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IL-17A and TNF-α-induced Dectin-1 expression may promote keratinocyte proliferation in psoriatic lesions

Background: Psoriasis is a common skin disease with a high recurrence rate. Aberrant keratinocyte proliferation is a significant pathogenic characteristic of psoriatic lesions, and studies have revealed that the development of psoriasis is significantly influenced by pro-inflammatory cytokines, such as IL-17A and TNF-α. Biologics targeting these cytokines have been widely used in psoriasis treatment and achieve remarkable effects, however, the underlying mechanism of how IL-17A and TNF-α specifically regulate keratinocyte proliferation has not been fully elucidated. Dectin-1 is an essential membrane protein that is directly related to the immune microenvironment and the proliferation of multiple cell types. *Objectives*: To elucidate how IL-17A and TNF-α may promote keratinocyte proliferation in psoriatic lesions and whether Dectin-1 is involved. *Materials & Methods:* The expression of Dectin-1 in keratinocytes from psoriatic lesions was detected by real-time PCR, western blot and immunofluorescence. Correlation analysis and cytological experiments were then performed to determine the relationship between Dectin-1 and IL-17A/TNF-α in psoriatic lesions. Finally, we investigated the signalling pathway through which Dectin-1 may promote keratinocyte proliferation. *Results:* Dectin-1 was significantly increased in keratinocytes from psoriatic lesions. Moreover, IL-17A and TNF-α effectively induced the expression of Dectin-1 in HaCaT cells, which was shown to activate the Syk/NF-κB signalling pathway and promote the proliferation of keratinocytes. *Conclusion:* IL-17A and TNF-α may promote the proliferation of keratinocytes in psoriatic lesions through induction of Dectin-1, indicating that Dectin-1 could be a potential therapeutic target for the treatment of psoriasis.

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Key words: psoriasis, keratinocyte, IL-17A, TNF-α, Dectin-1

Psoriasis is a common skin disease with typical clinical manifestations of scaly erythema, papules and plaques formed by abnormal proliferation and differentiation of keratinocytes [1]. To date, psoriasis remains associated with a high prevalence of morbidity and recurrence, and current clinical treatments are almost ineffective in preventing its recurrence, placing a heavy burden on patients [2, 3]. Therefore, it is necessary to further explore the pathogenesis of psoriasis and develop novel therapeutic targets.

Reports have indicated that a variety of immune cells, such as Th1 and Th17, are significantly increased in psoriatic lesions, resulting in elevated levels of local pro-inflammatory cytokines, such as IL-17A and TNF-α [4]. Moreover, IL-17A and TNF-α have been shown to play important roles in the pathogenesis of psoriasis [5, 6]. To date, a variety of biologics targeting these cytokines have been widely used in clinical practice, and have achieved excellent efficacy [7, 8]. However, these biologics still cannot completely prevent the recurrence of psoriasis, and some patients remain insensitive to these biologics [9, 10]. Therefore, further studies are required in order to elucidate the specific regulatory effect of IL-17A and TNF-α on the development of psoriasis, especially regarding their impact on the proliferation of keratinocytes.

Dectin-1, also called human C-type lectin domain family 7 member A (CLEC7A), is a widely expressed small type II membrane receptor glycoprotein [11, 12]. Dectin-1 is composed of an extracellular C-type lectin-like domain and a cytoplasmic domain with an immunoreceptor tyrosine-based activation motif [13, 14]. Dectin-1 acts as one of the pattern recognition receptors (PRRs) that can recognize multiple kinds of β-1,3-linked and β-1,6-linked glucans from fungi or plants, and therefore affect the innate immune response [15-17]. Research on skin wounds has shown that Dectin-1 activation can induce keratinocytes to proliferate, migrate, and promote wound re-epithelialization. This effect may be related to Dectin-1 sensing early damage signals, which can induce chemokine production and early neutrophil aggregation [18, 19]. To sum up, these findings suggest that Dectin-1

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might play an important role in the local immune microenvironment of psoriatic lesions and influence the proliferation of keratinocytes.
In this study, we found that Dectin-1 is significantly increased in keratinocytes from psoriatic lesions. Further study revealed that IL-17A and TNF-α can effectively induce the expression of Dectin-1 in HaCaT cells, which was shown to effectively promote proliferation. Mechanistically, enhanced Dectin-1 may boost the proliferation of keratinocytes via activation of the Syk/NF-κB signalling pathway.

Materials and methods

Patients and normal donor specimens

The diagnosis of psoriasis was based on the typical clinical manifestations and pathological examination in the Department of Dermatology, Tongji Hospital (Wuhan, China). All human tissue specimens were obtained from the Pathology Research Unit of the Department of Dermatology, Tongji Hospital, and were collected with the consent of patients and healthy donors.

Animal experiments

Eight-week-old female BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China) and were individually housed in a specific pathogen-free barrier facility. The mice received daily topical doses of 62.5 mg IMQ cream (Mingxin Pharmaceuticals, Sichuan, China) or Vaseline on shaved backs for seven consecutive days. Disease severity was assessed using a scoring system based on the clinical Psoriasis Area and Severity Index (PASI). More specifically, erythema, scaling, and thickening were scored independently on a scale from 0 to 4 (0 = none, 1 = slight, 2 = moderate, 3 = marker, 4 = very marked), and the cumulative score was used as a total score (scale: 0-12). Disease severity was assessed by two experienced researchers in a blinded manner. On day 8, serum samples and skin tissues were collected from the sacrificed mice for subsequent studies.

Cell lines

Human immortal keratinocyte HaCaT cells were purchased from the China Center for Type Culture Collection (CCTCC, Wuhan, China) and were cultured according to the instructions. Before the trial began, HaCaT cells were checked for mycoplasma, interspecies cross-contamination, and authenticity using short tandem repeat profiling and isoenzyme analysis at the CCTCC. HaCaT cells were cultured for a maximum of 15 passages.

Haematoxylin and eosin (H&E) staining

The skin tissues of mice were embedded in paraffin wax after being fixed in 4% paraformaldehyde solution. Following serial sectioning and haematoxylin and eosin (H&E) (Baso, Zhuhai, China) staining, these tissues were evaluated histologically. Epidermal thickness was measured using Image-pro Plus 6.0 software. Three photographs were taken randomly for each section under high magnification, and for each field of view, at least three measurements were taken.

RNA interference

siRNAs targeting the genes of interest and a negative control siRNA were purchased from RiboBio (Guangzhou, China). Details of the gene sequences are shown in *supplementary table 1*. Cells were transfected with siRNAs using Lipofectamine 2000 (Invitrogen, USA). The effect of silencing was verified by real-time PCR and western blot.

Cell counting

Different groups of HaCaT cells were inoculated in equal amounts in six-well plates, in two replicate wells. Each cell group was counted with a haematocyte counter after 24 h, 48 h and 72 h, respectively. Each well was counted three times using a manual counter, and the average value was taken for analysis.

Cell Counting Kit 8 (CCK-8) assay

Different groups of HaCaT cells were cultured at a density of 1×10^3 cells/well in 96-well plates at 37°C. After incubation for indicated time points, 10 μL of CCK-8 solution (DOJINDO, Japan) was added to each well, and cultured at 37°C with light protection for two hours. Optical density (OD) values were then recorded at 450 nm using an enzyme-labelled instrument.

Detection of cell apoptosis

Apoptosis of different HaCaT cell groups was detected using the Annexin-V/PI Apoptosis Detection Kit (KeyGen Biotech, China) according to the manufacturer's protocol and then analysed using an Accuri C6 flow cytometer (BD Biosciences, USA).

