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p38α deficiency ameliorates psoriasis development by downregulating STAT3-mediated keratinocyte proliferation and cytokine production

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Psoriasis is characterized by keratinocyte (KC) hyperproliferation and inflammatory cell infiltration, but the mechanisms remain unclear. In an imiquimod-induced mouse psoriasiform model, p38 activity is significantly elevated in KCs and p38α specific deletion in KCs ameliorates skin inflammation. p38α signaling promotes KC proliferation and psoriasis-related proinflammatory gene expression during psoriasis development. Mechanistically, p38α enhances KC proliferation and production of inflammatory cytokines and chemokines by activating STAT3. While p38α signaling in KCs does not affect the expression of IL-23 and IL-17, it substantially amplifies the IL-23/IL-17 pathogenic axis in psoriasis. The therapeutic effect of IL-17 neutralization is associated with decreased p38 and STAT3 activities in KCs and targeting the p38α-STAT3 axis in KCs ameliorates the severity of psoriasis. As IL-17 also highly activates p38 and STAT3 in KCs, our findings reveal a sustained signaling circuit important for psoriasis development, highlighting p38α-STAT3 axis as an important target for psoriasis treatment.

Psoriasis is a chronic inflammatory skin disease characterized by epidermis hyperproliferation and the infiltration of multiple inflammatory cells in the dermis, which affects ~125 million people globally and ~2–3% of the population worldwide[1–4]. Although the pathogenesis of psoriasis is still unclear, much progress has been made to uncover this disease pathogenesis by using imiquimod (IMQ)-[5] or interleukin-23 (IL-23)-[6] induced psoriatic mouse models. Currently, it is appreciated that the dysregulated interaction between keratinocytes (KCs) and immune cells is critical for psoriasis development[2,7].

KCs are the primary cell type in the epidermis, forming a barrier against environmental damage[8]. Various insults act on KCs to induce them to produce multiple inflammatory cytokines and chemokines, which further recruit and activate immune cells to initiate the inflammatory immune response[7]. One of recent pioneer studies demonstrates that triggers-stimulated KCs can secrete LL-37, binding with self-nucleic acids from these cells to form complexes[9,10]. These complexes further stimulate dendritic cells (DCs) to release polarized cytokines, such as IL-6 and IL-23, thereby activating the differentiation and/or expansion of IL-17-producing T cells (T17)[5]. T17 produce inflammatory molecules such as IL-17 and TNFα, acting on KCs and leading to their abnormal differentiation and hyperproliferation. The stimulated KCs can produce multiple chemokines to recruit various immune cells such as neutrophils into inflamed psoriatic plaques[11]. Moreover, these stimulated KCs can also synthesize and release certain cytokines, antimicrobial peptides, and growth factors, further enhancing the vicious cycles of inflammatory response[12,13]. Thus, targeting KCs is a promising therapeutic strategy for the treatment of psoriasis, highlighting an essential step to explore the signaling pathways that regulate the proliferation and inflammatory responses of KCs.

p38 mitogen-activated protein kinase (MAPK) is one of the most important signaling pathways to regulate the inflammatory immune

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responses[14,15]. Increased activation of p38 kinase in lesional compared to nonlesional psoriatic skin[16–18], as well as in CD45$^+$ immune cells in the skins in an IMQ-induced mouse psoriasiform model[19] has been reported. Improved psoriasis severity is accompanied with decreased activation of p38 kinase in Adalimumab therapy[18]. Accordingly, the expression of mitogen-activated protein kinase phosphatase 1 (MKP-1), the negative regulator of p38α, is decreased in mouse skin upon IMQ treatment[20] as well as in lesional psoriatic skin[21]. Our previous study indicates that MKP-1-deficient mice have severe skin inflammation upon IMQ-treatment[20]. Inhibition of p38 kinase suppresses the development of psoriasis-like lesions in a human skin transplant model of psoriasis[22]. Cutaneous activation of p38 kinase is sufficient to induce psoriasiform inflammation including epidermal thickening, neutrophil infiltration and proinflammatory cytokine expression in mice[23]. Our previous study has shown that p38α signaling in Langerhans cells (LCs), but not in other DCs or T cells, is essential for IMQ-induced psoriasiform[19], demonstrating the cell-type specific role of p38α in psoriasis pathogenesis. However, the role of p38α in KCs in vivo is still not well known.

In the current study, we found an increased p38 activity in KCs of IMQ-treated mice. To further examine the role of p38α in KCs in psoriasis in vivo, we crossed *Mapk14*$^{flox/flox}$ mice with K14-Cre transgenic mice, which have a human keratin 14 promoter/enhancer sequence, to obtain the mice with specific deletion of p38α in KCs. In the IMQ- or IL-23-induced mouse psoriasiform model, we found that p38α-signal transducer and activator of transcription 3 (STAT3) signaling axis in KCs significantly aggravated psoriasis severity by promoting KC proliferation and the production of proinflammatory cytokines and chemokines. Our study suggests that p38α-STAT3 axis is an important target for developing therapies for the treatment of skin inflammatory diseases.

Results

Deletion of p38α in KCs largely ameliorates IMQ-induced psoriasiform disease

An increased p38 activity has been reported in lesional psoriatic skin[16–18], but the role and underlying mechanisms of p38 in psoriasis pathogenesis are still unclear. In an IMQ-induced mouse psoriasiform model, we found that p38 activity was significantly elevated in epidermal tissue either by immunofluorescence (IF) staining (Fig. 1a). Further Western blot and flow cytometry analyses showed that the phosphorylation level of p38 was higher in KCs from IMQ-treated mice than control cream-treated mice (Fig. 1b, c and Supplementary Fig. 1a). These findings suggest an important role of p38 signaling in KCs during psoriasis pathogenesis. Considering that p38α is the main subunit of p38 MAPK in the regulation of inflammation, to delineate the specific role of KC-intrinsic p38 signaling in the pathogenesis of psoriasis in vivo, we generated *Mapk14*$^{flox/flox}$K14-Cre mice (referred to as "p38α^{ΔKC} mice" here). In p38α^{ΔKC} mice, we observed an efficient deletion of p38α in skin KCs both at mRNA level and protein level (Supplementary Fig. 1b, c). Notably, KC-specific ablation of p38α did not compromise mouse skin development and homeostasis (Supplementary Fig. 1d, e). In the IMQ-induced psoriasiform model, p38α^{ΔKC} mice had significantly decreased ear thickness and disease severity score compared with wild-type mice (Fig. 1d, e). Histological analysis showed less epidermal hyperplasia and inflammation in IMQ-treated skin of p38α^{ΔKC} mice than wild-type mice (Fig. 1f). In addition, the percentages and cell numbers of neutrophils and macrophages were largely reduced in skin tissues of p38α^{ΔKC} mice compared with wild-type control mice. However, the percentages and cell numbers of dendritic epidermal T cells (DETCs), γδ T cells, TCRβ$^+$ T cells and DCs were comparable in skin tissues of IMQ-treated wild-type mice and p38α^{ΔKC} mice (Fig. 1g). Collectively, these findings indicate a key role for KC p38α signaling in IMQ-induced psoriasiform skin disease.

p38α signaling in KCs promotes psoriasis-related inflammatory gene expression upon IMQ treatment

Consistent with the decreased disease severity in p38α^{ΔKC} mice after IMQ treatment, the skin tissues of IMQ-treated p38α^{ΔKC} mice had significantly reduced transcript levels of various psoriasis-related inflammatory cytokines and chemokines, such as *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, and *Cxcl2* (Fig. 2a). The mRNA expression of several genes encoding antimicrobial peptides such as *S100a7a*, *S100a8*, and *S100a9* was also decreased in IMQ-treated p38α^{ΔKC} mice compared with wild-type mice (Fig. 2a). Furthermore, the mRNA expression of *Krt6*, *Krt16*, and *Krt17*, encoding the keratins related to excessive KC proliferation, was significantly reduced in IMQ-treated p38α^{ΔKC} mice (Fig. 2a). However, the mRNA levels of *Il6*, *Il19*, *Il20*, *Il24*, *Il36a*, *Il36b*, *Il36g*, *Ifng*, *Cxcl5*, *Cxcl9*, *Cxcl10*, *Ccl20*, *Lcn2*, *Reg3g*, *Defb2*, *Krt1*, *Krt10*, and *Krt14* were comparable between wild-type mice and p38α^{ΔKC} mice (Fig. 2a and Supplementary Fig. 2a). Among these molecules, KRT14 is a basal layer-specific keratin and will be replaced by KRT1/KRT10 in suprabasal layers during KC differentiation[24], indicating that p38α might not be required for KC differentiation. IL-36α, IL-36β and IL-36γ secreted by KCs have been shown to be upregulated in human psoriatic skin and mouse models of psoriasiform skin disease and can activate DCs and neutrophils[25,26]. Notably, although IL-23/IL-17 axis and IL-17 family of cytokines play pivotal roles in the pathogenesis of psoriasis[5,27–30], the transcript levels of *Il23p19*, *Il22*, *Il17a*, *Il17c*, *Il17d*, *Il17e,* and *Il17f*, and the production of IL-17A from γδ T cells and CD4$^+$ T cells were comparable between IMQ-treated wild-type mice and p38α^{ΔKC} mice (Fig. 2a and Supplementary Figs. 2a, b and 3a). The percentages of γδ T cells, CD4$^+$ T cells, CD8$^+$ T cells, Th1 cells, Th2 cells, Treg cells, and IFNγ$^+$CD8$^+$ T cells were also similar in IMQ-treated wild-type mice and p38α^{ΔKC} mice (Supplementary Fig. 3a). Moreover, wild-type mice and p38α^{ΔKC} mice had comparable proportions of naïve γδ T cells, effector γδ T cells, naïve CD4$^+$ T cells and effector CD4$^+$ T cells upon IMQ treatment (Supplementary Fig. 3b, c). In line with these findings, there were no significant differences in the frequencies of total DCs, LCs, and dermal DCs, and the expression of activation markers CD40, CD80, and CD86 on LCs and dermal DCs in skin tissues of IMQ-treated wild-type mice and p38α^{ΔKC} mice (Supplementary Fig. 4a−c). In addition, the proportions of migratory DCs and resident DCs were also similar in draining lymph nodes of IMQ-treated wild-type mice and p38α^{ΔKC} mice (Supplementary Fig. 4d).

