mean of 3 to 4 independent experiments. (C) Chromatin immunoprecipitation–qPCR results quantifying the proximal fragment of the PPAR-γ2 promoter (270 to 430 bp upstream from the first exon) bound to Arid5a during day 3 of adipogenesis. Chip assay is performed twice. The data shown are the mean of duplicate qPCR data of each chip assay. Error bars represent means ± SEM (**P* < 0.05; ***P* < 0.01; *****P* < 0.0001; ns, not significant).

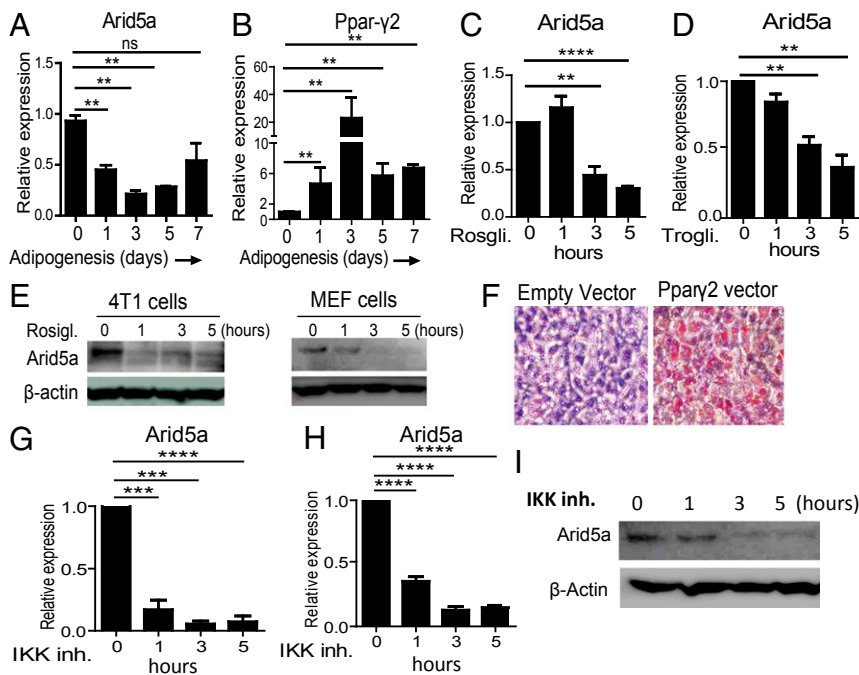


Fig. 5. Arid5a and Ppar- γ counterregulate each other. (A and B) mRNA expression of Arid5a (A) and Ppar- γ (B) during adipogenesis in 3T3-L1 cells measured at different time points during the adipogenesis process. (C and D) mRNA expression of Arid5a after treating 3T3-L1 cells with rosiglitazone (50 μ M) (C) and troglitazone (50 μ M) (D) for indicated time points. (E) Arid5a protein expression levels in 4T1 cancer cell lines or Wt MEF cells after treatment with rosiglitazone (50 μ M) for indicated time points. (F) Arid5a-overexpressing stable 3T3-L1 cells were transiently transfected with Ppar- γ -expressing vector and subjected to adipogenesis and analyzed qualitatively for adipocyte differentiation on day 7, as described in *Materials and Methods*. The images shown are 200 \times magnified cells and the red stain denotes the lipid droplets. (G and H) mRNA expression of Arid5a after treating 3T3-L1 cells (G) and MEF cells (H) with IKK inhibitor BMS-345541 (10 μ M) at indicated time points. (I) Arid5a protein expression level in the lysate of MEF cell lines for indicated time points. All experiments were performed at least twice. $n = 4$ to 5 for mRNA analysis. An unpaired 2-tailed Student's t test was performed for statistical analysis. Error bars show the means \pm SEM (** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant).

expected, the decreased expression of Arid5a by rosiglitazone was also observed at the protein level (Fig. 5E). These data indicate that Ppar- γ itself can inhibit the expression of Arid5a to enhance its up-regulation. Consistently, when Ppar- γ 2 was forcibly expressed in the Arid5a-overexpressing 3T3-L1 cell line, the inhibitory adipogenic effect was significantly reversed (Fig. 5F). These data indicate that Ppar- γ has the capacity to inhibit Arid5a and Arid5a-mediated inhibition of adipogenesis.

We next analyzed how Ppar- γ 2 could inhibit Arid5a expression. Previously, we showed that Arid5a is induced through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway during LPS stimulation and that the induced Arid5a is quickly degraded through the E3 ligase/ubiquitin pathway (10). It is well known that Ppar- γ inhibits the NF- κ B pathway, and it was recently discovered that Ppar- γ is an E3 ligase that can directly degrade the p65 component of the NF- κ B complex (20–22). Hence, we analyzed whether Ppar- γ could limit Arid5a by inhibiting NF- κ B signaling. Supporting our previous findings, upon inhibition of NF- κ B signaling by the I κ B kinase (IKK) inhibitor BMS-345541, the mRNA and protein expression of Arid5a were significantly decreased in 3T3-L1 and MEF cell lines (Fig. 5G–I). This result suggests that Arid5a expression in fibroblasts is maintained by NF- κ B and that Ppar- γ can inhibit Arid5a by inhibiting NF- κ B signaling.

