Lsd1 prevents age-programed loss of beige adipocytes

Delphine Duteil[a], Milica Tosic[a], Dominica Willmann[a], Anastasia Georgiadi[b,c], Toufike Kanouni[d], and Roland Schüle[a,e,f,1]

[a]Urologische Klinik, Zentrale Klinische Forschung, Universitätsklinikum Freiburg, Medizinische Fakultät, Albert-Ludwigs-University Freiburg, 79106 Freiburg, Germany; [b]Institute for Diabetes and Cancer, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, 85764 Neuherberg, Germany; [c]German Center for Diabetes Research, 85764 Neuherberg, Germany [d]Celgene Quanticel Research, San Diego, CA 92121; [e]BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University, 79106 Freiburg, Germany; and [f]Deutsche Konsortium für Translationale Krebsforschung, 79106 Freiburg, Germany

Edited by Pierre Chambon, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France, and approved March 31, 2017 (received for review February 15, 2017)

Aging is accompanied by major changes in adipose tissue distribution and function. In particular, with time, thermogenic-competent beige adipocytes progressively gain a white adipocyte morphology. However, the mechanisms controlling the age-related transition of beige adipocytes to white adipocytes remain unclear. Lysine-specific demethylase 1 (Lsd1) is an epigenetic eraser enzyme positively regulating differentiation and function of adipocytes. Here we show that Lsd1 levels decrease in aging inguinal white adipose tissue concomitantly with beige fat cell decline. Accordingly, adipocyte-specific increase of Lsd1 expression is sufficient to rescue the age-related transition of beige adipocytes to white adipocytes in vivo, whereas loss of Lsd1 precipitates it. Lsd1 maintains beige adipocytes by controlling the expression of peroxisome proliferator-activated receptor α (Ppara), and treatment with a Ppara agonist is sufficient to rescue the loss of beige adipocytes caused by Lsd1 ablation. In summary, our data provide insights into the mechanism controlling the age-related beige-to-white adipocyte transition and identify Lsd1 as a regulator of beige fat cell maintenance.

aging | Lsd1 | adipocyte | Ppara | beige fat

In mammals, adipose tissue is essential for the regulation of energy homeostasis. White adipose tissue (WAT) is deposited s.c. [i.e., inguinal WAT (ingWAT)] and around internal organs [i.e., epididymal WAT (epWAT)], whereas brown adipose tissue (BAT) is predominantly found between the scapulae of rodents and in the supraclavicular/thoracic regions of adult humans (1). Adipocytes that constitute WAT and BAT display distinguishable morphological features and functions. White adipocytes contain a single (unilocular) large lipid droplet and few mitochondria, and serve for lipid storage and adipokine production. In contrast, brown adipocytes have numerous smaller (multilocular) lipid droplets and abundant mitochondria, and are thermogenically active. They are defined by high expression of uncoupling protein-1 (Ucp1), a mitochondrial membrane protein that provides these cells with the unique ability to dissipate large quantities of energy as heat (2). The presence of Ucp1-positive brown-like adipocytes has been described in WAT. These cells were termed "brite" (brown in white) (3) or "beige" adipocytes (4). In mice, two types of beige adipocytes are formed, namely basal beige adipocytes that emerge during embryonic development (5, 6), and beige adipocytes that can be recruited upon environmental challenge (7). Basal and induced beige adipocytes prevail predominantly in ingWAT of young rodents, but are thought to have distinct origins. During embryonic development, a certain percentage of basal beige fat cells are derived from Myf5-positive precursor cells (8, 9) sharing common origins with brown adipocytes (10). In contrast, other studies suggest that basal beige cells originate from smooth muscle (11, 12). Appearance of induced beige adipocytes in WAT can be induced by adrenergic stimuli or exposure to cold (7). It is yet unclear whether the induced beige adipocytes preexist in WAT as "masked" unilocular cells that acquire their thermogenic properties and beige morphology upon cold or β3-adrenergic (β3AR) stimulation or develop by differentiation from precursor cells (9).

Aging is accompanied by dramatic metabolic changes and gradual weight gain as calorie-burning skeletal muscle is decreasing in mass and fat tissue is accumulating. In modern societies, age-related phenotypes are favored by increased lifespan, high-caloric diet, and sedentary lifestyle. In particular, changes in the morphology and function of adipose tissue have been reported in rodents and humans (13). Of note, beige adipocytes of young adult mice and human newborns are progressively lost during aging and replaced by white fat cells. In mice, expression of *Ucp1* transcript in ingWAT dramatically declines between 3 and 12 mo of age, and, at 12 mo of age, multilocular Ucp1-positive beige adipocytes are lost from WAT depots (14). Despite the high interest in understanding beige adipocyte development and metabolism, the molecular mechanism orchestrating age-related beige-to-white adipocyte transition remains unknown. We and others previously reported that lysine-specific demethylase 1 (Lsd1) is required for the differentiation of white and beige adipocytes, and that ubiquitously increased expression of Lsd1 promotes thermogenic and oxidative capabilities of WAT (15). Therefore, we questioned whether Lsd1 may also play a role in the age-dependent loss of beige fat cells. In the present study, we show that Lsd1 levels decrease with age in ingWAT concomitantly with the age-related loss of beige adipocytes. Maintenance of Lsd1 expression specifically in adipocytes of transgenic mice

Significance

Over time, beige adipocytes gain a white adipocyte morphology and lose their thermogenic activity. Here we show that levels of the epigenetic eraser lysine-specific demethylase 1 (Lsd1) decrease in aging inguinal white adipose tissue concomitantly with beige fat cell decline. Maintaining adipocyte-specific expression of Lsd1 in transgenic mice preserves the pool of beige adipocytes in old mice. Vice versa, using GFP reporter mice, we traced the fate of beige adipocytes and showed that adipocyte-specific loss of Lsd1 results in a premature beige-to-white adipocyte transition in vivo. Maintenance of beige adipocytes is mediated by the Lsd1 target gene peroxisome proliferator-activated receptor α (Ppara) and pharmacological activation of Ppara rescues the loss of beige adipocytes in Lsd1-KO mice. Together, we identified Lsd1 as a regulator of beige adipocyte maintenance.