To explore the potential mechanism by which KC p38α regulates the pathogenesis of IMQ-induced psoriasiform skin disease, we purified KCs from IMQ-treated skin tissues at different time points and analyzed the related gene expression. The results showed that IMQ treatment significantly increased the mRNA expression of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Ccl20*, *S100a7a*, *S100a8*, *S100a9*, *Krt6*, *Krt16*, *Krt17*, *Il36a*, *Il36b*, and *Il36g* in KCs (Fig. 2b and Supplementary Fig. 2c). Although the expression of *Ccl20*, *Il36a*, *Il36b*, and *Il36g* mRNA in KCs was comparable between IMQ-treated wild-type mice and p38α^{ΔKC} mice, p38α deletion in KCs largely reduced the transcript levels of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *S100a7a*, *S100a8*, *S100a9*, *Krt6*, *Krt16*, and *Krt17* in KCs upon IMQ treatment (Fig. 2b and Supplementary Fig. 2c). These results collectively demonstrate that deletion of p38α in KCs ameliorates psoriasis-related inflammatory gene expression, but does not affect IL-23/IL-17 axis during IMQ-treatment.

p38α signaling promotes KC hyperproliferation and psoriasis severity in a STAT3-dependent manner

KC hyperproliferation is a major pathogenic factor in psoriasis[13]. The decreased expression of keratin genes such as *Krt6*, *Krt16*, and *Krt17* in p38α^{ΔKC} mice, which mediate the abnormal proliferation of epidermal cells, prompted us to further examine whether p38α signaling can regulate KC hyperproliferation during psoriasis development. Immunohistochemical analysis showed reduced Ki-67 staining levels in epidermal cells of p38α^{ΔKC} mice treated with IMQ for 3 days (Fig. 3a) and 6 days (Fig. 3b) compared with wild-type mice. While the BrdU incorporation (indicating as cell proliferation) in KCs was similar in wild-type mice and p38α^{ΔKC} mice under steady state, there was significantly lower BrdU incorporation in KCs of p38α^{ΔKC} mice than that in wild-type mice upon IMQ treatment (Fig. 3c). The apoptosis of KCs has been shown to contribute to psoriasis pathogenesis[31]. Notably, active caspase-3 staining showed comparable

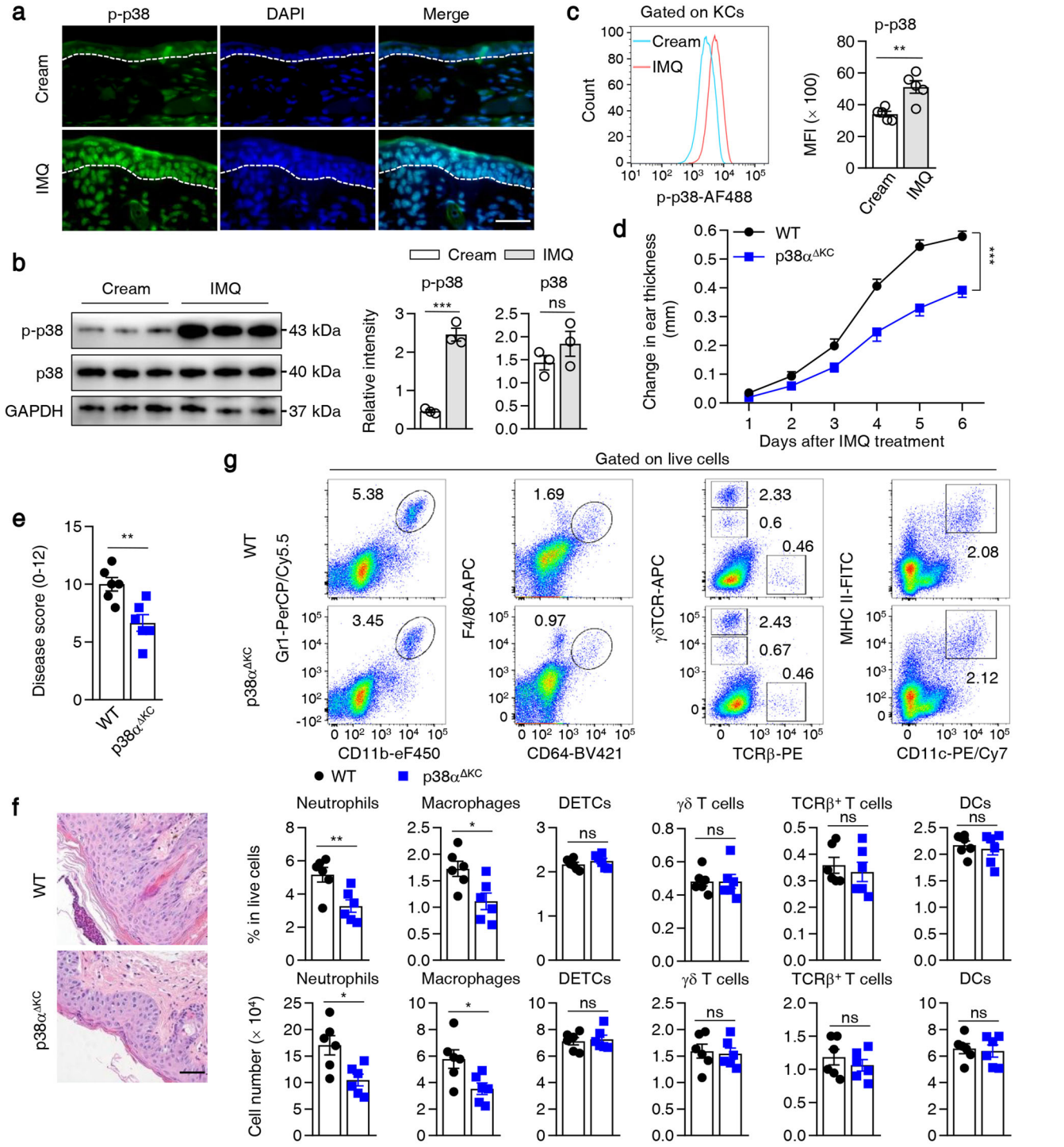


Fig. 1 | Deletion of p38α in KCs protects mice from IMQ-induced skin inflammation. a–c Wild-type (WT) mice were topically treated with imiquimod (IMQ)-containing or control cream for three consecutive days. The phosphorylation (p) of p38 in skin sections was examined by immunofluorescence staining (**a**). Scale bar: 50 μm. The abundance of p38 and p-p38 in keratinocytes (KCs) was analyzed by Western blot and the relative expression was normalized with GAPDH (**b**, $n = 3$). The activity of p38 in KCs was detected by flow cytometry (**c**, $n = 5$). **d–g** WT and p38α$^{\Delta KC}$ mice were topically treated with IMQ for six consecutive days ($n = 6$). Changes in ear thickness (**d**) and disease severity score (**e**) were recorded. Histopathological changes in skin sections were examined by hematoxylin and eosin (H&E) staining (**f**). Scale bar: 50 μm. The percentages and cell numbers of neutrophils, macrophages, dendritic epidermal T cells (DETCs), γδ T cells, TCRβ$^+$ T cells, and dendritic cells (DCs) in skin tissue were analyzed (**g**). All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-tailed Student's *t* tests (**b**, **c**, **e**, **g**) and two-way analysis of variance (ANOVA) (**d**) were performed. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns not significant.

apoptosis of KCs in both wild-type mice and p38α$^{\Delta KC}$ mice with or without IMQ treatment (Fig. 3d). These results demonstrate that p38α signaling promotes KC proliferation, but does not affect KC apoptosis during psoriasis development.

To explore the potential molecular mechanisms for p38α signaling in regulating KC proliferation and psoriasis-related inflammatory gene expression, we treated primary KCs with R848, an IMQ analog, for further analysis. Upon R848 stimulation, primary p38α-deficient KCs had

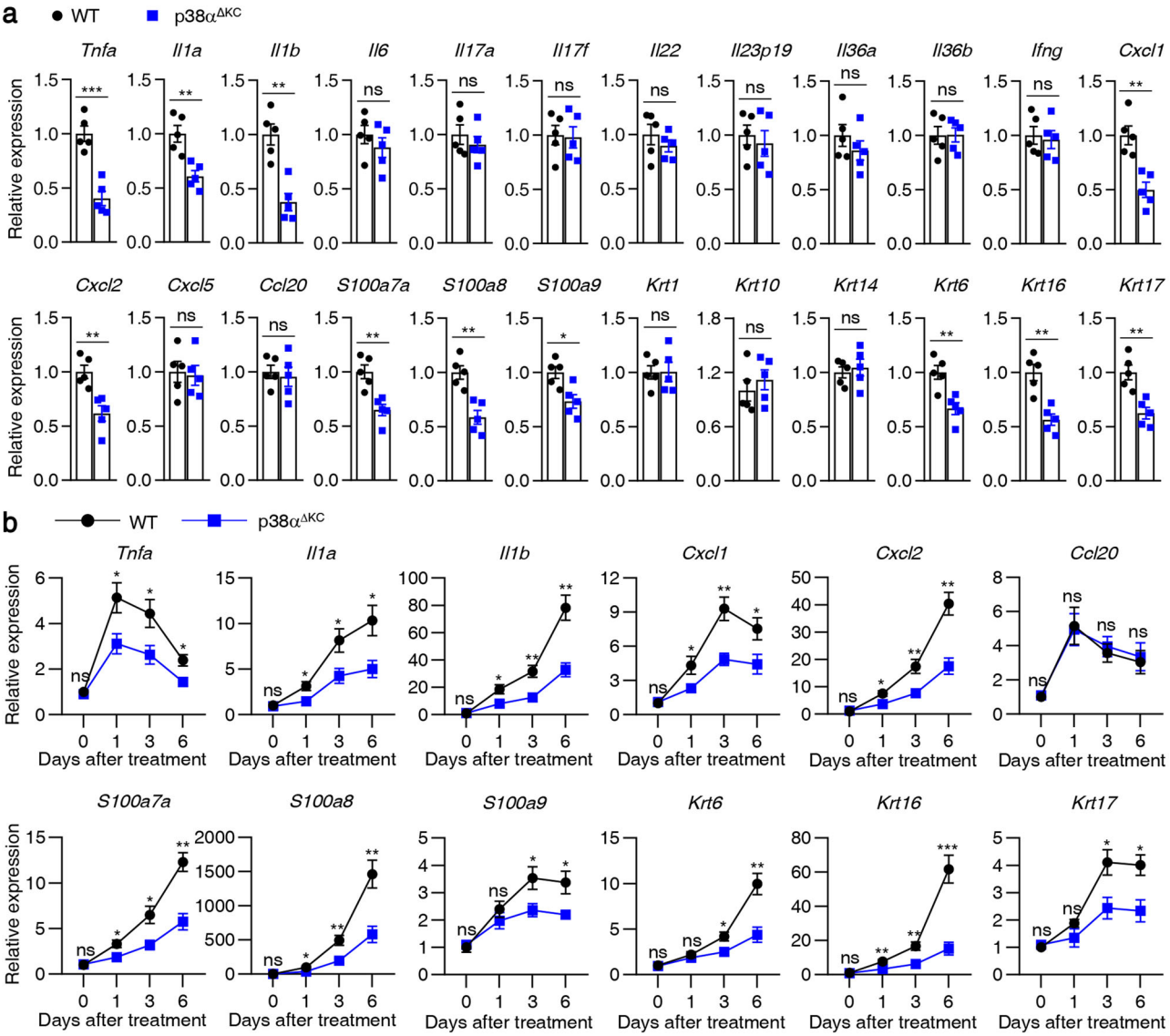


Fig. 2 | Mice with p38α deficiency in KCs have decreased skin inflammatory responses upon IMQ treatment. WT and p38α$^{\Delta KC}$ mice were topically treated with IMQ for six consecutive days. **a** Relative mRNA expression of inflammatory cytokines, chemokines, antimicrobial peptides, and keratins was measured in skin tissue ($n = 5$). **b** KCs were sorted from skin tissue at days 0, 1, 3, and 6 to examine the relative mRNA levels of inflammation-related genes ($n = 5$). All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-tailed Student's *t* tests were performed (**a**, each time point of **b**). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns not significant.

