

LETTER TO EDITOR

New therapeutic strategy for atopic dermatitis by targeting CHI3L1/ITGA5 axis

Dear Editor,

Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease that is difficult to treat despite the discovery of various disease targets and development of therapeutics. Previous clinical studies have shown that chitinase 3-like protein 1 (CHI3L1), also known as YKL-40, is associated with the onset and severity of AD.^{1–3} Recently, CHI3L1 has attracted attention as a new therapeutic target for treating various diseases, including cancer and autoimmune diseases.⁴ Our gene-disease network analysis also showed CHI3L1 is associated with various diseases, including inflammatory diseases (Figure S1). However, the role of CHI3L1 in AD pathogenesis is not well-understood. Previously, we provided the first direct evidence that inhibition of CHI3L1 by K284-6111, a novel CHI3L1 inhibitor, ameliorates AD-like skin inflammation.⁵ Here, we further investigated the role of CHI3L1 in AD pathogenesis and its underlying mechanism using CHI3L1 knockout (KO) mice and a CHI3L1-blocking antibody (CHI3L1-Ab), and based on outcomes, propose that a CHI3L1-targeted therapeutic strategy can be used to treat AD.

To determine if CHI3L1 plays a role in AD pathogenesis, CHI3L1-KO and wild-type (WT) mice were treated with phthalic anhydride (PA) to induce AD-like skin inflammation. WT mice displayed elevated CHI3L1 levels in the epidermal/dermal layers, particularly in epidermal keratinocytes, and blood serum, as well as typical AD-like symptoms such as erythema, edema, hyperkeratosis and epidermal thickening. In comparison, CHI3L1-KO mice showed significantly reduced symptoms and abrogated CHI3L1 levels (Figure 1). In addition, PA-induced pro-inflammatory marker protein (cyclooxygenase-2 and inducible nitric oxide synthase) expression, inflammatory immune cell infiltration, inflammatory cytokine and chemokine (interleukin [IL]-1 β , IL-4, IL-6, IL-13, thymic stromal lymphoprotein [TSLP] and C-C motif chemokine 22 [CCL22]) and IgE and histamine levels were decreased in the skin tissue or serum compared to those in WT

mice (Figure S2). Moreover, expression of genes encoding inflammatory cytokines and chemokines induced by treatment with a mixture of tumour necrosis factor (TNF)- α and interferon (IFN)- γ , an inflammatory stimulant that induces AD-like features, was inhibited by CHI3L1 knock-down, whereas it was up-regulated by CHI3L1 overexpression in HaCaT cells (Figure S3). Taken together, these findings indicate that CHI3L1 greatly contributes to the AD pathogenesis.

To explore the underlying mechanism of CHI3L1 in AD pathogenesis, we performed gene-network analysis using Humanbase, an interactive platform of data-driven predictions of gene regulation and interaction, which showed that CHI3L1 is linked to numerous genes of interest, including integrin beta2 (ITGB2) (Figure 2A). Integrins are transmembrane adhesion receptors with important roles in biological and pathological processes.⁶ Previous studies have shown that integrin alpha (ITGA) is overexpressed in atopic skin,⁷ and CHI3L1-mediated biological and pathological effects are through interactions with integrins.⁸ To determine the association between CHI3L1 and integrins including ITGB2, ITGA2, ITGA5 and ITGA6, we compared the mRNA expression levels of these integrins in the skin of the PA-induced AD model. Expression of all tested integrins increased in WT mice but decreased in CHI3L1-KO mice; particularly, ITGA5 expressions were significantly down-regulated by CHI3L1-KO (Figure 2B,C). Additionally, gene silencing and overexpression experiments in HaCaT cells demonstrated that ITGA5 expression was more affected by CHI3L1 compared to ITGA6 (Figure 2D,E). Differences in protein expression of ITGA5 were further confirmed in TNF- α /IFN- γ -treated HaCaT cells (Figure 2F) and skin tissues (Figure 2G). We further analysed the gene expression levels of pro-inflammatory mediators after knocking down ITGA5 expression in TNF- α /IFN- γ -treated HaCaT cells and found that the mRNA expressions of IL-1 β , IL-6, TSLP and CCL22 were significantly decreased (Figure 2H).

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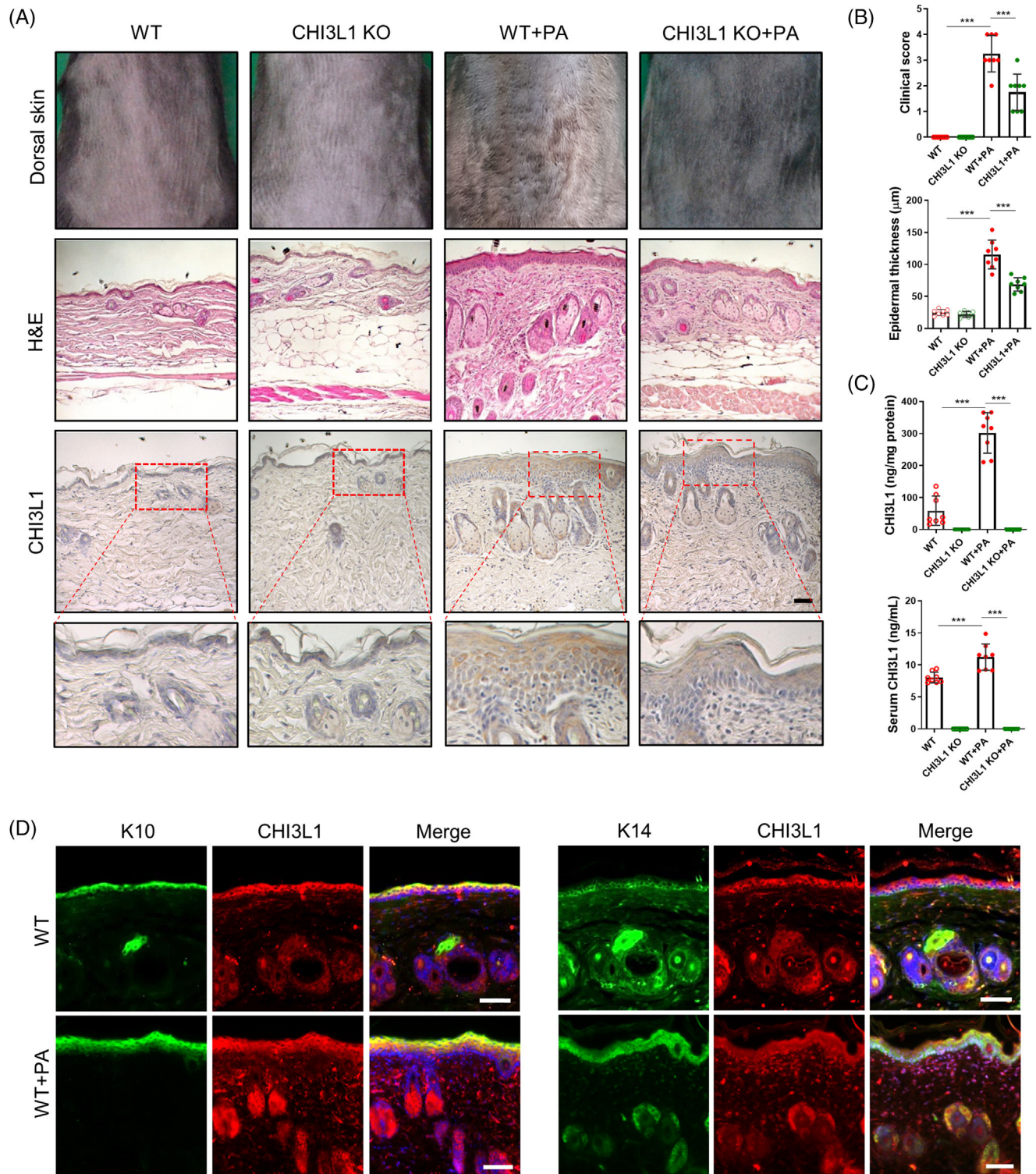


FIGURE 1 Chitinase 3-like protein 1 (CHI3L1) knockout (KO) inhibits atopic dermatitis (AD) development. Wild-type (WT) and CHI3L1 KO mice were treated with 5% phthalic anhydride (PA) for 4 weeks. (A) Morphological (upper images) and histological changes (middle images) in mice after 4-week treatment. Expression of CHI3L1 in PA-induced skin tissues by immunohistochemistry analysis (bottom images). The photographs are representative of each group of mice. Scale bar, 50 μ m. (B) Bar graphs indicate clinical score (upper panel) and epidermal thickness (lower panel). $n = 8$. (C) The concentration of CHI3L1 in mouse skin tissues (upper panel) and serum (lower panel). $n = 8$. (D) Representative immunofluorescence images showing CHI3L1, cytokeratin 10 (K10) and cytokeratin 14 (K14) stained cells in skin tissues. Scale bar, 50 μ m. Data are expressed as the mean $\pm$ standard deviation (SD). *** $p < .001$

