**β-actin contributes to open chromatin for activation of the adipogenic pioneer factor CEBPA during transcriptional reprogramming**

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**ABSTRACT** Adipogenesis is regulated by a cascade of signals that drive transcriptional reprogramming in adipocytes. Here, we report that nuclear actin regulates the chromatin states that establish tissue-specific expression during adipogenesis. To study the role of β-actin in adipocyte differentiation, we conducted RNA sequencing on wild-type and β-actin knockout mouse embryonic fibroblasts (MEFs) after reprogramming to adipocytes. We found that β-actin depletion affects induction of several adipogenic genes during transcriptional reprogramming. This impaired regulation of adipogenic genes is linked to reduced expression of the pioneer factor Cebpα and is rescued by reintroducing NLS-tagged β-actin. ATAC-Seq in knockout MEFs revealed that actin-dependent reduction of Cebpα expression correlates with decreased chromatin accessibility and loss of chromatin association of the ATPase Brg1. This, in turn, impairs CEBPB’s association with its Cebpα promoter-proximal binding site during adipogenesis. We propose a role for the nuclear β-actin pool in maintaining open chromatin for transcriptional reprogramming during adipogenic differentiation.

**INTRODUCTION**

In the nucleus, actin is required by all three eukaryotic RNA polymerases for both chromatin remodeling and transcription regulation (Virtanen and Vartiainen, 2010; Percipalle, 2013; Virtanen and Vartiainen, 2016; Xie and Percipalle, 2018). Actin-dependent epigenetic changes also significantly influence chromatin organization by facilitating heterochromatin segregation at the nuclear periphery and maintaining H3K9Me3-positive heterochromatin levels in the nucleus (Xie and Percipalle, 2018). We recently reported that these mechanisms have a significant impact on the expression of gene programs required during neurogenesis (Almuzzaini et al., 2016; Xie and Percipalle, 2018; Xie et al., 2018a). β-actin seems to perform these functions by regulating genomic deposition of Brg1 (Brahma-related gene 1), the ATPase subunit of the chromatin remodeling complex SWI/SNF or BAF (Brahma-associated factor). We found that β-actin-dependent loss of Brg1 binding at transcription start sites (TSS) of proneuronal and neuronal genes, along with promoter-specific H3K9me3 accumulation, accounts for loss of neuronal identity (Xie et al., 2018b). These findings suggest that β-actin may have a role in regulating activation of occluded genes during reprogramming to neurons. However, it remains unclear if β-actin-dependent Brg1 deposition is also required to maintain an open chromatin state during transcriptional reprogramming in other differentiation models such as adipogenesis.

During adipogenesis, preadipocytes differentiate into adipocytes, the main constituent of adipose tissue (Rosen et al., 2000; Rajala and Scherer, 2003; Kershaw and Flier, 2004).
Rosen and Macdougald, 2006; Adamczak and Wieck, 2013; McGown et al., 2014; Cinti, 2018). Adipocyte differentiation involves an interactive network of signaling cascades and transcription factors, including the peroxisome proliferator-activated receptor γ (PPARγ), members of the CCAAT/enhancer-binding protein (CEBP) family (Spiegelman and Flier, 1996; Farmer, 2006; Lowe et al., 2011), and the BAF complex (Siersbaek et al., 2012; Shapiro et al., 2017; Shapiro and Seale, 2019). PPARγ is a master regulator of adipogenesis (Spiegelman, 1998), whereas members of CEBP family, specifically CEBPA (Linhart et al., 2001; Rosen et al., 2002) play a role in adipocyte subtype specificity, promoting white adipocyte over brown adipocyte differentiation (Linhart et al., 2001). During differentiation, adipocytes adopt a rounded morphology for maximal lipid storage (Smas and Sul, 1995; Rosen et al., 2002), a configuration modulated by the actin cytoskeleton (Jaffe and Hall, 2005; Sordella et al., 2003; Mcbeath et al., 2004; Noguchi et al., 2007; Tanegashima et al., 2008; Horii et al., 2009; Nobusue et al., 2014).

