Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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ORIGINAL ARTICLE

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TFAP2C exacerbates psoriasis-like inflammation by promoting Th17 and Th1 cells activation through regulating *TEAD4* transcription

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Received 28 February 2023; Accepted 20 March 2023;
Available online 1 May 2023

KEYWORDS
mice;
psoriasis;
TEAD4;
TFAP2C;
Th1;
Th17

Abstract
Background: Psoriasis is one of the chronic and autoimmune skin diseases. It is important to uncover the mechanisms underlying the psoriasis. Transcription factor activator protein (TFAP-2) gamma, also known as AP2-gamma, is a protein encoded by the *TFAP2C* gene. Immune-mediated pathophysiological processes could be linked to psoriasis, but the mechanism is still unclear. Therefore, to date the cause of psoriasis has not been understood completely.
Materials and methods: Psoriasis is a complex disease triggered by genetic, immunological, and environmental stimuli. Keratinocytes play an important role in both initiation and maintenance phases of psoriasis. A psoriatic keratinocyte model was established by stimulating high sensitivity of human epidermal keratinocytes (HaCaT) to topoisomerase inhibitor cell lines using the accumulation of M5 cytokines comprising interleukin (IL)-17A, IL-22, oncostatin M, IL-1α, and tumor necrosis factor-α (TNF-α). The *TFAP2C* and *transcriptional enhanced associate domain 4* (*TEAD4*) genes expression was evaluated by reverse transcription-quantitative polymerase chain reaction. Western blot analysis was used to examine protein expression. Cell viability (quantitative) of keratinocytes, including cytotoxicity, proliferation, and cell activation, was evaluated by the MTT assay. The relative percentage values of interleukin (IL)-17a, interferon gamma, and IL-4+ cells were measured by flow cytometry. Accordingly, chromatin immunoprecipitation and luciferase reporter assays were applied to evaluate the binding affinity of *TFAP2C* and *TEAD4* promoter.
Results: Level of the *TFAP2C* gene was elevated in the lesional skin of psoriasis patients. On the other hand, silencing of the *TFAP2C* gene suppressed the proliferation and inflammatory response in M5-induced keratinocytes. In addition, inhibition of *TFAP2C* alleviated imiquimod (IMQ)-induced skin injury in mice model. We also observed that suppression of *TFAP2C* inhibited the activation of T-helper 17 (Th17) and Th1 cells in IMQ-induced mice model. Mechanically, *TFAP2C* promoted *TEAD4* transcriptional activation.
Conclusion: TFAP2C exacerbated psoriasis-like inflammation by increasing the activation of Th17 and Th1 cells by regulating *TEAD4* transcription. This finding clearly indicated that *TFAP2C* could be considered a valuable biomarker for the prevention and treatment for psoriasis.

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https://doi.org/10.15586/aei.v51i3.854

Introduction

Psoriasis is known as one of the chronic and immune-related skin diseases that severely threatens human lives globally. The global prevalence of psoriasis ranges from 0.27% to 11.43%.[1-3] The prevalence of psoriasis in China was reported to be about 3%, which seriously influenced people's quality of life.[4] Psoriasis is presented as erythema and scales in various parts of the body, including the face, head, scalp, hands, and nails.[5]. Psoriasis seriously affects the daily life of patients, and it is reportedly found to relapse even after treatment in some of the patients.[6,7] It is also reported that keratinocytes are excessively proliferated and abnormally differentiated, and inflammatory cells are infiltrated into the dermis and epidermis.[8] Although aberrant interactions between immune cells and keratinocytes have been identified to exercise an important role in the pathogenesis of psoriasis,[9] the detailed mechanism underlying psoriasis is still unclear. Therefore, to discover the mechanism underlying psoriasis is of great importance for the prevention and treatment of this disease.

It is reported that transcription factor activator protein-2 gamma (*TFAP2C*), also known as AP-2γ, is one of the members of AP-2 transcription factor family and is involved in DNA binding and regulation of transcriptional potential.[10,11] In the past, *TFAP2C* was revealed to participate in different biological processes. A study has established that *TFAP2C* accelerates long noncoding RNA (lncRNA) PCAT1 to suppress ferroptosis via c-Myc/miR-25-3p/SLC7A11 in prostate cancer.[12] Suppression of the *TFAP2C* gene changes estimated glomerular filtration rate (eGFR) and tumor necrosis factor-α (NF-κB) to behave normally and increases the sensitivity of bladder cancer cells to cisplatin.[13] Recently, *TFAP2C* has been discovered to regulate doxorubicin resistance in osteosarcoma, the most common malignancy of childhood.[14] Another study has shown that *TFAP2C* suppresses the growth arrest and DNA-damage-inducible beta (*GADD45B*) and phorbol-12-myristate-13-acetate-induced protein 1 (*PMAIP1*) genes to enhance non-small cell lung cancer cell (NSCLC) proliferation.[15] *TFAP2C* is also found to exercise an important role in the proliferation, migration, and invasion of breast cancer cells.[16] Different studies have established that *TFAP2C* is mainly distributed in the skin;[17-19] however, to the best of our knowledge it is still not clear whether *TFAP2C* is implicated in the development of psoriasis.

The data revealed that *TFAP2C* exacerbated psoriasis-like inflammation by promoting T-helper 17 (Th17) and Th1 cells activation by regulating *TEAD4* transcription. Hence, the purpose of this study was to probe the role of *TFAP2C* in the development of psoriasis and to understand its functioning in the prevention and treatment of the disease.