significantly decreased percentages of Edu^{+} cells compared with primary wild-type KCs, indicating a reduced proliferation of primary p38α-deficient KCs (Fig. 4a). Moreover, deletion of p38α in KCs substantially decreased the mRNA levels of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *S100a7a*, *S100a8*, *S100a9*, *Krt6*, *Krt16*, and *Krt17* in primary KCs stimulated with R848 (Fig. 4b), similar to the phenotype we obtained in vivo (Fig. 2b). Further Western blot analysis showed that p38α deficiency reduced the phosphorylation level of STAT3 but did not affect the activities of JNK, ERK, p65 and Akt in primary KCs stimulated with R848 (Fig. 4c and Supplementary Fig. 5a). IF analysis also demonstrated lower phosphorylation level of STAT3 in epidermis of IMQ-treated p38α$^{\Delta KC}$ mice (Fig. 4d).

To examine whether the decreased activity of STAT3 in primary p38α-deficient KCs contributed to the less psoriasiform disease severity in p38α$^{\Delta KC}$ mice, we treated wild-type mice and p38α$^{\Delta KC}$ mice with IMQ to induce psoriasiform skin disease and then intradermally injected STAT3 activator Colivelin or control vehicle. As expected, activation of STAT3 signaling significantly enhanced psoriasiform skin disease severity in both wild-type mice and p38α$^{\Delta KC}$ mice, indicated by ear swelling, disease severity score, epidermal hyperplasia, and neutrophil infiltration (Fig. 4e–h). More importantly, the activation of STAT3 signaling fully restored the less psoriasiform disease severity in p38α$^{\Delta KC}$ mice compared with wild-type mice (Fig. 4e–h). The decreased mRNA levels of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *S100a7a*, *S100a8*, *S100a9*, *Krt6*, *Krt16*, and *Krt17* in skin tissues and reduced proliferation in KCs of p38α$^{\Delta KC}$ mice were also completely reached to the levels of wild-type mice (Fig. 4i, j). Collectively, p38α signaling in KCs promotes mouse psoriasiform skin disease in a STAT3-dependent manner.

Deletion of p38α in KCs protects mice from IL-23-induced psoriasiform skin disease

To examine whether the critical role of p38α signaling in KCs in promoting mouse psoriasiform skin disease was unique to IMQ stimulation, we performed an IL-23-induced psoriasiform mouse model[32] and found that p38 activity was significantly elevated in KCs of IL-23-treated mice (Fig. 5a). Furthermore, we introduced IL-23 to wild-type mice and p38α$^{\Delta KC}$ mice and analyzed disease severity. Compared with IL-23-treated wild-type mice, IL-23-treated p38α$^{\Delta KC}$ mice had significantly less skin disease severity as

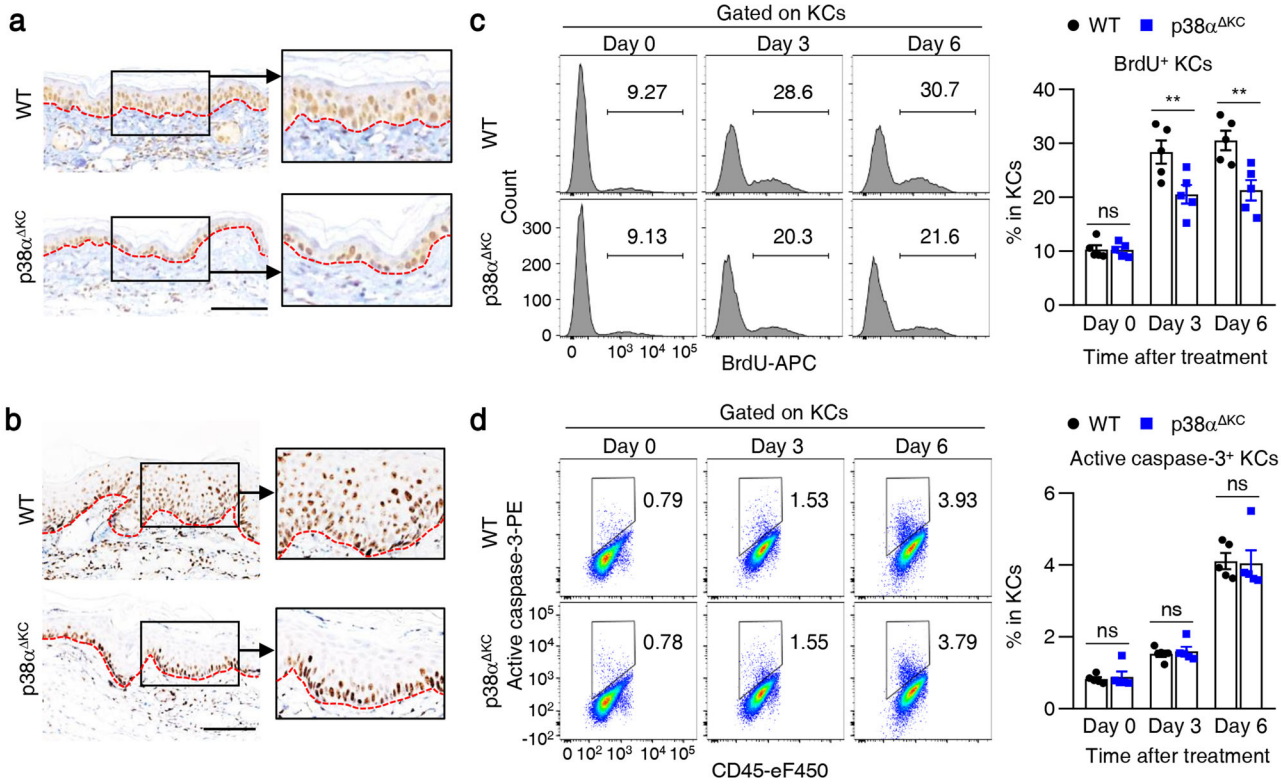


Fig. 3 | p38α activity is required for the hyperproliferation of KCs in IMQ-treated mice. a, **b** Immunohistochemistry analysis of Ki-67 in skin sections of WT and p38α$^{\Delta KC}$ mice treated with IMQ for 3 days (**a,** $n = 4$) and 6 days (**b**, $n = 4$). Scale bar: 100 μm. **c**, **d** The frequencies of BrdU$^+$ KCs (**c**) and active caspase-3$^+$ KCs (**d**) in skin tissue of IMQ-treated wild-type mice and p38α$^{\Delta KC}$ mice at indicated time points ($n = 5$). All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-way ANOVA with Bonferroni post-tests (**c**, **d**) were performed. $^{**}P < 0.01$; ns not significant.

indicated by ear swelling, disease severity score, epidermal hyperplasia, and neutrophil infiltration (Fig. 5b—e). In addition, the mRNA levels of inflammation-related genes, such as *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Krt6*, *Krt16*, and *Krt17*, were also significantly decreased in IL-23-treated p38α$^{\Delta KC}$ mice compared with wild-type mice (Fig. 5f). However, *Il6*, *Il17a*, *Il17f*, *Ifng*, *Il36a*, *Cxcl5*, *Ccl20*, and *Krt10* levels were comparable between wild-type mice and p38α$^{\Delta KC}$ mice upon IL-23 treatment (Fig. 5f). These results demonstrate that p38α signaling in KCs is also crucial for the regulation of IL-23-induced mouse psoriasiform skin disease.