While investigating the potential role of actin in Brg1 reprograming to adipogenic gene loci, we discovered that loss of nuclear β-actin leads to changes in the expression of adipogenic gene programs, alterations in heterochromatin regulation, and down-regulation of the Cebpa gene. ATAC-Seq analysis of wild-type (WT) and knockout (KO) MEFs shows that this is due to loss of chromatin accessibility at the TSS and promoter-proximal region of Cebpa, consistent with increased H3K9Me3 accumulation and loss of Brg1 binding. Excess chromatin compaction, in turn, impairs CEBPB binding to a specific regulatory motif upstream of the Cebpa TSS. Our results suggest that β-actin is required to maintain an open chromatin state during transcriptional reprogramming.

RESULTS AND DISCUSSION
Transcriptional reprogramming to adipocytes is dysregulated in the absence of β-actin
To find a potential role of β-actin in adipocyte differentiation, we performed gene ontology (GO) analysis on statistically significant genes (cutoff of p-value < 0.01) differentially expressed between WT and β-actin KO mouse embryonic fibroblasts (MEF-WT and MEF-KO; Xie et al., 2018a; Figure 1A). We identified GO terms for fat cell differentiation (GO:0045600) and adipose tissue development (GO:00060612; Figure 1A; Supplemental Table S1; Supplemental Table S2). Metric normalized (log count per million) heatmaps revealed down-regulation of Wif1, Frzb, Bmp2, Srfp1, and Wnt5b and up-regulation of Htr2a and Medag in the KO condition as compared with WT (Figure 1B). Among the genes involved in adipose tissue development, Ebf2 is heavily up-regulated, whereas genes such as Ppargc1a, Rorc, and Hmga2 are down-regulated in the KO condition (Figure 1C). These findings suggest that expression of genes related to adipocyte differentiation is dysregulated upon β-actin depletion.

We next induced adipocytes from both WT and KO MEFs as recently described (Cuaranta-Monroy et al., 2014). Following a six-day induction (Figure 2A), the induced adipocytes (MEF-iA) were probed for the formation of lipid triglyceride droplets around cell nuclei (Ramirez-Zacarias et al., 1992). Using Oil Red O (ORO) staining, a triglyceride-specific stain (Ramirez-Zacarias et al., 1992), we found that both MEF-WT and MEF-KO cells were positive for lipid droplets (Figure 2, B and C) after induction with adipogenic differentiation medium. Interestingly, although the production of lipid droplets seems lower in KO cells with respect to WT condition, cells lacking β-actin showed the presence of lipid droplets even in the undifferentiated stage. This phenotype combined with differential expression of key genes involved in adipocyte differentiation between KO and WT suggests that loss of β-actin affects adipogenic gene programs during reprogramming.

Transcriptome analysis reveals differential gene expression in adipocytes lacking β-actin
We performed RNA-Seq analysis on induced KO and WT adipocytes (MEF-iA) and compared their transcriptomes with their MEF counterparts (Figure 2). Principal component analysis (PCA) of the RNA-Seq datasets showed 48% total variance in the first principal component (PC1) and clearly separated uninduced MEFs from induced adipocytes (Supplemental Figure S1A). Pairwise comparisons between MEFs and corresponding MEF-iA in KO and WT conditions based on significance (p-value < 0.01) and log 2-fold change of greater than 0 (FC > 0) among differentially expressed genes revealed major transcriptome differences. In the two pairwise comparisons, MEF-WT-iA/MEF-WT and MEF-KO-iA/MEF-KO, we identified 4098 and 3330 differentially expressed genes (DEGs), which were further analyzed for GO enrichment (biological processes) using DAVID (Figure 2D). We observed a strong enrichment of genes regulating fat cell differentiation and subtype specificity. The GO-term “fat cell differentiation” (GO:0045444) had 24 genes associated with it in the MEF-WT-iA/MEF-WT comparison and 21 genes in the MEF-KO-iA/MEF-KO comparison. Only 12 of these genes were common to both comparisons, with the remaining being exclusively enriched in one condition (Figure 2E). A heat map of the normalized read counts of these genes revealed that the majority of these genes were up-regulated upon adipogenesis induction in both WT and KO MEFs (Figure 2F).

