

# TATA Element Modulatory Factor 1 Negatively Regulates Sodium Acetate Activated Milk Fat Synthesis Through SREBP1 Pathway in Bovine Mammary Epithelial Cells

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## Research

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# Abstract

**Background:** Sodium acetate is one of the important nutrients that regulate milk fat synthesis in bovine mammary epithelial cells (BMECs), and it regulates milk fat synthesis mainly through the SREBP1 pathway. Our previous study has showed that TATA element modulatory factor 1 (TMF1) may be interacts with SREBP1 and regulates the sodium acetate-dependent milk synthesis in BMECs, but the underlying mechanism is unclear. In the current study, the effect of TMF1 on sodium acetate activated milk fat synthesis in BMECs was assessed.

**Results:** Overexpressing or inhibiting TMF1 demonstrated that TMF1 negatively regulated sodium acetate activated sterol regulatory element-binding protein 1 (SREBP1) pathway and milk fat synthesis; Overexpressing or inhibiting SREBP1 showed that TMF1 inhibited sodium acetate activated milk fat synthesis through SREBP1 pathway; Co-immunoprecipitation analysis showed that TMF1 interacted with SREBP1; Nuclear localization of SREBP1 analysis showed that sodium acetate activated nuclear localization of SREBP1 was inhibited by TMF1; Depletion or supply sodium acetate demonstrated that sodium acetate negatively regulated expression of TMF1 and the interaction between TMF1 and SREBP1.

**Conclusions:** Together, these results indicate that TMF1 is a negative regulatory factor for sodium acetate activated milk fat synthesis, it induced expression by sodium acetate depletion, and interacts with SREBP1 in cytoplasmic, prevents the nuclear localization of SREBP1 and then suppresses the expression of SREBP1 target gene and subsequent milk fat synthesis in BMECs.

## Introduction

Milk fat is one of the important components of milk and in bovine mammary gland, synthesis of milk fat is one of the key steps of synthesis milk[1]. The synthesis of milk fat is controlled by genetics, hormones, nutrition and environment[2–5]. Short-chain fatty acids such as sodium acetate, sodium  $\beta$ -hydroxybutyrate, are important precursors for the synthesis of fatty acids, and they are also important for the synthesis of milk fat in bovine mammary epithelial cells (BMECs)[6, 7]. The sterol regulatory element binding protein 1 (SREBP1) pathway is one of the most important pathways that regulate milk fat synthesis in BMECs[8, 9]. SREBP1 in responses to short-chain fatty acids and enters the nucleus, and then promotes the expression of its target gene, such as Acetyl CoA carboxylase (ACC), Fatty acid synthesis (FAS), stearoyl-CoA desaturase (SCD) and fatty acid binding protein 3 (FABP3), and subsequent milk fat synthesis[10, 11]. But the mechanism of regulation of milk fat synthesis controlled by sodium acetate and SREBP1 pathway is poorly understood.

TATA element modulatory factor 1 (TMF1) also named as ARA160, is a Golgi-associated protein[12]. Studies have shown that the structure of TMF1 contain a coiled-coil forming domains, it mediates the interaction of TMF1 with many other cellular factors, and leads to the involvement of TMF in many cellular physiological functions[13–15]. In our previous study, we found that TMF1 may be interacts with SREBP1 and regulates the sodium acetate-dependent milk synthesis in BMECs (data is not show), but the pathway and mechanism of this regulation is unknown.

In this study, the regulation of TMF1 on sodium acetate activated milk fat synthesis and it mechanism in BMECs were explored. We find that TMF1 is an important negative regulator for the milk fat synthesis in BMECs. It prevents the nuclear localization of SREBP1 by interacting with SREBP1 in cytoplasmic, and then suppresses the expression of SREBP1 target gene and subsequent milk fat synthesis. This study enriches our understanding of

the regulatory pathways and mechanisms of milk fat synthesis, it provide the scientific data for the regulation of milk fat synthesis, and it is important for the development of dairy industry.

## **Materials And Methods**

### **Cell culture and treated**

BMECs were cultured with Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (11330032, Gibco, California, USA) containing 10% fetal bovine serum (FBS; 26140079, Gibco), Penicillin-Streptomycin Solution (100 U/ml each of penicillin and streptomycin) (C0222, Beyotime, Shanghai, China) as previous reports[16].

For the experiment of sodium acetate treatment, BMECs were planted into 6-well plates and cultured with DMEM/F12 containing 10% FBS. After 12 h, the cells were starved of FBS for 12 h, and then treated with sodium acetate supply (AS+, final concentration is 12.00 mmol/L) or sodium acetate depletion (AS-) for 24 h, the medium of each group was collected for the testing of TG secretion and the cells were collected for Western blotting (WB) or Co-Immunoprecipitation (Co-IP). For the experiment of gene function evaluation, BMECs were first treated with gene overexpression or silencing and then treated with AS + or AS-.

### **Western blotting (WB)**

The WB was completed with normally procedure. The total protein concentrations of each group sample was tested by BCA Protein Assay Kit (P0012S, Beyotime). About 30 µg of protein in each group sample was separated with 10% SDS-PAGE gel and then transferred onto the polyvinylidene fluoride (PVDF) membrane (FFP24, Beyotime). The PVDF membrane was blocked with TBSTw (ST671, Beyotime) solution containing 5% bovine serum albumin (BSA, ST025, Beyotime), and then incubated with primary antibody (diluted with blocking solution). The membrane was washed three times with TBSTw, and then incubated with HRP-conjugated secondary antibody (diluted with blocking solution). Then the membrane was washed three times with TBSTw, and visualized with Super ECL Plus (P0018M, Beyotime). The primary antibodies used in this experiment were as following: anti-β-Actin (1: 1000, #4970, Cell Signaling Technology, Massachusetts, USA), anti-SREBP1 (1: 1000, ab191857, Abcam, Cambridgeshire, UK), anti-ARA160 (TMF1) (1:500; sc-398411, Santa Cruz Biotechnology, California, USA), anti-ACC (1: 1000, #3662S, Cell Signaling Technology, USA), anti-FAS (1: 1000, ab22759, Abcam, UK), anti-SCD (1: 1000, ab23331, Abcam, UK), anti-FABP3 (1: 1000, ab45966, Abcam, UK), anti-Lamin B1 (1: 1000, ab16048, Abcam, UK), anti-β-Tubulin (1:500; sc-5274, Santa Cruz Biotechnology, USA), anti-Myc (1:1000, AM933, Beyotime), anti-Flag (1:1000, AF519, Beyotime).