p38α-STAT3 axis mediates IL-17 signaling in KCs

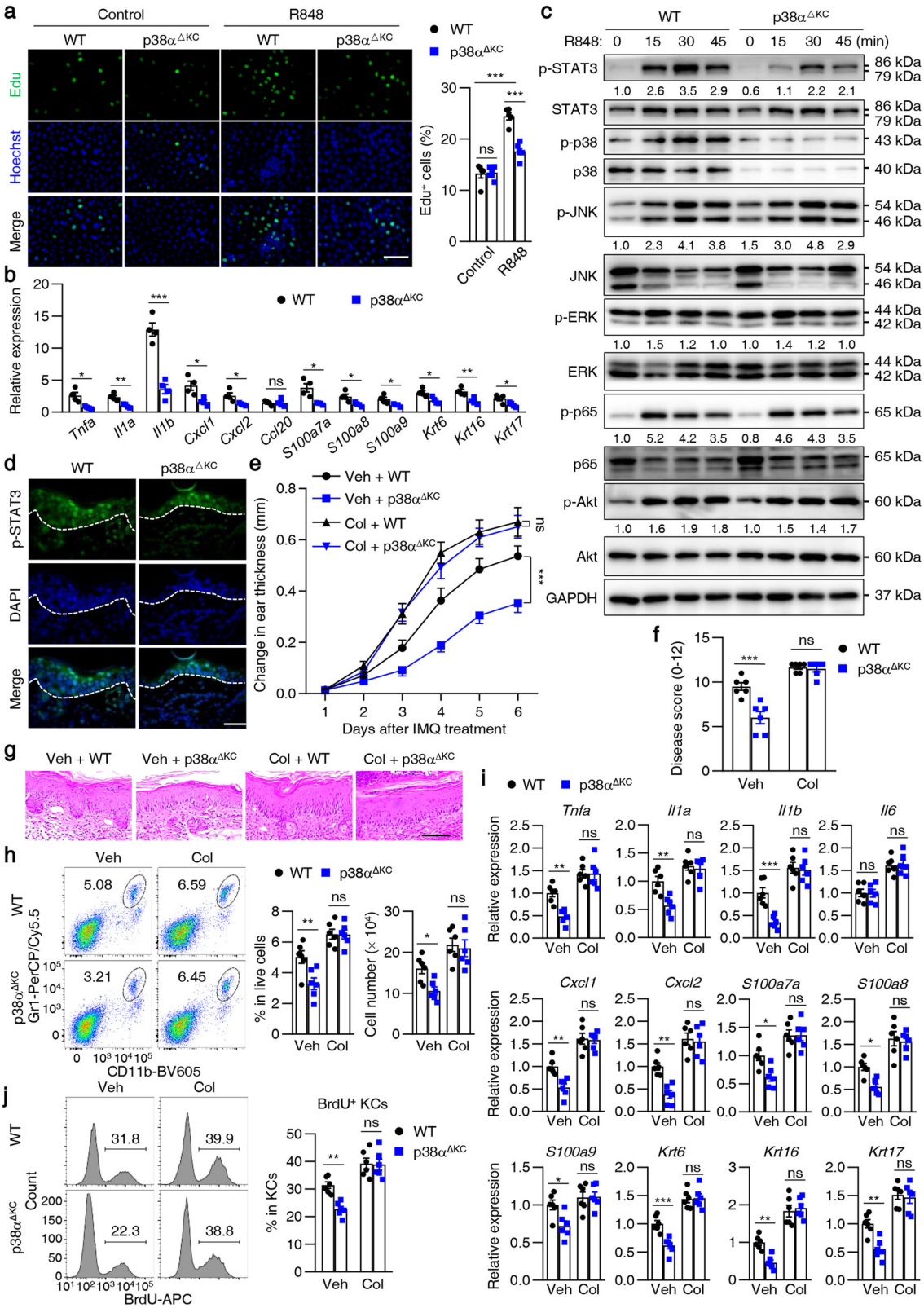
IL-17A plays an essential role in psoriasis pathogenesis, mainly through inducing the proliferation and secretion of inflammatory cytokines by KCs, further amplifying psoriasiform inflammation[33]. p38 activity in KCs was increased upon stimulation with IL-17A, which was associated with the increased phosphorylation of STAT3 (Fig. 6a and Supplementary Fig. 5b). However, upon deletion of p38α in KCs, the phosphorylation level of STAT3 was substantially reduced, without affecting the activities of JNK, ERK, p65 and Akt upon stimulation with IL-17A (Fig. 6a and Supplementary Fig. 5b). At the same line, IL-17A could largely induce KC proliferation, indicated by Edu$^+$ cells, however, the percentage of Edu$^+$ cells was significantly decreased in primary p38α-deficient KCs compared with primary wild-type KCs upon IL-17A treatment (Fig. 6b). We then explored whether certain psoriasis-related inflammatory cytokines in KCs would be affected by p38α signaling once stimulated with IL-17A. Our results showed that deletion of p38α in KCs significantly decreased the mRNA levels of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Krt6*, *Krt16*, and *Krt17* in primary KCs stimulated with IL-17A, but did not affect the mRNA level of *Ccl20* (Fig. 6c). Notably, STAT3 activator largely increased the proliferation and expression of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Ccl20*, *Krt6*, *Krt10*, *Krt16*, and *Krt17* in primary KCs and abolished the differences in proliferation and psoriasis-related inflammatory cytokine production between primary wild-type and p38α-deficient KCs upon IL-17A treatment (Fig. 6b, c).

To examine whether p38α signaling in KCs mediates the effector phase of IL-17-induced mouse psoriasiform inflammation in vivo, we intradermally injected IL-17A into the ear skin of wild-type mice and p38α$^{\Delta KC}$ mice to induce psoriasiform skin inflammation[34]. Upon IL-17A treatment, p38α$^{\Delta KC}$ mice had less ear swelling, disease severity score, and neutrophil infiltration than wild-type mice (Fig. 6d—f). Although the mRNA levels of *Ccl20* and *Krt10* were comparable between wild-type mice and p38α$^{\Delta KC}$ mice, the expression of *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Krt6*, *Krt16*, and *Krt17* in p38α$^{\Delta KC}$ mice was lower than that in wild-type mice (Fig. 6g). These results indicate that p38α deficiency in KCs significantly reduces the disease severity in the IL-17A-induced mouse psoriasiform disease. Taken together, p38α-STAT3 axis in KCs is important for amplifying the effector function of IL-17A during psoriasis development.

p38α-STAT3 axis in KCs is an important therapeutic target for the treatment of psoriasiform skin disease

IL-17A antagonist has been approved for psoriasis treatment since 2020[1]. To further determinate whether the p38α-STAT3 axis is a good target during IL-17A antagonist treatment in psoriasis, we intraperitoneally injected IMQ-treated wild-type mice with the antibody against IL-17A. Blockade of IL-17A largely reduced ear swelling, disease severity score and neutrophil infiltration in IMQ-treated wild-type mice (Supplementary Fig. 6a—c). More importantly, blockade of IL-17A suppressed the phosphorylation level of p38 and STAT3 in KCs (Fig. 7a).

Considering that p38α-STAT3 axis mediated IL-17 signaling in KCs (Fig. 6) and blockade of IL-17 largely reduced the activities of p38α and STAT3 (Fig. 7a), we then assessed whether p38α-STAT3 axis could be a good therapeutic target for the treatment of psoriasis. We intradermally injected STAT3 inhibitor S3I-201 or control vehicle to IMQ-treated wild-



type mice and p38α$^{\Delta KC}$ mice. The results showed that inhibition of STAT3 signaling largely alleviated ear swelling and disease severity of IMQ-treated mice (Fig. 7b, c). Furthermore, histological and flow cytometry analyses showed that inhibition of STAT3 signaling significantly reduced the epidermal hyperplasia and infiltration of neutrophils in skin tissue of IMQ-treated mice (Fig. 7d, e). Moreover, inhibition of STAT3 signaling also markedly decreased the relative expression of proinflammatory cytokines, chemokines, antimicrobial peptides and keratins, such as *Tnfa*, *Il1a*, *Il1b*, *Cxcl1*, *Cxcl2*, *Ccl20*, *S100a7a*, *S100a8*, *Krt6*, *Krt16*, and *Krt17*, in skin tissue of IMQ-treated mice (Fig. 7f). In addition, upon STAT3 inhibitor treatment, p38α$^{\Delta KC}$ mice exhibited similar psoriasiform skin disease severity as wild-type mice, including ear swelling, disease score, epidermal hyperplasia, and

Fig. 4 | p38α signaling in KCs promotes IMQ-induced skin inflammation in a SATA3-dependent manner. a–c Primary KCs of WT and p38α$^{\Delta KC}$ mice were stimulated with R848. R848 stimulation for 24 h to perform 5-ethynyl-2'-deoxyuridine (Edu) incorporation assay and calculate the frequency of Edu^{+} cells (**a**, $n = 5$-6). Scale bar: 100 μm. R848 stimulation for 5 h to determine the mRNA levels of inflammation-related genes and the relative expression was normalized with unstimulated KCs (**b**, $n = 4$). R848 stimulation for indicated times to examine the activities of STAT3, p38, JNK, ERK, p65 and Akt (**c**). The numbers below the lanes indicate the band intensity relative to total protein. **d** Immunofluorescence staining of p-STAT3 in skin sections of WT and p38α$^{\Delta KC}$ mice treated with IMQ for 3 days ($n = 4$). Scale bar: 50 μm. **e–j** WT and p38α$^{\Delta KC}$ mice topically treated with IMQ were intradermally injected with STAT3 activator Colivelin (Col) or control vehicle (Veh) ($n = 6$): changes in ear thickness (**e**); disease severity score (**f**); histopathological changes in skin sections (**g**); infiltration of neutrophils (**h**); and relative expression of inflammation-related genes (**i**) in skin tissue; the frequencies of BrdU^{+} KCs (**j**). Scale bar: 100 μm. All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-way ANOVA with Bonferroni post-tests (**a**, **f**, **h–j**), two-tailed Student's *t* tests (**b**), and two-way ANOVA (**e**) were performed. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns not significant.

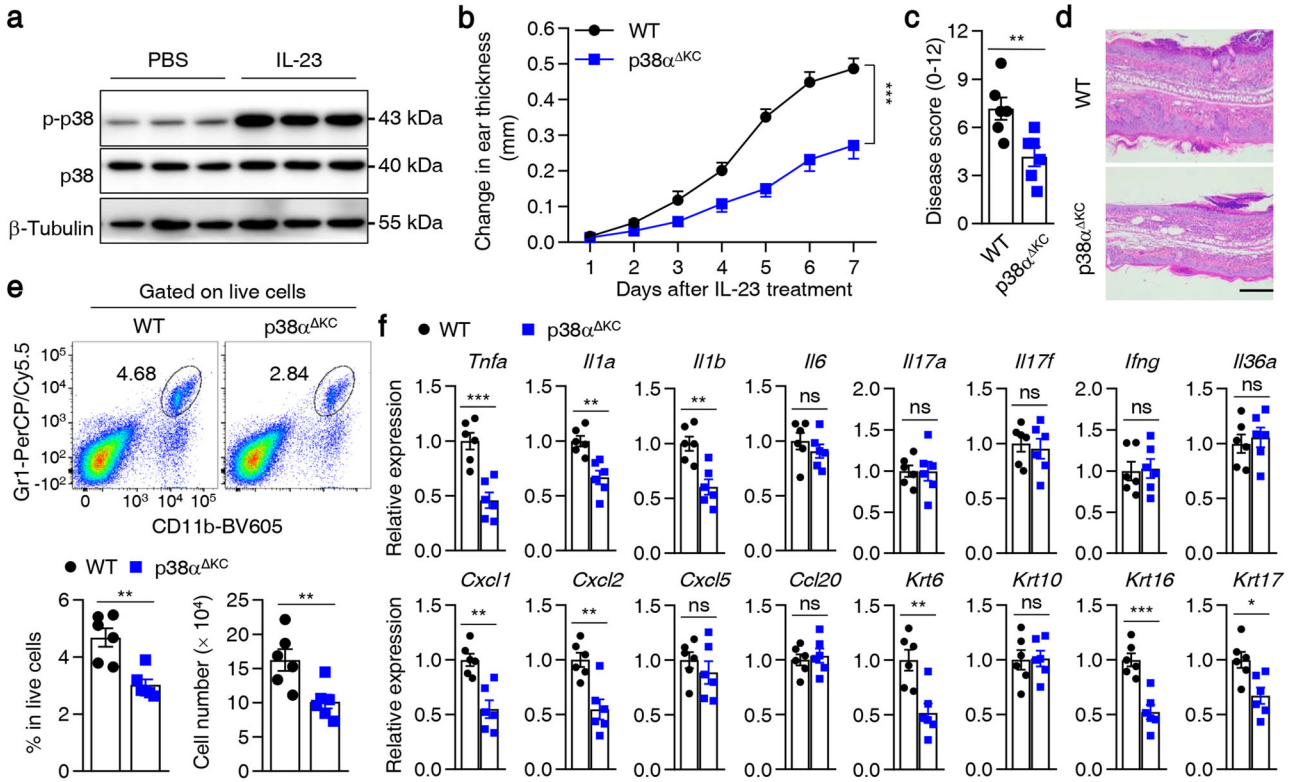


Fig. 5 | p38α deficiency in KCs attenuates IL-23-induced psoriasiform skin inflammation in mice. a The activity of p38 in KCs of WT mice intradermally injected with IL-23 or PBS every other day for two times. **b–f** WT and p38α$^{\Delta KC}$ mice were intradermally injected with IL-23 every other day for four times ($n = 6$): changes in ear thickness (**b**); disease severity score (**c**); histopathological changes in skin sections (**d**); infiltration of neutrophils (**e**); and relative expression of inflammation-related genes (**f**) in skin tissue. Scale bar: 200 μm. All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-way ANOVA (**b**) and two-tailed Student's *t* tests (**c**, **e**, **f**) were performed. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns not significant.

neutrophil infiltration (Fig. 7b—e). The mRNA levels of inflammation-related genes were also comparable between wild-type mice and p38α$^{\Delta KC}$ mice treated with STAT3 inhibitor (Fig. 7f).