Members of the CCAAT/enhancer-binding protein beta (Cebpβ) and delta (Cebpδ) that function as transcription factors in cellular differentiation (Scott et al., 1992; Wu et al., 1996; Tanaka et al., 1997), metabolism (Cardinaux and Magistretti, 1996) and immune responses (Zannetti et al., 2010) were induced under both conditions (Figure 2). Similarly, Socs1, Fcrr, Sox8, Smad6, Atf5, Egr2, and Id4, generally involved at multiple stages of adipogenesis were also up-regulated under both conditions (Figure 2F). However, Cebpa, Steap4, and Psmb8 were not up-regulated in KO adipocytes, in contrast to WT cells (Figure 2F). Steap4 encodes the six transmembrane epithelial antigen of the prostate-4, a plasma membrane protein that is associated with insulin sensitivity (Chen et al., 2010). Psmb8 encodes the proteasome subunit beta 8, an immunoproteasome that has been shown to be essential for adipocyte maturity during differentiation (Arimochi et al., 2016), whereas the Cebpa gene encodes the CCAAT/enhancer-binding protein alpha (CEBPA), a pioneer transcription factor known to induce adipogenesis through the PPARα pathway (Rosen et al., 2002). qPCR analysis on total RNA from induced adipocytes confirmed a significant drop in Cebpa expression under the KO condition. In contrast, Cebpβ and Fabp4, both of which are markers for mature adipocytes, showed significant up-regulation upon adipogenic induction (Moseti et al., 2016; Figure 3A). CEBPA is present in two alternatively spliced isoforms, CEBPA-p30 and CEBPA-p42 (Lane et al., 1996), and, in contrast to CEBPA-p42, during differentiation CEBPA-p30 is known to be up-regulated (Lin et al., 1993; Otto and Lane, 2005). While both WT and KO induced adipocytes showed CEBPA-p42 down-regulation, CEBPA-p30 expression was also significantly reduced in KO cells, consistent with the dysregulation of CEBPA expression in the absence of β-actin (Figure 3, B and C). In contrast, immunoblots of CEBPB and FABP4 did not reveal significant differences between WT and KO adipocytes.

To study if Cebpa expression is regulated by the nuclear β-actin pool during adipogenic induction, we differentiated MEF-KO cells constitutively expressing an NLS-tagged β-actin construct, referred
β-actin in heterochromatin regulation

We found that MEF-NA cells are also induced to adipocyte-like cells (MEF-NA-iA), as evidenced by the extensive formation of ORO-positive lipid droplets (Figure 3, D and E). We next performed qPCR analysis on total RNA from MEF-NA and MEF-KO cells before and after adipogenic induction and quantified the relative expression change of the Cebpa, Cebpb, and Fabp4 genes (Figure 3F). In both conditions, Cebpb and Fabp4 were differentially expressed upon induction to adipocytes. In contrast, the Cebpa gene, which does not exhibit differential expression between MEF-KO and the corresponding adipocyte condition, was significantly induced in MEF-NA after adipocyte induction (Figure 3F). In addition, while induced adipocytes from MEF-NA cells showed significantly higher levels of CEBPA-p30 expression compared with KO cells, immunoblots of CEBPB and FABP4 did not reveal significant differences between WT and KO adipocytes. These findings indicate that reintroduction

