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The transcription elongation factors Spt4 and Spt6 promote dermal adipocyte differentiation

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ABSTRACT

Regulation of adipogenesis has classically been viewed through the lens of transcription initiation driven by lineage defining transcription factors. In this study, we uncover transcription elongation as a critical and previously underappreciated regulatory layer controlling adipocyte cell fate. We demonstrate that the elongation factors Spt4 and Spt6 are indispensable for adipogenic differentiation, as their depletion severely impairs adipogenic gene induction and perilipin expression. Spt4 and Spt6 directly regulate the genes coding for core adipogenic transcription factors, including *Cebpa*, *Pparg*, *Krox20*, and *Stat3*, by promoting RNA polymerase II (Pol II) progression through their gene bodies. In the absence of these factors, Pol II becomes stalled at the transcriptional start sites of these adipogenic genes. These data support a post transcription initiation requirement for Spt4 and Spt6 in productive elongation rather than promoter loading. Our findings identify transcription elongation control as a key determinant of adipogenic fate.

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SPT4; SPT6; RNA polymerase II; transcriptional elongation; transcriptional pausing; Pparg; adipogenesis; adipocyte; NELFB

Introduction

Transcriptional output is governed not only by promoter activation but also by post- initiation checkpoints that control RNA polymerase II (Pol II) pausing and release into productive elongation [1]. After general transcription factors assemble at promoters and recruit Pol II to form the preinitiation complex, phosphorylation of the Pol II carboxy terminal domain (CTD) at Ser5 supports initiation and synthesis of a short nascent transcript. This facilitates recruitment of the pausing machinery, including DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) sensitivity inducing factor (DSIF), a complex containing Suppressor of Ty 4 (SPT4) and Suppressor of Ty 5 (SPT5), and negative elongation factor (NELF), which together stabilize promoter proximal pausing typically 20 to 60 nucleotides downstream of the transcription start site (TSS) [2]. Productive elongation is triggered when P-TEFb (CDK9 and Cyclin T) phosphorylates DSIF, NELF, and Pol II CTD Ser2, which promotes dissociation of NELF [3]. This enables Pol II Ser2 (phosphorylated) and DSIF to engage with elongation and chromatin associated factors such as the PAF1 complex (LEO1, CTR9, PAF1), SPT6, CDK12, CDK13, SSRP1, and SPT16 to facilitate transcription elongation [4–7]. Elongation can be enhanced by association with various members of the super elongation complex (SEC: ELL, ELL2, ELL3, EAF1, EAF2, AFF1, AFF4, AF9, ENL) which has been shown to be important for the expression of rapidly induced genes [1]. Promoter proximal pausing and regulated release into elongation constitute a major transcription control point in metazoans, enabling rapid transcriptional responses to developmental and environmental cues [8]. In the skin, preadipocyte specific deletion of *Nelfb* in mice prevented dermal white adipose tissue (dWAT) formation leading to neonatal lethality [9]. Nelfb promoted adipocyte differentiation by stabilizing the paused Pol II complex on key differentiation genes. In its absence, Pol II could no longer be maintained on critical differentiation genes such as *Pparg*, a master regulator that establishes and maintains adipocyte identity [10]. While the transcriptional pausing factor NELF is necessary for adipogenesis, it is

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unclear whether transcription elongation factors play any role. In addition, different transcription elongation factors have opposing roles even within the same cell type [11,12]. We therefore asked whether SPT4 and SPT6, elongation factors with established roles in Pol II processivity and chromatin regulation, are required for dermal preadipocyte differentiation and adipogenic gene activation, a process central to dermal white adipose tissue (dWAT) formation and cutaneous homoeostasis [13].

Results

To investigate the roles of Spt4 and Spt6 in dermal preadipocytes, we performed siRNA mediated knockdown in primary dermal preadipocytes isolated from the dorsal dermis of newborn (P0) mice by enzymatic digestion. Despite robust knockdown of Spt4 and Spt6 (Figure 1(A)), there were no differences in the morphology of the preadipocytes (Figure 1(B)) or their proliferation rates (Figure 1(C)). To determine if Spt4 or Spt6 have any roles in adipogenesis, control and knockdown cells were induced to differentiate in adipogenic medium for five days. Importantly, loss of Spt4 or Spt6 reduced the expression of adipogenic differentiation genes. Expression of *Stat3* and *Krox20* (Egr2), transcriptional regulators implicated in early adipogenic commitment, was significantly reduced upon *Spt4* or *Spt6* knockdown relative to control cells treated with non-targeting siRNAs (Figure 1(D)). Notably, expression of *Pparg* was markedly decreased, along with reduced expression of *Cebpa*, a key cooperating adipogenic transcription factor required for efficient maturation and maintenance of the adipocyte programme (Figure 1(D)) [14]. Consistent with impaired terminal differentiation, expression of downstream adipocyte markers *Fabp4* and *Adipoq*, which are essential for lipid handling and mature adipocyte identity, was also diminished (Figure 1(D)). These data support a requirement for Spt4 and Spt6 in activating or maintaining the transcriptional cascade that drives adipogenic gene induction and terminal adipocyte differentiation in dermal preadipocytes. To assess adipocyte maturation, we measured lipid accumulation and adipocyte marker expression in control and knockdown cells. Oil Red O staining, which detects neutral lipids, revealed a marked reduction in lipid containing cells in *Spt4* and *Spt6* depleted groups compared to controls (Figure 2(A)). Consistent with impaired adipocyte maturation, immunofluorescence staining showed reduced perilipin signal, indicating decreased formation of lipid droplets (Figure 2(B)). Together, these phenotypes indicate that depletion of *Spt4* and *Spt6* results in a decreased progression through adipogenic differentiation in primary dermal preadipocytes. To determine the mechanism by which Spt4 and Spt6 control adipogenic differentiation, CUT&RUN was performed to examine the binding profiles of Spt4 and Spt6 at adipogenic gene loci in control and knockdown cultures. Spt6 and Spt4 bound at the transcriptional start site (TSS) and gene body of *Cebpa*, *Pparg*, *Krox20*, and *Stat3* suggesting a role in promoting transcription elongation of these critical adipogenic genes (Figure 3(A–D)). Knockdown of each factor also led to decreased Spt6 or Spt4 binding at those sites confirming antibody specificity (Figure 3(A–D)). Since Spt6 and Spt4 are necessary for Pol II mediated transcription elongation, we tested how loss of these factors impacted Pol II binding. At TSS proximal regions, Pol II occupancy was not significantly different between controls and *Spt4* or *Spt6* depleted cultures, indicating that loss of either factor does not grossly impair Pol II recruitment or engagement at these sites (Figure 4(A,B)). In contrast, Pol II occupancy across the gene bodies of *Cebpa*, *Pparg*, *Krox20*, and *Stat3* was significantly reduced in *Spt4* and *Spt6* knockdown cells (Figure 4(C,D)). These results are consistent with a defect after initiation, such as impaired pause release and compromised transition into productive elongation. This redistribution of Pol II signal, preserved at the TSS but diminished through the gene body, supports a model in which transcription is initiated yet fails to efficiently elongate through downstream regions. Together, these data suggest that Spt4 and Spt6 function primarily at a post-initiation step to promote productive elongation and sustain robust transcriptional output during adipogenic differentiation.