Methods

Human tissue samples

All lesional skin (LS) tissue samples were collected from patients diagnosed with moderate or severe chronic plaque psoriasis in the Second Hospital of Hebei Medical University. A total of 78 chronic plaque psoriasis patients were enrolled for the study. Lesional (n = 39) and non-lesional skin (n = 39) tissue samples of the participants were collected to prepare 4-mm punch biopsies. No clinical treatments were prescribed to these patients during the past 1 month. Informed consent was obtained from all the participants. The experimental treatment of animals followed the 1964 Helsinki (Medical) Convention declaration, and the study protocol was approved by the Animal Experiment Committee of the Second Hospital of Hebei Medical University.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

TRIzol reagent (Invitrogen, USA) was used for extracting total RNA from tissues and cells. PrimeScript RT Master Mix (Takara, Japan) was applied for the synthesis of complementary DNA. RT-qPCR protocol was executed through 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA) with SYBR Green kit (Takara). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as a negative control, and the delta-delta-Ct algorithm ($2^{-\Delta\Delta ct}$) method was used to calculate gene levels.[20,21] The primer sequences of genes are displayed in Table 1.

Table 1 The sequence of primers.

TFAP2C (Human):	
F: TCAGTCCCTGGAAGATTGTCG	R: CCAGTAACGAGGCATTTAAGCA
TEAD4 (Human):	
F: GAACGGGGACCCTCCAATG	R: GCGAGCATACTCTGTCTCAAC
GAPDH (Human):	
F: TCAAGGCTGAGAACGGGAAG	R: TGGACTCCACGACGTACTCA
Tfap2c (Mouse):	
F: ATCCCTCACCTCTCCTCTCC	R: CCAGATGCGAGTAATGGTCGG
Tead4 (Mouse):	
F: CAACCTGGAACATCCCACGAT	R: GAAAGCCGAGAACTCCAACAT
Gapdh (Mouse):	
F: AGGTCGGTGTGAACGGATTTG	R: TGTAGACCATGTAGTTGAGGTCA

Western blot analysis

Protocol for preparation of sample for cell lysis and efficient protein extraction from cultured tissues and cells for subsequent Western blot analysis was followed by using radioimmunoprecipitation assay (RIPA) lysis buffer (Abcam, China) and dichloroacetate (DCA) protein assay kit (Bio-Rad, China). Transfering of proteins from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or native gels to nitrocellulose or polyvinylidene fluoride (PVDF) membranes (Sigma-Aldrich, China) was done and sealed with milk for 1 h at room temperature. Then the membranes were incubated with appropriate dilutions of primary antibody at 4°C overnight. Soaking the membranes at 4°C overnight helped solve some persistent background issues. Later the membranes were grown with specific secondary antibodies for 2 h and washed with tris buffered saline with tween (TBST). Signals were observed using enhanced chemiluminescent (ECL) kit.[22,23] The following primary antibodies were included: anti-*TFAP2C* (ab110635, 1:1000; Abcam), anti-*TEAD4* (ab97460, 1:3000; Abcam), anti-T-bet (ab307193, 1:1000; Abcam), anti-RORγt (ab111174, 6 µg/Ml; Abcam), and anti-*GATA3* (ab182747, 1:1000; Abcam).

Immunohistochemistry (IHC) analysis

In histology laboratory, the histotechs were dewaxed in xylene during tissue processing before staining, followed by recycling in turn with primary alcohol and antigen water. The samples were extracted at 95°C for 10 min using conventional microwave-assisted closed vessel and then dissolved in ethylenediaminetetraacetic acid (EDTA). Furthermore, the samples were boiled in 3% hydrogen peroxide for 30 min, followed by sealing with goat serum (20%) for 40 min. Primary antibody incubation was carried out in serum (1:100) at 4°C overnight. Horseradish peroxidase (HRP) binding protein secondary antibody was added and grown for an hour, followed by staining with 2-aminobenzidine and hematoxylin.[24,25]

HaCaT keratinocytes culture and M5 treatment

High sensitivity of human epidermal keratinocytes (HaCaT) to topoisomerase inhibitors cell lines were obtained from the Cell Bank of Type Culture Collection (Chinese Academy of Sciences, China). Dulbecco's modified Eagle's medium (DMEM) with Fetal Bovine Serum (FBS; 10%) was used for *in vitro* cell culture. Different concentrations of M5 (IL-17A [0 ng/mL], IL-22 [1 ng/mL], oncostatin M [2.5 ng/mL], IL-1α [5 ng/mL], and tumor necrosis factor-α [TNF-α]) were supplemented in HaCaT keratinocyte cell lines to construct a psoriasis-like keratinocytes model.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

MTT assay kit was used to check the viability of HaCaT keratinocyte cell lines cultured in 96-well plates for 24, 48, and 72 h (Sigma-Aldrich). This was followed by incubation for 4 h. The medium was discarded, and 100-µL dimethyl sulfoxide (DMSO) was filled in each well and further incubated for 20 min in dark at 20–25°C before use. Absorbance was observed at 570 nm through an enzyme-labelling measuring instrument.

5-ethynyl-2'-deoxyuridine (EdU) assay

Cell growth was carried out by culturing HaCaT keratinocyte cell lines, and proliferation was measured through EdU assay. HaCaT keratinocyte cell lines were cultured in 96-well plates followed by fixation and permeabilization. After incubating with 50-µM EdU (Sigma) for 3 h, the cells were cultured in 1-µg/mL 4',6-diamidino-2-phenylindole (DAPI; Sigma) for 10 min. Fluorescence microscopy (Leica, Germany) was applied to observe and capture the images of cultured cells.

Enzyme-linked immunosorbent serologic assay (ELISA)

The plasma levels of TNF-α, IL-1ß, and IL-18 were determined with a commercial ELISA kit (Abcam, China). Cell supernatant and HRP were supplemented in the antibody-coated plate and cultured at 37°C for 1 h. Then, 50-µL reaction solution was supplemented into each well and incubated for 30 min followed by filling in stop solution (50 µL). Finally, the absorbance was evaluated at 450 nm.

Imiquimod (IMQ)-induced psoriasis model

The IMQ-induced psoriasis mice model was established with 6-week-old female BALB/c mice (n = 6), fed under controlled conditions. The mice were treated with 5% IMQ cream (62.5 mg/day; Sichuan Med-Shine Pharmaceutical, H20030128, Sichuan, China) on shaved back for seven consecutive days. For control group (n = 6) experiment, the mice were treated with a vehicle cream (62.5 mg/day).

