IN VIVO *39*: 1378-1393 (2025)
doi: 10.21873/invivo.13941

Over-expression of Truncated IK Ameliorates Dinitrochlorobenzene-Induced Allergic Contact Dermatitis Lesions in BALB/c Mice

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Abstract

Background/Aim: Allergic contact dermatitis (ACD) is a delayed-type hypersensitivity reaction mediated by hapten-specific T cells. Dinitrochlorobenzene (DNCB)-induced mouse models are widely used to investigate the pathogenesis of contact dermatitis. Inhibitor K562 (IK) cytokine suppresses IFN-γ-induced MHC class II expression on B cells by increasing cAMP levels. Previously, we reported that truncated IK (tIK) expression in transgenic (Tg) mice ameliorated rheumatoid arthritis by suppressing $CD4^+$ T helper cells (Th)-1 and Th17 cell differentiation, as well as macrophage activation. However, its role in hypersensitivity diseases such as ACD remains underexplored. This study aimed to evaluate whether tIK Tg mice exhibit reduced susceptibility to DNCB-induced ACD and passive systemic anaphylaxis (PSA).

Materials and Methods: ACD was induced in BALB/c and tIK Tg mice through repeated DNCB application. Ear thickness and scratching behavior were assessed. Serum IgE levels and mast cell-associated gene expression were analyzed. Th cell differentiation was evaluated using flow cytometry. PSA, an experimental model used to study systemic allergic reactions, was induced by IgE sensitization followed by antigen challenge, and hypothermia, serum IgE, and mast cell activation were measured.

Results: DNCB-treated BALB/c mice developed severe dermatitis, including increased ear thickness and scratching behavior, whereas tIK Tg mice exhibited milder symptoms. tIK over-expression also led to lower serum IgE levels and reduced mast cell-associated gene expression. T cell analysis revealed suppressed Th2 and Th17 differentiation, while Tregs and Th1 cells remained unaffected. Beyond ACD, tIK Tg mice exhibited attenuated PSA responses, with less severe hypothermia, lower serum IgE levels, and reduced mast cell activation compared to wild-type controls.

Conclusion: tIK suppresses both localized and systemic hypersensitivity by modulating Th cell differentiation and mast cell activity. tIK may serve as a potential therapeutic target for allergic and inflammatory diseases.

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Received February 13, 2025 | Revised February 27, 2025 | Accepted March 4, 2025

Keywords: Inhibitor K562, allergic contact dermatitis, dinitrochlorobenzene, mast cell, T cell differentiation.

Introduction

Allergic contact dermatitis (ACD) is a prevalent inflammatory skin disease characterized by itchiness, redness, blistering, and dryness (1, 2). It falls under the category of delayed-type hypersensitivity (DTH) reactions, mediated specifically by hapten-specific T cells (3). The immunogenicity of haptens is induced through their interaction with molecules in the skin, leading to the sensitization of individual skin cells and subsequent priming of lymph nodes. Immune cells are then recruited to the skin through the afferent and efferent phases of the reaction. ACD involves a complex interplay between innate and adaptive immune responses influenced by genetic, environmental, pharmacological, and psychological factors (4, 5).

ACD is characterized by the excessive inflammatory cytokines production, including TNF-α, IL-4, 5, 6, 13, and IL-17, and leukocyte infiltration (6). Mast cells, eosinophils, neutrophils, and Th cells are dominant in the inflammatory skin lesions of ACD (7). Mast cells are key initiators, activated *via* high-affinity IgE receptors, leading to the release of proinflammatory mediators such as prostaglandins, leukotrienes, and cytokines (8). Additionally, cytokines secreted by infiltrating $CD8^+$ and $CD4^+$ T cells activate keratinocytes to release cytokines and chemokines, resulting in the massive recruitment of activated immune cells that infiltrate the dermis (9). T cell differentiation plays a central role in ACD pathogenesis. While an imbalance between Th1 and Th2 cells is a well-established driver (10, 11), recent studies have identified Th17 cells as additional key players, providing novel insights into the Th1/Th2 paradigm (12). IL-17, a proinflammatory cytokine central to autoimmune diseases such as rheumatoid arthritis, contributes to chronic skin inflammation in ACD. Increased IL-17-producing cells have also been observed in atopic dermatitis (AD), suggesting overlapping immune mechanisms between AD and ACD (13, 14).

The incidence of ACD is rising in industrialized countries and is particularly prevalent among children, although it can also occur in adulthood (15). ACD is primarily triggered by environmental allergens, such as nickel and rubber, which provoke inflammatory responses upon skin contact (16). Current treatments, including oral antihistamines, topical steroids, and non-steroidal anti-inflammatory drugs, provide only partial relief, emphasizing the need for more targeted and effective therapies (17). Given the central role of T cell differentiation and immune dysregulation in ACD, targeting key immunomodulatory pathways could provide novel therapeutic strategies.

Inhibitor K562 (IK), first isolated from the culture medium of K562 leukemia cell line (18), has been identified as a novel regulator of MHC class II expression through the induction of interferon-γ (IFN-γ) (19, 20). Interestingly, previous studies suggest that IK plays a role in immune evasion during viral infections. For example, in Coxsackie virus B3 (CVB3)-infected mice, IK mRNA expression was upregulated, and the expressed IK protein interacted with G protein-bound receptors, leading to increased cAMP levels and suppressed MHC class II expression (21, 22). This mechanism suggests that IK may act as an immune modulator beyond viral infections.

Emerging evidence suggests that IK may also regulate immune responses in autoimmune diseases. Dysregulated MHC class II expression has been implicated in autoimmune disorders, and given IK's role in immune modulation, its therapeutic potential has been explored in diseases such as rheumatoid arthritis (23, 24). Given the regulatory effects of IK on MHC molecule expression and T cell differentiation, its therapeutic potential has been explored in autoimmune diseases (25-27). Notably, truncated IK (tIK) cytokine has demonstrated efficacy in alleviating inflammatory arthritis by suppressing Th1 and Th17 differentiation and macrophage activation (26). This suggests that IK has a broader immunomodulatory role,

potentially extending to other inflammation-related diseases such as ACD.

In this study, we investigated the effects of tIK overexpression on ACD using tIK transgenic (Tg) mice. By evaluating its impact on immune responses in ACD, we aimed to enhance our understanding of ACD pathogenesis and explore the therapeutic potential of IK-derived biologics in allergic and inflammatory skin diseases.

Materials and Methods

Animals. Female BALB/c mice aged 6 weeks were purchased from Samtako, Inc. (Osan-si, Republic of Korea), and tIK transgenic mice in a BALB/c background were kindly provided by Dr. Jea-Hwan Nam, The Catholic University of Korea, Seoul, Republic of Korea. Mice were housed in a controlled environment, with a steady temperature (22-24˚), humidity (45-55%), and a 12-h light/dark cycle. All mice experiments were handled according to the guidelines from the Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University (Approval number: CBNUA-24-0102-02).

Passive systemic anaphylaxis (PSA) in mice. PSA was induced by an intraperitoneal challenge with 500 μg DNP-HAS (Albumin, dinitrophenyl; Sigma-Aldrich, St Louis, MO, USA) in 8-week-old male mice, which were passively sensitized with 5 μg DNP-specific IgE (Monoclonal Anti-mouse IgE antibody; Sigma-Aldrich) by intravenous injection 24 h prior. Anaphylaxis was monitored by measuring body temperature every 10 min and based on body temperature reduction.

ELISA for mouse mast cell protease 1. To assess mast cell activation, serum was collected from BALB/c mice 80 min post-induction of passive systemic anaphylaxis, and mouse mast cell protease 1 (mMCP-1) levels were quantified using an ELISA kit (mMCP-1 ELISA; Invitrogen, Carlsbad, CA, USA). Absorbance at 490 nm was measured using a microplate reader (Bio Tek Instruments, Inc., Winooski, VT, USA).