Discussion

Traditionally, the field of adipogenesis has focused on defining the transcription factors involved in the differentiation process [14,15]. The master regulators PPARγ and C/EBPα have long been recognized as the central drivers of adipocyte identity, with PPARγ being both necessary and sufficient for adipogenesis *in vitro* and *in vivo* [16]. C/EBPα cooperates with PPARγ to maintain the mature adipocyte phenotype, while early adipogenic factors such as Krox20 and Stat3 have been implicated in initiating the adipogenic cascade

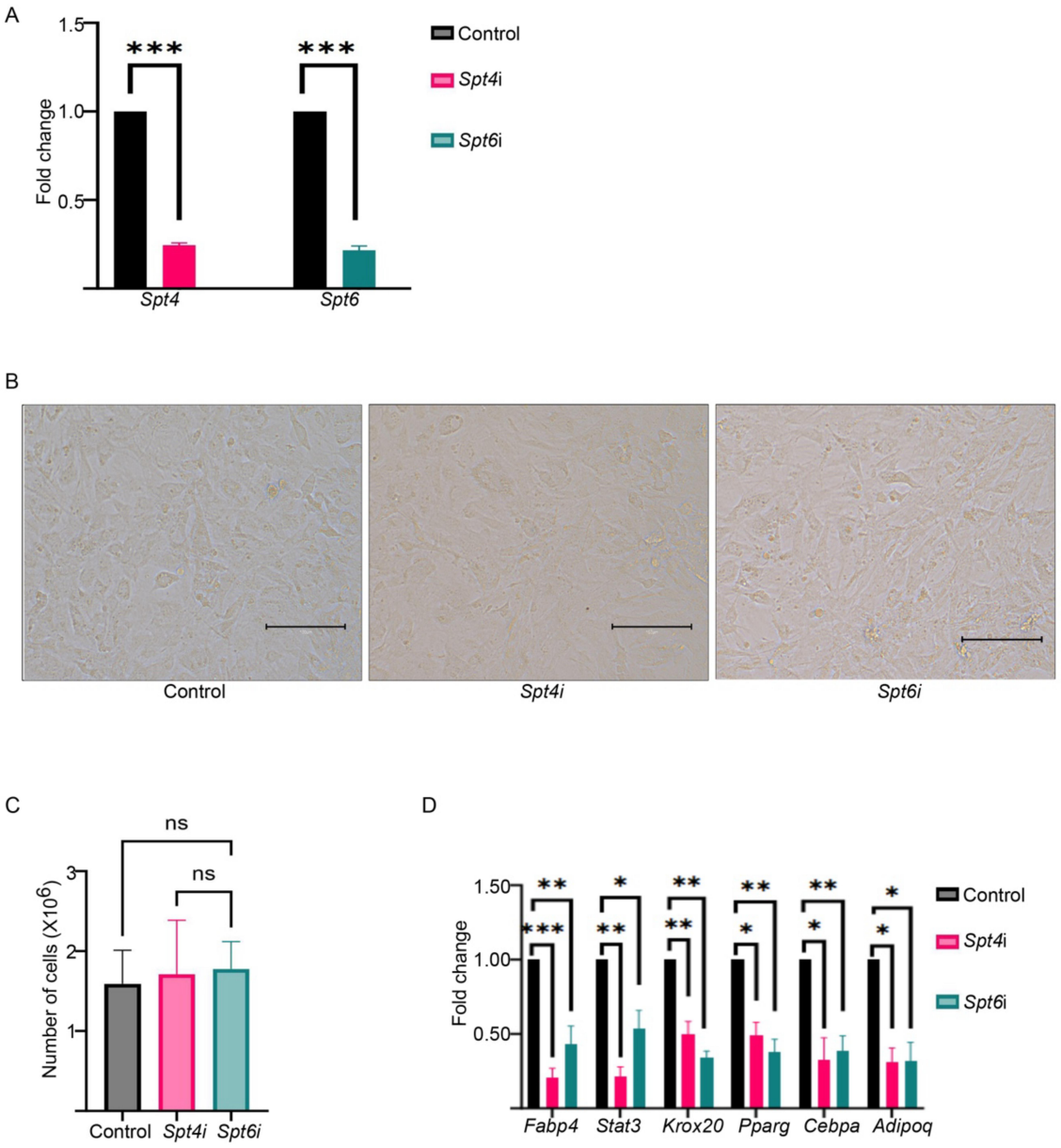


Figure 1. Spt4 and Spt6 are required for adipogenic gene induction. (a) RT-qPCR validation of siRNA mediated knockdown of *Spt4* (*Spt4*i) and *Spt6* (*Spt6*i) in primary dermal preadipocytes relative to non-targeting siRNA control. Expression was normalized to *Gapdh* and plotted as Fold change relative to control. $N = 4$. (b) Light microscopy images of control, *Spt4*i, and *Spt6*i preadipocytes 6 days after transfection. Scale bar = 150 µm. (c) cells were normalized and then counted 6 days after transfection with siRnas. $N = 3$. (d) RT-qPCR analysis of adipogenic regulators and markers (*Fabp4, Stat3, Krox20, Pparg, Cebpa, Adipoq*) in control, *Spt4*i, and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. Expression was normalized to *Gapdh* and plotted as Fold change relative to control. Mean values are shown with error bars = SEM. $N = 4$ independent biological replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (t-test relative to control).