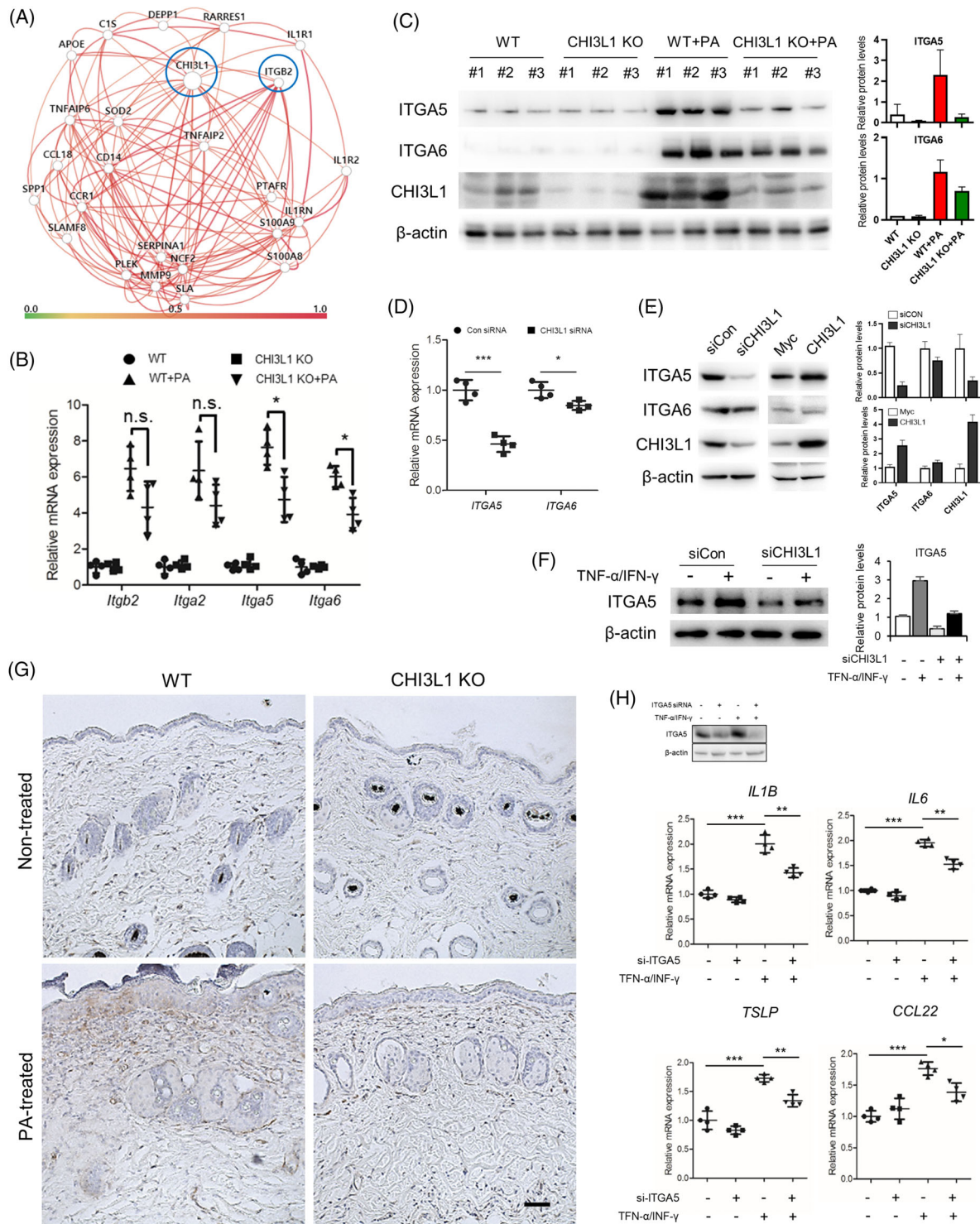


FIGURE 2 Chitinase 3-like protein 1 (CHI3L1) regulates ITGA5 expression. (A) The gene-network map of CHI3L1 and its predicted gene interactions. (B) mRNA expression of *Itgb2*, *Itga2*, *Itga5* and *Itga6* in animal model skin tissues determined by quantitative PCR (qPCR). $n = 4$. (C) Expression of ITGA5, ITGA6 and CHI3L1 in phthalic anhydride (PA)-induced skin tissues detected by Western blot analysis. $n = 3$. (D) HaCaT cells were transfected with human CHI3L1 siRNA (20 nM) for 24 h. mRNA expression of *ITGA5* and *ITGA6* was determined using qPCR. $n = 4$. (E) Expression of ITGA5 and ITGA6 in CHI3L1 knockdown or CHI3L1-overexpressing HaCaT cells detected by Western blot analysis. (F) HaCaT cells were transfected with CHI3L1 siRNA (20 nM). After 24 h, the cells were treated with tumour necrosis factor (TNF)- α and interferon (IFN)- γ (20 ng/ml) for 4 h. Expression of ITGA5 was analysed by Western blot analysis. (G) Expression of ITGA5 in PA-induced skin tissues detected using immunohistochemistry. Scale bar, 50 μ m. (H) HaCaT cells were transfected with human ITGA5 siRNA (20 nM). After 24 h, the cells were treated with tumour necrosis factor (TNF)- α and IFN- γ (20 ng/ml) for 4 h. Knockdown efficiency was determined by Western blot analysis, and mRNA expression of interleukin (IL)1B, IL6, thymic stromal lymphoprotein (TSLP) and *CCL22* was determined using qPCR. $n = 4$. Data are expressed as the mean $\pm$ SD. * $p < .05$, ** $p < .01$ and *** $p < .001$