FIGURE 1: Sets of adipocyte-related genes are differentially expressed between wild-type (MEF-WT) and β-knockout (MEF-KO) mouse embryonic fibroblasts. (A) Gene ontology on MEF-KO/MEF-WT pairwise comparison reveals enrichment of genes involved in adipose or fat development, actin cytoskeleton, and transcriptional mechanisms. GO enrichment terms are based on significance of p-value < 0.05 (normalized to -log10) cutoff. Numbers at the end of each bar reflect the numbers of genes identified in each GO-term. (B, C) Metric heat-map clustering of expression levels of genes associated with GO term: positive regulation of fat cell differentiation (GO:0045600) and adipose tissue development (GO:0060612). Genes are selected when they are differentially expressed by at least twofold in WT vs. KO comparisons. Clustering is based on the center values (CVs) of mean gene expression levels. Scale bar: log2 CPM. Data presented are based on three biological replicates (n = 3). See Materials and Methods for full description.
FIGURE 2: Direct reprogramming of MEFs into induced adipocytes. (A) Schematic illustration highlighting the differentiation protocol to induce MEFs (MEF-WT and MEF-KO) to adipocyte-like cells termed MEF-iA (MEF-WT-iA and MEF-KO-iA). GM = growth medium, Ins = insulin, DEX = dexamethasone, IBMX = 3-isobutyl-1-methylxanthine, AsA = ascorbic acid. (B) Lipid droplet detection in WT, KO MEFs and corresponding induced adipocytes by ORO staining at day 0 (basal condition) and day 6 (differentiation condition). Scale bar: 125 μm. (C) Quantification of oil red staining isolates via spectrophotometry comparing MEF and MEF-iA conditions for WT and KO with a wavelength optical density (O.D.) of 514 nm (n = 3; * p-value < 0.05) (D) Directional GO enrichment analysis on MEF-WT-iA/MEF-WT (Top) and MEF-KO-iA/MEF-KO (Bottom) identifies GO terms associated with adipocyte-related biological processes. The p-value < 0.05 (normalized by -log10) was defined to be statistically significant and is plotted on the x-axis. Numbers at the ends of bars reflect the number of genes identified in each GO-term. (E) The Venn diagram...
of β-actin in the cell nucleus in MEF-KO cells rescues Cebpa expression.

**β-actin regulates CEBPA expression by maintaining an open chromatin state**

To investigate if β-actin-dependent chromatin remodeling influences adipogenic gene programs, we then used ATAC-Seq to analyze chromatin accessibility around the TSSs and promoter-proximal upstream regulatory regions of genes linked to adipocyte differentiation in WT and KO cells (Figure 2E). Average ATAC-Seq signal at TSS of adipogenic genes did not show a significant difference between WT and KO conditions (Supplemental Figure S2). Similarly, analysis of H3K9Me3, H3K27Me3, and Brg1 ChIP-Seq profiles, together with ATAC-Seq profiles of specific adipogenesis-related genes such as Cebpb, Fabp4, Psmb8, and Steap4, did not reveal a clear link between chromatin accessibility and differential expression (Supplemental Figure S2, A–D). However, we observed a significant reduction in chromatin accessibility at the TSS and promoter-proximal regulatory region of Cebpa (Figure 4; Supplemental Figure S2E). Furthermore, cells heterozygous for β-actin showed an accessibility profile identical to that of WT cells, suggesting that even reduced levels of β-actin can maintain Cebpa in an accessible state (Supplemental Figure S2E). Remarkably, sequence analysis of the Cebpa promoter-proximal region also revealed that the loss of chromat in accessibility observed in KO cells correlated with a CEBP-binding motif present approximately 800 bp upstream of the Cebpa TSS (Figure 4A). To study if CEBPβ binding to this region is compromised during adipogenic induction, we performed chromatin immunoprecipitation combined with RT-qPCR on chromatin isolated from WT and β-actin KO MEFs before and after induction to adipocytes (MEF-IA) with antibodies to CEBPβ and H3K9me3 (Figure 4, B–D). RT-qPCR analysis was performed with several primers targeting multiple regions (R1–R7) upstream of the Cebpa gene TSS, including a CEBPβ binding motif, as well as a region (R6) located inside the Cebpa gene (Figure 4A). Our results show that under the WT condition, CEBPβ is specifically enriched with primers amplifying R5, a unique region encompassing a putative CEBPβ consensus binding site, but not in other regions (Figure 4, B–D; Supplemental Figure S2, F–I). Compatible with the fact that there is a putative CEBPβ binding site (see Figure 4A), strong CEBPβ association with R5 is maintained in differentiated adipocytes (MEF-IA) but is completely lost under the KO condition in both MEF and MEF-IA cells (Figure 4D). In addition, loss of CEBPβ binding correlates with increased H3K9me3 levels under both MEF and induced adipocyte conditions (Figure 4D), implying that an actin-dependent chromatin-based mechanism facilitates CEBPβ access to a specific site to enhance Cebpa gene expression. This is consistent with the rescue of CEBPβ association and loss of H3K9Me3 binding upon reinduction of β-actin into nuclei of KO cells in both MEF and induced adipocytes (Figure 4D) and with enhanced CEBPβ expression upon reinduction of actin in the cell nucleus. Our results suggest that β-actin is required for the maintenance of chromatin accessibility in the promoter-proximal region of Cebpa during adipogenic reprogramming. This mode of regulation is compatible with the role of CEBPβ as a pioneer transcription factor operating upstream of adipogenesis genes such as Psmb8 and Steap4.