Real-time PCR

Total RNA of sample cells was extracted using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocols. Real-time PCR analysis was performed using the SYBR Green PCR mix (Toyobo, Japan) on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Canada). Comparative quantitative mRNA levels of cells were normalized to the housekeeping gene, β-actin. Primers for real-time PCR were synthesized by Tsingke Biotechnology (Beijing, China). The primer sequences are shown in *supplementary table 2*.

Immunofluorescence

Wax blocks of tissue specimens were prepared as outlined above and serial sectioning was performed. Before staining, the sections were subjected to antigen retrieval in sodium citrate buffer (0.01 M, pH 6.0) at 100°C for 15 minutes and incubated with 10% normal goat serum

for one hour, followed by incubation overnight at 4°C with a target protein antibody. Anti-CLEC7A was purchased from ABclonal (Wuhan, China), and Alexa Fluor® 488-conjugated goat anti-rabbit IgG (Servicebio, Wuhan, China) was used as a secondary antibody. After staining the nuclei with DAPI for 15 minutes, the sections were prepared to observe immunofluorescence using an OLYMPUS BX51 fluorescence microscope.

Western blot analysis

Cell samples were lysed using a combination of NP40, a protease inhibitor, and a phosphatase inhibitor. Antibodies for western blot analysis included: anti-β-actin (ABclonal), anti-CLEC7A (ABclonal), anti-NF-κB p65 (Abmart, Beijing, China), anti-NF-κB Pho-p65 (Abmart), anti-Syk (Cell Signal Technology, USA), and anti-Pho-Syk (Cell Signal Technology) antibodies at the recommended dilutions.

Statistical analysis

Datasets from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) were analysed using R software (v.4.0.1). All experimental data were analysed by t-tests using GraphPad Prism 9.0 software, and then expressed as mean ± SEM. Differences were regarded as statistically significant at $p<0.05$.

Results

WGCNA analysis of psoriasis-related datasets from the GEO database

Previous studies have found that, compared to the normal epidermis, Dectin-1 expression is increased in psoriatic lesions [20, 21]. To more precisely investigate the expression of Dectin-1 in psoriatic skin lesions, we first searched for psoriasis-related sequences in the GEO database and integrated the datasets based on the GPL570 platform (GSE13355, GSE14905 and GSE78097). The expression profiles with complete follow-up information were normalized (*supplementary figure 1A, B*). The expression density plot also revealed that the batch effect of the GPL570 meta-cohort had been clearly removed (*supplementary figure 1C, D*). Finally, the Uniform Manifold Approximation and Projection (UMAP) analysis showed the distribution of each dataset before and after removal of batch effect (*supplementary figure 1E, F*). WGCNA was performed using the expression profiles in the GPL570 meta-cohort. The soft threshold power in the GPL570 meta-cohort was 7 (*figure 1A, B*). Subsequently, dynamic module identification was performed for the different cohorts, with the number of genes per module no lower than 30 (*figure 1C*). For the GPL570 meta-cohort, 18 co-expression modules were clustered, with the black module having the strongest positive correlation with psoriasis clinical traits (*figure 1D*). In the black module, positive correlations were observed between module membership (MM) and gene significance (GS) (*figure 1E*). In total, 102 genes in the black module were screened as potential psoriasis-related genes using MM > 0.9 and GS > 0.8 as thresholds. Based on limma analysis, we obtained 720 differentially expressed genes (DEGs) including 467 up-regulated DEGs and 253 down-regulated DEGs, which were partially shown on the heatmap (*supplementary figure 2*). Finally, based on the intersection of 467 up-regulated DEGs with 102 hub genes in the black gene module and protein-protein interaction (PPI) network analysis (*supplementary figure 3*), we identified Dectin-1 for further study on the basis that it may play an important role in the pathogenesis of psoriasis.

Dectin-1 expression in keratinocytes from psoriatic lesions

We first analysed the expression of Dectin-1 in the GPL570 meta-cohort and found that Dectin-1 was significantly increased in psoriatic lesions (*figure 2A*). We then analysed the expression of Dectin-1 in GSE78097 and found that Dectin-1 was significantly upregulated in mild and severe plaque psoriatic lesions, but there was no significant difference between mild and severe plaque psoriatic lesions (*figure 2A*). Moreover, Dectin-1 was also upregulated in palmoplantar pustular psoriasis (PPPP) compared with healthy palm skin (*figure 2A*). The expression of Dectin-1 in psoriatic lesions was significantly inhibited by anti-IL-17A (secukinumab) treatment in GSE137218 or etanercept treatment in GSE106992 (*figure 2B*). We then performed real-time PCR to verify the expression of Dectin-1. As expected, markedly higher levels of Dectin-1 were observed in psoriatic lesions compared to healthy skin (*figure 2C*). Subsequent immunofluorescence analysis showed that the expression of Dectin-1 in keratinocytes from psoriatic lesions was noticeably higher than that in healthy skin tissue (*figure 2D*).

To further confirm this finding, we used imiquimod (IMQ) to construct a psoriasis-like dermatitis mouse model, and Vaseline was used as a control (*figure 3A*). Epidermal thickness and PASI score of both groups were then evaluated (*figure 3B, C*). Western blot analysis revealed that the expression of Dectin-1 was apparently upregulated in the psoriasis-like lesions of the IMQ mouse model compared to the control group (*figure 3D*). Immunofluorescence analysis showed the same results (*figure 3E*). Furthermore, the mRNA expression level of Dectin-1 was also significantly higher in the psoriasis-like lesions of the IMQ mouse model compared to the control group (*figure 3F*). Collectively, these studies demonstrated that the expression level of Dectin-1 was significantly increased in lesions from either psoriasis patients or the mouse model.

Effect of IL-17A and TNF-α on Dectin-1 expression in keratinocytes

Previous reports have indicated that numerous pro-inflammatory cytokines play important roles in the pathogenesis of psoriasis [22, 23]. Pro-inflammatory cytokines, such as IL-17A and TNF-α, can effectively induce abnormal hyperactivity of the local immune response, which in turn induces abnormal proliferation and differentiation of keratinocytes [1, 24]. However, how IL-17A and TNF-α specifically