Induction of ACD. All mice were randomly assigned to four groups (n=5/group): the BALB/c control group, the BALB/c dinitrochlorobenzene (DNCB; Sigma-Aldrich) treatment group, the tIK control group, and the tIK DNCB treatment group. To induce AD-like symptoms and skin lesions, DNCB was applied to the dorsal skin and both ears. One day after complete dorsal hair removal, 200 μl of 1% DNCB dissolved in a 3:1 (v/v) mixture of acetone (Junsei Chemical Co., Ltd., Tokyo, Japan)/olive oil (Sigma-Aldrich) was applied to the dorsal skin, while 20 μl was applied to both ears on days 1 and 4. Five days after dorsal hair removal, sensitization was followed by a challenge phase, in which 0.4% DNCB in the same acetone oil mixture (3:1 v/v) was applied to the dorsal skin (200 μl) and both ears (20 μl each) three times per week for three weeks. Body weight was measured every 5 days.

Assessment of scratching behavior and ear thickness. Scratching behavior was assessed by placing each mouse in a cage for 20 min once per week. Ear thickness was measured and recorded for each mouse using a micrometer (Mitutoyo Corporation, Kawasaki, Japan). To minimize variability, all measurements were performed by a single investigator.

Histopathological analysis. Dorsal skin lesions and ear tissues from each mouse were collected, fixed in 10% neutral-buffered formalin, and embedded in paraffin. Tissue sections of 6 μm thickness were stained with hematoxylin (YD-diagnostics, Yongin, Republic of Korea) and eosin (Sigma-Aldrich) (H&E) or Toluidine Blue O (Sigma-Aldrich) to assess epidermal thickness and inflammatory cell infiltration. Histopathological evaluation of all skin and ear sections was performed in a blind manner. All samples were observed using an inverted microscope (Nikon Corporation, Tokyo, Japan), and the data are representative of three independent observations.

Measurement of serum IgE. Mice were sacrificed on day 28 and whole blood was collected. Serum obtained from whole blood was stored at −80˚C until use. Serum IgE levels

were measured using ELISA kits (Invitrogen) in accordance with the manufacturer's instructions. Absorbance was measured at 490 nm using a microplate reader (Bio Tek Instruments, Inc.).

Real-time polymerase chain reaction. Total RNA was extracted from ear tissue using RNAiso Plus (Takara Bio Inc., Kusatsu, Shiga, Japan) reagent according to the instructions of the manufacturer. cDNA was synthesized using ReverTra Ace® qPCR RT Kit (TOYOBO Co., Ltd., Osaka, Japan). Real-time polymerase chain reaction (PCR) was performed using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Enzynomics, Daejeon, Republic of Korea) on a Rotor-Gene Q (Qiagen, Hilden, Germany). The reaction conditions were followed in accordance with the manufacturer's protocol. The mRNA expression levels were normalized to the β-actin using 2-ΔΔCt method. Primers for IL-4, IL-13, IL-17, IL-6, IFN-γ, TNF-α, c-Kit, TSLP, GATA1, GATA2, MCP-5, and MCCPA were designed based on publicly available sequence information.

Western blot analysis. Ear tissues were homogenized in lysis buffer (T-per Buffer; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with a protease and phosphatase inhibitor tablet (Thermo Fisher Scientific). Equal concentrations of protein were separated *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane (Merck Millipore Ltd., Burlington, MA, USA). The membranes were blocked with 5% skim milk for 1 h at room temperature (RT) and then treated with primary antibody (Anti-TSLP antibody, Abcam, Cambridge, UK) overnight at 4˚C. Thereafter, the membranes were incubated with HRP-conjugated secondary antibodies for 1 h at RT. TSLP protein was detected using West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) or West save gold (YOUNG IN FRONTIER, Seoul, Republic of Korea) and analyzed using the Luminescent Image Analyzer System, LAS-4000 (FUJIFILM Corporation, Tokyo, Japan). Bands were quantified using ImageJ software (NIH, Bethesda, MD, USA).

Flow cytometry analysis. Spleens and draining lymph nodes (dLN) were obtained from sacrificed mice on day 28. A single-cell suspension was prepared in PBS and cells were treated with red blood cell lysis buffer (Invitrogen). Cells were suspended in RPMI-1640 medium (Cytiva, Marlborough, MA, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HI-FBS; Capricorn Scientific, Ebsdorfergrund, Germany), and 1% (v/v) penicillin-streptomycin (10,000 U/ml penicillin and 10,000 μg/ml streptomycin; Gibco, Waltham, MA, USA). For intracellular cytokine staining (ICS), cells were restimulated with PMA/ION (Cell stimulation cocktail; Invitrogen) and Brefeldin A (Invitrogen) at 37˚C incubator. After 4 h, cells were washed with FACS buffer (3% HI-FBS in PBS with 0.02% sodium azide) and stained with cell surface marker, anti-mouse CD4 (PerCP/Cy5.5, Invitrogen) and CD25 (PE, Invitrogen). Cells were fixed, permeabilized, and stained with anti-mouse IFN-γ (FITC, Invitrogen), IL-17A (PE, Invitrogen), IL-4 (Biotin, Invitrogen), Streptavidin (APC, Invitrogen), or Foxp3 (FITC, Invitrogen). Results were analyzed using BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

Statistical analysis. All data are presented as the mean±standard error of the mean (SEM) and were analyzed using GraphPad Prism version 10 (GraphPad Software, Inc., Boston, MA, USA). Statistical significance was determined using an unpaired *t*-test for comparisons between two groups. For experiments involving multiple time points or conditions, a two-way ANOVA test followed by Tukey's post hoc test was performed. A p-value of <0.05 was considered statistically significant.

Results

tIK over-expression alleviates ACD symptoms in mice. To assess the impact of tIK over-expression on ACD, we induced ACD in BALB/c and tIK Tg mice using DNCB sensitization and challenge over three weeks (Figure 1A). DNCB-treated BALB/c mice exhibited typical ACD