### **Triglyceride (TG) secretion**

The secretion of TG was evaluated by measuring the concentration of TG in the collected medium. The content of triglyceride (TG) in the medium was tested using the Triglyceride test kit (BC0625, Solarbio Science & Technology Co., Ltd, Beijing, China) according to the manufacturer's instructions.

### **Immunofluorescence (IF)**

The experiment of IF was completed as previously reported[17]. The cells were planted on cover slips in 6-well plates and normal cultured. After 12 h, the cells treated with AS + or AS- or/and gene overexpression or silencing. The cell crawling were made as previously reported[18]. The primary antibodies used in this study were anti-

TMF1 and anti-SREBP1 and the secondary antibodies were goat-anti-mouse Alexa fluor 488-conjugated IgG (1:500, bs-0296G-AF488, bioss, Beijing, China) and goat-anti-rabbit Alexa fluor 647-conjugated IgG (1:500, bs-0295G-AF647, bioss, China). The nucleus is stained using 4', 6-diamidino-2-phenylindole (DAPI; C1002, Beyotime) or Propidium Iodide (PI; P3566, Invitrogen, California, USA). The fluorescence was observed with laser scanning confocal microscopy (LEICA, Germany).

## Plasmid construction and cell transfection

The overexpression plasmid of TMF1-Myc or SREBP1-Flag was constructed as previously reported[19]. The specific primers using in this research were showed in Table 1. The eukaryotic expression plasmids used in this study were pCMV-C-Flag (D2632, Beyotime) and pCMV-C-Myc (D2672, Beyotime).

Table 1  
Primer sequences used for plasmid construction

Gene name	Primer sequence (5'-3')	
	Forward primer	Reverse primer
TMF1	TCCCCCGGGATGAGCTGGTTCAACGCCTCCCAG (the <i>Sma</i> I site is underlined)	GCTCTAGAACTGAGCCTTTGTCTTAGAAGTTC (the <i>Xba</i> I site is underlined)
SREBP1	TCCCCCGGGATGGACGAGCCACCCTTCAACG (the <i>Sma</i> I site is underlined)	GCTCTAGAGCTGGAGGTCACAGTGGTCCCAC (the <i>Xba</i> I site is underlined)

For the experiment of gene overexpression, BMECs were planted into 6-well plates and normal cultured. After 12 h, the medium was changed with OPTI-MEM medium (11058021, Gibco, USA), and the overexpression plasmid (SREBP1-Flag or TMF1-Myc) was transfected with Lipo6000™ Transfection Reagent (C0526, Beyotime) according to the manufacturer's instructions. The empty plasmids of pCMV-C-Flag or pCMV-C-Myc were also transfected as control.

## Small interfering RNA (siRNA) transfection

The specific siRNA of TMF1 gene, SREBP1 gene and negative control (NC) used in this study were synthesized by GenePharma Co.,Ltd (GenePharma, Shanghai, China). The sequences of siRNA used in this research were shown in Table 2. The si-TMF1, si-SREBP1 and NC were transfected using Lipo6000™ Transfection Reagent. The experimental process was the same as that of overexpression plasmid transfection and it was according to the manufacturer's instructions of Lipo6000™ Transfection Reagent.

Table 2  
siRNA sequences

Gene name	siRNA sequence (5'-3')	
TMF1	Sense	GCCCAGAAGUCUAUUGACATT
	Antisense	UGUCAAUAGACUUCUGGGCTT
SREBP1	Sense	CCUAUUUGACCCACCCUAUTT
	Antisense	AUAGGGUGGGUCAAAUAGGTT
Negative control	Sense	UUCUCCGAACGUGUCACGUTT
	Antisense	ACGUGACACGUUCGGAGAATT

## Nuclear and cytoplasmic protein extraction

The treated cells were harvested and the nuclear and cytoplasmic protein were extracted using Nuclear and Cytoplasmic Protein Extraction Kit (P0027, Beyotime) according to the manufacturer's instructions. The Lamin B1 and  $\beta$ -Tubulin were used as the marker of nuclear protein and cytoplasmic protein, respectively.

## Co-Immunoprecipitation (Co-IP)

BMECs were treated with no transfected, transfected with TMF1-Myc or/and SREBP1-Flag. Then the cell lysis of each group was collected with cell lysis buffer for western and IP (P0013, Beyotime) containing 1 mM Phenylmethanesulfonyl fluoride (PMSF) (ST506, Beyotime). The experiment of Co-IP was completed using the Protein A + G Agarose (P2012, Beyotime) according to the manufacturer's instructions. The primary antibody used for Co-IP in this research was anti-TMF1 or anti-Myc and the antibodies used to test the immunoprecipitate were as follows: anti-TMF1, anti-Myc, anti-Flag and anti-SREBP1.

## Statistical analysis

The data was analyzed using GraphPad Prism 7 software and reported as mean  $\pm$  standard deviation ( $n = 3$ ).  $p < 0.05$  was considered statistically significant. Grey-scale scanning of WB band and the co-localization of IF were analyzed with ImageJ2X software. All data of this study were averaged from three independent experiments.

## Results

### TMF1 negatively regulates sodium acetate activated milk fat synthesis

To investigate the effect of TMF1 on the regulation of sodium acetate on milk fat synthesis, cells were co-treated with TMF1 overexpression or silencing and sodium acetate supply or depletion. The expression of TMF1 and secretion of TG of BMECs were tested. The result showed that compared with sodium acetate depletion group, the expression of TMF1 was significantly decreased in cells only treated with sodium acetate supply, but when cells pre-treated with TMF1 overexpression, and then treated with sodium acetate supply, the expression of TMF1 was no decreased (Fig. 1A-1B). Compared with sodium acetate depletion group, the secretion of TG was

significantly increased in cells only treated with sodium acetate supply, but when cells pre-treated with TMF1 overexpression, and then treated with sodium acetate supply, the secretion of TG was no increased (Fig. 1C). Conversely, compared with sodium acetate supply group, the expression of TMF1 was significantly increased in sodium acetate depletion group, but when cells pre-treated with TMF1 silencing, and then treated with sodium acetate depletion, the expression of TMF1 was no decreased (Fig. 1D-1E). The secretion of TG was significantly decreased in sodium acetate depletion group, but when cells pre-treated with TMF1 silencing, and then treated with sodium acetate depletion, the secretion of TG was no decreased (Fig. 1F).

These results indicate that sodium acetate can promote the synthesis of milk fat and inhibit the expression of TMF1. TMF1 may be a negative regulator for sodium acetate dependent milk fat synthesis.