Scoring severity of skin inflammation

The inflammation severity of the back skin with psoriasis was scored in line with the clinical Psoriasis Area and Severity Index (PASI). Erythema, scaling, and thickening were scored from 0 to 4 (0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked). The erythema level was scored through a scoring table with red taints. The total score ranged from 0–12.

Hematoxylin and eosin (H&E) staining

Fixed in formalin, the collected mouse skin tissues were embedded in paraffin and cut into 6-µm sections. Tissue sections were stained in H&E to study the general histological structure of skin tissues. For measuring acanthosis,

the epidermal area was outlined and its pixel size was measured using the lasso tool in Adobe Photoshop CS4.

The relative area of the epidermis = pixels/(horizontal resolution × vertical resolution).

The number of infiltrating cells was analyzed in the selected six areas of the samples.

Flow cytometry analysis

Isolated CD4 cells were incubated for 5 h with ionomycin, phorbol 12-myristate 13-acetate, and monensin. Anti-mouse cluster of differentiation 4 (CD4) cells was employed for staining of cell surface markers and incubated at 4°C for 15 min. Following rinsing, fixing, and permeabilizing using a cytofix/cytoperm kit (BD, Germany), intracellular cytokines were stained with anti-IL-17A (Biolegend, San Diego, CA), anti-interferon gamma (IFNγ; Biolegend), and anti-IL-4+ at 4°C for 30 min. CD4 cells were obtained through a 2-laser flow cytometer (BD FACSCalibur; San Jose, CA), and the FlowJo software (FlowJo, LLC, Ashland, OR) was applied for data analysis. The process of fixation and permeabilization allows the antibody to pass through plasma membrane and move inside the cell while leaving the morphological characteristics used to separate intact cells.

Chromatin immunoprecipitation (ChIP) assay

The EZ-Magna ChIP kit (Abcam, UK) was applied in ChIP assay. HaCaT keratinocyte cell lines were fixed by using 1% formaldehyde followed by lysing and sonicating for 10 cycles of 10 s ON/20 s OFF and 50% audio power amplifier board (AMPL) through Sonics VCX130 (Sonics & Materials, Newtown, CT). Anti-*TFAP2C* (Thermo Fisher, USA) and rabbit immunoglobulin G (IgG; Thermo Fisher) were employed for immunoprecipitation. The relative enrichment of *TEAD4* was measured by RT-qPCR.

Luciferase reporter assay

HaCaT keratinocyte cell lines were cultured in 24-well plates and transfected with pGL4.70-*TEAD4* and pcDNA3.1-*TFAP2C* or empty plasmid cloning DNA (pcDNA) 3.1 vector. Following 48 h at room temperature, luciferase activity was measured using luciferase reporter assay (Promega, Madison, WI, USA). The luminescence intensities were measured through a microplate reader.

Statistical analysis

GraphPad Prism 6.0 was applied for statistical analysis, with the data displayed as mean ± standard deviation (SD). Comparisons between two groups were subjected to Student's *t*-test, and one-way analysis of variance (ANOVA) with post hoc tests was applied for multiple comparisons. Association between *TFAP2C* and *TEAD4* was analyzed by Pearson's correlation analysis. Normality and variance homogeneity were checked in all cases. $P < 0.05$ was considered statistically significant.

Results

TFAP2C *level was boosted in the lesional skin of psoriasis patients*

In this study, we investigated the role of *TFAP2C* in the development of psoriasis. *TFAP2C* was characterized by elevated expression in LS and normal skin of psoriasis patients. As shown in Figure 1A, *TFAP2C* level was boosted in the LS of psoriasis patients. Similarly, *TFAP2C* protein level was also elevated in the LS group, compared to the control group (Figure 1B). Moreover, the image of IHC analysis depicted that *TFAP2C* expression was higher in the LS group than in the control group (Figure 1C). On the whole, *TFAP2C* level was boosted in psoriasis patients.

Silencing of TFAP2C suppressed proliferation and inflammatory response in M5-induced keratinocytes

In this study, we explored the role of *TFAP2C* in the proliferation and inflammation of HaCaT cell line stimulation with M5, a cocktail of cytokines. HaCaT keratinocyte cell lines were treated with different concentrations of M5 (0 ng/mL, 1 ng/mL, 2.5 ng/mL, and 5 ng/mL); RNA and protein expression of *TFAP2C* were elevated in 2.5-ng/mL and 5-ng/mL groups (Figures 2A and 2B). We discovered that the range of concentrations at 2.5-ng/mL and 5-ng/mL M5 treatments tended to increase HaCaT keratinocyte cell lines viability, and HaCaT keratinocyte cell lines viability in 2.5-ng/mL group was higher than that in the 5-ng/mL group; therefore, M5 (2.5 ng/mL) was selected for subsequent assays (Figure 2C). Furthermore, we performed gene silencing analysis, and found that *TFAP2C* obviously decreased the level of *TFAP2C* in M5-treated HaCaT keratinocyte cells (Figure 2D). Our *in vitro* experiment revealed that the increased viability of HaCaT keratinocyte cells at the concentration of 2.5-ng/mL M5 treatment was reversed by *TFAP2C* downregulation (Figure 2E). EdU assay indicated that the proliferation of HaCaT keratinocyte cell lines in 2.5-ng/mL M5-treated group was decreased by the suppression of *TFAP2C* (Figure 2F). In addition, *TFAP2C* inhibition alleviated M5-induced elevation with concentrations of TNF-α, IL-1β, and IL-18 (Figure 2G). Our observation confirmed that silencing of *TFAP2C* suppressed the proliferation and inflammatory response of M5-induced keratinocytes.

