

IkappaBzeta in immune cells controls Th17-Th1 balance in imiquimod-induced psoriasis models

Yusuke Inada

Wakayama Medical University

Takashi Baba

Wakayama Medical University

Yutaka Inaba

Wakayama Medical University

Masatoshi Jinnin

Wakayama Medical University

Norimitsu Inoue

`inoue-no@wakayama-med.ac.jp`

Wakayama Medical University

Article

Keywords: IkBz, psoriasis, Th1 cell, Th17 cell, IL-1 β , TNF α

Posted Date: October 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11022984/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Psoriasis is a chronic inflammatory skin disease in which the IL-23/IL-17 axis and TNF α play crucial roles in disease initiation and persistence. I κ Bz is a transcriptional regulator that is involved in the expression of the psoriasis-associated genes in keratinocytes. In this study, The aim of this study was to elucidate the contribution of I κ Bz in immune cells to psoriatic inflammation. We demonstrated that I κ Bz critically regulates the balance between Th17 and Th1 inflammation in imiquimod-induced psoriasis-model mice. Hematopoietic cell-specific *Nfkbiz* deficiency (Tie2-Cre) failed to improve clinical severity and epidermal hyperplasia, whereas keratinocyte-specific *Nfkbiz* deficiency resulted in a marked reduction of these features. Tie2-Cre mice exhibited a shift toward the Th1-dominant inflammatory profile, which was characterized by greatly increased transcription of Th1 cell marker gene *Tbx21* as well as the Th1 cell-inducing cytokine *Il12a* and inflammatory cytokines *Il1b*, and *Tnf*, accompanied by reduced expression of *Il17a*. The expression of Th1 cytokine gene, *Ifng*, also showed an upward trend. Therapeutic blockage with anti-TNF α and anti-IL-1 β antibodies effectively ameliorated the Th1-skewed inflammation observed in Tie2-Cre mice treated with imiquimod. Combined inhibition of TNF α and IL-1 β is suggested by these findings to represent a potential therapeutic strategy for psoriasis with Th1-dominant inflammatory features.

Introduction

Psoriasis vulgaris is a chronic, recurrent, immune-mediated skin disease that is characterized by excessive epidermal proliferation and abnormal keratinization^{1,2}. The initiation and persistence of psoriatic inflammation involve complex interactions among keratinocytes and various resident and infiltrating immune cells at the dermo-epidermal junctions.

The IL-23/IL-17 axis and TNF α play pivotal roles in psoriasis pathogenesis. The clinical efficacy of monoclonal antibodies against IL-12/23p40, IL-23p19, IL-17A/F, IL-17 receptor A, and TNF α has demonstrated the critical involvement of the IL-23/IL-17 pathway and TNF α in this disease^{1,3}. Genome-wide association studies have identified psoriasis-associated SNPs in genes encoding IL-23 (*IL23A* and *IL12B*) or its downstream signaling components, such as *IL23R*, *TYK2*, *STAT3*, and *TRAF31P2*⁴⁻⁷. IL-23 is produced by specific subsets of dendritic cells in psoriatic lesions, such as CD11c⁺BDCA⁻ cells, or IL4I1⁺CD200⁺CCR7⁺ cells^{6,8}. It promotes the stabilization of Th17 cells or Tc17 cells through induction of the transcription factor ROR γ t⁹⁻¹¹. This stabilization exacerbates IL-17 production, which acts on keratinocytes to induce the transcription of psoriasis-associated genes, including antimicrobial peptides, chemokines, and inflammatory cytokines¹². TNF α is produced in monocytes, macrophages, and dendritic cells and acts synergistically with IL-17A (which is produced by T cells) on keratinocytes, thereby exacerbating psoriatic inflammation¹³.

Transcription of psoriasis-associated genes downstream of IL-17 receptors in keratinocytes critically depends on the *Nfkbiz* genes. I κ Bz, which is encoded by the *Nfkbiz* gene, is an atypical nuclear I κ B

protein that interacts with NF- κ B subunit p50 and regulates the transcription of a subset of NF- κ B-dependent genes. In imiquimod-induced psoriasis models, *Nfkbiz*-deficient mice exhibit marked reductions in epidermal thickness, inflammatory cytokines, and antimicrobial peptide expression¹⁴. Keratinocyte-specific *Nfkbiz* deficiency is sufficient to suppress psoriasiform skin inflammation induced by imiquimod or IL-36¹⁵. *Nfkbiz* deficiency in CD4⁺ T cells has also been shown to impair Th17 development and to reduce IL-17A expression, thereby protecting mice from experimental autoimmune encephalomyelitis¹⁶. In macrophages, I κ Bz induces secondary response genes including IL-6 and IL-12p40, in response to Toll-like receptor agonists or IL-1 β ¹⁷. Despite these established roles of I κ Bz in both keratinocytes and immune cells, the extent to which immune cell-specific I κ Bz contributes to the initiation and polarization of psoriatic inflammation remains unclear.

In this study, we analyzed the role of I κ Bz in immune cells in the development of psoriatic inflammation using hematopoietic cell-specific *Nfkbiz*-deficient mice.

Results

Pathogenic features of imiquimod-induced psoriasis in keratinocyte-specific or hematopoietic cell-specific *Nfkbiz* deficient mice

To elucidate the roles of I κ Bz in immune cells in the psoriasis pathogenesis, we compared phenotypical severity and epidermal thickness in imiquimod-treated skin lesions from hematopoietic cell-specific *Nfkbiz* deficient (Tie2-Cre) mice (*Nfkbiz*^{flox/flox};Tie2-Cre) and keratinocyte-specific *Nfkbiz* deficient (K5-Cre) mice (*Nfkbiz*^{flox/flox};K5-Cre) with those of control mice (*Nfkbiz*^{flox/flox}). K5-Cre mice exhibited marked improvements in erythema and desquamation following imiquimod treatment (Fig. 1a), as previously reported¹⁴. In contrast, there was no apparent improvement in phenotypic appearance in Tie2-Cre mice (Fig. 1b). Histological analysis revealed a significant reduction in epidermal thickness in K5-Cre mice compared with control mice (Figs. 1c and 1e) but there was no difference in epidermal thickness between Tie2-Cre mice and control mice (Figs. 1d and 1f). The I κ Bz deficiency in immune cells was suggested to be insufficient to attenuate the phenotypic and histological features of imiquimod-induced psoriasiform dermatitis.