## **TMF1 negatively regulates sodium acetate activated SREBP1 pathway**

Previous studies have shown that short-chain fatty acids regulate milk fat synthesis by SREBP1 pathway[20, 21]. To investigate whether the TMF1 affects sodium acetate activated SREBP pathway, cells were co-treated with TMF1 overexpression or silencing and sodium acetate supply or depletion. The expression of SREBP1 pathway-associated protein, ACC, FAS, SCD and FABP3 in BMECs were tested. The result showed that compared with sodium acetate depletion group, the expression of ACC, FAS, SCD and FABP3 was significantly increased in cells treated with sodium acetate supply, but when cells pre-treated with TMF1 overexpression, and then treated with sodium acetate supply, the expression of these proteins was no decreased (Fig. 2A-2B). Conversely, compared with sodium acetate supply group, the expression of ACC, FAS, SCD and FABP3 was significantly decreased in sodium acetate depletion group, but when cells pre-treated with TMF1 silencing, and then treated with sodium acetate depletion, the expression of these proteins was no decreased (Fig. 2C-2D).

These results suggest that TMF1 is a negative regulator for sodium acetate dependent activation of SREBP1 pathway, and the activation of SREBP1 pathway controlled by sodium acetate can be suppressed by TMF1.

## **TMF1 negatively regulates milk fat synthesis through SREBP1 pathway**

To investigate whether the SREBP1 pathway is involved in the regulation of TMF1 on milk fat synthesis, cells were co-treated with TMF1 overexpression or silencing and SREBP1 overexpression or silencing. The expression of SREBP1 and secretion of TG of BMECs were tested. The result showed that the expression of SREBP1 (Fig. 3A-3B) and the secretion of TG (Fig. 3C) were significantly decreased in cells treated with TMF1 overexpression, but when cells pre-treated with SREBP1 overexpression, and then treated with TMF1 overexpression, this decrease was restored (Fig. 3A-3C). Conversely, the expression of TMF1 (Fig. 3D-3E) and the secretion of TG (Fig. 3F) were significantly increased in TMF1 silencing group, but when cells pre-treated with SREBP1 silencing, and then treated with TMF1 silencing, this increase was blocked (Fig. 3D-3F).

These results indicate that SREBP1 pathway is involved in the regulation of TMF1 on milk fat synthesis, and TMF1 negatively regulates milk fat synthesis through SREBP1 pathway.

## **TMF1 negatively regulates the nuclear localization of SREBP1**

Previous studies have shown that sodium acetate promotes the nuclear localization of SREBP1. To explore whether the nuclear localization of SREBP1 is regulated by TMF1, cells were treated with TMF1 overexpression or silencing, the nuclear localization of SREBP1 was tested. The result showed that the nuclear localization of SREBP1 was significantly decreased and the cytoplasmic localization of SREBP1 was significantly increased in cells treated with TMF1 overexpression (Fig. 4A-4E). Conversely, the nuclear localization of SREBP1 was significantly increased and the cytoplasmic localization of SREBP1 was significantly decreased in cells treated with TMF1 silencing (Fig. 4F-4J).

These results suggest that TMF1 is a negative regulator for nuclear localization of SREBP1, and the nuclear localization of SREBP1 is blocked by TMF1.

## **TMF1 direct interacts with SREBP1**

To explore the mechanism of TMF1 inhibiting the nuclear localization of SREBP1, the interacting proteins of TMF1 were identified. The result showed that TMF1 was direct interacted with SREBP1 (Fig. 5A). To confirm the specificity of the interaction between TMF1 and SREBP1, BMECs were transfected with TMF1-Myc plasmid and/or SREBP1-Flag plasmid. The interaction between TMF1 and SREBP1 was tested by Co-IP with Myc antibody and Flag antibody. The result showed that only in the group that the cells co-transfected with TMF1-Myc plasmid and SREBP1-Flag plasmid, the SREBP1-Flag was detected with Flag antibody or SREBP1 antibody (Fig. 5B-5C). This result suggests that TMF1 specifically interacts with SREBP1. In addition, the co-location of TMF1 and SREBP1 was identified, the result showed that TMF1 and SREBP1 were co-located, and their co-localization only existed in cytoplasm (Fig. 5D).

These results suggest that TMF1 is direct interacts with SREBP1, and this interaction can block the the nuclear localization of SREBP1.

## **Sodium acetate inhibits the expression of TMF1 and interaction between TMF1 and SREBP1**

To investigate whether the expression of TMF1 and interaction between TMF1 and SREBP1 are affected by sodium acetate, BMECs were treated with sodium acetate supply or depletion. The expression of TMF1 and interaction between TMF1 and SREBP1 were tested. The result showed that the expression of TMF1 was significantly decreased in cells treated sodium acetate supply (Fig. 6A-6B). The interaction between TMF1 and SREBP1 tested by Co-IP (Fig. 6C-6D) and co-location of TMF1 and SREBP1 tested by IF (Fig. 6E-6F) were also significantly decreased in cells treated sodium acetate supply.

These results indicate that sodium acetate suppresses the expression of TMF1 and interaction between TMF1 and SREBP1.

## **Discussion**

Sodium acetate is one of the most important precursors of fatty acid synthesis in cell [6, 22, 23]. Studies have shown that the milk fat synthesis of mammary gland cells in vitro can be promoted by the addition of sodium acetate in medium [7, 24, 25]. In this study, the secretion of TG was significantly increased in BMECs treated with sodium acetate supplementation. This result is consistent with the conclusions of previous research.

TMF1 is a DNA binding factor that preferentially binds to the TATA element in the human immunodeficiency virus 1[12, 26]. In eukaryotic cells, TMF1 widely involved in a variety of cell signaling pathways and physiological processes. Studies have shown that TMF1 is dispersed in the cytoplasm of stressed cell and exerts E3 ubiquitin ligase activity[27]; TMF1 is a key regulator of morphogenesis of mature sperm[28]; TMF1 mediates the degradation of Stat3[27]; TMF1 down-regulates proangiogenic genes and attenuates the progression of PC3 xenografts[29]; TMF1 mediates the protein sorting and transport of proteins in Golgi apparatus[30]. In our previous study, we found that TMF1 may be involved in the regulation milk fat synthesis in BMECs. The current study demonstrated that TMF1 negatively regulates milk fat synthesis in response to sodium acetate depletion. Co-treated with sodium acetate supply or depletion and silencing or overexpression of TMF1 in BMECs discovered that sodium acetate depletion negatively regulated milk fat synthesis through TMF1.