upstream of PPARγ induction [16–18]. Despite extensive characterization of these transcriptional activators, the post-initiation mechanisms that ensure their robust and sustained expression during differentiation have remained largely unexplored. Here, we demonstrate that the transcription elongation factors Spt4 and Spt6 are indispensable for adipogenic differentiation in dermal preadipocytes, establishing productive transcription elongation as an active and previously unknown regulatory checkpoint governing adipocyte cell fate. Our findings are consistent with an emerging body of literature demonstrating that promoter-proximal pausing and regulated pause release constitute a major transcriptional control point in metazoans, particularly during rapid or signal-responsive gene activation [19,20]. Genome-wide studies in Drosophila and

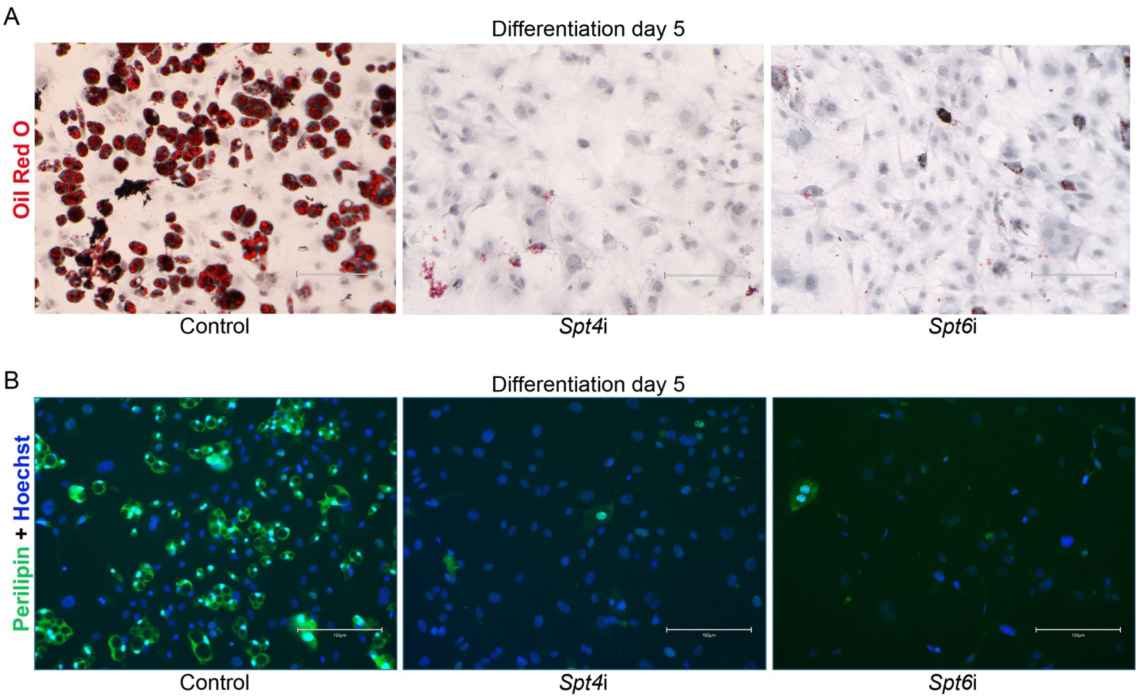


Figure 2. Spt4 and Spt6 are necessary for adipocyte differentiation. (a) Representative Oil Red O staining in control, *Spt4*i, and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. (b) Representative immunofluorescence for perilipin (green) with nuclear counterstain Hoechst (blue). Scale bar = 150 µm, $n = 4$ independent biological replicates.

mammalian systems have established that a large fraction of developmental genes harbour paused Pol II at their promoters, poised for rapid induction upon receipt of appropriate differentiation signals [21]. The present study extends this paradigm specifically to the adipogenic context, demonstrating that four key adipogenic regulatory genes - *Cebpa*, *Pparg*, *Krox20*, and *Stat3* require Spt4 and Spt6 for efficient Pol II elongation through their gene bodies. The preservation of TSS-proximal Pol II occupancy in the absence of Spt4 or Spt6, combined with the significant reduction in gene body occupancy, provides direct evidence that elongation rather than promoter loading or initiation, is the rate-limiting step controlled by these factors at adipogenic loci. This mechanism is importantly distinct from, yet complementary to, the role we previously described for NELF in dermal adipogenesis [9]. Loss of NELF caused complete destabilization of Pol II from adipogenic gene loci, including at the TSS, suggesting that NELF functions at an earlier checkpoint – stabilizing the promoter-proximal paused Pol II complex and maintaining a transcriptionally poised state at key differentiation genes. In contrast, loss of Spt4 or Spt6 preserves TSS-proximal Pol II while eliminating gene body occupancy, indicating a specific failure at the pause-release and elongation transition downstream of the pausing step itself. Taken together, these data support a sequential two-checkpoint model of adipogenic transcription regulation: NELF-dependent stabilization of paused Pol II at the promoter constitutes the first checkpoint, ensuring that key adipogenic genes are held in a poised state; Spt4/Spt6-dependent release and processive elongation through the gene body constitutes the second checkpoint, ensuring that paused Pol II is productively converted into full-length transcript upon differentiation induction. Disruption of either checkpoint is independently sufficient to block adipogenic differentiation, highlighting the sensitivity of the adipogenic transcriptional program to elongation control at multiple sequential levels. This two-checkpoint framework aligns with the broader literature on transcriptional pausing in development, where NELF and DSIF have been shown to have separable and temporally ordered functions during rapid gene activation in response to developmental cues [3,8,20,22]. Interestingly, Spt4 (component of DSIF) has dual roles in promoting transcription pausing and elongation [22]. Prior to phosphorylation by P-TEFb, it acts together with NELF to stabilize and pause Pol II at promoter proximal/TSS regions of genes. Following phosphorylation of DSIF, NELF, and the Pol II CTD at Ser2 by CDK9, NELF dissociates and DSIF transitions into a pro-elongation complex that travels with Pol II processively through the gene body. The accumulation of Pol II at TSS regions that we observe upon Spt4 knockdown rather than the complete loss