Inhibition of TFAP2C alleviated IMQ-induced skin injury in mice

In this study, we hypothesized that IMQ-induced dermatitis in mice model can serve as an illustration for analyzing pathogenic mechanism in psoriasis-like study evaluation. The RT-qPCR data confirmed that *TFAP2C* expression was highly expressed in IMQ-induced mice model (Figure 3A). In consistent with the RNA expression, the *TFAP2C* protein

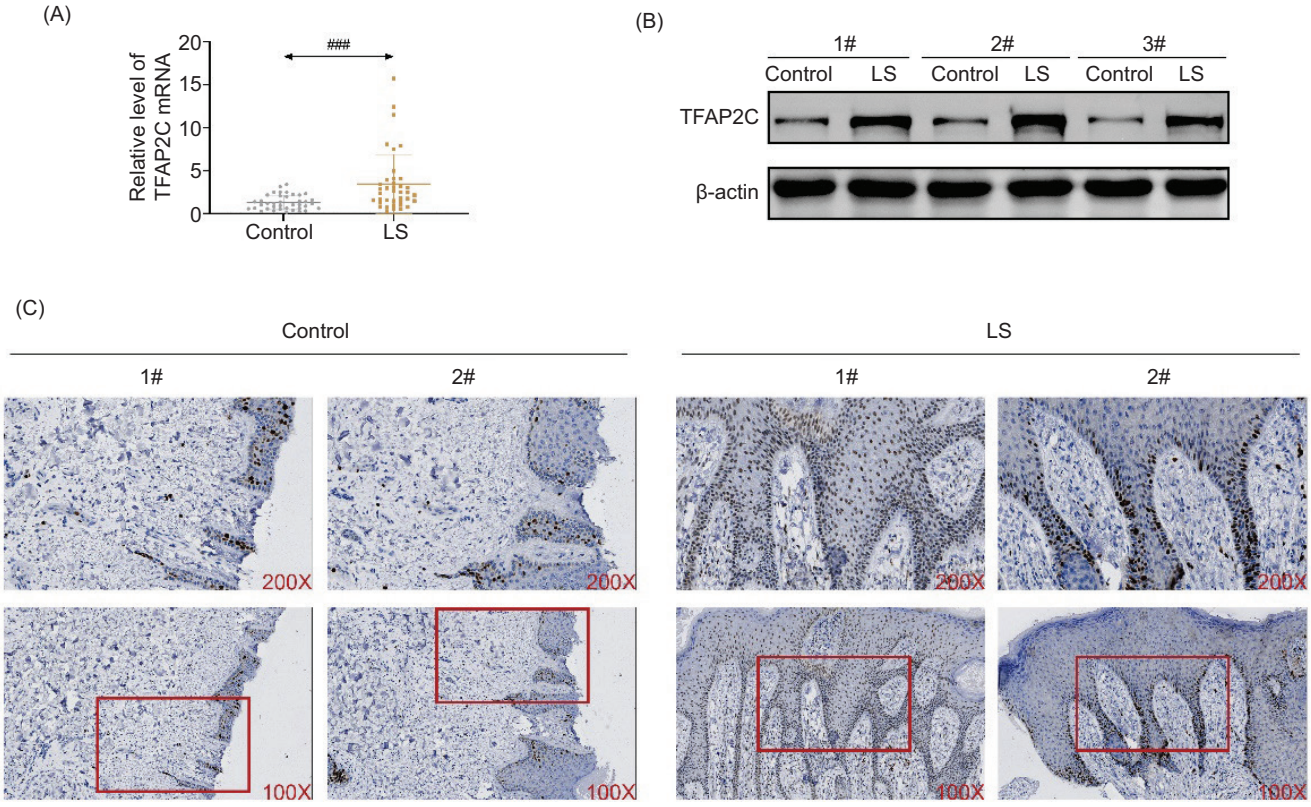

Figure 1 *TFAP2C* level was boosted in psoriasis patients. (A) RT-qPCR was employed to measure the expression of *TFAP2C* in skin tissues of psoriasis patients. (B) The protein level of *TFAP2C* in skin tissues of psoriasis patients was assessed via Western blot analysis. (C) IHC staining was applied to evaluate the expression of *TFAP2C*. ###P < 0.001 vs the control group.

expression was elevated in the IMQ-induced mice model (Figure 3B). Furthermore, we observed that *TFAP2C* was markedly decreased in the IMQ-induced mice model in the IMQ+*TFAP2C* knockout (KO) group (Figure 3C). H&E staining exposed that mice dealt with IMQ were associated with thicker epidermal layers than the control group, and *TFAP2C* depletion inversely changed these effects (Figure 3D). Moreover, the increased PASI score in the IMQ-induced mice model was inhibited after knockdown of *TFAP2C* (Figure 3E). In addition, the expression of KI67 was increased in the IMQ-induced mice model and decreased because of *TFAP2C* silencing (Figure 3F). Concisely, inhibition of TFAP2C eased IMQ-induced skin injury in mice.

Suppression of TFAP2C inhibited Th17 and Th1 cells activation in IMQ-induced mice model

In this study, we investigated whether *TFAP2C* was involved in the activation of Th17 and Th1 cells. IHC staining showed that CD4 expression was enhanced due to IMQ induction, while *TFAP2C* attenuation neutralized this effect (Figure 4A). The percentage of Th17 (CD4 + IL-17a+) and Th1 (CD4 + IFNγ+) was increased in the IMQ-induced mice model, but *TFAP2C* depression counteracted these effects; IMQ treatment decreased the percentage of Th2 (CD4 + IL-4+), but this effect was neutralized by *TFAP2C* downregulation (Figure 4B). The enhanced T-bet (Th1) and RORγt (Th17) protein expressions as well as decreased GATA3 (Th2) protein expression induced by IMQ were reversed by *TFAP2C* attenuation (Figure 4C). Taken together, suppression of *TFAP2C* inhibited the activation of Th17 and Th1 cells in the IMQ-induced mice model.