Next, we evaluated whether inhibition of p38 activity can attenuate psoriasis. In our previous study, we intraperitoneally injected p38 inhibitor SB203580 into the psoriatic mice and found this treatment could ameliorate psoriasiform inflammation[19]. In the current study, we intradermally injected SB203580 or control vehicle to wild-type mice pretreated with IMQ and analyzed disease severity. The results showed that the ear swelling and disease severity score were significantly alleviated in SB203580-treated mice compared with vehicle-treated mice (Supplementary Fig. 7a, b). We further observed decreased epidermal hyperplasia and infiltration of neutrophils in skin tissue of SB203580-treated mice (Supplementary Fig. 7c, d). Moreover, p38 inhibitor SB203580 reduced the relative expression of multiple key psoriasis-related cytokines, such as *Tnfa*, *Il1a*, *Il1b*, *Il17a*, *I17f*, *Cxcl1*, *Cxcl2*, *S100a7a*, *S100a8*, *S100a9*, *Krt6*, *Krt16*, and *Krt17*, but had no effect on the mRNA levels of *Il10*, *Tgfb1*, or *Ccl20* (Supplementary Fig. 7e). Collectively, these findings suggest that p38α-STAT3 axis could serve as a good target for the treatment of psoriasis.

Discussion

Although mounting evidence demonstrates an essential role of KCs in psoriasis pathogenesis, the underlying mechanisms by which KCs regulate the initiation and maintenance of skin inflammation remain incompletely understood. In this study, we identified the p38α-STAT3 axis as a key pathogenic factor in regulating the proliferation and inflammation-related cytokine production of KCs during psoriasis development. Selective deletion of p38α in KCs or inhibition of STAT3 signaling greatly alleviated the key features of disease severity. While p38α signaling in KCs did not affect the expression of IL-23 and IL-17, it substantially amplified the IL-23/IL-17 pathogenic axis in psoriasis. Our results thus establish a critical role of p38α-STAT3 axis in the pathogenesis of psoriasis.

Psoriasis is featured with KC hyperproliferation, abnormal differentiation and dysregulated DC-T cell interaction. The reduced expression of certain genes involved in excessive KC proliferation, such as *Krt6*, *Krt16*, and *Krt17*, in skin tissues, lower Ki-67 staining levels in epidermal cells and less BrdU positive KCs in IMQ-treated p38α$^{\Delta KC}$ mice reveal a critical role of p38α signaling in regulating KC proliferation. However, the comparable expression of certain genes regulating KC differentiation, such as *Krt1*,

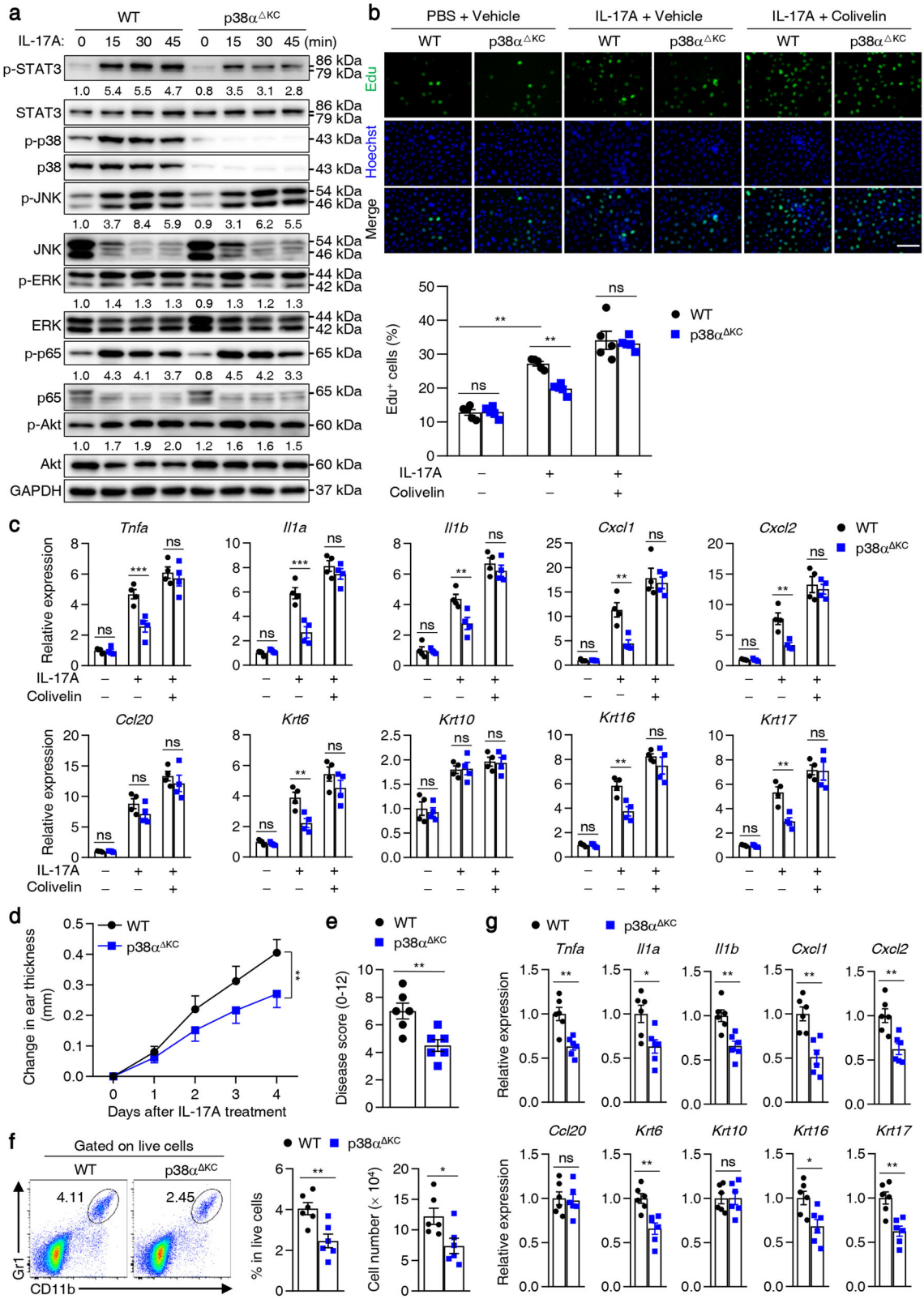


Fig. 6 | p38α-STAT3 axis mediates IL-17 signaling in KCs. a The activities of STAT3, p38, JNK, ERK, p65, and Akt were determined in WT and p38α$^{\Delta KC}$ primary KCs stimulated with IL-17A for indicated times. The numbers below the lanes indicate the band intensity relative to total protein. **b**, **c** Primary KCs of WT and p38α$^{\Delta KC}$ mice were pretreated with or without STAT3 activator Colivelin followed with or without stimulation of IL-17A. IL-17A stimulation for 24 h to perform the Edu incorporation assay and calculate the percentages of Edu^{+} cells (**b**, $n = 5$). Scale bar: 100 μm. IL-17A stimulation for 5 h to analyze the production of inflammation-related genes (**c**, $n = 4$). **d**–**g** WT and p38α$^{\Delta KC}$ mice were intradermally injected with IL-17A ($n = 6$): changes in ear thickness (**d**); disease severity score (**e**); infiltration of neutrophils (**f**) and relative expression of inflammation-related genes (**g**) in skin tissue. All the assays were replicated three times with consistent results. Data represent mean ± SEM. Two-way ANOVA with Bonferroni post-tests (**b**, **c**), two-way ANOVA (**d**) and two-tailed Student's *t* tests (**e**–**g**) were performed. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns not significant.