Immune cell infiltration in imiquimod-induced psoriasiform skin lesions of keratinocyte-specific and hematopoietic cell-specific *Nfkbiz*-deficient mice

To characterize immune cell infiltration in imiquimod-treated skin lesions of K5-Cre and Tie2-Cre mice, we quantified infiltrating immune cells in the dermal and epidermal compartments by immunohistochemistry. In K5-Cre mice, the numbers of CD4⁺ and CD8⁺ T cells were not different from those in control mice, whereas F4/80⁺ macrophages and Ly6G⁺ granulocytes were profoundly decreased in the K5-Cre mice (Fig. 2a, left and Fig. 2b). Tie2-Cre mice showed no clear differences from control mice in the numbers of CD4⁺, CD8⁺ T cells, or F4/80⁺ macrophages. There was increase in the number of Ly6G⁺ granulocytes (Fig. 2a, right and Fig. 2c). To assess Th1- and Th17-associated signatures in

psoriatic lesions, we measured the expression of *Tbx21* as the Th1-marker and *Rorc* as the Th17-marker in imiquimod-treated skin lesions by real-time RT-qPCR. K5-Cre mice did not alter the expression of *Tbx21* and *Rorc* (Fig. 2d, left). In contrast, Tie2-Cre mice resulted in the increased expression of both *Rorc* and *Tbx21* relative to control mice and the fold increase in *Tbx21* expression relative to controls was greater than that observed for *Rorc* (Fig. 2d, right). The expression of *Foxp3* mRNA as the regulatory T cell marker did not change in either Tie2-Cre or K5-cre mice (Supplementary Fig. 1).

Distinct cytokine expression profiles in imiquimod-induced psoriasiform skin of K5-Cre and Tie2-Cre mice

Next, we examined whether K5-Cre and Tie2-Cre mice have altered cytokine gene expression profiles in imiquimod-treated skin lesions. First, we compared the Th1/Th17 balance of skin-specific and hematopoietic cell-specific *Nfkbiz* knockout mice with their control mice. Although skin lesions in K5-Cre mice greatly improved, there were significant increases in expression of both *Il17a* and *Ifng*, whereas the expression levels of *Il23a* and *Il12a* (which are involved in the induction of Th17 and Th1 cytokines, respectively) were comparable to those in control mice (Fig. 3a). In contrast, Tie2-Cre mice exhibited a marked upregulation of *Il12a* and an upward trend of *Ifng* in skin lesions. Although *Il17a* expression significantly decreased, no corresponding reduction was observed in *Il23a* expression (Fig. 3b). The expression of Th2 cytokine gene *Il4* was significantly decreased in both K5-Cre (Fig. 3c) and Tie2-Cre mice (Fig. 3d). Next, we examined the expression of innate immune cytokine genes that are known to be involved in the psoriasis pathogenesis in K5-Cre (Fig. 3e), Tie2-Cre mice (Fig. 3f), and control mice. In K5-Cre mice, the expression of *Tnf* was unchanged, while the inflammatory cytokine gene *Il1b* and the psoriasis-associated gene *Il1f6*, which encode IL-36 α , were significantly reduced (Fig. 3e). Conversely, Tie2-Cre mice showed greatly increased expression of *Tnf* and *Il1b*, whereas there was no change in *Il1f6* expression (Fig. 3f). The elevated expression of Th1-associated and inflammatory cytokines, including IFN γ , IL-12, IL-1 β , and TNF α , is suggested by these findings to contribute to psoriasiform inflammation in Tie2-Cre mice, even when IL-17A expression is reduced.

Effect of stimulation with imiquimod and IL-1 β or TNF α on the *Nfkbiz* deficiency in splenocytes

Our *in vivo* experiments showed that the expression of *Il1b* and *Tnf* was greatly increased in Tie2-Cre mice following imiquimod treatment. This suggests that IL-1 β and TNF α should be related to the Th1-dominant pathogenesis of imiquimod-induced psoriatic inflammation in Tie2-Cre mice. To examine whether IL-1 β or TNF α synergistically promotes the expression of Th1-related cytokine genes upon imiquimod stimulation, we performed *ex vivo* experiments using splenocytes isolated from control and Tie2-Cre mice. Stimulation of control splenocytes by imiquimod in combination with IL-1 β or TNF α synergistically enhanced the expression of *Ifng*, and this synergistic effect was further augmented in Tie2-Cre splenocytes (Fig. 4a). IL-1 β and TNF α are suggested by these results to facilitate Th1 induction in imiquimod-treated splenocytes, and the deficiency of *Nfkbiz* in hematopoietic cells apparently further enhances this response.

We next examined whether the expression of IL-1 β and TNF α was differentially regulated in Tie2-Cre splenocytes following stimulation with imiquimod, IL-1 β , TNF α , or their combination. These stimuli did not induce the increased expression of *Tnf* in splenocytes of control mice (Fig. 4b). In contrast, imiquimod alone robustly induced *Tnf* expression in Tie2-Cre splenocytes, and co-stimulation with imiquimod and IL-1 β synergistically enhanced this effect (Fig. 4b, left). However, no such effect was observed with co-stimulation with imiquimod and TNF α (Fig. 4b, right). In both Tie2-Cre and control splenocytes, there tended to be slight increase in *Il1b* expression levels in response to imiquimod stimulation (Fig. 4c). IL-1 β further enhanced expression of *Il1b* induced by imiquimod in Tie2-Cre splenocytes (Fig. 4c, left), but the effect of TNF α on the expression of *Il1b* was limited (Fig. 4C, right), which suggests that IL-1 β acts upstream of TNF α and IL-1 β induction in this imiquimod-induced inflammatory cascade in Tie2-Cre mice.

Amelioration of psoriasis-like inflammation by suppression of IL-1 β and TNF α signaling in hematopoietic cell-specific Nfkbiz knockout mice

Ex vivo analysis demonstrated that stimulation with IL-1 β or TNF α synergistically enhanced imiquimod-induced Th1 cytokine production. To determine whether blockade of these pathways ameliorates psoriasis-like inflammation *in vivo*, we conducted therapeutic experiments by intraperitoneally injecting anti-IL-1 β , anti-TNF α antibodies, or a combination of both antibodies into control mice or Tie2-Cre mice treated with imiquimod. Co-administration of anti-IL-1 β and anti-TNF α antibodies markedly improved erythema and desquamation in both control and Tie2-Cre mice, whereas monotherapy of either anti-IL-1 β or anti-TNF α antibody alone failed to fully resolve these clinical symptoms (Fig. 5a). Histological analysis of H&E-stained skin sections revealed that combination treatment of anti-IL-1 β and anti-TNF α antibodies resulted in a significant reduction in epidermal thickness in both control and Tie2-Cre mice (Fig. 5b and 5c). We next examined the transcriptional expression of *Ifng* (Fig. 5d), *Tnf* (Fig. 5e), and *Il1b* (Fig. 5f) in imiquimod-treated skin lesions obtained from antibody-treated mice. Co-administration of anti-IL-1 β and anti-TNF α antibodies into Tie2-Cre mice resulted in a downward trend in the transcriptional levels of *Ifng*, *Tnf*, and *Il1b*. This is consistent with the observed improvement in phenotypical appearance and epidermal thickness (Figs. 5d, 5e, and 5f). The Th1-driven inflammation in Tie2-Cre mice is suggested by these results to be effectively attenuated by combined neutralization of IL-1 β and TNF α .