TFAP2C *promoted TEAD4 transcriptional activation*

In this study, the regulatory mechanism of *TFAP2C* was explored. As seen in Figure 5A, *TEAD4* level was enhanced in the LS of psoriasis patients. The expression of *TEAD4* was positively associated with *TFAP2C* (Figure 5B). *TFAP2C* level was dramatically decreased in *TFAP2C* KO group, and *TFAP2C* downregulation reduced the expression of *TEAD4* in mice model (Figures 5C and 5D). Besides, sh*TFAP2C* transfection evidently decreased the RNA and protein expressions of *TFAP2C* and *TEAD4* in HaCaT keratinocyte cells (Figures 5E and 5F). ChIP assay outlined that *TEAD4* expression was enriched in the *TFAP2C* group rather than IgG (Figure 5G), suggesting that *TFAP2C* could activate *TEAD4* transcription through binding its promoter region. Luciferase activity indicated that *TFAP2C* could bind to *TEAD4* promoter (Figure 5H). Our results further revealed that *TFAP2C* promoted *TEAD4* transcriptional activation.

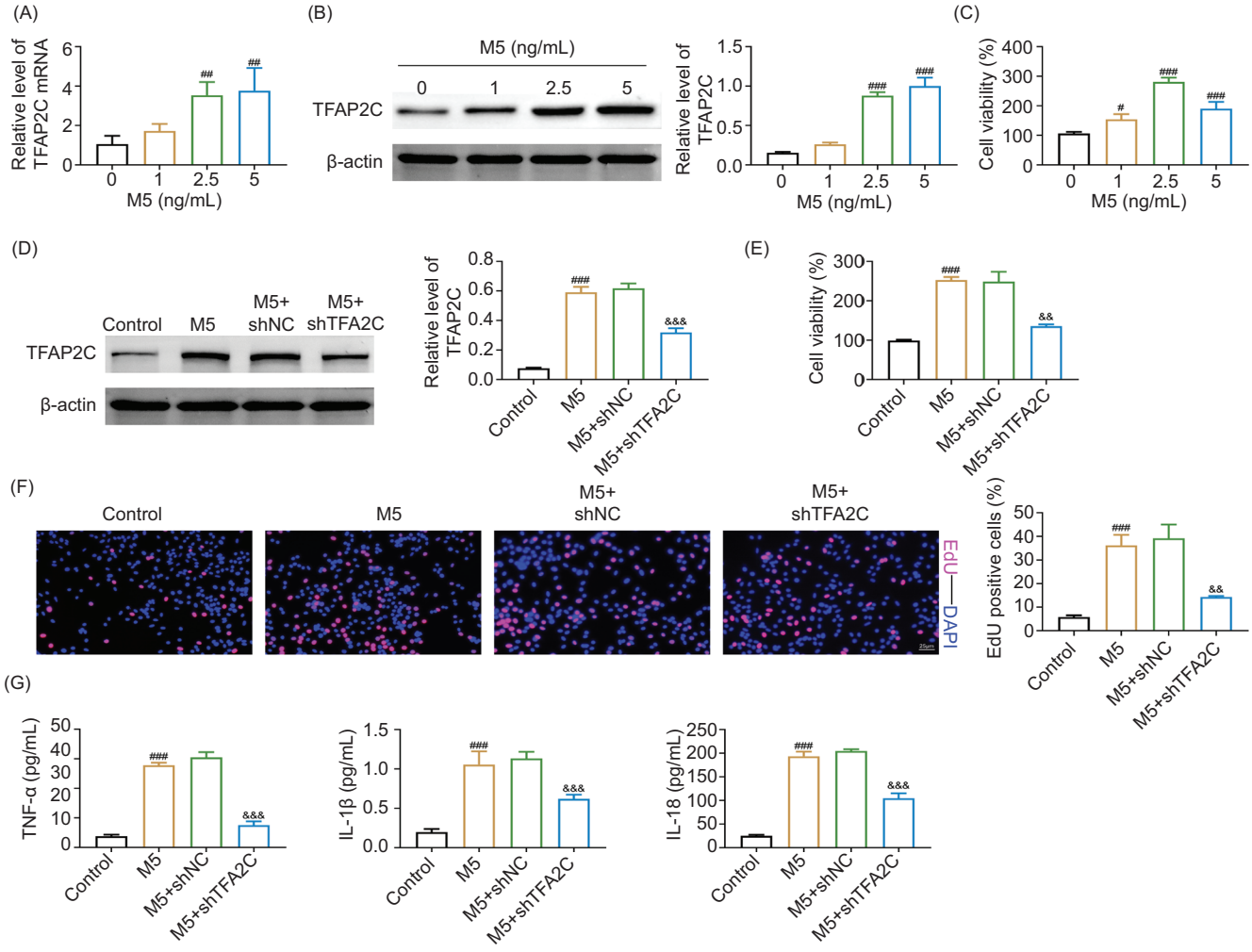

Figure 2 Silencing of *TFAP2C* suppressed proliferation and inflammatory response in M5-induced keratinocytes. (A) *TFAP2C* level in M5-induced keratinocytes was detected via RT-qPCR. ##P < 0.01 vs 0 ng/mL group. (B) The protein level of *TFAP2C* in M5-induced keratinocytes was measured by Western blot analysis. ###P < 0.001 vs 0-ng/mL group. (C) MTT assay evaluated the viability of keratinocytes. #P < 0.05, ###P < 0.001 vs 0-ng/mL group. (D) The protein level of *TFAP2C* in M5-induced keratinocytes was measured by Western blot analysis. ###P < 0.001 vs control group; &&&P < 0.001 vs M5+shNC group. (E) MTT assay was used to measure the viability of keratinocytes. ###P < 0.001 vs control group; &&P < 0.01, &&&P < 0.001 vs M5+shNC group. (F) EdU assay evaluated the proliferation of HaCaT keratinocytes. ###P < 0.001 vs control group; &&P < 0.01, &&&P < 0.001 vs M5+shNC group. (G) The concentrations of TNF-α, IL-1B, and IL-18 were evaluated by ELISA. ###P < 0.001 vs control group; &&&P < 0.001 vs M5+shNC group.