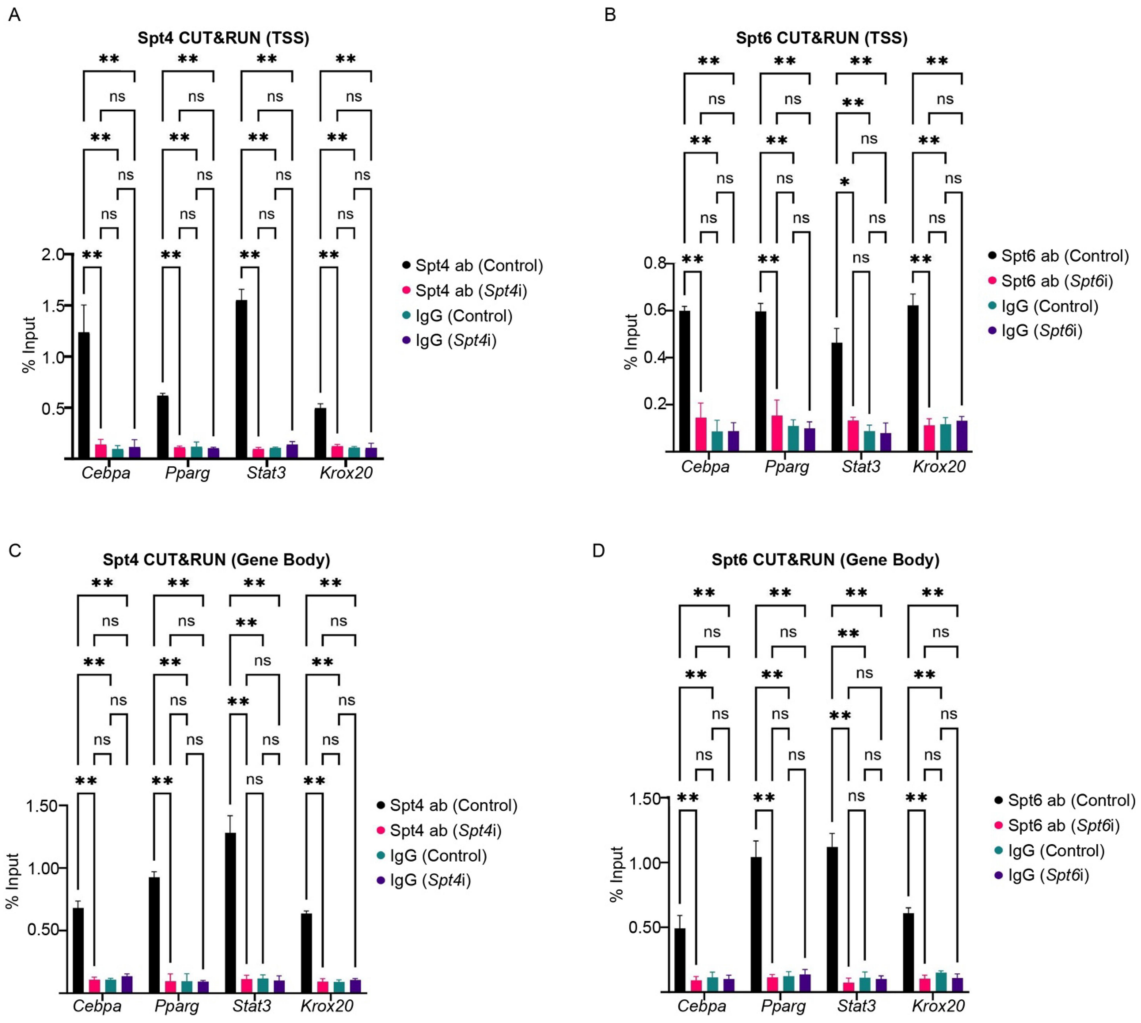


Figure 3. Spt4 and Spt6 bind to genes coding for adipogenic transcription factors. (a) CUT&RUN using an anti-Spt4 antibody and anti-IgG antibody in control and *Spt4*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on transcription start site (TSS) regions of *Cebpa, Pparg, Stat3*, and *Krox20*. signal was calculated as a percent of total input DNA. (b) CUT&RUN using an anti-Spt6 antibody and anti-IgG antibody in control and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on transcription start site (TSS) regions of *Cebpa, Pparg, Stat3*, and *Krox20*. (c) CUT&RUN using an anti-Spt4 antibody and anti-IgG antibody in control and *Spt4*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on gene body regions of *Cebpa, Pparg, Stat3*, and *Krox20*. (d) CUT&RUN using an anti-Spt6 antibody and anti-IgG antibody in control and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on gene body regions of *Cebpa, Pparg, Stat3*, and *Krox20*. $N = 4$ independent biological replicates. Mean values are shown with error bars = SEM. NS = not significant, $*p < 0.05$, $**p < 0.01$ (ANOVA).