Discussion

Psoriasis is an inflammatory skin disease for which drugs targeting the IL-23/IL-17A pathway are effective^{1,3}. I κ Bz enhances the production of IL-17A in T cells and is deeply involved in the pathogenesis of psoriasis. I κ Bz in keratinocytes has been shown to promote imiquimod-induced psoriasis-like inflammation through the induction of psoriasis-related genes, such as *DEFB4*, *CCL20*, *S100A7*, and *LCN2*¹⁴. Although I κ Bz is highly expressed in Th17 cells and it induces the development from naïve T cells to Th17 cells in cooperation with ROR γ t or ROR α , enhancing the expression of *Il17a*¹⁶, the contribution of I κ Bz in immune cells to the initiation and persistence of psoriatic inflammation has been unclear. In the present study, I κ Bz in immune cells was demonstrated to critically regulate the balance

between Th17 and Th1 inflammation in an imiquimod-induced psoriasis model. Tie2-Cre mice resulted in reduced *Il17a* expression in psoriatic lesions but concomitantly enhanced Th1-associated and inflammatory cytokines, including *Ifng*, *Il12a*, *Il1b*, and *Tnf*. As a consequence, the phenotypical severity and epidermal thickness in Tie2-Cre mice were comparable to those observed in control mice. Blockade therapies using anti-TNF α and anti-IL-1 β antibodies improved the skin inflammation and epidermal thickness in Tie2-Cre mice and showed a tendency to suppress the expression of IFN-g, a Th1 cytokine. Compensatory Th1-skewed responses are suggested to be involved in the development of psoriasiform pathology in hematopoietic cell-specific *Nfkbiz* deficiency despite suppression of IL-17A-mediated inflammation.

Tie2-Cre mice exhibited increased transcriptional levels of *Ifng* and the Th1-specific transcription factor *Tbx21*. Multiple mechanisms are likely to contribute to this Th1-skewing profile in the imiquimod-treated skin lesions in Tie2-Cre mice. Our *ex vivo* experiments using splenocytes indicated that TNF α and IL-1 β synergistically enhanced the expression of *Ifng* induced by the stimulation with imiquimod, and that this synergistic effect was further augmented by the *Nfkbiz* deficiency. Consistently, the expression of Th1-inducing cytokine gene *Il12a* was increased in the imiquimod-treated skin lesions in Tie2-Cre mice. *Nfkbiz* deficiency has been shown in previous studies to reduce IL-17 production in Th17 cells¹⁶, but it enhances IFN γ production in T cells¹⁸. In addition, TNF α signaling through TNFR2 on T cells not only contributes to T cell differentiation toward the Th1 and Th17 lineages, it also reportedly promotes inflammatory cytokine production in Th1 cells¹⁹. Taken together, these findings suggest that the Th1-dominant inflammation observed in Tie2-Cre mice results from the combined effects of altered cytokine signaling and changes in T cell development. Given the complexity of these mechanisms, further studies are required to fully elucidate how I κ B ζ in immune cells regulates Th1 polarization in psoriatic-like inflammation.

The expression levels of *Il12a*, *Tnf*, and *Il1b* showed a great increase in the imiquimod-treated lesion of Tie2-Cre mice. These cytokines are predominantly produced by dendritic cells or macrophages²⁰, but I κ B ζ in murine bone marrow-derived macrophages or peritoneal macrophages has been reported to positively regulate *Il1b* transcription or IL-12p40 production^{17,21}. Macrophages thus may not be the primary contributors to the elevated IL-1 β and IL-12 levels observed in Tie2-Cre mice treated with imiquimod. Notably, IL-1 β has been shown to colocalize predominantly with a neutrophil marker CD66b in psoriatic lesions from human patients²². Consistently, we detected the increased infiltration of Ly6G-positive cells into the imiquimod-treated skin of Tie2-Cre mice. Furthermore, stimulation of splenocytes with TNF α or IL-1 β resulted in increased expression levels of *Tnf* in Tie2-Cre mice. We propose that the enhanced neutrophil infiltration into imiquimod-treated lesions in Tie2-Cre mice leads to increased IL-1 β production, which subsequently amplifies TNF α expression.

Co-administration of neutralizing antibodies against TNF α and IL-1 β ameliorated imiquimod-induced and Th1-skewed psoriasiform inflammation in Tie2-Cre mice. A real-life study enrolling 50 patients who failed secukinumab, an anti-IL-17A monoclonal antibody, suggested that switching to adalimumab (anti-TNF α

monoclonal antibody) or ustekinumab (anti-IL-12/23 antibody) significantly improved the psoriasis area and severity index and dermatology life quality index^{23,24}. These clinical observations mirror our experimental models, in which TNF α blockade suppresses the sustained epidermal thickening in Tie2-Cre mice that exhibit reduced IL-17 expression. If anti-TNF α monoclonal antibodies are ineffective against patients with psoriasis who are refractory to IL-17-targeted therapies, combination therapy with anti-IL-1 β monoclonal antibody such as canakinumab may enhance the efficacy of anti-TNF α antibody monotherapy. To validate this therapeutic concept, further analyses using human psoriatic specimens are necessary to examine the Th1/Th17 balance in the affected area.

Conclusion

In this study, we demonstrated that the I κ B ζ deficiency in immune cells did not suppress imiquimod-induced psoriatic inflammation. The immune cell-specific *Nfkbiz* deficiency suppressed IL-17A expression but exacerbated the Th1-associated inflammatory response, thereby promoting epidermal hyperplasia. Moreover, combined therapeutic blockade of TNF α and IL-1 β ameliorates the Th1-skewed psoriatic inflammation. These findings reveal that I κ B ζ is a key regulator of the Th1/Th17 balance in immune cells in psoriasis mouse models.

Materials and Methods

Mice

C57BL/6J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Tie2-Cre and K5-Cre mice were kindly provided by Dr. Shizuo Akira (Osaka University) and Dr. Junji Takeda (Osaka University), respectively. *Nfkbiz*^{flox/flox} mice (RBRC06410) were provided by RIKEN BRC through the National BioResource Project of the MEXT/AMED, Japan²⁵. Cre-expressing male mice were mated with *Nfkbiz*^{flox/flox} female mice, and their offspring male mice as hematopoietic cell-specific knockout mice (*Nfkbiz*^{flox/flox};Tie2-Cre male mice) or keratinocyte-specific knockout mice (*Nfkbiz*^{flox/flox};K5-Cre male mice) and their control *Nfkbiz*^{flox/flox} male mice were used in *in vivo* experiments. Male mice aged 8–13 weeks were used for all experiments. All mice were maintained under specific pathogen-free conditions.