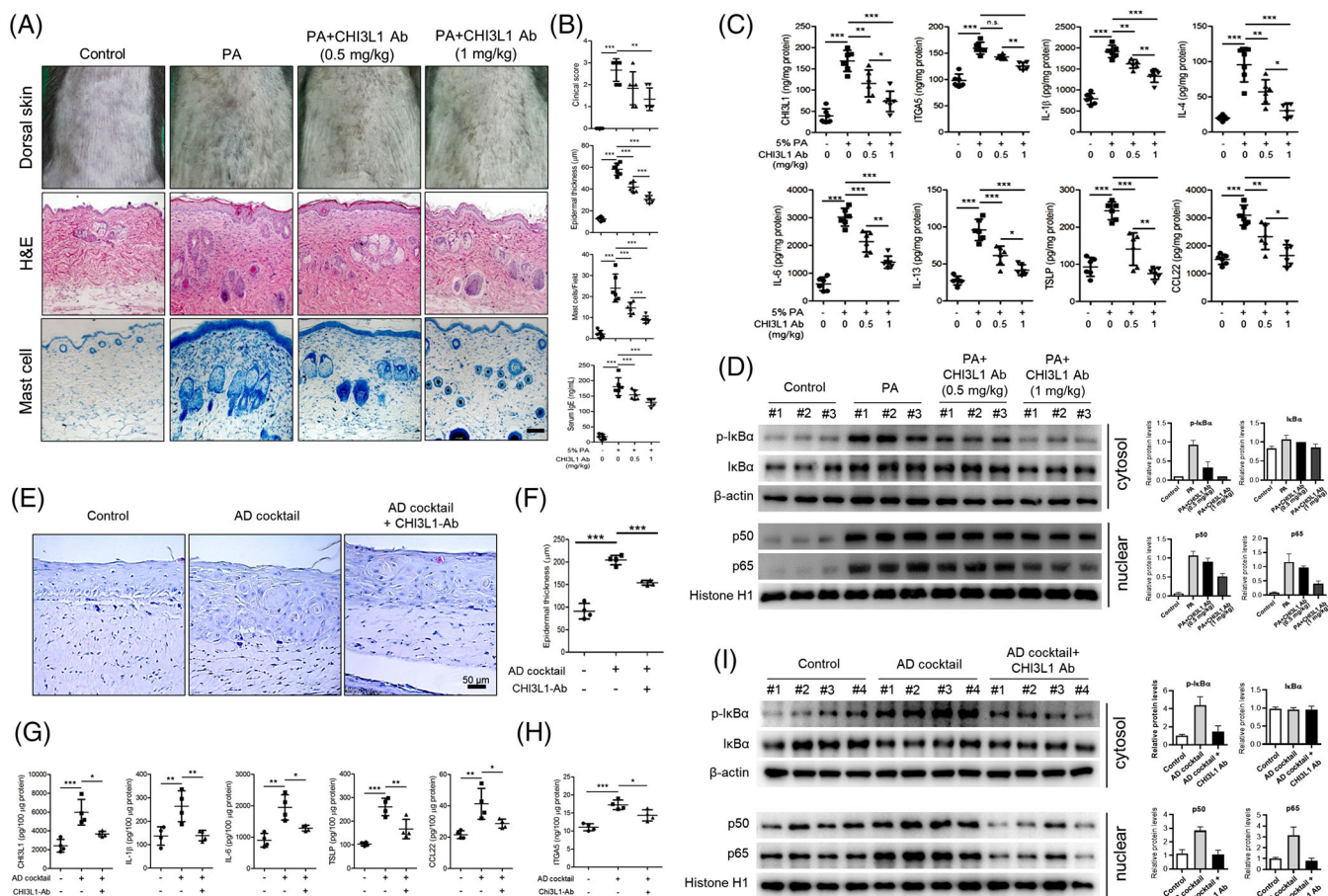


FIGURE 3 Blockade of chitinase 3-like protein 1 (CHI3L1) suppresses atopic dermatitis (AD) skin inflammation in phthalic anhydride (PA)-induced mouse model and AD-like reconstructed human skin (RHS) model. (A–D) Wild-type (WT) mice were treated with 5% PA for 4 weeks. From the third week, CHI3L1 antibody (CHI3L1 Ab; .5 and 1 mg/kg per mouse) was intravenously injected via the tail vein at 3 h after PA treatment. (A) Morphologic (upper images) and histologic changes (middle images) in the mice after CHI3L1 antibody therapy. Scale bar, 50 μ m. (B) Bar graphs indicate the clinical score, epidermal thickness, mast cell number and serum IgE levels. $n = 6$. (C) Protein levels of CHI3L1, ITGA5, interleukin (IL)-1 β , IL-4, IL-6, IL-13, thymic stromal lymphoprotein (TSLP) and CCL22 in PA-induced skin tissues. $n = 6$. (D) Expression of phosphorylated I κ B α in cytosolic fractions and nuclear translocation of p50 and p65 in nuclear fractions in PA-induced skin tissues by Western blot analysis. $n = 3$. (E–I) RHS model cells were cultured with AD cocktail with/without CHI3L1 Ab (500 ng/ml) for 6 days. (E) Histological changes in AD-RHS model. Scale bar, 50 μ m. (F) Bar graph presents epidermal thickness. $n = 4$. (G) Protein levels of CHI3L1, IL-1 β , IL-6, TSLP and CCL22 in AD-RHS model. $n = 4$. (H) Levels of ITGA5 in AD-RHS model. $n = 4$. (I) Expression of phosphorylated I κ B α in cytosolic fractions and nuclear translocation of p50 and p65 in nuclear fractions in AD-RHS model determined by Western blot analysis. $n = 3$. Data are expressed as the mean $\pm$ SD. * $p < .05$, ** $p < .01$ and *** $p < .001$

Notably, transcription factor prediction analysis showed that ITGA5 contains 13 putative nuclear factor (NF)- κ B binding sites (Figure S4A). Interestingly, ITGA5 expression was inhibited by not only NF- κ B p65 siRNA in TNF- α /IFN- γ -treated HaCaT cells (Figure S4B), but also in CHI3L1-overexpressing cells treated with an NF- κ B inhibitor (Figure S4C), indicating that NF- κ B is a key transcription factor regulating ITGA5 expression. As NF- κ B is a critical regulator in AD,⁹ and CHI3L1 is known to bind to the RAGE (receptor for advanced glycation end-products) receptor to contribute to NF- κ B activation, we investigated whether CHI3L1 modulates NF- κ B signaling.¹⁰ CHI3L1 overexpression promoted NF- κ B

signaling activation in resting and TNF- α /IFN- γ -treated HaCaT cells (Figure S5A,B). In contrast, CHI3L1 knockdown reduced both TNF- α /IFN- γ -induced phosphorylation of I κ B α and nuclear translocation of p50/p65 (Figure S5C,D). In addition, CHI3L1 knockdown together with the NF- κ B inhibitor synergistically inhibited IL-1 β , IL-6, TSLP and CCL22 production (Figure S5E). Notably, PA-induced NF- κ B activation in the skin tissues decreased in CHI3L1-KO mice compared to in WT mice (Figure S5F,G). These results suggest that CHI3L1 induces inflammatory responses by activating NF- κ B signaling, which may lead to AD-related skin inflammation. Collectively, our findings suggest that CHI3L1-related

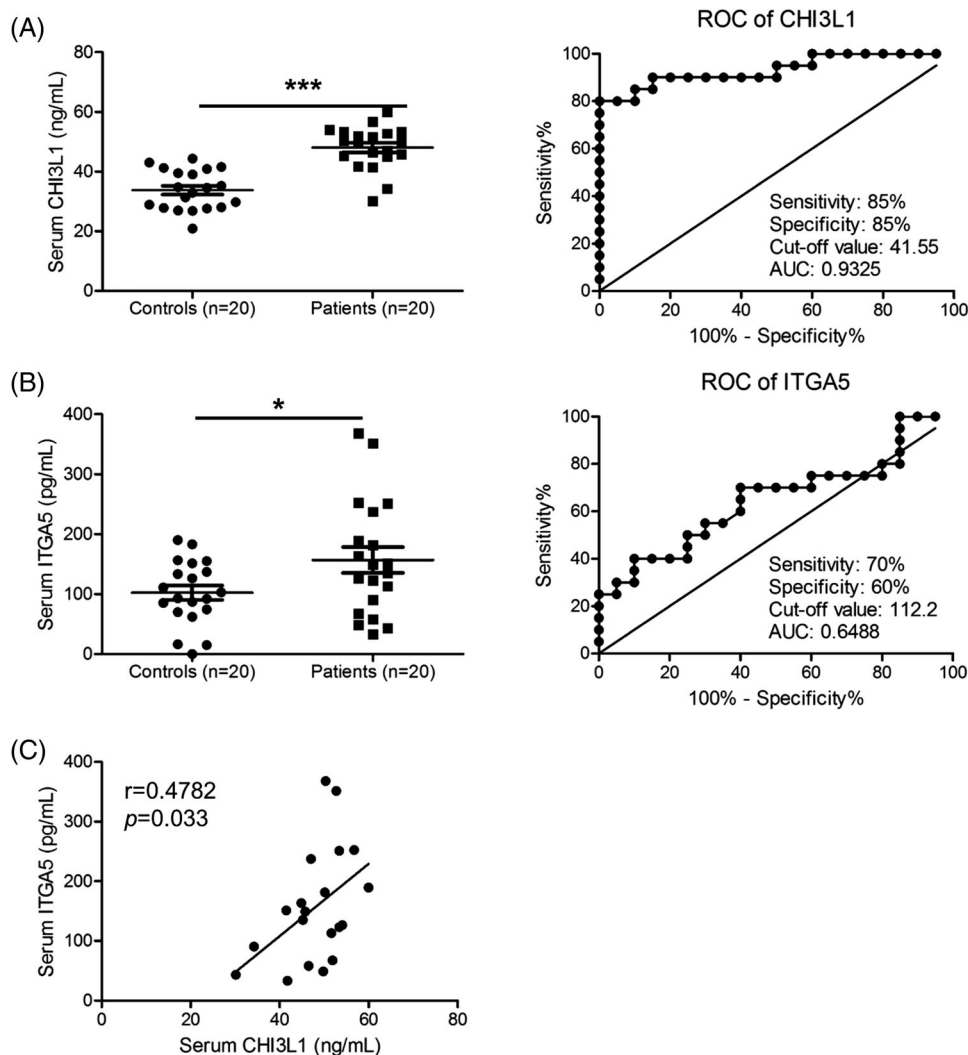


FIGURE 4 Clinical relationship between CHI3L1 and ITGA5 in patients with atopic dermatitis (AD). (A and B) Serum levels and receiver operating characteristic curve of chitinase 3-like protein 1 (CHI3L1) (A) and ITGA5 (B) in patients with AD and healthy controls. $n = 20$. (C) Correlation between CHI3L1 and ITGA5. R value indicates Spearman's correlation co-efficient. Data are expressed as the mean $\pm$ SD. * $p < .05$, and *** $p < .001$

AD pathogenesis is associated with the regulation of the NF- κ B/ITGA5 axis.

After confirming that CHI3L1 regulates AD-related skin inflammation, we examined the therapeutic effect of CHI3L1-antibody (Ab). Administration of a CHI3L1-Ab alleviated PA-induced AD development and the inflammatory response and suppressed the production of CHI3L1 and ITGA5 and activation of NF- κ B (Figure 3A–D). Furthermore, the analysis of the reconstructed human skin (RHS) tissue AD model confirmed that CHI3L1-Ab treatment reduced skin inflammation by inhibiting the CHI3L1/NF- κ B/ITGA5 axis (Figure 3E–I). Additionally, we demonstrated that ITGA5-Ab treatment reduced epidermal thickness in the same RHS AD model (Figure S6).