Author contributions: D.D. and R.S. designed research; D.D. and M.T. performed research; A.G. and T.K. contributed new reagents/analytic tools; D.D. and D.W. analyzed data; and D.D. and M.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE93293).

[1]To whom correspondence should be addressed. Email: roland.schuele@uniklinik-freiburg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1702641114/-/DCSupplemental.

rescues the age-related beige-to-white transition of ingWAT. Conversely, adipocyte-specific loss of Lsd1 induces a premature progressive whitening of ingWAT before 10 wk of age. Lsd1 ablation allows the emergence of basal beige adipocytes but prevents the formation of beige adipocytes induced upon cold exposure. Finally, basal and cold-induced beige adipocytes cannot be maintained after conditional Lsd1 ablation in WAT even in the presence of continuous cold stimulus. Progressive beige-to-white transition of ingWAT is mediated by the Lsd1 target gene peroxisome proliferator-activated receptor α (Ppara). In beige fat, Lsd1 is recruited to the Ppara promoter, boosting Ppara expression and promoting the beige phenotype. In summary, our data uncover Lsd1 as a key player controlling the age-related beige-to-white transition of ingWAT via direct control of Ppara expression.

Results

Lsd1 Is Essential for the Maintenance of Basal Beige Adipocytes. To evaluate whether Lsd1 might contribute to the age-programmed loss of basal beige adipocytes, we analyzed Lsd1 expression in WAT of mice at 10 wk (young adult) and 30 wk (old) of age. Lsd1 transcript and protein levels dramatically decreased in epWAT and ingWAT of old compared with young WT mice (Fig. 1 *A* and *B* and Fig. S1*A*), which was not observed in other tissues such as liver (Fig. 1*A* and Fig. S1*A*). H&E staining of WT ingWAT displayed an age-related conversion from beige to white adipocyte morphology (Fig. 1*C*) as previously reported (14). We thus hypothesized that Lsd1 might play a role in the maintenance of beige adipocytes and that the gradual loss of Lsd1 with age might be responsible for the morphological changes observed in ingWAT of older mice. To validate this hypothesis, we aimed to maintain Lsd1 expression selectively in adipocytes during aging. We engineered transgenic mice in which the flag-tagged human Lsd1 coding sequence followed by a floxed translational stop cassette is under the control of the chicken β-actin promoter (*Methods* and Fig. S1*B*). The transgenic mice were crossed with the well described adiponectin (Adipoq)-Cre deleter strain (16) to generate adipocyte-specific Lsd1-overexpressing mice, hereafter named Lsd1^{cTg} (Fig. S1*B*). Western blot analysis confirmed that Lsd1 levels remained high in 30-wk-old Lsd1^{cTg} mice but decreased with age in control mice (Fig. S1*C*). In ingWAT of young Lsd1^{cTg} mice, we did not observe any morphological changes compared with age-matched control mice (Fig. 1*C*). Importantly, the typical age-related phenotypic beige-to-white adipocyte transition was robustly impaired in ingWAT of old Lsd1^{cTg} mice (Fig. 1*C*), and old Lsd1^{cTg} mice conserved their pool of Ucp1-positive beige fat cells to a similar extent as young WT animals (Fig. 1*D* and Fig. S1*D*). Next, we tested whether the transcription levels of beige-selective markers (17) and genes involved in thermogenesis and oxidative metabolism were altered in ingWAT during aging. In accordance with the observed morphological changes, analyzed markers were strongly down-regulated with age in ingWAT of control mice (Fig. 1*E* and Fig. S1*C*). In contrast, this decrease was less severe in old Lsd1^{cTg} mice, and the expression pattern resembled that of young mice (Fig. 1*E*). As a control, the expression of general adipogenic markers was not altered (Fig. S1*E*). These data show that maintaining Lsd1 levels in ingWAT during aging is sufficient to preserve basal beige adipocyte morphology and gene expression program.

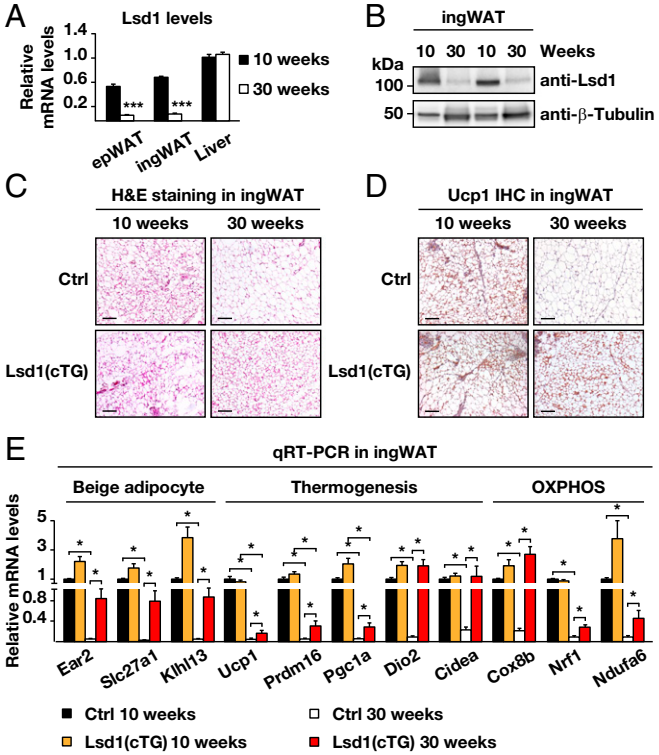

Fig. 1. Lsd1 prevents the age-programmed loss of beige adipocytes. (*A*) qRT-PCR analysis showing relative Lsd1 mRNA levels in epWAT and ingWAT and in liver of 10- and 30-wk-old WT mice (mean ± SEM; ***$P < 0.001$; 10-wk-old mice, $n = 9$; 30-wk-old mice, $n = 12$). (*B*) Western blot analysis of Lsd1 protein levels in ingWAT of 10- and 30-wk-old WT mice. β-Tubulin was used as a loading control. (*C*) H&E staining and (*D*) immunohistochemical detection of Ucp1 on representative sections of ingWAT from control (Ctrl) and adipose tissue-specific Lsd1 overexpressing (Lsd1^{cTg}) mice at 10 and 30 wk of age. (Scale bars: 200 μm.) (*E*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control and Lsd1^{cTg} mice at 10 and 30 wk of age (mean ± SEM; *$P < 0.05$; $n = 12$).