Discussion

Psoriasis is a common chronic inflammatory skin disease characterized by abnormal proliferation/differentiation of keratinocytes and excessive autoimmune response of the immune system accidentally attacking the body instead of protecting it.[26] Previous studies have proved that there is a strong evidence revealing that Th17 cells and Th17-related cytokines, such as IL-17A and IL-22, may be involved in the pathogenesis of psoriasis.[27] Psoriasis is a systemic inflammatory disease caused by the immune imbalance of T cells, resulting in excessive proliferation of keratinocytes and production of chemokines.[28,29] In some research studies, inhibition of IL-23 or IL-17A is reported to be implicated with psoriasis.[30,31] Similarly, Th1 cell activation is also validated to be associated with the pathogenesis of psoriasis.[32] Therefore, activated Th17 and Th1 cells can enhance the inflammatory response of keratinocytes in psoriasis. In the past, *TFAP2C* is verified to be implicated with the development of prostate cancer,[12] bladder cancer,[13] osteosarcoma,[14] NSCLC,[15] and breast cancer.[16] *TFAP2C* is also found to be related to the skin.[17-19] Nevertheless, whether *TFAP2C* modulated psoriasis-like inflammation through Th17 and Th1 cells activation remains unclear. In this study, *TFAP2C* expression was boosted in the LS tissues of psoriasis patients. In addition, silencing of *TFAP2C* suppressed proliferation and inflammatory response in M5-induced keratinocytes. In addition, we found that inhibition of *TFAP2C* alleviated IMQ-induced skin injury in mice model. More importantly, suppression of *TFAP2C* inhibited the activation of Th17 and Th1 cells in the IMQ-induced mouse model. In summary, *TFAP2C* exacerbated psoriasis-like inflammation by promoting Th17 and Th1 cells activation.

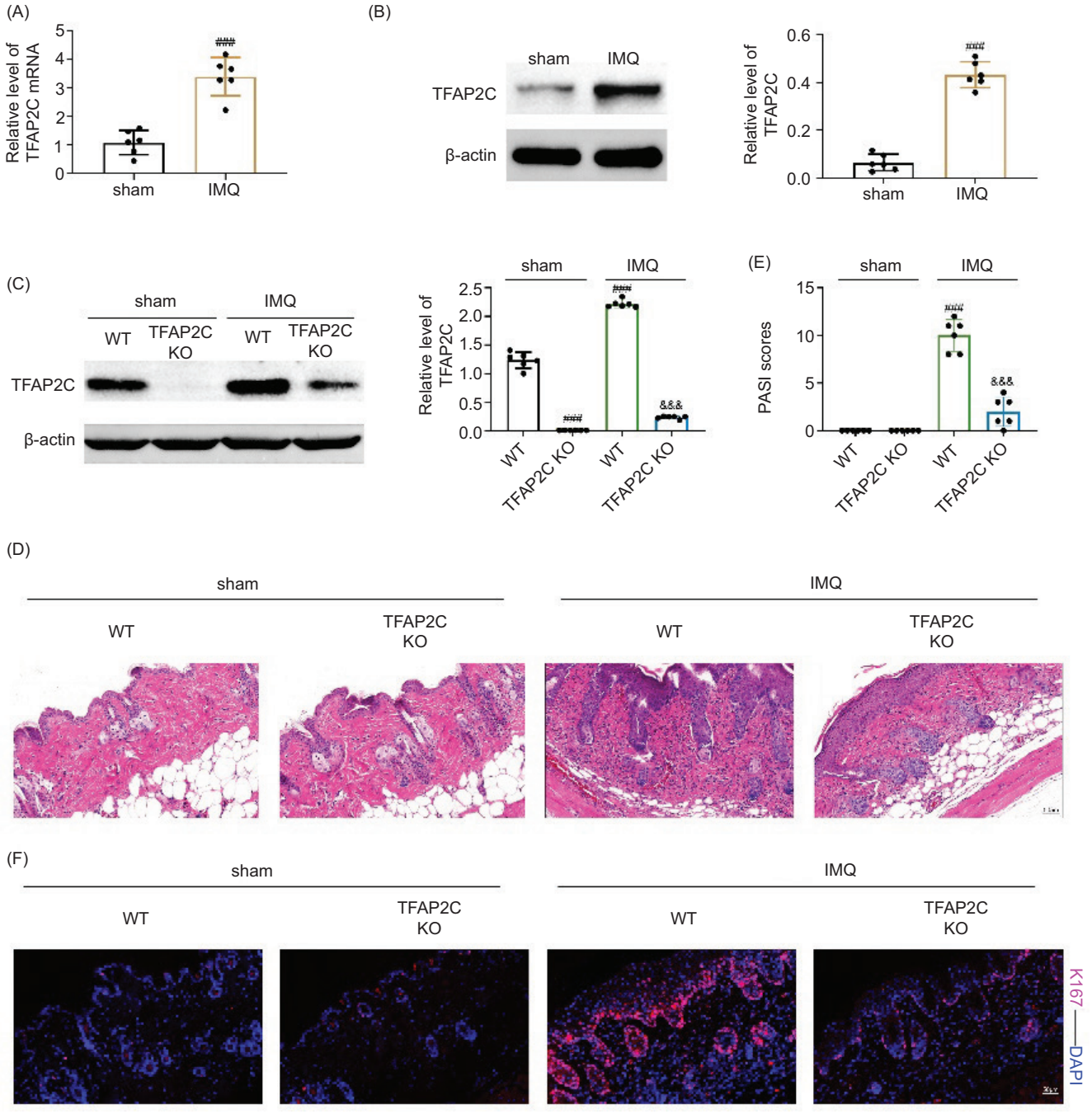

Figure 3 Inhibition of *TFAP2C* alleviated the IMQ-induced skin injury in mice. (A) RT-qPCR delineated *TFAP2C* expression in IMQ-induced mice. ###P < 0.001 vs sham group. (B) The *TFAP2C* protein level was evaluated in the IMQ-induced psoriasis-like mice model by Western blot analysis. ###P < 0.001 vs sham group. (C) The *TFAP2C* protein level was analyzed via Western blot analysis. ###P < 0.001 vs sham-WT group; &&&P < 0.001 vs IMQ-WT group. (D) H&E staining uncovered the histological features of skin tissues in the IMQ-induced mice model. (E) PASI score in the IMQ-induced mice model. ###P < 0.001 vs sham-WT group; &&&P < 0.001 vs IMQ-WT group. (F) IHC revealed the expression of KI67 in skin tissues in the IMQ-induced mice model.