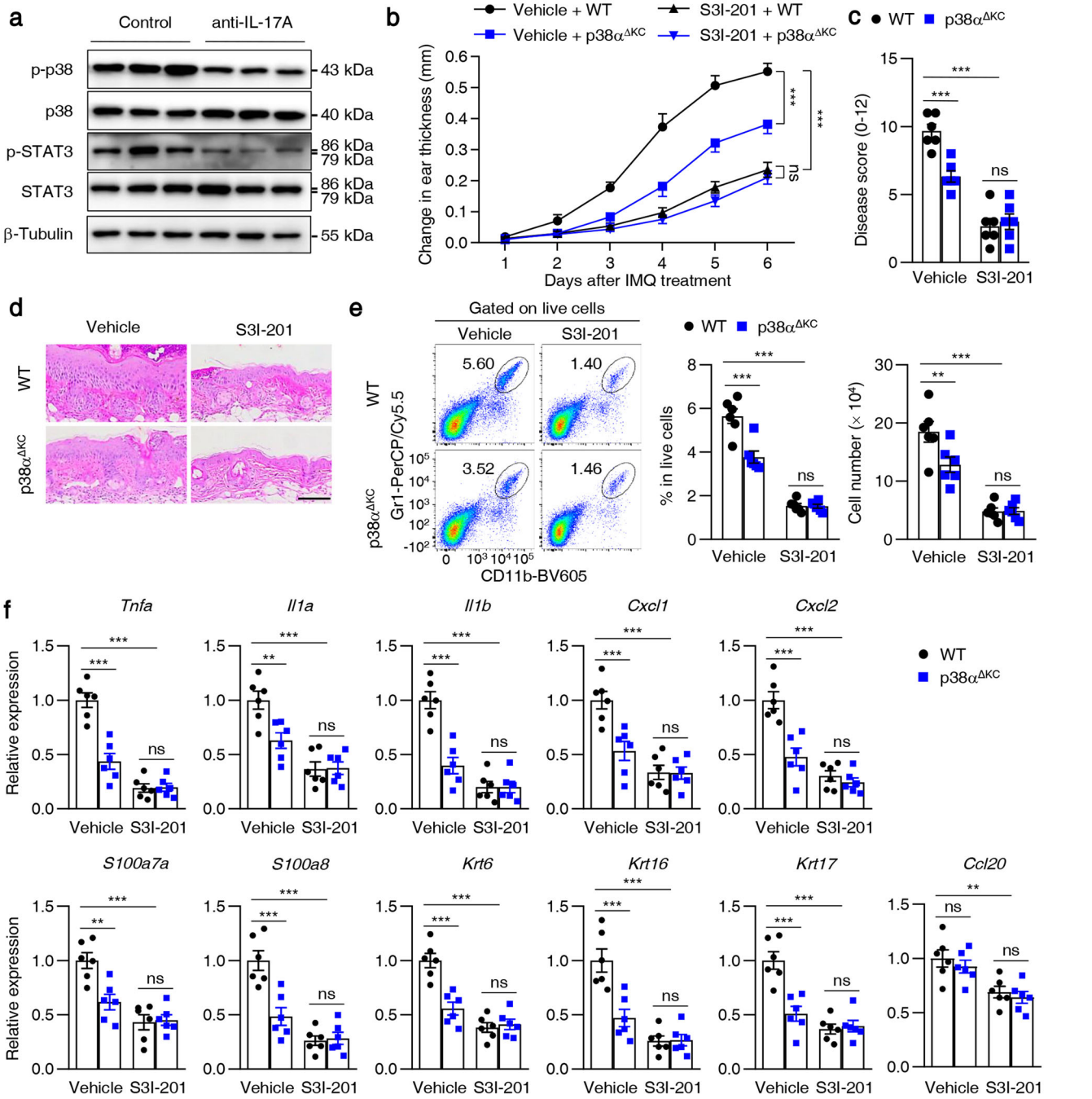


Fig. 7 | p38α-STAT3 axis in KCs is an important target for psoriasis treatment. **a** WT mice topically treated with IMQ for six consecutive days were intraperitoneally administered with the antibody against IL-17A (anti-IL-17A) or control IgG 4 h after IMQ treatment and the activities of p38 and STAT3 in KCs were analyzed by Western blot. **b**–**f** WT and p38α$^{\Delta KC}$ mice topically treated with IMQ for six consecutive days were intradermally injected with STAT3 inhibitor S3I-201 or control vehicle at days 1, 3, and 5 ($n = 6$): changes in ear thickness (**b**), disease severity score (**c**); histopathological changes in skin sections (**d**); infiltration of neutrophils (**e**) and relative expression of inflammation-related genes (**f**) in skin tissue. Scale bar: 100 μm. All the assays were replicated two times with consistent results. Data represent mean ± SEM. Two-way ANOVA (**b**) and two-way ANOVA with Bonferroni post-tests (**c**, **e**, **f**) were performed. $^{**}P < 0.01$; $^{***}P < 0.001$; ns not significant.

Krt10, and *Krt14*, between IMQ-treated wild-type mice and p38α$^{\Delta KC}$ mice indicates that p38α signaling might not be required for KC differentiation. Furthermore, p38α has no effect on KC apoptosis in psoriasis despite it participates in apoptosis in sunitinib-treated primary oral KCs[35]. In the current study, we show that during the initial phase of psoriasis, exogenous stimuli can activate p38-STAT3 axis in KCs to produce proinflammatory cytokines and chemokines, as well as damage-associated molecular patterns, which further promote the infiltration and activation of LCs and other immune cells. Moreover, exogenous stimuli can also directly activate p38 activity in LCs to produce IL-23 and IL-6, which promoting the secretion of IL-17 from T17 cells[19]. In the maintenance phase of psoriasis, IL-17 further activates p38-STAT3 axis in KCs to produce proinflammatory cytokines and chemokines, thereby amplifying the inflammatory response of psoriasis. Thus, p38α-mediated cross-talk between KCs and LCs generates the inflammatory and immune circuits responsible for the initiation, progression and persistence of the psoriasiform diseases.

The IL-23/IL-17 axis has been well studied in psoriasis pathogenesis and clinical application[1,5,33]. Biologics targeting IL-23, IL-17 and IL-17RA have been approved and widely used in the clinical treatment of psoriasis, which benefit most of psoriasis patients in the past decade[1,36]. Although the therapeutics have obtained excellent efficacy, there are still some unavoidable side effects, including nasopharyngitis, upper respiratory tract or systemic infections, injection-site reaction, and headache[1,36]. These side effects are largely due to the widespread expression of IL-17R in multiple cell types and the protective roles of IL-17 in other tissues and diseases. Therefore, it is necessary to develop drugs with higher specificity. Although IL-17 significantly influences phenotype determination in the IMQ-induced psoriasis model[5], p38α signaling in KCs does not affect the expression of IL-23 and IL-17 family cytokines, as well as the differentiation and recruitment of T17 cells. However, it specifically mediates the downstream inflammatory signals of IL-23/IL-17 axis in KCs in a STAT3-dependent manner. Inhibition of STAT3 signaling could alleviate disease severity and decrease neutrophil and macrophage recruitment, as well as IL-17 expression. Thus, it will be very interesting to elucidate the direct targets of p38α in KCs during psoriasis pathogenesis in future study. Moreover, certain chemokines such as CXCL1 and CXCL2, which critically guide neutrophil and macrophage recruitment, are regulated by p38α-STAT3 axis in KCs. Published papers have shown that p38 MAPK in other cell types can also controls neutrophil trafficking in different mechanisms[37–42]. Whether p38-STAT3 axis in KCs will take advantage of these mechanisms to mediate the pathogenesis of psoriasis will need further in-depth study in the future. Notably, although *Ccl20* is known as a factor upregulated by IL-17 and STAT3 in KCs[43], p38α signaling does not affect the gene expression of *Ccl20*. It has been shown that *Ccl20* promoter region contains AP-1 binding sites[44] and AP-1 activity can promote *Ccl20* production in human neonatal foreskin KCs[45] and primary normal human epidermal KCs[46]. Considering that p38α deficiency can upregulate the phosphorylation of c-Jun and thereby enhancing the transcriptional activity of AP-1 binding site[47], which might counteract the role of STAT3 in regulating the expression of *Ccl20*. All these data highlight a central role of the p38-STAT3 axis in KCs in the regulation of psoriasis pathogenesis and p38α-STAT3 axis in KCs might be as an effective therapeutic target to treat psoriasis.

Certain published papers have shown the activation of the epidermal p38 pathway by IMQ models or IL-17A is involved in psoriasis[48–50]. As the epidermal cells include many cell types such as KCs and immune cells, they might play different roles in psoriasis pathogenesis. Our previous study has demonstrated that p38α signaling in LCs, but not in dermal DCs or T cells, is critical for disease development[19], highlighting to further define the specific cell types of p38α in the pathogenesis of psoriasis is important. Considering that KCs is the predominant cells in epidermis and the cells expressing K14 can more accurately represent KCs than epidermis, in the current study, we have used the genetic model of *Mapk14*$^{flox/flox}$K14-Cre mice to better elucidate the cellular mechanism of p38α in psoriasis pathogenesis.

The activity of p38α is markedly increased in lesional psoriatic skin and animal models[16–19]. Inhibition of p38 can reduce the symptoms of psoriasis by either *intraperitoneally* injected p38 inhibitor prior to IMQ treatment or applied p38 inhibitor along with IMQ treatment[23,51]. To further examine whether p38 inhibitors would have a therapeutic effect on IMQ-induced psoriasis, we have *intraperitoneally* injected p38 inhibitor into the mice after the onset of diseases (3 days after IMQ treatment) and found that this treatment could alleviate the ongoing psoriasis development[19]. However, considering the potential liver toxicity caused by systemic application of SB203580, in the current study, we *intradermally* injection of lower doses of SB203580 into the mice after the onset of diseases (3 days after IMQ treatment) and found that this treatment can also achieve a good therapeutic effect, which is more in line with clinical application.

Until now, clinical application of p38 inhibitors is limited[52]. In addition to the liver toxicity, its cellular specificity is also a significant factor. The cell type-specific inflammatory function of p38α can coordinate pro- and anti-inflammatory gene expression in sodium dodecyl sulfate-induced chronic skin inflammation[53].Considering that KCs are the main cell type in skin tissues and the roles of KC p38α signaling in promoting inflammatory response and KC hyperproliferation both in initiation phase and in maintenance phase of psoriasis, inhibiting of p38α signaling pathway in KCs is a promising new approach to prevent psoriasis aggravation.

Methods

Experimental animals

Mapk14^{flox} mice have been described previously[54,55]. K14-Cre mice were kindly provided by Dr. Gang Ma (Shanghai Jiao Tong University, China)[56]. We purchased C57BL/6 mice from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China) and SPF Biotechnology Co., Ltd (Beijing, China). All mice were backcrossed to C57BL/6 background for at least 9 generations. Age- and sex-matched mice at 6 to 10 weeks of age were used for all experiments. Male and female were used in all experiments, as we have not observed sex difference in any of the measured endpoints. Wild-type or Cre^{+} littermate control mice were used as control. No adverse effects due to Cre expression itself were observed in these studies. All mice were bred and maintained in specific pathogen-free condition. This study was approved by the Experimental Animal Center of Guangdong Medical University and the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine. We have complied with all relevant ethical regulations for animal use.