Psoriasis mouse model

Mice were anesthetized by intraperitoneal injection of a mixed anesthetic cocktail containing medetomidine hydrochloride (0.75 mg/kg; Medetomin; Meiji Seika Pharma Co., Tokyo, Japan), midazolam (4 mg/kg; Dormicum; Astellas Pharma Inc., Tokyo, Japan), and butorphanol (5 mg/kg; Betolfar; Meiji Seika Pharma Co.). The dorsal hair was then shaved using an electric shaver. Starting the following day, only isoflurane (4%; Isoflurane; Viatri Healthcare G.K.) was used for anesthesia, and 5% imiquimod cream (Beselna® Cream; Mochida Pharmaceutical Co., Tokyo, Japan) was applied to the dorsal skin at a dose of 62.5 mg/mouse/day for six consecutive days. On Day 6, mice were euthanized

by cervical dislocation, and dorsal skin tissues were collected for hematoxylin and eosin (H&E) staining, immunofluorescence, and real-time RT-qPCR analysis.

Preparation and stimulation of splenocytes

Spleens were collected from 8–13-week-old male control (*Nfkbiz^{flox/flox}*) and hematopoietic cell-specific knockout (*Nfkbiz^{flox/flox};Tie2-Cre*) mice. The spleens were mechanically dissociated using glass slides in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cell suspensions were centrifuged at 1500 rpm for 5 min at 4°C, and red blood cells were lysed by 3 mL of red blood cell lysis buffer (pluriSelect Life Science, Leipzig, Germany) for 5 min at room temperature. After dilution with RPMI 1640, the cells were filtered through 40 µm cell strainers (Cat#352340, Corning Inc., Corning, NY). The isolated splenocytes were centrifuged again and seeded into round-bottom 96-well plates at 1×10^6 cells/well, followed by stimulation with imiquimod (1 µg/mL), IL-1β (10 ng/mL), TNFα (100 ng/mL), or combination of imiquimod with IL-1β or TNFα for 24 h. After stimulation, the splenocytes were collected for real-time RT-qPCR.

Real-time RT-qPCR analysis

Dorsal skin tissues were immediately frozen by liquid nitrogen and homogenized with RL buffer (Nippon Genetics, Tokyo, Japan) using a Multi-Beads Shocker (Yasui Kikai Co., Osaka, Japan). Total RNA was purified using the FastGene RNA Basic Kit (Nippon Genetics), and cDNA was synthesized using SuperScript™ IV VILO™ Master Mix (Thermo Fisher Scientific, Waltham, MA). Gene expression levels of *Il23a*, *Il17a*, *Il12a*, *Ifng*, *Il4*, *Tnf*, *Il1b*, *Il1f6*, *Foxp3*, *Rorc*, and *Tbx21* were measured using TaqMan probe-based assays (Thermo Fisher Scientific) with eukaryotic 18S rRNA (Cat# 4352930E, Thermo Fisher Scientific) as the internal control. Real-time RT-qPCR was performed using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA), and data were analyzed using the $\Delta\Delta CT$ method. The TaqMan probe IDs used in this assay are as follows: *Il23a* (Mm00518984_m1), *Il17a* (Mm00439619_m1), *Il12a* (Mm00434169_m1), *Ifng* (Mm01168134_m1), *Il4* (Mm00445259_m1), *Tnf* (Mm00457645_m1), *Il1b* (Mm00434228_m1), *Il1f6* (Mm00457645_m1), *Foxp3* (Mm00475162_m1), *Rorc* (Mm01261022_m1), and *Tbx21* (Mm00450960_m1).

Histological and immunofluorescence analysis

Dorsal skin tissues were fixed in 4% paraformaldehyde at 4°C for 48 h, and embedded in paraffin using HistoCore Arcadia embedding center (Leica Biosystems, Nussloch, Germany). Paraffin sections (6 µm) were prepared using rotary microtome RX-860 (Yamato Kohki Industrial, Niiza, Japan) for hematoxylin-eosin (H&E) staining or immunohistochemical staining. Antigen retrieval for immunohistochemical staining was performed by treating the sections at 121°C for 1 min in Tris-EDTA buffer (pH 9.0). For frozen sections, tissues were cryoprotected with 30% sucrose in phosphate-buffered saline, and embedded in OCT compound (Sakura Finetek, Tokyo, Japan). Sections (15 µm) were prepared using a

cryostat (Leica Biosystems). The sections were incubated with primary antibodies overnight at 4°C, followed by incubation with Alexa Fluor 488- and/or Alexa Fluor 568-conjugated secondary antibodies diluted in 0.1% bovine serum albumin at room temperature for 1 h. Autofluorescence from erythrocytes was reduced using the Vector TrueVIEW Autofluorescence Quenching Kit with 4,6-Diamidino-2-phenylindole Dihydrochloride (DAPI) (Vector Laboratories, Newark, CA). Images were captured using a BZ-X800 fluorescence microscope (Keyence, Osaka, Japan). Nine random fields per sample (20× magnification) were analyzed to determine the mean number of marker-positive cells per field. Only cells that were double-positive for DAPI and the marker were counted. The analyzed area extended from the epidermis to the top of the subcutaneous fat layer. All antibodies used in this study are listed in Supplementary Table 1.

Quantification of epidermal thickness

Epidermal thickness was measured on H&E-stained skin sections at nine randomly selected points per mouse, excluding areas containing hair follicles or sweat glands. The representative epidermal thickness for each mouse was comprised of the mean of these nine values. The length of epidermal thickness was quantitated using ImageJ software (NIH, Bethesda, MD).

Antibody-treated psoriasis mouse model

Imiquimod cream was applied to the shaved dorsal skin for six consecutive days. Anti-IL-1 β antibody, anti-TNF α antibody, and isotype control antibody were administered intraperitoneally at 100 μ g/mouse on Day 0 (the first day of imiquimod application) and on Day 3. Dorsal skin tissues were collected on Day 6 for H&E staining and RNA analysis.

Statistical analysis

Statistical analysis was performed using JMP statistical analysis software. Student's t-test was used for comparisons between two groups, and Tukey's post hoc test following two-way ANOVA was used for comparisons among three or more groups. $p < 0.05$ was considered statistically significant.

Declarations

Ethical Approval

All procedures and protocols were approved by the Wakayama Medical University Animal Care and Use Committee (No. 1179). Animal experiments were conducted in accordance with our institutional policies and regulations, which were prepared based on the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan) and the ARRIVE Guidelines.

Conflicts of Interest

The authors declare no competing interests.

Funding

This work was supported by JSPS KAKENHI Grant Numbers JP19H03529 and JP26K10090, and 2022 and 2023 Wakayama Medical University Special Grant-in Aid for Research Projects to N.I., and JSPS KAKENHI Grant Numbers JP22K07155 and JP25K10495, and Takeda Science Foundation to T.B.