Finally, we found that the serum levels of CHI3L1, ITGA5 and other AD biomarkers were significantly

increased in sera from patients with AD (Figure 4A,B [left] and Figure S7A). Receiver operating characteristic curve analysis indicated that CHI3L1 and ITGA5 are more suitable diagnostic markers for AD compared to other biomarkers (Figure 4A,B [right] and Figure S7B). Additionally, Spearman's correlation analysis showed that serum level of CHI3L1 significantly correlated with the serum level of ITGA5 (Figure 4C). These results suggest that CHI3L1 and ITGA5 are potent diagnostic and prognostic biomarkers for AD.

In conclusion, CHI3L1 mediates AD-like skin inflammation by regulating NF- κ B-dependent ITGA5 expression. Furthermore, the inhibition of CHI3L1 by CHI3L1-Ab suppresses AD-like symptoms and inflammatory responses by regulating CHI3L1, the NF- κ B/ITGA5 axis. Our findings provide experimental rationale for

developing therapeutic strategies for AD by targeting CHI3L1.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Supporting Information

New therapeutic strategy for atopic dermatitis by targeting CHI3L1/ITGA5 axis

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Supplemental Materials and Methods

Animals and ethical approval

The experimental protocols were carried out according to the guidelines for animal experimentation of the Institutional Animal Care and Use Committee of the Laboratory Animal Research Center at Chungbuk National University, Korea (ethical approval number: CBNUA-1206-18-01). All efforts were made to minimize animal suffering and reduce the number of animals used. All mice were housed with three mice per cage with automatic temperature control (21–25°C), relative humidity (45–65%), and 12-h light-dark cycle illumination, and were provided filtered tap water and rodent chow diet *ad libitum* throughout the experiment. Wild-type (WT) (C57BL/6) mice were purchased from DBL (Eumsung, Korea). CHI3L1 knockout (KO) mice were generated as described in a previous study.¹

PA-induced AD model

Age-matched male WT mice and CHI3L1 KO mice (8-week old) in the C57BL/6 background strain were used. To induce atopic dermatitis (AD) on the skin, 100 µL of 5% phthalic anhydride (PA) solution or vehicle (acetone: olive oil in a 4:1, v/v) was spread on the dorsum of the back skin three times per week for 4 weeks. Clinical scores were evaluated as none (0), mild (1), moderate (2), or severe (3) according to the AD symptoms, erythema (redness), scaling, and itching. For the CHI3L1 therapy experiment, anti-CHI3L1 antibody (0.5 or 1 mg/kg per mouse; polyclonal, Cat# ab180569, Abcam, Cambridge, UK) was intravenously injected via the tail vein at 3 h after PA treatment in the third week.

AD-like reconstructed human skin model

A reconstructed human skin (RHS) model was purchased from TEGO Science, Inc. (Neoderm®-ED, Seoul, Korea). The RHS model contains the epidermis and dermis and generates AD-like inflammation when an inflammatory cocktail is applied, as

previously described, with some modifications ^{2,3}. Briefly, an inflammatory cocktail (AD cocktail; IL-4: 30 ng/mL; IL-13: 30 ng/mL; TNF- α : 3.5 ng/mL; PeproTech, Inc., Rocky Hill, NJ, USA) was added with or without CHI3L1 antibody (500 ng/mL; Abcam) to the maintenance medium (TEGO Science) for 6 days. The culture medium was changed every 48 h. The culture medium was supplied by TEGO Science Inc.

Western blot analysis

Western blot analysis was performed as previously described.⁴ Total 10 μ g of proteins were subjected to SDS-PAGE for separation and then transferred to PVDF membrane. The membranes were incubated with the following specific primary antibodies: β -actin (Santa Cruz Biotechnology, Dallas, TX, USA); iNOS, COX-2, CHI3L1, ITGA5, and ITGA6 (Abcam), and p50, p65, p-IkBa, and IkBa (Cell Signaling Technology, Danvers, MA, USA). Information on the antibodies is presented in Table S1.

Immunohistochemistry

Back skins were removed from the mice, fixed with 10% formalin, embedded in paraffin wax, routinely processed, and then sectioned into 10- μ m-thick slices. Hematoxylin and eosin staining and immunohistochemistry were performed as previously described.⁴ Hematoxylin and eosin stained sections were viewed under a light microscope (Olympus, Tokyo, Japan). Toluidine blue was used to stain the mast cells. For immunohistochemistry, the skin tissue sections were blocked with 3% normal goat serum diluted in PBS, for 30 min; the sections were then incubated with antibodies for phospho-p65, CHI3L1, and ITGA5 (Abcam) and F4/80 (Cell Signaling Technology), at the appropriate dilution in blocking serum, for overnight at 4 °C. The slides were washed in PBS, followed by the avidin–biotin–peroxidase complex (#PK-6101, Vector Laboratories, Burlingame, CA). The slides were washed, and the peroxidase reaction was developed with diaminobenzidine and peroxide (#SK-4100, Vector Laboratories), mounted in Aqua-Mount, and evaluated under a light microscope (Olympus).

79

80 **Immunofluorescence**

81 Back skins were removed from the mice, fixed with 10% formalin, embedded in
82 paraffin wax, routinely processed, and then sectioned into 10- μ m-thick slices.
83 Primary antibodies were incubated for overnight at 4 °C and Alexa Fluor 488
84 (#A32723, #A32731, Invitrogen) or Texas Red (#T-862, #T-2767, Invitrogen)
85 conjugated secondary antibodies were incubated for 1h at room temperature. Fixed
86 cells were incubated with 1 μ g/ml of DAPI (#D9542, Sigma-Aldrich) for 5 min at room
87 temperature and then covered with Fluoromount-G Mounting Medium (#0100-01,
88 Southern Biotech, Birmingham, AL). Cells were visualized using Zeiss AxioObserver
89 (Carl Zeiss, Oberkochen, Germany) fluorescence microscope system. Digital images
90 were analyzed using the imageJ (NIH) or ZEN 2.1 (Carl Zeiss) software. The primary
91 antibodies were as follows: CHI3L1 and K10 (Abcam), K10 (Invitrogen, Carlsbad,
92 CA), and K14 (Santa Cruz Biotechnology). Information on the antibodies is shown in
93 Table S1.

94

95 **Quantitative real-time PCR**

96 Total RNA was extracted from the skin tissues or cells using the Ribo^{EX} RNA
97 Extraction Kit (GeneAll Biotechnology, Seoul, Korea), and cDNA was synthesized
98 using a HighCapacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA).
99 Quantitative real-time PCR was performed using specific primers with SYBR Green
100 master mix (ELPIS Biotech, Daejeon, Korea) in a StepOnePlusTM PCR System
101 (Applied Biosystems) (Table S2). The thermocycling conditions were as follows:
102 initial denaturation of 3 min at 94.0°C, followed by 40 cycles of 94.0°C for 1 min,
103 58.0°C for 30 s, and 72.0°C for 30 s according to the manufacturer's instructions.
104 The values obtained for target gene expression were normalized to GAPDH or 18S
105 and quantified relative to the expression in control samples.

106

107 **Enzyme-linked immunosorbent assay**

108 Blood samples were collected from the mice at the end of the study period. Mouse

cytokines (IL-1 β , IL-4, IL-6, IL-13, TSLP, and CCL22), ITGA5, and serum IgE levels were measured using enzyme-linked immunosorbent assay (ELISA) kits provided by KOMA Biotech (Seoul, Korea), MyBioSource (SanDiego, CA, USA), and R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. Mouse tissue and serum CHI3L1 and human serum CHI3L1 levels were analyzed using specific ELISA kits purchased from R&D Systems according to the manufacturer's instructions. Human cytokines (IL-1 β , IL-4, IL-6, IL-13, TSLP, and CCL22) and human ITGA5 levels were measured using ELISA kits provided by KOMA Biotech, Invitrogen (Carlsbad, CA, USA), and R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. Detailed information is provided in Supplementary Table S1.

Cell culture and transfection

HaCaT cells were kindly provided by Dr. Do Young Yoon (Konkuk University, Seoul, Korea). HaCaT cells were grown at 37°C in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin in a humidified atmosphere of 5% CO₂. HaCaT cells were transfected with human CHI3L1 siRNA, human ITGA5 siRNA (Origene Technologies, Inc., Rockville, MD, USA), human p65 siRNA (Santa Cruz Biotechnology), and its control siRNAs using Lipofectamine RNAiMAX reagent (Invitrogen), and then stimulated with TNF- α /IFN- γ (20 ng/mL; Peprotech) for the times indicated in the figure legends. To overexpress CHI3L1, HaCaT cells were transfected with the pcDNA3.1(+)-6xMyc-CHI3L1 vector or control vector using Lipofectamine 3000 transfection reagent (Invitrogen).

Luciferase assay

HaCaT cells were transfected with a luciferase-expressing NF- κ B plasmid vector (Promega, Madison, WI, USA) for 24 h. The cells were then transfected with human CHI3L1 siRNA for another 24 h. The transfected cells were treated with TNF- α /IFN- γ (20 ng/mL) for 24 h. Luciferase activity was measured using a luciferase assay kit (GeneCopoeia, Rockville, MD, USA) following the manufacturer's instructions.