**An actin-based mechanism controls chromatin accessibility during adipogenesis**

Establishment of an adipogenic phenotype requires a cohort of transcription factors including CEBPA, CEBPB, CEBPD, and PPARγ. Although they participate in a single pathway of fat cell development, PPARγ is the proximal effector, while CEBPβ works as the pioneer factor needed for robust adipocyte-specific gene expression through binding at PPARγ-adjacent binding sites (Rosen et al., 2002; Lefterova et al., 2008). CEBPβ and CEBPD are upstream of PPARγ and CEBPA. In the mouse 3T3-L1 preadipocyte cell line, PPARγ and CEBPA are regulated by CEBPβ and CEBPD, both rapidly induced within 4 h postdifferentiation. PPARγ and CEBPA are thought to activate each other’s expression, inducing a number of genes that define terminally differentiated adipocytes (Rosen et al., 2002; Rosen and Macdougald, 2006). We found that loss of β-actin does not affect CEBPβ, CEBPD, or PPARγ but leads to selective down-regulation of CEBPβ at both mRNA and protein level. Reinroduction of β-actin in the nucleus of KO cells rescues CEBPβ expression, suggesting that CEBPβ is induced through a nuclear actin-based mechanism, fundamentally different from that activating PPARγ. Consistent with the selective impairment of CEBPβ, GO terms on brown fat cell development in which CEBPβ is involved (Kajimura et al., 2010; Saely et al., 2012; Zhang et al., 2018; Shapira and Seale, 2019) are enriched. CEBPβ binds to DNA sites adjacent to PPARγ binding sites to regulate expression of genes that promote differentiation of preadipocytes to adipocytes (Lefterova et al., 2008; Madsen et al., 2014). Once bound, CEBPB functions as a pioneer factor, possibly by activating downstream effectors such as Psmb8 and Steap4 (Chen et al., 2010; Arimochi et al., 2016). Consistently, in β-actin KO cells, neither Psmb8 nor Steap4 is induced after adipogenic differentiation.

In preadipocytes, the CEBPβ gene locus is maintained in a “poised” chromatin state ready for active transcription upon induction (Matsumura et al., 2015). Our ChIP-Seq and ChIP qPCR results suggest that this poised configuration at TSS and promoter-proximal region is achieved by maintaining low H3K9me3 and high Brg1 levels in WT and is reversed in KO cells. Results from ATAC-Seq are compatible with these observations, showing loss of chromatin accessibility primarily at TSS and promoter-proximal region in the absence of β-actin. This indicates β-actin plays a key role in maintaining a chromatin state that ensures Cebpa gene expression during transcriptional reprogramming of MEFs into adipocytes (see Figure 4, E and F, for a speculative model). Seeing that both ATPase activity and genomic deposition of Brg1 require β-actin, β-actin seems to contribute to chromatin accessibility by regulating the activity of the
FIGURE 3: Expression of adipogenic genes requires nuclear β-actin. (A) Quantitative PCR analysis on total RNA isolated from WT and KO MEFs and the corresponding adipocytes using primers amplifying Cebpa (left chart), Cebpb (middle chart), and Fabp4 (right chart). The analysis was normalized against the housekeeping gene Nono. Conditions were normalized to MEF-WT to observe fold induction normalized to 1 (n = 3; *p-value < 0.05, **p-value < 0.01). (B) Immunoblots on lysates isolated from WT and KO MEFs and the corresponding induced adipocytes with antibodies against CEBPA (detecting p30 and p42 isoforms), CEBPB, FABP4, and α-tubulin as loading control. (C) Densitometric quantification of the immunoblot signals from three independent experiments (n = 3). Relative density values were generated via ImageJ software (n = 3; *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001). (D) Analysis of cell morphology and lipid droplet production in KO MEFs constitutively expressing β-actin in the cell nucleus (MEF-NA) and the corresponding induced adipocytes (MEF-NA-iA) by ORO staining at day 0 (basal condition) and day 6 (differentiation condition). Scale bar: 125 μm. (E) Quantification of oil red staining isolated via spectrophotometry comparing MEF-NA
MATERIALS AND METHODS