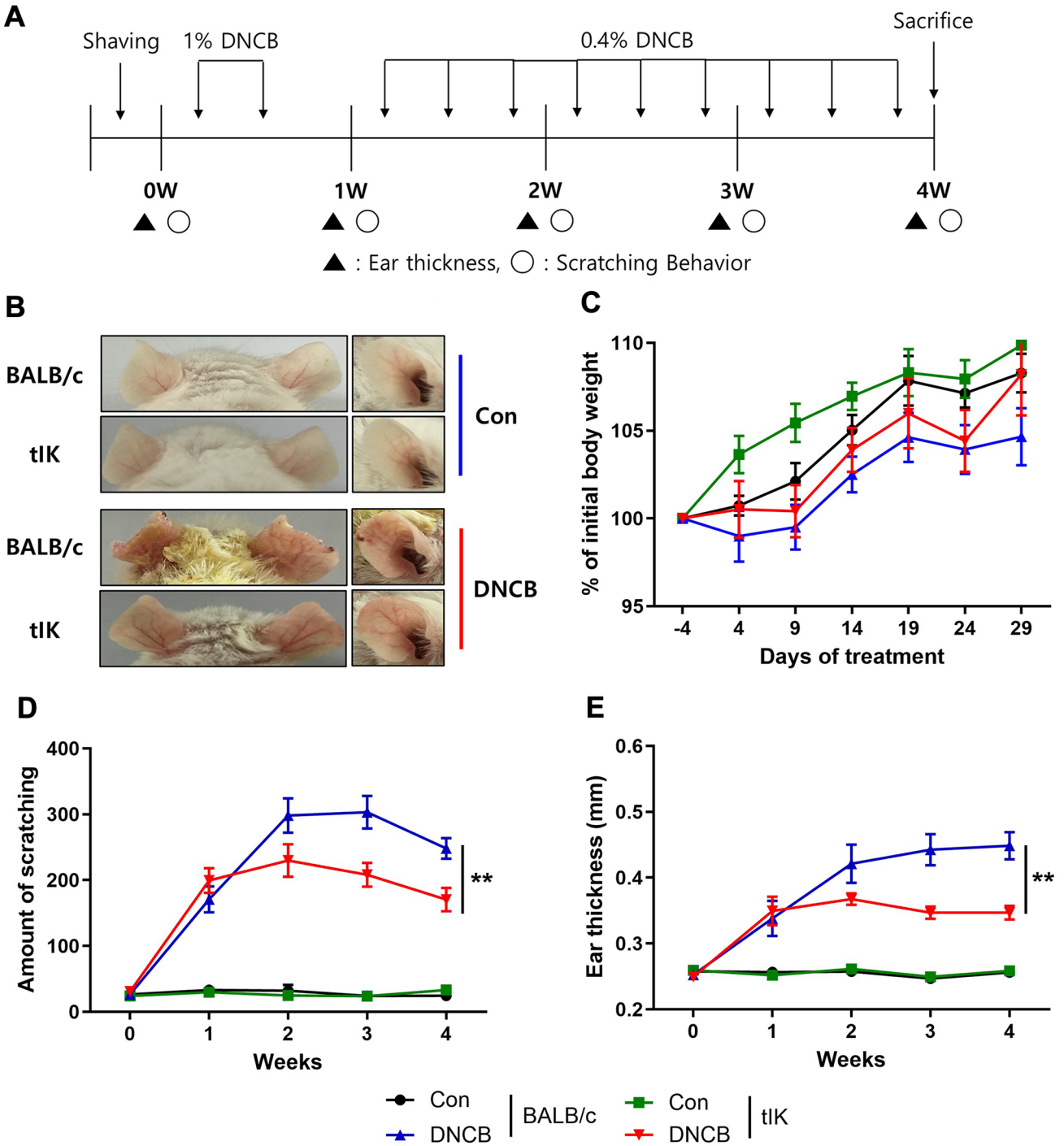
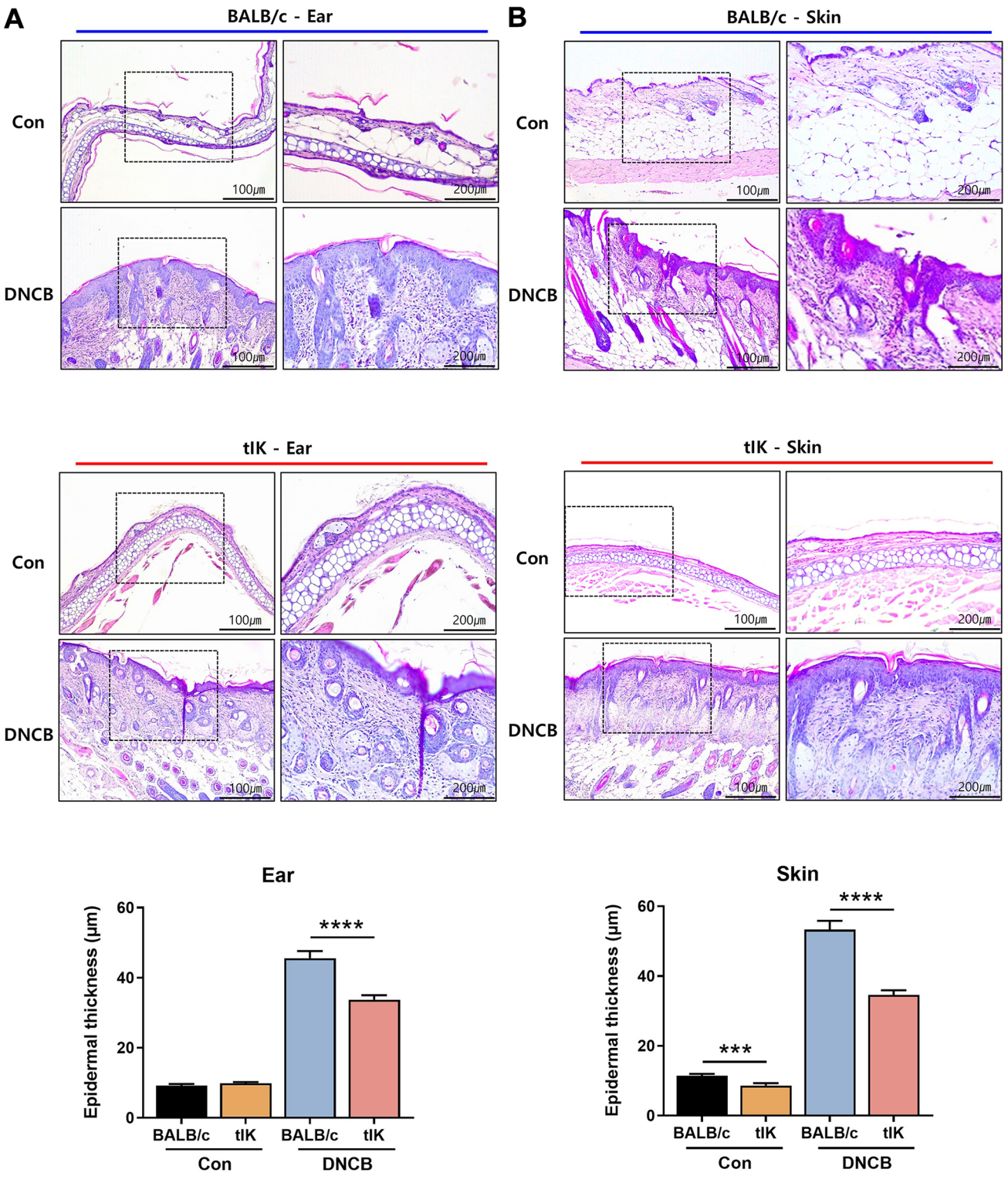


Figure 1. *Effects of tIK over-expression on dinitrochlorobenzene (DNCB)-induced allergic contact dermatitis (ACD) in mouse models. (A) Experimental design for DNCB-induced ACD in BALB/c and tIK Tg mice. Mice were sensitized with 1% DNCB and subsequently challenged with 0.4% DNCB over a period of three weeks. (B) Representative images of ears in BALB/c and tIK Tg mice. (C) Body weights during the experimental period were measured every five days. (D) Scratching behavior of mice was monitored for 20 min. (E) Variation in ear thickness during the ACD induction was measured with a digital thickness gauge. Data are presented as mean±SEM. (**p<0.01).*