Collectively, these data indicate that although Arid5a can repress the transcription of Ppar- γ 2, Ppar- γ 2 counterregulates Arid5a by controlling NF- κ B signaling during adipogenesis. Hence, for effective adipogenesis, Arid5a might be inhibited, which is accomplished by causing Ppar- γ 2 to induce itself and facilitate adipogenesis. In contrast, to limit excess adipogenesis and maintain homeostasis, Ppar- γ 2 should be checked, which is accomplished by Arid5a (*SI Appendix, Fig. S6*).

Discussion

Obesity is becoming an epidemic problem worldwide. Manipulation of energy programming by targeting the associated regulators is a promising therapeutic strategy to control obesity (23). Here, we report that Arid5a is an important regulatory protein of energy metabolism that can significantly modulate the process of adipogenesis in vitro and obesity in vivo.

Adipogenesis is a biologically evolved process to form adipocytes, which have several purposes, of which the main purpose is to store excess energy that is intended to be used during an energy crisis. Considering the increased nutrient intake and reduced energy expenditure in the modern world, our bodies have a propensity to store the maximum surplus energy, which might lead to obesity. Hence, a restriction mechanism is required to limit excess adipogenesis and maintain adipose tissue homeostasis (24). However, the precise regulatory mechanism of this adipose tissue homeostasis is poorly understood. Adipogenesis occurs in the stromal vascular environment where several types of cells, including immune cells (mainly macrophages and endothelial cells), are also present to a large extent. This study and several others have suggested that cytokines, including IL-6 secreted by immune cells and mature adipocytes, play a role in limiting adipogenesis and obesity (3, 25–27). Cytokines, mainly IL-6 and IL-17, induce Arid5a, which can further limit the differentiation of new fibroblasts to adipocytes by regulating Ppar- γ , hence maintaining adipose tissue homeostasis. Together with several previous reports, we also observed that IL-6 inhibited the differentiation of adipogenesis; however, IL-6 could not inhibit the differentiation in Arid5a^{-/-} cells, indicating that IL-6 utilized Arid5a to inhibit adipogenesis. We previously showed that Arid5a extends the half-life of IL-6 by stabilizing IL-6 mRNA and hence increasing the levels of IL-6 in vivo. Although the mechanism is not clear, a few studies have reported that IL-6-deficient mice develop adult-onset obesity, suggesting that IL-6 is directly or indirectly required to restrict the excessive formation of fat cells (6, 28, 29). As a strong inducer of Arid5a, it is highly likely that IL-6-deficient mice have insufficient activation of Arid5a, resulting in unrestricted adipogenesis and leading to obesity. Thus, our findings suggest that IL-6 inhibit adipogenesis and obesity by activating Arid5a.

One major finding of this paper is the negative regulation of Ppar- γ 2 expression by Arid5a. The obesity development in Arid5a^{-/-} mice, enhanced adipogenesis in Arid5a^{-/-} cells, and increased expression of the fat uptake molecules CD36 and FABP4 can be explained to a large extent by the known effects of Ppar- γ on lipid metabolism. Ppar- γ promotes lipid uptake and lipid biosynthesis, hence enhancing the adipogenesis program (30). Moreover, increased Ppar- γ activity is associated with weight gain in both humans and mice (31, 32). Patients taking Ppar- γ -inducing antidiabetic

thiazolidinediones have been reported to gain significant weight (31, 33). Recently, it was reported that neuronal Ppar- γ plays a role in promoting obesity in mice (32). Moreover, Ppar- γ is widely reported to exert anti-inflammatory effects in cases of septic shock, EAE, and bleomycin-induced lung injury (34–36). Interestingly, our previous findings have revealed the Arid5a^{-/-} mice are resistant to those diseases (12, 15, 16), suggesting a connection of Ppar- γ and Arid5a.

To date, we understand that Arid5a is localized in the nucleus under noninflammatory conditions, but it is translocated to cytoplasm after an inflammatory signal to exert RNA-stabilizing functions (17). The Ppar- γ -repressing activity of Arid5a appears to occur in the nucleus. Arid5a binds to the Ppar- γ promoter at the AATATT sequence adjacent to the CCAAT sequence, the binding site of CEBP, which is another master regulator of adipogenesis. Binding of CEBPs is important for Ppar- γ activation during adipogenesis (37). Arid5a binding to Ppar- γ might mask the binding of CEBP to the Ppar- γ promoter, preventing the activation of Ppar- γ transcription. It is likely that Arid5a forms a complex with other proteins to repress Ppar- γ transcription. Arid5a has been reported to bind physically to Sox9 and direct the process of chondrogenesis (38). Recently, it was reported that Sox9 must be inhibited for effective adipogenesis (39). These data indicate that a complex consisting of Arid5a, Sox9, and other unknown proteins might be necessary for the binding of Arid5a to DNA and repression of Ppar- γ under physiological conditions.