Author Contribution

Yusuke Inada: Investigation, Data curation, Writing-original draft, Writing – review and editing.

Takashi Baba: Conceptualization, Funding acquisition, Investigation, Data curation, Writing-original draft, Writing – review and editing.

Yutaka Inaba: Supervision, Writing – review and editing.

Masatoshi Jinnin: Conceptualization, Supervision, Writing – review and editing.

Norimitsu Inoue: Conceptualization, Supervision, Funding acquisition, Investigation, Data curation, Writing-original draft, Writing – review and editing.

Acknowledgement

We thank Dr. Shizuo Akira (Osaka University), Dr. Junji Takeda (Osaka University) and Dr. Takashi Maruyama (Chiba University) for kindly providing Tie2-Cre, K5-Cre, and Nfkbizflox/flox mice, respectively, Dr. Keiichi Katayama and Dr. Hiroshi Tsujimoto (Wakayama Medical University) for discussions. We also thank Kazuko Watase, Yukari Ikeda, and Fumiko Saito (Wakayama Medical University) for technical help. We acknowledge proofreading and editing by Benjamin Phillis, a Board-Certified Editor in the Life Sciences (BELS), at Wakayama Medical University.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Armstrong, A. W., Read, C. & Pathophysiology Clinical Presentation, and Treatment of Psoriasis: A Review. *JAMA* **323**, 1945–1960 (2020).
2. Goncalves, M. B. S., Fonseca, M., Mascarenhas-Melo, F. & Figueiras, A. Advancing insights into psoriasis: from pathogenesis to current and emerging therapies. *Int. Immunopharmacol.* **165**,

- 115429 (2025).
3. Ghoreschi, K., Balato, A., Enerback, C. & Sabat, R. Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis. *Lancet* **397**, 754–766 (2021).
 4. Cargill, M. et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am. J. Hum. Genet.* **80**, 273–290 (2007).
 5. Nair, R. P. et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat. Genet.* **41**, 199–204 (2009).
 6. Tsoi, L. C. et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* **44**, 1341–1348 (2012).
 7. Genetic Analysis of Psoriasis. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat. Genet.* **42**, 985–990 (2010).
 8. Sun, Y. et al. CCR7(+) dendritic cells expressing both IL-23A and IL-12B potentially contribute to psoriasis relapse. *Nat. Commun.* **16**, 7581 (2025).
 9. Fitch, E., Harper, E., Skorcheva, I., Kurtz, S. E. & Blauvelt, A. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. *Curr. Rheumatol. Rep.* **9**, 461–467 (2007).
 10. Liu, J. et al. Single-cell RNA sequencing of psoriatic skin identifies pathogenic Tc17 cell subsets and reveals distinctions between CD8(+) T cells in autoimmunity and cancer. *J. Allergy Clin. Immunol.* **147**, 2370–2380 (2021).
 11. Hipp, A. V., Bengsch, B. & Globig, A. M. Friend or Foe - Tc17 cell generation and current evidence for their importance in human disease. *Discov Immunol.* **2**, kyad010 (2023).
 12. Furue, M., Furue, K., Tsuji, G. & Nakahara, T. Interleukin-17A and Keratinocytes in Psoriasis. *Int J. Mol. Sci* **21** (2020).
 13. Chiricozzi, A. et al. Integrative responses to IL-17 and TNF-alpha in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *J. Invest. Dermatol.* **131**, 677–687 (2011).
 14. Johansen, C. et al. IkappaBzeta is a key driver in the development of psoriasis. *Proc. Natl. Acad. Sci. U S A.* **112**, E5825–5833 (2015).
 15. Lorscheid, S. et al. Keratinocyte-derived IkappaBzeta drives psoriasis and associated systemic inflammation. *JCI Insight* **4** (2019).
 16. Okamoto, K. et al. IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. *Nature* **464**, 1381–1385 (2010).
 17. Yamamoto, M. et al. Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta. *Nature* **430**, 218–222 (2004).
 18. Luger, D. et al. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J. Exp. Med.* **205**, 799–810 (2008).
 19. Alam, M. S. et al. TNF plays a crucial role in inflammation by signaling via T cell TNFR2. *Proc Natl. Acad. Sci. U S A* **118** (2021).

20. Arango Duque, G. & Descoteaux, A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front. Immunol.* **5**, 491 (2014).
21. Daly, A. E., Yeh, G., Soltero, S. & Smale, S. T. Selective regulation of a defined subset of inflammatory and immunoregulatory genes by an NF-kappaB p50-IkappaBzeta pathway. *Genes Dev.* **38**, 536–553 (2024).
22. Fanoni, D. et al. Evidence for a role of autoinflammation in early-phase psoriasis. *Clin. Exp. Immunol.* **198**, 283–291 (2019).
23. Damiani, G. et al. When IL-17 inhibitors fail: Real-life evidence to switch from secukinumab to adalimumab or ustekinumab. *Dermatol. Ther.* **32**, e12793 (2019).
24. Wang, Y., Yang, F., Wang, R. & Luo, S. Paradoxical psoriasis induced by IL-17 antagonists. *Indian J. Dermatol. Venereol. Leprol.* **90**, 623–631 (2024).
25. Okuma, A. et al. Enhanced apoptosis by disruption of the STAT3-IkappaB-zeta signaling pathway in epithelial cells induces Sjogren's syndrome-like autoimmune disease. *Immunity* **38**, 450–460 (2013).

Figures

Figure 1

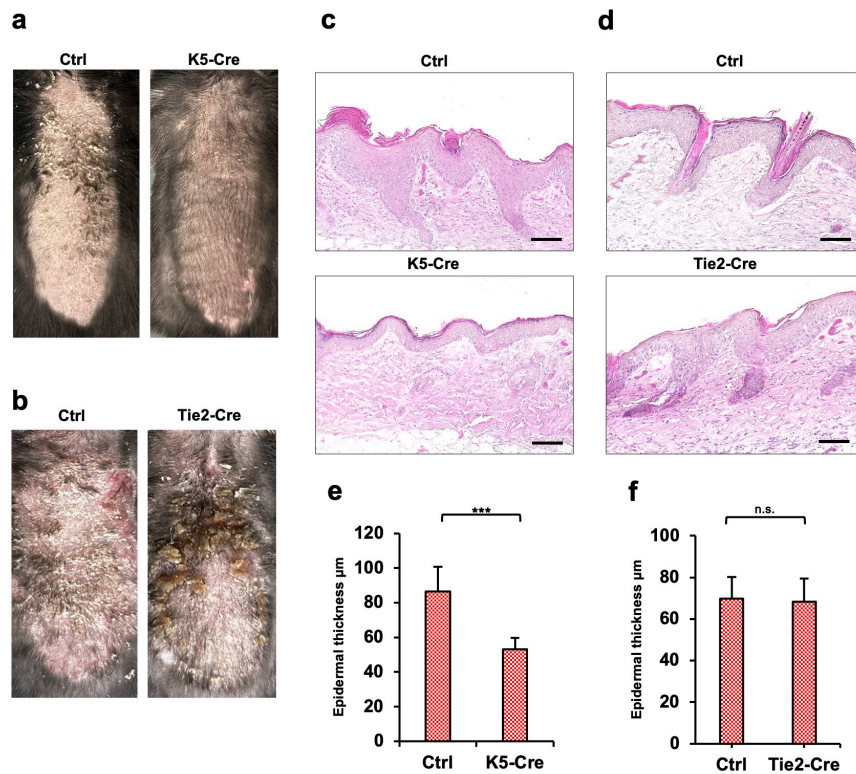


Figure 1

Pathogenic development in the imiquimod-induced psoriasis model in mice with skin- and hematopoietic cell-specific *Nfkbiz* deficiencies.