Antibodies

Mouse anti-CEBPA (catalogue number sc-365318) and rabbit anti-CEBPB (catalogue number sc-7962) antibodies were purchased from Santa Cruz Biotechnology and used at 1:200 and 1:400 dilution, respectively. The rabbit antibody to FABP4 (catalogue number ab66682) and the mouse antibody to α-tubulin (catalogue number ab6046) were purchased from AbCam and used at 1:500 dilutions. Horseradish peroxidase–conjugated goat anti-mouse and goat anti-rabbit secondary antibodies were obtained from JacksonImmunoresearch Laboratories and used at 1:2000 dilutions.

In vitro adipocyte differentiation

MEF-WT, MEF-KO, and MEF-NA were previously characterized (Almuzzaini et al., 2016; Xie et al., 2018a). They were maintained briefly in DMEM with high glucose supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Pen/Strp growth media) in treated tissue culture T25 flasks (ThermoScientific NUNC) at 37°C with 5% CO2. An adipocyte differentiation protocol was adapted from a previous study (Cuaranta-Monroy et al., 2014). Cell lines were split with trypsin, counted, and plated at a density of 1 × 105 cells per well in six-well tissue culture plates (ThermoScientific NUNC). After 48 h, the medium was changed to an adipogenic differentiation cocktail, which included the growth medium supplemented with 10 μg/ml insulin (Ins), 2 μM dexamethasone (Dex), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 25 μg/ml ascorbic acid (AsA; differentiation media; Cuaranta-Monroy et al., 2014). All supplements used were products of Sigma. Cells were allowed to differentiate for up to 6 d. The differentiation medium was changed every 2 d.

Lipid droplet staining

Medium from each well plate was aspirated and washed with 1 ml of 1x phosphate-buffered saline (PBS). Paraformaldehyde (PFA) in PBS (4%; 2.4 ml) was added to each well for 5 min and then was changed to a fresh 2.4 ml 4%PFA for 1 h. Fixative solution was aspirated and cells were washed once with 2.4 ml of 60% isopropanol. After being washed, plates were allowed to dry completely before addition of 1 ml of diluted working ORO solution (six parts ORO stock solution [Sigma]; four parts H2O2, after 10 min incubation at room temperature, staining solution was removed, and cells were washed four times with H2O to remove residual ORO. H2O2 was added (2 ml) to each well before the visualization under microscopy (EVOS FLOID Cell Imaging System Life Technologies). Visual assessment of ORO staining was conducted in four independent experiments. For quantification, ORO-stained particles were eluted with 100% isopropanol and analyzed using Thermo Scientific Varioskan Flash for spectrophotometry readings at 514 nm.