In conclusion, the findings in this paper introduce Arid5a as a novel and important component of energy homeostasis. Arid5a restricts the process of adipogenesis. Thus, to facilitate the formation of adipocytes, Arid5a is inhibited by Ppar- γ in the early stage of adipogenesis (SI Appendix, Fig. S6). Once sufficient adipocytes are formed, mature adipocytes along with infiltrated immune cells secrete IL-6 and other cytokines, which, through

inducing Arid5a, further limit the differentiation of adipocytes (SI Appendix, Fig. S6). Hence, the coordinated activity of Ppar- γ and Arid5a maintains metabolic homeostasis in adipose tissue.

Materials and Methods

Mice and Ethical Permissions. All mice were housed in the animal house of Frontiers of Biomedical Science (FBS), Osaka University. Mice were maintained in a specific pathogen-free facility with a 12 h/12 h light/dark cycle and free access to food and water. Unless mentioned otherwise, the mice were fed standard chow containing a total amount of 4.5% crude fat. Wt C57bl/6j mice were initially purchased from CLEA Japan and further bred at the FBS animal facility. Arid5a^{-/-} and Arid5a-overexpressing transgenic (Tg-Arid5a) mice were generated on the C57bl/6j background as previously described (12, 17). All experiments were performed in accordance with the guidelines approved by the institutional animal care and use committee of the Graduate School of Frontier Biosciences, Osaka University.

Statistical Analysis. All data were analyzed with Microsoft Excel and GraphPad Prism version 6. Data are expressed as the mean \pm SEM for in vitro experiments and mean \pm SD for in vivo experiments. For in vitro experiments, the difference between groups was determined using an unpaired 2-tailed Student's *t* test. For in vivo weight studies, the difference between groups was determined by a 2-tailed Mann-Whitney *U* test. For all statistical analyses, *P* values < 0.05 were considered statistically significant.

Extended methods and information about cell lines, plasmids, stable cell line preparation, histology, qRT-PCR, adipogenesis, red oil O staining, lipid quantification, high-fat diet studies, ITT, GTT, total steroid measurement and fatty acid uptake assay, Arid5a localization (immunofluorescence), FACS analysis, Western blot, luciferase activity, chromatin immunoprecipitation assay, and RNA sequencing are described in SI Appendix.