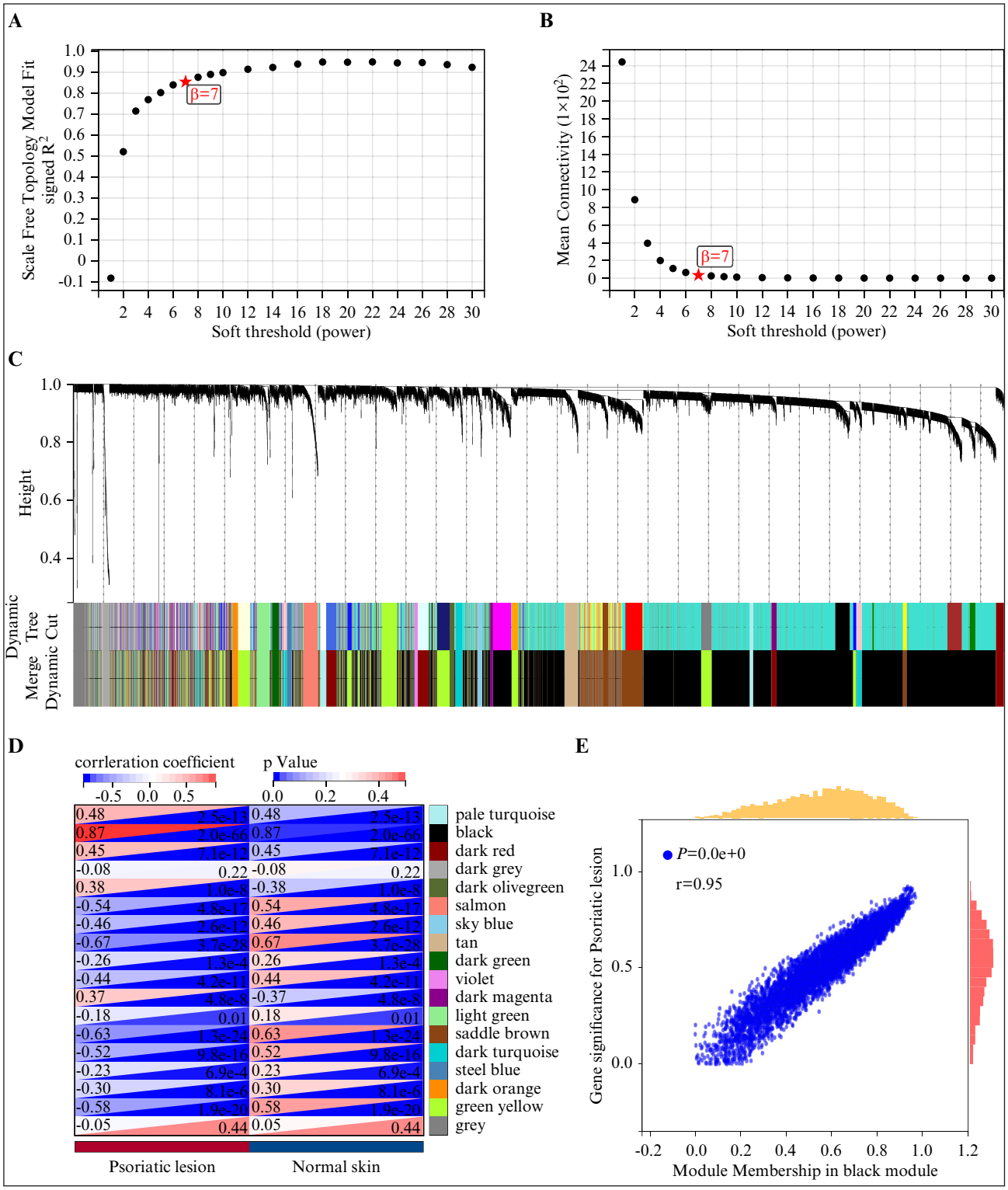

Figure 1. Bioinformatics analysis of important molecules in keratinocytes from psoriatic skin lesions. **A)** Scale independence in the GPL570 meta-cohort. **B)** Mean connectivity in the GPL570 meta-cohort. **C)** Gene dendrogram and modules after merging in the GPL570 meta-cohort. **D)** Pearson correlation analysis of merged modules and clinical traits in the GPL570 meta-cohort. **E)** Scatterplot of MM and GS from the black module in the GPL570 meta-cohort.

regulate the proliferation of keratinocytes in psoriatic lesions has not been fully elucidated.

As mentioned above, the expression of Dectin-1 was clearly shown to be increased in keratinocytes from psoriatic lesions. To further investigate the relationship between the expression levels of IL-17A/TNF-α and Dectin-1, we first performed a correlation analysis based on the GPL570 meta-cohort. A positive correlation was

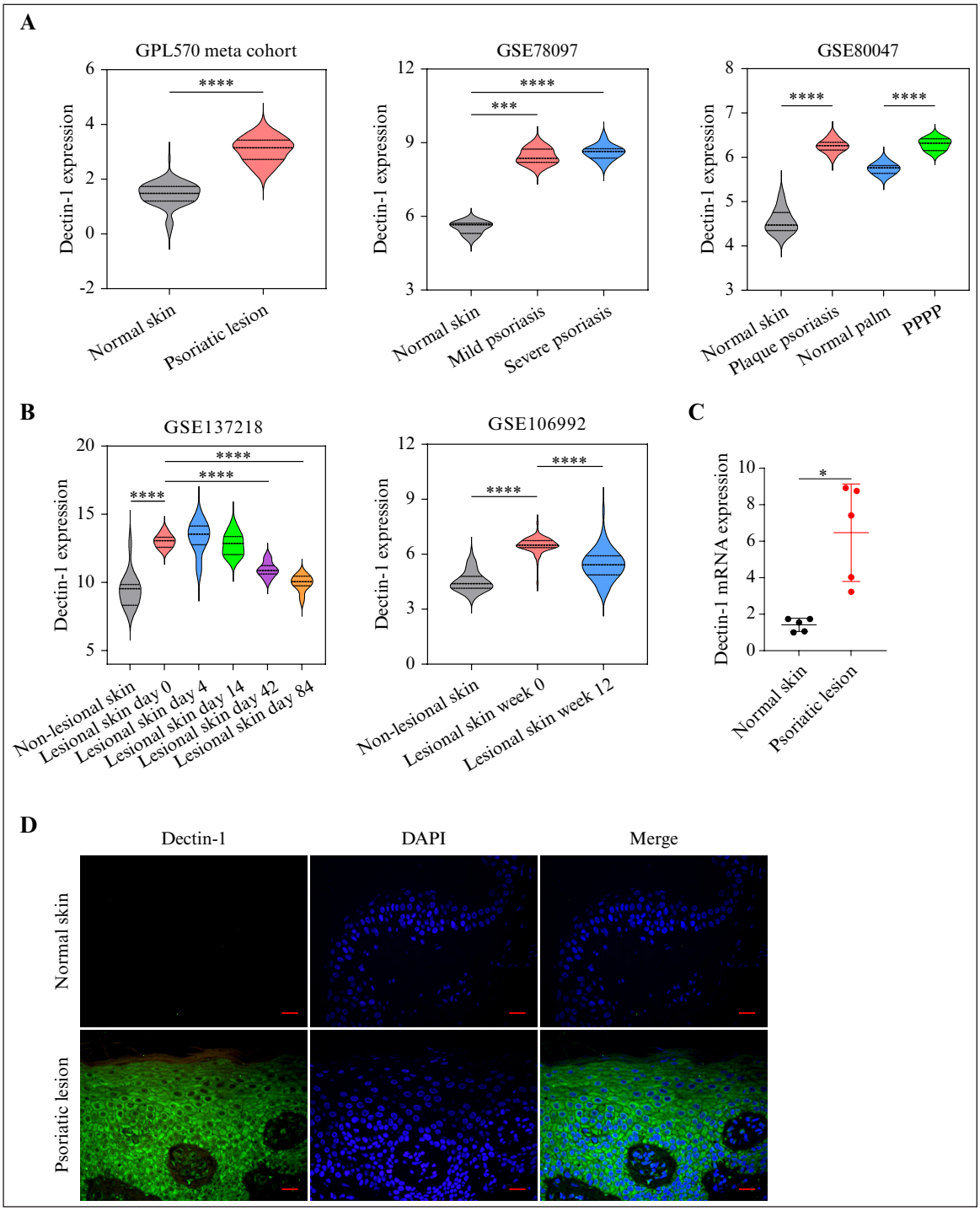

Figure 2. The expression of Dectin-1 is significantly increased in keratinocytes from psoriatic skin lesions**. A)** mRNA expression of Dectin-1 in the GPL570 meta-cohort, GSE78097 and GSE80047. **B)** mRNA expression of Dectin-1 after anti-IL-17A (secukinumab) treatment in GSE137218 and etanercept treatment in GSE106992. **C)** Comparison of mRNA expression of Dectin-1 in keratinocytes isolated from psoriatic lesions. **D)** Immunofluorescence analysis of Dectin-1 in psoriatic lesions and normal skin. Bars indicate 50 µm. Data are expressed as means ± SEM. $*p<0.05$, $***p<0.001$, $****p<0.0001$.