from gene loci seen with NELF deletion is mechanistically consistent with Spt4 functioning predominantly at the elongation stage in the context of adipogenic genes, after the pausing complex has already been assembled. This raises the important and unresolved question of whether P-TEFb activity itself is rate-limiting or regulated during adipogenic differentiation [8]. Whether analogous P-TEFb activation events occur specifically at adipogenic gene loci during differentiation, and whether Spt4 and Spt6 recruitment or activity is dependent on this upstream event, remain important mechanistic questions for future investigation. Our findings also build on and extend the broader context of transcription elongation in skin biology established by our laboratory. SPT6 and CDK12 are required for epidermal differentiation, while ELL promotes epidermal proliferation and self-renewal, each through regulation of transcription elongation at lineage-specific target genes [11,12,23]. Notably, SSRP1 and SPT16 have no impact on epidermal growth or differentiation but are required for the skin's inflammatory transcriptional response, illustrating that each elongation factor has unique functions even within the same cell type [24]. The present study extends this framework to the

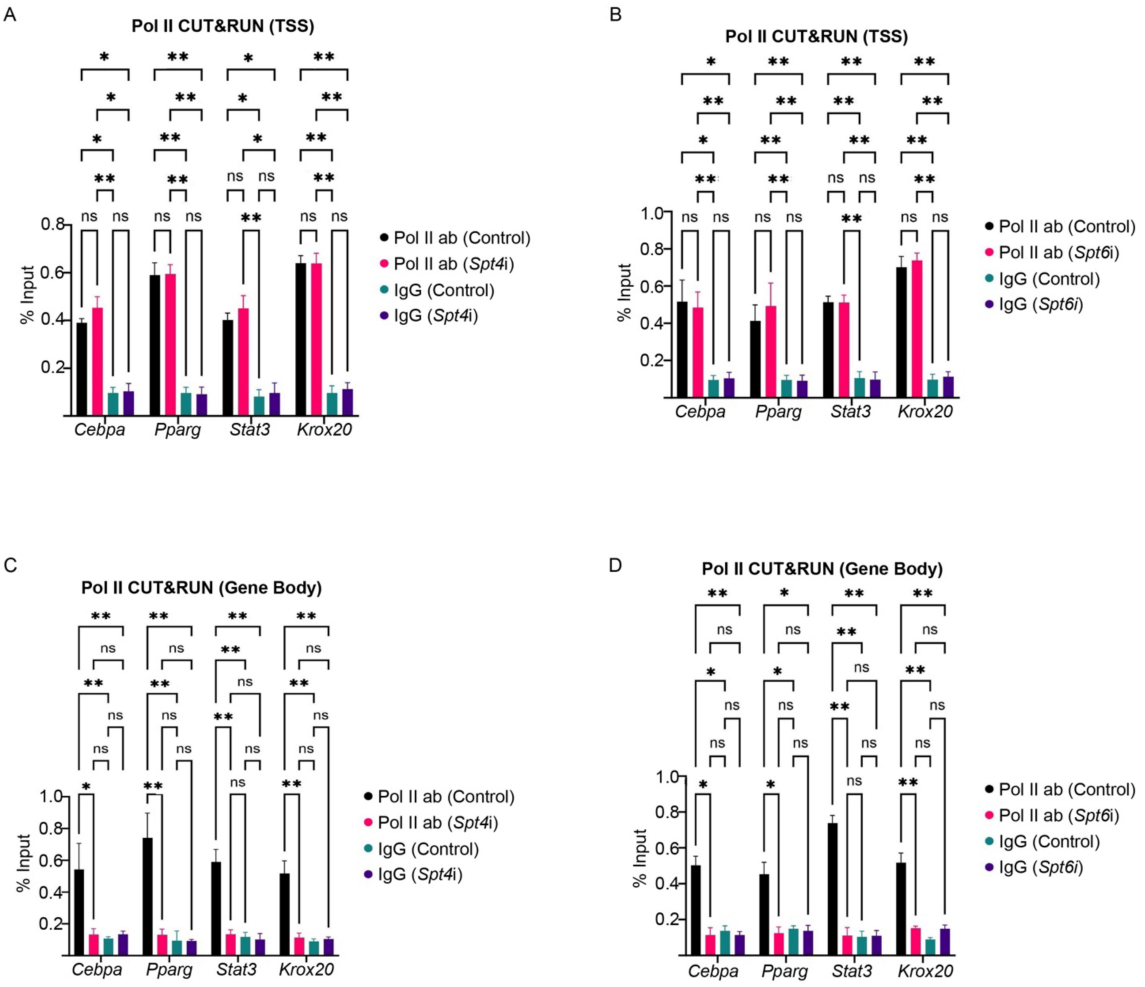


Figure 4. Spt4 and Spt6 promote the transcription elongation of genes coding for adipogenic transcription factors. (a) CUT&RUN using an anti-RNA polymerase ii (Pol ii) antibody and anti-IgG antibody in control and *Spt4*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on transcription start site (TSS) regions of *Cebpa, Pparg, Stat3*, and *Krox20*. signal was calculated as a percent of total input DNA. (b) CUT&RUN using an anti-RNA polymerase ii (Pol ii) antibody and anti-IgG antibody in control and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on transcription start site (TSS) regions of *Cebpa, Pparg, Stat3*, and *Krox20*. (c) CUT&RUN using an anti-RNA polymerase ii (Pol ii) antibody and anti-IgG antibody in control and *Spt4*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on gene body regions of *Cebpa, Pparg, Stat3*, and *Krox20*. (d) CUT&RUN using an anti-RNA polymerase ii (Pol ii) and anti-IgG antibody in control and *Spt6*i cells cultured in adipocyte differentiation medium for 5 days. qPCR analysis was conducted on gene body regions of *Cebpa, Pparg, Stat3*, and *Krox20*. $N = 4$ independent biological replicates. Mean values are shown with error bars = SEM. NS = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$ (ANOVA).