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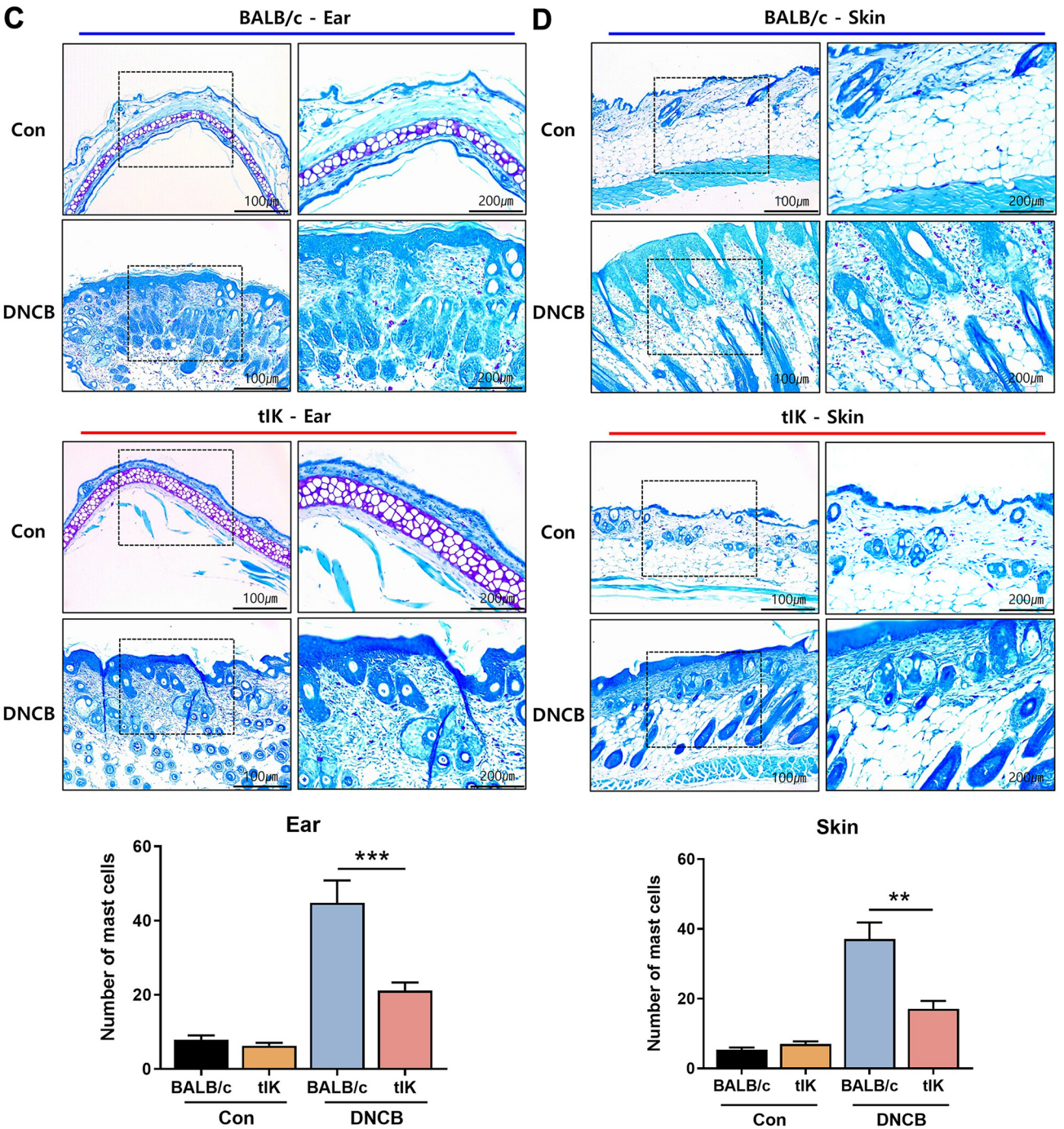

Figure 2. *Histological analysis of allergic contact dermatitis-induced ear and dorsal skin lesions in mice. (A, B) Representative hematoxylin and eosin (H&E) staining of dinitrochlorobenzene (DNCB)-treated ear and dorsal skin lesions. Skin sections show the epidermis and dermis at day 28. Scale bar: 100 μm and 200 μm. Epidermal thickness in H&E-stained sections was evaluated as the average total length across 15 fields per individual. (C, D) Representative Toluidine Blue staining of DNCB-treated ear and dorsal skin lesions. Scale bar: 100 μm and 200 μm. Mast cells are visible as dark blue-stained granulated cells in Toluidine Blue-stained sections. Mast cell numbers were determined in three randomly selected fields per individual. Data are presented as mean±SEM (**p<0.01, ***p<0.001, ****p<0.0001).*

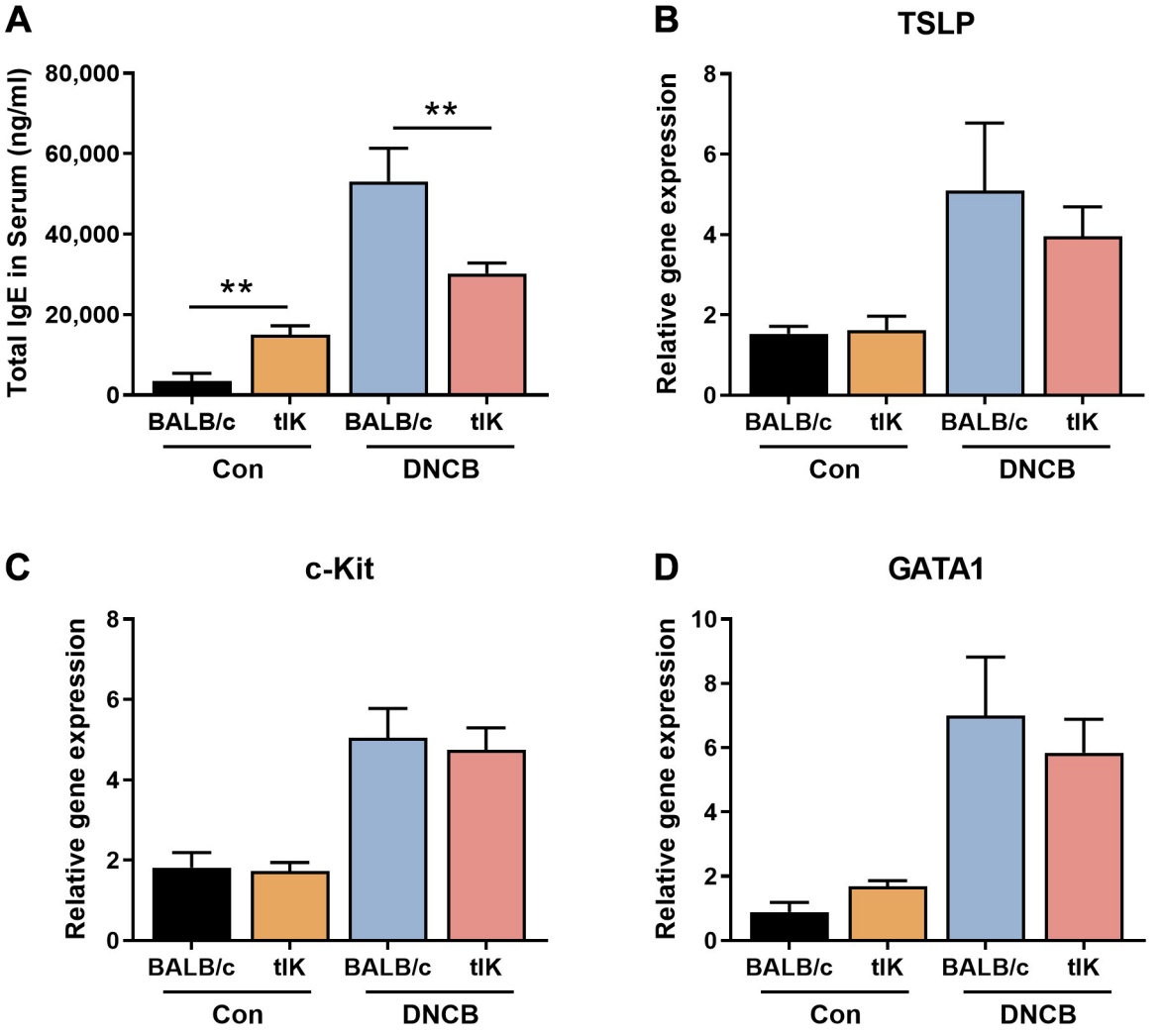

Figure 3. *Continued*

symptoms, including crusting, epidermal thickening, erythema, and dryness, while tIK Tg mice showed noticeably milder symptoms (Figure 1B). By day 14, scratching behavior was significantly reduced in tIK Tg mice compared to BALB/c controls (Figure 1D), along with a notable reduction in ear thickness (Figure 1E). Furthermore, DNCB-induced weight loss was observed in both BALB/c and tIK Tg mice; however, tIK Tg mice exhibited less weight reduction, suggesting a protective role against DNCB-induced stress (Figure 1C). These findings indicate that tIK over-expression mitigates ACD severity by reducing clinical symptoms and physical stress in affected mice.