demonstrated between the level of Dectin-1 (CLEC7A) and IL-17A (*figure 4A*), and Dectin-1 (CLEC7A) expression also positively correlated with TNF-α (*figure 4B*). Following this, we stimulated HaCaT cells with IL-17A or TNF-α cytokine, revealing that this stimulation boosted the expression of Dectin-1(*figure 4C-F*). These findings demonstrated that IL-17A and TNF-α could effectively induce Dectin-1 expression in keratinocytes from psoriatic lesions, suggesting that Dectin-1 might be a crucial element through which IL-17A and TNF-α affect keratinocytes. A number of reports have indicated that the activation and effect of Dectin-1 is dependent

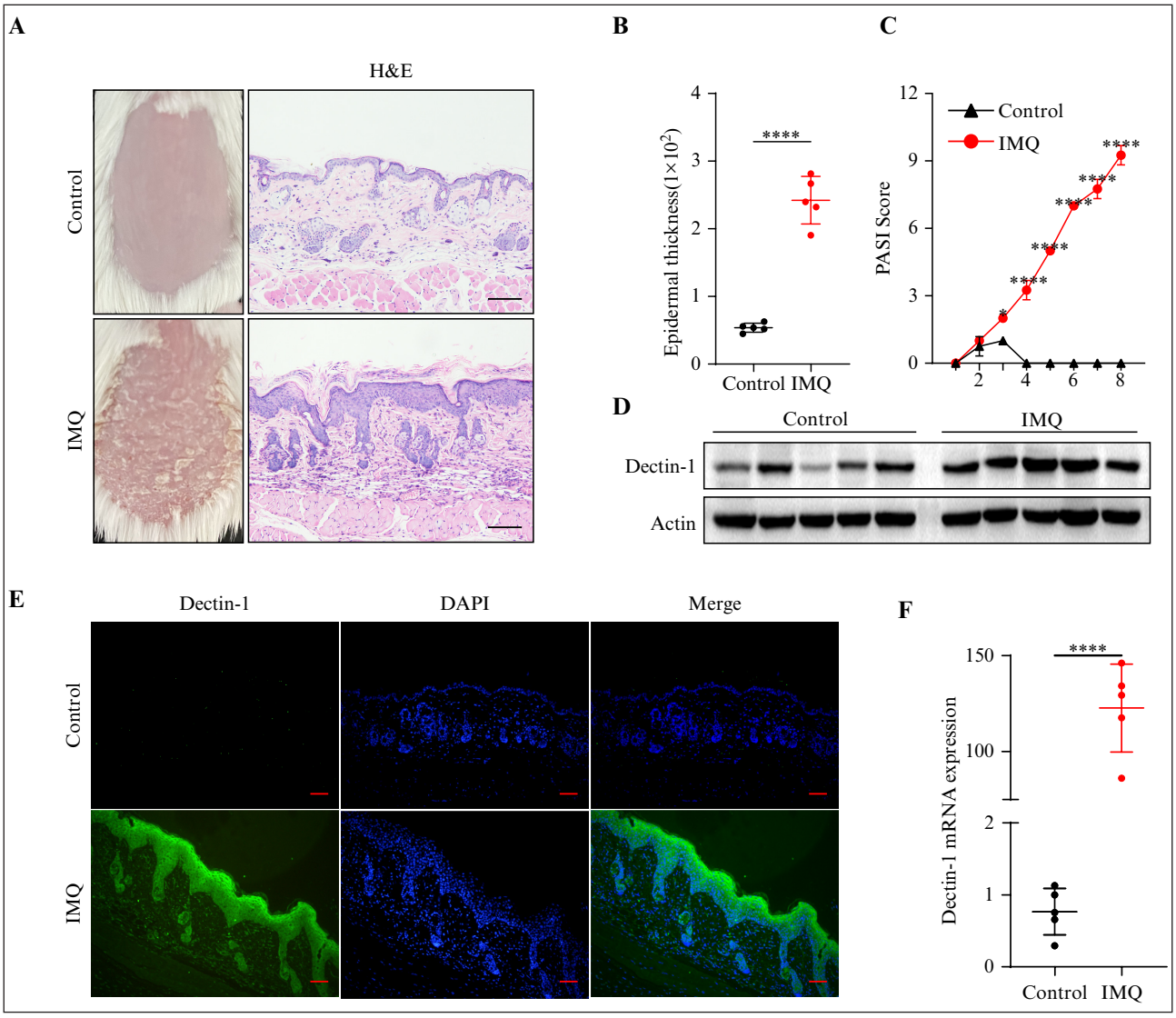

Figure 3. Dectin-1 is significantly increased in keratinocytes from mouse psoriatic skin lesions. **A-C)** Phenotypic presentation and H&E staining (for H&E staining, bars indicate 100 µm) **(A)**, epidermal thickness **(B)** and PASI score **(C)** of the control and IMQ group. **D)** Protein levels of Dectin-1 in the control and IMQ group. **E)** Immunofluorescence analysis of Dectin-1 in the control and IMQ group (bars indicate 50 µm). **F)** Comparison of mRNA expression of Dectin-1 in keratinocytes isolated from both groups. Data are expressed as means ± SEM. $*p<0.05$, $***p<0.001$, $****p<0.0001$.

on the presence of its endogenous ligands [13]. According to several bioinformatics analyses, we found that the expression of endogenous ligands, such as Galectin-9 and Annexin A13, was upregulated in psoriatic lesions based on the GPL570 meta-cohort (*figure 4G, H*). To further investigate the relationship between the expression levels of IL-17A/TNF-α and Galectin-9/Annexin A13, we performed a correlation analysis based on the GPL570 meta-cohort. The level of Galectin-9 showed a positive correlation with that of TNF-α (*figure 4I*), suggesting that the endogenous ligands might be involved in the regulatory effect of TNF-α on Dectin-1. However, the expression level of Annexin A13 did not significantly correlate with that of IL-17A and TNF-α (*figure 4J*), indicating that other intermediate molecules, such as important transcription factors, are also involved in IL-17A/TNF-α-mediated Dectin-1 expression, and further research is required to elucidate this.

Effect of IL-17A and TNF-α-induced Dectin-1 on keratinocyte proliferation

Pro-inflammatory cytokines, IL-17A and TNF-α, are reported to effectively promote the proliferation of keratinocytes in psoriatic lesions [25-27]. However, our cytological study found that while IL-17A indeed significantly promoted the proliferation of HaCaT keratinocytes, TNF-α did not exhibit a significant pro-proliferative effect (*figure 5A, B*). We speculated that this phenomenon might be related to the fact that TNF-α could also promote apoptosis of keratinocytes [28, 29], which we demonstrated to be the case (*figure 5C, D*). Our data