Human samples

Human serum samples from patients with AD and normal controls were obtained from the Chungbuk National University Hospital Biobank and the Korea Institute of Radiological & Medical Sciences Radiation Biobank. All studies using human samples were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of Chungbuk National University Medical Center (IRB No. CBNU-201902-BR-786-01).

Web-based analysis

The gene-disease network of CHI3L1 was analyzed using the Open Targets platform (<https://www.opentargets.org/>) and Disease-Connect web server (<http://www.disease-connect.org>). The Open Targets platform is based on rare and common disease genetics, somatic mutations in cancer, transcriptomics, approved drugs and clinical candidates, animal models, biochemical pathways, and text mining from the medical literature. The Disease-Connect web server is based on various sources, genome-wide association studies, the Online Mendelian Inheritance in Man, GeneRIF, and GeneWays. The CHI3L1-related gene network was analyzed using Humanbase (biological process-specific interactions set as global; <https://hb.flatironinstitute.org/>). Humanbase provides data-driven predictions of gene expression, function, regulation, and interactions in humans, particularly in the context of specific cell types/tissues and human diseases.

Statistical analysis

The experiments were conducted in triplicate, and all experiments were repeated at least three times and showed similar results. All statistical analyses were performed using GraphPad Prism 4 software (GraphPad, Inc., San Diego, CA, USA). Group differences were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test. All values are presented as the mean $\pm$ standard deviation. Significance was set at $p < 0.05$.

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186 **Supplementary Tables**

187 Table S1. Information of antibodies, kits, and reagents

Name	Cat. No.	Application	Supplier
Antibody			
CHI3L1	ab180569	WB, IHC-P	Abcam
ITGA5 [EPR7854]	ab150361	WB, IHC-P	Abcam
ITGA6	A3236	WB	Abclonal
iNOS	ab15323	WB	Abcam
COX-2 [EPR12012]	ab179800	WB	Abcam
Phospho-p65	ab86299	IHC-P	Abcam
Histone H1	ab61177	WB	Abcam
Phospho-IkBα	5209	WB	Cell Signaling
IkBα	4814	WB	Cell Signaling
p65 (D14E12)	8242	WB	Cell Signaling
F4/80 (D2S9R)	70076	IHC-P	Cell Signaling
Phospho-ERK (D13.14.4E)	4370	WB	Cell Signaling
ERK (137F5)	4695	WB	Cell Signaling
Myc-tag (71D10)	2278	WB	Cell Signaling
p50 (NLS)	sc-114	WB	Santa Cruz Bio
Cytokeratin 10	MA1-06319	IF	Invitrogen
Cytokeratin 14 (LL001)	Sc-53253	IF	Santa Cruz Bio
β-actin (C4)	sc-47778	WB	Santa Cruz Bio
Kits			
Human Chi3L1 ELISA Kit	DY2599	ELISA	R&D systems
Human ITGA5 ELISA Kit	DY1864-05	ELISA	R&D systems
Human CCL22 ELISA Kit	DY336	ELISA	R&D systems
Human IL-4 ELISA Kit	BMS225-2	ELISA	Invitrogen
Human IL-13 ELISA Kit	BMS231-3	ELISA	Invitrogen

Human IL-1 β ELISA Kit	K0331800HS	ELISA	KOMA Biotech
Human IL-6 ELISA Kit	K0331194HS	ELISA	KOMA Biotech
Mouse Chi3L1 ELISA Kit	DY2649	ELISA	R&D systems
Mouse ITGA5 ELISA Kit	MBS2887565	ELISA	MyBioSource
Mouse IL-1 β ELISA Kit	K0331231	ELISA	KOMA Biotech
Mouse IL-4 ELISA Kit	K0331144	ELISA	KOMA Biotech
Mouse IL-6 ELISA Kit	K0331230	ELISA	KOMA Biotech
Mouse IL-13 ELISA Kit	K0331201	ELISA	KOMA Biotech
Mouse IgE ELISA Kit	K3231082	ELISA	KOMA Biotech
Mouse TSLP ELISA Kit	MTLP00	ELISA	R&D systems
Mouse CCL22 ELISA Kit	MCC220	ELISA	R&D systems
qPCR 2x Master Mix (SYBR Green)	EBT-1802	qPCR	ELPIS Biotech
High-Capacity cDNA Reverse Transcription Kit	4368813	qPCR	Applied Biosystems
VECTASTAIN® Elite ABC-HRP Kit	PK-6101	IHC-P	Vector Laboratories
NovaUltra Toluidine Blue Stain Kit	IW-3013	IHC-P	IHC World
Secrete-Pair™ Dual Luciferase Assay Kit	LF033	Luciferase assay	GeneCopoeia
Reagents			
Recombinant human TNF- α	300-01A		PeproTech
Recombinant human IFN- γ	300-02		PeproTech
Recombinant human IL-4	200-04		PeproTech
Recombinant human IL-13	200-13		PeproTech
Lipofectamine® 3000	L3000015	Transfection	Invitrogen
Lipofectamine® RNAiMAX	13778150	Transfection	Invitrogen
Phthalic anhydride	320064		Sigma Aldrich
BAY 11-7082 (NF- κ B inhibitor)	B5556		Sigma

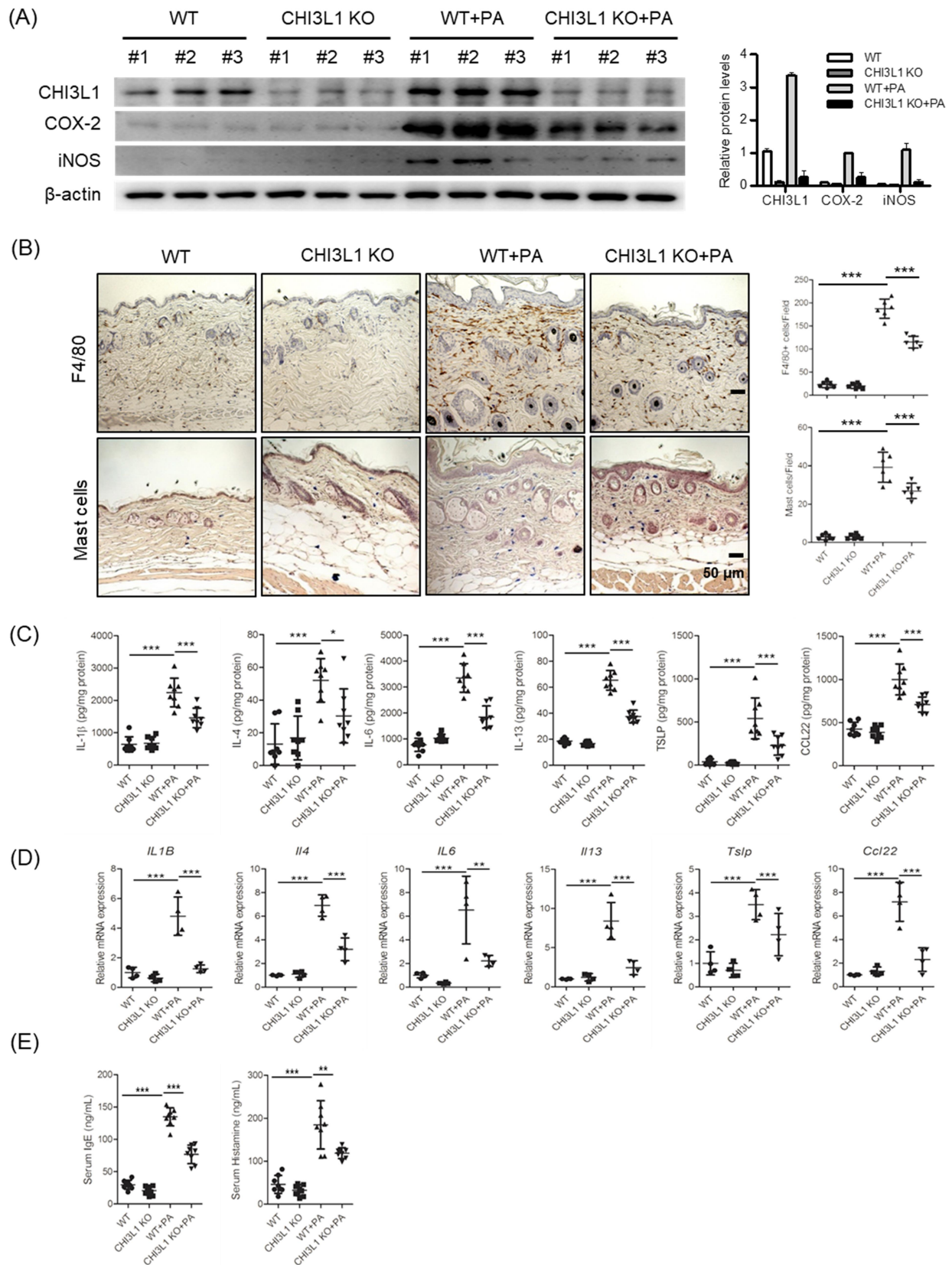
189 Table S2. Primer sequence information