tIK over-expression reduces epidermal thickening and immune cell infiltration. ACD is characterized by epidermal thickening, immune cell infiltration, and mast cell accumulation (8, 28). To assess how tIK affects these pathological changes, we performed H&E and Toluidine Blue staining on skin samples. By day 28, DNCB-treated BALB/c mice exhibited significantly thickened dermis and epidermis with extensive immune cell infiltration, as

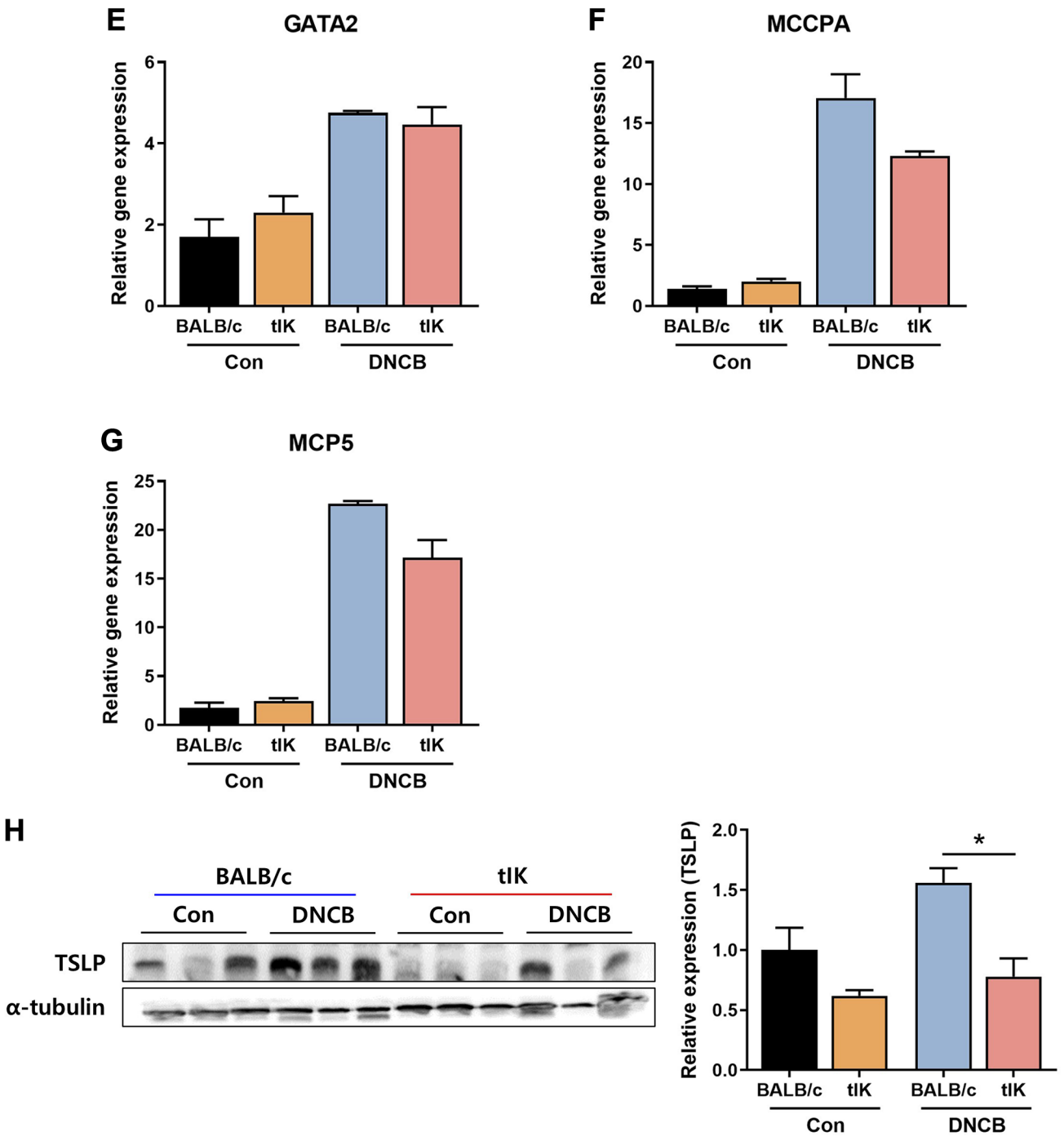

Figure 3. *tIK over-expression reduces IgE levels, mast cell-related gene expression, and TSLP protein expression in dinitrochlorobenzene (DNCB)-induced allergic contact dermatitis (ACD). (A) Total serum IgE levels in BALB/c and tIK Tg mice with or without DNCB treatment were measured using ELISA kit. (B-G) ACD was induced in BALB/c and tIK Tg mice after DNCB treatment. Relative mRNA expression of mast cell-related genes, including (B) TSLP, (C) c-Kit, (D) GATA1, (E) GATA2, (F) MCCPA, and (G) MCP5, in ear tissue of BALB/c and tIK Tg mice. (H) TSLP protein expression in ear lesions of mice, as detected through western blotting. α-tubulin was used as a loading control. Data are presented as mean±SEM (*p<0.05, **p<0.01).*