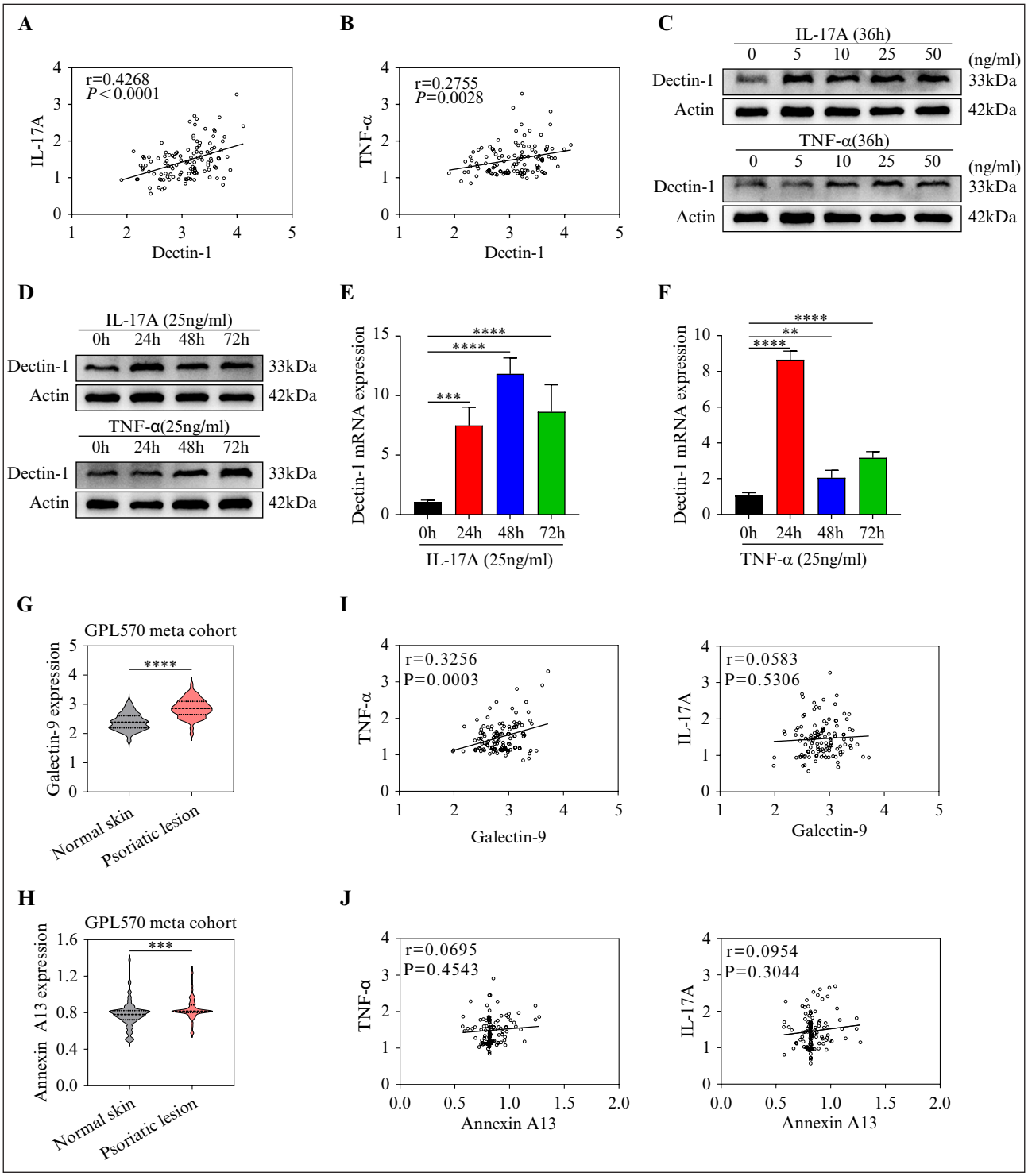

Figure 4. IL-17A and TNF-α induce the expression of Dectin-1 in HaCaT keratinocytes. **A, B)** Relationship between the expression level of Dectin-1 and IL-17A **(A)** or TNF-α **(B)** of the combined dataset using Pearson correlation. **C)** Protein levels of Dectin-1 in HaCaT cells upon treating with IL-17A or TNF-α were detected at different concentration. **D)** Protein level of Dectin-1 in HaCaT cells upon treating with 25 ng/mL IL-17A or TNF-α at indicated time points. **E)** mRNA expression level of Dectin-1 in HaCaT cells upon treating with 25 ng/mL IL-17A at indicated time points. **F)** mRNA expression level of Dectin-1 in HaCaT cells upon treating with 25 ng/mL TNF-α at different time points. **G)** mRNA expression level of Galectin-9 in the GPL570 meta-cohort. **H)** mRNA expression level of Annexin A13 in the GPL570 meta-cohort. **I)** Relationship between the expression level of Galectin-9 and TNF-α or IL-17A of the combined dataset using the Pearson correlation. **J)** Relationship between the expression level of Annexin A13 and TNF-α or IL-17A of the combined dataset using the Pearson correlation. Data are expressed as means ± SEM. ***p*<0.01, ****p*<0.001, *****p*<0.0001.

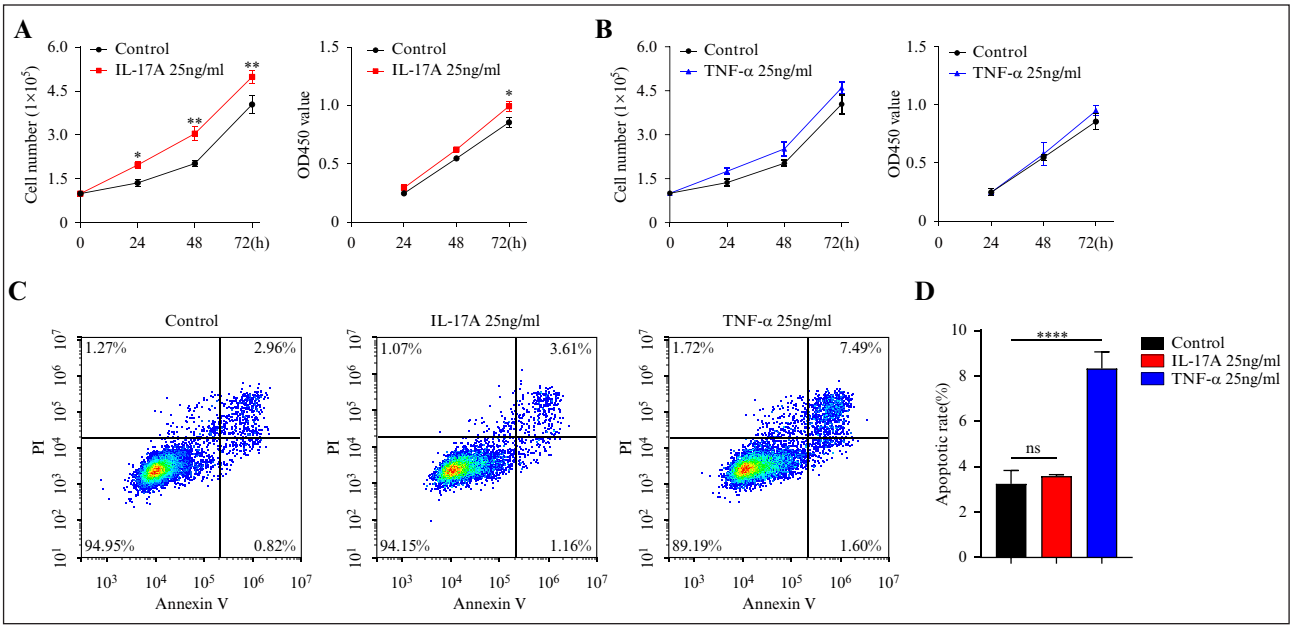

Figure 5. IL-17A and TNF-α promote the proliferation of HaCaT keratinocytes. **A**) Direct cell count and CCK-8 assay of HaCaT cells upon treating with 25 ng/mL IL-17A at indicated time points. **B)** Direct cell count and CCK-8 assay of HaCaT cells upon treating with 25 ng/mL TNF-α at different time points. **C, D)** Flow cytometry to detect apoptosis of HaCaT cells after treatment with 25n g/mL IL-17A or TNF-α for 24 hours. Data are expressed as means ± SEM. $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$, ns : not significant.

therefore indicate that IL-17A and TNF-α effectively induce the expression of Dectin-1 in keratinocytes. To further confirm the effect of Dectin-1 on the proliferation of keratinocytes, we silenced the expression of Dectin-1 in HaCaT cells using siRNAs (*figure 6A*) and then analysed cell proliferation *in vitro.* The results showed that after Dectin-1 silencing, there was no significant change in apoptosis of HaCaT cells (*figure 6B, C*), however, proliferation of HaCaT cells significantly decreased (*figure 6D, E*). The above results demonstrate that increased Dectin-1 induced by IL-17A and TNF-α could effectively promote the proliferation of keratinocytes.