dermal adipocyte lineage, with Spt4 and Spt6 emerging as elongation factors required for the adipogenic program. Importantly, the target genes regulated by Spt4 and Spt6 in dermal preadipocytes, *Cebpa*, *Pparg*, *Krox20*, and *Stat3* are the same genes whose elongation was disrupted upon NELF loss in our prior study [9], suggesting that these adipogenic regulators are particularly dependent on elongation control for their expression, and represent a convergence point for multiple elongation regulatory mechanisms. Whether additional elongation complex components, such as PAF1 complex members or super elongation complex subunits, play complementary or opposing roles specifically at these adipogenic loci is an important open question that warrants further investigation [25]. While this study provides compelling evidence for a role of Spt4 and Spt6 in dermal adipocyte differentiation, we acknowledge there are limitations to the study. Our experiments were all performed in cultured primary dermal preadipocytes. Extension to in vivo models, such as preadipocyte-specific conditional knockout mice, would further solidify the physiological significance of these findings in the intact skin, analogous to approaches used in our prior study [9]. Our study also focused

on a limited set of adipogenic factors including Cebpa, *Pparg*, *Krox20*, and *Stat3*. Future genome-wide approaches such as RNA-seq and CUT&RUN-seq would complement these findings and may reveal additional target genes that further illuminate the mechanisms described here. Finally, the upstream signals governing Spt4 and Spt6 recruitment to adipogenic loci remain an exciting open question that our laboratory is actively pursuing. Finally, our work shown here has potential relevance to human disease, though it is important to note that the diseases discussed below are complex, multi-tissue conditions and the present data do not establish direct causality. Defects in elongation-dependent activation of *Pparg*, *Cebpa*, and related adipogenic genes could in principle contribute to lipodystrophy – a spectrum of disorders in which failure of adipose tissue development leads to severe metabolic consequences including insulin resistance, hypertriglyceridaemia, and hepatic steatosis though whether elongation factor dysfunction contributes to lipodystrophic phenotypes in humans remains to be established [26]. In the dermal context, dWAT serves functions beyond energy storage, including support of hair follicle cycling, cutaneous wound healing, and innate immune defence through production of antimicrobial peptides such as cathelicidins [27–29]. Impairment of transcription elongation-dependent adipogenic programmes in dermal preadipocytes may therefore compromise these protective cutaneous functions. In summary, our findings demonstrate that adipogenesis is controlled not only through transcription factor mediated transcription initiation but also through distinct post-initiation checkpoints. Specifically, NELF regulates promoter-proximal Pol II engagement, whereas Spt4 and Spt6 promote productive elongation across key adipogenic genes.

Methods

Neonatal mice

All experiments performed in this work had an $N = 4$ independent biological replicates. This means that one neonatal pup per group was harvested for dermal fibroblast isolation at four separate times. Thus 4 independent neonatal mice were used per experimental group for a total of 12 mice per experiment (Control siRNA, Spt4i, Spt6i).

Neonatal fibroblasts isolation

Dermal fibroblasts were isolated from the dorsal skin of P0 neonatal mice (C57BL/6J) as we previously described [9]. Dorsal skin was placed into Eppendorf tubes containing 2 ml of 3 mg/ml dispase dissolved in EpiLife media (Life Technologies, #MEPI500CA), supplemented with EDGS (Life Technologies, #S0015) and penicillin/streptomycin (pen/strep). The tubes were incubated overnight at 4°C on a tube rocker. Following incubation, the dorsal skin was removed from the tubes, and the epidermis was peeled away while keeping the dermis side down. The dermis was washed in PBS, then minced using a scalpel in a 10 cm dish containing 1–2 ml of filtered HBSS buffer supplemented with 20 mg/ml bovine serum albumin (BSA) (Sigma-Aldrich, #A7906) and 1× pen/strep, kept on ice. The minced dermal tissue was transferred into a 15 ml conical tube, and 5× Collagenase D solution (2.5 mg/ml, Roche, #11088882001) and 1 mg/ml DNase I (1:30, Sigma-Aldrich, #D5025) were added. The 15 ml conical tubes were warmed in a 37°C water bath for 5 min, then incubated at 37°C with rotation for 1–2 h. After incubation, the tubes were vortexed and placed on ice for 10 min. The suspension was then passed sequentially through 100 µm and 40 µm filters to remove hair, fat and cell clumps. The flow-through was centrifuged at 1400 rpm (368 g) for 10 min at 4°C, and the supernatant was discarded. The pellet was resuspended in complete DMEM media (Gibco, #10–013-CV) supplemented with 10% foetal bovine serum (FBS; Gibco, #A5256701) and pen/strep. A 10 µl aliquot of the cell suspension was taken for cell counting, and the remaining cells were plated in 12 ml of DMEM media in a 10 cm dish. The media was replenished 24 h later. Once the cells reached 70% confluency, they were trypsinized, counted and plated into 12-well for future experiments.

Knockdown of Spt6 and Spt4 and adipocyte differentiation

Neonatal fibroblasts were cultured until they reached ~60–70% confluency. For transfection, Lipofectamine™ RNAiMAX reagent (Thermo Fisher Scientific, #13778030) was diluted in Opti-MEM™

medium (Thermo Fisher Scientific, #31985070); 9 µl Lipofectamine was added to 150 µl Opti-MEM per well (Solution A; total volume 159 µl/well). Separately, Spt4, Spt6 or non-targeting control siRNAs (Invitrogen) were each diluted in Opti-MEM (6 µl siRNA +150 µl Opti-MEM per well; Solution B; total volume 156 µl/well). Solutions A and B were combined at a 1:1 ratio, gently mixed, and incubated for 20 min at room temperature to allow complex formation. The siRNA – lipid complexes were then added dropwise to the appropriate wells containing DMEM supplemented with 10% FBS. Plates were incubated at 37°C for 24 h, after which the medium was replaced with DMEM media. The medium was replaced again with adipocyte differentiation medium (Stem Cell Technologies: #05507) 24 h later, and cells were differentiated for 5 days. Differentiated cells were harvested for RNA extraction, staining, and CUT&RUN

RNA isolation and RT-qPCR

Total RNA was extracted from cells using the GeneJET RNA Purification Kit (Thermo Fisher Scientific, #K0732) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer. For cDNA synthesis, 1 µg of total RNA was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, #K1642) following the manufacturer's protocol. Quantitative PCR was performed using SYBR Green chemistry on a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad). Each reaction was carried out in a 14 µL reaction volume containing cDNA template, gene-specific primers, and SYBR Green master mix. The thermal cycling conditions were as follows: an initial denaturation at 95°C for 10 min, followed by 40 amplification cycles consisting of 95°C for 15 s, 60°C for 30 s, and 72°C for 45 s, with fluorescence acquisition after each cycle. Gapdh and Hprt were used as the internal reference genes for normalization. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primers were designed using the Primer3 online programme (https://primer3.ut.ee) and synthesized through Integrated DNA Technologies (IDT). Primers/amplicons were validated using melting curve analysis. The amplicon size, melting temperature, and primer sequences are listed in Table S1.