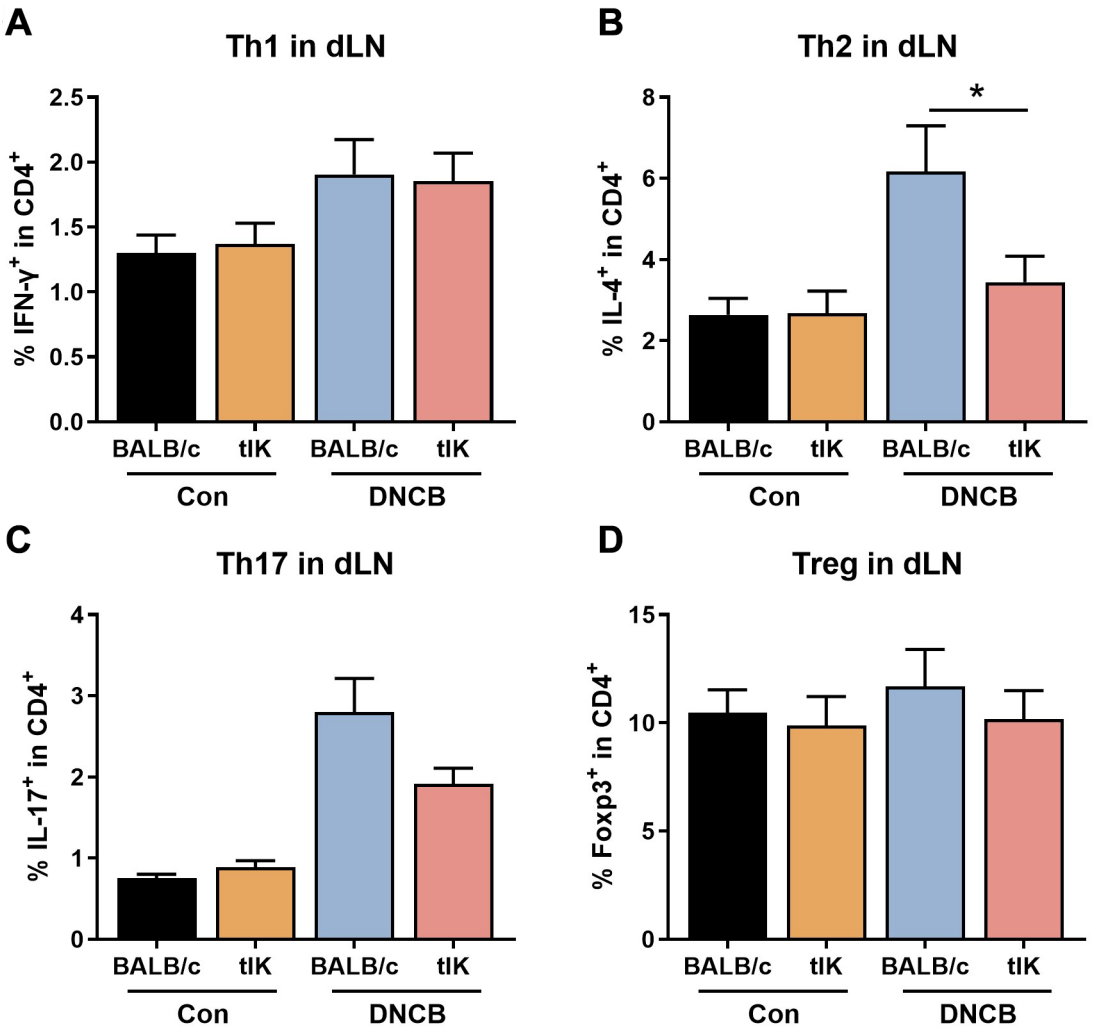

Figure 4. *Continued*

shown in H&E-stained sections. In contrast, tIK Tg mice displayed thinner epidermis and reduced inflammatory cell infiltration, with a 12 μm reduction in ear epidermis and 19 μm reduction in dorsal skin (Figure 2A and B). Toluidine Blue staining revealed a notable increase in mast cell accumulation and degranulation in DNCB-treated BALB/c mice. However, in tIK Tg mice, mast cell numbers were reduced by over 50% in both the ear and dorsal skin, and degranulation was significantly lower (Figure 2C and D). These findings suggest that tIK attenuates ACD progression by reducing immune cell recruitment, epidermal thickening, and mast cell activation.

tIK over-expression suppresses IgE levels, mast cell activation, and TSLP expression in DNCB-induced ACD. Mast cells and IgE play a critical role in allergic responses, with elevated IgE levels and mast cell infiltration being key features of ACD and other allergic disorders (7). DNCB-treated BALB/c mice exhibited a threefold increase in serum IgE levels compared to untreated controls. However, tIK Tg mice showed significantly lower serum IgE, suggesting modulation of IgE production as a mechanism of ACD alleviation (Figure 3A). To further investigate tIK's role in mast cell regulation, we analyzed the expression of mast cell-related genes in ear tissues.

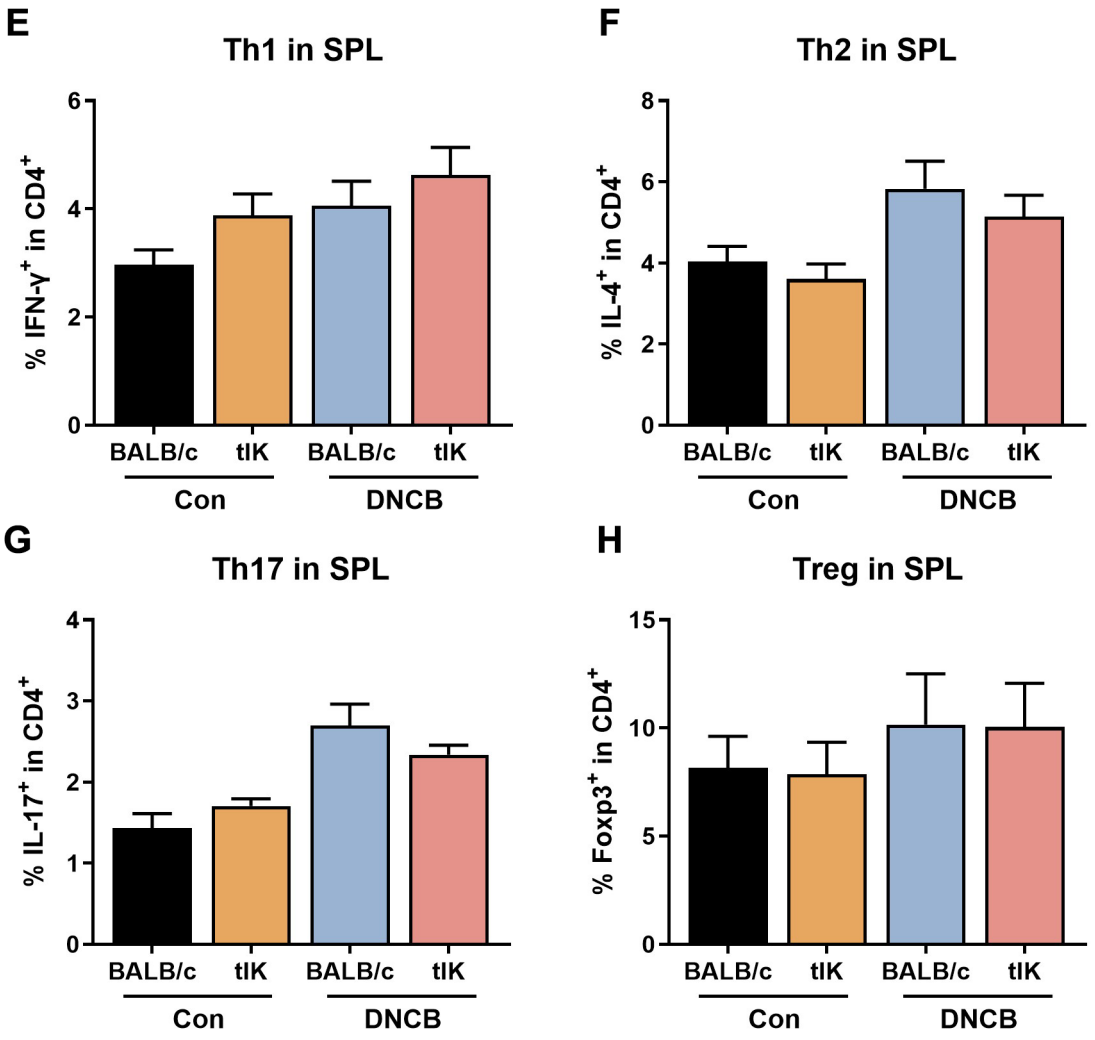

Figure 4. *Helper T cell subpopulations in draining lymph nodes and spleens from BALB/c and tIK Tg mice treated with dinitrochlorobenzene (DNCB). Single-cell suspensions from allergic contact dermatitis-induced mice were stained with anti-CD4 antibodies, followed by intracellular cytokine staining for various cytokines. Cells were then analyzed by flow cytometry. Flow cytometry analysis of CD4+ T cell subsets (A-D) in dLN and (E-H) in the spleen. Data are presented as mean±SEM (*p<0.05).*

DNCB treatment significantly up-regulated TSLP, c-Kit, GATA1, GATA2, MCCPA, and MCP5 expression, while tIK Tg mice exhibited a marked reduction in these markers (Figure 3B-G). Additionally, thymic stromal lymphopoietin (TSLP), a cytokine highly expressed by keratinocytes in atopic dermatitis (7, 8), was analyzed by western blotting using ear tissue. TSLP expression was markedly up-regulated in DNCB-treated mice compared to untreated mice. However, consistent with previous findings, tIK Tg mice exhibited lower TSLP protein levels than BALB/c mice with ACD (Figure 3H). These results suggest that tIK alleviates ACD by down-regulating TSLP expression and IgE-mediated mast cell activation, thereby mitigating allergic skin inflammation. Taken together, these findings indicate that tIK mitigates ACD progression by suppressing mast cell infiltration, IgE production, and TSLP expression, which collectively contribute to allergic inflammation.

tIK over-expression modulates T cell differentiation in DNCB-induced ACD. ACD is driven by altered T cell differentiation, particularly enhanced Th2 and Th17 responses, leading to IgE overproduction and chronic inflammation (7, 8). To determine how tIK modulates immune cell profiles, we analyzed T cell subsets in draining lymph nodes (dLN), spleen, and ear lesions. Flow cytometry showed that DNCB exposure increased Th1 (IFN-γ), Th2 (IL-4), and Th17 (IL-17) $CD4^+$ T cells in dLN and the spleen of BALB/c mice. Treg ($Foxp3^+$) cell frequencies remained unchanged. In tIK Tg mice, Th2 and Th17 frequencies were reduced in dLN, with a significant decrease in IL-4^+ $CD4^+$ T cells, though reductions in IL-17^+ cells were not statistically significant (Figure 4A-H).