Protein isolation and Western blotting analysis

Cell lysates were prepared using in-house-prepared radioimmuno precipitation assay (1XRIPA) buffer, followed by incubation on ice for 30 min and vortexing at 5-min intervals. Lysates were then centrifuged at 15,000 rpm for 30 min and supernatant was collected. Protein concentrations were measured using a Pierce BCA protein assay kit (ThermoScientific). Samples were mixed with sodium dodecyl sulphate (SDS) loading buffer and boiled at 95°C for 5 min. From each sample, 20 μg was loaded and separated on 10% SDS–PAGE gels, followed by transferring proteins to polyvinylidene difluoride membranes at 90 V for 50 min. Membranes were then blocked for 1 h with 5% nonfat milk in PBS-T (0.05% Tween-20 in PBS). The membranes were incubated overnight at 4°C with primary antibodies in 1% nonfat milk in PBS. Membranes were washed with PBS and then incubated with secondary antibodies for 1 h at room temperature. Protein bands were developed using a Pierce ECL Western blot substrate kit (ThermoScientific) and were imaged using a Syngene GeneGnome system. Densitometry analysis was performed using ImageJ 1.x software (Schneider et al., 2012).

RNA isolation and purification

Total RNA was extracted using a combination of TriZol (Life Technologies) and an RNAeasy Mini Kit (Qiagen) with modifications from the manufacturer’s protocols. MEF cell lines in each well were washed once with 1 ml of 1XPBS before the addition of 1 ml of TriZol reagent. TriZol lysates were added to a fresh 1.5-ml tube. A volume of 0.2 ml of chloroform was added per ml of TriZol and was centrifuged at a speed of 12,000 × g at 4°C for 15 min. The upper aqueous phase (~400 μl of RNA) was transferred and added to a fresh 1.5-ml tube. One volume of 70% ethanol was mixed with the RNA and the downstream purification was performed using an RNAeasy Mini spin column (Qiagen) according to the manufacturer’s protocol. RNA concentrations and integrity (RNA Integrity Number—RIN) were determined using NanoDrop (ThermoScientific) and an RNA Nanochip BioAnalyzer 2100 (Agilent), respectively.
cDNA synthesis and quantitative PCR
An amount of 0.1 μg of isolated RNA was used as a template for synthesizing complementary DNA (cDNA) using a 1st Strand Synthesis Kit (Invitrogen) according to the manufacturer's protocol. To conduct quantitative PCR (qPCR), the cDNA product synthesized was diluted 1:50 before being used as a template for the SYBR-Green reaction (ThermoScientific). A total volume of 10 μl was prepared for the SYBR-Green qPCR reaction (2.5 μl diluted cDNA or nH₂O, 2.5 μl 2 μM Forward+Reverse primers [Integrated DNA Technologies] mix, and 5 μl of SYBER-Green). The qPCR thermal cycle was run on an Applied Biosystems StepOnePlus real-time PCR System. The qPCR thermal cycle included a cycle of 50°C for 2 min and 95°C for 10 min followed by 44 cycles of 95°C for 15 s and 60°C for 1 min. Values of expression of gene targets were normalized to housekeeping gene Noneo and determined via ΔΔCt. Primers for qPCR analysis were designed against the following genes: Cebpa (forward, AAAAAGCGCAACGTGGAGAG; reverse, GGATGGCAGAAAAGAGGACCC), CebpB (forward, ATCGACTTACGCCCTACCT; reverse, TAGTCGTCGGCGAAGAGG), Fabp4 (forward, GGATGGCAGAAAAGAGGACCC), Tggaagaacgtcagctttcatat, Noneo (forward, GCGAAGAAGCCTTGGTAC; reverse, TATCAAGGGAAGATTGCGCA).

RNA-secreorying library preparation and sequencing
Using a ThermoScientific NanoDrop 2000 and an Agilent BioAnalyzer 2100, the quantity and quality (RIN > 8) of total RNA were assessed before library preparation. RNA sequencing (RNA-Seq) libraries were prepared using an Illumina TruSeq Stranded mRNA prep kit. Using the manufacturer's LS protocol, samples were barcoded, multiplexed, and sequenced (100 bp pair-end) in a single lane on the Illumina NextSeq 550 platform at the New York University Abu Dhabi (NYUAD) Genomic Core facility (Abu Dhabi, U.A.E).