Primer	Species	Sequence (Forward)	Sequence (Reverse)
qPCR			
<i>18S</i>		AGGAATTGACGGAAGGGCACCA	GTGCAGCCCCGGACATCTAAG
<i>Il1b</i>	mouse	CCTTCCAGGATGAGGACATGA	TGAGTCACAGAGGATGGGCTC
<i>Il4</i>	mouse	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
<i>Il6</i>	mouse	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
<i>Il13</i>	mouse	CCTGGCTCTTGCTTGCCTT	GGTCTTGTGTGATGTTGCTCA
<i>Tslp</i>	mouse	ACGGATGGGGCTAACTTACAA	AGTCCTCGATTGCTCGAACT
<i>Ccl22</i>	mouse	CTCTGCCATCACGTTTAGTGAA	GACGGTTATCAAAACAACGCC
<i>Itgb2</i>	mouse	CAGGAATGCACCAAGTACAAAGT	CCTGGTCCAGTGAAGTTCAGC
<i>Itga2</i>	mouse	TGTCTGGCGTATAATGTTGGC	CTTGTGGGTTCGTAAGCTGCT
<i>Itga5</i>	mouse	CTTCTCCGTGGAGTTTTACCG	GCTGTCAAATTGAATGGTGGTG
<i>Itga6</i>	mouse	TGCAGAGGGCGAACAGAAC	GCACACGTCACCACTTTGC
<i>GAPDH</i>	human	GACAAGCTTCCCGTTCTCAG	CAATGACCCCTTCATTGACC
<i>IL1B</i>	human	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
<i>IL6</i>	human	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTTCAGGTTG
<i>TSLP</i>	human	TAGCAATCGGCCACATTGCC	CTGAGTTTCCGAATAGCCTG
<i>CCL22</i>	human	GTTGTCCTCGTCCTCCTTGC	GGAGTCTGAGGTCCAGTAGAAGTG
<i>ITGA5</i>	human	GGCTTCAACTTAGACGCGGAG	TGGCTGGTATTAGCCTTGGGT
<i>ITGA6</i>	human	GGCGGTGTTATGTCCTGAGTC	AATCGCCCATCACAAAAGCTC
Genotyping			
<i>Chi3l1</i>	WT	TGACACTGGATGGCACAACT	TGCACAGGAAGGTTGGAT
<i>Chi3l1</i>	KO	CTTACCAGACGCCATCCTTCGT	CATGTTACCATCCCAACACAGC

193

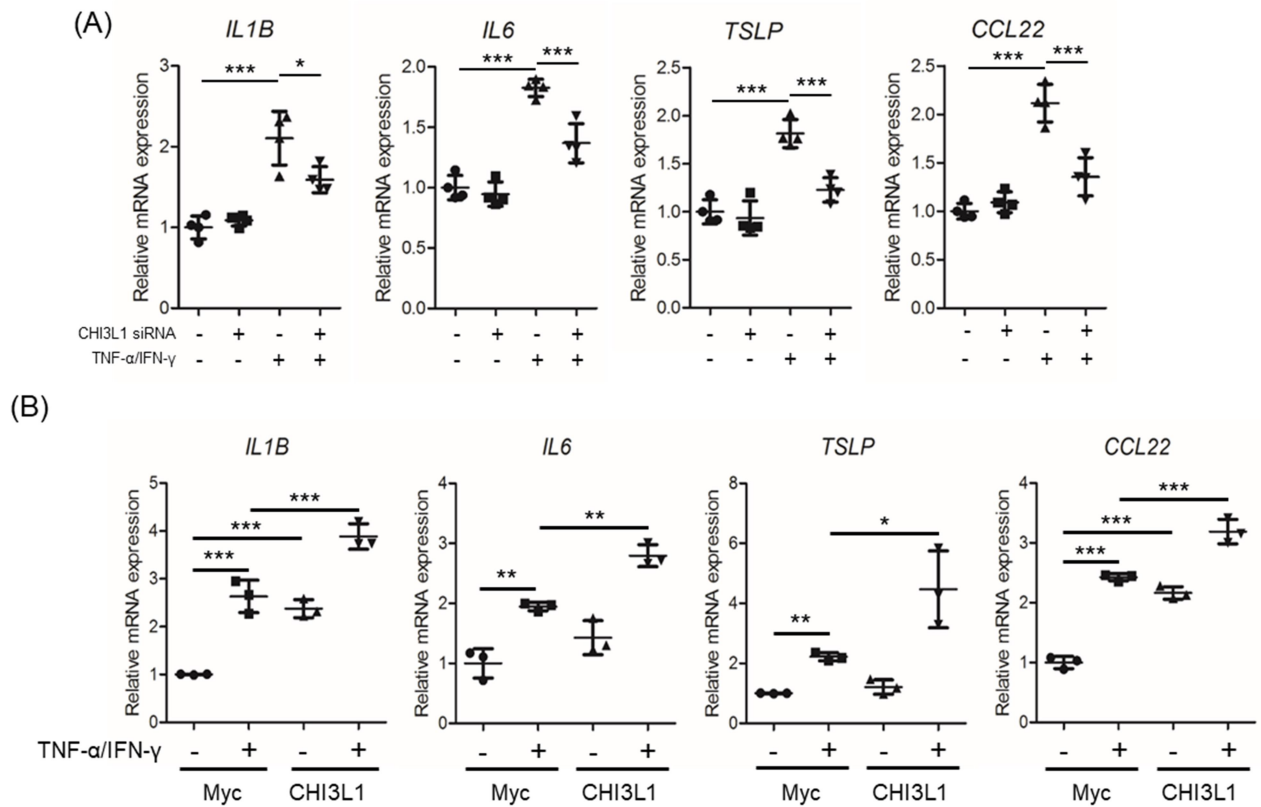


195



Supplementary Figure S2. CHI3L1 KO suppresses expression of inflammation markers and inflammatory cell infiltration in PA-induced mouse model. WT and CHI3L1 KO mice were treated with 5% PA for 4 weeks. (A) Expression of inflammation

200 markers, COX-2 and iNOS, in PA-induced skin tissues of WT and CHI3L1 KO mice. (B)
201 Representative immunohistochemistry images showing F4/80 (macrophage) and toluidine
202 blue (mast cell) stained cells in PA-induced skin tissue sections. Scale bar, 50 μ m. Bar
203 graphs indicate stained cells quantified by counting the number of positive cells in the field.
204 $n = 6$. (C) Levels of AD-related inflammatory cytokines and chemokines, IL-1 β , IL-4, IL-6,
205 IL-13, TSLP and CCL22, in skin tissues. $n=8$. (D) The mRNA expression of AD-related
206 inflammatory cytokines and chemokines, *Il1b*, *Il4*, *Il6*, *Il13*, *Tslp* and *Ccl22*, in skin tissues.
207 $n=4$. (E) Serum IgE and histamine levels in PA-induced mice. $n=8$. Data are expressed as
208 the mean $\pm$ S.D. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.



Supplementary Figure S3. CHI3L1 regulates skin inflammatory cytokines and chemokines. (A) HaCaT cells were transfected with CHI3L1 siRNA (20 nM). After 24 h, the cells were treated with TNF- α /IFN- γ (20 ng/ml). After 24 h, the cells were treated with TNF- α /IFN- γ for 4 h. mRNA expression of *IL1B*, *IL6*, *TSLP*, and *CCL22* was analyzed using qPCR. $n=4$. (B) HaCaT cells were transfected with CHI3L1 plasmid vector. After 24 h, the cells were treated with TNF- α /IFN- γ for 4 h. mRNA expression of *IL1B*, *IL-6*, *TSLP*, and *CCL22* was analyzed using qPCR. $n = 3$. Data are expressed as the mean $\pm$ S.D. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

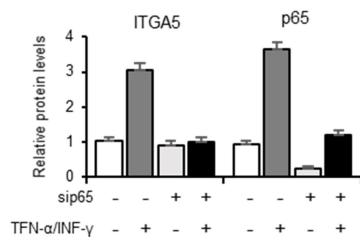
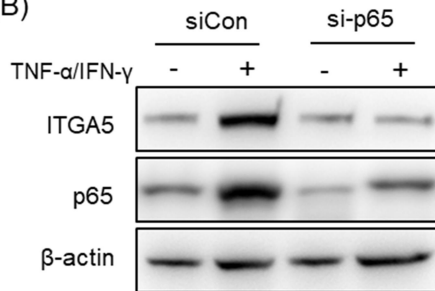
(A)

Prediction of transcription factors for ITGA5 (-1954 to 46)