Psoriasis mouse model

IMQ-induced mouse psoriasiform skin disease model was indued by topically treating both sides of the ear of each mouse with 25 mg IMQ cream (5%) (3 M Pharmaceuticals or MedShine) daily for six consecutive days from day 0 to day 5. IL-23-induced experimental psoriasis model was established by intradermally injecting 500 ng IL-23 (eBioscience) to the ear of each mouse every other day on days 0, 2, 4, and 6. IL-17A-induced experimental psoriasis model was established by intradermally injecting 500 ng IL-17A (R&D) to the ear of each mouse daily for four consecutive days from day 0 to day 3. Ear thickness was measured daily by a micrometer. The severity of erythema, scaling, and thickening were independently scored as follows: 0 = none, 1 = slight, 2 = moderate, 3 = marked, and 4 = very marked, and their cumulative score served as the disease severity score (scale, 0 to 12). In some experiments, IMQ-treated mice were intradermally injected with STAT3 activator Colivelin (Selleck) at a dose of 0.1 mg/kg body weight or STAT3 inhibitor S3I-201 (Selleck) at a dose of 0.5 mg/kg body weight on days 1, 3 and 5. For pharmacological inhibition of p38, IMQ-treated mice were intradermally injected with p38 inhibitor SB203580 (Merck Calbiochem) at a dose of 30 μg/kg body weight on days 3-5. Liquid Colivelin dissolved in water, and liquid S3I-201 and liquid SB203580 dissolved in dimethyl sulfoxide (DMSO) were diluted with phosphate-buffered saline (PBS) for injection into mice. Control mice were injected with same volume of water or DMSO diluted with PBS on the same schedule. For IL-17A blockade, antibody against IL-17A (2 mg/kg body weight, Bio-X-Cell) or control IgG was diluted with PBS and intraperitoneally administered to mice 4 h after IMQ treatment.

Skin cell isolation

Skin cells were isolated as described previously with minor modification[19]. Briefly, mouse ears were collected from euthanized mice and divided into dorsal and ventral halves. After carefully removing cartilage and fat tissue, the ear tissue was spread on the surface of 0.5% trypsin (w/v) (Gibco) and incubated at 37°C for 45 min for separating the epidermal and dermal tissues. The separated epidermal and dermal tissues were cut into pieces and digested respectively in 1 mg/ml and 1.5 mg/ml type IV Collagenase (Gibco) for 90 min, respectively. Then the cells were thoroughly mixed with a pipette and filtered through a nylon membrane to obtain single-cell suspension.

Primary keratinocyte (KC) culture and analysis

Neonatal mice were euthanized for isolating the entire skin to be divided into four parts and suspended on 1 mg/ml Dispase II (Sigma-Aldrich) at 4 °C overnight in dark. Gently the epidermis was separated and placed on 0.05%

Tryspin-EDTA (w/v) (Gibco) at 37 °C for 10 min. Then the cells were mix thoroughly and filtered through a 40 μm cell strainer. After centrifugation, the cells were resuspended with 154CF medium (Gibco) containing Human Keratinocyte Growth Supplement (Gibco) and cultured in the culture plate pretreated with rat tail collagen. The primary KCs were stimulated with 10 μM R848 (Invivogen) or 100 ng/ml IL-17A (R&D system) for indicated time points for protein analysis and 5 h for mRNA analysis. In Western blot analysis, the band intensity of phosphorylated protein relative to total protein was calculated by ImageJ. In 5-ethynyl-2'-deoxyuridine (Edu) incorporation assay, primary KCs were stimulated with 10 μM R848 (Invivogen) or 100 ng/ml IL-17A (R&D system) for 24 h and subjected to Edu at last 2 h with further staining by EdU Cell Proliferation Kit (Beyotime) according to the manufacturer's instructions. Cells were observed under a fluorescence microscope and Edu^+ positive cells were counted.

Flow cytometry and cell purification

For analysis of surface markers, cells were stained with surface marker antibodies in PBS with 2% fetal bovine serum for 30 min on ice. For intracellular cytokine staining (ICS), cells were pre-stimulated with 1 μM ionomycin (Sigma-Aldrich) and 50 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences) and GolgiPlug (BD Biosciences) for 5 h and ICS was performed with Intracellular Fixation & Permeabilization Buffer Set (eBioscience). Intracellular phosphorylation antibody staining was performed with Lyse/Fix Buffer and Perm Buffer III (BD Biosciences). Staining of transcriptional factor, active caspase-3 and BrdU was performed with Foxp3/Transcription Factor Staining Buffer Set (eBioscience), APC BrdU Flow Kit (BD Biosciences, Cat No. 552598, used at 1 μl/test), and PE Active Caspase-3 Apoptosis Kit (BD Biosciences, Cat No. 550914, used at 10 μl/test), respectively. Antibodies used in this study include: Fixable Viability Dye (FVD, eBioscience, Cat No. 65-0865-18, 1:1000), anti-CD45 (clone: 30-F11, eBioscience, Cat No. 48-0451-82, used at 1 μg/ml; BD Biosciences, Cat No. 564279, used at 0.5 μg/ml), anti-EpCAM (clone: G8.8, eBioscience, Cat No. 12-5791-82 or 17-5791-82, used at 0.5 μg/ml), anti-CD11b (clone: M1/70, eBioscience, Cat No. 48-0112-82, used at 1 μg/ml; BD Biosciences, Cat No. 563015, used at 0.5 μg/ml), anti-F4/80 (clone: BM8, eBioscience, Cat No. 17-4801-82, used at 0.5 μg/ml), anti-γδTCR (clone: eBioGL3, eBioscience, Cat No. 17-5711-82 or 25-5711-82, used at 0.5 μg/ml), anti-TCRβ (clone: H57-597, eBioscience, Cat No. 12-5961-82 or 45-5961-82, used at 0.5 μg/ml), anti-CD4 (clone: RM4-5, eBioscience, Cat No. 48-0042-82, used at 1 μg/ml), anti-CD8α (clone: 53-6.7, BD Biosciences, Cat No. 563152, used at 0.5 μg/ml), anti-CD11c (clone: N418, eBioscience, Cat No. 25-0114-82, used at 0.5 μg/ml), anti-MHC II (clone: M5/114.15.2, eBioscience, Cat No. 11-5321-85, used at 1 μg/ml), anti-CD40 (clone: 1C10, eBioscience, Cat No. 17-0401-81, used at 0.5 μg/ml), anti-CD80 (clone: 16-10A1, BD Biosciences, Cat No. 740888, used at 0.5 μg/ml), anti-CD86 (clone: GL1, eBioscience, Cat No. 48-0862-82, used at 1 μg/ml), anti-CD44 (clone: IM7, eBioscience, Cat No. 17-0441-82, used at 0.5 μg/ml), anti-CD62L (clone: MEL-14, eBioscience, Cat No. 12-0621-82, used at 0.5 μg/ml), anti-IL-17 (clone: eBio17B7, eBioscience, Cat No. 25-7177-82, used at 2 μg/ml), anti-IFNγ (clone: XMG1.2e, eBioscience, Cat No. 11-7311-82, used at 2 μg/ml), anti-IL-4 (clone: 11B11, eBioscience, Cat No. 12-7041-82, used at 2 μg/ml), anti-Foxps3 (clone: FJK-165, eBioscience, Cat No. 12-5773-82, used at 2 μg/ml), anti-Gr1 (clone: 1A8, BD Biosciences, Cat No. 560602, used at 0.5 μg/ml); anti-CD64 (clone: X54-5/7.1, Biolegend, Cat No. 139309, used at 0.5 μg/ml); anti-phospho-p38 (clone: 28B10, CST, Cat No. 4551, 1:50). Flow cytometry was performed with BD LSR Fortessa X-20 and analyzed with FlowJo-v10. FVD^- cells were gated as live cells. $CD45^-EpCAM^+$ cells of skin cells were gated as KCs[57] (Supplementary Fig. 1a). KCs of skin tissue were sorted with Beckman MoFlo XDP.

Hematoxylin and eosin, immunohistochemistry, and immunofluorescence staining

Skin issues were fixed in formalin, embedded in paraffin, and cut into 6 μm thickness longitudinal sections of for histological studies. The mouse skin paraffin sections were stained with hematoxylin and eosin (Beyotime) for hematoxylin and eosin assay, and anti-Ki-67 (clone: Sp6, Abcam, Cat No. ab1667, 1:200) for immunohistochemistry assay according to standard procedures. The histological images were taken with Leica ICC50 W Microscope. For IF analysis, skin tissue was frozen and embedded in Neg-50 (Epredia). The mouse skin frozen sections were prepared as 8 μm thickness and stained with anti-phospho (p)-p38 (clone: D3F9, CST, Cat No. 4511, 1:1000) and anti-p-STAT3 (clone: D3A7, CST, Cat No. 9145, 1:200) according to standard procedures. Samples were observed using a Leica TCS SP8 Laser Confocal Microscope.

Protein and RNA analyses

Skin tissue and cells were lysed and quantified as described previously. Western blot analysis was performed with the following primary antibodies: p38 (clone: D13E1, CST, Cat No. 8690, 1:1000), p-p38 (clone: D3F9, CST, Cat No. 4511, 1:1000), STAT3 (clone: D3Z2G, CST, Cat No. 12640, 1:1000), p-STAT3 (clone: D3A7, CST, Cat No. 9145, 1:2000), JNK (clone: 56G8, CST, Cat No. 9258, 1:1000), p-JNK (clone: 81E11, CST, Cat No. 4668, 1:1000), ERK (clone:137F5, CST, Cat No. 4695, 1:1000), p-ERK (clone: D13.14.4E, CST, Cat No. 4370, 1:2000), p65 (clone: D14E12, CST, Cat No. 8242, 1:1000), p-p65 (clone: 93H1, CST, Cat No. 3033, 1:1000), Akt (clone: 11E7, CST, Cat No. 4685, 1:1000), p-Akt (clone: D9E, CST, Cat No. 4060, 1:2000), GAPDH (clone: 14C10, CST, Cat No. 2118, 1:1000), and β-Tubulin (clone: 9F3, CST, Cat No. 2128, 1:1000). The relative expression in Western blot analysis was calculated with IntDen in Image J. RNA isolation and reverse transcription were performed as described previously[19]. Quantitative real-time PCR (qRT-PCR) analysis was performed by using Hieff qPCR SYBR Green Master Mix (Yeasen) in a QuantStudio 5 Real time fluorescence quantitative PCR system (Applied Biosystems) with the primers listed in Supplementary Table 1.

Statistics and reproducibility

Data were analyzed with Graph Prism 8 and presented as mean ± SEM from at least three repeats. Two-way analysis of variance (ANOVA) with Bonferroni post-tests was used for multiple comparisons, and two-tailed Student's *t* test was used when two conditions were compared. The number of experimental and technical replicates are specified in the figure legends. *P* values were indicated and $P < 0.05$ was considered significant. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant. "*n*" indicates biological replicates for in vitro experiments and number of mice for in vivo studies.

Data availability

All data are available in the paper and its supplementary information files. All source data in this study are available upon reasonable request and the source data for graphs in figures are provided in Supplementary Data file. Uncropped blots are shown in Supplementary Fig. 8.