To further investigate local cytokine expression, we quantified mRNA levels of T cell-related cytokines in ear skin lesions using qRT-PCR. DNCB-treated BALB/c mice exhibited significantly elevated expression levels of Th1 (IFN-γ, and TNF-α), Th2 (IL-4, IL-6, IL-13), and Th17 (IL-17) cytokines. In contrast, tIK Tg mice displayed reduced expression of these cytokines compared to DNCB-treated BALB/c mice (Figure 5A-F). Taken together, these findings indicate that tIK over-expression modulates systemic and local immune responses, highlighting its potential role in mitigating ACD pathogenesis through immunomodulation.

Effects of tIK over-expression on antigen-specific IgE-induced passive systemic anaphylaxis. Anaphylaxis is a severe, systemic hypersensitivity reaction mediated by mast cell degranulation and IgE signaling (29). To determine whether tIK's immunomodulatory effects extend beyond ACD, we investigated its role in antigen-specific IgE-induced PSA using anti-DNP IgE and DNP-HSA antigen stimulation (Figure 6A). Body temperature monitoring revealed that tIK Tg mice exhibited significantly less hypothermia following antigen challenge, particularly in the early phase, compared to BALB/c controls (Figure 6B). To assess mast cell degranulation, we measured serum mMCP-1 levels, a specific marker of mast cell activation (30). DNCB-treated BALB/c mice had significantly higher mMCP-1 levels (80.3 ng/ml), whereas tIK Tg mice exhibited a substantial reduction (49.9 ng/ml) (Figure 6C). These results indicate that tIK suppresses IgE-mediated mast cell activation not only in ACD but also in systemic anaphylaxis, demonstrating its broader immunomodulatory effects beyond localized allergic inflammation.

Discussion

Allergic contact dermatitis (ACD) is a chronic inflammatory skin disease with an increasing prevalence, particularly in industrialized countries (31). It is classified as a delayed-type hypersensitivity (DTH) reaction driven by hapten-specific T cells, leading to immune cell infiltration, epidermal thickening, and mast cell activation (3). Current treatments, including antihistamines and topical steroids, provide limited relief, highlighting the need for novel therapeutic strategies targeting immune regulation (29). In this study, we demonstrated that tIK over-expression alleviates ACD symptoms in a DNCB-induced mouse model. tIK Tg mice exhibited reduced ear thickness, scratching behavior, and weight loss, suggesting a protective role against allergic skin inflammation. These clinical improvements were accompanied by reduced epidermal thickening, mast cell infiltration, and inflammatory cytokine expression, highlighting tIK's immunomodulatory effects in ACD pathogenesis.

ACD is primarily mediated by Th2-associated immune responses, including the activation of macrophages, eosinophils, and mast cells, along with TSLP up-regulation (5). Mast cells, which are activated *via* IgE binding, release histamine, cytokines, and chemokines, amplifying allergic inflammation (32, 33). Notably, our results showed that tIK Tg mice exhibited significantly lower serum IgE levels and reduced mast cell infiltration in skin lesions. This reduction in IgE-mediated mast cell activation suggests that tIK influences the early sensitization and effector phases of ACD, likely by modulating Th2 cytokine production and TSLP expression. TSLP, a key Th2-driving cytokine, is highly expressed by keratinocytes and primes dendritic cells (DCs) to induce Th2 differentiation (34-36).

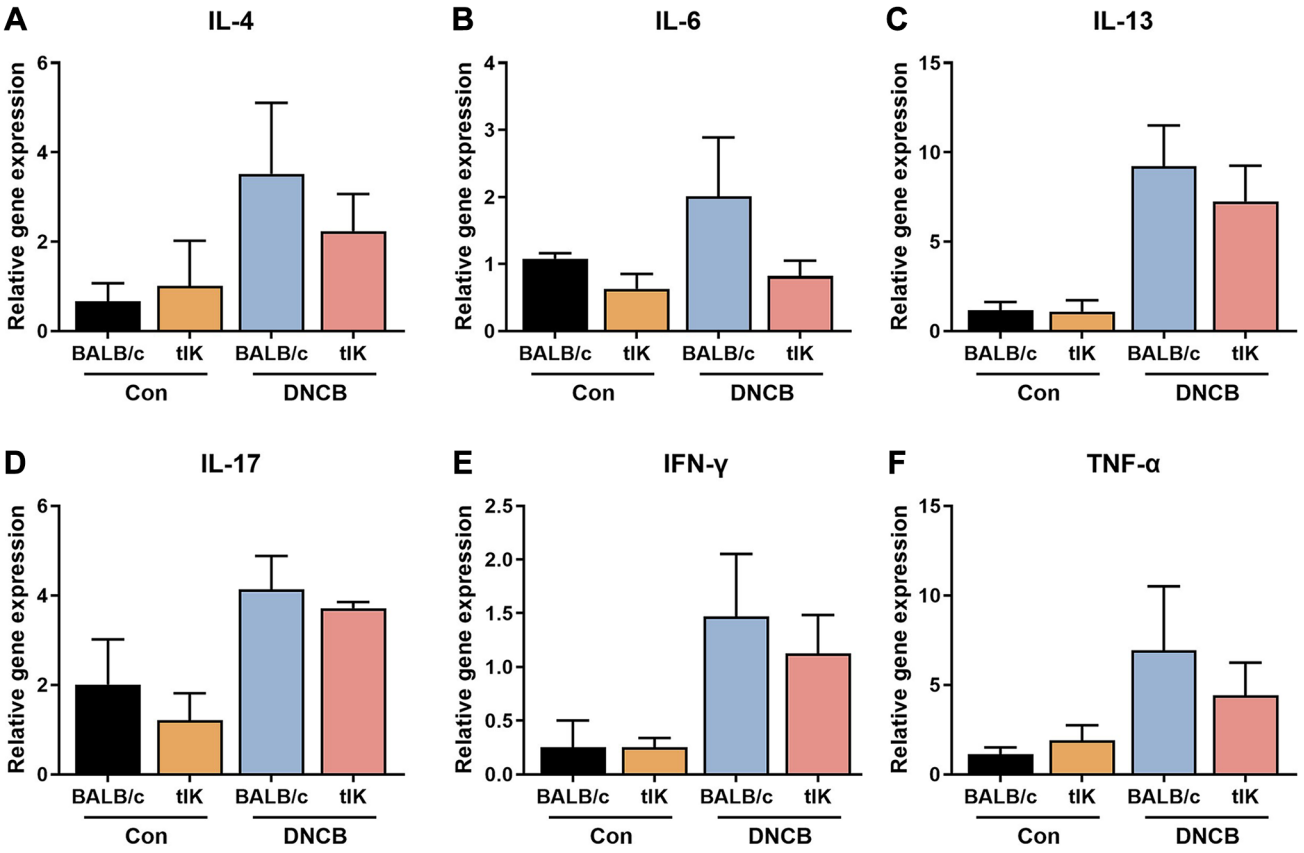

Figure 5. *mRNA expression levels of T cell-related cytokines in the ear lesions of BALB/c and tIK Tg mice treated with dinitrochlorobenzene (DNCB). Allergic contact dermatitis was induced in BALB/c and tIK Tg mice following DNCB treatment. Relative mRNA expression levels of T cell-related cytokines, including (A) IL-4, (B) IL-6, (C) IL-13, (D) IL-17, (E) IFN- γ, and (F) TNF-α, were measured in ear tissue using qRT-PCR. Data are presented as mean±SEM (*p<0.05).*

Our study revealed that tIK over-expression suppresses TSLP expression, further supporting its role in down-regulating Th2-driven inflammation in ACD.