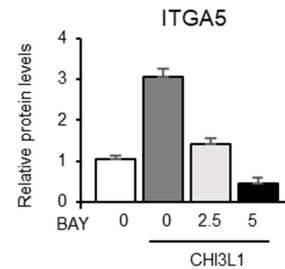
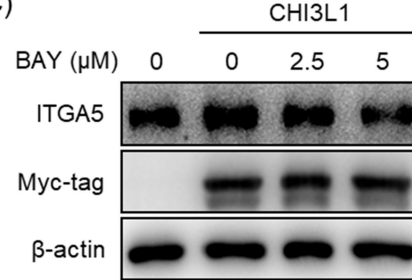
0 C/EBPbeta [T00581]	1 NF-1 [T00539]	2 c-Myc [T00140]	3 USF1 [T00874]
8 p53 [T00671]	9 TFII-I [T00824]	10 TFIID [T00820]	11 SRY [T00997]
16 NF-kappaB [T00590]	17 NF-kappaB1 [T00593]	18 c-Ets-1 [T00112]	19 RelA [T00594]
24 T3R-beta1 [T00851]	25 ER-alpha [T00261]	26 RXR-alpha [T01345]	27 RAR-alpha1 [T00719]
32 AP-1 [T00029]	33 c-Fos [T00123]	34 IRF-1 [T00423]	35 NF-AT1 [T00550]
40 MAZ [T00490]	41 Elk-1 [T00250]	42 PU.1 [T02068]	43 EBF [T05427]
48 PXR-1:RXR-alpha [T05671]	49 c-Myb [T00137]	50 PEA3 [T00685]	51 LEF-1 [T02905]
56 AR [T00040]	57 Sp1 [T00759]	58 Egr-3 [T00243]	59 HNF-3alpha [T02512]
64 E2F [T00221]	65 HNF-4alpha [T03828]	66 RAR-beta:RXR-alpha [T05420]	67 USF2 [T00878]
72 ELF-1 [T01113]			
4 GR-beta [T01920]	5 GR-alpha [T00337]	6 AP-2alphaA [T00035]	7 Pax-5 [T00070]
12 TCF-4E [T02878]	13 NF/CTF [T00094]	14 GR [T05076]	15 STAT4 [T01577]
20 YY1 [T00915]	21 Ik-1 [T02702]	22 XBP-1 [T00902]	23 ENKTF-1 [T00255]
28 PPAR-alpha:RXR-alpha [T05221]	29 c-Jun [T00133]	30 C/EBPalpha [T00105]	31 c-Ets-2 [T00113]
36 FOXP3 [T04280]	37 PR B [T00696]	38 PR A [T01661]	39 IRF-2 [T01491]
44 NF-AT2 [T01945]	45 E2F-1 [T01542]	46 GATA-1 [T00306]	47 VDR [T00885]
52 NF-AT1 [T01948]	53 STAT1beta [T01573]	54 RAR-beta [T00721]	55 ATF3 [T01313]
60 POU2F2 (Oct-2.1) [T00646]	61 WT1 [T00899]	62 ETF [T00270]	63 AhR:Arnt [T05394]
68 ME2A [T01005]	69 HOXD9 [T01424]	70 HOXD10 [T01425]	71 GATA-2 [T00308]

NF-kB: 4 sites / NF-kB1: 4 sites / RelA: 5 sites

(B)

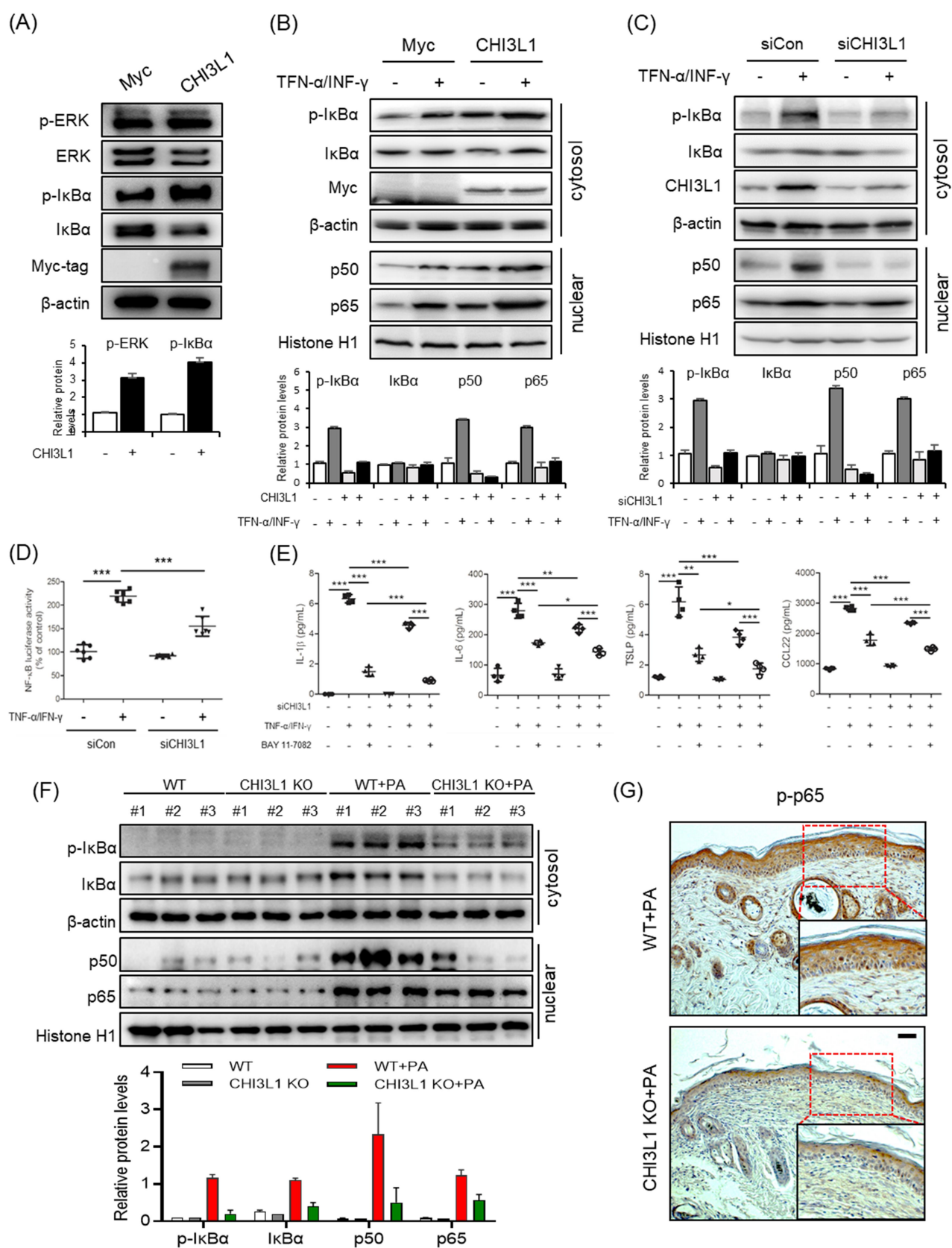


(C)



218

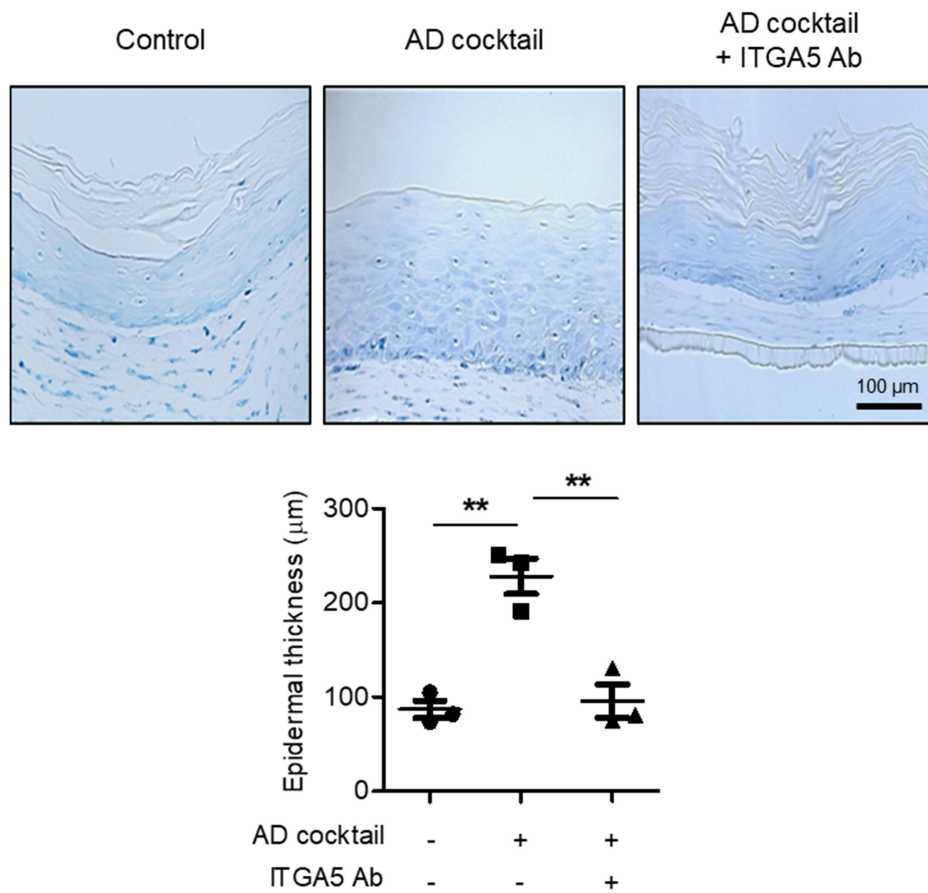
219 **Supplementary Figure S4. ITGA5 is regulated by NF- κ B signaling.** (A) Prediction of
220 transcription factor binding sites at ITGA5 promotor region. (B) HaCaT cells were
221 transfected with human p65 siRNA (20 nM). After 24 h, the cells were treated with TNF- α
222 and IFN- γ (20 ng/mL) for 4 h. Expression of ITGA5 was analyzed by Western blot analysis.
223 (C) HaCaT cells were transfected with CHI3L1 plasmid vector for 24 h. Cells were treated
224 with BAY 11-7082 (BAY; NF- κ B inhibitor). Expression of ITGA5 was analyzed by Western
225 blot analysis. Data are expressed as the mean $\pm$ S.D.