(a and b) Representative clinical appearances of the dorsal skin from control (ctrl) and *Nfkbiz*^{flox/flox};K5-Cre (K5-Cre) mice (a), and from ctrl and *Nfkbiz*^{flox/flox};Tie2-Cre (Tie2-Cre) mice (b). Mice were treated with

imiquimod cream on the dorsal skin for six consecutive days.

(c and d) H&E-stained sections of dorsal skin from the imiquimod-treated mice corresponding to those shown in panels a and b. Scale bar = 100 μ m.

(e and f) Quantitative analysis of epidermal thickness from H&E-stained sections shown in panels c and d. Epidermal thickness was measured at nine randomly selected sites per sample. Data represent mean $\pm$ SD. *** $p < 0.001$. (e) Ctrl and K5-Cre mice (n = 8 per group). (f) Ctrl and Tie2-Cre mice (n = 10 per group).

Figure 2

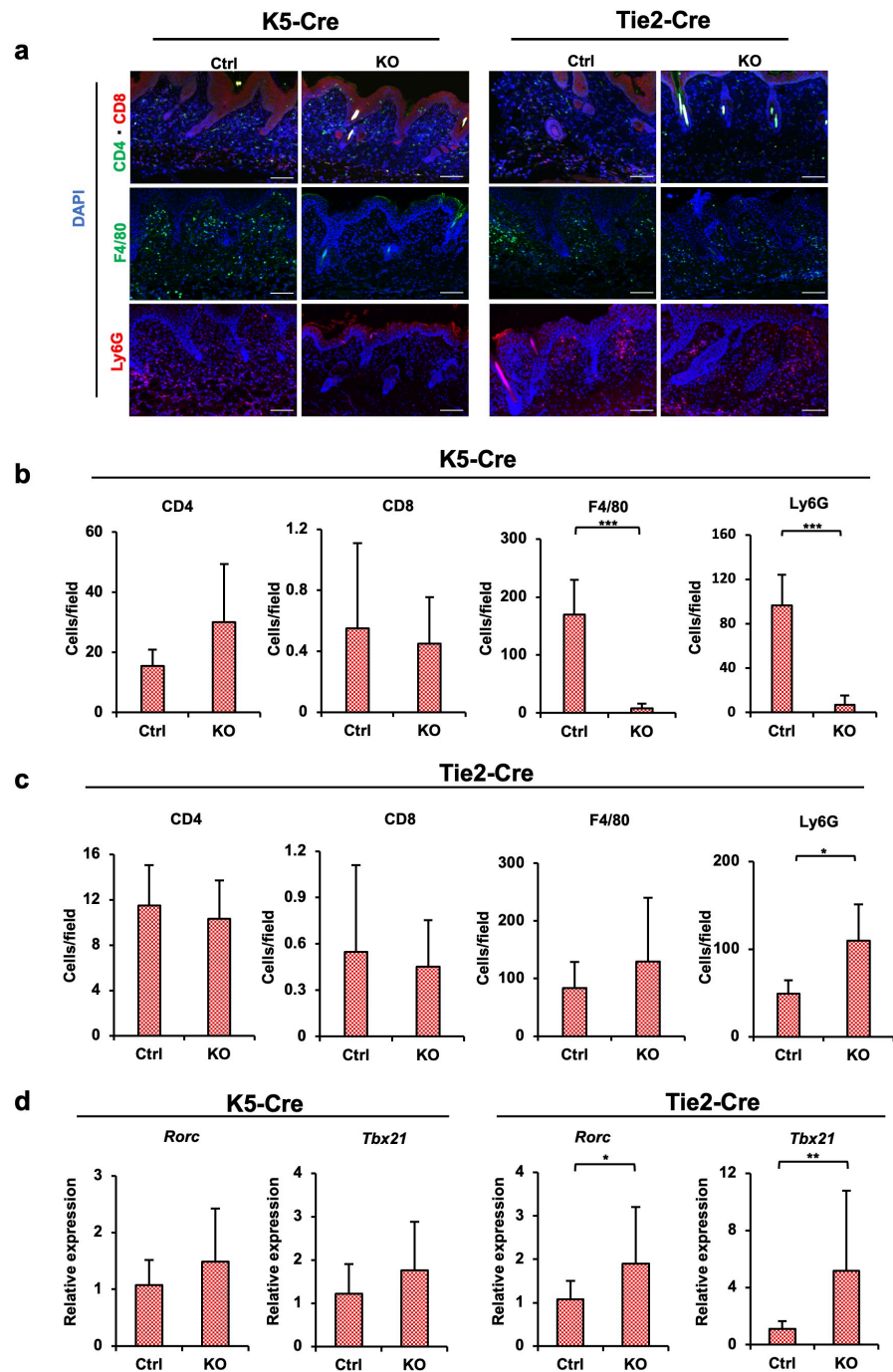


Figure 2

Th1-skewed immune profile in hematopoietic cell-specific *Nfkbiz*-deficient mice.

(a) Representative immunofluorescence images of dorsal skin sections from control (ctrl), *Nfkbiz*^{flox/flox};K5-Cre (K5-Cre) mice (KO), and ctrl and *Nfkbiz*^{flox/flox};Tie2-Cre (Tie2-Cre) mice (KO). These dorsal skin sections were harvested from the mice treated with imiquimod for 6 days and stained with DAPI and antibodies against CD4 and CD8 (Paraffin section), F4/80 (Frozen section), or Ly6G (Frozen section). Scale bar = 100 μ m.

(b and c) Quantification of CD4⁺, CD8⁺, F4/80⁺, and Ly6G⁺ cells in dorsal skin sections corresponding to panels a. For each mouse (n = 5 per group), nine random fields per section were analyzed to calculate mean cell counts.

(d) Quantitative real-time qPCR analysis of *Rorc* or *Tbx21* expression in dorsal skins after six days of imiquimod treatment. The number of mice was as follows: Ctrl and K5-Cre mice (n = 10 per group), and Ctrl and Tie2-Cre mice (n = 15 per group).