SREBP1 pathway is one of most important pathways involved in milk fat synthesis. It responds to short-or long-chain fatty acids and then promotes fat synthesis[31]. In this study, sodium acetate supply promote the expression of SREBP1 and its target gene related to milk fat synthesis, such as ACC, FAS, SCD and FABP3. In addition, silencing or overexpression of TMF1 in BMECs demonstrates that TMF1 negatively controls the expression of SREBP1 and its target gene, and the increase of the expression of these genes regulated by sodium acetate supply is suppressed by overexpression of TMF1. These results suggest TMF1 is involved in sodium acetate dependent milk fat synthesis, it is an important negative regulator in this regulatory process in BMECs.

As a main regulator of fat synthesis, SREBP1 responds to fatty acids, and then transfers into cell nucleus and promotes the expression of its target gene and subsequent milk fat synthesis[32]. In our study, the regulation of TMF1 on nuclear localization of SREBP1 was tested, and the result showed that the nuclear localization of SREBP1 was significantly blocked by the overexpression of TMF1.

Because of the central and C-terminal parts contain coiled-coil forming domains, TMF1 can interacts with many other proteins, such as Golgi and acrosome-associated variant of the tyrosine kinase Fer-FerT[13, 33], Rab6 GTPbinding protein[34], ATPase subunits of the human chromatin remodeling complex SNF/SWI -hbrm/hSNF2a and BRG-1/hSNF2b[14, 15, 35]. And this combination enables it to participate in a wide range of cellular physiological processes and functions. In current study, the experiments of CO-IP indicate that TMF1 can interacts with SREBP1, and the co-localization of IF demonstrates that TMF1 and SREBP1 interacts in cytoplasmic rather than in nuclear. Combined with the previous results in this study, we show that the TMF1 interacts with SREBP1 directly in cytoplasmic, and then suppresses the nuclear localization of SREBP1 and expression of SREBP1 target gene (ACC, FAS, SCD and FABP3) and subsequent milk fat synthesis in BMECs.

In addition, the effect of sodium acetate on the expression of TMF1 and the interaction between TMF1 and SREBP1 was investigated in this study, and the result indicates that supplement of sodium acetate, can suppress the expression of TMF1 and the interaction between TMF1 and SREBP1. It suggest that TMF1 is an important negative regulator for sodium acetate dependent milk fat synthesis in BMECs.

## Conclusions

In summary, the current study finds that TMF1 is one of the important regulator for the regulation of sodium acetate activated milk fat synthesis in BMECs. In BMECs, the expression of TMF1 is promoted in response to the sodium acetate depletion, and the TMF1 prevents the nuclear localization of SREBP1 by interacting with SREBP1

in cytoplasmic and then suppresses the expression of SREBP1 target genes and subsequent milk fat synthesis. The results of this study will help to increase our understanding for the regulation mechanism and pathway of milk fat synthesis, and it is important for the development of dairy industry.

## Abbreviations

BMECs: bovine mammary epithelial cells; TMF1: TATA element modulatory factor 1; SREBP1: sterol regulatory element binding protein 1; ACC: Acetyl CoA carboxylase; FAS: Fatty acid synthesis; SCD: stearoyl-CoA desaturase; FABP3: fatty acid binding protein 3; NC: negative control; EV: empty vector; DAPI: 4', 6-diamidino-2-phenylindole; PI: Propidium Iodide; Co-IP: Co-Immunoprecipitation; WB: western blotting; IF: Immunofluorescence; TG: triglyceride; DMEM/F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; FBS: fetal bovine serum

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

CCL and NL conceived and designed the experiments, performed the experiments and collected and analyzed the experimental data and written the manuscript. QZW analyzed a party of the experimental data and revised the manuscript. CJL conceived and designed the experiments and revised the manuscript. All authors read and approved the final manuscript.