Effect of Dectin-1 expression on activation of the Syk/NF-κB signalling pathway

The aforementioned investigations reveal that increased Dectin-1, induced by IL-17A and TNF-α, promotes the proliferation of keratinocytes, however, the exact mechanism remains unknown. To further explore how Dectin-1 may promote the proliferation of keratinocytes, we silenced the expression of Dectin-1 in HaCaT cells using siRNAs, and investigated activation of the Syk/NF-κB signalling pathway. Upon Dectin-1 silencing, activation of the Syk/NF-κB signalling pathway was considerably reduced (*figure 7A, B*). Additionally, we exposed HaCaT cells to curdlan, a small molecule activator of Dectin-1, and found that curdlan induced the expression of Dectin-1 in HaCaT cells and enhanced their proliferation (*figure 7C*). In addition, curdlan stimulation also significantly activated the Syk/NF-κB signalling pathway (*figure 7D*).

In conclusion, these studies indicate that increased IL-17A and TNF-α in psoriatic lesions may induce the expression of Dectin-1 in keratinocytes, which in turn may activate the Syk/NF-κB signalling pathway, thereby promoting the proliferation of keratinocytes.

Discussion

As a common chronic skin disease, the incidence and recurrence rate of psoriasis is high [30]. Despite the fact that there have been numerous studies on psoriasis in recent years and many biological agents have been widely used, the high recurrence rate of psoriasis continues to be a challenge for researchers. To date, many studies have found that aberrant immune responses are directly associated with the onset and progression of psoriasis [31-33]. Among them, pro-inflammatory cytokines, such as IL-17A and TNF-α, play important roles in the aetiology of psoriasis, and monoclonal antibodies targeting these cytokines are widely used in clinical treatment, achieving excellent efficacy [34, 35].

However, the intrinsic mechanism by which IL-17A and TNF-α affect the proliferation of keratinocytes in psoriatic lesions is still not completely understood. In addition, numerous commonly used monoclonal antibodies for the treatment of psoriasis have their drawbacks, including inadequate efficacy, drug resistance, and recurrence [36-38]. Therefore, our understanding of the precise regulatory effects of IL-17A and TNF-α on keratinocytes of psoriatic lesions requires further study,

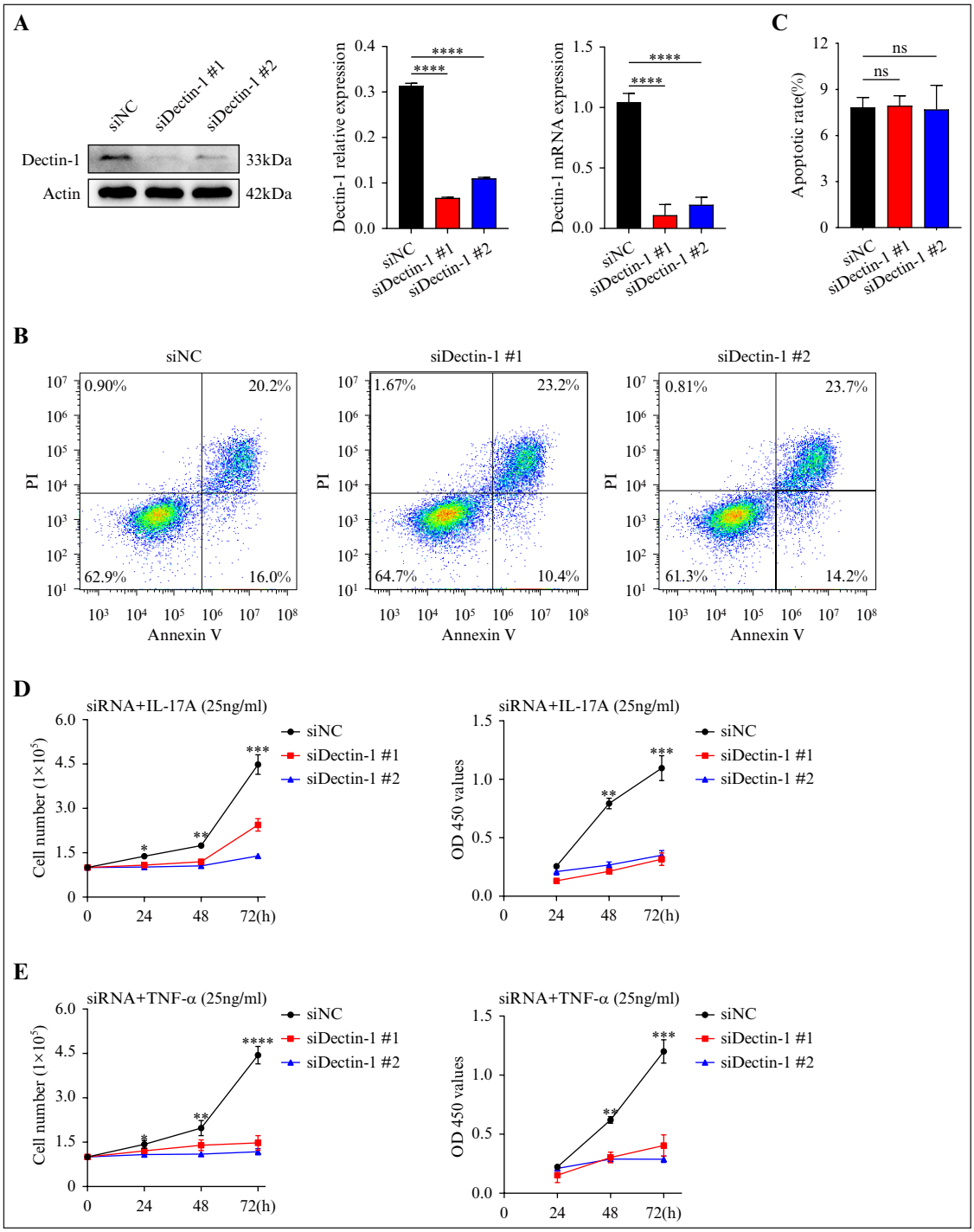

Figure 6. Knockdown of Dectin-1 inhibits the proliferation of HaCaT keratinocytes induced by IL-17A and TNF-α. **A)** HaCaT cells were transfected with Dectin-1-specific siRNAs, and real-time PCR and western blotting were performed to confirm Dectin-1 silencing. **B, C)** HaCaT cells were transfected with Dectin-1-specific siRNAs, and flow cytometry was used to detect the cell apoptosis rate. **D, E**) HaCaT cells were transfected with Dectin-1-specific siRNAs, followed by treatment with 25 ng/mL IL-17A or TNF-α. Cell counting and a CCK-8 assay were then performed at indicated time points. Data are expressed as means ± SEM. $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$, ns : not significant.

which is of particular importance regarding optimizing current therapy or developing novel biological agents. In this study, we found that the expression of Dectin-1 was significantly increased in keratinocytes in psoriatic lesions, and Dectin-1 expression significantly positively correlated with IL-17A and TNF-α levels in these lesions. Moreover, the expression levels of the endogenous ligands, Galectin-9 and Annexin A13, were also upregulated in psoriatic lesions, and the expression level of Galectin-9 positively correlated with that of