Loss of Lsd1 in Adipocytes Causes Premature Beige-to-White Transition of ingWAT. As the presence of Lsd1 protein seems to be essential to maintain basal beige fat cells in ingWAT, we hypothesized that loss of Lsd1 in adipocytes would lead to a premature loss of the beige fat cells. Therefore, we crossed mice harboring conditional Lsd1 alleles (Lsd1$^{p/p}$ mice) (18) with the Adipoq-Cre deleter strain, resulting in Cre-mediated loss of Lsd1 selectively in adipocytes, hereafter named Lsd1^{cKO} mice (Fig. S2 *A* and *B*). Of note, beige adipocytes emerged in ingWAT of control and Lsd1^{cKO} mice. However, already at 6 wk of age, ingWAT of Lsd1^{cKO} mice morphologically resembled that of 10-wk-old control mice (Fig. S2*C*). More importantly, at 10 wk of age, ingWAT of Lsd1^{cKO} mice elicited uniformly white adipocyte appearance (Fig. 2*A*). Consistently, Ucp1 protein levels were decreased (Fig. 2*B* and Fig. S2 *B* and *D*), and transcript levels of beige fat-selective markers were down-regulated in 10-wk-old Lsd1^{cKO} mice compared with their control littermates and were similar to the levels of 30-wk-old control mice (Fig. 2*B* and Fig. S2*E*). Of note, the loss of beige adipocytes in Lsd1^{cKO} mice was not caused by apoptosis as shown by transcript levels of *Bax* and *Bcl2* and protein levels of Parp and Casp3 (Fig. S2 *E* and *F*). In summary, beige adipocytes emerge in ingWAT in the absence of Lsd1. However, beige adipocytes in young Lsd1^{cKO} mice alter their morphology and transcriptional repertoire prematurely to resemble mature adipocytes of old WT mice. These data indicate that Lsd1 loss contributes to the age-related depletion of beige adipocytes.

To unravel the destiny of beige adipocytes in Lsd1^{cKO} mice, i.e., to determine whether Ucp1-positive beige fat cells are progressively converted to white adipocytes with age, we crossed Lsd1^{cKO} mice with the previously published Ucp1-diphtheria toxin receptor (Dtr)-GFP reporter strain (19) to generate Lsd1$^{cKO\text{-}GFP}$ mice. These mice express GFP fused to a transmembrane protein, the Dtr, under the control of the *Ucp1* promoter.

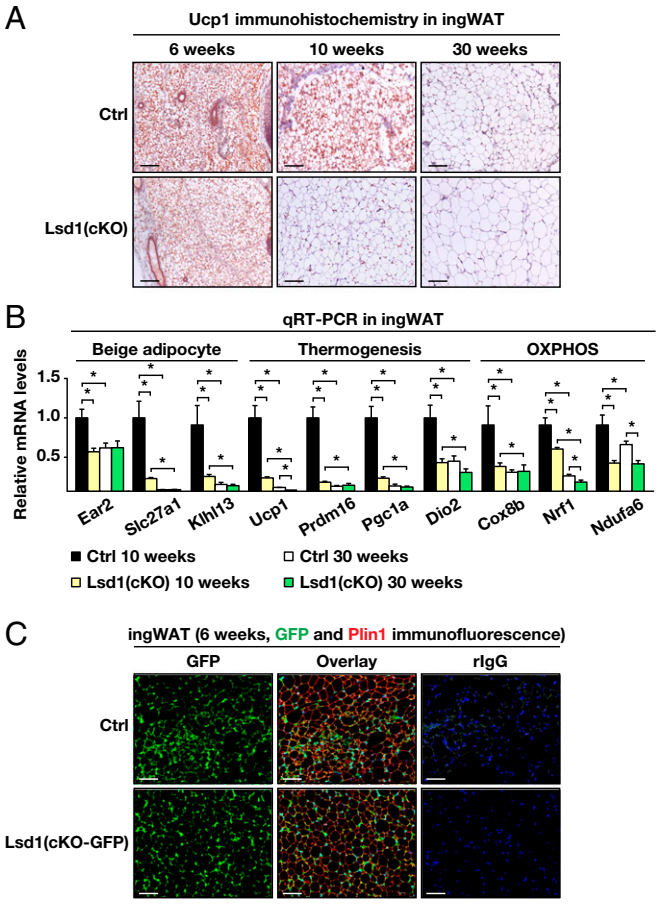

Fig. 2. Loss of Lsd1 in adipocytes accelerates the beige-to-white transition of ingWAT. (*A*) Immunohistochemical detection of Ucp1 on ingWAT from control (Ctrl) and adipocyte-specific Lsd1 KO (Lsd1^{cKO}) mice at 6, 10, and 30 wk of age. (Scale bars: 200 μm.) (*B*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control and Lsd1^{cKO} mice at 10 and 30 wk of age (mean ± SEM; $*P < 0.05$; $n = 10$). (*C*) Immunofluorescence detection using anti-GFP and anti-perilipin (Plin1) antibodies on representative sections of ingWAT of control and Lsd1$^{cKO\text{-}GFP}$ mice containing Ucp1-Dtr-GFP fusion construct, which enables detection of membrane-localized GFP protein in Ucp1-positive cells and their descendants. (Scale bars: 100 μm.)