While Th2-mediated inflammation is central to ACD, recent studies have identified Th1 and Th17 cells as additional contributors to chronic skin inflammation (12, 37). Th1 cells secrete IFN-γ and TNF-α, exacerbating inflammation, while Th17 cells produce IL-17, which is linked to keratinocyte activation and chronic immune cell infiltration (12, 13). Our findings indicate that tIK over-expression reduces Th1 and Th17 cytokine expression in both skin lesions and lymphoid organs, suggesting that tIK modulates T cell differentiation beyond Th2 suppression. This is consistent with previous reports demonstrating that tIK inhibits STAT3 signaling, a pathway essential for Th17 differentiation (29). Furthermore, tIK has been shown to suppress macrophage activation, reducing pro-inflammatory cytokine release (27). These mechanisms collectively contribute to tIK's broader anti-inflammatory effects in ACD. Additionally, previous studies suggest that tIK enhances regulatory T cell (Treg) populations, which play a critical role in immune homeostasis (3, 38). Although Treg levels were unchanged in our study, the observed reduction in pro-inflammatory Th subsets suggests that tIK indirectly fosters immune tolerance by shifting the inflammatory milieu toward resolution.

Beyond its effects on localized ACD lesions, our study revealed that tIK also attenuates passive systemic

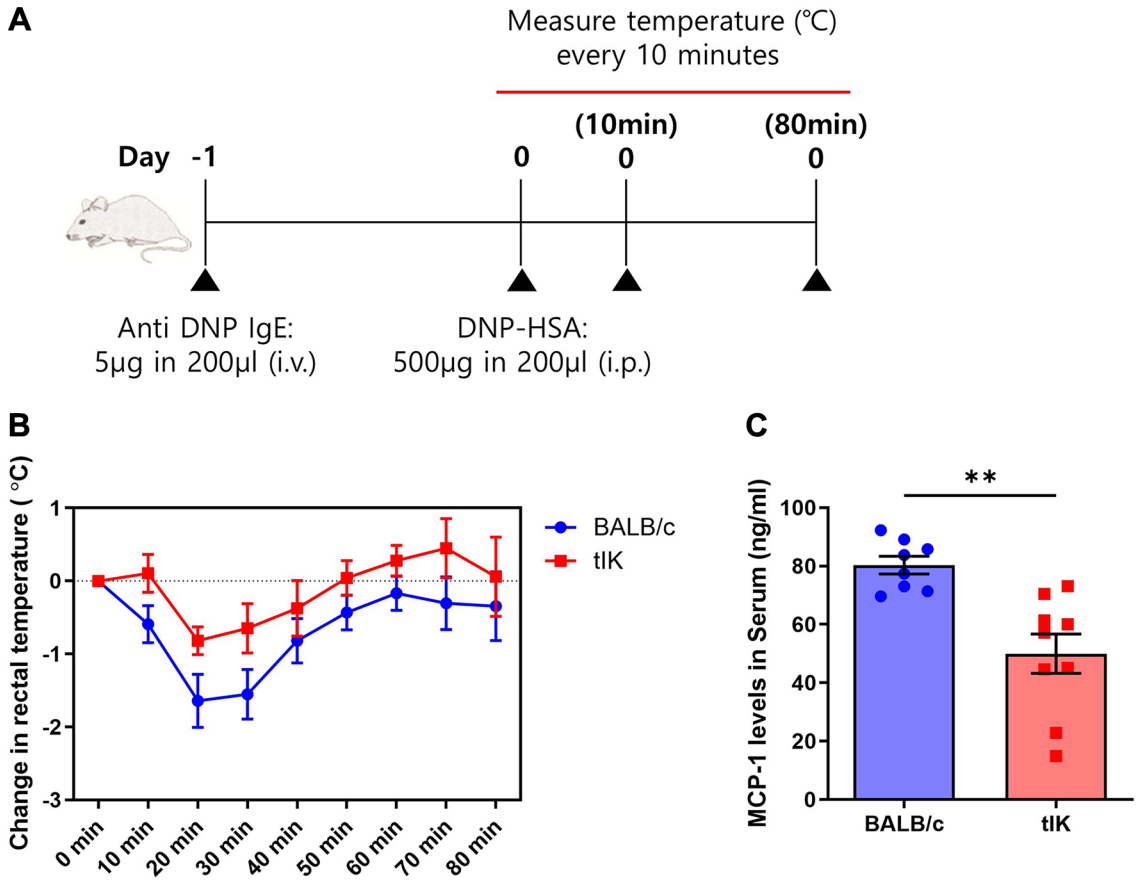

Figure 6. *Significant reduction in IgE-dependent passive systemic anaphylaxis in tIK Tg mice. (A) BALB/c and tIK Tg mice were intravenously injected with 5 μg of anti-DNP IgE. After 24 h, systemic anaphylaxis was induced by intraperitoneal injection of 500 μg of DNP-HSA. (B) Body temperature was measured at 10-min intervals following an antigen challenge. (C) Serum mMCP-1 levels were measured 80 min after PSA induction. Data are presented as mean±SEM (**p<0.01).*

anaphylaxis (PSA), a mast cell-driven hypersensitivity reaction. tIK Tg mice exhibited less hypothermia and lower serum mMCP-1 levels, indicating reduced mast cell degranulation. These findings suggest that tIK's immunoregulatory effects extend beyond ACD, potentially influencing systemic allergic reactions. Although ACD and PSA are distinct hypersensitivity models—ACD being a T cell-driven, delayed-type reaction, while PSA is an IgE/mast cell-mediated immediate hypersensitivity—our findings indicate a common regulatory mechanism. tIK likely attenuates PSA *via* suppression of IgE production and mast cell activation, similar to its effects in ACD. This raises the possibility that tIK could be a promising therapeutic candidate for a broader spectrum of allergic disorders, including systemic anaphylaxis and atopic dermatitis.

Conclusion

Our findings demonstrate that tIK over-expression mitigates ACD progression by modulating Th cell differentiation, reducing mast cell activation, and suppressing TSLP expression. Additionally, tIK's ability to attenuate IgE-mediated systemic anaphylaxis suggests a broader role in allergic disease regulation. Future studies should focus on elucidating the precise molecular mechanisms by which tIK regulates Th2/Th17 differentiation and mast cell function,

as well as exploring its potential as a therapeutic agent, including recombinant tIK protein or gene therapy approaches. Collectively, our study provides new insights into tIK's immunomodulatory potential, suggesting that it may serve as a novel therapeutic strategy for ACD and other allergic conditions.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

J.S. and E.Y.O. performed the experiments and contributed to writing the manuscript. S.P. analyzed the data. S.-M.L. supervised the research, designed the experiments, and drafted and revised the manuscript. All Authors read and approved the final version of the manuscript.

Funding

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (#NRF-2019R1A2C2010432) and a grant of the Korea Health Technology R&D Project through the Korea Heath Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (#RS 2022-KH128441). Additionally, this work was supported by the Technology Innovation Program (20019452) funded by the Ministry of Trade, Industry & Energy (MOTIE, Republic of Korea).

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