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- H. Wang, J. Ye, Regulation of energy balance by inflammation: Common theme in physiology and pathology. *Rev. Endocr. Metab. Disord.* **16**, 47–54 (2015).
- A. Rodríguez, S. Ezquerro, L. Méndez-Giménez, S. Becerril, G. Frühbeck, Revisiting the adipocyte: A model for integration of cytokine signaling in the regulation of energy metabolism. *Am. J. Physiol. Endocrinol. Metab.* **309**, E691–E714 (2015).
- J. Mauer, J. L. Denson, J. C. Brüning, Versatile functions for IL-6 in metabolism and cancer. *Trends Immunol.* **36**, 92–101 (2015).
- K. C. Fearon, D. J. Glass, D. C. Guttridge, Cancer cachexia: Mediators, signaling, and metabolic pathways. *Cell Metab.* **16**, 153–166 (2012).
- S. Younis *et al.*, Weight change during pharmacological blockade of interleukin-6 or tumor necrosis factor- α in patients with inflammatory rheumatic disorders: A 16-week comparative study. *Cytokine* **61**, 353–355 (2013).
- V. Wallenius *et al.*, Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* **8**, 75–79 (2002).
- J. Ye, O. P. McGuinness, Inflammation during obesity is not all bad: Evidence from animal and human studies. *Am. J. Physiol. Endocrinol. Metab.* **304**, E466–E477 (2013).
- D. Wilsker, A. Patsialou, P. B. Dallas, E. Moran, ARID proteins: A diverse family of DNA binding proteins implicated in the control of cell growth, differentiation, and development. *Cell Growth Differ.* **13**, 95–106 (2002).
- N. Amaty *et al.*, IL-17 integrates multiple self-reinforcing, feed-forward mechanisms through the RNA binding protein Arid5a. *Sci. Signal.* **11**, eaat4617 (2018).
- K. K. Nyati *et al.*, TLR4-induced NF- κ B and MAPK signaling regulate the IL-6 mRNA stabilizing protein Arid5a. *Nucleic Acids Res.* **45**, 2687–2703 (2017).
- T. H. Huang *et al.*, Repression by a differentiation-specific factor of the human cytomegalovirus enhancer. *Nucleic Acids Res.* **24**, 1695–1701 (1996).
- K. Masuda *et al.*, Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 9409–9414 (2013).
- H. Hanieh *et al.*, Arid5a stabilizes OX40 mRNA in murine CD4⁺ T cells by recognizing a stem-loop structure in its 3'UTR. *Eur. J. Immunol.* **48**, 593–604 (2018).
- K. Masuda *et al.*, Arid5a regulates naive CD4⁺ T cell fate through selective stabilization of Stat3 mRNA. *J. Exp. Med.* **213**, 605–619 (2016).
- M. M. Zaman *et al.*, Arid5a exacerbates IFN- γ -mediated septic shock by stabilizing T-bet mRNA. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11543–11548 (2016).
- P. K. Dubey *et al.*, Arid5a-deficient mice are highly resistant to bleomycin-induced lung injury. *Int. Immunol.* **29**, 79–85 (2017).
- M. Higa *et al.*, Regulation of inflammatory responses by dynamic subcellular localization of RNA-binding protein Arid5a. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E1214–E1220 (2018).
- P. Tontonoz, E. Hu, B. M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**, 1147–1156 (1994).
- T. Mello, M. Materozzi, A. Galli, PPARs and mitochondrial metabolism: From NAFLD to HCC. *PPAR Res.* **2016**, 7403230 (2016).
- A. H. Remels *et al.*, PPARgamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **297**, E174–E183 (2009).
- Y. Hou, F. Moreau, K. Chadee, PPAR γ is an E3 ligase that induces the degradation of NF- κ B/p65. *Nat. Commun.* **3**, 1300 (2012).
- H. Ruan, H. J. Pownall, H. F. Lodish, Troglitazone antagonizes tumor necrosis factor- α -induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. *J. Biol. Chem.* **278**, 28181–28192 (2003).
- Y. H. Tseng, A. M. Cypess, C. R. Kahn, Cellular bioenergetics as a target for obesity therapy. *Nat. Rev. Drug Discov.* **9**, 465–482 (2010).
- D. Mosefi, A. Regassa, W. K. Kim, Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. *Int. J. Mol. Sci.* **17**, E124 (2016).
- G. van Hall *et al.*, Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J. Clin. Endocrinol. Metab.* **88**, 3005–3010 (2003).
- A. S. Wedell-Neergaard *et al.*, Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial. *Cell Metab.* **29**, 844–855.e3 (2019).
- A. L. Carey *et al.*, Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* **55**, 2688–2697 (2006).
- K. Wallenius, V. Wallenius, D. Sunter, S. L. Dickson, J. O. Jansson, Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem. Biophys. Res. Commun.* **293**, 560–565 (2002).
- D. Chida, T. Osaka, O. Hashimoto, Y. Iwakura, Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. *Diabetes* **55**, 971–977 (2006).
- M. Ahmadian *et al.*, PPAR γ signaling and metabolism: The good, the bad and the future. *Nat. Med.* **19**, 557–566 (2013).
- M. Lehrke, M. A. Lazar, The many faces of PPARgamma. *Cell* **123**, 993–999 (2005).
- M. Lu *et al.*, Brain PPAR- γ promotes obesity and is required for the insulin-sensitizing effect of thiazolidinediones. *Nat. Med.* **17**, 618–622 (2011).
- K. Hermansen, L. S. Mortensen, Bodyweight changes associated with antihyperglycaemic agents in type 2 diabetes mellitus. *Drug Saf.* **30**, 1127–1142 (2007).
- Y. Aoki *et al.*, Pioglitazone, a peroxisome proliferator-activated receptor gamma ligand, suppresses bleomycin-induced acute lung injury and fibrosis. *Respiration* **77**, 311–319 (2009).
- B. Zingarelli, J. A. Cook, Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation. *Shock* **23**, 393–399 (2005).
- P. D. Drew, J. Xu, M. K. Racke, PPAR-gamma: Therapeutic potential for multiple sclerosis. *PPAR Res.* **2008**, 627463 (2008).
- J. E. Lee, K. Ge, Transcriptional and epigenetic regulation of PPAR γ expression during adipogenesis. *Cell Biosci.* **4**, 29 (2014).
- K. Amano *et al.*, Arid5a cooperates with Sox9 to stimulate chondrocyte-specific transcription. *Mol. Biol. Cell* **22**, 1300–1311 (2011).
- O. Gulyaeva, H. Nguyen, A. Sambeat, K. Heydari, H. S. Sul, Sox9-Meis1 inactivation is required for adipogenesis, advancing Pref-1(+) to PDGFRalpha(+) cells. *Cell Rep.* **25**, 1002–1017.e14 (2018).