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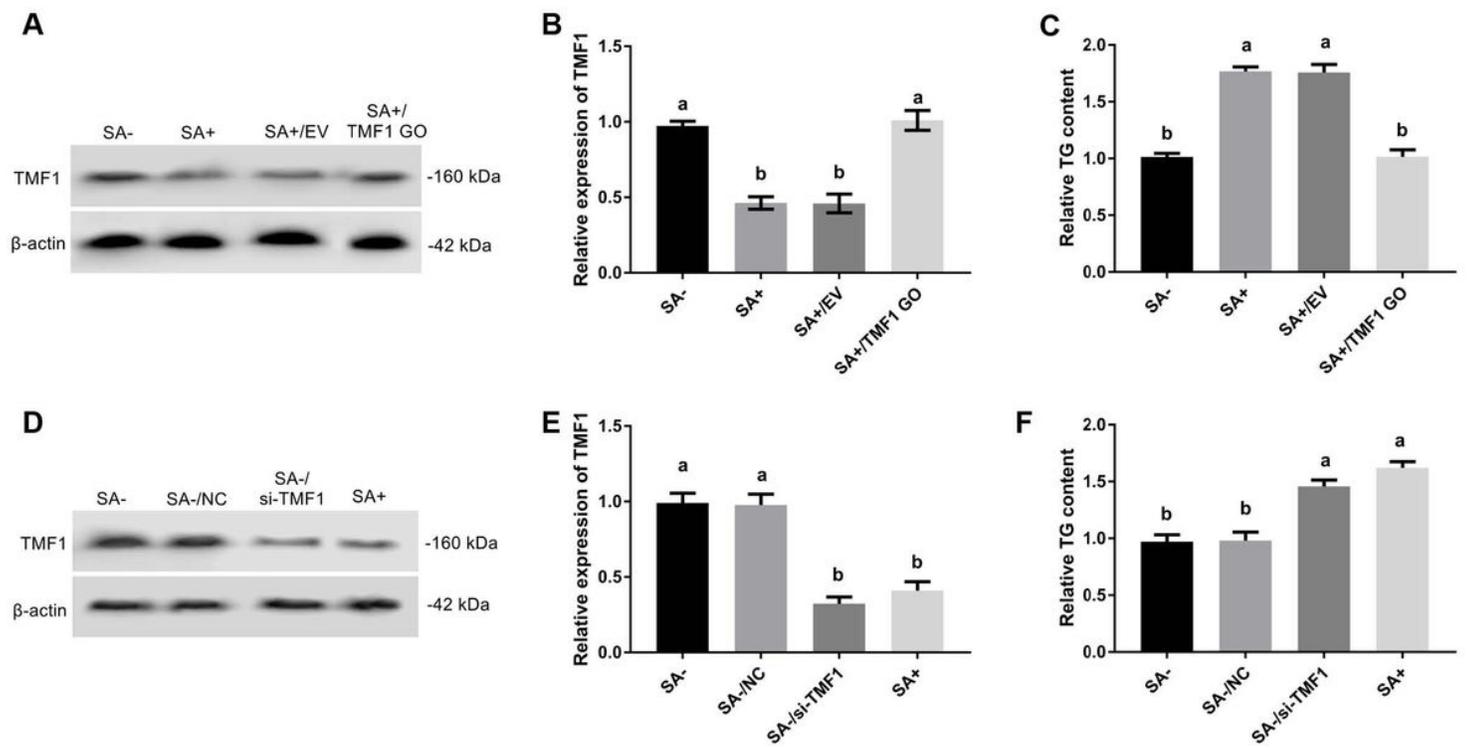
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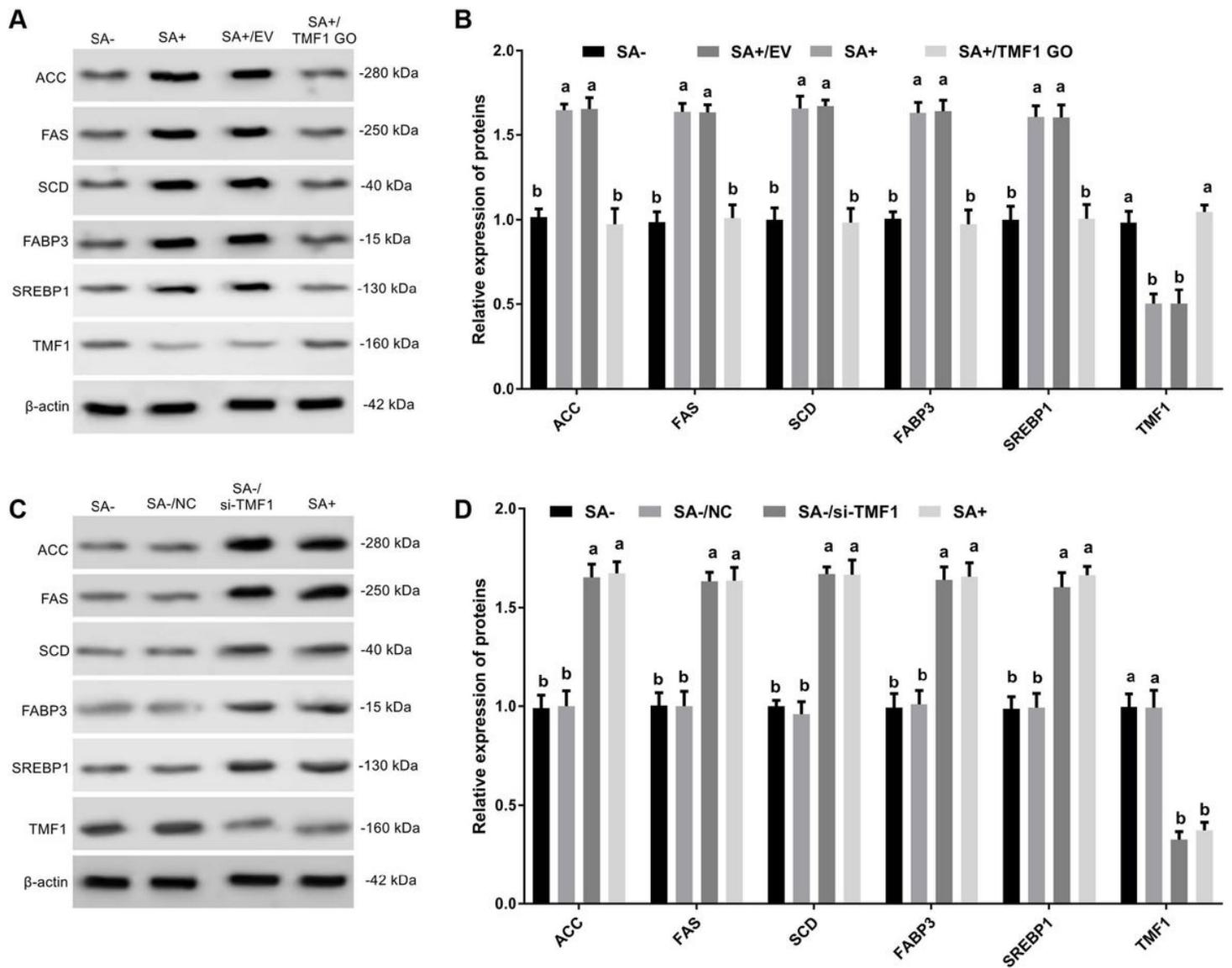
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## Figures