Data represent mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3

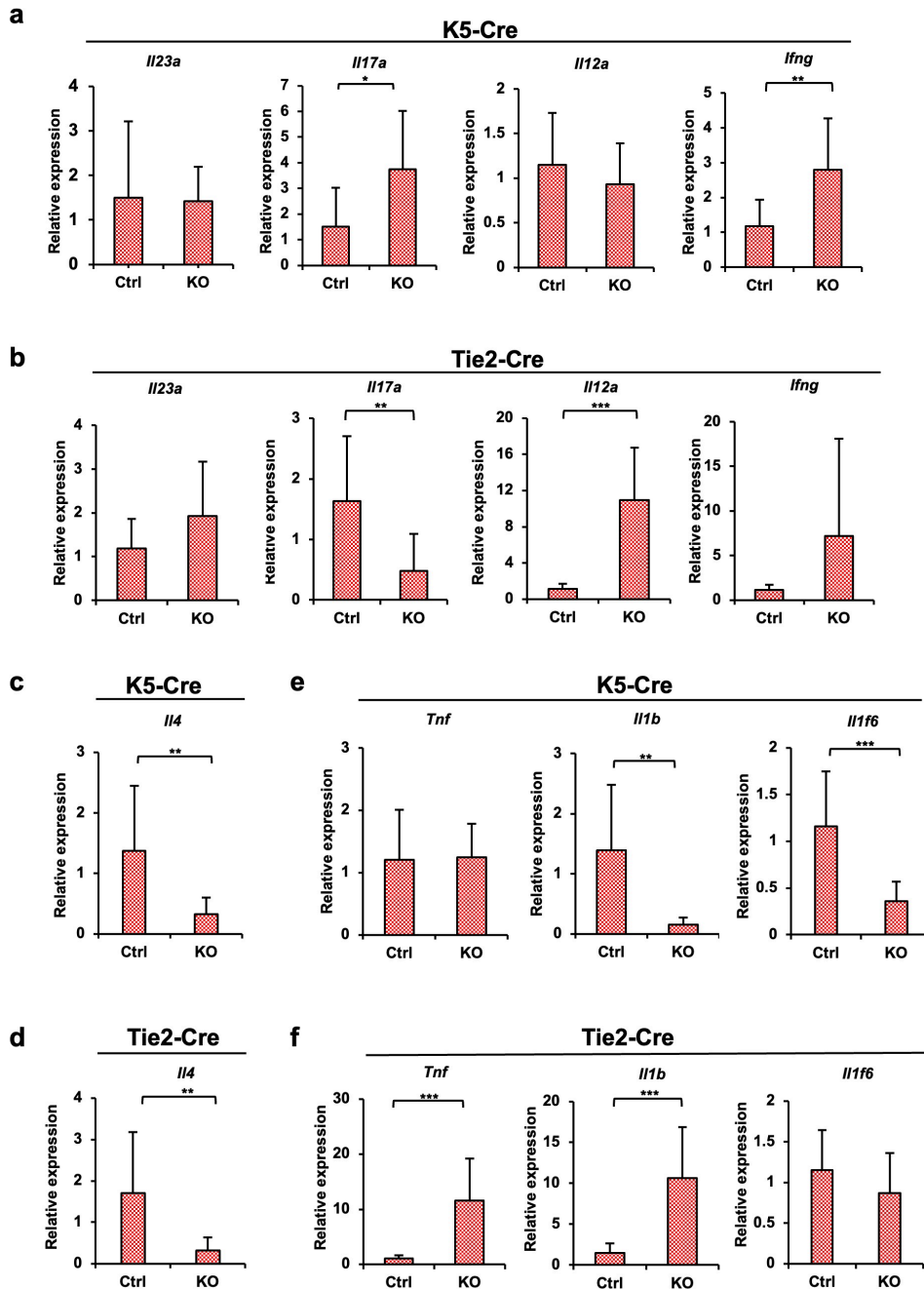


Figure 3

Selective upregulation of Th1-inducing or Th1-derived cytokines in hematopoietic cell-specific *Nfkbiz*-deficient mice treated with imiquimod.

(a-f) Quantitative real-time qPCR analysis of the expression of the indicated inflammatory cytokines in dorsal skins. Control (Ctrl) and *Nfkbiz*^{flox/flox};K5-Cre (K5-Cre) mice (a, c and e), and Ctrl and *Nfkbiz*^{flox/flox};Tie2-Cre (Tie2-Cre) mice (b, d and f) were treated with imiquimod on the dorsal skin for six

consecutive days, and then their dorsal skins were harvested from the mice. The number of mice was as follows: Ctrl and K5-Cre mice (n = 10 per group), and Ctrl and Tie2-Cre mice (n = 15 per group). Data represent mean $\pm$ SD. * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 4

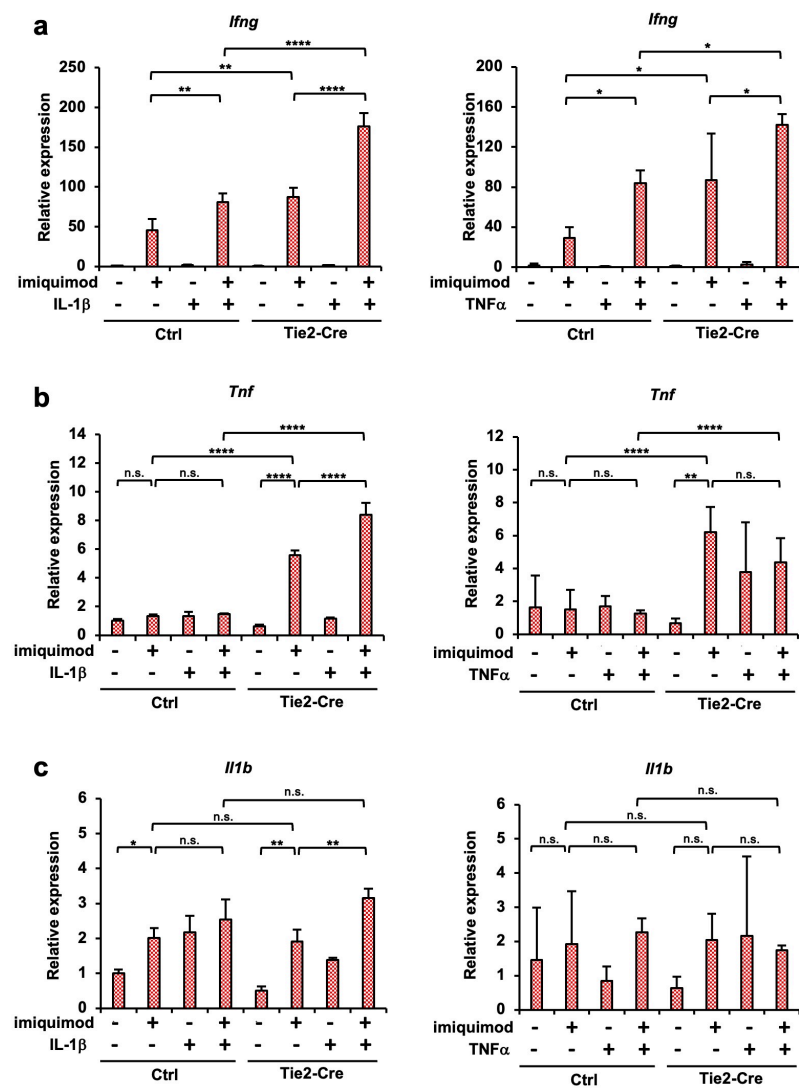


Figure 4

Enhancement of Th1-type polarization of *Nfkbiz*-deficient splenocytes upon IL-1 β and TNF α stimulation