Immunofluorescence staining

For immunofluorescence staining of cultured cells, control, Spt4i, Spt6i cells were differentiated in adipocyte differentiation media (Stem Cell Technologies: #05507) for 5 days in 12-well plates then fixed in 4% PFA for 10 min. After three washes with PBS containing 0.1% Tween (3 min per wash), cells were permeabilized in 0.2% Triton X-100 in PBS for 10–20 min. Blocking was performed in PBS with 5% goat serum for 1 h. Cells were then incubated overnight at 4°C with primary antibodies against Perilipin (Cell Signaling Technology, #D1D8, 1:100). Following three washes with PBS +0.1% Tween, the secondary antibody Alexa 488-conjugated donkey anti-rabbit IgG was applied at 1:500 for 45 min at room temperature. Cells were then washed three times with PBS, counterstained with Hoechst for 2 min and rinsed briefly with PBS followed by MilliQ water to remove residual salts. Fluoromount-G was used for mounting and circular coverslips were placed over the wells. Imaging was performed using the EVOS M5000 Imaging System.

Oil Red O staining

For Oil Red O staining of cultured cells: control, Spt4i, Spt6i cells grown in adipocyte differentiation media (Stem Cell Technologies: #05507) for 5 days in 12-well plates were fixed in 10% formalin for 15 min, followed by a 5-min incubation in distilled water. Cells were then treated with 60% isopropanol for 15 min before staining with Oil Red O for 15 min. The stock solution was prepared by dissolving 0.5 g of Oil Red O (Sigma-Aldrich, #O0625) in 100 ml of 100% isopropanol (Sigma-Aldrich, #I9030-4 L). For working solution preparation, 15 ml of the stock was diluted with 10 ml of distilled water, incubated at room temperature for 10 min and filtered into a Coplin jar. After staining, cells were washed ten times in 60% isopropanol, followed by two 3-min washes in distilled water. Hematoxylin (VectorLabs, #H3401) staining was performed for 3–5 min, followed by two 5-min washes in distilled water. Fluoromount-G was used for mounting and circular coverslips were placed over the wells. Imaging was performed under bright field using the EVOS M5000 Imaging System.

CUT&RUN

Control, Spt6i, and Spt4i cells were cultured in adipocyte differentiation medium (Stem Cell Technologies: #05507) for 5 days: ~120,000–140,000 cells/reaction were trypsinized, counted and pelleted. The CUT&RUN kit used for these experiments was purchased from Cell Signaling Technology (#91931). Pellets were fixed in 16% PFA for 2 min, and cross-linking was stopped using 10× glycine for 5 min at room temperature. Cells were washed with 1× Wash Buffer after centrifugation (3000× g, 3 min, 4°C). Each reaction received 100 µl 1× Wash Buffer and resuspended pellets. Concanavalin buffer (10 µl per 10 µl magnetic beads/reaction) was added, incubated for 5 min, and removed using a magnetic rack. Cell suspensions were incubated with antibodies in 100 µl Antibody Binding Buffer overnight at 4°C on a rotator. Antibodies used were: RNA Pol II (Active Motif, #39097), Spt4 (Cell Signalling, #64828S), Spt6 (Bethyl, #A300-801A). After washing with Digitonin Buffer, 50 µl pAG-MNase pre-mix was added to each reaction and incubated at 4°C for 1 h. MNase activation was triggered with 3 µl cold CaCl2, incubated at 4°C for 30 min, and terminated by adding 150 µl Stop Buffer and 5 µl Spike-In DNA (this was added for sample normalization) per sample followed by 10 min incubation at 37°C. DNA fragments were collected by centrifugation (16,000 g at 4°C for 2 min) and the supernatant transferred to new tubes. Samples were incubated at 65°C for 2 h with SDS and Proteinase K to reverse crosslinks. DNA was purified using DNA purification buffers and spin columns kit (Cell Signalling Technology, #14209). Input samples were mixed with Extraction Buffer (Proteinase K+RNAse A) and incubated at 55°C for 1 h. After cooling on ice for 5 min, chromatin was fragmented via sonication (three cycles: 7.5 min total, 0.5 s off, 1 s on). Lysates were clarified (18,500× g, 10 min, 4°C) and DNA was purified. Gel electrophoresis (2–4% agarose) confirmed sheared DNA fragments between 200–400 bp.

Primers and siRNA sequences

A list of all RT-qPCR and CUT&RUN primers are listed in Table S1. Spt4 and Spt6 siRNA sequences are also listed in Table S1.

Statistical analysis

Data are presented as the mean ± SEM of 4 independent biological experiments. Significant changes ($p < 0.05$) were determined by unpaired two-tailed t-test (groups of 2) or one way analysis of variance (ANOVA) with Tukey's post hoc test (groups > 2). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data were confirmed to have normal distribution through Q-Q plots. Statistical tests were calculated using GraphPad Prism 11 (GraphPad Software, San Diego, CA, USA).

Author contributions

CRediT: **Julian Gomez:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing; **Samiksha Mahapatra:** Investigation, Methodology, Writing – review & editing; **Uyanga Batzorig:** Investigation, Methodology, Writing – review & editing; **Ye Liu:** Investigation, Methodology, Writing – review & editing; **Neha Quadir:** Investigation, Methodology, Writing – review & editing; **George L. Sen:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Disclosure statement

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Data availability statement

The primary data generated or analysed during this study are included in this article in Table S2.

Ethical approval

The study protocols were approved by the Institutional Animal Care and Use Committee from the University of California, San Diego (Approval number S11051).