226

227 **Supplementary Figure S5. CHI3L1 controls NF- κ B activation.** (A) HaCaT cells were
 228 transfected with CHI3L1 plasmid vector for 24 h. Expression of phosphorylated ERK (p-

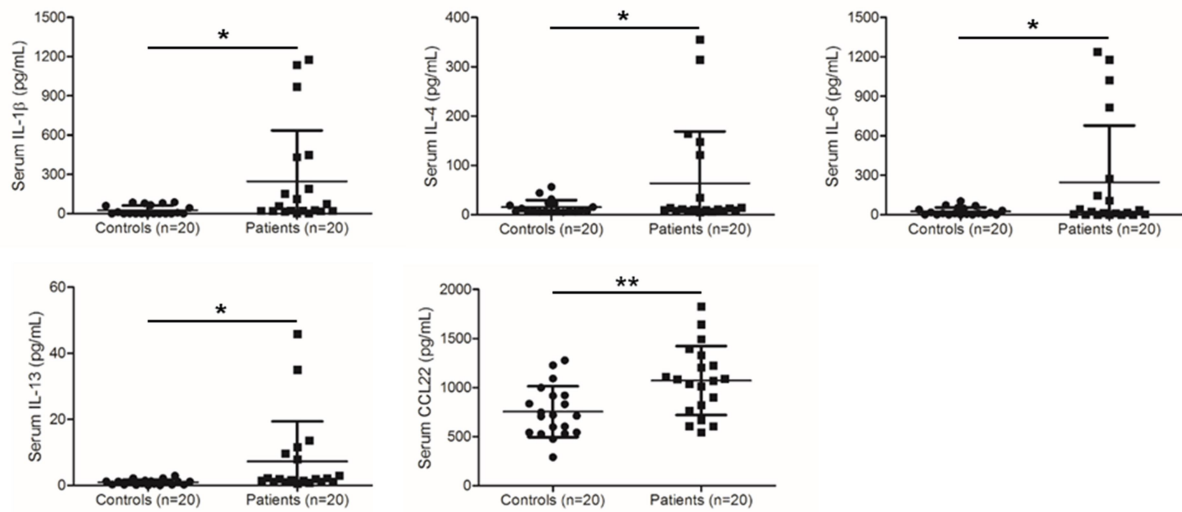
229 ERK) and phosphorylated I κ B α (p-I κ B α) determined by Western blot analysis. (B) HaCaT
230 cells were transfected with CHI3L1 plasmid vector. After 24 h, the cells were treated with or
231 without TNF- α /IFN- γ for 4 h. Expression of I κ B α in cytosolic fractions and nuclear
232 translocation of p50 and p65 in nuclear fractions determined in Western blot analysis. (C
233 and D) HaCaT cells were transfected with CHI3L1 siRNA (20 nM). After 24 h, the cells were
234 treated with TNF- α /IFN- γ (20 ng/ml) for 24 h. Expression of phosphorylated I κ B α in the
235 cytosolic fractions and nuclear translocation of p50 and p65 in nuclear fractions determined
236 in western blot analysis (C). NF- κ B transcriptional activity was measured in a luciferase
237 assay using transfection of luciferase-expressing NF- κ B plasmid vector (D). $n = 6$. (E)
238 HaCaT cells were transfected with human CHI3L1 siRNA (20 nM). After 24 h, the cells were
239 treated with TNF- α /IFN- γ with BAY 11-7082 (2.5 μ M; NF- κ B inhibitor) for 24 h. The levels of
240 pro-inflammatory mediators IL-1 β , IL-6, TSLP, and CCL22 were analyzed using ELISA. $n =$
241 4. (F and G) WT and CHI3L1-KO mice were treated with 5% PA for 4 weeks. Expression of
242 phosphorylated I κ B α in cytosolic fractions and nuclear translocation of p50 and p65 in
243 nuclear fractions in PA-induced skin tissues by western blot analysis (F). Expression of
244 phosphorylated p65 (p-p65) in PA-induced skin tissues by IHC analysis (G). The
245 immunohistological images are representative of each group of PA-induced skin tissues.
246 Scale bar, 50 μ m. Data are expressed as the mean $\pm$ S.D. ** $p < 0.01$ and *** $p < 0.001$.



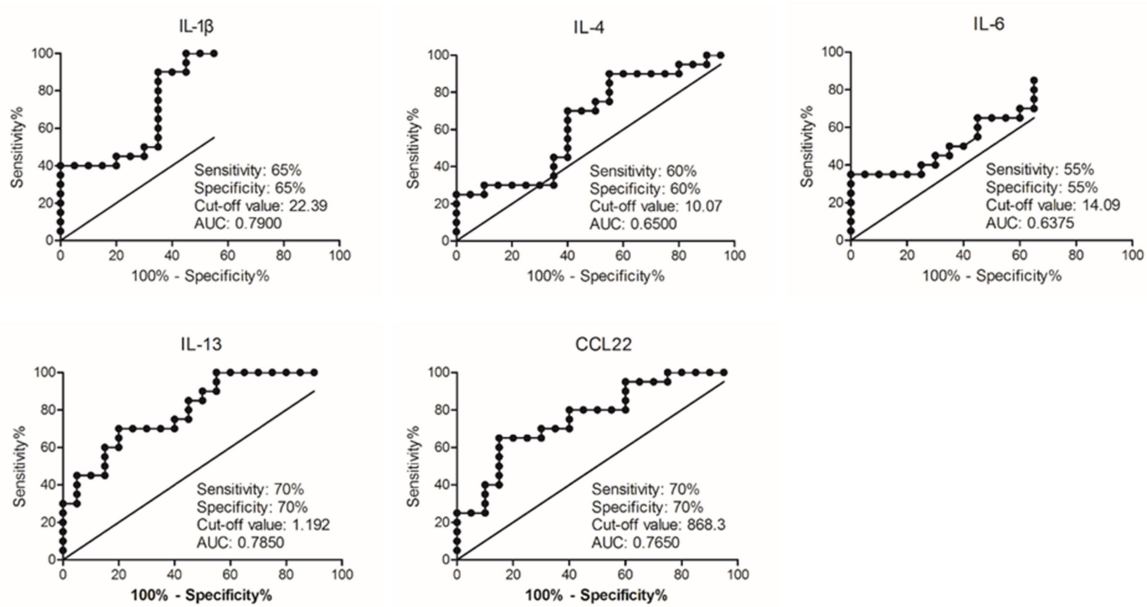
247

248 **Supplementary Figure S6. Depletion of ITGA5 reduces epidermal thickness in AD-like**
 249 **RHS model.** RHS model cells were cultured with AD cocktail with/without ITGA5 antibody
 250 (1 μg/mL) for 6 days. Histological changes in AD-RHS model. Bar graph presents
 251 epidermal thickness. $n = 3$. Scale bar, 100 μm. Data are expressed as the mean $\pm$ S.D. * $p <$
 252 0.05 and ** $p < 0.01$.

(A)



(B)



253

254 **Supplemental Figure S7. Serum analysis of AD-related biomarkers in patients with**
255 **AD.** (A and B) Serum levels (A) and ROC curves (B) of IL-1 β , IL-4, IL-6, IL-13, and CCL22
256 in patients with AD and healthy controls. $n = 20$. Data are expressed as the mean $\pm$ S.D. * p
257 < 0.05 and ** $p < 0.01$.