(a–c) Quantification of mRNA levels of *Ifng* (a), *Tnf* (b), or *Il1b* (c) in splenocytes by RT-qPCR. Splenocytes from control (Ctrl) and *Nfkbiz*^{fox/fox};Tie2-Cre mice (Tie2-Cre) were stimulated for 24 h with imiquimod, IL-1 β , TNF α , or a combination of imiquimod with IL-1 β or TNF α . Data represent mean $\pm$ SD (n=3). **p* < 0.05, ***p* < 0.01, *****p* < 0.0001.

Figure5

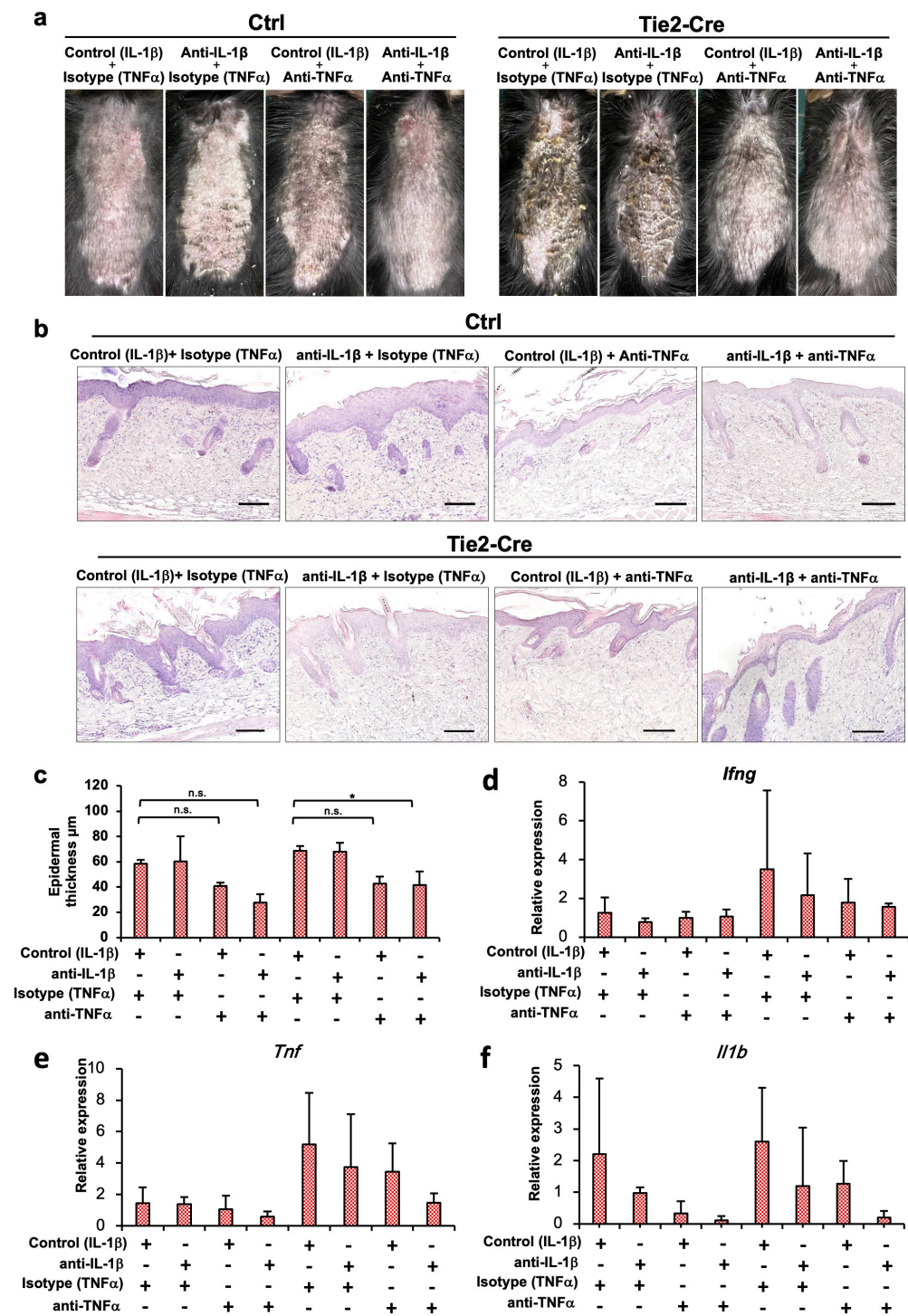


Figure 5

Therapeutic effect of anti-IL1b and anti-TNF α antibodies on imiquimod-induced psoriasis in *Nfkbiz*-deficient mice.

Ctrl and *Nfkbiz*^{flox/flox};Tie2-Cre (Tie2-Cre) mice were treated with imiquimod on the dorsal skin for six consecutive days. In parallel, anti-IL-1 β antibody (100 μ g/mouse), anti-TNF α antibody (100 μ g/mouse) and isotype control antibodies [control antibody for anti-IL-1 β antibody (100 μ g/mouse) or isotype control antibody for anti-TNF α antibody (100 μ g/mouse)], were peritoneally injected in the imiquimod-treated mice on Day 0 and Day 3, according to the antibody combinations shown in panel a.

Representative clinical images of the dorsal skin are shown in panel a. Dorsal skin samples from antibody-injected mice was further analyzed by H&E staining (b), epidermal thickness quantification (c), and RT-qPCR targeting *Ifng* (d), *Tnf* (e), or *Il1b* (f) .

(b) Representative H&E-stained dorsal skin sections, Scale bar = 100 μ m.

(c) Quantitative analysis of epidermal thickness. For each group (n = 3), epidermal thickness was measured at nine random sites on H&E-stained sections to calculate mean values.

(d-f) Quantitative real-time qPCR analysis of *Ifng*, *Tnf*, or *Il1b* expression in dorsal skin (n = 3). Data represent mean $\pm$ SD. * p < 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterialnada.docx](#)