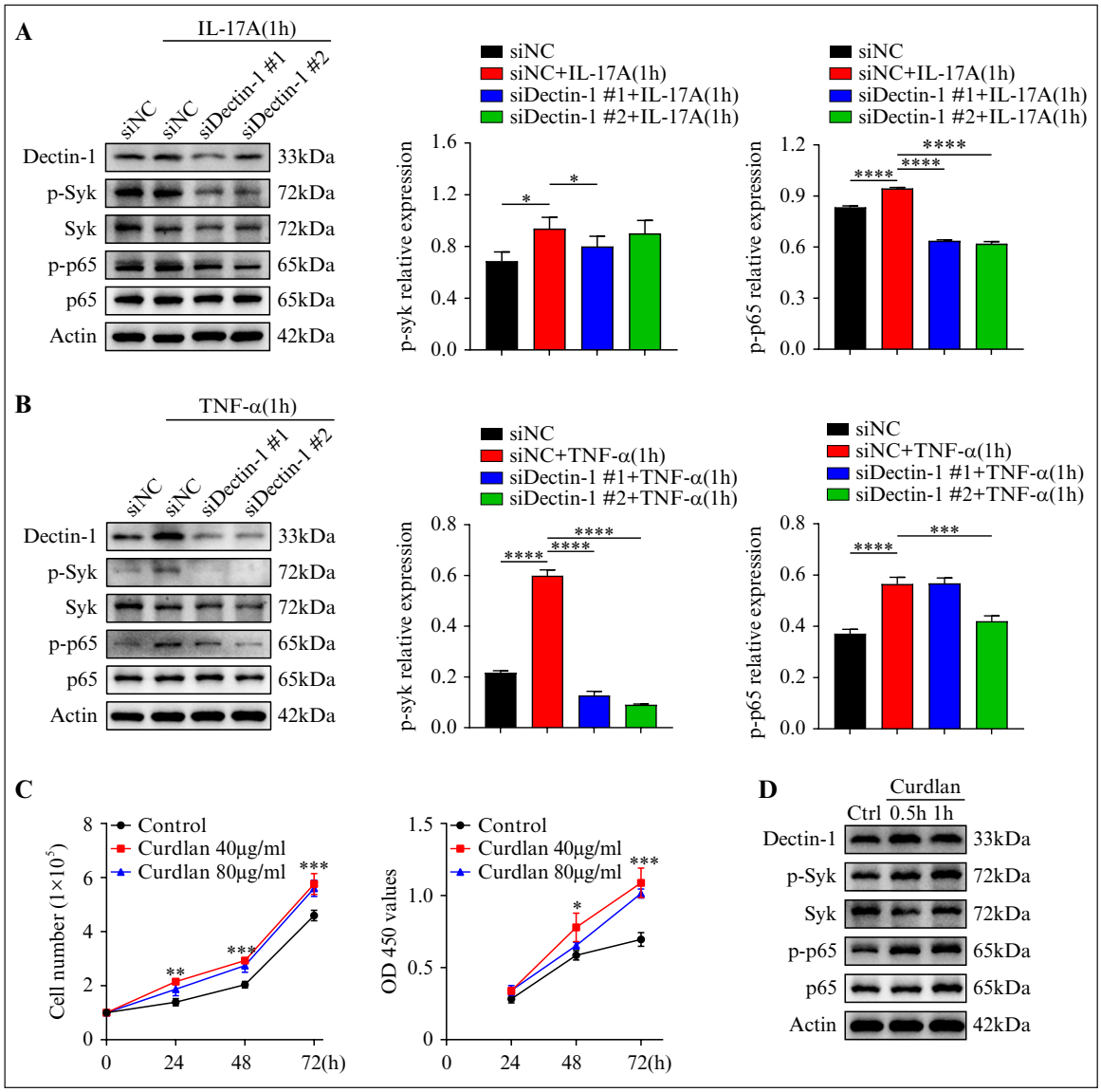

Figure 7. Increased Dectin-1 promotes HaCaT keratinocyte proliferation by activating the Syk/NF-κB signalling pathway. **A, B**) HaCaT cells were transfected with Dectin-1-specific siRNAs for 36 h, followed by treatment with 25 ng/mL IL-17A or TNF-α for 1 h, and then the protein levels of Dectin-1, Syk, p-Syk, p-p65 and p65 were detected. **C)** HaCaT cells were treated with curdlan at indicated concentrations, and a direct cell count and CCK8 assay were performed at indicated time points. **D)** The protein levels of Dectin-1, Syk, p-Syk, p-p65 and p65 in HaCaT cells upon treatment with 80 μg/mL curdlan were detected at indicated time points. Data are expressed as means ± SEM. ∗p<0.05, ∗∗p<0.01, ∗∗∗p<0.001, ∗∗∗∗p<0.0001.

TNF-α, suggesting that Galectin-9 might be involved in the effect of TNF-α on Dectin-1. We then found that IL-17A and TNF-α effectively induced the expression of Dectin-1 in keratinocytes *in vitro*, and the proliferation of keratinocytes was significantly decreased after Dectin-1 silencing. Furthermore, we showed that Dectin-1 expression activates the Syk/NF-κB pathway in keratinocytes. Previous research has demonstrated that Syk and NF-κB are crucial for keratinocyte proliferation [39, 40]. However, the specific mechanisms through which IL-17A and TNF-α can promote the expression of Dectin-1 are still unclear. To date, there are few reports on the specific regulatory mechanisms underlying the expression of Dectin-1. Studies indicate that granulocyte-macrophage colony-stimulating factor and cytokines, such as IL-4 and IL-13, can promote the expression of Dectin-1 in mouse peritoneal macrophages, and IL-33 induces the production of Dectin-1 in rat monocytes [41]. However, reports have also demonstrated that there are differences in the open regulatory sites of the Dectin-1 gene in different cell types, and the specific regulatory mechanisms remain unknown [42].

In conclusion, our study shows that elevated IL-17A and TNF-α effectively induce the expression of Dectin-1 in keratinocytes, thereby activating the Syk/NF-κB pathway and promoting keratinocyte proliferation, which in turn may promote the development of psoriasis.

Although it is unclear how IL-17A and TNF-α promote the expression of Dectin-1 in keratinocytes, we believe our findings offer a novel mechanism through which IL-17A and TNF-α can affect the proliferation of keratinocytes in psoriatic lesions, and indicate that Dectin-1 might become a new target for optimizing current biological agent therapy. ■

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Conflicts of interest: *none.*

References

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker J. Psoriasis. *Lancet* 2021 ; 397 : 1301-15.

2. Mehrmal S, Uppal P, Nedley N, Giesey RL, Delost GR. The global, regional, and national burden of psoriasis in 195 countries and territories, 1990 to 2017: a systematic analysis from the Global Burden of Disease Study 2017. *J Am Acad Dermatol* 2021 ; 84 : 46-52.

3. Takeshita J, Grewal S, Langan SM, *et al.* Psoriasis and comorbid diseases: implications for management. *J Am Acad Dermatol* 2017 ; 76 : 393-403.

4. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. *JAMA* 2020 ; 323 : 1945-60.

5. Blauvelt A, Chiricozzi A. The immunologic Role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. *Clin Rev Allergy Immunol* 2018 ; 55 : 379-90.

6. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol* 2014 ; 32 : 227-55.

7. Jiang Y, Chen Y, Yu Q, Shi Y. Biologic and small-molecule therapies for moderate-to-severe psoriasis: focus on psoriasis comorbidities. *BioDrugs* 2023 ; 37 : 35-55.