Arrive guidelines

The work described in this manuscript adhered to the ARRIVE guidelines.

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References

[1] Chen FX, Smith ER, Shilatifard A. Born to run: control of transcription elongation by RNA polymerase II. Nat Rev Mol Cell Biol. 2018;19:464–478. doi: 10.1038/s41580-018-0010-5
[2] Liu X, Kraus WL, Bai X., et al. Ready, pause, go: regulation of RNA polymerase II pausing and release by cellular signaling pathways. Trends Biochem Sci. 2015;40(9):516–525. doi: 10.1016/j.tibs.2015.07.003
[3] Saunders A, Core LJ, Lis JT. Breaking barriers to transcription elongation. Nat Rev Mol Cell Biol. 2006;7(8):557–567. doi: 10.1038/nrm1981
[4] Vos SM, Farnung L, Urlaub H, et al. Structure of paused transcription complex Pol II-DSIF-NELF. Nature. 2018;560 (7720):601–606. doi: 10.1038/s41586-018-0442-2
[5] Vos SM, Farnung L, Boehning M, et al. Structure of activated transcription complex Pol II-DSIF-PAF-SPT6. Nature. 2018;560(7720):607–612. doi: 10.1038/s41586-018-0440-4
[6] Greenleaf AL. Human CDK12 and CDK13, multi-tasking CTD kinases for the new millenium. Transcription. 2019;10 (2):91–110. doi: 10.1080/21541264.2018.1535211
[7] Belotserkovskaya R, Saunders A, Lis JT, et al. Transcription through chromatin: understanding a complex fact. Biochim Biophys Acta. 2004;1677(1–3):87–99. doi: 10.1016/j.bbaexp.2003.09.017
[8] Smith E, Shilatifard A. Transcriptional elongation checkpoint control in development and disease. Genes Dev. 2013;27(10):1079–1088. doi: 10.1101/gad.215137.113
[9] Mahapatra S, Gomez J, Batzorig U, et al. Nelfb promotes dermal white adipose tissue formation through RNA polymerase II-mediated adipogenic gene regulation. Development. 2025;152(20). doi: 10.1242/dev.204976
[10] Rosen ED, Sarraf P, Troy AE, et al. Ppar gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell. 1999;4(4):611–617. doi: 10.1016/s1097-2765(00)80211-7
[11] Li J, Xu X, Tiwari M, et al. Spt6 promotes epidermal differentiation and blockade of an intestinal-like phenotype through control of transcriptional elongation. Nat Commun. 2021;12(1):784. doi: 10.1038/s41467-021-21067-w
[12] Li J, Bansal V, Tiwari M, et al. Ell facilitates RNA polymerase II mediated transcription of human epidermal proliferation genes. J Invest Dermatol. 2020. doi: 10.1016/j.jid.2020.09.024
[13] Kruglikov IL, Scherer PE. Dermal adipocytes: from irrelevance to metabolic targets? Trends Endocrinol Metab. 2016;27(1):1–10. doi: 10.1016/j.tem.2015.11.002
[14] Hilton C, Karpe F, Pinnick KE. Role of developmental transcription factors in white, brown and beige adipose tissues. Biochim Biophys Acta. 2015;1851(5):686–696. doi: 10.1016/j.bbalip.2015.02.003
[15] Ali AT, Hochfeld WE, Myburgh R, et al. Adipocyte and adipogenesis. Eur J Cell Biol. 2013;92(6–7):229–236. doi: 10.1016/j.ejcb.2013.06.001
[16] Rosen ED, Hsu CH, Wang X, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev. 2002;16(1):22–26. doi: 10.1101/gad.948702
[17] Chen Z, Torrens JI, Ana Nd A, et al. Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. Cell Metab. 2005;1(2):93–106. doi: 10.1016/j.cmet.2004.12.009
[18] Stephens JM, Morrison RF, Pilch PF. The expression and regulation of STATs during 3T3-L1 adipocyte differentiation. J Biol Chem. 1996;271(18):10441–10444. doi: 10.1074/jbc.271.18.10441
[19] Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat Rev Genet. 2012;13:720–731. doi: 10.1038/nrg3293

[20] Core L, Adelman K. Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. Genes Dev. 2019;33(15–16):960–982. doi: 10.1101/gad.325142.119
[21] Zeitlinger J, Stark A, Kellis M, et al. Rna polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. Nat Genet. 2007;39:1512–1516. doi: 10.1038/ng.2007.26
[22] Yamaguchi Y, Shibata H, Handa H. Transcription elongation factors DSIF and NELF: promoter-proximal pausing and beyond. Biochim Biophys Acta. 2013;1829(1):98–104. doi: 10.1016/j.bbagrm.2012.11.007
[23] Li J, Tiwari M, Chen Y, et al. Cdk12 is necessary to promote epidermal differentiation through transcription elongation. STEM Cells. 2022;40:435–445. doi: 10.1093/stmcls/sxac002
[24] Sawada Y, Nakatsuji T, Dokoshi T, et al. Cutaneous innate immune tolerance is mediated by epigenetic control of MAP2K3 by HDAC8/9. Sci Immunol. 2021;6(59). doi: 10.1126/sciimmunol.abe1935
[25] Luo Z, Lin C, Shilatifard A. The super elongation complex (SEC) family in transcriptional control. Nat Rev Mol Cell Biol. 2012;13:543–547. doi: 10.1038/nrm3417
[26] Akinci B, Meral R, Oral EA. Phenotypic and genetic characteristics of lipodystrophy: pathophysiology, metabolic abnormalities, and comorbidities. Curr Diab Rep. 2018;18:143. doi: 10.1007/s11892-018-1099-9
[27] Festa E, Fretz J, Berry R, et al. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. Cell. 2011;146:761–771. doi: 10.1016/j.cell.2011.07.019
[28] Schmidt BA, Horsley V. Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development. 2013;140:1517–1527. doi: 10.1242/dev.087593
[29] Zhang LJ, Guerrero-Juarez CF, Hata T, et al. Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science. 2015;347:67–71. doi: 10.1126/science.1260972