TEAD4 factor plays a significant role in development and tissue regeneration and is reported as one of the transcriptional enhanced associate (TEA) domains containing transcription factors.[33] *TEAD4* contains a TEA DNA binding domain that binds the promoters of target genes near a hippo pathway effector YES-associated protein (YAP)-binding domain at C- and N-terminuses.[34,35] *TEAD4* is implicated with the expressions of several genes and modulated various biological and disease processes. For example, *TEAD4* is involved in the development of embryo.[36] It has been shown that *TEAD4* interacts with YAP to participate in the *CK2* gene-mediated cooperation of the *HHEX* (hematopoietically expressed homeobox) gene in colorectal tumorigenesis.[37] *TEAD4* is implicated with HSP110 in the hypoxia-induced pulmonary hypertension in a mice model.[38] MiR-4269- and miR-1343-3p-regulated *TEAD4*

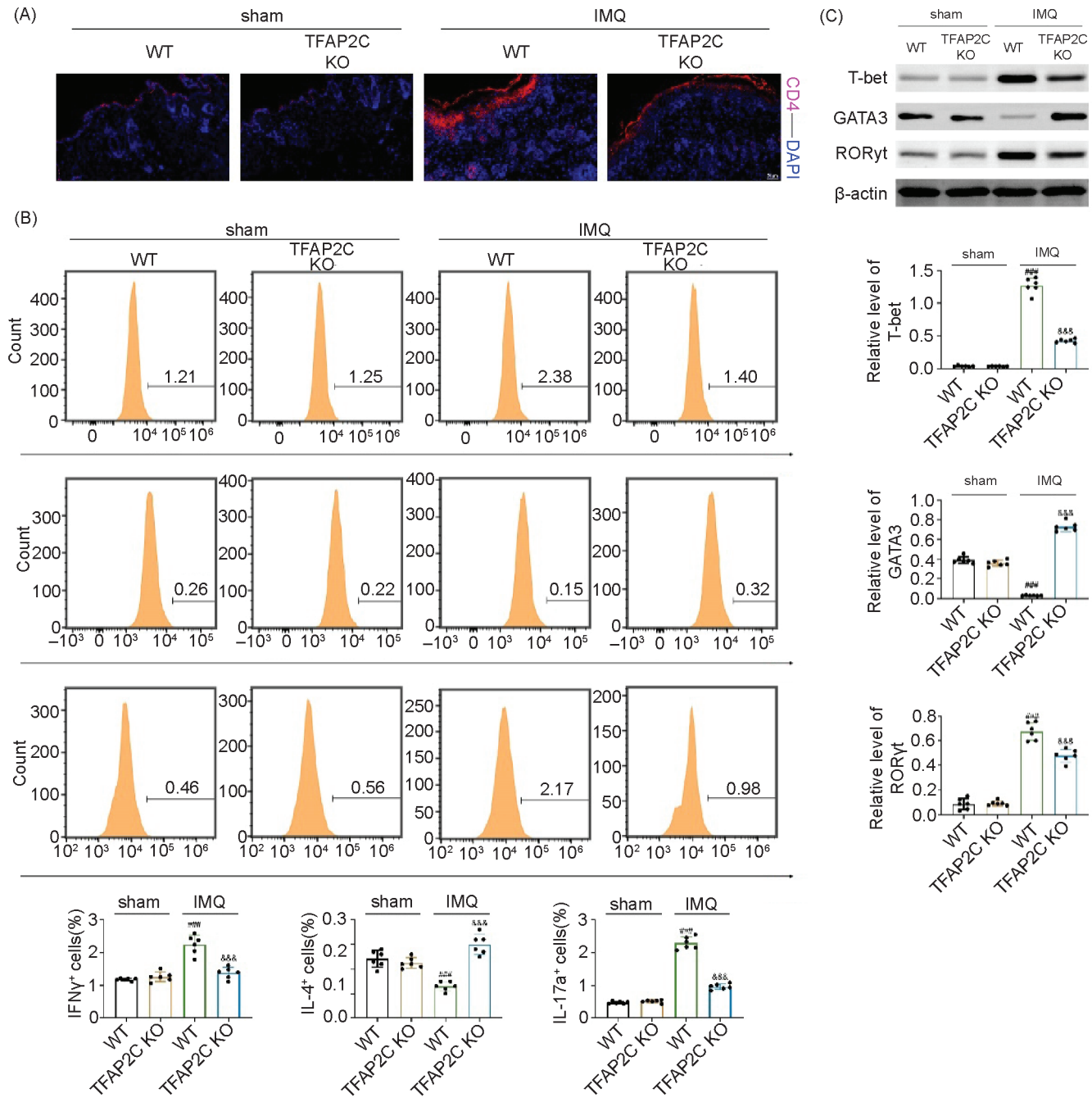

Figure 4 Suppression of *TFAP2C* inhibited the activation of Th17 and Th1 cells in IMQ-induced mice model. (A) IHC staining showed the expression of CD4 in the IMQ-induced mice model. (B) Flow cytometry analysis analyzed the percentage values of IL-17a, IFNγ, and IL-4+. $^{###}P < 0.001$ vs sham-WT group; $^{&&&}P < 0.001$ vs IMQ-WT group. (C) Western blot analysis showed the protein levels of T-bet, GATA3, and RORγt. $^{###}P < 0.001$ vs sham-WT group; $^{&&&}P < 0.001$ vs IMQ-WT group.

nuclear localization is involved in the development of colorectal carcinoma.[39] To the best of our knowledge, few studies have reported the role of *TEAD4* in psoriasis and its possible regulatory mechanism. In the present study, we reported that *TFAP2C* could bind to *TEAD4* transcriptional promoter, suggesting that *TFAP2C* promoted *TEAD4* transcriptional activation.