8. van de Kerkhof PC. From empirical to pathogenesis-based treatments for psoriasis. *J Invest Dermatol* 2022 ; 142 : 1778-85.

9. Carrasquillo OY, Pabon-Cartagena G, Falto-Aizpurua LA, *et al.* Treatment of erythrodermic psoriasis with biologics: a systematic review. *J Am Acad Dermatol* 2020 ; 83 : 151-8.

10. Egeberg A, Ottosen MB, Gniadecki R, *et al.* Safety, efficacy and drug survival of biologics and biosimilars for moderate-to-severe plaque psoriasis. *Br J Dermatol* 2018 ; 178 : 509-19.

11. Deerhake ME, Shinohara ML. Emerging roles of Dectin-1 in noninfectious settings and in the CNS. *Trends Immunol* 2021 ; 42 : 891-903.

12. Fan Q, Tao R, Zhang H, *et al.* Dectin-1 contributes to myocardial ischemia/reperfusion injury by regulating macrophage polarization and neutrophil infiltration. *Circulation* 2019 ; 139 : 663-78.

13. Mata-Martinez P, Bergon-Gutierrez M, Del Fresno C. Dectin-1 signaling update: new perspectives for trained immunity. *Front Immunol* 2022 ; 13 : 812148.

14. Tone K, Stappers MHT, Willment JA, Brown GD. C-type lectin receptors of the Dectin-1 cluster: physiological roles and involvement in disease. *Eur J Immunol* 2019 ; 49 : 2127-33.

15. Kalia N, Singh J, Kaur M. The role of dectin-1 in health and disease. *Immunobiology* 2021 ; 226 : 152071.

16. Iliev ID, Funari VA, Taylor KD, *et al.* Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science* 2012 ; 336 : 1314-7.

17. Drummond RA, Desai JV, Hsu AP, *et al.* Human Dectin-1 deficiency impairs macrophage-mediated defense against phaeohyphomycosis. *J Clin Invest* 2022 ; 132.

18. Yamaguchi K, Kanno E, Tanno H, *et al.* Distinct roles for Dectin-1 and Dectin-2 in skin wound healing and neutrophilic inflammatory responses. *J Invest Dermatol* 2021 ; 141 : 164-76 e8.

19. van den Berg LM, Zijlstra-Willems EM, Richters CD, Ulrich MM, Geijtenbeek TB. Dectin-1 activation induces proliferation and migration of human keratinocytes enhancing wound re-epithelialization. *Cell Immunol* 2014 ; 289 : 49-54.

20. de Koning HD, Rodijk-Olthuis D, van Vlijmen-Willems IM, *et al.* A comprehensive analysis of pattern recognition receptors in normal and inflamed human epidermis: upregulation of dectin-1 in psoriasis. *J Invest Dermatol* 2010 ; 130 : 2611-20.

21. Caputo V, Strafella C, Termine A, *et al.* Overview of the molecular determinants contributing to the expression of Psoriasis and Psoriatic Arthritis phenotypes. *J Cell Mol Med* 2020 ; 24 : 13554-63.

22. Boehncke WH, Schon MP. Psoriasis. *Lancet* 2015 ; 386 : 983-94.

23. Ghoreschi K, Balato A, Enerback C, Sabat R. Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis. *Lancet* 2021 ; 397 : 754-66.

24. de Alcantara CC, Reiche EMV, Simao ANC. Cytokines in psoriasis. *Adv Clin Chem* 2021 ; 100 : 171-204.

25. Yu Z, Yu Q, Xu H, *et al.* IL-17A promotes psoriasis-associated keratinocyte proliferation through ACT1-dependent activation of YAP-AREG axis. *J Invest Dermatol* 2022 ; 142 : 2343-52.

26. Ogawa E, Sato Y, Minagawa A, Okuyama R. Pathogenesis of psoriasis and development of treatment. *J Dermatol* 2018 ; 45 : 264-72.

27. Furue M, Furue K, Tsuji G, Nakahara T. Interleukin-17A and keratinocytes in psoriasis. *Int J Mol Sci* 2020 ; 21.

28. Wang Z, Zhou H, Zheng H, *et al.* Autophagy-based unconventional secretion of HMGB1 by keratinocytes plays a pivotal role in psoriatic skin in fl ammation. *Autophagy* 2021 ; 17 : 529-52.

29. Zimmermann M, Koreck A, Meyer N, *et al.* TNF-like weak inducer of apoptosis (TWEAK) and TNF-alpha cooperate in the induction of keratinocyte apoptosis. *J Allergy Clin Immunol* 2011 ; 127 : 200-7.

30. FitzGerald O, Ogdie A, Chandran V, *et al.* Psoriatic arthritis. *Nat Rev Dis Primers* 2021 ; 7 : 59.

31. Girolomoni G, Strohal R, Puig L, *et al.* The role of IL-23 and the IL-23/T(H) 17 immune axis in the pathogenesis and treatment of psoriasis. *J Eur Acad Dermatol Venereol* 2017 ; 31 : 1616-26.

32. Greb JE, Goldminz AM, Elder JT, *et al.* Psoriasis. *Nat Rev Dis Primers* 2016 ; 2 : 16082.

33. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. *Lancet* 2018 ; 391 : 2273-84.

34. Gupta RK, Gracias DT, Figueroa DS, *et al.* TWEAK functions with TNF and IL-17 on keratinocytes and is a potential target for psoriasis therapy. *Sci Immunol* 2021 ; 6 : eabi8823.

35. Magnolo N, Kingo K, Laquer V, *et al.* A phase 3 open-label, randomized multicenter study to evaluate efficacy and safety of secukinumab in pediatric patients with moderate to severe plaque psoriasis: 24-week results. *J Am Acad Dermatol* 2022 ; 86 : 122-30.

36. Merola JF, Landewe R, McInnes IB, *et al.* Bimekizumab in patients with active psoriatic arthritis and previous inadequate response or intolerance to tumour necrosis factor-alpha inhibitors: a randomised, double-blind, placebo-controlled, phase 3 trial (BE COMPLETE). *Lancet* 2023 ; 401 : 38-48.

37. Syversen SW, Jorgensen KK, Goll GL, *et al.* Effect of therapeutic drug monitoring vs standard therapy during maintenance infliximab therapy on disease control in patients with immune-mediated inflammatory diseases: a randomized clinical trial. *JAMA* 2021 ; 326 : 2375-84.

38. Jeon C, Sekhon S, Yan D, Afifi L, Nakamura M, Bhutani T. Monoclonal antibodies inhibiting IL-12, -23, and -17 for the treatment of psoriasis. *Hum Vaccin Immunother* 2017 ; 13 : 2247-59.

39. Lu PH, Wang JY, Chiu LY, Huang YT, Hung CF, Wu NL. Spleen tyrosine kinase regulates keratinocyte inflammasome activation and skin inflammation induced by UVB irradiation. *Free Radic Biol Med* 2022 ; 180 : 121-33.

40. Na J, Lee K, Na W, *et al.* Histone H3K27 demethylase JMJD3 in cooperation with NF-kappaB regulates keratinocyte wound healing. *J Invest Dermatol* 2016 ; 136 : 847-58.

41. Agier J, Brzezińska-Błaszczyk E, Różalska S, *et al.* Mast cell phenotypic plasticity and their activity under the influence of cathelicidin-related antimicrobial peptide (CRAMP) and IL-33 alarmins. *Cell Immunol.* 2021 ; 369 : 104424.

42. Yong HM, Gour N, Sharma D, *et al.* Epigenetic regulation of epithelial dectin-1 through an IL-33-STAT3 axis in allergic disease. *Allergy.* 2022 ; 77 : 207-17.