Transcriptome data computational analysis
Transcriptional data from MEF-KO and MEF-WT cells were available from a previous study. The data sets are stored in the publicly available Gene Expression Omnibus (GEO) repository under GSE95830. The RNA-Seq data obtained on induced adipocytes were assessed before library preparation. RNA sequencing (RNA-Seq) libraries were prepared using an Illumina TruSeq Stranded mRNA prep kit. Using the manufacturer's LS protocol, samples were barcoded, multiplexed, and sequenced (100 bp pair-end) in a single lane on the Illumina NextSeq 550 platform at the New York University Abu Dhabi (NYUAD) Genomic Core facility (Abu Dhabi, U.A.E).

Chromatin immunoprecipitation and qPCR analysis
Chromatin immunoprecipitation was previously described in Xie et al. (2018a). Briefly, chromatin isolated from WT MEFs and β-actin KO MEFs was subjected to immunoprecipitations with an anti-CEBPB (catalogue number sc-7962) and anti-H3K9me3 (ab10812) antibodies. Analysis was performed by qPCR with primers amplifying overlapping regions covering approximately 2 kb upstream from the Cebba gene TSS (regions R1–R5), a sequence within the gene coding region (R6), and a distant upstream sequence (R7). The following primers were used for qPCR analysis: R1 (forward, AACTGTGCTTACCGCC; reverse,CTCGGGAGAAAAGAGCCTAAA), R2 (forward, AGCAATCTATCGCTCTGG; reverse, GCTCTCACGAGTATGGGC), R3 (forward, CTCCCCGTTGGCGGC), R4 (forward, ACCGTCTTGGAAAGTGCAAGG; reverse, CGCGGGGCGCTTTTATAAC), R5 (forward, GGCTTGCGGTCGTGCC; reverse, ACTCTCATGGGGAGATGTA), R6 (forward, AGAAAAGCCTTCCCCACCC; reverse, CCACCAACAAACCCCTGT), R7 (forward, CGATCTCCTGCACACCAG; reverse, TCGGTTCGAGAGCCAATTTAAA).

Chromatin immunoprecipitation sequencing
Data of chromatin immunoprecipitation sequencing (ChiP-Seq) for WT MEFs and β-actin KO MEFs using Brg1 and H3k9me3 antibodies were from previous study in Xie et al. (2018a), which was deposited in the GEO repository with accession number GSE100096.
Statistical analysis

Statistical analysis was carried out using Student’s t tests analysis using Microsoft Excel. Results were represented as means of at least three independent experiments. A p-value < 0.05 was considered significant.

ATAC-Seq

50,000 cells per sample were used for the preparation of ATAC-Seq libraries. Cell samples in frozen medium (DEEM with 50% FBS and 10% DMSO) were shipped on dry ice to Novogene (Beijing, China). Subsequent processing, ATAC-seq library construction, and sequencing were performed by Novogene (Beijing, China). Briefly, cell nuclei were isolated from the cell samples and mixed with Tn5 Transposase with two adapters, and tagmentation was performed for 30 min at 37 °C. The fragmented DNA was purified and amplified with a limited PCR cycle using index primers. Libraries were sequenced using NovaSeq6000, paired-end 150 cycles. Raw reads were assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and quality trimmed using Trimmomatic (Bolger et al., 2014) to trim low-quality bases, systematic base calling errors, and sequencing adapter contamination. The specific parameters used were “trimmomatic_adapter.fa:2:30:10 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36.” Surviving paired reads were then aligned against the mouse reference genome (GRCm38) using a Burrows–Wheeler Aligner BWA-MEM 9 (Li and Durbin, 2010). The resulting BAM alignments were cleaned, sorted and deduplicated (PCR and Optical duplicates) with PICARD tools (http://broadinstitute.github.io/picard). Processed alignments were analyzed using the computeMatrix function of DeepTools2 (Ramirez et al., 2016) to plot the average signal around regions of interest. ChiP and ATAC-Seq profiles of genes of interest were visualized using IGV (Robinson et al., 2011). The ATAC-seq data are deposited in the GEO repository with accession number GSE133196 and are publicly available.

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