Conclusion

The present study initially identified that *TFAP2C* regulates *TEAD4* transcription to exacerbate psoriasis-like inflammation by promoting activation of Th17 and Th1 cells. Nevertheless, some limitations existed in this work, such as lack of other phenotypes (autophagy, oxidative stress, and

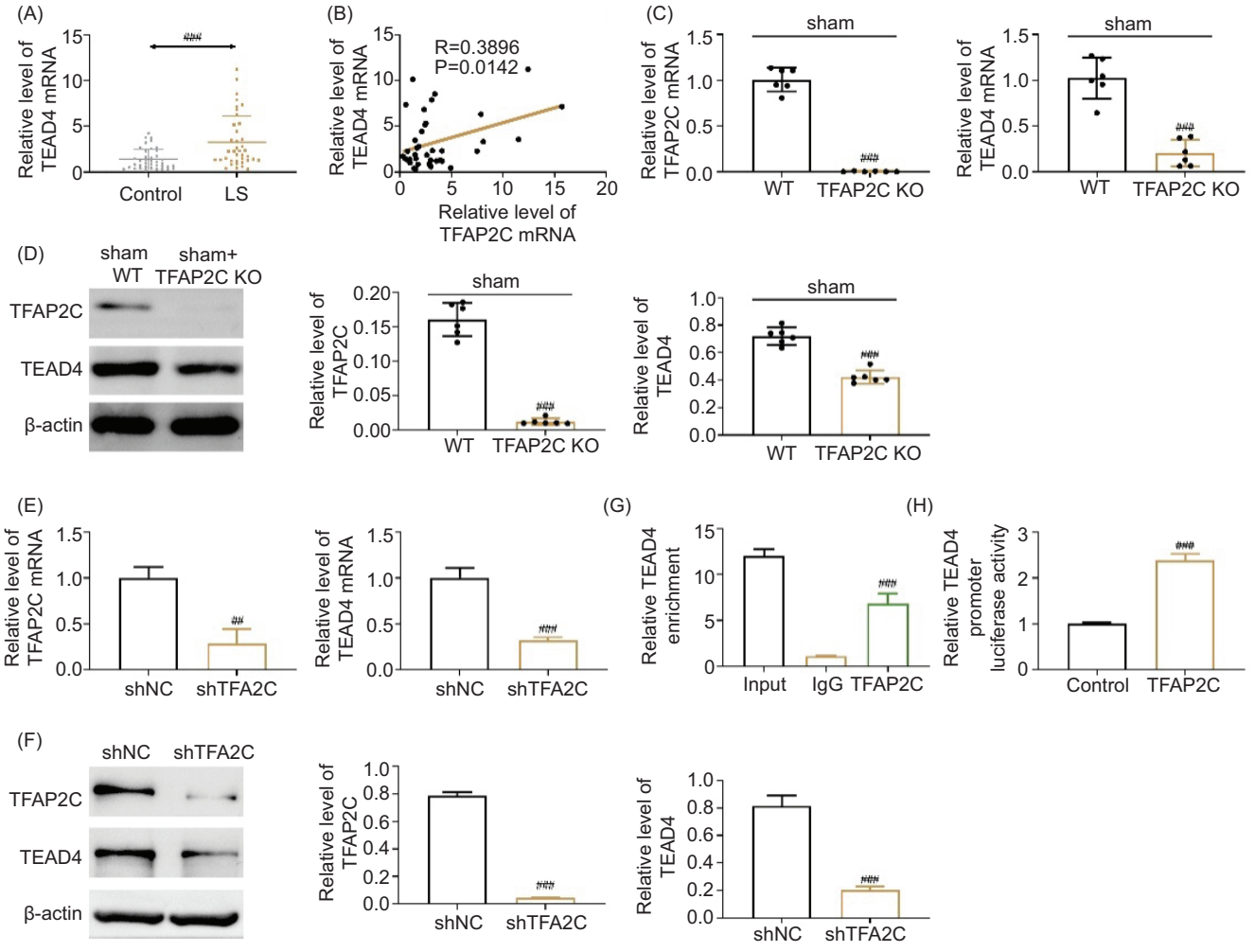

Figure 5 *TFAP2C* promoted *TEAD4* transcriptional activation. (A) RT-qPCR evaluated the *TEAD4* expression. ###P < 0.001 vs control group. (B) Association between *TFAP2C* and *TEAD4* was analyzed by Pearson's correlation analysis. (C and D) RT-qPCR and Western blot analysis were employed for evaluating the expression of *TFAP2C* and *TEAD4* in mice. ###P < 0.001 vs sham-WT group. (E and F) The expression of *TFAP2C* and *TEAD4* in HaCaT keratinocytes was measured by RT-qPCR and Western blot analysis. ##P < 0.01, ###P < 0.001 vs shNC group. (G and H) ChIP and luciferase reporter assays were applied to evaluate the binding ability of *TFAP2C* and *TEAD4* promoter. ###P < 0.001 vs IgG group.

M1/M2 polarization). The findings could highlight the function of *TFAP2C* in the prevention and treatment for psoriasis in the future studies.

Funding

This work was supported by the Precision Medicine Joint Fund Cultivation Project of Hebei Natural Science Foundation (Grant No. H2021206053).

Availability of Data

All data generated or analyzed in this study are included in the published article. The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Conflict of interest

The authors stated that there were no conflicts of interest to disclose.

Statement of Informed Consent

Written informed consent was obtained from legally authorized representative(s) for anonymized patient information to be published in this study.

Authors' Contributions

Huanhuan Zhang did conceptualization, methodology, and writing of original draft. Cuimin Ren carried out formal analysis, resources, and investigation. Qiang Liu did formal

analysis, visualization, and data curation. Qing Wang did project administration, supervision, and validation. Dahu Wang carried out validation, supervision, and writing, including review and editing, of the manuscript. All authors read and approved the final manuscript.

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