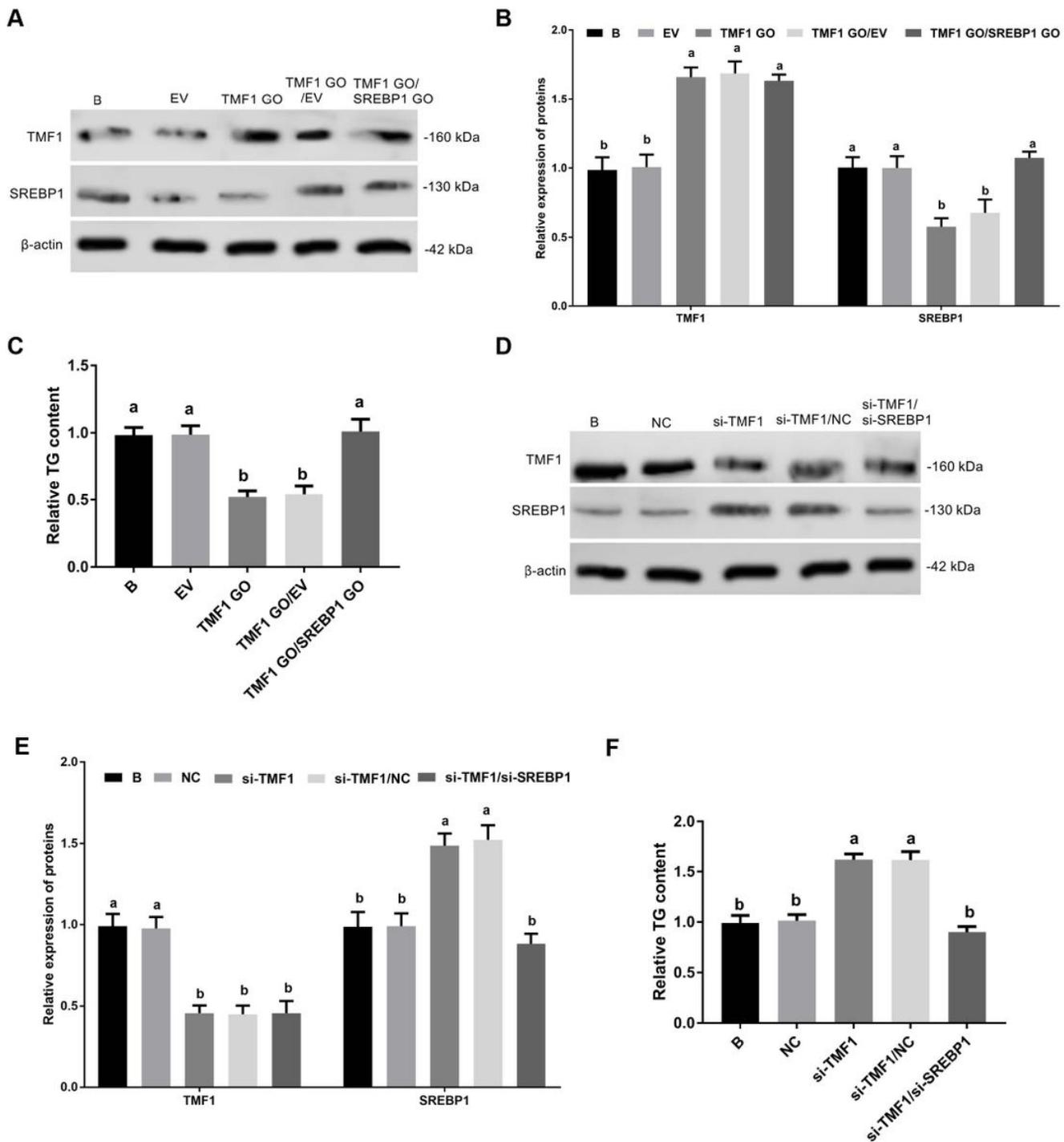
**Figure 1**

TMF1 negatively regulates sodium acetate activated milk fat synthesis. (A-B) Expression of TMF1 in BMECs treated with SA-, SA+ and SA+/TMF1 GO was tested by WB. (C) The secretion of TG in BMECs treated with SA-, SA+ and SA+/TMF1 GO was tested by Kit. (D-E) Expression of TMF1 in BMECs treated with SA-, SA+, and SA-/si-TMF1 was tested by WB. (F) The secretion of TG in BMECs treated with SA-, SA+, and SA-/si-TMF1 was tested by Kit. In A-B and D-E,  $\beta$ -actin was used as a loading control, and the expression of TMF1 in BMECs treated with SA- was set to "1". In C and F, the concentration of TG in BMECs treated with SA- was set to "1". SA-: cells were cultured in DMEM/F12 no adding FBS and sodium acetate. SA+: cells were cultured in DMEM/F12 lacking FBS but adding sodium acetate. SA+/EV or TMF1 GO: cells were transfected with empty vector or TMF1 overexpression vector and then cultured in DMEM/F12 lacking FBS but adding sodium acetate. SA-/NC or si-TMF1: cells were transfected with NC siRNA or TMF1 siRNA and then cultured in DMEM/F12 no adding FBS and sodium acetate. In the bar charts, different superscript lowercase letters indicate significant differences ( $p < 0.05$ ), while the same letters represent no significant difference ( $p > 0.05$ ).



**Figure 2**

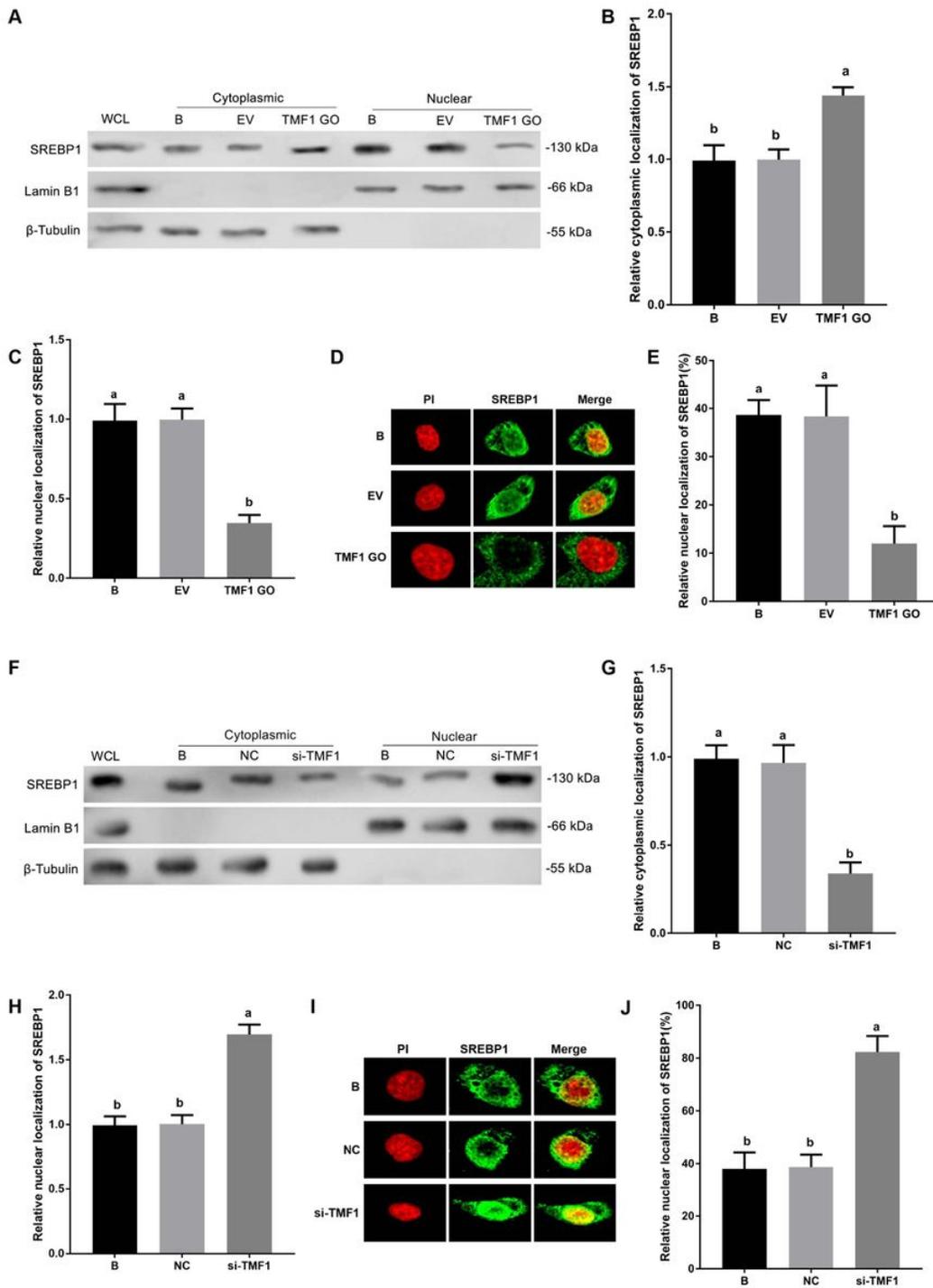
TMF1 negatively regulates sodium acetate activated SREBP1 pathway. (A-B) Expression of ACC, FAS, SCD, FABP3 and TMF1 in BMECs treated with SA-, SA+ and SA+/TMF1 GO was tested by WB. (C-D) Expression of ACC, FAS, SCD, FABP3 and TMF1 in BMECs treated with SA-, SA+, and SA-/si-TMF1 was tested by WB.  $\beta$ -actin was used as a loading control, and the expression of these proteins in BMECs treated with SA- was set to "1". The abbreviation of SA-, SA+, SA+/EV, SA+/TMF1 GO, SA-/NC and SA-/si-TMF1 has the same meaning as Fig. 1. In the bar charts, different superscript lowercase letters indicate significant differences ( $p < 0.05$ ), while the same letters represent no significant difference ( $p > 0.05$ ).