Thus, GFP is selectively expressed and addressed to the membrane of Ucp1-positive cells, allowing us to trace the fate of Ucp1-expressing beige adipocytes. Lsd1$^{p/p}$ mice expressing Ucp1-Dtr-GFP were used as controls. Importantly, a population of white adipocytes present in ingWAT of Lsd1$^{cKO\text{-}GFP}$ mice stained positive for GFP (Fig. 2*C*), demonstrating that, in the absence of Lsd1, Ucp1-expressing basal beige adipocytes are formed but converted into cells with white adipocyte appearance.

Lsd1 Is Required for the Maintenance of Beige Adipocytes in Adult Mice. To address whether Lsd1 is required for the maintenance of basal beige adipocytes at adult stage, we generated tamoxifen (Tam)-inducible Lsd1$^{iKO\text{-}GFP}$ mice by crossing Lsd1$^{p/p}$ mice with the Adipoq-CreER^{T2}deleter (20) and the Ucp1-Dtr-GFP reporter strains (Fig. S2*G*). Lsd1$^{p/p}$ mice expressing Ucp1-Dtr-GFP were used as controls. In Lsd1$^{iKO\text{-}GFP}$ mice, adipocyte-specific Lsd1 ablation is induced upon Tam administration at 7 wk of age (Fig. S2 *H–J*). Consistent with the data obtained from Lsd1^{cKO} mice, we observed a beige-to-white adipocyte transition in ingWAT of Lsd1$^{iKO\text{-}GFP}$ mice 3 wk after depletion of Lsd1 (Fig. S2*K*). Of note, GFP tracing revealed that Lsd1-depleted white adipocytes in ingWAT of Lsd1$^{iKO\text{-}GFP}$ mice emerged from preexisting beige fat cells (Fig. S2 *L* and *M*), confirming that Lsd1 is required to maintain the pool of basal beige fat cells in young adult mice. Taken together, our Lsd1-proficient and -deficient mouse models present Lsd1 as an important regulator of beige-to-white adipocyte transition in ingWAT.

Lsd1 Is Required for the Formation and Maintenance of Cold-Induced Beige Adipocytes. To address whether Lsd1 is required for emergence and maintenance of induced beige adipocytes, we administered Tam to 7-wk-old control and Lsd1$^{iKO\text{-}GFP}$ mice. At 9 wk of age, mice were exposed for 10 d to cold (Fig. S3*A*). Although the appearance of cold-induced beige adipocytes was evident in control mice (Fig. S3*A*), very few cold-induced beige adipocytes emerged in ingWAT of Lsd1$^{iKO\text{-}GFP}$ mice (Fig. S3*A*). To rule out the possibility that induced beige adipocytes were formed in Lsd1$^{iKO\text{-}GFP}$ mice but immediately converted into white adipocytes, we traced GFP-positive cells. Upon cold exposure, the number of GFP-positive beige adipocytes strongly increased in control mice, whereas no GFP-positive adipocytes displaying a beige morphology were detected in Lsd1$^{iKO\text{-}GFP}$ mice (Fig. S3*B*). To test whether the impairment of cold-induced beige adipocyte formation affects the functional properties of ingWAT, we assayed mitochondrial respiration. Cold-induced respiratory capacities of ingWAT of Lsd1$^{iKO\text{-}GFP}$ mice were significantly hampered, contrary to that of control mice (Fig. S3*C*), indicating an impaired thermogenic response. In accordance, expression of oxidative and thermogenic markers was only slightly elevated in Lsd1$^{iKO\text{-}GFP}$ mice exposed to cold (Fig. S3*D*), unlike their control littermates, which responded to cold by robust up-regulation of these markers. Finally, we inquired whether Lsd1 also governs the maintenance of inducible beige adipocytes. Therefore, control and Lsd1$^{iKO\text{-}GFP}$ mice were exposed to cold to induce appearance of beige fat cells (Fig. 3*A*). After 10 d of cold treatment, mice were injected with Tam in the presence of the continuous cold stimulus and kept at 10 °C for another 2 wk (in total, 25 d of cold exposure; Fig. 3*A*). Control mice exposed to cold for 25 d developed significantly more beige fat cells in ingWAT compared with mice exposed for 10 d (Fig. 3*A*). On the contrary, upon Lsd1 loss, cold-induced beige adipocytes in ingWAT of Lsd1$^{iKO\text{-}GFP}$ mice underwent a massive beige-to-white adipocyte transition (Fig. 3*A*). Importantly, white adipocytes observed in mice with Lsd1 ablation were GFP-positive, indicating that they originated from Ucp1-expressing beige adipocytes (Fig. 3*B*). Loss of Lsd1 caused decreased mitochondrial respiration (Fig. S3*E*) and down-regulation of beige fat-selective markers (Fig. 3*C*), confirming that adipocytes from ingWAT have reduced thermogenic capacities. In summary, our data show that development and maintenance of cold-induced beige adipocytes critically depend on Lsd1 (Fig. S3 *F* and *G*).

Lsd1 Targets Ppara to Maintain Beige Adipocytes in ingWAT. To identify Lsd1 target genes that contribute to the maintenance of beige adipocytes, we determined the transcriptome of Lsd1^{cKO} and control mice at 6 wk of age, when beige adipocytes prevail in ingWAT of WT mice. We identified 727 differentially expressed genes (DEGs) in Lsd1^{cKO} compared with control mice. Among these 727 DEGs, 449 were up- and 278 were down-regulated (Fig. S4*A*). Pathway analysis of the 449 up-regulated transcripts uncovered genes related to glucose metabolism (Table S1), similar to what we observed in BAT (21). Ppar signaling was one of the most enriched pathways among the 278 down-regulated genes (Fig. S4*B* and Table S1). Ppars are ligand-regulated nuclear receptors that have been shown to play important roles in fat differentiation and metabolism (22, 23). In particular, Ppara is expressed at substantially higher levels in brown relative to white adipocytes (24, 25). Interestingly, our RNA sequencing (RNA-seq) data revealed a 50% decrease in transcript levels of