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References

1. Ghoreschi, K., Balato, A., Enerback, C. & Sabat, R. Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis. *Lancet* **397**, 754–766 (2021).
2. Zhou, X., Chen, Y., Cui, L., Shi, Y. & Guo, C. Advances in the pathogenesis of psoriasis: from keratinocyte perspective. *Cell Death Dis.* **13**, 81 (2022).
3. Lowes, M. A., Suarez-Farinas, M. & Krueger, J. G. Immunology of psoriasis. *Annu. Rev. Immunol.* **32**, 227–255 (2014).
4. Dainichi, T. et al. The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis. *Nat. Immunol.* **19**, 1286–1298 (2018).
5. van der Fits, L. et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* **182**, 5836–5845 (2009).

6. Zheng, Y. et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* **445**, 648–651 (2007).
7. Albanesi, C., Madonna, S., Gisondi, P. & Girolomoni, G. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front. Immunol.* **9**, 1549 (2018).
8. Trompette, A. & Ubags, N. D. Skin barrier immunology from early life to adulthood. *Mucosal Immunol.* **16**, 194–207 (2023).
9. Lande, R. et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564–569 (2007).
10. Ganguly, D. et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *J. Exp. Med.* **206**, 1983–1994 (2009).
11. Hawkes, J. E., Chan, T. C. & Krueger, J. G. Psoriasis pathogenesis and the development of novel targeted immune therapies. *J. Allergy Clin. Immunol.* **140**, 645–653 (2017).
12. Morizane, S. & Gallo, R. L. Antimicrobial peptides in the pathogenesis of psoriasis. *J. Dermatol.* **39**, 225–230 (2012).
13. Greb, J. E. et al. Psoriasis. *Nat. Rev. Dis. Prim.* **2**, 16082 (2016).
14. Arthur, J. S. & Ley, S. C. Mitogen-activated protein kinases in innate immunity. *Nat. Rev. Immunol.* **13**, 679–692 (2013).
15. Mavropoulos, A., Rigopoulou, E. I., Liaskos, C., Bogdanos, D. P. & Sakkas, L. I. The role of p38 MAPK in the aetiopathogenesis of psoriasis and psoriatic arthritis. *Clin. Dev. Immunol.* **2013**, 569751 (2013).
16. Johansen, C. et al. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br. J. Dermatol.* **152**, 37–42 (2005).
17. Yu, X. J. et al. Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin. *Exp. Mol. Pathol.* **83**, 413–418 (2007).
18. Soegaard-Madsen, L., Johansen, C., Iversen, L. & Kragballe, K. Adalimumab therapy rapidly inhibits p38 mitogen-activated protein kinase activity in lesional psoriatic skin preceding clinical improvement. *Br. J. Dermatol* **162**, 1216–1223 (2010).
19. Zheng, T. et al. p38alpha signaling in Langerhans cells promotes the development of IL-17-producing T cells and psoriasiform skin inflammation. *Sci. Signal.* **11**, eaao1685 (2018).
20. Zhao, W. et al. MAPK phosphatase-1 deficiency exacerbates the severity of imiquimod-induced psoriasiform skin disease. *Front. Immunol.* **9**, 569 (2018).
21. Kjellerup, R. B., Johansen, C., Kragballe, K. & Iversen, L. The expression of dual-specificity phosphatase 1 mRNA is downregulated in lesional psoriatic skin. *Br. J. Dermatol.* **168**, 339–345 (2013).
22. Mihara, K., Elliott, G. R., Boots, A. M. & Nelissen, R. L. Inhibition of p38 kinase suppresses the development of psoriasis-like lesions in a human skin transplant model of psoriasis. *Br. J. Dermatol.* **167**, 455–457 (2012).
23. Sakurai, K. et al. Cutaneous p38 mitogen-activated protein kinase activation triggers psoriatic dermatitis. *J. Allergy Clin. Immunol.* **144**, 1036–1049 (2019).
24. Sumer, C., Boz Er, A. B. & Dincer, T. Keratin 14 is a novel interaction partner of keratinocyte differentiation regulator: receptor-interacting protein kinase 4. *Turk. J. Biol.* **43**, 225–234 (2019).
25. Tortola, L. et al. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J. Clin. Invest.* **122**, 3965–3976 (2012).
26. Sugiura, K. Role of interleukin 36 in generalised pustular psoriasis and beyond. *Dermatol. Ther. (Heidelb.)* **12**, 315–328 (2022).
27. Watanabe, H. et al. Functional characterization of IL-17F as a selective neutrophil attractant in psoriasis. *J. Invest. Dermatol.* **129**, 650–656 (2009).
28. Johnston, A. et al. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. *J. Immunol.* **190**, 2252–2262 (2013).
29. Senra, L. et al. Keratinocyte-derived IL-17E contributes to inflammation in psoriasis. *J. Invest. Dermatol.* **136**, 1970–1980 (2016).
30. Ni, X. et al. IL-17D-induced inhibition of DDX5 expression in keratinocytes amplifies IL-36R-mediated skin inflammation. *Nat. Immunol.* **23**, 1577–1587 (2022).
31. Kastelan, M., Prpic-Massari, L. & Brajac, I. Apoptosis in psoriasis. *Acta Dermatovenerol. Croat.* **17**, 182–186 (2009).
32. Singh, T. P., Zhang, H. H., Hwang, S. T. & Farber, J. M. IL-23- and imiquimod-induced models of experimental psoriasis in mice. *Curr. Protoc. Immunol.* **125**, e71 (2019).
33. Martin, D. A. et al. The emerging role of IL-17 in the pathogenesis of psoriasis: preclinical and clinical findings. *J. Invest. Dermatol.* **133**, 17–26 (2013).
34. Veras, F. P. et al. Pyruvate kinase M2 mediates IL-17 signaling in keratinocytes driving psoriatic skin inflammation. *Cell Rep.* **41**, 111897 (2022).
35. Fukada, S. et al. Sunitinib promotes apoptosis via p38 MAPK activation and STAT3 downregulation in oral keratinocytes. *Oral Dis.* **30**, 639–649 (2022).
36. Kolli, S. S., Kepley, A. L., Cline, A. & Feldman, S. R. A safety review of recent advancements in the treatment of psoriasis: analysis of clinical trial safety data. *Expert Opin. Drug Saf.* **18**, 523–536 (2019).
37. Smolen, J. E. et al. L-selectin signaling of neutrophil adhesion and degranulation involves p38 mitogen-activated protein kinase. *J. Biol. Chem.* **275**, 15876–15884 (2000).
38. Tandon, R., Sha'afi, R. I. & Thrall, R. S. Neutrophil beta2-integrin upregulation is blocked by a p38 MAP kinase inhibitor. *Biochem. Biophys. Res. Commun.* **270**, 858–862 (2000).
39. Yan, W. et al. Role of p38 MAPK in ICAM-1 expression of vascular endothelial cells induced by lipopolysaccharide. *Shock* **17**, 433–438 (2002).
40. Hidalgo, A., Peired, A. J., Wild, M., Vestweber, D. & Frenette, P. S. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. *Immunity* **26**, 477–489 (2007).
41. Liu, X. et al. Bidirectional regulation of neutrophil migration by mitogen-activated protein kinases. *Nat. Immunol.* **13**, 457–464 (2012).
42. Molinar-Inglis, O. et al. Phosphoproteomic analysis of thrombin- and p38 MAPK-regulated signaling networks in endothelial cells. *J. Biol. Chem.* **298**, 101801 (2022).
43. Bae, H. C. et al. RIP4 upregulates CCL20 expression through STAT3 signalling in cultured keratinocytes. *Exp. Dermatol.* **27**, 1126–1133 (2018).
44. Zhao, L., Xia, J., Wang, X. & Xu, F. Transcriptional regulation of CCL20 expression. *Microbes Infect.* **16**, 864–870 (2014).
45. Kanda, N. et al. Prolactin enhances basal and IL-17-induced CCL20 production by human keratinocytes. *Eur. J. Immunol.* **39**, 996–1006 (2009).
46. Li, H. et al. Cyr61/CCN1 induces CCL20 production by keratinocyte via activating p38 and JNK/AP-1 pathway in psoriasis. *J. Dermatol. Sci.* **88**, 46–56 (2017).
47. Zheng, T. et al. Protein kinase p38alpha signaling in dendritic cells regulates colon inflammation and tumorigenesis. *Proc. Natl Acad. Sci. USA* **115**, E12313–E12322 (2018).
48. Kanemaru, K. et al. Phospholipase Cdelta1 regulates p38 MAPK activity and skin barrier integrity. *Cell Death Differ.* **24**, 1079–1090 (2017).
49. Mose, M., Kang, Z., Raaby, L., Iversen, L. & Johansen, C. TNFalpha- and IL-17A-mediated S100A8 expression is regulated by p38 MAPK. *Exp. Dermatol.* **22**, 476–481 (2013).
50. Johansen, C., Bertelsen, T., Ljungberg, C., Mose, M. & Iversen, L. Characterization of TNF-alpha- and IL-17A-mediated synergistic induction of DEFB4 gene expression in human keratinocytes through IkappaBzeta. *J. Invest. Dermatol.* **136**, 1608–1616 (2016).
51. Lee, J. H. et al. NJK14047 inhibition of p38 MAPK ameliorates inflammatory immune diseases by suppressing T cell differentiation. *Int. Immunopharmacol.* **130**, 111800 (2024).

52. Schindler, J. F., Monahan, J. B. & Smith, W. G. p38 pathway kinases as anti-inflammatory drug targets. *J. Dent. Res.* **86**, 800–811 (2007).
53. Kim, C. et al. The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. *Nat. Immunol.* **9**, 1019–1027 (2008).
54. Nishida, K. et al. p38alpha mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol. Cell. Biol.* **24**, 10611–10620 (2004).
55. Huang, G. et al. Signaling via the kinase p38alpha programs dendritic cells to drive TH17 differentiation and autoimmune inflammation. *Nat. Immunol.* **13**, 152–161 (2012).
56. Huang, S. et al. Involvement of epithelial Wntless in the regulation of postnatal hair follicle morphogenesis. *Arch. Dermatol. Res.* **307**, 835–839 (2015).
57. Sakamoto, K., Goel, S., Funakoshi, A., Honda, T. & Nagao, K. Flow cytometry analysis of the subpopulations of mouse keratinocytes and skin immune cells. *STAR Protoc.* **3**, 101052 (2022).

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Author contributions

T.Z. performed experiments, analyzed the data, prepared figures, and drafted the manuscript. J.D., J.W., S.X., H.H., J.S., L.Z., H.C., and J.L. performed experiments. Y.W., S.O., and M.Y. contributed to data analysis and manuscript revising. K.O. provided mouse models. X.L. provided reagents. G.H. designed experiments, analyzed the data, revised the manuscript, and provided overall direction. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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