**Figure 3**

TMF1 negatively regulates the milk fat synthesis through SREBP1 pathway. (A-B) Expression of TMF1 and SREBP1 in BMECs treated with TMF1 GO and TMF1 GO/SREBP1 GO was tested by WB. (C) The secretion of TG in BMECs treated with TMF1 GO and TMF1 GO/SREBP1 GO was tested by Kit. (D-E) Expression of TMF1 and SREBP1 in BMECs treated with si-TMF1 and si-TMF1/si-SREBP1 was tested by WB. (F) The secretion of TG in BMECs treated with si-TMF1 and si-TMF1/si-SREBP1 was tested by Kit. In A-B and D-E, β-actin was used as a loading control, and the expression of TMF1 and SREBP1 in BMECs of B group was set to "1". In C and F, the concentration of TG in BMECs of B group was set to "1". B: cells were no treated. EV: cells were transfected with empty vector. NC: cells were transfected with NC siRNA. TMF1 or SREBP1 GO: cells were transfected with TMF1

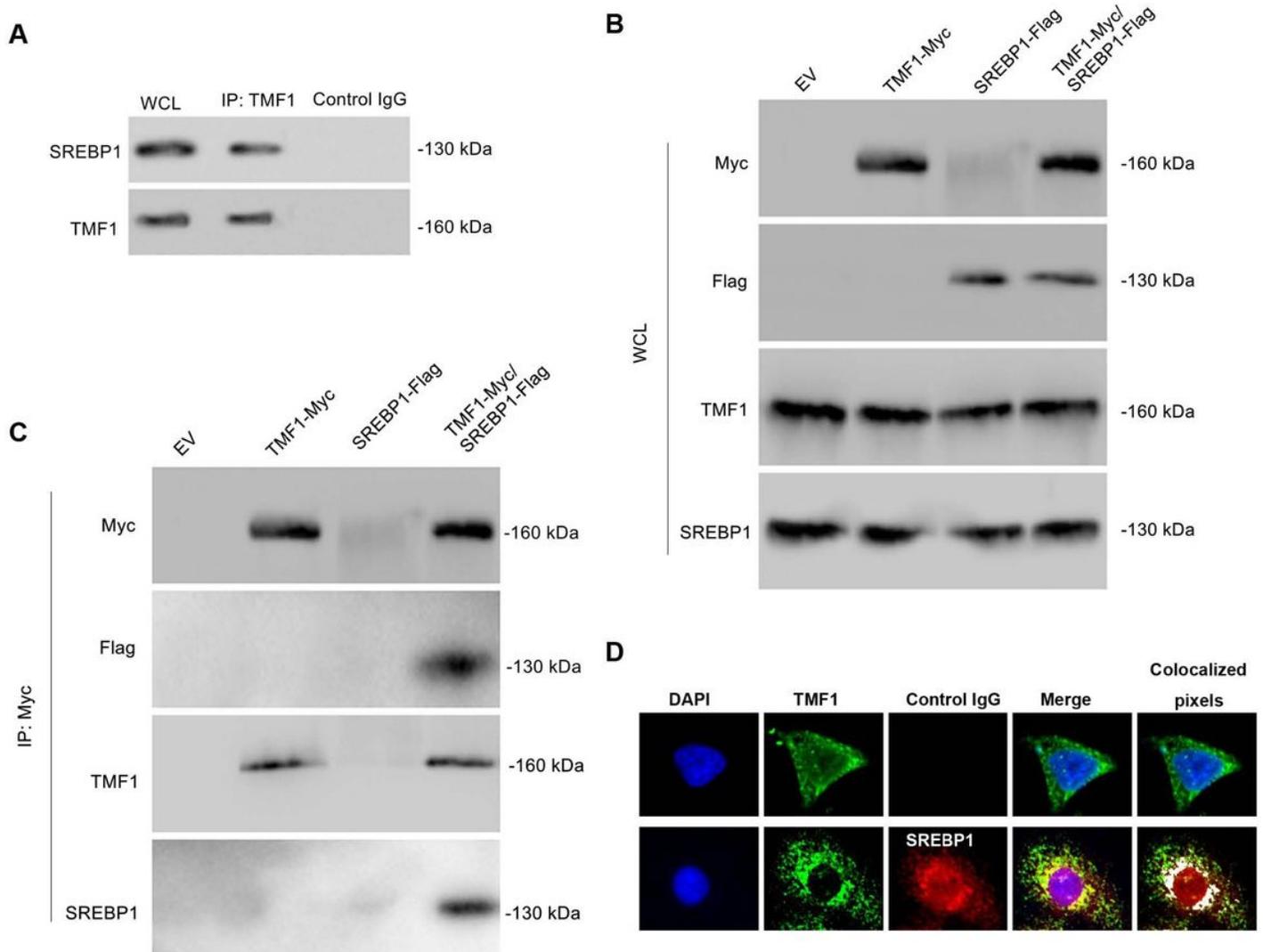
or SREBP1 expression vector. si-TMF1 or SREBP1: cells were transfected with TMF1 or SREBP1 siRNA. TMF1 GO/SREBP1 GO: cells were co-transfected with TMF1 and SREBP1 expression vector. si-TMF1/si-SREBP1: cells were co-transfected with TMF1 or SREBP1 siRNA. In the bar charts, different superscript lowercase letters indicate significant differences ( $p < 0.05$ ), while the same letters represent no significant difference ( $p > 0.05$ ).