PHYSIOLOGY

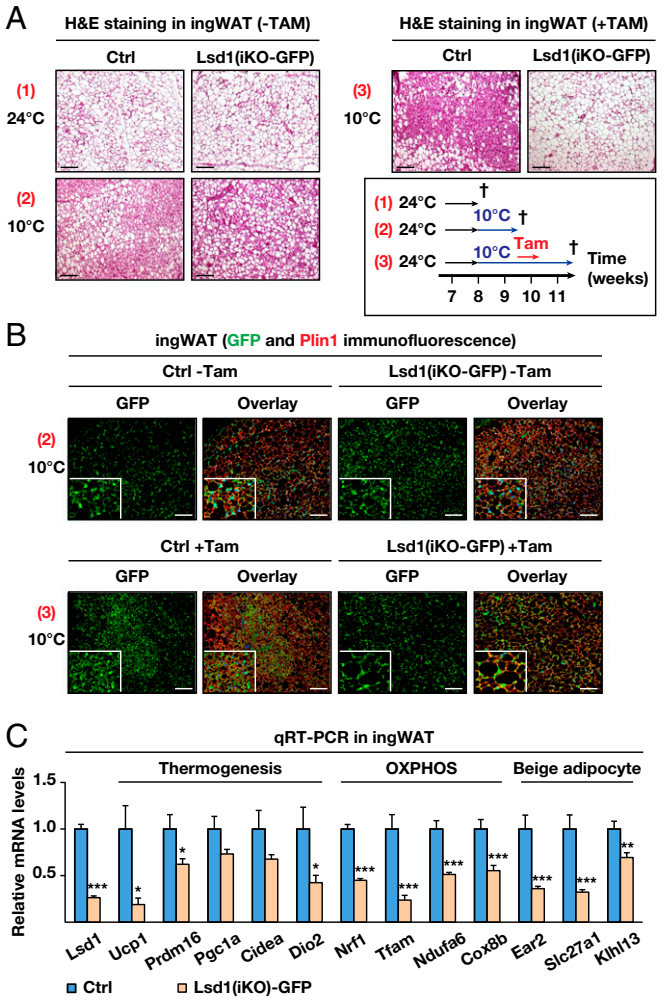

Fig. 3. Lsd1 is required for development and maintenance of cold-induced beige adipocytes. (*A*) H&E staining and (*B*) immunofluorescence detection using anti-GFP and anti-Plin1 antibodies on representative sections of ingWAT of control (Ctrl) and Lsd1$^{iKO\text{-}GFP}$ mice in the absence or presence of Tam treatment. Time scale indicates age of the mice and start and end of Tam and cold treatment. Dagger indicates the time point at which mice were killed. (Scale bars: 200 μm.) (*C*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT extracts from control and Lsd1$^{iKO\text{-}GFP}$ mice treated as described in *A* (mean ± SEM; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001; *n* = 7).

Ppara in Lsd1^{cKO} compared with control mice. We therefore hypothesized that Lsd1, at least in part, might exert its action by regulating Ppara.

To investigate whether Lsd1 associates with the *Ppara* locus, we used C3H-10T1/2 multipotent mesenchymal cells (26, 27) and immortalized preadipocytes isolated from the stromal vascular fraction from ingWAT, which can be differentiated into beige or white adipocytes (*Methods*). Upon treatment with mixtures promoting beige or white adipogenesis, immortalized preadipocytes acquired the transcriptional repertoire of beige and white adipocytes, respectively (Fig. S4*C*). In C3H-10T1/2 cells, beige adipocyte properties were stimulated by treating the cells with the β3AR agonist CL316,243 (15, 28, 29). In both cell lines, *Ppara* expression increased in beige compared with white adipocytes (Fig. S4*D*). This induction was efficiently abolished by treating the cells with Lsd1-specific inhibitor QC6688 [Lsd1(i)] (21) (Fig. S4 *C* and *D*). To unravel whether Lsd1 directly controls *Ppara* expression, both cell lines were subjected to ChIP followed by quantitative PCR (qPCR). In the presence of stimuli promoting beige adipogenesis, Lsd1 was enriched at the promoter of Ppara in both differentiated C3H-10T1/2 and immortalized adipocytes (Fig. 4*A* and Fig. S4*E*). Increased Lsd1 occupancy was accompanied by recruitment of the coactivator protein p300 and increased PolII binding. Furthermore, we observed increased levels of the active histone marks histone H3 acetylated at lysine 27 (H3K27ac) and histone H3 di- and trimethylated at lysine 4 (H3K4me2 and H3K4me3; Fig. 4*A* and Fig. S4*E*), and decreased levels of the repressive histone marks histone H3 di- and trimethylated at lysine 9 (H3K9me2 and H3K9me3; Fig. 4*A* and Fig. S4*E*). Of note, these marks were not enriched at an unrelated chromatin region (Fig. S4 *F* and *G*) or when IgG was used as a control (Fig. 4*A* and Fig. S4*E*). Next, we addressed whether the role of Lsd1 catalytic activity is involved in the regulation of Ppara expression. The increase in the levels of Lsd1, p300, PolII, H3K27ac, and H3K4me3/me2 in beige adipocytes was abolished by treating the cells with Lsd1(i) (Fig. 4*A*). Conversely, treatment with Lsd1(i) increased the levels of

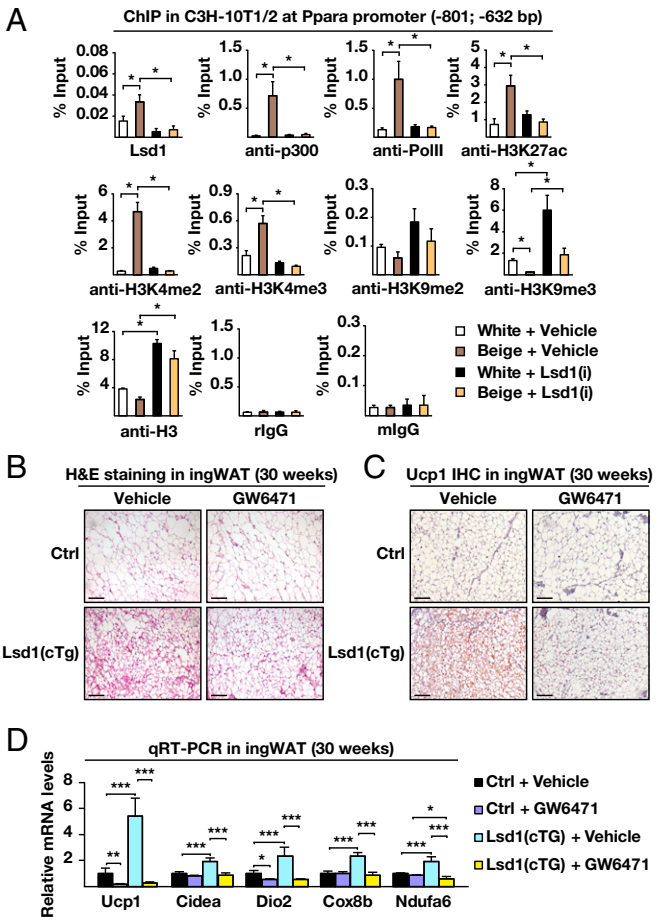

Fig. 4. Lsd1 targets Ppara to maintain beige adipocytes. (*A*) ChIP-qPCR analysis of the Ppara promotor (−801 to −632 bp) performed with anti-Lsd1, anti-p300, anti-PolII, anti-H3K27ac, anti-H3K4me2, anti-H3K4me3, H3K9me2, H3K9me3, anti-H3 antibodies, and rabbit (rIgG) or mouse IgG (mIgG) in differentiated C3H-10T1/2 adipocytes treated with Lsd1-specific inhibitor QC6688 [Lsd1(i)] or vehicle. The precipitated chromatin was quantified by qPCR using primers flanking the −801 to −632 bp region of the Ppara gene (mean ± SEM; **P* < 0.05 and ***P* < 0.01; *n* = 3). (*B*) H&E staining and (*C*) immunohistochemical detection of Ucp1 on representative sections and (*D*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control (Ctrl) and Lsd1^{cTg} mice at 30 wk of age treated for 3 d with vehicle or the Ppara antagonist GW6471 (*D*) (mean ± SEM; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001; *n* = 6). (Scale bars: 200 μm.)

the repressive marks H3K9me3/me2 (Fig. 4*A*) and that of histone H3 in white and beige adipocytes, indicating chromatin compaction (Fig. 4*A*). Together, our data demonstrate that Lsd1 enzymatic activity is essential for induced expression of Ppara in beige adipocytes.

Lsd1-Dependent Maintenance of Beige Adipocytes Is Mediated by Ppara. To demonstrate whether Lsd1-dependent maintenance of beige adipocytes is mediated by Ppara in vivo, we analyzed Ppara protein levels in ingWAT of young and old WT mice. Similar to Lsd1, the expression of Ppara strongly decreased between 10 and 30 wk of age (Fig. S4*H*). Thus, we used GW9578, a potent synthetic Ppara agonist, to activate the residual Ppara protein present in 10 wk-old Lsd1^{cKO} mice. Of note, treatment with GW9578 was sufficient to alleviate the premature beige-to-white adipocyte transition we observed in young Lsd1^{cKO} mice (Fig. S4*I* and S4*J*) and to increase the transcript levels of beige fat markers in control mice (Fig. S4*K*). More importantly, in GW9578-treated Lsd1^{cKO} mice, levels of these markers were comparable to those of vehicle-treated control mice (Fig. S4*K*). These data show that the Ppara agonist GW9578 efficiently rescues the loss of beige adipocytes caused by Lsd1 depletion in ingWAT.

To further corroborate that Lsd1-dependent maintenance of beige adipocytes is mediated by Ppara, we treated 30-wk-old adipocyte-specific Lsd1-overexpressing Lsd1^{cTg} mice with the Ppara antagonist GW6471. H&E staining and Ucp1 immunohistochemistry analysis revealed that, upon GW6471 treatment, maintenance of beige adipocytes is abrogated, and beige adipocytes were barely detectable in old Lsd1^{cTg} mice (Fig. 4 *B* and *C*). In addition, the increased transcription of beige fat markers observed in old Lsd1^{cTg} mice was completely abolished upon GW6471 treatment, demonstrating that Ppara inhibition suffices to deactivate the Lsd1-dependent expression program of beige adipocytes (Fig. 4*D*). In conclusion, our data show that maintenance of beige adipocytes is mediated by the Lsd1 target gene Ppara.

Discussion

Understanding the development and maintenance of beige adipocytes provides exciting insights in establishing novel therapies against obesity and obesity-associated disorders. Despite the broad interest these cells sparked in recent years, the process of progressive age-related beige-to-white transition (14) remains unclear. In the present study, we identified Lsd1 as a regulator of beige adipocyte maintenance. We show that, in WAT, Lsd1 mRNA and protein levels decrease concurrently with aging. In accordance, adipocyte-specific Lsd1 ablation led to an untimely beige-to-white transition in early adulthood. Conversely, adipocyte-specific elevation of Lsd1 expression was sufficient to maintain the pool of beige fat cells. We previously suggested that ubiquitous Lsd1 overexpression induces appearance of beige fat cells in WAT (15). Our findings unravel that adipocyte-specific Lsd1 expression is essential to maintain beige adipocytes. Furthermore, our tracing experiments provide evidence that Lsd1 loss in beige adipocytes results in a beige-to-white adipocyte transition. Maintaining Lsd1 levels in adipocytes would therefore prevent the age-related beige-to-white transition that occurs in adipose tissue.