**Figure 4**

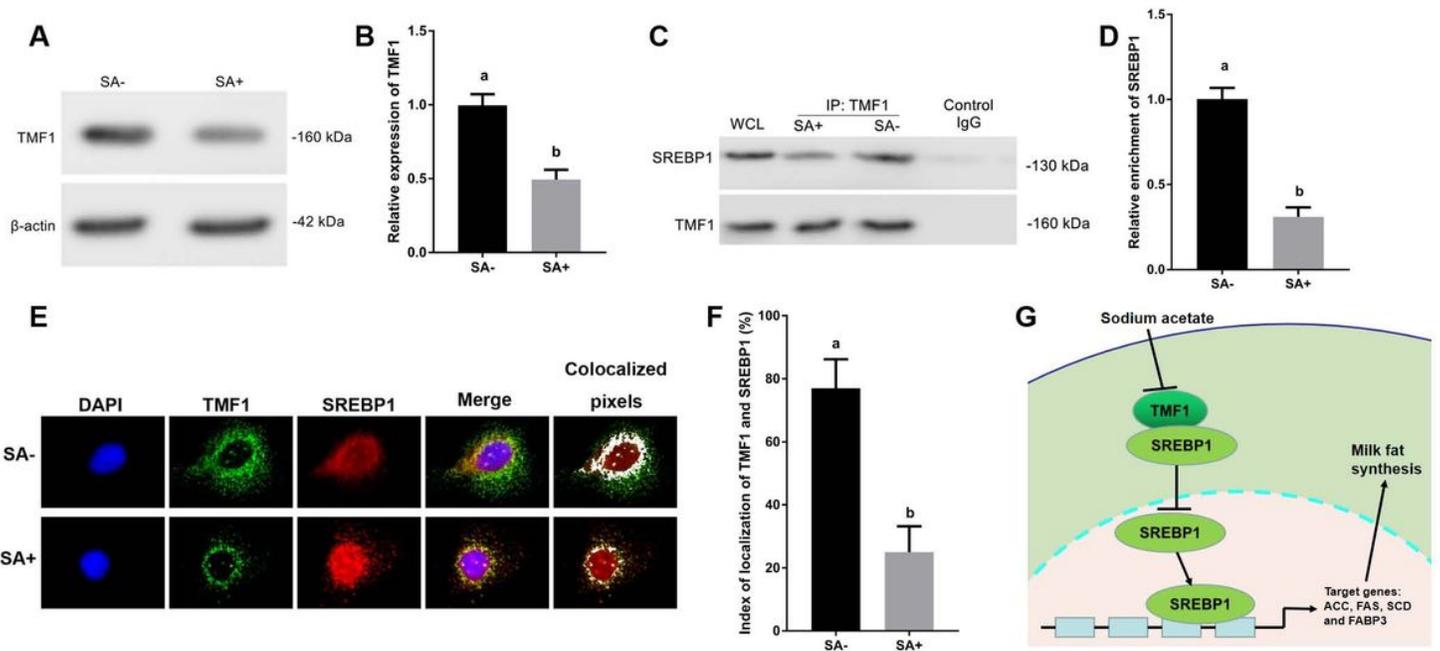
TMF1 negatively regulates the nuclear localization of SREBP1. (A-C) The cytoplasmic localization (A and B) and nuclear localization (A and C) of SREBP1 in BMECs treated with TMF1 GO was tested by WB. (D-E) The nuclear localization of SREBP1 in BMECs treated with TMF1 GO was tested by IF. (F-H) The cytoplasmic localization (F and G) and nuclear localization (F and H) of SREBP1 in BMECs treated with si-TMF1 was tested by WB. (I-J) The

nuclear localization of SREBP1 in BMECs treated with si-TMF1 was tested by IF. In B and G, The cytoplasmic localization of TMF1 in BMECs of B group was set to "1". In C and H, The nuclear localization of TMF1 in BMECs of B group was set to "1". The abbreviation of B, EV, NC, TMF1 GO and si-TMF1 has the same meaning as Fig. 3. In the bar charts, different superscript lowercase letters indicate significant differences ( $p < 0.05$ ), while the same letters represent no significant difference ( $p > 0.05$ ).



**Figure 5**

TMF1 direct interacts with SREBP1. (A) Co-IP of TMF1 was completed and the immunoprecipitate was tested with SREBP1 antibody. IgG was used as a control. (B-C) BMECs were transfected with TMF1-Myc or/and SREBP1-Flag, and then the Co-IP of Myc was completed, the WCL (B) and immunoprecipitate (C) were tested with Myc, Flag, TMF1 and SREBP1 antibody. (D) The co-localization of TMF1 and SREBP1 was tested by IF. WCL: whole cell lysate.



**Figure 6**

Sodium acetate negatively regulates the expression of TMF1 and the interaction between TMF1 and SREBP1. (A-B) Expression of TMF1 in BMECs treated with SA- or SA+ was tested by WB. (C-D) the interaction between TMF1 and SREBP1 in BMECs treated with SA- or SA+ was tested by WB. (E-F) The co-localization of TMF1 and SREBP1 in BMECs treated with SA- or SA+ was tested by IF. (G) Schematic model of TMF1 negatively regulates sodium acetate activated milk fat synthesis through SREBP1 pathway in BMECs. In B, The expression of TMF1 in BMECs treated with SA- was set to "1". In C, The enrichment of TMF1 in BMECs treated with SA- was set to "1". WCL: whole cell lysate. SA-: sodium acetate depletion. SA+: sodium acetate supply. In the bar charts, different superscript lowercase letters indicate significant differences ( $p < 0.05$ ), while the same letters represent no significant difference ( $p > 0.05$ ).