In the present study, we also analyzed the role of Lsd1 in the development and maintenance of the population of beige adipocytes that are induced upon external stimuli. We found that ablation of Lsd1 robustly impaired the thermogenic response of ingWAT to cold stimulation. In addition, Lsd1 expression was necessary to maintain inducible beige adipocytes and prevent them from acquiring white adipocyte morphology. In previous studies, Rosenwald et al. performed tracing experiments showing in mice that cold-induced beige adipocytes are converted into white adipocytes upon warm adaptation (19). By using a similar mouse model, we traced Ucp1-positive cells and showed that induced beige adipocytes are not formed in Lsd1-ablated ingWAT, contrary to the formation of basal beige adipocytes. To date, it remains unclear whether basal and induced beige adipocytes represent the same cell population, or if they emerge from different precursor cells (30–32). Our findings unravel that Lsd1 expression is required for the development of only induced beige adipocytes, whereas basal ones are formed in ingWAT even upon Lsd1 ablation. Potential explanations for distinct Lsd1 functions might be that basal and induced beige adipocytes originate from different precursors or that their formation is regulated by diverse signaling pathways. However, Lsd1 is necessary to prevent the progressive age-related beige-to-white transition in basal and induced beige adipocytes.

Lsd1 exerts its function in ingWAT by targeting a set of genes involved in thermogenesis, oxidative phosphorylation, and genes identified as beige fat markers (17). In particular, our transcriptome analysis in Lsd1^{cKO} mice identified the nuclear receptor Ppara as an Lsd1 target. Of note, Ppara-null mice present a phenotype close to what we observed in Lsd1^{cKO} mice, as they also showed a reduced number of beige adipocytes (33). A detailed analysis of the *Ppara* locus by ChIP revealed that Lsd1 is recruited in a beige adipocyte-dependent manner. By using a pharmacological approach, we confirmed that Ppara mediates the effects of Lsd1 in mice to maintain beige adipocytes. The Ppara agonist GW9578 was already shown to reduce adiposity in vivo (34, 35) and to positively regulate the expression of Ucp1 in brown adipocytes in cell culture (6). However, to our knowledge, we provide first evidence that the Ppara agonist GW9578 promotes expression of the thermogenic gene program in beige adipocytes and rescues the untimely beige-to-white fat transition phenotype induced by loss of Lsd1. Conversely, our data show that treatment of 30-wk-old Lsd1^{cTg} mice with the Ppara antagonist GW6471 significantly impairs Lsd1-dependent expression program and counteracts the ability of Lsd1 to maintain beige adipocytes. Our findings give pioneering insights in the potential use of pharmacological Ppara modulators to maintain beige adipocytes and prevent age-related beige-to-white fat transition. However, because we applied systemic treatment of both Ppara agonist and antagonist, the observed effects might not be fully mediated by Ppara in adipose tissue. Therefore, it would be of interest to cross Lsd1^{cTg} and Lsd1^{cKO} mice with adipocyte-specific Ppara-KO or -overexpressing mice, respectively.

Adipose tissue is a plastic organ that can easily adapt to environmental cues. In this report, and in our former studies (15), we show that levels of the epigenetic modifier Lsd1 are affected by environmental stimuli in a two-way direction. Cold exposure and treatment with β3-adrenergic agonists enhance Lsd1 expression, which in turn will activate Ppara and a battery of thermogenic genes. On the contrary, with progressing age or upon a high-caloric diet, Lsd1 levels decrease and beige adipocytes lose their thermogenic functions and accumulate lipids. In this respect, Lsd1 might be considered as a sensor of metabolic requirements in beige adipocytes. Thus, it will be important to better understand the regulation of Lsd1 expression to control maintenance and function of beige adipocytes to potentially fight obesity.

Methods

All mice were housed in a pathogen-free barrier facility of the University Medical Center Freiburg in accordance with institutional guidelines and approved by the Regierungspräsidium Freiburg.

Generation of Conditional Lsd1-KO Mice. All experiments were performed in C57/Bl6N background. The targeting strategy for the conditional deletion

of the first exon of Lsd1 (Lsd1tm1Schüle) is available upon request (18). Briefly, conditional Lsd1 mice were mated with Adipoq-Cre (16) or Adipoq-CreERT2 (20) mice to selectively ablate Lsd1 in adipose tissue. Homozygous conditional mice were used as controls. Mice were genotyped with primers for detection of conditional Lsd1 alleles and Cre recombinase (Table S2).

RNA Preparation and qRT-PCR. RNA was isolated with TRIzol Reagent (Invitrogen) and processed as described previously (15). qRT-PCR data were analyzed by using the standard curve method (39). 36b4 or Hprt were used for normalization. Primer sequences used are given in Table S3.

RNA-Seq. Total RNA was sequenced by the standard Illumina protocol to create raw sequence files (.fastq files). We annotated these reads to the mm10 build of the mouse genome by using TopHat version 2. The aligned reads were counted with Homer software (analyze RNA), and DEGs were identified by using EdgeR and DESeq version 1.8.3. Differentially regulated genes (reads > 50, $P < 10^{-3}$, and fold change >1.3 or <0.77) were further used for pathway analysis in WebGestalt (36, 37). RNA-seq data are available from the Gene Expression Omnibus database (accession no. GSE93293).

ChIP. ChIP experiments were performed by using anti-Lsd1 [R.S. laboratory (15)], anti-H3K27ac (C15410196; Diagenode), anti-p300 (ab14984; Abcam), anti-PolII (sc-899, N-20; Santa Cruz), anti-H3K4me1 (ab8895; Abcam), anti-H3K4me2 (CS-035-100; Diagenode), anti-H3K4me3 (ab1012; Abcam), anti-H3K9me2 (Mab-154-050; Diagenode), anti-H3K9me3 (C15410056; Diagenode), and anti-H3 (ab1791; Abcam) antibodies, or a rabbit or mouse IgG-negative control on protein G-Sepharose 4B beads (GE Healthcare) as described previously (38). ChIPed DNA was used for qPCR analyses with the primers described in Table S4.

ACKNOWLEDGMENTS. The authors thank F. Pfefferle for providing excellent technical assistance, J.M. Müller for helpful discussions, and Prof. Marcel Scheideler (Helmholtz Zentrum Muenchen) for the gift of immortalized preadipocytes. This work was supported by European Research Council Ad Grant 322844 (to R.S.) and Deutsche Forschungsgemeinschaft Grants SFB 992, 850, and 746 (to R.S.).

1. Cypess AM, et al. (2009) Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 360:1509–1517.
2. Cannon B, Nedergaard J (2004) Brown adipose tissue: Function and physiological significance. *Physiol Rev* 84:277–359.
3. Petrovic N, et al. (2010) Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* 285:7153–7164.
4. Ishibashi J, Seale P (2010) Medicine. Beige can be slimming. *Science* 328:1113–1114.
5. Vitali A, et al. (2012) The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. *J Lipid Res* 53:619–629.
6. Galmozzi A, et al. (2014) ThermoMouse: An in vivo model to identify modulators of UCP1 expression in brown adipose tissue. *Cell Reports* 9:1584–1593.
7. Himms-Hagen J, et al. (2000) Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am J Physiol Cell Physiol* 279:C670–C681.
8. Sanchez-Gurmaches J, Guertin DA (2014) Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. *Nat Commun* 5:4099.
9. Sanchez-Gurmaches J, Hung CM, Guertin DA (2016) Emerging complexities in adipocyte origins and identity. *Trends Cell Biol* 26:313–326.
10. Seale P, et al. (2008) PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454: 961–967.
11. Long JZ, et al. (2014) A smooth muscle-like origin for beige adipocytes. *Cell Metab* 19: 810–820.
12. McDonald ME, et al. (2015) Myocardin-related transcription factor A regulates conversion of progenitors to beige adipocytes. *Cell* 160:105–118.
13. Graja A, Schulz TJ (2015) Mechanisms of aging-related impairment of brown adipocyte development and function. *Gerontology* 61:211–217.
14. Rogers NH, Landa A, Park S, Smith RG (2012) Aging leads to a programmed loss of brown adipocytes in murine subcutaneous white adipose tissue. *Aging Cell* 11: 1074–1083.
15. Duteil D, et al. (2014) LSD1 promotes oxidative metabolism of white adipose tissue. *Nat Commun* 5:4093.
16. Eguchi J, et al. (2011) Transcriptional control of adipose lipid handling by IRF4. *Cell Metab* 13:249–259.
17. Wu J, et al. (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150:366–376.
18. Zhu D, et al. (2014) Lysine-specific demethylase 1 regulates differentiation onset and migration of trophoblast stem cells. *Nat Commun* 5:3174.
19. Rosenwald M, Perdikari A, Rülicke T, Wolfrum C (2013) Bi-directional interconversion of brite and white adipocytes. *Nat Cell Biol* 15:659–667.
20. Sassmann A, Offermanns S, Wettschureck N (2010) Tamoxifen-inducible Cre-mediated recombination in adipocytes. *Genesis* 48:618–625.
21. Duteil D, et al. (2016) Lsd1 ablation triggers metabolic reprogramming of brown adipose tissue. *Cell Reports* 17:1008–1021.
22. Picard F, et al. (2002) SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell* 111:931–941.
23. Seale P (2015) Transcriptional regulatory circuits controlling brown fat development and activation. *Diabetes* 64:2369–2375.
24. Rachid TL, et al. (2015) Fenofibrate (PPARalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. *Mol Cell Endocrinol* 402:86–94.
25. Wang L, et al. (2013) PPARα and Sirt1 mediate erythropoietin action in increasing metabolic activity and browning of white adipocytes to protect against obesity and metabolic disorders. *Diabetes* 62:4122–4131.
26. Tang QQ, Otto TC, Lane MD (2004) Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci USA* 101:9607–9611.
27. Tseng YH, et al. (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454:1000–1004.
28. Seale P, et al. (2011) Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest* 121:96–105.
29. Mottillo EP, Granneman JG (2011) Intracellular fatty acids suppress β-adrenergic induction of PKA-targeted gene expression in white adipocytes. *Am J Physiol Endocrinol Metab* 301:E122–E131.
30. Lee YH, Petkova AP, Konkar AA, Granneman JG (2015) Cellular origins of cold-induced brown adipocytes in adult mice. *FASEB J* 29:286–299.
31. Lee YK, Cowan CA (2013) White to brite adipocyte transition and back again. *Nat Cell Biol* 15:568–569.
32. Wang QA, Tao C, Gupta RK, Scherer PE (2013) Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat Med* 19:1338–1344.
33. Li P, Zhu Z, Lu Y, Granneman JG (2005) Metabolic and cellular plasticity in white adipose tissue II: Role of peroxisome proliferator-activated receptor-alpha. *Am J Physiol Endocrinol Metab* 289:E617–E626.
34. Guerre-Millo M, et al. (2000) Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* 275:16638–16642.
35. Harrington WW, et al. (2007) The effect of PPARalpha, PPARdelta, PPARgamma, and PPARpan agonists on body weight, body mass, and serum lipid profiles in diet-induced obese AKR/J mice. *PPAR Res* 2007:97125.
36. Heinz S, et al. (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38:576–589.
37. Wang J, Duncan D, Shi Z, Zhang B (2013) WEB-based GEneSeTAnaLysis Toolkit (WebGestalt): update 2013. *Nucleic Acids Res* 41:W77–83.
38. Metzger E, et al. (2008) Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation. *Nat Cell Biol* 10:53–60.
39. Bookout AL, Cummins CL, Mangelsdorf DJ, Pesola JM, Kramer MF (2006) High-throughput real-time quantitative reverse transcription PCR. *Curr Protoc Mol Biol*, 10.1002/0471142727.mb1508s73.
40. Duteil D, et al. (2010) The transcriptional coregulators TIF2 and SRC-1 regulate energy homeostasis by modulating mitochondrial respiration in skeletal muscles. *Cell Metab* 12:496–508.
41. Metzger E, et al. (2016) Assembly of methylated KDM1A and CHD1 drives androgen receptor-dependent transcription and translocation. *Nat Struct Mol Biol* 23:132–139.
42. Todaro GJ, Green H (1963) Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J Cell Biol* 17:299–313.